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Review Papers

Edited by Prof. Niamh Nic Daéid

Centre for Forensic Science, University of Strathclyde, Glasgow, UK

TABLE OF CONTENTS

Drug Evidence

Review 2010 – June 30, 2013

Jeffrey H. Comparin
Robert F.X. Klein

U.S. Department of Justice
Drug Enforcement Administration
Office of Forensic Sciences
8701 Morrissette Drive
Springfield, VA 22152 USA
(Coordinating Office)

and

Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166 USA
(Coordinating Laboratory)

TABLE OF CONTENTS

Preface Notes:

1. With the exception of synthetic cannabinoids and cannabimimetics, all references are subdivided by individual drug, drug group or class, or general topic, then chronologically, and finally alphabetically within each year (first author's last name). Individual synthetic cannabinoids and cannabimimetics are included in that drug group (i.e., not as individual drugs). In addition, and in contrast to past reports from this laboratory, references are organized as much as is practical by specific drug or drug group/class. This change is necessary because of the large numbers of similar types of "designer drugs," most notably the synthetic cannabinoids and cannabimimetics, the cathinones and related amphetamine-type-stimulants, and the methylenedioxyphenethyl-amines and related hallucinogens.

2. References from January 1, 2010 to June 30, 2010 are included because many were either not cited in the last review (because they had not yet been abstracted or printed), or were cited as "Ahead of Print" (i.e., without volume, issue, or page numbers). Some of the references from January 1, 2013 to June 30, 2013 in this report are similarly cited as "Ahead of Print;" all such references were still in "Ahead of Print" status as of June 30, 2013. Readers should be aware that the year listed with "Ahead of Print" may not reflect the eventual year of publication; however, the article's author(s), article title, and journal should remain the same regardless of the actual year of publication, allowing the full citation to be easily found by Internet searching.

3. Note that the following reference is law enforcement restricted, and is not available to the general public: *Journal of the Clandestine Laboratory Investigating Chemists Association* (all years). All other references cited in this report were acquired from the "Forensic Chemistry" sections of *Chemical Abstracts*, and to the author's knowledge are non-restricted. [Please also note that the second quarterly issue of the 2013 *Journal of the Clandestine Laboratory Investigating Chemists Association* (i.e., 2013; 23(2)) had not been published by the reference cutoff date, June 30, 2013.]

1 General Overview:

Production, trafficking, and use of illicit drugs are not static situations, but rather are undergoing continuous change. The worldwide situation is significantly different since the last Symposium, and dramatically different versus 10 years ago. The most noteworthy change over the past decade has been the explosive expansion of so-called "designer drugs" (aka "legal highs"); i.e., substances that mimic the effects of controlled substances but that are not themselves controlled upon first appearance. Produced by semi-legitimate or rogue laboratories, widely sold over the Internet, and marketed under deliberately innocuous and misleading labels such as "research chemicals / not for human use," or as "smoking blends," "bath salts," "plant foods," etc., such drugs can either be structurally similar analogues of a controlled substance, or structurally dissimilar but still mimicking the effects of

a controlled substance. While nearly all such substances are eventually controlled, the legal processes for doing so are slow in comparison with their appearance and recognition as drugs of abuse – and new substances often appear immediately upon the control of existing substances, in obvious, direct response to the scheduling action(s).

In considering this situation from a law enforcement perspective, it is important to recognize that *most* controlled substance statutes (worldwide) specifically name every compound being scheduled; therefore, it would be quite challenging – though not impossible – to craft general statutes that would control abused substances based on their pharmacological / physiological effects as opposed to their names or structures. To date, however, no nation has enacted or to the author's knowledge even attempted to draft such legislation. In the U.S., the Controlled Substance Analogue Act (1986) and the Positional Isomer clause for Schedule I hallucinogens (2007) were efforts towards broader control of abused substances based on structural similarities; however, both are somewhat subjective with respect to interpretation and enforcement, often result in lengthy legal proceedings upon prosecution, and (more importantly) cannot address structurally dissimilar mimic compounds. Analogous statutes in other nations are either overly broad or restrictively narrow – and are similarly subjective. As a whole, this situation is now a major issue for forensic laboratories tasked with analyzing drugs of abuse. Long predicted as the wave of the future, "designer drugs" have arrived – and are here to stay.

Although a wide variety of "designer drugs" have appeared over the past decade, the most notable are the synthetic cannabinoids and cannabimimetics – of which over a hundred have already been identified. Others include the cathinones, the methylenedioxy-, dimethoxy-, and trimethoxy- phenethylamines, and the piperazines; these latter drugs, however, are for the most part utilized at only low to moderate levels.

Synthetic cannabinoids are compounds that are structurally similar to delta-9-tetrahydrocannabinol (THC), the active component of marijuana. Cannabimimetics are compounds with chemical structures that bear no resemblance to THC but that mimic the pharmacological / physiological effects of THC in the body. While many law enforcement personnel and forensic chemists use the terms interchangeably, the distinction is important because most synthetic cannabinoids are automatically controlled under U.S. law, whereas virtually all cannabimimetics are not controlled upon initial appearance and must be scheduled on a case-by-case basis (which is, as noted above, a process that can take many months and in some cases several years).

Although these compounds are occasionally seized in bulk quantities (i.e., as pure chemicals), in the vast majority of cases they are encountered as so-called "synthetic marijuana;" that is, in trace to low-level quantities laced onto mixtures of plant materials, intended for smoking similarly as marijuana. Such materials are commonly marketed in small foil packets with attractive labeling and naming. As an additional complication, it is routine for such products to

contain mixtures of synthetic cannabinoids and/or cannabimimetics, and further a few submissions have been found to consist of synthetic cannabinoids and/or cannabimimetics laced onto marijuana, Salvia divinorum, Kratom, or other psychoactive plant materials.

Nearly all of these compounds were originally developed by legitimate scientists who were researching the CB1 and CB2 cannabinoid receptors in the human body. In many / most cases, they are significantly more potent than THC – and in some cases far more potent. For this reason, their use was initially difficult (and for the cannabimimetics, impossible) to detect via standard marijuana drug screening tests. This, along with the non-controlled status of the cannabimimetics when first marketed, were quickly recognized and widely publicized by the drug-using communities, resulting in explosive growth in the use of "synthetic marijuana" type products. This use has since begun to level off, due to the passage of laws that controlled known compounds, the publication of numerous cases of negative and sometimes bizarre experiences resulting from their use, new drug tests, and a major, continuing law enforcement effort (in the U.S.) against the manufacturers and suppliers. New compounds and products are appearing continuously, however, and this situation is expected to continue for many years to come.

Actual marijuana use continues to grow steadily throughout the U.S., with both massive imports and domestic production filling an appetite among domestic consumers. The average potency (percent THC) of federally-seized marijuana continues to increase, and in 2012 was around 15 percent (due to budgetary contraints, however, this figure does not include any state or local seizures, which are usually of significantly lower potency).

The second most noteworthy change over the past decade has been the dramatic increase in the abuse of pharmaceutical opioids, especially those containing oxycodone, hydrocodone, and hydromorphone. Very widely prescribed for a variety of physical injuries, and improperly considered as "safe" with respect to abuse potential, their use has resulted in a large population of addicts, and many thousands of deaths. Efforts to restrict production and use (including the development of "abuse-resistant" time-release formulations) have resulted in a thriving black market, with genuine pharmaceutical products selling for extreme markups (as much as $50 USD *per tablet* for high-dose products). Clandestinely-produced mimic tablets containing heroin, fentanyl, or other narcotics are sold nationwide, expanding the problem and resulting in additional overdoses and deaths from multi-drug intoxications. In addition, "traditional" heroin abuse is rising very rapidly, as street-level heroin is easily obtained and at lower expense versus prescription opioids. As a result, heroin overdose deaths are rapidly increasing throughout the U.S., and several surges in fentanyl overdose deaths have also occurred over the past decade (including some involving more potent fentanyl derivatives).

The production and use of methamphetamine continues at a high level in the U.S. Over the past decade, high-purity, Mexican-produced methamphetamine has essentially taken over the U.S. markets. Small-scale

domestic laboratories are still in widespread operation, but their percentage of the domestic market is, on a relative basis, tiny. Across the U.S., the percentage of bills (currency) contaminated by methamphetamine is approaching and in some areas exceeding that by cocaine.

The domestic use of Ecstasy continues at recent levels, but with a notable difference in tablet composition. Although the term “Ecstasy” has historically referred to tablets containing 3,4-methylenedioxymethamphetamine (MDMA), or to a lesser extent 3,4-methylenedioxyamphetamine (MDA), the continuous increase over the past 15 years of combination or mimic tablets containing any number of active ingredients has effectively rendered this term almost meaningless. Indeed, it is now common for forensic laboratories to receive “Ecstasy Tablets” that contain no MDMA or MDA whatsoever. For this reason “Ecstasy Tablets” are now more properly categorized as “Polydrug.”

Other rising drugs of abuse in the U.S. include Attention Deficit/Hyperactivity Disorder (ADHD) pharmaceuticals (Adderall, Ritalin, Vyvanse, etc.), erectile dysfunction (ED) pharmaceuticals (Cialis (tadalafil), Levitra (vardenafil), and Viagra (sildenafil), etc.) and their many counterfeits, heroin, Kratom, phencyclidine (PCP), and “poppy tea.” The abuse of ADHD medications (as a study aid) is widespread among college students, and to a lesser extent among high school students, especially during examination time periods. In addition to their intended use – which constitutes a huge and quite lucrative market – ED medications are commonly abused as performance enhancing drugs in various sports, are commonly (illegally) added to “traditional” aphrodisiacs and similar folk remedies, and are widely counterfeited worldwide, sometimes with controlled substances, including with amphetamine and other ATSs, methylenedioxyphenethylamines, and/or similar designer drugs.

Drugs that appear to have leveled off somewhat in the U.S. over the past decade include clandestinely-produced amphetamine, GHB/GBL, khat, MDA, and Psilocybe mushrooms. In most such cases, however, the leveling is due to abusers turning to other less expensive or more easily obtained substitutes. Regardless, usage spikes and dips are routine with these and other, more obscure drugs.

Of some encouragement, however, while few drugs ever disappear completely, the use of certain drugs of abuse has faded somewhat in the U.S. over the past decade. Some of these include ayahuasca “tea”, flunitrazepam, LSD, Salvia divinorum, and two of the pro-drugs for GHB, i.e., 1,4-butanediol (BD) and tetrahydrofuran (THF). The ongoing scarcity of LSD dates from the seizure of a large-scale clandestine laboratory in Wamego, Kansas in 2000, which appears to have driven the major LSD production and trafficking group in the U.S. into deep seclusion – and given the passage of time, to have possibly faded altogether. In addition, the rise of many highly potent substitute hallucinogens has quite probably reduced the demand for LSD, since most of these substances are far more easily synthesized, at lower personal risk and and at much lower costs. The reduction in the use of Salvia divinorum is due to a combination of factors, including overharvesting in

Mexico and the U.S. desert southwest, the steadily increasing availability of high-potency marijuana, the continuing availability of synthetic cannabinoids and cannabimimetics, the rise of Kratom, and the negative (sometimes highly negative) experiences reported by some users in on-line chat-rooms and websites.

An interesting development for monitoring illicit drug use in a community is the analysis of municipal wastewater (sewage) for trace levels of abused drugs and their metabolites. First reported approximately 15 years ago as a mechanism for determining contamination of the environment by pharmaceuticals and their metabolites, such analyses were a curiosity until about 5 years ago, when focused interest and advances in isolation techniques and analytical sensitivity allowed for identification of select compounds associated with controlled substances. However, evaluation of the data from such analyses is somewhat subjective – consider, for example, whether the presence of cocaine metabolites in the wastewater stream of a small city is the result of 1,000 “hard-core” addicts or 25,000 “casual” users, or the effects of different water purification chemicals, co-contaminants, time, temperature, bacteria, algae, etc., on such metabolites.

Significant advances in instrumentation have also been reported. The use of near-infrared (NIR) and/or Raman based instrumentation for non-destructive, non-invasive “stand-off” analyses are quickly becoming mainstream techniques, particularly for identification of counterfeit medications and for quality control in pharmaceutical production. Of particular note, the ability of Raman to analyze substances enclosed within clear plastic packaging or glass containers (even those made of dark glass) is of particular utility for law enforcement screening of suspect products. Portable, high quality NIR and Raman instruments are now widely available. In addition, specialized techniques such as Surface-Enhanced Raman (SERS) and Attentuated Total Reflection – Fourier Transform Infrared (ATR/FTIR) allow for analyses of minute amounts of material. In the laboratory, the development of Ultra-High Performance Liquid Chromatography (UHPLC, aka UPLC) offers resolution approaching capillary GC, and tandem LC/MS techniques (HPLC/MS, CE/MS, UHPLC/MS) and tandem LC-MS/MS techniques, enable mass spectral analyses of thermally sensitive compounds that do not survive heated injection ports. Similarly, a number of ambient pressure mass spectrometry instruments (API, DART, DESI, etc.) allow for very rapid screening of materials, even trace amounts on surfaces or on wipes. Finally, although still hindered by a lack of authentics, isotope ratio and stable element analyses are slowly becoming mainstream in source determination programs, for determining both geographic and/or synthetic origins.

1.1 Routine and Improved Analyses of Abused Substances

Improved methods of analysis, i.e., faster, more discriminatory, more sensitive, less costly, etc., are needed for all abused substances. Additionally, standard analytical data are required for previously unknown or rarely encountered substances and/or new "designer drugs."

Drug seizures and clandestine laboratory operations are continuously monitored to provide a comprehensive overview of new developments. Ongoing research in the forensic community, as well as in the general fields of analytical chemistry and toxicology, provide new and/or improved methods of analysis for abused substances. Reports providing standard analytical data for new drugs of abuse and/or improved analytical protocols for known drugs of abuse are generated for the forensic and enforcement communities.

1.A – General Reviews and Overviews
1.B – Individual Compounds or Substances
1.C – Common Groups or Classes of Compounds or Substances
1.D – Polydrug A: Mixed or Unrelated Named Compounds or Substances

1.A – General Reviews and Overviews

2010 INTERPOL Triennial Report on forensic science (1); brief overview (2); **2011** *Analytical Chemistry* biannual review of forensic science (3); brief, conversational overview (4).

1.B – Individual Compounds or Substances
(except individual synthetic cannabinoids and cannabimimetics)

Alprazolam: 2011 analysis by DART-TOF-MS (5);

Amphetamine: **2010** 2H and 13C isotope ratios in amphetamine synthesized from benzaldehyde and nitroethane (6); impurity profiling (7); **2011** by Raman and SERS, with spectral analyses by ab initio calculations (8);

1-Benzyl-4-methylpiperazine: 2012 identification by MS, after derivatization with trifluoroacetic anhydride, and by NMR (9);

Buphedrone (2-(methylamino)-1-phenylbutan-1-one):
2013 characterization with GC/MS, HPLC-DAD, and LC-MS/MS (10);

Buprenorphine: 2011 by GC/MS (11);

2-(4-Chloro-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine (25C-NBOMe): 2013 characterization by GC-EI-MS (with and without derivatization with TFAA), LC-ESI-QTOF-MS, FTIR, and NMR (12);

meta-Chlorophenylpiperazine (m-CPP): **2011** characterization by easy ambient sonic-spray ionization, XRF, IMS, and NMR (13);

Citalopram: **2012** determination by chromatographic and spectrophotometric methods (14);

Cocaine: **2010** detection on clothing using Raman (15); transacetylation of benzocaine by acetylsalicylic acid to create N-acetylbenzocaine in cocaine (16); comparison of corona discharge ionization-IMS versus AP-CI-MS for detection of cocaine (17); a 20 year survey of cocaine seized in France (year range not specified in the abstract) (18); detailed evaluation of the mass spectrum of cocaine (19); **2011** detection of cocaine solutions in sealed bottles of (nominal) alcoholic beverages by Raman (20); determination on banknotes using an aptamer-based electrochemiluminescence biosensor (21); detection of 2,6-diisopropylnaphthalene as an adulterant in cocaine by GC/MS (22); detection of cocaine solutions in wine bottles by 1H-NMR (23); detection by TLC and cobalt thiocyanate (24); detection based on strand-displacement polymerization and fluorescence resonance energy transfer (25); analysis and classification using GC/IRMS to determine d13C values (26); use of the gold chloride microcrystalline test to identify cocaine and certain adulterants (27); temperature-dependent elimination of benzoic acid during pyrolysis of cocaine (28); analysis by TLC coupled to easy ambient sonic-spray ionization MS (29); use of metastable state nanoparticle-enhanced Raman for highly sensitive detection of cocaine (30); **2012** determination of phenyltetrahydroimidazothiazole enantiomers (present in cocaine) by chiral GC (31); detection by structure-switch aptamer-based CZE (32); determination of the time lag between coca leaf harvest and the seizure and analysis of illicit cocaine (33); analysis using differential mobility spectrometry-MS (34); by electrochemical detection (35); detection using a specialized fluorescence sensor (36); analysis of cocaine smuggled by dissolution in polyvinyl alcohol in a dance pad (37); quantification of binary mixtures of cocaine and adulterants using dispersive Raman, FTIR, and Principal Component Regression (38); analysis of Brazilian "oxi" cocaine (analytical methods not specified in the abstract) (39); **2013** by electrochemical determination (40); by GC/FID (41); detection of hygrine and cuscohygrine as possible markers (to distinguish coca chewing from cocaine abuse) by GC/MS (42); comparative analysis of solvent impurity profiles obtained by HS-GC/MS (43);

Diazepam: **2010** detection in spiked alcoholic beverages by fluorimetry (44);

3,4-Dimethylmethcathinone (3,4-DMMC): **2012** characterization by GC/MS, LC/MS, 1D- and 2D-NMR, IR, and UV (45);

2,5-Dimethoxy-3,4-dimethyl-beta-phenethylamine (2C-G): 2012 by GC-EI/MS (including after derivatization with trifluoroacetic anhydride), LC-ESI/QTOF-MS, LC-ESI/QTOF-MS/MS, FTIR, and 1H- and 13C-NMR (46);

2,5-Dimethoxy-4-nitro-beta-phenethylamine (2C-N): 2012 characterization by GC-EI/MS, LC/ESI-QTOFMS, FTIR, and NMR (including after derivatization with trifluoroacetic anhydride) (47);

2-(Diphenylmethyl)pyrrolidine: **2011** by GC-EI/CI-ion trap-MS and HPLC/DAD-ESI-MS (48);

N-Ethyl-alpha-ethylphenethylamine: 2013 characterization by GC/MS, LC-TOF-MS, and 1D- and 2D-NMR (49);

Ethylphenidate: **2011** characterization by MS, IR, and 1H- and 13C-NMR (50);

Fentanyl: **2012** impurity profiling using UHPLC-MS/MS (51);

Flunitrazepam: **2011** detection using a photocatalytic reaction with ZnO particles with monitoring by UV-Vis (52); **2012** detection in alcoholic beverages by DESI-MS (53);

Glaucine: **2010** detection in “legal highs” (54);

Heroin: **2010** a probabilistic approach to heroin signatures (55); profiling and classification of illicit heroin by GC/MS of acidic and neutral manufacturing impurities (56); by optimized GC/FID (57); analysis by FTIR (58); **2011** identification of levamisole and lidocaine acetylation reaction impurities in heroin (59); rapid and semi-quantitative presumptive testing (60); converting GC/MS heroin profiling to a UHPLC-MS/MS method (61); identification of adulterants and diluents in heroin by IR and/or Raman (62); **2012** analysis of trace elements by ICP-MS (63); comparative evaluation using a simplified clustering analysis (64); impurity profiling by GC (65); by GC (66); analysis of heroin containing aspirin, paracetamol, caffeine, theophylline, codeine, acetyl codeine, and monoacetylmorphine, by GC/MS (67); purification of street samples by prep-HPLC (68); analysis by ICP/MS (69); by reflectance NIR (70); impurity profiling based on the major alkaloids (acetylcodeine, 6-monoacetylmorphine, papaverine, noscapine, codeine, and morphine) (71);

Human Growth Hormone (HGH): **2010** analysis by CE-ESI-TOF/MS (72);

Ketamine: **2010** study of the fragmentation pattern of ketamine-heptafluorobutyramide by GC/MS (73); **2012** detection in beverage residues by LC/MS and MS/MS (74); (see also Methoxetamine, below, and Reference # 528);

Khat (Catha edulis): **2010** preservation of cathinone in khat via drying (75); **2012** qualitative and quantitative analysis of cathinone, cathine, and phenylpropanolamine by GC/MS and GC/FID (76); **2013** analysis by CE (77);

Kratom: **2012** quantitative analysis of mitragynine, codeine, caffeine, chlorpheniramine, and phenylephrine in a kratom cocktail using HPLC (78); by

HPLC/ESI-MS (with comparison of 3 different extraction techniques) (79); **2013** by HPLC- DAD (80);

LSD: **2010** quantitation by HPLC (81); **2012** LSD (and 9,10-dihydro-LSD) – by color testing, TLC, EASI-MS, HPLC-UV (82);

Marijuana and Marijuana-Derived Cannabinoids: **2010** tracing geographic and temporal trafficking patterns for marijuana in Alaska using stable isotopes (83); differentiation of fibre- and drug type seedlings by GC/MS and chemometrics (84); tracing retail cannabis in the U.S. using hydrogen and carbon isotope ratios to determine geographic origins, cultivation parameters, and trafficking patterns (85,86); evaluation of an experimental indoor hydroponic cannabis grow operation using the Screen of Green method (87); evaluation of an experimental indoor hydroponic Cannabis grow operation, using the "Screen of Green" yield estimation program, THC analysis, and DNA analysis (88); survey of the potency trends of THC and other cannabinoids in marijuana from 1993 to 2008 (89); analysis of marijuana seized in Novi-Sad, Serbia in 2008 (90); determination of THC, CBD, and CBN in edible oils by UHPLC-MS/MS (91); **2011** use of DNA collection cards for in-the-field sampling (92); differentiation of seedlings by GC/MS and Linear Discriminant Analysis, Partial Least Squares Discriminant Analysis, Nearest Neighbor Classification, Learning Vector Quantization, Radial Basis Function Support Vector Machines, Random Forest, and Artificial Neural Networks (93); a survey of cannabinoid ratios in marijuana seized in California from 1996 to 2008 (94); profiling and source determination by GF AAS and ICP OES (95); differentiation of drug and non-drug marijuana using a single nucleotide polymorphism assay (96); analysis of THC in industrial hemp crops in Morocco (97); differentiation of drug-type and fiber-type by multiplex PCR analysis (98); determination of the long term stability of select cannabinoids (method not reported in the abstract) (99); a formula for determining the yield and quality of indoor grow operations (100); semi-prep scale isolation of tetrahydrocannabinolic acid A (THCA) using two flash chromatography systems (101); **2012** determination of THC by voltammetry (102); investigation of potential interferences by other drugs with the Fast Blue B and Duquenois-Levine color tests (103); a survey of the potency of marijuana grown in Albania (survey range not listed in the abstract) (104); isomerization of CBD and THC under positive ESI conditions (105); an investigation into the hypothesis of transgenic (genetically modified) marijuana (106); a PCR assay for the relative quantification of THCA synthase gene (107); analysis of DNA by CE for geo-sourcing (108); differentiation between very young drug- and hemp-type cannabis seedlings and cuttings by determination of select cannabinoids by HPLC-DAD (109); classification of cultivars based on analysis of cannabinoids and terpenoids (110); preliminary analysis of genetic diversity of hemp cultivars based on ISSR molecular markers (111); use of delta13C isotope ratios for differentiation of samples (112); a study of the effects of electrical lighting power and irradiance on indoor-grown marijuana potency and yield (113); by LC/API-MS and LC/API-MS/MS (114); determination of THC, CBD, and CBN in marijuana grown in northern Thailand, by GC/FID (115); a study of the long-term storage and stability of hash oil (methods not listed in the abstract) (116); a study of the long-term

storage and stability of “cannabis resin” (methods not listed in the abstract) (117); identification and characterization of hybrid and/or high potency marijuana (methods not specified in the abstract) (118); a survey of the potency of marijuana seized in Japan in 2010 (methods not listed in the abstract) (119); use of ultrasound for improved extraction of cannabinoids for HPLC analysis (120); evaluation of the uncertainty of THC determined by HPLC (121); **2013** by HPLC-UV following cloud point extraction (122); by DNA analysis (123); by laser-ablation inductively-coupled plasma MS (LA-ICP-MS) – a review, covering many other applications (124); a study of marijuana potency from the 1970s to the 2000s (125); characterization of seeds by DNA analysis (126);

Mephedrone (4-Methylmethcathinone): 2010 by color testing, GC/MS, and FTIR (127); by LC (128); **2011** by GC/MS following derivatization with 2,2,2-trichloroethyl chloroformate (129); characterization of 2-, 3- and 4-methylmethcathinone (i.e., mephedrone and its two positional isomers) by GC/MS, NMR, and IR (130); synthesis and characterization (synthetic route and analytical methods not specified in the abstract) (131); an overview and literature review (132); **2012** determination of isotopic fractionation to link precursor to product in the synthesis of (±)-mephedrone (133); a literature review (134); a study of the degradation in alkaline solutions (135); **2013** by SERS with a portable Raman (136);

Mescaline/Peyote: 2013 analysis of “peyote tea” by GC/MS and GC/MS/MS in PCI mode (137);

Methamphetamine: 2010 enantio-discrimination of methamphetamine by circular dichroism using a porphyrin tweezer (138); an overview of law enforcement efforts against methamphetamine production in New Zealand (139); isotope fractionation during precipitation (140); recovery and identification of trace methamphetamine and pseudoephedrine on impermeable surfaces in clandestine laboratories (141); identification of three byproducts found in methamphetamine synthesized by the Emde route (142); identification of iodine and red phosphorus using AccuTOF-DART (143); use of phosphorous acid flakes in the reduction of (pseudo)ephedrine to methamphetamine (144); screening of methamphetamine/methyl sulfone exhibits using Raman spectroscopy (145); **2011** analysis by UFLC (Ultra-Fast-LC) (146); an (unsuccessful) attempted synthesis by electrolytic reduction of pseudoephedrine (147); enantioseparation and identification of methamphetamine and the ephedrines using using trifluoroacetic anhydride derivatization and chiral GC/MS (148); analysis using by highly fluorescent polyfluorenes with NH2-terminated side chains (149); chiral analysis by CE with added cyclodextrins (150); a urea – based “one-pot” methamphetamine synthesis (151); chiral separation with CE using dynamically coated capillaries (includes “related compounds”) (152); chiral analysis of the enantiomers of ephedrine, pseudoephedrine, chlorinated intermediates, and methamphetamine by derivatization with fluorinated acid anhydrides followed by GC on a cyclodextrin stationary phase, for impurity profiling of methamphetamine synthesized by the Emde method (153); a study of the efficacy of wipe sampling to determine contamination at clandestine

laboratories (with analyses by LC/MS or GC/MS) (154); **2012** comparative analysis of impurity profiles from GC/FID (155); the environmental fate of clandestine laboratory waste (156); impurity profiling of Iranian seizures using GC/MS and LC/MS (157); an overview of abuse, treatment, and U.S. law (158); identification of (1S,2S)-1-methylamino-1-phenyl-2-chloropropane as a route specific marker impurity for methamphetamine synthesized from ephedrine via chloroephedrine (159); impurity profiling of methamphetamine synthesised by the Birch method (160); impurity profiling of methamphetamine synthesized using the Nagai method (161); critical evaluation of LLE and SPME methods for impurity profiling (162); detection of trace ephedrine and pseudoephedrine in high-purity methamphetamine by HPLC (163); degradation of 1-(1',4'-cyclohexadienyl)-2-methylaminopropane in soils (164); degradation of methamphetamine production precursors and byproducts in soils (165); chiral analysis of chlorinated intermediates of methamphetamine (from the Emde synthesis) by 1D- and 2D-NMR and GC/MS (166); analysis of a sample cut with diphenylmethane, by GC/MS (167); a study of the effects of synthetic conditions on the d13C, d15N, and d2H isotope ratios of the final product (168); determination of synthetic route via impurity profiling using GC/MS (169); preparation and certification of reference quality material (170); **2013** detection of pharmaceutical impurities in methamphetamine by GC/FID and GC/MS (171); impurity profiling of methamphetamine by CE using a highly sulfated gamma-cyclodextrin as a chiral selector (includes methamphetamine, amphetamine, ephedrine, pseudoephedrine, norephedrine, and norpseudoephedrine) (172); screening of methamphetamine, pseudoephedrine, and ephedrine by a portable lab-on-a-chip instrument (173); evaluation of the use of IMS in remediation of clandestine laboratories (174); influence of precursor solvent extraction on stable isotope signatures of methamphetamine prepared from OTC pharmaceuticals using the Moscow and hypophosphorous syntheses (175); impurity profiling of methamphetamine synthesized from P2P prepared from phenylacetic acid (or its esters) (176);

Methiopropamine: 2011 characterization by IR, MS, and 1H- and 13C-NMR (177); (see also Reference # 250);

Methorphan: 2012 chiral analysis by GC/MS following derivatization with (-)-menthyl chloroformate (includes MS and NMR analyses of the derivatives) (178);

Methoxetamine: 2012 by NMR, MS, and IR (with comparisons with ketamine) (179);

2-(5-Methoxy-1-benzofuran-3-yl)-N,N-dimethylethanamine (5-MeO-BFE) (and its N-ethyl analog): 2012 characterization by MS, NMR, and IR (180);

4-Methoxyphencyclidine: 2011 characterization by MS, IR, and NMR (181);

4'-Methoxyphenyl–2–propanone: 2012 clandestine synthesis and characterization (182);

alpha–Methyl–3,4–methylenedioxyphenylpropionamide (MMDPPA): 2013 identified in Australia as an intermediate from helional to MDA (183; see also 184);

Methylenedioxyamphetamine (MDA): 2013 from helional (185); (see also alpha–methyl–3,4–methylenedioxyphenylpropionamide);

3,4-Methylenedioxy-N-benzyl cathinone (BMDP): 2013 characterization by LC/high res QTOF-MS, EI-MS, IR, and 1D- and 2D- 1H- and 13C-NMR (186);

Methylenedioxymethamphetamine (MDMA): 2010 use of stable isotope ratios to differentiate MDMA according to synthetic route (187); identification of some tertiary amines related to MDMA by GC- IRD (188); determination of synthetic route by ICP-MS (189); impurity profiles of MDMA prepared by four different methods (190); **2011** use of impurity profiling, stable isotope analyses, and pattern recognition techniques for characterization and sourcing (191); a historical overview (192); determination of volatile components of MDMA tablets with LC/MS and HS-SPME-GC/MS, for development of canine training aids (193); determination of volatiles by HS-SPME followed by GCxGC and GCxGC-TOFMS (194); by SERS using modified Silver nanoparticles (195); **2012** impurity profiling of MDMA prepared from piperine versus vanillin (196); isolation of MDMA using a specialized SPME cartridge with analysis by GC/MS (197); comparative analysis by GC×GC-TOF-MS (198); **2013** enantiomeric purification by batch chromatography with a cyclodextrin chiral selector (199); impurity profiling of sassafras oils by GC×GC-TOF-MS (200);

Methylenedioxypyrovalerone (MDPV): 2010 characterization by GC/MS, NMR, FTIR, and UV (201);

4-Methylethcathinone (4-MEC): 2013 by GC/MS, HPLC-DAD, and LC-MS/MS (202);

N-Methylphthalimide: 2011 characterization by GC/MS, FTIR, and NMR (203);

4'-Methyl-alpha-pyrrolidinohexanophenone (MPHP): 2011 analysis by GC/MS, HPLC/DAD, and GC/FID (toxicological focus) (204);

3,4-Methylenedioxyphenylacetone (MDP2P): 2010 differentiation of methoxy methyl phenylacetones related to MDP2P by GC/IRD (205);

3,4-Methylenedioxypyrrolidinobutyrophenone (MDPBP): 2011 characterization by IR, MS, and 1D- and 2D- 1H- and 13C- NMR (206);

4-Methylthioamphetamine (4-MTA): 2012 impurity profiling of 4-MTA produced by the nitropropene route (207); identification of by-products produced by the Leuckart method, using MS, 1H- and 13C-NMR, IR, and crystallography (208);

Morphine: 2012 analysis by FTIR and Raman, with density functional theory (DFT) calculations (209); extraction from poppy seeds, with analysis by GC/MS and GC/FID (210); quantitation in a Chinese traditional medication, by HPLC (211); analysis by cyclic voltammetry, chronoamperometry, and differential pulse voltammetry (212);

Naphyrone (naphthylpyrovalerone, 1-naphthalen-2-yl-2-pyrrolidin-1-ylpentan-1-one): 2010 isomer determination by GC- ion trap-EI/CI-MS and 1D/2D NMR spectroscopy (213); **2012** an overview and literature review (214);

Oxycodone: 2010 analysis of pyrolysis products by GC and GC/MS (215);

Phencyclidine (PCP): 2013 false-positive immunoassay caused by MDPV (216);

Psilocybe Mushrooms: 2010 comparative analysis of hallucinogenic mushrooms using ATR and transflection IR (217); **2011** by DNA analysis (a review, also including some non-hallucinogenic, poisonous mushrooms) (218);

alpha-Pyrrolidinopentiophenone: 2012 by MS, NMR, and IR (219);

Salvia divinorum: 2010 thermal degradation products from Salvia divinorum smoke (220); **2012** differentiation from other Salvia species by GC/MS with principal components analysis (221); analysis of "spiked" plant materials by GC/MS (222); **2013** identification of Salvinorin A in Salvia divinorum (but not in 612 related Salvia species) by GC/MS (223); differentiation from marijuana and tobacco by DNA analysis (224);

Sibutramine: 2012 by TLC and TLC-densitometry (225); **2013** detection of illicit adulteration of botanical food supplements, by color tests, TLC, HPLC-DAD, MS, and NMR (226);

Zolpidem: 2012 by HPLC and MS (includes a degradation study) (227);

Miscellaneous Drugs: 2011 characterization of RTI-126 (228).

1.C – Common Groups or Classes of Compounds or Substances

Amphetamine-Type Stimulants (ATSs) and Related Phenethylamines (PEAs): 2010 analysis of ring and side chain regioisomers of ethoxyphenethylamines related to the controlled substances MDEA, MDMMA, and MBDB by GC/MS and GC/IRD (229); methamphetamine, 4-fluoro-, 4-chloro-, 4-bromo-, 4-iodo-, and 4-nitromethamphetamine – analysis by GC/MS following trifluoroacetyl derivatization (230); differentiation of regioisomeric ring-substituted fluorophenethylamines by product ion spectrometry (231); "Fly" and "Dragonfly" Compounds – synthesis and charaterization by GC/MS, LC/MS, and LC-MS/MS (232); **2011** GC/MS and GC/IRD studies on the ring

isomers of N-methyl-2-methoxyphenyl-3-butanamines (MPBA) related to 3,4-MDMA (233); 4-methylthioamphetamine, 4-fluoroamphetamine, 4-methylamphetamine, 3-trifluoromethylamphetamine, MDA, 2,5-dimethoxyamphetamine, and 2,4,5- and 3,4,5-trimethoxyamphetamines – mass spectrometric properties and identification of some N,N-di-(beta-arylisopropyl)formamides (synthetic impurities) (234); 5- and 6-(2-aminopropyl)-2,3-dihydrobenzofuran – characterization by MS, IR, and NMR (235); amphetamine and methamphetamine – detection by digital image-based colorimetric tests (236); identification of (unspecified) ATSs by GC/MS and GC/FTIR (237); general classification of amphetamines versus non-amphetamines based on GC/FTIR and GC/MS with Principal Component Analysis coupled with Artificial Neural Networks (238); amphetamine, methamphetamine, pseudoephedrine, and five "amphetamine analogs" (not specified in the abstract) – field analysis using the Agilent Bioanalyzer (239); novel syntheses of ATS precursors (240); a review of methods for the chiral determination of ATSs (241); aminoindanes – a review (242); **2012** 4- and 5-iodo-2-aminoindan – by MS, NMR, and IR (243); 2-, 3- and 4-methylmethamphetamine and 2-, 3- and 4-methylamphetamine – analysis by GC/MS, acetylation, and GC/IRD (244); "amphetamine-type illicit drugs" by a miniaturized gas sensor system using surface ionization (245); DOB and positional isomers – differentiation of various perfluoroacylated derivatives by GC/MS and GC/IRD (246); amphetamine, methamphetamine, ephedrine, pseudoephedrine, norephedrine, and norpseudoephedrine – enantioseparation by CE with contactless conductivity detection (247); a review of the chiral analysis of amphetamine "and related compounds" by CE and NMR (248); 25D-NBOMe, 25E-NBOMe, and 25G-NBOMe – characterization by GC-EI-MS (with and without derivatization with trifluoroacetic anhydride), LC-ESI-QTOF-MS (and MS/MS), FTIR, and NMR (249); **2013** methiopropamine and its 3-thienyl isomer – synthesis and analysis/differentiation by GC (250); o-, m-, p-chloro- and o-, m-, p-fluoro-amphetamine – by CE-LIF, following derivatization with fluorescein isothiocyanate (includes comparisons against CZE-UV, sweeping-MEKC-UV, and LC-Q-TOF-MS) (251); diethylpropion, fenproporex, and sibutramine – in counterfeit tablets, by ATR/FTIR (252); unspecified amphetamines and precursors – by a portable instrument combining miniaturized GC and IR Absorption Spectroscopy (253); 2-, 3-, and 4-methylamphetamine – synthesis and characterization by GC/MS, HR-ESI-MS, NMR, and IR (254); methamphetamine, MDMA, and other unspecified ATSs – by GC/MS after derivatization with iso-Bu chloroformate and SPME (toxicological focus) (255); methamphetamine, MDMA, amphetamine, DMA, and PMA – a review of impurity profiling and syntheses (256);

Anions: **2010** identification via complexation with meso-octamethylcalix(4)pyrrole and detection using EI-MS (257); **2011** by CE (258,259);

Barbiturates: **2010** mephobarbital, pentobarbital, and secobarbital – by MEKC-MS (toxicological focus) (260); **2011** spectrophotometric determination of barbituric acid in pharmaceuticals (261);

Benzodiazepines: **2011** determination of pK values by potentiometric titration (262); diazepam, estazolam, chlordiazepoxide, and triazolam – analysis by RP-HPLC (263); **2012** clotiazepam, clozapine, and pinazepam – analysis by micellar liquid chromatography (toxicological focus) (264);

Cathinones: **2010** mephedrone, butylone, 4-methyl-N-ethylcathinone, flephedrone, MDPV, and naphyrone – by GC-ion trap-MS (both EI and CI) and NMR (265); mephedrone, methylone, and bk-MBDB – characterization by FTIR, FT-Raman, 1H NMR, 13C NMR, GC/MS, and EI-HRMS (266); **2011** 4-fluoromethcathinone, pentylone, MDPBP, MDPV, and MPPP – by GC-(EI/CI)-MS and NMR (267); 4'-methylethcathinone (4-MEC) and 6 other methcathinone analogs (not specified in the abstract) by LC-MS/MS (268); analysis of isomeric byproducts and related impurities in mephedrone and ethylcathinone (269); synthesis and analysis of various methylenedioxycathinones, including bk-DMBDB (270); by Raman (271); methylone, bk-MBDB, and bk-MDEA – a review, including analyses by GC/MS, LC/MS, and LC-MS/MS (toxicological focus) (272); **2012** 10 homologous and regioisomeric aminoketones related to MDPV – analysis by GC-EI-MS (273); 3,5-difluoromethcathinone and 3,5-dichloromethcathinone – synthesis and characterization by GC/MS, NMR, IR, and GC/IRD (274); the 2,3-isomers of MDPV, butylone, and methylone – synthesis and characterization by GC, IR, GC/MS, and 1H and 13C NMR (275); 4'-methyl-N-ethylcathinone (4-MEC) and 4'-methyl-N-benzylcathinone (4-MBC) – characterization (methods not specified in the abstract (276); buphedrone and pentedrone – synthesis and characterization by FTIR, Raman, 1H- and 13C-NMR, GC/MS, and ESI-HRMS (277); mephedrone, methedrone, and 17 others not specified in the abstract – chiral separation by cyclodextrin-modified CZE (278); methcathinone and 17 other cathinones (not specified in the abstract) – chiral analysis by GC/MS following derivatization with trifluoroacetyl-L-prolyl chloride (279); 22 cathinones (not specified in the abstract) – by positive ESI MS with in-source CID (280); cathinone, methcathinone, 4-methylmethcathinone, dimethylcathinone, and 4-methoxymethcathinone – by color testing (281); screening identification of methcathinone and 5 other cathinones by portable ATR/FTIR (282); 4-methylmethcathinone, three positional isomers of fluoromethcathinones, 4-methoxymethcathinone, N-ethylcathinone, N,N-dimethylcathinone, buphedrone, and pentedrone – by GC/MS (283); "synthetic cathinones" – detection and screening using a portable ion trap DESI-MS (284); differentiation of isomeric N-alkylated fluorocathinones by GC-MS/MS (285); pentedrone and pentylone – characterization by MS, 1D- and 2D-, 1H- and 13C-NMR, and IR (286); **2013** mephedrone, methylone and MDPV – by ambient ionization MS using arrays of low-temperature plasma probes, and also following injection of trifluoroacetic anhydride directly into the plasma stream for online derivatization (287);

Ephedrines: **2010** N–acetylpseudoephedrine and N–acetylephedrine – synthesis and characterization by GC–MS, NMR, FTIR, LC–MS, and UPLC–MS (288); **2012** phenylpropanolamine, cathine, ephedrine, pseudoephedrine, and methylephedrine – analysis by HILIC, with comparison versus RPLC (289); chiral separation of enantiomers of ephedrine and pseudoephedrine in

ATSs using achiral modifiers in the gas phase (290); synthesis of alpha-aminoalcohols via the Akabori–Momotani reaction (291); **2013** comparison of RP-UHPLC and HILIC for quantitation, with medium-resolution accurate MS (292);

Erectile Dysfunction Drugs – Cialis (tadalafil), Levitra (vardenafil), and Viagra (sildenafil): **2010** detection of counterfeits by FTIR, NIR, and Raman (293); identification of (-)-trans-tadalafil, tadalafil, and sildenafil in counterfeit Cialis (294); **2011** development of "classification trees" based on infrared spectroscopic data to discriminate between genuine and counterfeit medicines (295); identification of counterfeits by impurity profiling (296); detection of counterfeits by Raman (297); **2012** differentiation of legitimate and counterfeit medications by chemometrics and chromatography (298); detection of counterfeits by image processing and statistical analysis (299); analysis of counterfeit Cialis tablets using Raman microscopy and multivariate curve resolution (300); fingerprinting of sildenafil citrate and tadalafil tablets by XRF (301); identification of sildenafil and/or vardenafil using ESI-LC/MS (302); detection of adulteration of capsule shells (a novel and unusual "smuggling" technique) by HPLC-DAD, HPLC/MS, microscopy, and Raman (303); **2013** differentiation between counterfeit and authentic Cialis and Viagra by ATR/FTIR with PCA (304); analysis and profiling by UPLC/MS (305);

Ergot Alkaloids (see also LSD): **2012** quantitative analysis using electronic absorption, fluorescence, IR, Raman, CD, ESI-MS, and MALDI-MS (specific compounds not listed in the abstract) (306);

Fentanyl Derivatives: **2012** identification of trace level fentanyl derivatives with nonaqueous CE-ESI-MS/MS (307);

***gamma*-Hydroxybutyric acid (GHB) and *gamma*-Butyrolactone (GBL):** **2010** use of IRMS to discriminate between seizures of GBL and for source determination (308); detection of GHB in solutions using a colorimetric sensor array (309); **2011** a study of the spontaneous formation of GHB from GBL in tap water (310); screening for gamma-hydroxybutyrate by ion chromatography (with comparison versus GC/MS) (311); detection of GHB and GBL in adulterated beverages, using 1H-NMR (312); **2012** sodium, potassium, magnesium and calcium salts of gamma-hydroxybutyrate – synthesis and characterization by FTIR, elemental analysis, X-ray powder diffraction analysis, color testing, and microcrystal testing (313); field testing for GHB with a rapid enzymic test (also includes commentary on MDMA, flunitrazepam, and ketamine) (314); **2013** a comprehensive study of the worldwide distribution of GBL using internet monitoring, comparison of packaging, and carbon isotopic measurements (315); in dietary supplements and foods, by GC/MS (using isotopologues for quantitation) (316);

Methylenedioxyphenethylamines and Related Compounds (note that methylenedioxysubstituted cathinones are categorized under "Cathinones")**:** **2010** identification of side chain regioisomers related to MDEA, MDMMA, and MBDB (317); **2011** methylenedioxy-2-aminoindans – synthesis and analysis of the 4,5 and 5,6 isomers by GC/MS, ATR/FTIR, and 1H- and 13C-NMR

(318); **2012** MDA, alpha-methyl-3,4-methylenedioxyphenylpropionamide (and 2-chloro-4,5-methylenedioxyamphetamine) – characterization by GC/MS, GC/IRD, ATR/FTIR, and NMR (319);

Papaver and Opium: **2010** by cyclodextrin-modified CE following ultrasound-assisted extraction of Papaver (320); identification of opium poppies using 10 genetic markers (321); **2011** differentiation of P. somniferum, P. rhoeas, and P. setigerum by GC/MS and multivariate statistical analyses (322); identification of expressed sequence tag (EST) and simple sequence repeat (SSR) markers (323); determination and analysis of opium alkaloids and crude heroin in complex mixtures by surface-ionization MS (324); **2012** Papaver setigerum by genetic and chemical components analysis (325); opium – determination of 14N and 15N isotopes by proton induced gamma-ray emission (326);

Piperazines: **2010** differentiation of methylenedioxybenzylpiperazines by GC/IRD and GC/MS (327); BZP, mCPP, MeBP, MeOPP, MePP, and TFMPP – detection in "Legal Highs" by GC/MS and HPLC-DAD (328); **2011** differentiation of methylenedioxybenzylpiperazines and methoxymethylbenzylpiperazines by GC/IRD and GC/MS (329); BZP and TFMPP – analysis by ATR/FTIR and GC/MS (330); **2012** methoxybenzoylpiperazines (OMeBzPs) and methylenedioxybenzylpiperazines (MDBPs) – differentiation using GC/MS, GC-TOF-MS, and GC/IRD (both underivatized and as perfluoroacylated derivatives (331); **2013** BZP – a review (social focus, but includes "analytical methodologies for the identification of BZP in forensic settings") (332);

Plant Materials: **2010** a review of poisonous plants (includes drugs) (333); **2011** use of cellulose d18O as an index of leaf-to-air vapor pressure difference in tropical plants (334); **2012** analysis of alkaloids from psychoactive plants by nonaqueous CE/MS (specific plants not listed in the abstract) (335); plant DNA fingerprinting – listed applications include "investigation of trade in illicit drugs" (336); **2013** identification of plant materials used as supporting matrices for pharmaceuticals, nutritional supplements, and illicit drugs, by DAD, evaporative light scattering detection, and MS (337); analysis of the plant materials used as support matrices, by DNA analysis, GC/MS, and LC/MS (338); (see also Reference Number 352);

Steroids: **2010** correlation of the product ion profiles from ESI MS/MS with molecular structures (339); analysis by GC- microchip-AP-photoionization-MS (toxicological focus) (340); identification of anabolic steroids and derivatives using bioassay-guided fractionation and UHPLC/TOFMS analysis (341); **2011** testosterone – IRMS of various black-market products collected in Austria (342); a review of the literature from 2004-2010 (343); analysis by GC/MS using hydrogen as the carrier gas (toxicological focus) (344); **2012** prediction of GC relative retention times of trimethylsilylated derivatives (345); identification of methyltestosterone in counterfeit 4-chlorodehydromethyltestosterone products, by RP-HPLC-ESI-MS (346); elucidation of the m/z 97 ion from androst-4-en-3-one-based steroids by ESI-CID and IRMPD (347); **2013** (primarily) stanozolol, testosterone and

nandrolone – a study of authentic and counterfeit products seized in Brazil from 2006 to 2011 (348);

Synthetic Cannabinoids and Cannabimimetics: [Notes: To aid searching for specific compounds, all compounds in this section are listed in alphabetical order within their individual citation (but not within the section). In addition, compounds are listed either by their acronym or full name as was specified in their respective abstract – no effort was made to transcribe acronyms to full chemical names or vice versa. Articles that include both synthetic cannabinoids and/or cannabimimetics with other drugs are detailed in the next section.] **2010** JWH–018 and JWH–073 – by color testing, TLC, GC/MS, and FTIR (349); a survey of synthetic cannabinoids and/or cannabimimetics containing products obtained from June 2008 to September 2009 in Germany/Europe (350); analysis of "Spice Gold" with GC/MS and solid probe MS (351); identification of the plants used as the base materials for products containing synthetic cannabinoids and cannabimimetics (352); JWH-018 – detection by TLC and GC/MS (353); analysis and identification of cannabicyclohexanol, CP-47,497, JWH-018, JWH-073, and oleamide in herbal products by GC/MS and LC/MS (354); an overview of synthetic cannabinoids and cannabimimetics (355); **2011** JWH-203 – characterization by LC/MS, GC/MS, LC with UV detection, NMR, and high-res MS (356); JWH–018, JWH–073, and 9 other unspecified synthetic cannabinoids – a survey of 33 smoking blend products, with analysis by GC/MS (357); JWH-015, JWH-018, JWH-019, JWH-020 JWH-073, JWH-081, JWH 200, JWH-250, WIN 55,212-2 and methanandamide – by LC-MS/MS (toxicological focus) (358); JWH-122 – characterization by NMR, "spectroscopy," and MS (359); JWH–201, JWH–250, and JWH–302 – differentiation by GC/MS fragment ion ratio comparisons (360); an overview and review of synthetic cannabinoids and cannabimimetics, including some GC/MS and LC-MS/MS data (361); (unspecified) analog of a CP 47,497-C8 type compound – by off-line LC-DAD-NMR (362); AM-694, AM-2201, JWH-122, RCS-4, and (2-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone (a positional isomer of RCS-4) – analysis by LC/MS, GC/MS, and NMR (363); AM-694, JWH-019, JWH-122, JWH-210, and (4-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone – analysis by LC/MS, GC/MS MS, and NMR (364); JWH-250 – identification and quantitation by GC/MS, LS/MS, high-res MS, and NMR (365); 1-pentyl-3-(1-naphthoyl)indole, 1-butyl-3-(1-naphthoyl)indole, 1-hexyl-3-(1-naphthoyl)indole, and 3-[4-(1,1-dimethyloctyl)-2-hydroxyphenyl]cyclohexan-1-ol – by "chromatography-mass spectrometry" (chromatographic method(s) not specified in the abstract) (366); JWH-018 and JWH-073 – detection by GC/MS (367); JWH–018, JWH–018 N–(2–methylbutyl) isomer, JWH–018 N–(3–methylbutyl) isomer, JWH–201, JWH–250, JWH–302 – isomer differentiation by GC/MS retention times (368); cannabipiperidiethanone – identification and characterization by GC/MS, LC/MS, high-res MS, and NMR (369); JWH- 015, JWH-073, JWH-081, JWH-200, JWH-250, JWH-251 – identification and quantitation by GC/MS, LS/MS, high-res MS, and NMR (370); JWH-018 and JWH-073 – detection by GC/MS (371); cannabicyclohexanol (CP-47,497-C8-homolog), JWH-018, JWH-073 – determination by GC/MS (372); **2012** AM2201, JWH-018, and JWH-022 – JWH- 018 and JWH-022 identified as combustion products of AM2201, as

determined by GC/MS and Accu-TOF-DART (373); JWH-018 – by DART-TOF-MS (374); JWH-307 – characterization by NMR, GC-HRMS, ESI-MS/MS, UV, and IR (375); JWH-018 and JWH-073 – purity levels of materials from three different on-line suppliers, as determined by HPLC-UV (376); "synthetic cannabinoids" (specific compounds not listed in the abstract) – analysis by MEKC-DAD (377); AM-694, JWH-018, JWH-019, JWH-073, JWH-081, JWH-210, and JWH-250 – analysis by GC/MS and MALDI-TOF MS (378); AM-679 and 1-pentyl-3-(1-adamantoyl)indole – by LC-UV-MS/MS, LC-TOF-MS, GC/MS, and NMR (379); AM-2201, JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-203, JWH-210, JWH-307, and RCS-4 – analysis by LC-ESI-MS/MS (toxicological focus) (380); AM-694, AM-2201, JWH-018, JWH-019, JWH-081, JWH-122, JWH-203, JWH-210, JWH-250, JWH-307, MAM-2201, and RCS-4 – by LC/ESI-MS/MS (toxicological focus) (381); AM-1220 and (N-methylazepan-3-yl)-3-(1-naphthoyl)indole – by TLC, GC/MS, high-res MS, LC-HR-MS/MS, and NMR (382); 3-(1-adamantoyl)-1-pentylindole – identification by GC/MS, TLC, NMR, high-res MS, and GC-MS/MS (383); AM-694, AM-2201, CP 47,497 (C=8) (cannabicyclohexanol), JWH-018, JWH-019, JWH-073, JWH-081, JWH-200, JWH-210, JWH-250, RCS-4, and RCS-8 – analysis by TLC, GC/MS, HPLC, and LC-TOF-MS (384); 1-[(5-fluoropentyl)-1H-indol-3yl]-(4-methylnaphthalen-1-yl)methanone and JWH-412 – separation by flash chromatography and analysis by GC/MS and NMR (385); "synthetic cannabinoids" (five compounds not specified in the abstract) by DART-MS with collision-induced dissociation (386); AM-251 and JWH-015 – analysis by DART-MS (387); color testing for 24 (unspecified) indole-based cannabimimetics (388); an overview (389); naphthoylindoles – by ESI-QTOFMS (390); N-(1-adamantyl)-1-pentyl-1H-indole-3-carboxamide (APICA), N-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide (APINACA), AM-1220, AM-1241, AM-1248, AM-2233, and CB-13 (CRA-13) – analysis by LC/MS, GC/MS, high-res MS, and NMR (391); 1-butyl-3-(1-(4-methyl)naphtoyl)indole – synthesis and characterization with GC/FID, 1H- and 13C-NMR, DSC, GC/MS, and elemental analysis (392); an overview and review (393); JWH-073 and its 4-methylnaphthoyl analogue – by TLC, NMR, GC/MS, and LC/MS (394); JWH-018, JWH-081, and 10 other (unspecified) "synthetic cannabinoids" – by GC/MS (395); JWH-018 – by GC/MS (396); **2013** JWH-018, JWH-019, JWH-073, and JWH-250 – by GC/MS (397); 5F-UR-144 and UR-144 – by GC/MS, LC-TOF-MS, and 1D- and 2D-NMR (398); AM-2201, JWH-203, JWH-210 and RCS-4 – by LC, high-res MS, LC-QTOF-MS, and NMR (399); 28 (unspecified) "synthetic cannabinoids" – by LC/ESI-MS/MS (toxicological focus) (400); cis- and trans- CP-47,497-C8 (and others not specified in the abstract) – extraction from plant materials by flash chromatography (401); azepane isomers of AM-1220 and AM-2233, AM-2233, and URB-597 – by LC/MS, GC/MS, "accurate MS," and NMR (402); unspecified "cannabimimetics" bearing 2,2,3,3-tetramethylcyclopropanecarbonyl moieties – by GC/MS, LC/MS, and NMR (403); JWH-213 – by LC-PDA-MS, GC/MS, high-res MS, and NMR (404); N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1H-indazole-3-carboxamide (AB-PINACA) and N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide (AB-FUBINACA) – by LC/MS, GC/MS, high-res MS, and NMR (405); cannabicyclohexanol, JWH-018, JWH-073, JWH-081, JWH-

122, JWH-210, JWH-250, and RCS-4 – by GC/MS, LC-QTOF-MS, and HPLC (406);

Synthetic Cannabinoids and Cannabimimetics with Other Drugs: 2012 1-butyl-3-(4-methoxybenzoyl)indole, JWH-018, JWH-073, JWH-122, JWH-250, 1-pentyl-3-(4-methoxybenzoyl)indole, and phenazepam – detection in plant materials (analytical methods not specified in the abstract) (407); 12 "synthetic cannabinoids and cannabimimetics" (not specified in the abstract) and THC – by nano-LC/MS and nano-LC-MS/MS (408); AM-2201, AM-2202, JWH-019, JWH-203, JWH-210, mitragynine (Kratom), (1-(4-pentenyl)-1H-indol-3-yl)(naphthalen-1-yl)methanone – analysis by LC/MS, GC/MS, high-res MS, and NMR (409); **2013** AB-001, AM-2232, APINACA, N,5-dimethyl-N-(1-oxo-1-(p-tolyl)butan-2-yl)-2-(N'-(p-tolyl)ureido)benzamide, (4-ethylnaphtyl)-AM-2201 (EAM-2201), 5-fluoropentyl-3-pyridinoylindole, 5FUR-144 (synonym: XLR11), 4-hydroxy-diethyltryptamine (4-OH-DET), JWH-213, JWH-307, JWH-030, 4-methylbuphedrone, (4-methylnaphtyl)-AM-2201 (MAM-2201), (4-methylnaphtyl)-JWH-022 [synonym: N-(5-fluoropentyl)-JWH-122], N-(4-pentenyl)-JWH-122, UR-144, and URB-754 – detection on plant materials (methods not specified in the abstract) (410); (see also References Numbers 424, 432, 441, 467, 469, and 470);

Tryptamines (see also Psilocybe Mushrooms): 2010 a review of the analyses of psychoactive N,N-dialkylated tryptamines (411); characterization of the byproducts from the synthesis of DMT by reductive amination, using GC- ion trap-MS (412); profiling psychoactive tryptamine-drug syntheses by MS (to identify route specific impurities) (413); **2011** preparation and analytical characterization of twelve 5-ethoxy-N,N-dialkyl-tryptamines and their deuterated analogues (414); **2012** 5-methoxy-2-methyl-N,N-dialkylated tryptamines – synthesis and characterization by 1H and 13C NMR, GC-EI-IT-MS, and CI-IT-MS/MS (415); quantitation of substituted N,N-dimethyl-tryptamines in the presence of natural type XII alkaloids by HPLC, ESI-MS, MS/MS, MALDI-MS, and Raman (416); **2013** AMT (3-(2-aminopropyl)indole) and 5-IT (5-(2-aminopropyl)indole) – characterization using 1H- and 13C-NMR, GC-EI/CI-ion trap-MS, U/HPLC-DAD, and HPLC/MS (417).

1.D – Polydrug A: Mixed or Unrelated Named Compounds or Substances

2010 amphetamines, cocaine, codeine, heroin, and morphine – by CEC-ESI ion trap MS (418); 4-methylmethcathinone, 2-fluoromethamphetamine, alpha-phthalimidopropiophenone, and N-ethylcathinone by GC/MS, NMR, FTIR, and GC/IRD (419); 1,4-benzodiazepines and amfepramone – determination as adulterants in phytotherapeutic formulations by adsorptive cathodic stripping voltammetry (420); separation and detection of seven amphetamines, amphetamine, dextroamphetamine, methamphetamine, and MDMA by CZE with capacitively coupled contactless conductivity detection (421); hallucinogenic mushrooms and khat by cation exchange LC (422); morphine, morphine HCl, cocaine HCl, codeine phosphate, papaverine HCl, pethidine

HCl, and thebaine – differentiation with THz time domain spectroscopy (423); piperazines, phenethylamines (2Cs and FLYs), 4-substituted amphetamines, beta-keto-amphetamines (cathinones), 2,5-dimethoxyamphetamines, pyrrolidinophenones, and synthetic cannabinoids – a review of their analyses (toxicological focus) (424); MDMA, MDA, and methamphetamine in Ecstasy tablets by GC/FID (425); marijuana, cocaine, heroin, MDMA, amphetamine, methamphetamine (and other unspecified drugs) – detection using spectral fluorescence signatures (426); **2011** diazepam, flunitrazepam, and methadone – by FT-NIR (427); cocaine and MDMA – detection on textiles using micro-Raman (428); evaluation of the fragmentation pathways of various drugs of abuse (cannabinoids, ketamine, amphetamine, ATSs, cocaine, and opioids) by LC-QTOF MS/MS and MSE accurate-mass spectra (429); sibutramine, modafinil, ephedrine, norephedrine, metformin, theophylline, caffeine, diethylpropion, and orlistat – identification and quantification in diet aids by UHPLC-DAD (430); cocaine and heroin – an evaluation of impurity profiling for comparative analysis (431); herbal products [khat, Psilocybe mushrooms, opium, and “Spice”], designer drugs in tablet and powder form [e.g., mCPP, 3-fluoromethamphetamine (3-FMA), MDPV, and methylone], and anabolic steroids in oil and tablets – by DAPPI-MS (432); MDMA, ketamine, phenmetrazine, ephedrine, pseudoephedrine, caffeine, tramadol (possibly others not listed in the abstract) – analysis of Ecstasy tablets seized in Iran from 2007 to 2008, by physical characteristization, color testing, TLC, anion testing, residual solvent analysis, GC/MS, and LC/MS (433); methamphetamine, amphetamine, MDMA, MDEA, MBDB, MDA, and BDB – by GC/MS following derivatization with trifluoroacetic anhydride (434); heroin, dl-methamphetamine, dl-MDMA, and dl-ketamine – application of dispersive liquid-liquid microextraction and CE with UV detection for chiral separation and determination (toxicological focus) (435); cocaine and heroin – analysis of “crack” cocaine in Iran by TLC and GC/MS (proving that most such samples actually contained heroin) (436); benzodiazepines, beta-blockers, angiotensin-converting enzyme inhibitors, phenothiazines, dihydropyridine calcium channel blockers, diuretics, local anesthetics, vasodilators, anti-diabetic, antidepressant, analgesic, and antihistaminic drugs – by LC-MS/MS (toxicological focus) (437); methamphetamine, MDMA, pseudoephedrine, N-formylmethylamphetamine, and 1-benzyl-3-methylnaphthalene – a study of their degradation in soil (438); analysis of “Happy Water” (containing methamphetamine, caffeine, ketamine, and other components) – by GC/MS and GC/FID (439); morphine, codeine, and hydrocodone – by SERS (440); p-fluoroamphetamine, mephedrone, flephedrone, PPP (alpha-pyrrolidinopropiophenone), MDPV, bk-MBDB, pFBT (3-(p-fluorobenzoyl)-tropane), JWH-073, methylone (3,4-methylenedioxymethcathinone), and N-ethylcathinone – by GC/MS, UPLC-QTOF-MS, and NMR (441); m-CPP and MDMA tablets, cocaine, and LSD – by easy ambient sonic-spray ionization MS (442); Ecstasy Tablets – MDMA, methamphetamine, MDEA, MDA, amphetamine, caffeine, and lidocaine – by TLC and EASI-MS (443); methamphetamine, methamphetamine analogs, and MDMA – a theoretical study of the energetics of the synthesis of various ATS and MDMA (including reactants, products and by-products) (444); cocaine and heroin – a survey of seizures in Luxembourg from 2005 to 2010 (445); bunitrolol, caffeine, cocaine, codeine, diazepam, doxepin, haloperidol, 3,4-methylendioxyamphetamine,

morphine, nicotine, and zolpidem – impact of solvent choice on the analysis of basic drugs by micro-LC/MS (toxicological focus) (446); methamphetamine, MDA, MDMA, and ketamine – detection by 2D THz signatures and spectral dynamics analysis (447); **2012** methandrostenolone, sildenafil, tamoxifen, quinine, clomiphene, dehydroepiandrosterone, anastrazole, clenbuterol, stanozolol, oxandrolone, liothyronine, finasteride, and melatonin in counterfeit drugs and pharmaceutical preparations seized from the black market among bodybuilders – RPLC-DAD and GC/MS (448); antidepressant drugs (sertraline, paroxetine, citalopram, venlafaxine, and fluoxetine) – determination by spectrofluorometry (449); mephedrone, BZP, MDAI, and TFMPP – by microcrystal testing, FTIR, and GC/MS (450); MDA, MDMA, methadone, cocaine, morphine, codeine and 6-monoacetylmorphine – analysis with CZE-TOF-MS (451); MBDB, MMDA-2, and D2PM (and possibly others not specified in the abstract) – enantiomeric separation after derivatization with (R)-(-)-DBD-Py-NCS by UHPLC, with fluorescence and MS detection (452); lidocaine and benzocaine – detection by HPLC with amperometric detection (453); MDMA, ketamine, cocaine, diazepam, phenobarbital, and barbital – analysis using a deep UV/Vis reflected optical fiber sensor (454); cocaine, codeine, nicotine, methadone, phenmetrazine, pentylenetetrazole, niketamide, fencamfamine, and caffeine – by GC/high-res-TOF-MS with a soft ionization source (455); atenolol, salbutamol and cocaine – detection of drug vapors using an ion funnel interface for secondary ESI-MS (456); acetaminophen, phenylephrine, glucose, and caffeine – noninvasive, quantitative analysis of simulated drug mixtures using SORS and multivariate statistical analysis (457); constituents of “legal highs” – MPDV, caffeine, butylone, TFMPP, lidocaine, 4-MEC, mephedrone, pFPP, BZP, and MDPBP – by GC/MS, LC-QTOF-MS, HPLC, and NMR (458); **2013** flunitrazepam, ketamine, and MDMA – detection by IMS (toxicological focus) (459); methoxetamine, 3-methoxyeticyclidine and 3-methoxyphencyclidine – characterization by GC- and CI- MS, NMR, and HPLC-DAD-ESI-MS/MS (toxicological focus) (460); 1,4-benzobenzodiazepines (clonazepam, flurazepam, alprazolam, midazolam, bromazepam, chlordiazepoxide, lorazepam, and diazepam) and antidepressants (bupropion, sertraline, paroxetine, and fluoxetine) – identification as adulterants in phytotherapeutic dieting formulations by voltammetry (461); anorexics (amfepramone, fenproporex, sibutramine), benzozodiazepinic anxiolytics (clonazepam, flurazepam, alprazolam, midazolam, medazepam, chlordiazepoxide, diazepam), antidepressants (bupropione, fluoxetine, sertraline, paroxetine), diuretics (hydrochlorothiazide, furosemide, chlortalidone, amiloride, spironolactone), and hypoglycemics (glimepiride, chlorpropamide, glibenclamide) – differentiation by a solid state electrochemical method (462); mephedrone, 5,6-methylenedioxy-2-aminoindane (MDAI), and MDMA – by SERS on copper coins coated with deposited silver (463); Psilocybe mushrooms, 5MeO-DIPT, tryptamine, MDMA and related compounds, and synthetic cannabinoids and cannabimimetics – an overview (464).

2. Instrument Focus

Forensic Chemists must maintain familiarity with updates in current instrumental techniques and become versant in new, improved methods of analysis.

Improved/existing and new technologies are reviewed and applied to both routine and specialized analyses of drugs. In cases where improved performance is observed, case reports are generated for the forensic community.

2.A – Polydrug B: Mixed or Unrelated Groups of Compounds or Substances

Named Groups of Compounds: 2011 opioids, tranquilizers, stimulants, and hallucinogens – analysis by flow-analysis methods with chemiluminescence or electrochemiluminescence detection (465); a review of the analytical methodologies used to determine adulterants in slimming phytotherapeutic formulations (466); designer cathinones, tryptamines, phenethylamines, and synthetic cannabinoids and cannabimimetics – an overview and review (467); phenethylamine, amphetamine, and tryptamine imine by-products – characterization by GC/MSD, IR, and NMR (468); **2012** (unspecified) synthetic cannabinoids, cannabimimetics, and cathinones – by DART-TOF-MS (469); cathinones, pyrrolidinophenones, tryptamines, and synthetic cannabinoids and cannabimimetics – a review of analytical methods (toxicological focus) (470); 24 phenylethylamines (including 8 cathinones), 3 piperazines, and 3 tryptamines (only MDA, MDMA, ethylamphetamine, and AMT were listed in the abstract) – cross- reactivity in immunosorbent assays (471); phenethylamines, tryptamines, piperazines and cathinones – a review of analyses by GC-EI/MS, LC-ESI/QTOF-MS, and (in some cases) by NMR and FTIR (472); **2013** cathinones, phenethylamines, tryptamines, and piperazines – by LC-QQQ-MS/MS in the MRM mode (toxicological focus) (473);

"Ecstasy Tablets": 2010 impurity profiling of tablets seized in Vietnam using GC and GC/MS (474); **2011** variation in likelihood ratios for same- and different-batch comparisons (specific compounds and analytical methods not specified in the abstract) (475); microwave-assisted extraction of tablets for improved impurity profiling (476); chemical profiling by analysis and identification of residual solvents by static headspace (477); **2012** detection of amines in Ecstasy tablets using a fluorogenic probe (478);

Abused Drugs and Pharmaceuticals in Municipal Wastewater Streams: 2010 by isotopic-dilution direct injection RP-LC-MS/MS (location not specified in the abstract) (479); from a wastewater treatment plant located in "the mid-Atlantic U.S.," by solid phase extraction and GC/MS (480); an overview and review of current methodologies (481); in Paris, France using HPLC-MS/MS after SPE extraction (482); in three Canadian cities (method not specified in the abstract) (483); in Zagreb, Croatia using LC-MS/MS (484); **2011** by SPE

and LC/MS, including a critical evaluation and verification of methodologies (485); a historical review (486); in Australia (methodologies not specified in the abstract) (487); a sampling strategy for sport villages to monitor doping (488); refining the estimation of illicit drug consumptions from wastewater analysis (489); for estimating total drug consumption in small, semi-enclosed population (methodologies not listed in the abstract) (490); **2012** by Mixed-Mode SPE and LC-QTOF-MS (491); for estimating cocaine consumption in the Brazilian Federal District (492); **2013** a study of the uncertainty associated with the estimation of community illicit drug consumption via analysis of sewage (493); by online-SPE-LC/MS (494);

"Illicit Drugs" – Including "Controlled Substances," "Drugs of Abuse," "Illicit Drugs," "Narcotics," "Seized Drugs" (and similar generic terms): 2010 a sensor for "drugs of abuse" (495); screening for "drugs of abuse" by LC-DAD (496); detection of "drugs" using neutron computerized tomography and artificial intelligence techniques (497); detection of "narcotics" using IMS (498); rapid analyses of "illicit drugs" by FTIR and GC/MS (499); rapid field air sampling and analysis of "illicit drugs" using dynamic planar SPME-IMS (500); determination of "illicit drugs" by UHPLC/MS (501); "illicit drug salt forms" by LC/MS (502); qualitative analysis of "narcotics" using Raman and chemometrics (503); identification of "illicit drugs" by teraHertz spectroscopy (504); detection of "illicit drugs" using a tagged neutron inspection system (505); QSAR study on GC/MS Retention Times of "illicit drugs" (506); **2011** "drugs of abuse" and pharmaceuticals – identification of active ingredients by AP glow discharge MS (507); a review and overview of adulterants in "illicit drugs" and their effects (508); acquiring LC/MS or GC/MS analyses following dissolution of microcrystalline test products from "drugs of abuse" (509); detection of "illicit drugs" on surfaces using DART-TOF-MS (510); detection of drugs by proton exchange reaction MS (511); analysis of "narcotics" by Raman (512); detection of "controlled substances" in tablets by ATR/FTIR (and LC-ESIMS) (513); analysis of "seized drugs" by HILIC (514); analysis of banknotes (Euros) from the Canary Islands for "illicit drugs" by LC and MS (515); analysis of "illicit drugs" by GCxGC (516); detection of packaged or concealed "illicit drugs" by spatially offset Raman (517); detection and identification of "illicit drugs" using neutron based techniques (518); detection of "street drugs" by 3-dimensional Spectral Fluorescent Signatures (519); analysis of "multicomponent illicit drugs" by IMS (520); recovery of "illicit drugs" from surfaces using electrostatic lifting and nanomanipulation, with analysis by nanospray ionization mass spectrometry (521); a review of analysis of "drugs of abuse" by Raman (522); screening for "illicit drugs" on banknotes by LC-MS/MS (523); **2012** a review of hyphenated LC techniques (listed applications include "drugs of abuse in alternative matrixes") (524); use of gold-plated Mylar lift films for Raman of "drug residues" (525); 18 (unspecified) "illegal adulterants" in herbal medicines and health foods for male sexual potency – by LC-EI-MS/MS (526); screening of "narcotic drugs" using MECC on a microfluidic device (527); fabrication and use of silver nanoneedles array for SERS and their application in rapid detection of "narcotics" (stated to be especially sensitive for ketamine) (528); **2013** "forensic drug analysis" by microfluidic devices – an overview (529); an evaluation of the results of impurity profiling of "illicit drugs" from different

analytical methods and/or from different laboratories (530); analysis of “seized drugs” by LC-ESI/MS/MS and AP-MALDI-MS/MS, with comparisons of the two techniques (531); an overview of advanced analytical instrumentation and methods for “drugs of abuse” (toxicological focus) (532);

Pharmaceuticals/Counterfeits (with a focus on differentiation of legitimate versus counterfeit products, or for monitoring quality control for legitimate pharmaceutics)**:** **2010** use of portable Raman for identification of tablets and capsules (533); detection of counterfeits using hand-held Raman, infrared, and NIR spectrometers (534); an overview of the analysis of multi-component formulations by spectrophotometric methods (535); imaging pharmaceutical tablets and screening counterfeit drugs by infrared laser ablation metastable-induced chemical ionization (IR-LAMICI) (536); analysis by NIR chemical imaging (537); a review of the use of NIR imaging for pharmaceutical production and counterfeit detection (538); an overview and review of detection of counterfeits using portable NIR and Raman spectrometers (539); a review of the use of FTIR and ATR/FTIR imaging in pharmaceutical production (540); NIR hyperspectral unmixing for chemometric characterization of counterfeit tablets (541); an overview of the detection of counterfeit drugs using LC, CE, and NIR (542); overview and review of the detection of counterfeit drugs, using artemisinin derivatives to illustrate advances in the field (543); analysis by CE (544); identification by NIR (545); detection of counterfeits by NIR (546); an overview of the use of Raman in the pharmaceutical industry (547); application of 2D and 3D optical microscopy in the examination of suspect counterfeit tablets (548); identification by NIR and NIR chemical imaging (549); detection by NIR (550); identification of tablets by Raman and chemometrics (551); a review of the determination of drugs by TLC (552); tracing the origin of complex pharmaceutical preparations using surface desorption AP-CI-MS (553); detection of counterfeits by NIR (554); **2011** detection of counterfeit drugs by NIR (555); comparison of laboratory and handheld Raman for the identification of counterfeits (556); detection and identification of counterfeits by NIR (557); discrimination between legitimate and counterfeit products using NIR, Raman, GC/MS, and FTIR, with application of supervised classifiers (k-Nearest Neighbors, Partial Least Squares Discriminant Analysis, Probabilistic Neural Networks, and Counterpropagation Artificial Neural Networks) (558); a review of non-invasive analyses of turbid samples using deep Raman (559); isotopic finger-printing of active pharmaceutical ingredients by 13C-NMR (560); by portable Raman (561); use of DART-MS to screen tableted pharmaceuticals and detect counterfeits (562); detection and profiling of counterfeits by Raman and chemometrics (563); use of isotope-labeled excipients to identify legitimate and counterfeit products (564); an overview of “poor quality” drugs (565); detection by DOSY-NMR (566); detection of counterfeits by NIR diffuse reflectance spectroscopy (567); detection of counterfeits by quantitative NMR and DOSY NMR (568); analysis by TLC with AccuTOF-DART MS (569); overview of detection using a portable NIR spectrometer (570); detection and analysis of counterfeit pharmaceutical tablet cores by ATR/FTIR and micro-ATR/FTIR imaging (571); discrimination of illicit tablets by surface granularity (572); identification of the components in drugs by near-infrared hyperspectral unmixing of tablets (573); an overview of counterfeit drugs (574); a review of

rapid, noninvasive characterization of pharmaceuticals and counterfeits in packaging or containers using Raman (575,576); determination of the elemental distributions in tablets by confocal micro-XRF (577); invisible labeling of pharmaceuticals for identification and verification of authenticity (578); a review of chiral analyses of drugs (579); detection of counterfeits by vibrational spectroscopy (580); a review of methods used to detect counterfeits or confirm authenticity (581); overview and review of Raman for analysis of pharmaceuticals (582); an overview and review of counterfeiting (583); analysis of pharmaceuticals with hyperspectral Raman imaging and various chemometric methods (584); analysis of pharmaceuticals by DART-AccuTOF-MS following TLC separation (585); **2012** comparison of handheld to benchtop Raman instruments for the identification of authentic versus counterfeit tablets (586); detection of counterfeit tablets by transmission Raman (587); quality control screening and counterfeit detection using portable Raman (588); evaluation of differently manufactured pharmaceutical tablets (including illicit drugs and counterfeits) Raman hyperspectral images (589); use of laser-induced breakdown spectroscopy and support vector machines for classification of pharmaceuticals and counterfeits (590); by DART-MS – an overview (listed applications include “screening of counterfeit drugs”) (591); analysis of “soft” pharmaceuticals and counterfeits (suppositories, etc.) by DART-MS (592); analysis of tablet packaging by Raman microscopy and 2D-correlation spectroscopy (593); monitoring and detection using NIR (594); analysis of residual solvents in counterfeits by GC/MS (595); differentiation of legitimate versus counterfeit drugs by NIR and chemometrics (596); 14 unspecified “sedative-hypnotic drugs” – detection in health foods and traditional Chinese medicines by GC/MS (597); **2013** a review of a paper-based test for screening for counterfeits (598); an overview of chromatographic and spectroscopic detection methods (599); by Raman (600); a review, focusing on HPLC and MS, but also discussing color testing, TLC, GC, Raman, NIR, FTIR, and NMR, using antimalarial drugs and sildenafil (Viagra) as illustrative examples (601); an overview of the use of GC/MS for “forensic substance identification” (602).

2.B – New and/or Improved Instrumental Techniques

Atomic Absorption Spectroscopy: **2012** a review, focusing on pharmaceuticals (listed applications include “forensic”) (603);

Capillary Electrophoresis (and Related Techniques, including Tandem Techniques): **2011** CE – a review of the literature from 2006-2010 (focus is “natural products; ” listed applications include pharmaceuticals and “toxicological compounds of interest to forensics”) (604); **2012** evaluation and optimization of CZE for common drugs of forensic interest in aqueous matrices (605); CE – a review of the literature from 2009 to 2011 (listed focus includes illicit and abused drugs, ions, and small molecules of forensic interest) (606); **2013** a review of recent advances in electrodriven enantioseparations (listed applications include “pharmaceutical” and “forensic”) (607);

Gas Chromatography: 2012 a review (listed applications include "bulk drugs") (608);

Infrared Spectroscopy: 2012 ATR/FTIR – a review (includes select chemical, pharmaceutical, and forensic applications) (609); IR of solid–dosage drug substances – an overview (610);

Infrared and Raman Spectroscopy: 2012 in Forensic Science (Reference Text) (611);

Ion Spectroscopy: 2012 IMS with an orthogonal acceleration sector TOF mass analyzer (designed for "forensic applications") (612);

Mass Spectrometry: 2010 identification of active compounds in tablets by flow-injection data-dependent tandem mass spectrometry combined with library searching (613); differentiation of structural isomers of "drug substances" using LC/Q-TOFMS and fragmentation prediction (614); **2011** ESI-MS – use of wooden toothpicks for facile loading and ionization of samples (615); ambient ionization mass spectrometry – an overview and review, including discussions of counterfeit and illicit drugs (616); DART-MS – a review (listed applications include pharmaceuticals and forensics) (617); a review of the applications of DESI-MS (includes "drugs," pharmaceuticals, and "forensics") (618); **2012** ambient desorption/ionization MS (ADI-MS) – an overview and review (listed applications include "forensics") (619); identification of unknowns utilizing accurate MS data and ChemSpider (620); an overview of recent advances (621); identification of unknowns using an API MS/MS library (622); **2013** ambient mass spectrometry – a review, including DESI, DART, and extractive ESI (listed applications include "forensic identification") (623); DESI-MS (listed applications include "illicit drugs") (624);

Microscopy: 2010 an overview (625);

Nuclear Magnetic Resonance Spectroscopy: 2012 high-precision 1H-qNMR – for determination of the purity of standards (626);

Raman: 2010 non-contact, in-the-field analysis of "hazardous materials" by portable Raman operating in various modes (627); **2011** a review (includes forensic science applications) (628); **2012** multi-wavelength excitation Raman spectrometers and microscopes (listed applications include "narcotics identification") (629);

Solvent-Microextraction: 2013 a review (listed applications include forensic and pharmaceutical) (630);

Stable Isotope Analyses: 2010 recent advances (includes drugs) (631); position specific 13C analysis for determination of source and the natural attenuation of contaminants (632); a review of the use of stable isotopes in forensic science (633); **2011** an overview of the use of IRMS, proposing a 6-step methodological approach for application to specific forensic issues (634); a general review of the use of stable isotopes to determine source (635); **2012**

an overview of the signature value of isotope deltas (636); **2013** a review of inter-laboratory comparability (637); tracking authentic pharmaceuticals by 2H- and 13C-NMR (638);

Thin Layer Chromatography: **2011** a review of TLC/MS (639); **2012** quantitative HPTLC-densitometry – converting TLC screening for counterfeit pharmaceuticals to HPTLC (640);

X-Ray Techniques: **2012** wavelength-dispersive XRF – for analysis of very small samples (listed applications include "forensic analysis") (641).

3. Miscellaneous Topics

Clandestine Laboratories – Appraisals and Safety: **2012** comparison of first responder decontamination procedures (642); testing of fire resistant fabrics after the application of flammable solvents (643); therapeutic detoxification of law enforcement personnel suffering from chronic occupational exposure to methamphetamine (644);

Education: **2011** analysis of a simulated drug sample by GC/MS and FTIR (645); analysis of a simulated drug sample by TLC and GC/MS (646); **2013** use of forensic science to teach method development in undergraduate analytical laboratories (647);

Legal Issues: **2010** legal issues (648); **2011** legal issues (649); **2012** brief news release concerning counterfeits (650); reference text (651);

Packaging: **2011** identification of plastic packaging used by body packers, by IR (652); **2012** a review of the use of SEM/EDS and FTIR to identify counterfeit pharmaceutical packaging (653); analysis of polyethylene cling film (commonly used for packaging illicit drugs) by ATR/FTIR (654);

Quality Assurance: **2010** measurement uncertainty in forensic/analytical testing (655); the uncertainty in measurement of the total mass of a substance packaged in numerous containers (656); **2011** comparison of the stability of stock solutions of drugs at freezer, refrigerator, and ambient temperatures (657); measurement uncertainty in sampling and analysis of illicit drugs (658); **2013** use of a software tool ("Drugs WorkBook") for the quantification of illicit drugs (659);

Sampling Plans: **2010** an Excel based sampling calculator (660); a probability-based sampling approach for the analysis of multiple containers of cocaine, heroin, or marijuana (661);

Soil: **2011** determination of source by XRF (662); **2012** analysis by Raman following oxidative sample preparation (663); an overview of forensic analysis for determining geographical source (664);

Other: **2010** an informal classification scheme for "designer drugs" in Israel (665); **2011** an overview of drug production and use in New Zealand (666);

synthetic chemist David Nichols discusses his research on psychedelic compounds, commenting on how his products have been abused (667); **2012** Laboratory Information Management System (LIMS) – an overview and review (668).

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Toxicology

Review 2010 - 2013

Wai-ming Tam, Ph.D.; Lai-chu Chim, Ph.D.;
Wing-sum Chan, Ph.D.; Tai-wai Wong, M.Phil.; Kit-mai Fung, Ph.D.;
Wing-cheong Wong, Ph.D.; Wai-kit Lee, Ph.D.; Wing-sze Lee, Ph.D.;
Kit-man Fan, M.Phil
Government Laboratory, Hong Kong Special Administrative Region, CHINA

Presented by
Fu-chiu Kwok, Ph.D.

All correspondence should be addressed to Dr. Fu-chiu Kwok, Acting Assistant Government Chemist, Government Laboratory, 7/F, Homantin Government Offices, 88 Chung Hau Street, Ho Man Tin, Kowloon, Hong Kong SAR, China.
e-mail : fckwok@govtlab.gov.hk
http://www.govtlab.gov.hk/

TABLE OF CONTENTS

1 Abstract

The rapid development of forensic toxicology in recent years is evidenced by the proliferation of professional societies, growth in the awareness for the need of accreditation and quality assurance, and publication of a large number of research articles encompassing a variety of toxicology disciplines. Undeniably, advancement of forensic toxicology has been largely driven by the development of highly sophisticated instruments and improved methodologies. These breakthroughs lead to a remarkable enhancement in sensitivity and specificity of detection that render the detection of drugs and poisons at very trace level and in a wide range of biological specimens possible. Nevertheless, the continual appearance of new designer drugs has posed serious challenges to both analytical and interpretative abilities of forensic toxicologists since the chemical structures of these drugs are continuously modified in order to obscure their detection and evade legislative control.

The purpose of this paper is to review the scientific literature from 2010 to 2013. This review is divided into two parts, namely “Current Toxicological Issues” and “Advances in Toxicological Analysis” capturing the significant progress and development in the field of toxicology over the past three years by making reference to hundreds of articles and papers published in the international journals and symposiums.

2 Introduction

Toxicology is not a science that simply studies the toxic and harmful effects of chemicals, drugs and poisons; it has to draw upon knowledge, theories and techniques from diverse scientific fields such as biochemistry, chemistry, epidemiology, pharmacology and pathology in order to deal with the ever increasing complexity in this discipline. Forensic toxicologists are tasked with the challenges in detecting and identifying alcohol, drugs and poisons in bodily fluids, tissue samples and related items, and, whenever necessary, offering professional opinion to aid the medico-legal investigation of death, poisoning and drug-facilitated criminal offences in the interest of justice.

3 Current Toxicological Issues

3.1 Driving Under the Influence

Undoubtedly impaired driving caused by the influence of alcohol or drugs has led to a very large number of accidents and casualties every year worldwide since the intake of alcohol and drugs directly impairs the driving abilities, response time and judgment of the drivers as well as affects their coordination of cognitive and psychomotor functions during driving. In a clinical research using the technique of functional magnetic resonance imaging (fMRI), impaired driving behavior is associated with disruptions in functional network connectivity (1).

3.1.1 Driving under the influence of alcohol (DUIA)

Various bodily specimens may be considered for measuring the concentration of alcohol in an individual. The two most popular specimens for alcohol testing are blood and breath. Since blood alcohol analysis is invasive, expensive and time-consuming, breathalysers, which are non-invasive, become the most prevalent devices worldwide to assist the law enforcement nowadays.

That blood and breath analyses are interchangeable is based on the presumption that there is a stable relationship between the blood and breath alcohol levels. Grubb D *et al* have studied their relationship during the absorption, distribution and elimination phases of alcohol metabolism with particular emphasis on the absorption phase (2). Even though sampled blood is stored in the presence of preservative and anticoagulant, it is imperative that blood alcohol analysis should be performed as soon as possible because studies have shown that blood alcohol concentrations decreased over long term storage both under refrigeration and at room temperature (3). Besides measurements using conventional devices, recent studies have been undertaken to develop a novel non-invasive biological sensor for detecting individuals driving under the influence of alcohol by measuring biosignals (4).

As a defence argument to evade justice, drivers may allege that consumption of alcohol took place after driving by a tactic commonly known as the hip-flask defence. A research was undertaken by Jones AW on human pharmacokinetics with a major focus on elimination rate of blood alcohol (5). The study facilitated back calculation for cases in which the courts of law want

to know the defendants' blood alcohol concentration at some earlier time, such as the time of driving.

Apart from enforcement, public education and publicity are of equal importance to raise the awareness of the legal implications as well as the dangers of driving while intoxicated. High concentrations of blood alcohol (≧0.8g/L) significantly increase the risk of severe injuries while driving (6). It has been reported that educational programmes in Brazil should be targeted at specific groups in order to increase their awareness about the legal blood alcohol concentration limit and its consequence (7). A study has analyzed local drink-driving patterns by a cluster analysis approach to model the spatial-temporal variation of drink-driving distribution in Hong Kong (8). The results indicated that drivers in rural areas tend to consume more alcohol than those in urban areas. Another study (9) investigated the trend of drink driving in Hong Kong after the implementations of random breath testing and alcohol tax reductions. It was concluded that the problem of drink drinking could be combated by strategies such as random breath testing, awareness-raising campaigns and increased penalties.

It is well understood that combined consumption of alcohol and illicit drugs can have detrimental effects on driving beyond those of alcohol alone. Studies have shown that the effect of alcohol and cannabis taken simultaneously is indeed additive leading to increased risk of traffic accidents (10,11,12). It was found in reference (13) that blood concentrations of tetrahydrocannabinol (THC), the principal psychoactive ingredient of cannabis, would be higher when THC is consumed with alcohol. According to this study, this explains why drivers were more impaired in cannabis and alcohol combined conditions.

3.1.2 Driving under the influence of drugs (DUID)

It is known that use of drugs can impair driving. However the extent of impairment can be difficult to measure, predict or quantify. Furthermore, DUID is often under-reported or unrecognized. Effort has been made to investigate what types of drugs and their associated limits in blood that should be specified in DUID (14) and a consultation in this regard has been launched in the UK (15). In the USA, a national survey on drug use and health revealed that 9.4 million persons or 3.7% of the population aged 12 or older had driven under the influence of illicit drugs in 2011 (16).

Toxicological investigations of drivers killed in road traffic accidents in Norway during 2006-2008 showed that 17.9% (of 196 cases analyzed) of the fatally injured drivers had drugs or alcohol/drug concentrations above the proposed legal limit in the blood. The extent of impairment was comparable to a blood alcohol concentration (BAC) of 0.02% (17). Similar inference was found from a study in the Netherlands, which indicated patients who have been exposed to psychoactive medications, especially anxiolytics or selective serotonin re-uptake inhibitors (SSRIs), are more likely to be involved in traffic accidents (18).

Stimulants, depressants, hallucinogens and sedatives are among the frequently encountered drugs in drug-impaired drivers. Many of the drugs that affect central nervous system (CNS) produce characteristic effects. Depressants tend to slow reactions and reduce concentration. Drivers under the influence of marijuana may find complex driving situations more difficult to negotiate. Stimulants might make drivers over-confident and aggressive, while those under the influence of hallucinogens might react erratically to imaginary obstacles or sounds. In Switzerland, results from a nationwide study on DUID indicated that cannabinoids and cocaine were the most prevalent classes of drugs (besides alcohol) among DUID offenders in 2005 (48%, N = 2,291 and 25%, N = 1,184 respectively) (19). Prevalence studies conducted in different countries have demonstrated that drug-impaired driving (20,21,22,23,24), cannabinoids in particular (25,26), is a serious problem worldwide.

3.1.3 Detection of DUID

Identification of DUID drivers is generally based on two approaches, namely the impairment approach and the drug presence approach.

3.1.3.1 Impairment approach

Impairment approach utilizes standardized tests, based on a variety of observable signs and symptoms, and divided attention tests, to identify driving impairment associated with the consumption of drug. However, the relationship between the use of psychoactive drugs and degree of impairment is an extremely complex subject.

Several studies have been carried out to establish the impairment effect of amphetamine type stimulants (ATS) drugs on driving (27,28,29). In one of the studies (28), 8,709 cases of DUID plasma samples were analysed and 1,857

of them were positive to ATS, with cannabinoids being the most common (59.7%) co-consumption drugs. For those cases positive to ATS only, there is no correlation between impairment symptoms and plasma ATS concentration.

In another investigation (29), a dose of 0.42 mg/kg *d,l*-methamphetamine (MA) was taken orally by 20 healthy recreational illicit stimulant users. The mean levels *d,l*-MA detected in blood and saliva were found to be 95 ng/mL and 475 ng/mL respectively after 3 hours of drug administration. Participant's simulated driving performance was evaluated at 150 minutes post consumption. These drug levels were found not to significantly impair or improve the driving performance in the simulated test.

Standardized Field Sobriety Tests (SFSTs) are physical tests designed to assess psychomotor and cognitive functioning and divided attention component. These are believed to be accurate indicators of driving behavior following the consumption of alcohol or drugs. Evaluation of the effectiveness of SFSTs in identifying impaired driver who consumed *d*-MA and *d,l*-3,4-methylenedioxymethamphetamine (MDMA) was the main subject of the study (30).

In order to facilitate judicial process, impairment based legislative limits for DUID was suggested in Norway (31). Legislative limits for six classes of drugs (benzodiazepines, cannabis, CNS stimulants, γ-hydroxybutyric acid, hallucinogens, and opioids), which would cause degrees of impairment comparable to BAC of 0.02% and to BACs of 0.05% and 0.12 %, were proposed as impairment limits and as limits for graded sanctions respectively.

3.1.3.2 Drug presence approach

Even though collection of blood sample is considered invasive and requires the assistance of qualified healthcare professionals, who may not be available at roadside, blood remains the preferred specimen because it provides a direct evidence of the presence of drug(s) in the body and information about the respective drug(s) concentration in blood. Specific and efficient screening can be achieved by using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (32,33), which has the potential to analyse large drug panels. A fully automated method was developed which is capable of quantifying 31 illicit and medicinal drugs and metabolites, including commonly abused drugs such as amphetamines and cocaine, in whole blood (32). The

published method (33), developed in a bid to both facilitate high-throughput screening and replace immunoassay, simultaneously detected 28 drugs/metabolites in 0.5 mL of whole blood in 9 minutes. Alternatively, the use of time-of-flight mass spectrometry (TOF-MS) was able to resolve isobaric compounds and identify unknown analytes by accurate mass measurement (34).

A study (35) in Sweden examined 1,000 blood samples from drivers suspected of DUID. Blood concentrations of diazepam and nordiazepam were assessed against the upper therapeutic limit of diazepam (0.83 mg/L in blood). 9% (N=90) of the cases had blood concentrations of diazepam above 0.83 mg/L, where 27% (N=267) of them were above that limit if the combined concentrations of diazepam and nordiazepam were considered. According to a study aiming at quantifying the concentrations of drugs in blood collected from suspected drugged drivers in England and Wales (36), diazepam was the second most common drug of abuse.

To combat drug driving, the use of oral fluid (OF) as an alternative specimen to detect the presence of drugs has accelerated in recent years and effort has been made to compare drug concentrations in blood and OF (37,38). Drugs concentration ratios between OF and blood (OF/B) varied considerably from drugs to drugs and patients to patients. The median OF/B ratios found for zopiclone, amphetamine, THC, MDMA, codeine, and MA were 3.8, 7.1, 4.7, 4.6, 5.4 and 2.9 respectively. On the other hand, benzodiazepines included in the study had low OF/B ratios (mean <0.5) and this can be explained by their high protein binding ability. The considerable variation in drug concentration ratios between OF and blood indicated it might not be possible to estimate drug concentration in blood precisely from that in OF. Nonetheless, OF/B ratios have been used to estimate the prevalence of drug concentrations in blood above specified limits (39).

OF is gaining popularity as an alternative matrix for drug testing in different fields, especially in roadside drug testing because of the ease and less intrusive protocol of sample collection. The performance of various on-site OF drug testing devices was assessed (40,41,42,43), where effort has been focused on sensitivity and specificity of the devices. In one of the studies (40), eight on-site OF drug screening devices for enforcement purposes were evaluated in Belgium, Finland and the Netherlands, as a part of the European

collaborative project named “Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID)” that was carried out between October 2006 and October 2011. OF screening results were assessed against the DRUID cut-off concentrations. Overall, no device reached the 80% goal set for sensitivity, specificity and accuracy for all of the separate tests that they comprised.

Besides on-site screening devices, collection tools can have a major impact on the concentrations of drugs present in OF. Stability of two collection devices, Intercept® and StatSure Saliva Sampler™, were compared by using authentic OF samples with different substances (44). According to the study, drugs showed greater stability in StatSure than in Intercept® for storage at 4 ºC or ambient temperature for one week. Recovery of zopiclone was particularly problematic (Intercept®: 6% and StatSure: 56% after one week room temperature storage). As a result, freezing after sampling was advised. In a study focused on THC (45), recoveries of THC from on-site collectors were unsatisfactory due to the problem of drug adsorption onto the collectors.

A recent research (46) evaluated cross-reactivities of three commercial OF immunoassays: amphetamine direct enzyme-linked immunosorbent assay (ELISA) kit, MA direct ELISA kit, and Oral-View Saliva multidrug of abuse test for detection of ATS. None of the ELISA kits showed significant cross-reactivities with *d,l*-fenproporex (FEN), *d,l*-diethylpropion (DIE) and *d,l*-threo-methylphenidate (MPH) (Amphetamine ELISA: < 0.01%, < 0.006% and < 0.006% respectively; MA ELISA: All < 0.02%). Oral-View did not cross react with these drugs at 10 folds of the cutoff concentrations (50 ng/mL). It should be noted that MPH and DIE are commercialized in the United States, while FEN is used as an anorectic in Brazil and Chile.

Due to the volume of OF collected is usually relatively low, simultaneous analysis of multiple drugs in OF is expected. Recently, an ultra-high-performance liquid chromatography (UHPLC) MS/MS method was published (47) for the detection of opiates, amphetamines, cocaine, ketamine, and cannabinoids in a single 11-minute run. 466 on-site residual OF samples were collected. 250 µL of OF was spiked with deuterated internal standard and injected to UHPLC-MS/MS directly. No sample preparation was needed. Of the 466 samples, 74 samples showed the presence of cocaine and its metabolites, THC was detected in 49 samples, MDMA was detected in 11 samples and ketamine in four samples and two samples showed codeine and morphine. In

contrast, a sophisticated gas chromatography-mass spectrometry (GC-MS) method (48) was developed to simultaneously detect and quantify 50 drugs of abuse and medicinal drugs in OF, including cannabinoids, cocaine, amphetamines, opioids, benzodiazepines and other psychoactive medicines. Altogether, 4,183 OF samples were collected on-site with StatSure SalivaSampler™ device in Finland. These were analyzed with the aforesaid method as a part of the EU project DRUID. THC was found to be the most prevalent drug.

To evaluate the performance of an OF drug screening device, confirmatory results done by using liquid chromatography-MS/MS (LC-MS/MS) are needed. Evaluation of DrugWipe® benzodiazepine on-site test was carried out in Finland with whole blood specimens (49). Use of OF on-site screening tests and blood confirmatory analyses mimics the real scenario in many countries. In a total of 224 DrugWipe® OF positive cases from the Finnish police, 181 were positive for one or more benzodiazepines in the whole blood analysis. DrugWipe® OF screening device was able to report positive benzodiazepine results in OF from cases that contained only single benzodiazepine with relatively low concentration in whole blood analysis. In one of those screened-positive cases, clonazepam (therapeutic range: 20-60 ng/mL) with a concentration of 11 ng/mL in whole blood was detected.

Confirmation analysis in DUID cases has been continuously proven to be challenging in light of emerging new designer drugs and a variety of drugs affecting the CNS (50,51,52). Synthetic cannabinoids, for instance, often lead to driving impairment similar to that caused by cannabis (53) and could go undetected by routinely used drug screenings. Two suspects were arrested for DUID with amphetamine-like impairment. Target confirmatory analyses of their urine samples, which previously tested positive for amphetamines in an immunoassay screening, were found negative. A GC-MS method was thereby established for the analysis of 4-fluoroamphetamine (4-FA) in serum with a limit of detection (LOD) of 1 ng/mL. Using the new method, 4-FA was detected in serum at concentrations of 350 ng/mL and 475 ng/mL in the two subjects respectively. Another designer drug, 3,4-methylenedioxypyrovalerone (MDPV), emerged in Finland since 2008 (52). Blood samples from 3,000 drivers suspected of DUID were screened for MDPV using an LC-MS/MS method with a LOD of 3 µg/mL. 259 of them were tested positive for MDPV, accounted for 5.7% of the confirmed DUID cases in Finland from August 2009 to August

2010.

3.2 Drug-facilitated Sexual Assault (DFSA)

In recent years, there has been an increase in the number of reports involving the administration of drug(s), sometimes in conjunction with alcohol, to render a victim physically incapacitated or helpless and thus incapable of giving or withholding consent. If an individual takes advantage of such situation and has non-consensual sexual relations with the victim, it should be considered a case of DFSA. Victims may be unconscious during all or parts of the sexual assault and, upon regaining consciousness, may experience anterograde amnesia which means individuals may not recall events they experienced while under the influence of drug.

3.2.1 Detection of drugs

There has been an increase in reported DFSA cases over the last 15-20 years (54,55). In a separate report, 135 cases of DFSA in the Netherlands were studied from January 2002 until December 2006 (56). The study showed that alcohol was the most commonly found substance in DFSA cases in the Netherlands followed by non-opiate analgesics and illicit drugs (of which the most frequently encountered drugs were cocaine, MDMA, THC or their metabolites, followed by amphetamine and benzodiazepines). In the same report, it was found that blood specimens collected within 12 hours of the alleged assault were all tested positive for alcohol or drugs while those collected more than 24 hours after the alleged sexual assault were all tested negative. When urine samples were available, only 36% of the cases with collection time longer than 24 hours had negative toxicological results. It was therefore concluded that if sexual assault took place 24 hours or longer, urine rather than blood would be a more suitable specimen for collection.

Ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC-TOF-MS) was used for the screening of 46 medicinal drugs and abused drugs (including amphetamines, cocaine, benzodiazepines and opioids) in 167 whole blood samples obtained from victims of alleged sexual assault cases in the Aarhus area, Denmark (57). The whole blood samples were extracted using a mixed mode solid phase extraction procedure and the estimated limits of quantification for the drugs ranged from 0.06 to 27 ng/g. Ethanol, barbiturates, THC and its metabolites were analyzed using other

methods. It was concluded that only a small percentage of all cases seemed to be genuine DFSA cases. It was also notable that victims tested positive of medicinal/abused drugs did not undergo a timely medical examination.

3.2.2 Drugs detected in DFSA cases

Two cases of DFSA using tetrahydrozoline (THZ), an ingredient in over-the-counter eye drops, were reported (58). THZ was detected in the urine samples by GC⁄MS at levels of 114 ng/mL and 150 ng/mL, respectively, in these two cases. However, THZ was not detectable in the blood for both cases. It was shown that the use of GC⁄MS was successful in identifying THZ in the 100 ng⁄mL range up to 20 hours post-exposure. Stillwell *et al.* also reported a case with THZ at a level of 1.481 ng/mL in the urine approximately 7 hours after the victim was reportedly being sexually assaulted, even though no symptoms was observable in the emergency department (59).

Gamma-hydroxybutyrate (GHB) has frequently been implicated in a number of DFSA cases. In this regard, the possibility of maintaining long term stability of GHB in both post-mortem and ante-mortem whole blood samples was investigated in reference (60). Cut-off level in the study was 10.3 mg/L and GHB concentrations were found to be stable for several years in both post-mortem and ante-mortem samples when stored at -20 ℃ with fluoride preservation. The maximum changes in GHB concentrations were 32.4% for ante-mortem and 34.4% for post-mortem samples.

Cut-off values of exogenous GHB remained an active area in research. A study of in vitro production of GHB in blood and serum samples suggested that the 5 μg/mL cut-off for exogenous GHB could be lowered significantly if the whole blood sample is frozen immediately after collection with procedure well documented (61).

As for urine specimens, a study of urinary GHB concentrations in samples taken from 1,126 healthy female volunteers supported the use of 10 mg/L urinary GHB as the cutoff (54).

γ-butyrolactone (GBL) was known to be metabolized into GHB. Pharmacokinetics study of GHB after single uptake of a low dose of GBL showed that the GHB concentration in serum decreased below 1 μg/mL after 4-5 hour and further diminished to less than 1 μg/mL within 8 - 10 hours (62).

γ-valerolactone (GVL) is reported to be a substance that can be used as a legal substitute for GHB. But unlike GBL and 1,4-butanediol, GVL is not metabolized to GHB. Instead, the lactone ring of GVL is split to form gamma-hydroxyvaleric acid (GHV or 4-methyl-GHB) by lactonase. Andresen-Streichert *et al.* reported the detection of GVL in three cases (63). The study results indicated that GVL can be used as an alternative to GHB and its precursors, i.e. GBL and 1,4-butanediol. With one of the three cases being probably a DFSA incident, the use of GVL should be taken seriously. It was advised that GVL or GHV should be included routinely in toxicological analysis, particularly in DFSA cases.

3.2.3 Summary

When drugs are used to facilitate an assault, the victims, medical professionals and law enforcement officers are relying on the forensic toxicologist to conduct the best possible testing of the available specimens. It is imperative that adequate volumes of blood and urine samples be collected from the victims as soon as practicable. This is particularly pertinent for drugs that are eliminated quickly such as GHB and its related compounds. At the same time, forensic toxicology laboratories should properly preserve the drugs in the specimens to prevent them from deterioration, develop validated analytical procedures, and employ sophisticated instruments whenever necessary so as to improve the detection limits in their drug screening as some drugs may be present at very low levels in DFSA cases.

3.3 Workplace Drug Testing

Federal Workplace Drug Testing Programme was firstly introduced in the United States in 1988 aiming at establishing a drug free environment in workplace through a mandatory requirement for all relevant executive-level and civil-service federal employees to pass urine drug tests for drugs of abuse (64). Now, Substance Abuse & Mental Health Services Administration (SAMHSA) of the Department of Health and Human Services is authorized to promulgate scientific and technical guidelines for drug testing programme.

Meanwhile, pre-employment and workplace drug testing in the field of safety-critical and security-sensitive jobs has increased rapidly over the last decade in many European countries including Italy and Turkey

(65,66,67,68,69,70,71,72,73). Since the outcomes of testing can have serious consequences for the employees, the European Workplace Drug Testing Society (EWDTS) has formulated guidelines in order to ensure that the whole drug testing process is of high quality, accredited, and defensible, hence giving accurate and reliable information about employees' drug use profiles while respecting their privacy. Furthermore, the testing laboratories must adhere to national and international quality standards (ISO/IEC 17025) (67).

3.3.1 Urine for workplace drug testing

Urine remains the most commonly used specimen for drug testing because the technology used in urine testing is well developed and has withstood legal challenges. Drugs in urine are normally detectable several days after the last intake (74). A positive urine test result can serve as an evidence of recent use, but does not necessarily mean that an individual was impaired at the time of being tested (64).

Careful attention should be exercised at the time of collecting urine specimen from donor in order to avoid tampering by adulteration, substitution or dilution which may circumvent the purpose of drug testing. Aiming to evade detection, potassium nitrite is an effective urine adulterant due to its oxidizing potential, and has been shown to mask the presence of many drugs of abuse. A study (75) has revealed the possibility of using LC-MS to detect two stable reaction products, i.e. 2-nitro-morphine and 2-nitro-morphine-6-glucuronide in an attempt to indirectly infer morphine and morphine-6-glucuronide in urine once the specimens are suspected to be adulterated with nitrite. Since dilution of urine specimen is another deceitful tactic to avert drug detection, a study (76) has examined the effectiveness of creatinine normalization on urine drug concentrations of 5 substances (amphetamines, cocaine, marijuana, opiates, and phencyclidine) and the test results indicated that the proportion of reported positives would be affected.

Workplace urine drug testing usually adopts a two-step approach for the positive identification of drugs. This involves both a screening test and a confirmatory test. Immunoassay is commonly used as a screening tool because the method is fast, inexpensive and reasonably cost-effective. A urine specimen once presumptively screened positive by immunoassay must be subject to confirmatory testing by mass spectrometry techniques in order to eliminate false-positive results that may arise from cross reactivity in immunoassay (77,78).

Generally, immunoassay can screen for most common drugs of abuse, but fail to detect a number of emerging designer drugs. In contrast, direct analysis using LC-MS/MS offers an attractive way forward for the development of a rapid routine screen for new psychoactive substances (79). It was also reported that a multi-target screening method that allows the simultaneous detection and identification of 700 drugs and metabolites in biological fluids by using a hybrid triple-quadrupole linear ion trap mass spectrometer in a single analytical run was successfully developed. With the assistance of software program to achieve automated acquisition and library searching, the time for evaluation and interpretation of the test results could be drastically reduced (80).

THC remains one of the most frequently encountered drugs in workplace drug testing. Therefore, there is a great demand for sensitive, rapid and reliable methods for confirming the presence of this drug or its metabolite in biological samples including urine. A newly developed method employing LC-MS/MS for direct analysis and simultaneous determination of THCA and THCA-glucuronide in urine, without the need of hydrolyzing/derivatising the samples has been validated and proved to be accurate, precise and sensitive with a LOD of 5 ng/mL for both analytes. The developed method had been applied to several authentic samples of urine which were tested positive in immunoassay screening and 98% of them were confirmed (81). As a marker for detection of cannabis abuse in urine, THCA needs to be present at a concentration exceeding 15 ng/mL for a positive result to be reported. A research team presented a method (82) combining a GC-MS/MS method with a fast sample preparation procedure using microwave assisted derivatisation. This method was proven to be selective, linear over the range 5-100 ng/mL, along with excellent precision and trueness.

Another study demonstrated the use of a newly developed method employing GC-MS technique for the quantitative analysis of the new designer drug MDPV along with common stimulants including amphetamine, methamphetamine, and MDMA in urine (83).

The ongoing epidemic of prescription opioid abuse in the United States has prompted interest in semi-synthetic opioids in the federal workplace drug testing program. Cone *et al.* initiated a study characterizing the metabolism and disposition of oxycodone (OC) in human urine (84). Twelve healthy adults were administered a single oral 20 mg dose of OC in a controlled clinical

setting. Their urine specimens were collected at regular time intervals and analyzed by liquid chromatography-tandem mass spectrometry for OC and its metabolites. The data of this study provided information in facilitating the selection of appropriate test parameters for OC in urine and interpretation of test results.

3.3.2 Oral fluid for workplace drug testing

As an alternative specimen to urine for workplace drug testing, oral fluid is increasingly used because the concentrations of many drugs in oral fluid seemingly correlate well with blood/plasma concentrations. However a study indicated that cannabinoid concentrations in oral fluid cannot predict respective concurrent concentrations in plasma (85). Advancement of instrumental sensitivity makes oral fluid a suitable alternative to blood. Oral fluid is getting popular because its collection is easy, convenient and non-invasive. Furthermore, adulteration is inherently difficult (86). Even though SAMSHA is still actively seeking comments about the use of oral fluid as an alternative specimen in Federal Workplace Drug Testing Programs, EWDTS has outlined guidelines for oral fluid drug testing that suggested the maximum cut-off concentrations acceptable under the workplace drug testing programme. The recommended cut-off values may be subject to change as advances in technology or other considerations warrant identification of these substances at different concentrations (87).

As oral fluid often contains drugs in low concentrations and volumes of specimen collected are small, it is therefore necessary to have a sensitive, multi-component method for drug detection. Through a research study (44), such objective has been fulfilled by successful development of a method employing an UPLC-MS/MS with 32 drugs of abuse being determined with a cycle time of 9 minutes. Furthermore, stability of drugs in oral fluid before analysis was evaluated and test results showed that 6-acetylmorphine, cocaine and zopiclone were the least stable drugs. Therefore, samples of oral fluid should be analysed as soon as possible after collection, and the specimens should be kept frozen if immediate analysis is not possible.

Since cannabis abuse has long been a concern in workplace, many studies were undertaken to test for the abilities of different drug screening devices in detecting cannabinoids in oral fluid including the characterization of assay performance and limitations (88,89), as well as establishing the detection windows and cutoff concentrations of different cannabinoids in oral fluid (90).

In response to the concern about potentially false-positive results arising from passive exposure, Scheidweiler *et al.* proposed using 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH) as a marker of cannabis intake since it is not present in cannabis smoke and was not measureable in oral fluid collected from subjects passively exposed to cannabis (91). However, THCCOOH concentrations are in the pg/mL range in oral fluid and pose considerable analytical challenges. A method employing HPLC-MS/MS triple quadrupole system was successfully developed and validated for quantifying THCCOOH with limit of quantification at the level of <15 pg/mL.

MDMA is another drug gaining popularity of being abused in workplace. However, little is known about MDMA detection window in oral fluid. Study showed that MDMA was first observed in oral fluid 0.25-1.25 hours after administration of a recreational dose and MDA was subsequently detected at 0.5-1.75 hours. In general, the windows of detection for MDMA and MDA were 47 and 29 hours, respectively, although a few specimens were positive up to 71 and 47 hours (92).

3.3.3 Hair for workplace drug testing

Hair is an excellent specimen for pre-employment drug testing because of its ability to provide historical information on drug intake of an individual from months to years through a much longer window of detection (74). In contrast to providing short-term drug abuse profile through blood and urine testing, hair drug testing provides complementary information about the long-term drug abuse history of a donor. Furthermore, sampling head hair specimen is considered non-invasive and drugs incorporated in the hair remain stable and bound for a long time leading to little concern about specimen adulteration.

In Lombardy Region, Italy, individuals undergoing hair testing for workplace drug testing can choose one of the eleven analytical laboratories accredited for forensic proposes to conduct the analyses. An inter-laboratory exercise was therefore performed to verify the level of standardization of hair testing for drugs of abuse in these accredited laboratories. Nine out of these eleven laboratories participated in this exercise. Sixteen hair strands coming from different subjects were longitudinally divided in 3-4 aliquots and distributed to participating laboratories, which were requested to apply their routine methods for testing for drugs of abuse. Results demonstrated good qualitative

performance for all participants, since no false positive results were reported by any of them (93).

Incorporation of drugs in hair varies greatly between different classes of drugs and is subject to influence of melanin affinity, lipophilicity and membrane permeability. An article has deliberated the importance of whether the analytical procedure employed for hair drug testing was sensitive enough to identify traces of drugs; this is particularly important when the urine sample(s) of the subject was positive and the hair sample(s) was negative. It was concluded that until laboratories have sensitive enough methodologies to detect a single use of drug, care should be taken to compare urine and hair findings because the negative hair findings can cast doubt on the positive urine analysis, resulting in substantial legal debate and various consequences for the subject (94).

A scientific publication reported that a simple procedure was developed and validated for qualitative and quantitative analysis of several opiates (morphine, 6-acetylmorphine, codeine, 6-acetylcodeine) and tramadol in hair by GC-MS through selected ion monitoring mode (95). Intra- and inter-day precision and trueness were in conformity with the criteria normally accepted in bioanalytical method validation. Furthermore, 6-acetylmorphine was not significantly hydrolyzed to morphine in the course of incubation.

In order to effectively monitor multiclass abused drugs in hair, a simple procedure that allows the simultaneous determination of a series of commonly abused drugs or their metabolites would be highly desirable. A method employing UPLC-MS/MS instrument for simultaneous quantitative determination of 13 drugs of abuse and their metabolites including THC, along with high sample-throughput, excellent sensitivity and selectivity was successfully developed and fully validated in a study (96). These qualities, combined with minimal sample treatment, make the cost of this screening affordable for most private and public administrations to undertake routine hair analyses for workplace drug testing.

3.4 Emergence of New Designer Drugs

The increasing popularity of new designer drugs is a growing challenge for law enforcement agencies worldwide. Emerged in early 1990’s, designer drugs

generally refer to analogues or derivatives of controlled psychoactive drugs that exert similar pharmacological effects. Their chemical structures are modified to varying degrees in order to obscure their detection and evade legislative control (97,98). However, some designer drugs identified in recent years are of entirely different chemical structures when compared to the psychoactive drugs they mimic. Though, they still affect the same receptors in the central nervous system. Normally, these drugs are designed such that they would circumvent legislative control of the existing drug ordinances.

3.4.1 Synthetic Cathinones

'Synthetic cathinones' refers to derivatives of cathinone, which is a beta-keto phenylethylamine mostly from khat plant. They are often considered "legal highs" and sold as "bath salts" or "plant food" and labeled "not for human consumption" to circumvent controlled drugs legislation. MDPV was recently classified as a Class I drug by Racing Commissioners International, indicating that it is a banned substance in equine athletes because it lacks therapeutic value in horses (98). With psychostimulant effect similar to that of amphetamines and cocaine, these recently emerged compounds have been marketed over the Internet and gained popularity among drugs abusers. Some of the synthetic cathinones, including mephedrone and naphyrone, have already entered the illicit drug market (99,100,101,102,103,104).

Detection and determination of 25 designer cathinones and their related ephedrines in blood sample using LC-MS/MS method was reported (100). The method used only 100 μL of blood and employed liquid-liquid extraction with 1 mL of 1-chlorobutane containing 10% of isopropanol. The lower limits of quantification (LLOQs) for this method were reported to be 10 ng/mL for all the compounds.

Studies of 3-bromomethcathinone and 3-fluoromethcathinone metabolism in rat urine and human liver microsomes using GC-MS and LC-HRMS found that the main metabolic steps were N-demethylation, reduction of the keto group to the corresponding alcohol, hydroxylation of the aromatic system and combinations of these steps (105).

A rapid with high sensitivity method for determining 32 cathinone derivatives and designer drugs of the phenethylamine, tryptamine and piperazine classes in serum using liquid chromatography triple quadrupole tandem mass

spectrometry (LC-QQQ-MS/MS) was reported. The limits of quantitation (LOQ) were reported to be in range of 1-10 ng/mL for each compound with LOD close to 10 pg/mL (106).

New designer drugs containing β-ketone analogues of 3,4-methylenedioxymethcathinone (βk-MDMA, 'methylone') were reported in New Zealand (107). In addition, the synthesis and analytical data for β-ketone-N,N-dimethyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (βk-DMBDB) were reported for the first time in the publication.

Fornal *et al.* also reported the use of high performance liquid chromatography-quadrupole time of flight mass spectrometry (LC-ESI-Q/TOF) for six 3,4- methylenedioxy derivatives including methylone, butylone, pentylone, MDPBP, MDPV and BMDP (108).

3.4.2 Reported fatal cases in association with the abuse of synthetic cathinones

There is a reported case of death of a 40-year-old male who injected and snorted "bath salts" containing MDPV (109). Another case of psychosis involving a 23-year-old male insufflated a bath salt product containing MDPV and 4-fluoromethcathinone (flephedrone) has been reported (110). The MDPV levels in serum and urine of the male were found to be 186 and 136 ng/mL, respectively. Flephedrone levels were reported to be 346 and 257 ng/mL in serum and urine, respectively. The bath salt product was found to contain 143 μg of MDPV and 142 μg of flephedrone per milligram of powder. Kesha *et al.* also reviewed MDPV related death cases (111).

Three fatal intoxications due to methylone, a designer cathinone were reported (112). The peripheral blood methylone concentrations in the three fatal cases were reported to be 0.84, 3.3 and 0.56 mg/L. Distribution of methylone in four post-mortem cases was also reported (113). The methylone heart blood concentrations were found to be 0.740, 0.118, 0.060 and 1.12 mg/L. The average liver-to-blood ratio was found to be 2.68.

Wyman *et al.* also reported the distribution of MDPV in a case of an exposure of a 39-year-old male to MDPV. MDPV was found uniformly distributed among multiple tissues (blood, brain, muscle, cerebrospinal fluid and lung) at concentrations of approximately 0.4 to 0.6 μg/mL. Tissue and fluids

responsible for detoxification/ excretion had higher concentrations of MDPV (kidney, liver and bile > 0.8 µg/mL). A blood concentration ≥ 0.4 µg/mL was judged sufficient to cause death (114).

3.4.3 Synthetic cannabinoids

Synthetic cannabinoids have been abused as new designer drugs since 2004 (115). They can be divided into seven major structural groups: 1) naphthoylindoles (such as JWH-018 and JWH-073); 2) naphthylmethylindoles; 3) naphthoylpyrroles, 4) naphthylmethylindenes; 5) phenylacetylindoles (such as JWH-250); 6) cyclohexylphenols (such as CP47,497); and 7) classical cannabinoids (such as HU-210) (116). Several synthetic cannabinoids, including JWH-018, JWH-073, JWH-200, CP 47-497, and CP 47-497C8 homologue, were given schedule I status by the US Drug Enforcement Administration (DEA) in early 2011 (116).

Detection and quantification of 25 synthetic cannabinoids, including WIN 48.098, AM-1241, WIN-55212-2, RCS-4 C-4 homolog, RCS-4 2-methoxy homolog, JWH-030, JWH-015m JWH-302, RCS-4, RCS-4 3methoxy homolog, JWH-250, JWH-073, JWH-251, JWH-203, JWH-018, JWH-081, JWH-007, CP 47497, JWH-019, RCS-8, CP 47,497 C-8 homolog, JWH-398, JWH-210 and HU-210 in human blood sample using LC-MS/MS were reported (115). The extraction efficiencies ranged from 30-101% and the matrix effects from 67-112%. Analysis of 30 synthetic cannabinoids in serum by liquid chromatography-electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) was also reported (117).

Detection of JWH-018 and JWH-073 in post-mortem whole blood by UPLC-MS/MS was also reported (118). The LOD for each analyte was 0.01 ng/mL with a linear dynamic range of 0.05-50 ng/mL.

Two new types of synthetic cannabinoids, N-(1-adamantyl)-1-pentyl-1H-indole-3-carboxamide (APICA) and N-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide (APINACA) together with five synthetic cannabinoids, AM-1220, AM-2233, AM-1241, CB-13(CRA-13) and AM-1248 in illegal products were identified in Japan (119).

An analysis of first and second generation legal highs for synthetic

cannabinoids and synthetic stimulants by UPLC-TOFMS showed that many of the banned substances are no longer used and have been replaced by other derivatives that are federally legal in the US (120).

There are also some publications about the analysis for designer drugs and/or their metabolites in urine, for example, the analysis for CP 47,497 in human urine using LC-MS/MS (121); the detection of the urinary metabolite of 3-[(adamantan-1-yl)carbonyl]-1-pentylindole (AB-001) (122) as well as 1-[(5-fluoropentyl)-1H-indol-3-yl]-(2-iodophenyl)methanone (AM-694) (123) by GC-MS. The urinary metabolites of JWH-018, JWH-073, JWH-081, JWH-122, JWH-210, JWH-250 and RCS-4 were studied by LC-MS/MS. The major metabolic pathway was found to be monohydroxylation either at the N-alkyl side chain, the naphthyl moiety or the indole moiety. Moreover, metabolites with carboxylated alkyl chains were also identified for some of the compounds (124). Sixteen urinary metabolites of 3-(4-methoxybenzoyl)-1-pentylindole (RCS-4) were identified by GC-MS. The O-demethylated metabolites were found to be the most useful metabolic markers for the identification of RCS-4 ingestion (125).

In addition to LC-MS/MS, UPLC-TOFMS and GC-MS, solid-phase microextraction headspace gas chromatography-mass spectrometry (SPME-HS-GC-MS) was also used for the analysis of synthetic cannabinoids in herbal products (126).

JWH-018, JWH-073, JWH-200, CP47,497, JWH-250, HU-210 and cannabicyclohexanol (CP-47,497 C8) were determined in OF specimens collected with the Quantisal^{TM} device using SPE and LC-MS/MS (127). The method was applied to specimens taken from two individuals and found respectively a peak concentration of JWH-018 of 35 µg/L 20 minutes after smoking “Blueberry Posh” and 5 µg/L 20 minutes after smoking “Black Mamba”. It was noted that JWH-018 was still detectable 12 hours after a single intake of “Blueberry Posh” while JWH-018 was not detectable 12 hours after intake of “Black Mamba”.

Gottard R *et al.* used LC-QTOF-MS for the screening of new psychoactive substances in hair. 435 samples were screened for the presence of 50 different synthetic cannabinoids, cathinones and phenthylamines, where 8 samples were found positive for JWH-018, JWH-073, JWH-081, JWH-250,

JWH-122, in a broad range of concentrations (0.010-1.28 ng/mg) (128).

3.4.4 Methoxetamine

Long-term use of ketamine has been reported to be associated with severe symptomatic urinary tract problems. Methoxetamine (MXE), an arylcyclohexylamine derivative of ketamine, is marketed as a “bladder safe” derivative of ketamine. It presents new healthcare threat because of its easy accessibility via the Internet, and lack of legal restrictions in many countries. A low dose of MXE is claimed to be cause for “peace and serenity”, although higher dose may act the opposite. Cases of MXE abuse by injection intramuscularly have been reported (129). A series of cases involving three individuals with acute toxicity related to the use of MXE was confirmed analytically. Their serum concentrations ranged from 0.09 to 0.2 mg/L (130). Another case of MXE abuse was also reported (131).

3.4.5 Other synthetic drugs

Direct analysis of benzylpiperazine, methylone, 5,6-methylenedioxy-2-aminoindane (MDAI), fenproporex, 4-fluoroamphetamine (4-FA), 4-methyl-N-ethylcathinone (4-MEC), 4-methylamphetamine (4-MA), methylbenzodioxolylbutanamine (MBDB), mephedrone, methylthioamphetamine (MTA), MDPV, mefenorex, nabilone, furfenorex, clobenzorex, JWH-200, AM 694, JWH-250, JWH-073, JWH-018, JWH-019, JWH-122, HU 210 and CP 47,497 in OF by liquid chromatography–electrospray ionization–tandem mass spectrometry (UHPLC-ESI-MS/MS) has also been reported (132). 250 µL OF sample was diluted with 250 µL of mobile phase and the chromatographic run time is 9 minutes. LODs of the method vary from 1 ng/mL to 20 ng/mL and the linearity ranges from the LOD to 1000 ng/L.

3.5 Survey on Trend of Common Drugs of Abuse

3.5.1 Opiates and opioids

3.5.1.1 Heroin

Heroin is the most rapidly acting opiate drug. It is highly addictive and hence is one of the most popularly abused substances. Heroin associated fatalities have been widely reported in the world because of its strong potency. A survey studying the deaths caused by illegal drugs in East Germany between 1995

and 2004 revealed that opiates, especially heroin, caused majority of the deaths, and the average age of the victims were 24 years with males accounting for 85% of all fatalities (133).

In another epidemiological study on all poisoning deaths in Epirus, Greece, in the period from 1998 to 2010 (134), a total of 126 poisoning fatalities were recorded and heroin was the most frequently detected substance.

Similarly, a study (135) on medico-legally examined fatal poisonings cases in 2007 among drug addicts in the five Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden) revealed that heroin/morphine was still the main intoxicant in Norway and Sweden. However, methadone was the main intoxicant in Denmark while only a few cases were due to heroin/morphine in Iceland. Finland differed from other Nordic countries in having a high number of poisonings caused by buprenorphine and just very few caused by heroin/morphine.

Through a study on a total of 149 drug abuse deaths of teenagers aged 13-19 years from 1991 to 2006 in Maryland (136), it was reported that the increase in teenager drug abuse deaths occurred in 1999 and since then remained at a high rate. Further analysis revealed that such increase was attributable to a large degree to narcotic drugs, particularly heroin/morphine.

3.5.1.2 Methadone

Methadone has a long and successful history in the treatment of opioid addiction. However, in recent years, it has also become popular as a potent and inexpensive analgesic for patients suffering from chronic pains. Over the years, the numbers of methadone related deaths have seen a significant growth in the United States including Vermont, Western Virginia, rural southwestern Virginia, Oklahoma, Wisconsin and etc. (137,138,139,140,141,142). Such findings were also widely reported in European countries/cities and Australian state including Zurich, Montpellier of France, Ghent of Belgium, United Kingdom, Denmark, Norway and Victoria of Australia (143,144,145,146,147,148,149). The great number of reported methadone related deaths should therefore be a matter of concern especially about the source of supply such as the improper taking of the medication by

patients, diversion of the drug from the patient to someone else, or other means.

3.5.1.3 Oxycodone

A cross-sectional study analysing prescriptions for morphine and oxycodone in relation to oxycodone-related mortality data was conducted in Australia (150). The study results revealed that the prescriptions for morphine declined, while those for oxycodone increased and 465 oxycodone-related deaths were recorded during 2001-2009. Furthermore, it was concluded that in comparison to heroin, the morbidity and mortality associated with oxycodone are relatively low in Australia.

In view of the toxicity concern of oxycodone, all fatal oxycodone toxicity cases presented to the New South Wales Department of Forensic Medicine of Australia from 1999 to 2008 were retrieved with a total of 70 cases identified and studied (151). It was found that in 30% of the cases, oxycodone had not been prescribed to the decedent. Furthermore, psychoactive substances other than oxycodone were also detected, most frequently hypnosedatives (68.6%), other opioids (54.3%), antidepressants (41.4%), and alcohol (32.9%).

In the United States, unintentional poisonings were the second leading cause of injury death (after motor-vehicle crashes) with most of them caused by drug overdose. In a survey studying the drug overdose deaths in Florida from 2003 to 2009 (152), it was found that the death rate for prescription drugs increased 84.2%. The greatest increase was observed in the death rate from oxycodone (264.6%), followed by alprazolam (233.8%) and methadone (79.2%).

3.5.1.4 Fentanyl

Fentanyl is a potent, synthetic opioid analgesic and is an increasingly common drug of abuse. Fatalities in relation to fentanyl overdoses are common. A toxicology-based review of fentanyl-related deaths in New Mexico from 1986 to 2007 was undertaken (153). Amongst 154 cases identified with fentanyl present in the post-mortem samples, 96 cases were concluded as fentanyl-related drug overdoses. The number of fentanyl-related deaths has increased over the past 20 years, corresponding to both statewide increases in the medical use of fentanyl and the abuse of prescription opioids.

Similarly, a study of fentanyl in drug-related deaths in Philadelphia 2004-2006 was undertaken by reviewing data from the Philadelphia Medical Examiner's Office (154). In comparison to 2004 and 2005 data, there was a statistically significant increase in the number of drug related deaths with fentanyl tested positive in 2006. It was postulated that the change may be related to increase in the abuse of fentanyl and lack of general public awareness that fentanyl is a potent opoid.

3.5.2 Amphetamine type stimulants

Amphetamine is a major drug of abuse in Sweden. Through a study on forensic blood samples from 2001 to 2010, it was found that the mean (median) concentrations of amphetamine in blood were 1.25 (0.40) mg/L in autopsy cases and 0.61 (0.40) mg/L in users of illicit drugs (155). The major co-ingested drugs were benzodiazepines, cannabis, opiates and alcohol. In an overview of amphetamine-type stimulant mortality data in the United Kingdom from 1997 to 2007 (156), 832 amphetamine/methamphetamine and 605 ecstasy (mostly MDMA and MDA)-related deaths were respectively identified. Furthermore, it was noted that ecstasy was more typically identified in victims who were young, healthy, and less likely to be known as drug users.

Deaths involving MDMA and the concomitant use of pharmaceutical drugs in Victoria of Australia from 2002 to 2008 were investigated (157). In all, 106 fatalities were identified, of which 43 cases involved the concomitant use of MDMA with other drugs, including pharmaceuticals that were likely to result in an adverse drug reaction or varying risks.

A severe outbreak of paramethoxymethamphetamine (PMMA) and paramethoxyamphetamine (PMA) resulting in 24 fatalities in Israel was reported in a publication (158) and stimulant co-exposures may have contributed to the severity of the poisoning. The PMMA epidemic in Norway involving 12 fatal intoxications during a 6 month period (July 2010-January 2011) was also studied with evaluation on the cause of death (159).

3.5.3 Cocaine

A review of cocaine-related deaths in Bexar County, Texas was undertaken (160). The data obtained showed that cocaine was toxic over a large range

with deaths occurring at concentrations ranging from 0.01 to 78 mg/L. The analyses also indicated lethality increases when cocaine is used in combination with ethanol, heroin, opiates, and antidepressant/antipsychotic medications.

The use of cocaine in Australia has risen steadily since the late 1990s. A study was launched to identify all deaths occurring in Victoria of Australia, from 2000 to 2011. There were 49 cases of death where cocaine, benzoylecgonine, ecgonine methyl ester, methylecgonine or cocaethylene, were detected (161).

A review on the temporal and geographic shifts in urban and nonurban cocaine-related fatal overdoses in British Columbia, Canada from 2001 to 2005 was published (162). A total of 904 illicit drug overdoses were recorded, including 369 (40.8%) in nonurban areas and 532 (58.9%) related to cocaine consumption. In another publication, 21 cases of cocaine-related sudden death in south-west Spain from November 2003 to June 2006 were reported (163).

3.5.4 Gamma-hydroxybutyrate (GHB)

All death cases with GHB detected during 2000-2007 in the region of western Sweden were studied (164). Twenty-three cases were diagnosed as deaths due to GHB overdose.

Another research group in Sweden also studied the concentrations of the GHB in femoral venous blood and urine obtained at autopsy in a series of GHB-related deaths (165). Considerable poly-drug use was evident in these GHB-related deaths including ethanol, amphetamine, and various prescription medications (benzodiazepines, opiates, and antidepressants) in other cases.

3.5.5 Antidepressant and hypnotic

The contributory and incidental blood concentrations in deaths involving citalopram in New South Wales of Australia from 2001 to 2010 were investigated (166). A total of 348 cases were identified. Citalopram contributed to death in 21.0% and was incidental in 79.0%.

The toxicology and characteristics of deaths involving zolpidem in New South Wales of Australia from 2001 to 2010 were studied (167). A total of 91 cases were identified. Zolpidem was a factor contributing to death in 35 cases, of which 31 involved zolpidem toxicity.

3.6 Quality Assurance

3.6.1 Proficiency test

While forensic laboratories are required to estimate uncertainties of measurements for those quantifications reported to the end users of the information, the procedures for such estimations have been hardly discussed in the forensic literature. An article illustrated how proficiency test results provide the basis for estimating uncertainties in three instances: (i) breath alcohol analyzers, (ii) blood alcohol and (iii) toxicology. It was claimed that data from proficiency tests enable estimates of uncertainty that are empirical, simple, thorough, and applicable to a wide range of concentrations (168).

The International Interlaboratory Quality Control Program for Measurement of Antiretroviral Drugs in Plasma was initiated by Radboud University Nijmegen Medical Center of the Netherlands in 1999, and later the Dutch Association for Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology collaborated in the Program. The Program provides a proficiency testing program in which laboratories are alerted to potential analytical errors while performing therapeutic drug monitoring in HIV-infected patients (169).

The organization of the first international proficiency test (PT) programme on ketamine (K) and norketamine (NK) in hair samples has been discussed (170). The primary objective of the programme was to evaluate the analytical capability of participating laboratories on hair analysis for K and NK via comparison of results. Authentic samples, instead of spiked samples were used in the programme to mimic the analysis of incorporated illicit drugs in real-life situations.

The conditions of measurement required to evaluate bias in analytical results, as illustrated by the use of data from a multi-round, blind-duplicated, proficiency test, was reported (171). Results of a six-round blind-duplicated interlaboratory proficiency program for creatinine in urine showed that bias was present in each individual run with components from that batch as well as and from the laboratory over the rounds of the program. It was concluded that bias should be determined in each batch run under repeatability conditions. Measurement of laboratory bias alone is not sufficient to account for effects in each batch run.

3.6.2 Establishing the measurement uncertainty

The calculation and verification of blood alcohol measurement uncertainty for headspace gas chromatography were reported (172). The uncertainty sources, in order of decreasing magnitude, were method reproducibility, linear calibration, recovery, calibrator preparation, reference material, and sample preparation. A large set of reproducibility data was evaluated (n = 15,433) in order to encompass measurement variability across multiple conditions, operators, instruments, concentrations and timeframes. The relative, combined standard uncertainty was calculated as ±2.7%, with an expanded uncertainty of ±8.2% (99.7% level of confidence, k = 3). Bias was separately evaluated through a recovery study using standard reference material from a national metrology institute. The uncertainty estimate was verified through the use of proficiency test (PT) results.

An approach was proposed for the estimation of measurement uncertainty for analytical methods based on one-point calibration (173). The approach was applied to the estimation of measurement uncertainty for the quantitative determination of ketamine (K) and norketamine (NK) at a 100 ng/mL threshold concentration in urine. The expanded uncertainties (k = 2) were estimated to be 10 and 8 ng/mL for K and NK, respectively.

Several established and well-documented methods are available to determine and report the uncertainty in blood alcohol measurement (174). A straightforward bottom-up approach is presented that includes: 1) specifying the measurand, 2) identifying the major components of uncertainty, 3) quantifying the components, 4) statistically combining the components and 5) reporting the results. A hypothetical example is presented that employs reasonable estimates for forensic blood alcohol analysis using headspace gas chromatography.

3.6.3 Quality control materials

Quality control (QC) used in routine analysis needs to be stable and matrix-matched if practicable. However, it may be difficult to find representative and low-cost QC materials, especially for specific analytes in biological tissue. The preparation of four caprine liver pools for use as internal QC materials for trace element measurements in biological tissue was reported (175). Analytes of interest include essential and non-essential trace elements and the lanthanide series elements.

The Federal Institute for Materials Research and Testing in Germany has issued a series of large volume ethanol in water. These certified reference materials (CRMs) were primarily developed for the calibration of evidential breath alcohol analyzers in Germany. The certified parameter is the ethanol mass concentration at 20 °C. When used in a wet bath simulator, the solutions deliver gas samples that meet the requirements set by the Organization of Legal Metrology for calibration of breathalyzers (176).

An example of the use of the multivariate statistical analysis for the certification of metronidazole and captopril was demonstrated (177). The technique was quick, easy and readily provided an evaluation of the homogeneity. Through the use of statistical tools, it was possible to reduce the standard uncertainty due to between-bottle inhomogeneity and consequently the combined standard uncertainty of the certified reference materials with 95% confidence level. Metronidazole and captopril in the study are used as pharmaceutical reference materials.

Internal standards play critical roles in ensuring the accuracy of an analysis. In a publication, the use of internal standards for quantitative LC-MS bioanalysis was discussed in detail (178).

Any high-quality analytical result should include information about the associated measurement uncertainty, and the purity uncertainty of the reference is a parameter which always appears in the overall measurement uncertainty calculation of the measurand (such as the concentration or content of an analyte). A publication postulates that the purity and the uncertainty of all reference materials must be known (179).

4 Advances in Toxicological Analysis

4.1 Development of LC-MS Techniques

Over the past few years, diversified development has been found in the applications of liquid chromatography coupled with tandem mass spectrometry for the determination of drugs and their metabolites in various biological specimens. In the field of forensic toxicology, mass spectrometry (MS) has been traditionally playing a key role in the identification of drugs and their

metabolites. The development of High-resolution Mass Spectrometry (HRMS) instrumentation with improved accuracy and stability, along with new data processing techniques, has further improved the quality and productivity of metabolite identification processes.

LC-MS/MS is an increasingly important tool in therapeutic drug monitoring as it offers increased sensitivity and specificity compared to other methods (180). However, sample preparation technique, column selection, use of proper internal standard and optimization of instrumental conditions are also important issues when accurate drug measurement is to be achieved. Furthermore, technological advances such as the development of pipetting robots and online solid phase extraction greatly prompt LC-MS/MS becoming an attractive and convenient automated system for therapeutic drug monitoring in clinical laboratories.

Applications of liquid chromatography tandem mass spectrometry has proliferated at a fast pace over the past few years and several reviews have been published (128,181,182,183). In addition, drug metabolite profiling and identification by HRMS has also seen a major progress. In a review (184), HRMS-based targeted and non-targeted acquisition methods and data mining techniques (*e.g.* mass defect, product ion, and isotope pattern filters and background subtraction) that facilitate metabolite identification were examined. Methods involving multiple metabolite identification tasks with a single LC/HRMS platform and/or analysis were also presented.

Liang *et al.* have published a review on the development in liquid chromatography/mass spectrometry and emerging technologies for metabolite identification (185). In this article, the classical and practical mass spectrometry-based techniques, such as low resolution MS (quadruple, ion trap, linear ion trap, etc), high resolution MS (time-of-flight, hybrid time-of-flight instruments, Orbitrap, Fourier transform ion cyclotron resonance MS, etc) and the corresponding post acquisition data processing and mining modes (precursor ion filtering, neutral loss filtering, mass defect filter, isotope-pattern-filtering, etc) were described comprehensively.

Recent advances on metabolite identification and quantitative bioanalysis by LC-Q-TOF MS have also been studied by another team of researchers (186). The key properties of the Q-TOF MS system, including mass accuracy,

resolution, scan speed and dynamic range, were discussed. The performance and versatility of LC-Q-TOF MS were thoroughly illustrated by its applications in metabolite identification and quantitative bioanalysis. Future perspectives were also discussed in the article.

Wissenbach *et al.* have studied transferring a linear ion trap (LIT) LC-MS(n) screening approach and reference library to an LC-MS/MS system with a quadrupole-LIT hybrid mass analyzer using SmileMS, a sophisticated search algorithm (187). Modified library sets were generated to improve the detection of a compound by the used search algorithm. The data presented showed that the LIT screening approach and reference library could be used successfully on a QTRAP instrument with some limitations that could be overcome by further optimizations on settings and modifications of library.

Roman *et al.* also reported a validated liquid chromatography/time-of-flight mass spectrometry method for targeted toxicological screening of post-mortem blood samples. Separation was achieved within 12 minutes by high resolution gradient chromatography (188).

Another study has reported the successful detection and identification of 700 drugs by multi-target screening with a QTRAP LC-MS/MS system (80). Identification of the compounds in the samples was accomplished by searching the MS/MS spectra against a library developed from the electrospray ionization-MS/MS spectra of over 1,250 compounds. Data acquisition and library searching are integrated and automated by the software program.

Liu *et al.* reported the successful development of a method performing rapid screening and confirmation of drugs and toxic compounds in biological specimens using liquid chromatography/ion trap tandem mass spectrometry and automated library search in a single analytical step (189). The established method was found highly effective when applied to the analyses of post-mortem specimens (blood, urine, and hair) and external proficiency test samples provided by the College of American Pathology (CAP).

In the field of urinalysis, a published article has reported an automated determination of 21 therapeutic drugs and 21 abused drugs in human urine (190). According to the article, their analyses could simultaneously identify and

quantify the 42 drugs in human urine through an automated online solid phase extraction ultra high performance liquid chromatography method coupled with tandem mass spectrometry (SPE UHPLC-MS/MS).

Another novel analytical toxicology method has been developed for urinalysis by using a high resolution and high mass accuracy hybrid linear ion trap-Orbitrap mass spectrometer (LTQ-Orbitrap-MS), with 65 compounds analysed within a run time of 20 minutes (191).

Nakamura conducted a review on the procedures for multi-analyte single-stage LC-MS and LC-MS/MS using different mass analyzers for the screening, identification and/or quantification of drugs, poisons and/or their metabolites in blood, plasma, serum or urine published since 2001 (192).

d-Amphetamine is extensively used in drug research and forensic toxicology investigation. A research study on a specific and high-throughput quantitative method, with minimal sample preparation, for routine analysis of d-amphetamine in biological samples using MS^3 scan mode on a hybrid triple quadrupole-linear ion trap mass spectrometer (LC-MS/MS/MS) has been published (193). This method was successfully applied to evaluate the pharmacokinetics of d-amphetamine in rat.

Time of flight mass spectrometry provides accurate molecular mass and isotope pattern and hence determination of the molecular formula of a substance directly becomes possible. However, there are frequently a large number of possible isomers, the differentiation of which requires additional evidence. Broecker *et al.* reported their study on the combined use of LC-hybrid quadruple time-of-flight mass spectrometry (LC-QTOF-MS) and high performance liquid chromatography with photodiode array detector (HPLC-DAD) in systematic toxicological analysis (194).

LC-MS/MS has also found its application in the detection of a number of new psychoactive drugs (legal highs) (195). The method validation demonstrated limited interference from urine matrix, linear response within the measuring range (0.1 – 10 mg/mL), and acceptable imprecision in quantification (CV < 15%).

4.2 Development of Extraction Techniques

Novel extraction techniques such as on-line solid phase extraction had been introduced during the period under the present review. One of the studies reported using protein precipitation with extraction (PPE) in acetonitrile instead of the tedious liquid-liquid extraction in the quantification of 25-hydroxyvitamin D (a marker of vitamin D). Combined with a 96-well plate filtration system, the entire separation process becomes much more efficient (196). The rapid extraction was then followed by an on-line solid phase extraction (SPE) using a selective chromatographic separation. Furthermore, a trapping column was used to enhance the lifespan of the analytical column.

Savolainen *et al.* also employed an on-line solid phase extraction liquid chromatography-tandem mass spectrometry in their analysis of testosterone in serum samples (197). When compared with their previous routine LC-MS/MS method using liquid-liquid extraction with tert-butyl methyl ether for the pre-purification of the samples, the precision of the new method was notably better, especially in the lower concentration range. Therefore, the researchers concluded that the on-line SPE-pre-purification technique tested in long-term use offered a rapid and reliable technique in the LC-MS/MS analysis of serum testosterone and was a valuable tool in the improvement of efficiency in the laborious steroid analytics.

The successful application of LC-MS/MS for immunosuppressant therapeutic drug monitoring has been published (198). Authors in the article claimed that online sample clean-up with either a single analytical column or with 2D chromatography significantly reduced manual handling, minimized matrix effects and maximized specificity. It was concluded that LC-MS/MS was an attractive and versatile technique that facilitates rapid development of analytical methods.

Wang *et al.* have reported a one-step membrane extraction for the determination of 8-hydroxy-2'-deoxyguanosine in human plasma by a combination of on-line SPE and LC-MS/MS (199). Another study by Emara *et al.* also reported an on-line sample cleanup and enrichment chromatographic technique for the determination of ambroxol in human serum (200). Fernández *et al.* published a study reporting a chromatographic determination of drugs of

abuse in vitreous humor using solid-phase extraction (201).

A sensitive method using capillary electrophoresis with online large-volume sample stacking for the determination of barbiturates in biological matrix has been published (202). The technique involved injecting a large volume of sample into a capillary and removing the sample matrix plug out of the capillary by reversing the polarity. The method was satisfactorily applied to real forensic specimens.

Turbulent flow chromatography (TFC) was introduced in the mid-1990s for online sample processing in bioanalysis. It combines 'size exclusion' and traditional stationary phase column chemistry to separate macromolecules, such as proteins, from smaller molecules and analytes of interest in biological fluids. Several articles have been published relating to TFC (203,204,205). One of them is an overview of TFC in bioanalysis (203). The article aimed at reviewing the chromatographic theory of TFC and illustrating, using examples from recent literature, the application of this technique to a range of analytes in different biological matrices. Bunch *et al*. have reported a fast and simple assay for busulfan in serum or plasma by liquid chromatography-tandem mass spectrometry using turbulent flow online extraction technology (206).

Serdi *et al.* have published a paper reporting a novel low-voltage electrically-enhanced microextraction for simultaneous extraction of acidic and basic drugs from biological fluids (207). The research team termed the technique electromembrane extraction at low voltages followed by high performance liquid chromatography with ultraviolet detection. They anticipated that their techniques could have a wide application in different complicated matrices.

Testing for illicit drugs in hair has been gaining attention. Sergi *et al.* have studied on a pressurised-extraction for determination of illicit drugs in hair by LC-MS/MS (208). Their procedure, in conjunction with a decontamination step, enabled the detection of all the analytes in pg/mg level.

4.3 Analysis of Specific Drugs

4.3.1 Toxic and volatile gases

4.3.1.1 Cyanide

Cyanide is a powerful chemical asphyxiant found in some forensic cases following voluntary (suicide) or involuntary ingestion (fire, accidental exposure). A quantification method for cyanide by headspace gas chromatography coupled to mass spectrometry using a GS-GASPRO column on an HP-6890 gas chromatograph with an HP-5973N mass detector has been developed (209). Identical calibration curves were obtained when blood, gastric contents and aqueous solutions were used as the calibration standard matrix. Furthermore, this method was also successful in quantifying cyanide in gastric contents, one of most variable biological fluids.

A LC-MS/MS method using cyanide isotope $^{13}C^{15}N$ as internal standard and coupled to online extraction has been developed for cyanide determination in blood (210). The method was simple and time saving using small volume of blood sample. Hence, it is very suitable for cyanide determination in blood and could be useful in forensic toxicology.

In addition, an electrospray ionization tandem mass spectrometric (ESI-MS/MS) method has been developed for the determination of cyanide (CN^-) in blood. CN^- could be measured in the quantification range of 2.60 to 260 µg/L with the limit of detection at 0.56 µg/L in blood (211).

An analytical method utilizing chemical ionization gas chromatography-mass spectrometry has been developed for the simultaneous determination of cyanide and thiocyanate in plasma (212). Sample preparation for this analysis required essentially one step by combining the reaction of cyanide and thiocyanate with pentafluorobenzyl bromide and simultaneous extraction of the product into ethyl acetate facilitated by a phase-transfer catalyst, tetrabutylammonium sulfate. The LOD for cyanide and thiocyanate were 1 µM and 50 nM, respectively.

Cyanide concentrations vary among different types of post-mortem specimens,

and this is very important in interpreting the cause of death in post-mortem forensic toxicology. 21 cases related to cyanide intoxication by oral ingestion were studied in which heart blood, peripheral blood and gastric contents were analyzed colorimetrically for cyanide. From the difference and ratio of cyanide concentration in different types of post-mortem specimens, post-mortem redistribution of cyanide and death could be distinguished from oral ingestion (213).

Assigning a level of significance to cyanide concentrations found in the blood of fire victims is often hampered by the fact that cyanide is inherently unstable in cadavers and in stored blood samples. The effect of sodium fluoride on the stability of cyanide in post-mortem blood samples from fire victims has been studied (214). It was found that samples treated with sodium fluoride showed virtually no overall change in blood cyanide levels over a 25-30 day period whereas the unconditioned control samples showed a significant average increase of 35%. Based on the findings of this study, it is recommended that 2% sodium fluoride be added to blood samples obtained from fire victims to reduce cyanide instability due to bacteriological activity.

4.3.1.2 Carbon Monoxide (CO)

Measurement of carboxyhemoglobin (COHb) is crucial to recognizing CO as a contributor in deaths involving fires, exposure to automobile exhaust, aircraft accidents, and residential exposures. Interferences, including lipid-caused turbidity, MetHb, sulfhemoglobin, microcoagulates, putrefaction, and contamination, have called into question the accuracy of COHb measurements obtained by CO-oximetry. The reliability of post-mortem COHb measurement by CO-oximetry was discussed through a case study (215). It was concluded that CO-oximetry, with the appropriate multiwavelength technology, can be a reliable and accurate method for post-mortem COHb measurement.

An innovative headspace-gas chromatography-mass spectrometry (HS-GC-MS) method applicable for the routine determination of blood CO concentration in forensic toxicology laboratories has been developed (216). A labelled internal standard gas (^{13}CO) formed by the reaction of labelled formic acid ($H^{13}COOH$) with sulfuric acid was generated in a vial in situ. This method allows for the precise measurement of blood CO concentrations from a small

amount of blood (10 µL). It was applied to measure the CO concentration of intoxicated human blood samples from autopsies.

In a published article (217), Nowicka *et al.* reviewed various analytical methods used for the determinations of carbon monoxide in post-mortem blood. The advantages, disadvantages and the cause of errors resulting from the specificity were discussed.

4.3.1.3 Volatile organic compounds

Dynamic measurement of volatile organic compounds (VOCs) in exhaled breath under exercise conditions has been studied by a team of researchers in Austria (218). They presented an experimental setup combining breath-by-breath analyses with proton transfer reaction mass spectrometry (PTR-MS). Their data reflected the behaviour of major hemodynamic and respiratory parameters. Furthermore, a methodology for complementing continuous VOC profiles obtained by PTR-MS with simultaneous SPME/GC-MS measurements is outlined.

Rasanen *et al.* presented the successful development of a novel headspace in-tube extraction gas chromatography-mass spectrometry (ITEX-GC-MS) approach for broad-scale analysis of low molecular weight organic compounds in blood and/or urine (219). From the results of 11 representative compounds, it was demonstrated that ITEX was more sensitive than the corresponding static headspace method for analysis of volatile organic compounds.

A fast and simple screening procedure using solid-phase micro-extraction and gas chromatography-mass spectrometry (SPME-GC-MS) in full-scan mode for the determination of volatile organic compounds (VOC) was presented in a published study (220). To simulate the screening procedure, eight VOC with different chemical characteristics were chosen. The limits of detection ranged from 2.9 µg/L (xylene) to 37.1 µg/L (isoflurane) and the recoveries varied from 7.9% (chloroform) to 61.5% (benzene).

A study to investigate using the scent profile of human urine as potential source of chemical markers of human presence in collapsed buildings after

natural or man-made disasters was launched (221). The study aimed at building a library of potential biomarkers of human urine to be used for the detection of entrapped victims and to further examine their evolution profile in time. A library of potential markers of human urine was created that would be verified in further field studies using portable and sensitive instruments.

4.3.1.4 Others

It is difficult to obtain toxicological evidence inferring the cause of death being resulted from inert gas asphyxiation. Helium, due to its low atomic mass and high diffusivity, is particularly challenging in this respect. A rapid and simple gas chromatography-thermal conductivity detection method to qualitatively screen a variety of post-mortem biological specimens for the presence of helium was described in a study in which application of this developed method has been successfully demonstrated with three case examples, encompassing an array of different biological matrices (222).

A novel method was developed to measure methane in tissues (223). The method used labeled CDH_3 that was produced in-situ, resulting in reliable and precise quantification of methane content in the post-mortem samples of two victims that assisted to determine the explosion origin.

A gas chromatography-mass spectrometry (GC-MS) method for the determination of ketone bodies (β-hydroxybutyrate, acetone, and acetoacetate) in blood was presented in a study (224). The method was based on enzymatic oxidation of D-β-hydroxybutyrate to acetoacetate, followed by decarboxylation to acetone, which was then quantified by the use of headspace GC-MS using acetone-$^{13}C_3$ as an internal standard.

4.3.2 Chemical warfare agents

Organophosphorus (OP) nerve agents and sulphur or nitrogen mustard are among the most toxic organic compounds known. They are continually a threat for both military and anti-terrorist personels. Since some OP compounds can be hydrolysed, degradation products may remain and even predominate in samples acquired in the field. A team of researchers has successfully employed ESI-MS/MS in analysing non-volatile OP compounds and their degradation products (225).

An analytical method for determining OP nerve agents sarin, soman and VX adducts with tyrosine residue of albumin in rat plasma has been developed and validated using liquid chromatography-isotope dilution tandem mass spectrometry (LC-IDMS/MS). The LOD were 0.01 ng/mL for sarin and soman adducts and 0.05 ng/mL for the VX adduct with recoveries ranged from 86-111% (226).

It was known that acetylcholinesterase (AChE) enzyme activity in red blood cells (RBCs) could be used as a biomarker for monitoring the exposures to OP pesticides and chemical nerve agents. Immuno-capture /electrochemical assay of AChE activity offers an opportunity that acted as a sensitive, selective and rapid AChE activity assay for biomonitoring the exposure to OPs with a linear response obtained over standard AChE concentration ranged from 0.1 to 10 nM (227).

4.3.3 Toxic mushrooms

Many plants and animals are known to contain toxins that may be harmful to human. In recent years, a number of toxicology cases related to mushrooms poisoning have been reported in various countries (228,229,230,231,232,233,234,235). In particular, an increase of poisoning by tropical mushrooms in Japan has also been reported (236). Mushrooms poisoning can often be proved by microscopic examination of their spores in the stomach or intestinal contents. Such method has been used for detection of A. pantherina or A. muscaria poisoning (237). Two forensic toxicology reviews on mushroom toxins were published (238,239). Mushroom toxins are tabulated according to mushroom species, symptoms, toxicities and analytical methods. A method for analysing amatoxins, the most virulent mushroom toxins, by LC-TOFMS was also reported (238).

4.3.4 Chinese medicines

Aconite poisonings following the use of aconite roots are commonly encountered in Asia (240,241). Aconite roots are widely used in traditional medicines and homeopathic medicines as analgesic, anti-inflammatory and cardiotonic agents. Aconitine, mesaconitine, hypaconitine, and other Aconitum alkaloids are known cardiotoxins and neurotoxins found in all parts of the Aconitum species, especially in their roots and root tubers (aconite roots) (240,241,242,243). The Aconitum alkaloids are highly toxic and have a very

narrow safety range; they easily induce ventricular tachycardia and fibrillation even at therapeutic dose levels (244). There was a report on seven cases relating to fatal aconite poisoning in China (245). Furthermore, there were three fatal poisoning cases reported in Austria that suicide was committed through ingestion of this highly toxic herb (246).

A review on herb-induced aconite poisoning indicated that poor post-harvest processing of aconite roots, use of greater than the recommended doses and inadequate boiling of processed aconite roots during decoction preparation were important contributory factors in herb-induced aconite poisoning (247). Data on the distribution of the Aconitum alkaloids in the body in cases of aconite poisoning was reported (248). Relevant reports on percutaneous absorption of Aconitum alkaloids and aconite poisoning are reviewed (249). It was found that aconite tincture and raw aconite roots can be absorbed through the skin into systemic circulation to cause fatal and non-fatal aconite poisoning.

Strychnine and brucine, another kind of alkaloids, are the predominant active constituents present in many traditional herbal medicines such as *Strychnos nux-vomica*, which is frequently used for the treatment of nervous diseases or vomiting, as a tonic or as an aphrodisiac (250,251). Chen *et al.* has reported a simultaneous analysis of strychnine and brucine and their major metabolites in rat liver by liquid chromatography-electrospray ionization-ion trap mass spectrometry (LC-ESI-ITMS) (251). The limits of detection for strychnine and brucine were both 0.008 μg/mL. The linearity ranges of strychnine and brucine were 0.020 to 8.0 μg/mL and 0.020 to 8.5 μg/mL, respectively.

Determination of strychnine and brucine in human urine by capillary electrophoresis with field-amplified sample stacking was also reported (252). Wu *et al.* developed a method for simultaneous determination of six toxic alkaloids including aconitine, hypaconitine, gelsemine, raceanisodamine, strychnine and brucine in blood and urine using a hydrophilic interaction liquid chromatography (HILIC)-ESI-MS/MS (253). Simultaneous determination of six toxic alkaloids including brucine, strychnine, atropine sulfate, anisodamine hydrobromide, scopolamine hydrobromide and anisodine hydrobromide in human plasma and urine using capillary zone electrophoresis coupled to time-of-flight mass spectrometry was also reported in another publication (254).

4.3.5 Doping Control

Not only restricted to professional athletes, the use of doping agents has nowadays become a problem of public health since it also concerns young people and non-competing amateurs in different sports. A publication has reviewed utilizing UHPLC/MS in determining and profiling prohibited steroids in human biological matrices (255). The advantages and limitations of this technique in human sports drug testing have also been discussed in another review (256).

With a recent increasing trend of abuse of synthetic cannabinoids, a study for the use of the synthetic cannabinoids, JWH-018 and JWH-073, was conducted. 5,946 urine samples collected from U.S. athletes were tested. Metabolites of JWH-018 and/or JWH-073 were detected in 4.5% of the tested samples. It was suggested that these compounds should remain a priority for anti-doping programs (257). A detection method was developed and validated in accordance with conventional screening protocols based on enzymatic hydrolysis, liquid-liquid extraction, and liquid chromatography/electrospray tandem mass spectrometry analysis. The method was applied to approximately 7,500 urine doping control samples yielding two JWH-018 findings and demonstrated its capability for a sensitive and selective identification of JWH-018 and its metabolites in human urine (258).

A study was conducted to investigate the plasma and urine profiles ofΔ^9-tetrahydrocannabinol (THC) and its metabolites 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in male volunteers after they smoked cannabis (259). The author suggested that THC and THC-OH should also be used as target analytes in additional to THC-COOH for doping urine analysis.

Thevis *et al.* have published reviews for the substances banned annually between October 2009 and September 2010 (260) and between October 2010 and September 2011 (261), with the purpose to improve the quality of doping controls by reporting emerging and advancing methods that focus on detecting known and recently outlawed substances.

Since January 2009, the list of prohibited substances and methods of doping as established by the World Anti-Doping Agency (WADA) has included new

therapeutics such as the peroxisome-proliferator-activated receptor (PPAR)-delta agonist GW1516, which is categorized as a gene doping substance. A method to detect the new target GW1516 in sports drug testing samples was developed in accordance with conventional screening procedures based on enzymatic hydrolysis and liquid–liquid extraction followed by liquid chromatography, electrospray ionization, and tandem mass spectrometry (262). The authors later reported a synthetic method for GW1516 and two oxidized metabolites (263).

Clomiphene is a selective estrogen receptor modulator that is prohibited by WADA, both out-of-competition and in-competition. Lu *et al.* have identified and characterized seven unreported urinary metabolites of clomiphene arising from a new metabolic pathway (hydrogenation) by liquid chromatography–quadrupole time-of-flight mass spectrometry (LC–QTOFMS) (264).

A screening method based on matrix-assisted laser desorption/ionization time-of flight mass spectrometry (MALDI-TOF/TOF) for the qualitative determination of doping agents as well as drugs of potential abuse was reported (265). The LOD for the analysis of target doping compounds in horse samples was reported to be 100 ng/mL, while that for the analysis of cocaine and its metabolite in human urine samples was 50 ng/mL.

4.4 Alternative Specimens

Blood and urine have long been and remain the most widely used biological specimens for forensic toxicological examination as well as routine drug testing. Blood is widely used for drug testing in clinical and emergency toxicology because it offers the best correlation between drug level and pharmacological impairments to the body. On the other hand, urine testing has been playing an important role in facilitating the judicial sentencing of drugs abusers in courts and drug surveillance programmes of inmates under custodial detention.

Following the advancement of testing technology, the use of alternative specimens in the field of toxicology has gained attention along with a number of studies published. Since the application of oral fluid and hair in workplace drug testing has been discussed in detail in the previous sections, this section will focus on other alternative specimens which have attracted less attention in the past.

4.4.1 Skeletal tissue

Skeletal tissue could be useful in forensic toxicology especially for heavily decomposed sample. A review of bone marrow analysis in forensic toxicology has summarized the analytical conditions and quantification results of 45 compounds from bone marrow samples and concluded that further experimental data and validated analytical assays are required for reliable determination and quantitative interpretation (266).

Watterson *et al.* examined the effects of burial on ketamine and diazepam detection and found that fresh tissue sample may not be representative of decomposed samples in terms of skeletal tissue drug levels (267). Later in another study (268), they reported the relative distribution of ketamine and norketamine in skeletal tissue with various decomposition periods and that the decomposition time was significantly related to the drug/metabolite level ratio (DMLR).

Watterson *et al.* also examined whether different patterns of drug exposure could be discriminated through toxicological analysis of decomposed skeletal tissues. The result suggested that acute and repeated exposures to ketamine may be discriminated on the basis of the levels of ketamine and norketamine in bone as well as the ratio of ketamine level to norketamine level (269). Apart from ketamine, norketamine and diazepam, relative distribution of amitriptyline and its metabolite, nortriptyline, and that of citalopram and its metabolite, desmethylcitalopram, in skeletal tissue following outdoor decomposition were also studied (270).

4.4.2 Brain tissue

To study the persistence of drugs in brain tissue over plasma, Sampedro *et al.* developed a simultaneous screening and determination of the 17 most commonly used antipsychotic drugs using LC-MS/MS (271). The linear ranges for calibration curves prepared in the spiked brain tissue were 20-8,000 ng/g for all the drugs studied except olanzapine and the LOQ ranged between 2 ng/g and 80 ng/g.

4.4.3 Meconium

Ethanol exposure during pregnancy can have negative effect on newborns (272,273,274). Fatty acid ethyl esters (FAEEs), products of non-oxidative ethanol metabolism, have been measured in meconium and acted as reliable

markers of intrauterine exposure to ethanol (275,276,277). Roehsig *et al.* reported an optimized and validated method for the simultaneous determination of eight FAEEs by headspace solid phase microextraction (HS-SPME) and GC-MS, with synthesized deuterated d5-ethyl esters used as internal standard (278). The LOQ and LOD for each analyte were reported to be <150 and <100 ng/g, respectively.

Hutson *et al*. developed another method for the determination of FAEEs in meconium using HS-SPME/GC-MS with improved LODs ranging from 6.3-11.9 ng/g and LOQs ranging from 18.8 – 35.8 ng/g because this method was able to produce clean chromatograms (279). Although analysis for FAEEs is a validated method for identifying heavy prenatal ethanol exposure, false-positive for FAEEs result was reported for meconium sample delayed in collection. Median time to appearance of FAEE-positive samples was 59.2 hours postpartum and four of the 30 babies excreted FAEE-positive meconium in less than 24 hours postpartum (280).

Another suitable marker for the detection of recent alcohol consumption is ethyl glucuronide (EtG) and ethyl sulfate (EtS), direct metabolites of ethanol (272). Studies of EtG in hair and meconium were reported (274,281). A study of EtG and EtS in meconium and hair samples from mothers and their newborns was conducted. The result showed that neither maternal nor neonatal hair was a good predictor of gestational ethanol consumption and subsequent fetal exposure in these mother–infant dyads. The authors concluded that meconium is so far the best matrix in evaluating intrauterine exposure to ethanol, with EtG and EtS being potentially good alternative biomarkers to FAEEs (274). Bakdash *et al.* performed a study on the determination of FAEEs and EtG in meconium (282). The FAEEs were measured by HS-SPME in combination with GC-MS, while EtG was quantified by LC-MS-MS. The authors suggested that combined use of FAEE and EtG in meconium as markers for fetal alcohol exposure essentially increases the accuracy of the interpretation and helps to avoid both false-positive and false-negative results.

4.4.4 Placenta

Placenta could be an alternative to urine for drugs of abuse testing during the first trimester of gestation. Joya *et al.* reported a GC/MS method for the quantification of drugs of abuse in human placenta including amphetamine,

methamphetamine, MDMA, methadone, cocaine, benzoylecgonine, cocaethylene, morphine, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol, nicotine, and cotinine with drug concentration ranges of 5–500 ng/g (283).

Huestis *et al.* reported a study on the correlations on the placental disposition of methadone and its metabolite [2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)] of pregnant women with maternal methadone dose and neonatal outcomes. The subject women were methadone-maintained opioid-dependent and the objective was to test the ability to detect in utero exposure to illicit drugs (284). Huestis *et al.* also compared placenta and matched meconium concentrations and investigated the relationships between maternal buprenorphine dose, placenta concentrations, and neonatal outcomes following controlled administration during gestation (285).

4.4.5 Dried Blood Spots (DBS)

The introduction of LC-MS/MS instrumentation enabled the development of assays using micro quantities of blood and serum with good sensitivity and precision (286). Drug analyses using DBS have the advantages that less blood is required and the collection of sample is less invasive (287). Determination of drugs, such as rufinamide (288), gabapentin (289), fluoxetine, norfluoxetine, reboxetine, paroxetine (290), cyclosporine A and tacrolimus (291) in DBS using LC-MS/MS was reported.

Saussereau *et al.* also reported the determination of illicit drugs, including opiates (morphine and its 3- and 6-glucuronide metabolites, codeine, 6-acetylmorphine) cocainics (ecgonine methylester, benzoylecgonine, cocaine, cocaethylene) and amphetamines (amphetamine, methamphetamine, MDA, MDMA, MDEA) in DBS (287). The method required 30 μL of whole blood spotted in a Whatman card 903 and dried overnight at room temperature. LODs for the drugs ranged from 0.5 to 5.0 ng/mL.

4.4.6 Vitreous humor

A study for the determination of opiates, including free morphine, 6-acetylmorphine and codeine, in blood and vitreous humor after trimethylsilyl derivatization by GC-MS was reported (292). The average recoveries were 82% for whole blood and 100% for vitreous humor. This method was applied to a case study and the concentrations of morphine and codeine detected in the

vitreous humor samples were lower than those in the whole blood samples.

Analysis of insulin is difficult in post-mortem blood sample because of the rapid degradation of insulin by insulin-degrading enzyme. Nonetheless, Thevis *et al.* have developed a method for the determination of insulin in human vitreous humor by LC-MS/MS (293).

4.5 Interpretation of Toxicological Results

Post-mortem toxicology analyses represent one of the effective tools to facilitate forensic pathologists in determining the cause and manner of death in fatalities cases. This is accomplished by performing tests on body fluids (i.e. blood, urine and vitreous humor) and tissues samples (i.e. liver, stomach, lung and etc.) and then offering interpretation of the findings. However, reliable interpretation of the level of drugs in post-mortem specimens especially blood is difficult and complicated by a number of factors including post-mortem redistribution, simple diffusion after death from a drug depot such as the gastric content and drug stability in specimen.

4.5.1 Post-mortem redistribution

Interpretation of the analytical results constitutes one of the biggest challenges in forensic toxicology because drugs in a post-mortem blood sample may have been subjected to post-mortem changes from the time of death until samples are collected; thus, the drug concentration in post-mortem blood may not reflect the actual drug concentration in blood at the time of death. A literature review by Gisela (294) pointed out that formation of new entities as well as degradation of drugs may occur, especially in putrefied corpses. In addition, body fluids and tissues may be severely affected by autolysis and putrefaction. Therefore, specimens should be selected based on individual case history and on their availability.

Post-mortem redistribution (PMR) of drugs is one of the post-mortem changes that affects drug concentration in blood. Evaluations of PMR phenomena for commonly encountered drugs were reported (113,295,296,297,298,299,300,301,302). Post-mortem drug concentrations showed variations depending on sampling sites and characteristics of the drugs. 76 drugs found in 129 drug-related cases were studied (295). 76 drugs including psychotropic drugs, antidepressants and sedatives were

simultaneously quantified in cardiac and peripheral blood by GC-MS or LC-MS/MS.

Post-mortem redistribution of ten commonly prescribed antipsychotic drugs including 9-OH-risperidone (paliperidone), amisulpride, chlorpromazine, clozapine, haloperidol, olanzapine, promethazine, quetiapine, risperidone, and zuclopenthixol was also investigated (296). The changes in blood concentrations after admission to the mortuary can increase by 112% (for chlorpromazine and olanzapine) but might also decrease by 43% (for 9-OH-risperidone). The large standard deviations between sample pairs and substantial day-to-day unpredictable changes highlighted the difficulty in the interpretation of drug concentrations post-mortem.

A study between sertraline concentrations and postmortem redistribution was reported (297). The study involved a total of nine cases with marked post-mortem redistribution. A study involving 19 medical examiner cases (16 males and 3 females) which screened positive in cannabinoid urine immunoassay indicated that THC and its metabolites 11-OH-THC and THCA undergo only modest PMR, much less than expected based on the lipophilic nature and the high volume of redistribution (Vd) of the cannabinoids. Average central:peripheral (C:P) ratios for all analytes were less than 2.0 (299).

Andresen *et al.* conducted a comparison of the blood concentrations of fentanyl in 118 post-mortem cases with serum levels of fentanyl in 27 living persons after therapeutic administration of fentanyl patches (303). The study revealed that the post-mortem fentanyl blood concentrations were on average up to nine times higher than *in vivo* serum levels at the same dose. Gill *et al.* carried out yet another study on the post-mortem fentanyl concentrations which involved 92 decedents who had one or more fentanyl transdermal patches on their body and had fentanyl detected in their post-mortem toxicology analysis (304). Among 37 accidental fentanyl intoxication deaths, 32 involved substance abuse. The substance abuse deaths had a mean fentanyl blood concentration (26.4 ng/mL) that was over twice that of the natural group (11.8 ng/mL). The analysis also suggested a relationship between total patch dosage and mean post-mortem fentanyl concentration up to the 100-µg/h dose.

4.5.2 Drug stability in blood

4.5.2.1 Stability of zopiclone in blood

Apart from PMR, other factors such as pre-storage condition of the samples prior to examination may also affect the detected drug levels. Differences in the stability of zopiclone between spiked and authentic whole blood from subjects dosed with zopiclone were studied (305). It was found that the degradation of zopiclone in authentic blood was equal to that from spiked blood at the temperatures and times studied. The stability of zopiclone was less than 1 day at 20 °C, less than 2 weeks at 5°C but stable for 3 months at -20 °C.

4.5.2.2 Stability of GHB in blood

The stability of GHB in blood and serum samples under various storage conditions was evaluated (61). GHB was found to be stable at least for weeks in serum samples separated immediately after blood withdrawal and in whole blood samples frozen immediately after blood collection. Another study on long-term stability of GHB in post-mortem samples and samples from living persons, stored at –20 °C, using fluoride preservatives was reported (60). Re-analyses of 59 forensic whole blood samples stored several years (ranged from 0.4 to 7.2 years) at -20 °C with fluoride preservation showed that GHB concentrations did not change significantly for the interpretation of toxicological findings.

4.5.2.3 Stability of benzodiazepines in blood

Study of the stability of benzodiazepines, including lorazepam, estazolam, chlordiazepoxide and ketazolam, in post-mortem blood, bile and vitreous humor stored at different temperatures over six months has shown that benzodiazepine concentrations remained almost stable in all samples at -20°C and -80°C. Among the benzodiazepines studied, estazolam appeared to be the most stable while ketazolam being the least, totally degraded in methanolic solutions over 1 or 2 weeks at room temperature and over 8 or 12 weeks at 4 °C (306).

Karinen *et al.* conducted a study on the stability of stock solutions of a variety of illegal and medicinal drugs stored in freezer (at -20°C), refrigerators (at 4-6°C) and at ambient temperature for up to one year (307). The study indicated that lorazepam and promethazine showed significant concentration

losses after 1 month of storage at ambient temperature. Olanzapine was found to be unstable after one month of storage at ambient temperature, after three months in the refrigerator and had disappeared completely upon one year of storage. In contrast, some drugs demonstrated an increase in concentrations after one year of storage. For example, tramadol and carbamazepine concentrations increased significantly when stored in refrigerator or at ambient temperature for one year.

4.5.2.4 Stability of alcohol in blood

Ethanol analysis in biological samples is the most common test in forensic toxicology laboratories. Kelly and Mozayani published a literature review (308) to give an overview of alcohol testing and result interpretation. This review covered pharmacokinetics including absorption, distribution, and elimination of ethanol, methods for the detection of ethanol, the effect of ethanol on human performance, the role of alcohol in injuries and fatalities, and information regarding the interactions that may occur between alcohol and other drugs. An explanation on how to interpret alcohol levels as well as the extrapolation and calculation of blood alcohol levels at times prior to sample collection was also discussed. Gullberg has presented a paper in regard to the estimation of measurement uncertainty in forensic blood alcohol analysis using a simple bottom-up model (174). The coefficient of variation based on the combined uncertainty in forensic blood alcohol analysis is approximately 1-3%.

A study of blood alcohol stability in forensic ante-mortem blood samples was reported (3). 32 whole blood case samples (each with two tubes of blood) were used for this study. The blood samples were analyzed on blood alcohol concentration (BAC) before and after storage (ranging from 13 to 39 months). 25 samples demonstrated various losses in BAC in both tubes. The same blood samples were then stored at room temperature for 6 months followed by 38 °C for 7 and 28 days and analyzed for BAC at the end of each storage time period. Six months of storage at room temperature decreased BAC further for both tubes of the alcohol positive cases with a mean loss of 0.014 g/dL. Further storage at 38 °C for 7 days did not cause any significant change in BAC. Storage at 38 °C for 28 days caused some loss in BAC which was determined to be significant by statistical analysis.

4.5.3 Toxic fumes in fire-related fatalities

Carbon monoxide (CO) and hydrogen cyanide (HCN) are the most toxic fumes generated in fire-related fatalities. In February 2009, 173 persons were killed by the incident of Victorian Bushfire in Australia. Blood samples, available from 30 deceased (aged 3-80), were tested for degree of COHb saturation (309). Another study based on the data collected from deceased fire victims during 1992-2009 from two Swedish nationwide forensic databases (ToxBase and RättsBase) revealed that 17% of the victims had lethal or life-threatening blood cyanide levels (>1 µg/g), 32% had lethal COHb levels (>50% COHb) and over 31% had cyanide levels above 0.5 µg/g (310).

Since CO may be the cause of more than half of the fatal poisoning reported in many countries, an accurate and reliable analytical method to measure the COHb levels is essential for correct diagnosis. Hao *et al.* have developed a technique employing headspace-gas chromatography-mass spectrometry (HS/GC/MS) for determining CO and COHb% which are crucial to the investigation of deaths potentially related to CO exposure (311). Furthermore, Fujihara J *et al.* also evaluated the usefulness of the AVOXimeter 4000 (AVOX), a portable CO-oximeter, in measuring the HbCO% in post-mortem blood (312).

A study on the quantitative evaluation of volatile hydrocarbons in post-mortem blood of 37 fire-related deaths revealed that the concentrations of volatile hydrocarbons in post-mortem blood could be used to classify the cases into three types of fires: construction fires, gasoline- and kerosene-related fires (313). Quantitative analysis of blood revealed that the benzene and styrene concentrations were positively correlated to the COHb concentration, indicating that the deceased inhaled the hydrocarbons and carbon monoxide simultaneously.

A study on the trend in suicide by CO inhalation involving 158 cases in King County, Washington, United States during 1996-2009 was reported (314). Furthermore, carbon monoxide poisoning in Krakow during year 2002-2010 (315) and in United Arab Emirates during 2007-2009 were also shared in publications (316).

4.5.4 Intoxication by cyanide and inert gases

A suicide case involving a 48-year-old man by oral ingestion of potassium cyanide and inhalation of hydrogen cyanide was reported (317). At autopsy, hemorrhages and erosions of the mucosa of the respiratory tract, esophagus and stomach were found. Concentrations of cyanide were 0.2 mg/L in stomach contents, 0.96 mg/kg in brain tissue, 2.79 mg/kg in lungs, and 5.3 mg/L in blood.

There has been recently an increasing trend of suicide cases that involved insufflations of helium using suffocating plastic bags (318). There are two separate reports on suicide cases by asphyxiation using helium and/or argon (319,320).

4.5.5 Intoxication by drugs of abuse

The first case of fatality due to concomitant consumption of GHB and mephedrone was reported in which 43-years-old man was found dead during a drugs-based party (321). The authors aimed to bring to the attention in the emerging role of new drugs of abuse, and highlighted problems in identifying these drugs with commonly-used immunoassay screening test.

Since amphetamine is a major drug of abuse in Sweden and in other Nordic countries, a survey has studied the demographics of amphetamine abusers in Sweden and the concentrations of this stimulant in forensic blood samples, including 1,183 amphetamine-related deaths, for 10 years in the period of 2001-2010 (155). The authors found that the deaths were mostly results of the toxicity of coingested drugs or adverse drug-drug interaction.

Since abuse of illicit drugs could cause sudden cardiac death, a recent published article has conducted a review on the prevalence of major abused drugs in Europe back in 2009 (322).

A study of 385 toxicology reports related to non-natural deaths of pregnant women in Florida from 1999-2005 revealed that 54% involved prescription drugs (mostly opioids) and 46% involved illicit drugs (323). Such deaths might be intervened and prevented through more interactions with healthcare providers.

Amongst the data on poisoning deaths collected from the autopsy reports in

Estonia from 2000 to 2009, 21.5% cases were found to be poisoned by illicit drugs (324). In addition, deaths from abusing fentanyl increased sharply and remained at a high level since 2002 and the high death toll was attributed to the easy availability of illegal drugs.

5 Conclusions

Over the past three years, significant development and progress have been achieved in the field of forensic toxicology. Recent advances in analytical techniques and increased availability of state-of-the-art hyphenated mass spectrometry instruments have much enhanced the laboratories' capabilities in detecting a wider scope of drugs and/or their metabolites at very low levels in both conventional and alternative specimens. Such advancement has led to evolutionary development in driving under the influence, drug-facilitated sexual assaults and workplace drug testing.

On the other hand, the continual emergence of new drugs of abuse, particularly designer drugs, has posed challenges to toxicologists. Because of shortage of systematic pharmacological and toxicological studies on these new drugs, toxicologists would be difficult to assess their potential risks to human and evaluate their harmful effects from pharmacokinetic and pharmacodynamic perspectives. In addition, the lack of reference standards of these new drugs, in particular their metabolites, have greatly hampered the development of sensitive and effective analytical methods for their identification.

Over the past decade, forensic toxicology has been developing at fast pace with growing complexity. Professionals, experts and practitioners in this discipline should unify and work in collaboration to contend with the changes and challenges ahead through undertaking research and development studies, sharing views and experience, and promoting international cooperation. Under our united and concerted effort, it is expected that the development of forensic toxicology should be sustainable and prosperous in the coming years.

6 References

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