Post-Mortem-Drug-Screening in Dental Hard Tissue Samples by LC-QToF MS

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INTRODUCTION

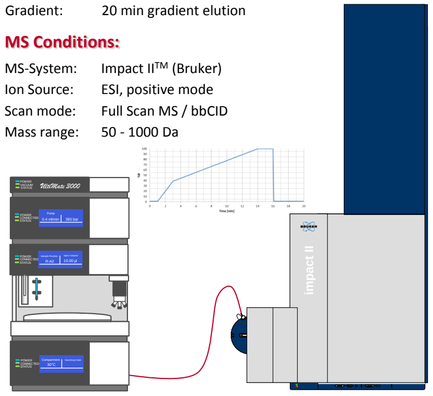
When dealing with burnt, severely putrefied or skeletonized bodies, traditional sample materials for toxicological analysis (e.g. blood, tissue, urine) are often unavailable. In these cases dental hard tissue is one of the remaining materials applicable for post-mortem toxicology. Full scan based screening methods using LC-QToF-MS are a valuable tool for forensic analysis of these materials due to the possibility of qual/quant and retrospective data evaluation. In this study the applicability of a screening workflow - initially developed for the detection and identification of xenobiotics in human serum and urine samples[1] - to dental hard tissue as an alternative matrix for post-mortem toxicology was evaluated. The findings were confirmed using LC-QqQ-MS methods. Results were compared with those obtained from routine post-mortem toxicological analysis of urine and blood.

LC Conditions:

LC-System:	Dionex UltiMate 3000 LC-System
Eluent A:	0.01 % HCOOH + 5 mM $NH_4^+COO^-$ + 10 % MeOH
Eluent B:	MeOH + 5 mM $NH_4^+COO^-$ + 0.01 % HCOOH
Column:	Acclaim® RSLC 120 C18 2,2 µm 120 A 2.1x100 mm
Total flow:	Flow gradient 200 to 480 µL/min
Injection vol.:	2 µL
Gradient:	20 min gradient elution

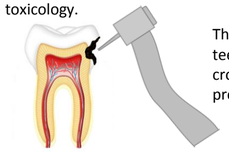
MS Conditions:

MS-System:	Impact II™ (Bruker)
Ion Source:	ESI, positive mode
Scan mode:	Full Scan MS / bbCID
Mass range:	50 - 1000 Da



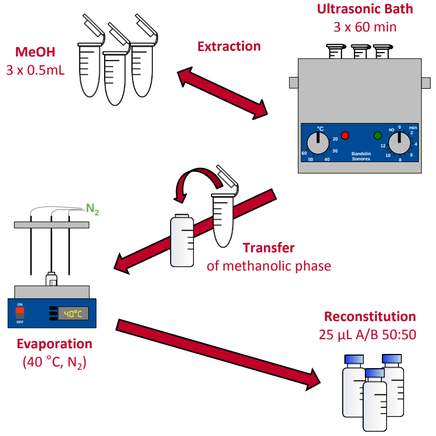
METHODS

In death cases with a known history of drug intake one whole tooth was obtained during the autopsy in addition to the body fluids and tissue samples taken for routine post-mortem toxicology.



The pulp was removed, and the teeth separated into enamel, crown and root dentin, and if present carious dentin.

The dental tissues were powdered using a diamond burr and subsequently extracted three times for 1 h with methanol under ultrasonication.



The extracts off all dental tissue samples were analyzed on an LC-QToF-MS-system to estimate the assay's detection limits in authentic samples. All compounds detected in the screening were subsequently quantified by LC-MS/MS in MRM mode. The results were compared to those of routine toxicological analysis of femoral blood, cardiac blood, urine, stomach contents, and hair to evaluate the performance of the QToF-screening.

ANALYTICAL FINDINGS

Case	Analyte	Dental Hard Tissue ToF Screening	Dental Hard Tissue QqQ (conc. [pg/mg])	Routine Post Mortem Findings (blood, urine, hair)
1	Methadone	✓	9.0	✓
	EDDP	✓	1.4	✓
	Diazepam	✓	2.7	✓
	Nordazepam	✓	×	✓
	Doxepin	✓	1.8	×
	Lidocaine	✓	20	×
	Caffeine	✓	×	✓
2	6-Acetylmorphine	✓	~ 200	✓
	Morphine	✓	~ 130	✓
	Normorphine	×	34	✓
	Codeine	✓	13	✓
	Diazepam	✓	~ 220	✓
	Nordazepam	✓	10	✓
	Oxazepam	✓	36	✓
	Temazepam	✓	6.1	✓
	Tetrazepam	✓	33	×
	Promethazine	✓	~ 3000	✓
	Chlorprotixene	✓	5.7	×
	Buprenorphine	✓	~ 130	✓
	Norbuprenorphine	✓	×	×
	Phenobarbital	×	×	✓
	Doxepin	✓	1.1	×
	Mirtazapine	✓	0.3	×
	Quetiapine	✓	2.8	×
	Caffeine	✓	×	✓
	Papaverine	×	×	✓
	Noscapine	✓	×	✓
	Meconine	×	×	✓
	Nicotine	✓	×	✓
3	Amphetamine	×	×	✓
	Diazepam	✓	2.8	✓
	Nordazepam	✓	2.8	✓
	Oxazepam	×	×	✓
	Temazepam	×	×	✓
	Methadone	✓	10	✓
	EDDP	✓	1.3	✓
	Diphenhydramine	×	×	✓
	Acetaminophen	✓	×	✓
	Caffeine	✓	×	✓
	Doxepin	✓	0.8	×
4	Methadone	✓	35	✓
	EDDP	✓	×	✓
	Buprenorphine	✓	×	✓
	Norbuprenorphine	×	×	✓
	Fentanyl	✓	13	✓
	Norfentanyl	×	×	✓
	Mirtazapine	✓	3.8	✓
	Desmethylmirtazapine	✓	4.7	✓
	Diazepam	✓	20	✓
	Nordazepam	✓	10	✓
	Oxazepam	×	×	✓
	Temazepam	×	×	✓
	THC-COOH	×	×	✓
	Bisoprolol	✓	×	✓
	Naloxone	×	×	✓
	Nicotine	×	×	✓
5	MDMB-CHMICA	✓	49	✓
	Mirtazapine	✓	1.2	✓
	Desmethylmirtazapine	✓	1.6	✓
	THC-COOH	×	×	✓
	Cocaine	✓	×	✓
	Coffeine	✓	×	✓
	Nicotine	✓	×	✓

Case	Analyte	Dental Hard Tissue ToF Screening	Dental Hard Tissue QqQ (conc. [pg/mg])	Routine Post Mortem Findings (blood, urine)
6	Fentanyl	✓	1.0	✓
	Norfentanyl	×	×	✓
	Diazepam	✓	×	✓
	Nordazepam	×	×	✓
	Lorazepam	✓	180	×
	Risperidone	×	41	×
	9-OH-Risperidone	✓	×	✓
	Citalopram	✓	47	✓
	Melperone	✓	110	✓
	Haloperidol	✓	7.2	✓
	Acetaminophen	✓	×	✓
	Promethazine	✓	14	×
	Caffeine	✓	×	✓
	Lidocaine	✓	4.1	×
7	6-Acetylmorphine	×	×	✓
	Morphine	✓	1.6	✓
	Codeine	×	0.7	✓
	Diazepam	✓	3.5	✓
	Nordazepam	✓	13	✓
	Oxazepam	×	×	✓
	Temazepam	×	×	✓
	THC-COOH	×	×	✓
	Noscapine	✓	×	✓
	Papaverine	×	×	✓
	Nicotine	✓	×	✓
	Buprenorphine	✓	220	×
	Norbuprenorphine	✓	×	×
	Cocaine	✓	×	×
	Benzoylecgonine	✓	1.6	×
8	Methadone	✓	10	✓
	EDDP	✓	×	✓
	Cocaine	✓	~ 0.2	×
	Benzoylecgonine	✓	1.3	×
	Diazepam	✓	4.9	✓
	Nordazepam	✓	1.4	✓
	Oxazepam	×	×	✓
	Temazepam	×	×	✓
	Fentanyl	✓	0.8	×
	Carbamazepine	✓	47	✓
	Promethazine	✓	53	✓
	Metamizole-Met.	✓	✓	✓
	Clomethiazole	✓	12	✓
	Valproic Acid	×	×	✓
	Doxepin	✓	2.1	×
	Mirtazapine	✓	2.9	×
	Pregabalin	×	×	✓
	Morphine	✓	×	✓
	Chlorprothixene	✓	34	×
	Nicotine	✓	×	✓
	Acetaminophen	✓	×	✓
9	Morphine	✓	130	✓
	Normorphine	×	10	✓
	Lidocaine	✓	26	✓
	Metamizole-Met.	✓	✓	✓
	Haloperidol	×	17	✓
	Lorazepam	×	×	✓
	Diazepam	✓	0.6	✓
	Nordazepam	✓	1.7	✓
	Olanzapine	×	×	✓
	Trimipramine	✓	23	✓
	Aripiprazole	✓	69	✓
	Methylphenidate	×	×	✓
	Zuclopenthixole	✓	✓	×
	Chlorprothixene	✓	14	×
	Pregabalin	✓	×	✓

RESULTS and DISCUSSION

Drugs and drugs of abuse found in body fluids, tissue samples, and hair could mostly be detected in dental tissues with the applied HRMS assay. Substances detected by the QToF screening that previously had not been detected during routine post-mortem analysis, were labelled as 'tentative' if the identification criteria (retention time, parent and qualifier ion present, isotope ratio) were fulfilled.

In dental tissue samples, several opioids (6-acetylmorphine, buprenorphine, codeine, EDDP, fentanyl, methadone, morphine, and norfentanyl), cocaine, and its metabolite benzoylecgonine, numerous benzodiazepines (diazepam, lorazepam, nordazepam, oxazepam, temazepam, and tetrazepam), carbamazepine, chlorprothixene, citalopram, desmetylmirtazapine, doxepin, haloperidol, melperon, metamizol metabolites (4-AA, 4-AAA, and 4-FAA), mirtazapine, pregabalin, promethazine, quetiapine, risperidone, trimipramine, zuclopenthixol, and - in one case - the synthetic cannabinoid MDMB-CHMICA were detected.

In all cases included in this study, substances that supposably contributed to death could be reliably identified with this approach. The screening's overall sensitivity seems comparable to targeted MRM methods since the assay was able to detect compounds at concentrations between LOD and LOQ determined for the LC-MS/MS method used[2] but in contrast, Full scan MS and broad band CID mode (bbCID) of the QToF also allows for retrospective data analysis.

CONCLUSION

The preliminary results of this comparative study are promising so far. Analysis of dental hard tissue represents a useful alternative matrix for post mortem toxicology, especially if there is no other material available. Based on a study conducted earlier, the incorporation of medical and illicit drugs into dental hard tissue depends on the compound's physico-chemical properties and seems to occur mainly via the blood stream.

However, further investigations, especially regarding the significance of quantitative data but also technical details like matrix effects, will be needed to completely implement the assay in routine post-mortem analysis.

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