Targeted toxicological screening for acidic, neutral and basic compounds in post- and ante mortem whole blood using simple protein precipitation and UPLC-TOF-MS (bbCID)

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Introduction

The aim of this work was to develop and evaluate a simple, rapid and efficient UPLC-TOF-MS screening analysis in whole blood for the detection of acidic, neutral and basic drugs in one single extraction and injection. The method is used routinely in our laboratory on various samples from forensic autopsy-, clinical forensic- and DUID cases.

Methods

Workflow for multi target screening:

The extraction is described in detail in the abstract PE_{41}. Separation was done in 13.5 minutes by gradient elution on an ACQUITY BEH C18 (100 mm x 2.1 mm, 1.7 µm) column (flow rate: 0.6 mL/min) with mobile phases A and B consisting of 0.1 % formic acid and acetonitrile, respectively. The maXis Impact UHR TOF MS instrument (Bruker) equipped with an electrospray ionisation source was operated in positive mode using a m/z calibration range of 50-1000. The acquisition rate was 10 Hz and the MS/MS analysis was carried out using bbCID (broadband collision-induced dissociation), undirected fragmentation. Calibration was performed in the first 0.2 min of each injection using mixed sodium formate/acetate clusters.

Identification of the compounds was based on the following criteria:

- Retention Time
- Mass accuracy, Err [ppm]
- True Isotopic Patern (mSigma), intensity ratio and mass distances
- Qualifier Ions for confirmation in bbCID for selected compounds
- Individual area thresshold for each compound

Results

The method presently includes 246 compounds of forensic interest (incl. beta- and gamma hydroxybutyrate (BHB and GHB)) - all detected in positive mode as $[M+H]^+$. For each compound a minimum required performance level (MRPL) was set and tested in negative human blood samples. Figure 1 shows the separation of the compounds by retention time and precursor ion m/z ratios.

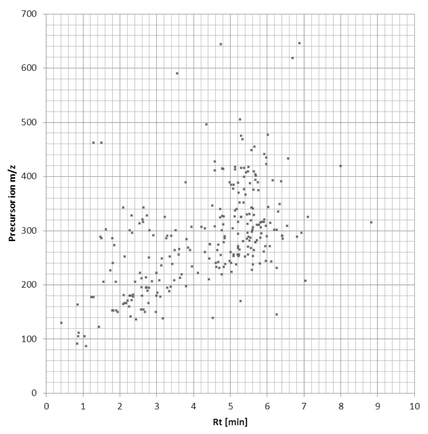

Figure 1: Separation of compounds by rentention time (R_t) and precursor ion m/z ratios.

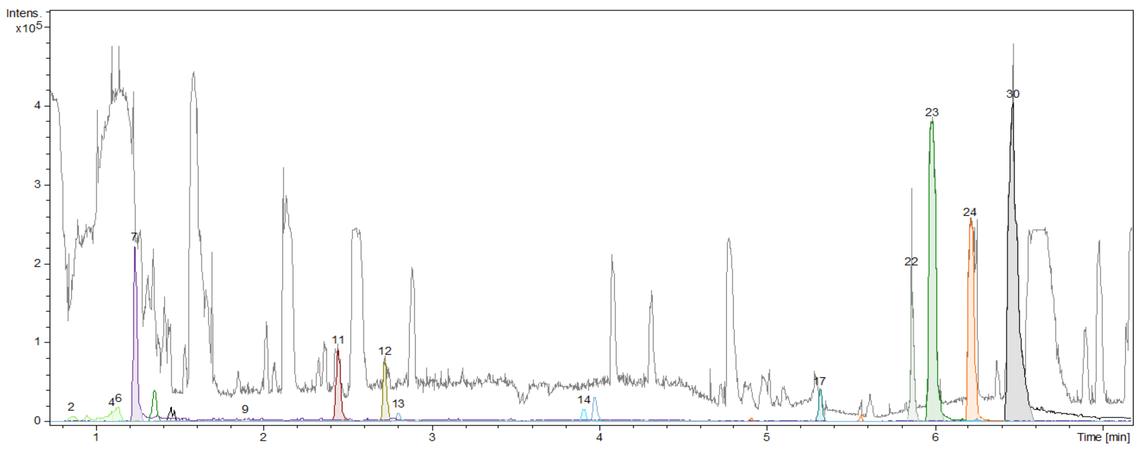

Figure 2: Base peak chromatogram and extracted ion chromatogram, overlaid view. Peak identification: Amphetamine (11); BHB (beta-Hydroxybutyric acid) (4,6); Citalopram (17); Caffeine (12); Cotinine (7); Diazepam (30); Nicotine (2); Nordazepam (23); Oxazepam (22); Paracetamol (acetaminophen) (9); Temazepam (24); Tramadol (14); O-desmethyltramadol (13)

The method was developed to detect compounds at sub-therapeutic as well as at lethal levels. High concentrations resulted in unacceptable high mass errors. In order to overcome this, the primary mass detection window used for rough mass detection was set wide (15 ppm). A secondary mass detection window was used to score the findings according to the mass error. Drugs at high concentrations were then detected and reported, but with a low score. Additionally the presence of qualifier ions were used as further identification for some compounds. Figure 2 and table 1 show the chromatogram and the search results from a DUID case containing high concentrations of diazepam and nordazapam.

Table 1: Search result from TargetAnalyses showing compounds found in a DUID case. Diazepam and nordazepam were reported with a low score [++] and a high Err [ppm] and mSigma. The concentration of diazepam and nordazepam were 3.0 mg/kg and 1.8 mg/kg, respectively.

Score	Cmpd.Name	Formula	deltaRT [min]	Err [ppm]	mSigma	Area	# Exp. Qualifiers	# Found Qualifiers	QI 1 accepted
++++	Amfetamin	C 9 H 13 N 1	-0.01	3.4	5.2	174530	1	1	yes
+++	BHB	C 4 H 8 O 3	-0.03	1.4	2.5	51192	0	0	n.a.
++++	Citalopram	C 20 H 21 F 1 N 2 O 1	-0.01	-1.3	11.2	61568	1	1	yes
+++	Coffein	C 8 H 10 N 4 O 2	0.00	3.8	8.6	129282	0	0	n.a.
+++	Cotinin	C 10 H 12 N 2 O 1	-0.02	4.0	2.4	339479	0	0	n.a.
++	Diazepam	C 16 H 13 Cl 1 N 2 O 1	-0.03	8.6	298.0	1566000	1	1	yes
+++	Nicotin	C 10 H 14 N 2	-0.00	0.1	7.2	24592	0	0	n.a.
++	Nordazepam	C 15 H 11 Cl 1 N 2 O 1	-0.02	10.4	165.8	1126780	1	1	yes
++++	Oxazepam	C 15 H 11 Cl 1 N 2 O 2	-0.02	4.1	17.6	253257	1	1	yes
+++	Paracetamol	C 8 H 9 N 1 O 2	-0.01	-0.3	36.6	13256	0	0	n.a.
++++	Temazepam	C 16 H 13 Cl 1 N 2 O 2	-0.02	-4.4	66.1	627380	1	1	yes
+++	Tramadol	C 16 H 25 N 1 O 2	0.00	1.5	13.4	33351	0	0	n.a.
+++	Tramadol. O-demethyl-	C 15 H 23 N 1 O 2	-0.01	-0.5	12.4	20687	0	0	n.a.
+++	zz_Amfetamin-D5	C 9 H 8 D 5 N 1	-0.01	-2.5	8.7	162397	0	0	n.a.
+++	zz_Cocain-D3	C 17 H 18 D 3 N 1 O 4	-0.01	-1.9	25.1	348863	0	0	n.a.
+++	zz_Diazepam-D5	C 16 H 8 Cl 1 D 5 N 2 O 1	-0.01	1.2	26.6	125676	0	0	n.a.
+++	zz_GHB-D6	C 4 H 2 D 6 O 3	0.01	-0.5	50.0	1716	0	0	n.a.
+++	zz_Phenobarbital-D5	C 12 H 7 D 5 N 2 O 3	0.02	0.4	80.0	9300	0	0	n.a.

The compounds included in the method were extracted by protein precipitation; due to the use of this simple extraction technique, suppression or enhancement from matrix would be expected to be extensive for a large part of the compounds. Also the matrix effects will vary between samples due to the biological diversity and quality of the samples. The matrix effects and the true recoveries were investigated by spiking all compounds in 30 negative post mortem whole blood samples and 10 negative ante mortem whole blood samples from DUID cases. The matrix effects as mean area suppression/enhancement ranged from 171 % enhancement to 98 % suppression. The true recovery ranged from 29 % to 175 %. Table 2 shows matrix effects and true recoveries for selected compounds.

Table 2: Matrix effects and true recoveries for selected compounds determined in different postmortem and antemortem blood samples.

			Post mortem blood samples					Ante mortem blood samples				
	MRPL [a]	Level[b]	Matrix effect[c] (%)		True recovery[d] (%)			Matrix effect[c] (%)		True recovery[d] (%)		
Substance	(mg L^{-1})	(mg L^{-1})	Mean	SD	Mean	SD	n[d]	Mean	SD	Mean	SD	n[e]
4-fluoroamphetamine	0.001	0.03	19	19	96	12	30	11	14	86	6	10
Amphetamine	0.001	0.03	18	14	90	9	28	11	10	93	8	10
Barbital	0.3	0.3	29	14	88	11	28	26	11	89	13	10
Benzoylecgonine	0.001	0.03	-16	16	106	14	29	-25	13	108	5	10
Bromazepam	0.001	0.03	37	9	90	20	30	42	7	120	9	10
Bromo-Dragonfly	0.0002	0.03	86	3	66	15	29	81	3	70	9	9
Cocaine	0.001	0.03	-12	11	106	7	29	-9	24	112	5	10
Lidocaine	0.002	0.03	-3	16	105	13	29	-9	13	101	8	10
LSD	0.0005	0.03	9	30	96	29	26	36	26	83	16	9
Mephedrone	0.001	0.03	14	14	98	8	28	10	14	85	9	10
Methadone	0.001	0.03	73	1	110	9	26	69	2	109	11	10
Norbuprenorphine	0.0005	0.03	29	17	92	29	29	40	17	74	17	9
Oxycodone	0.002	0.03	-4	14	95	6	30	-18	11	103	8	10
Pregabalin	1.0	0.03	31	13	94	12	30	29	9	110	12	10
Risperidone	0.004	0.03	-171	53	168	25	29	-158	81	169	33	10
Valproate	2.0	0.12	38	10	43	16	28	29	11	76	28	9

[a]The MRPLs were based on National laws, TIAFT drug concentrations list, Baselt and other relevant literature. [b]Level = concentration levels in the matrix and recovery experiments. [c]Matrix effect (+ ion suppression , – ion enhancement) calculated as (Responce of standard solution minus Responce of sample spiked after extraction) * 100 divided by (Responce of standard solutiuon). [d]True recoveries calculated as (Response of sample spiked before extraction*100) divided by (Responce of sample spiked after extraction). [e]n = number of replicates.

For the vast majority of the compounds, the MRPLs were met even when taking matrix effects and recoveries into account. The rest of the compounds could all be detected below toxic levels or by detecting metabolites. In the case of the important semi-synthetic opioid buprenorphine, the screening was only successful at the low therapeutic level after adding the metabolites norbuprenorphine, buprenorphine glucuronide and nor-buprenorphine glucuronide to the compound list.

Conclusions

The method has proved to be a fast and reliable procedure for targeted screening of post-mortem and ante-mortem forensic cases using 300 µL whole blood. The procedure can easily be automated and upgraded to include more compounds.

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