

An LC-QToF-MS Assay for Post-Mortem Drug Screening in Dental Hard Tissue Samples

Laura M. Huppertz¹, Miriam Klima^{1, 2}, Miriam C. Kualess³, Markus J. Altenburger³, Volker Auwärter¹, and Merja A. Neukamm¹

¹Institute of Forensic Medicine, Medical Center – University of Freiburg, University of Freiburg, Germany; ²Hermann Staudinger Graduate School, University of Freiburg, Germany;

³Center for Dental Medicine, Department of Operative Dentistry and Periodontology, Medical Center – University of Freiburg, University of Freiburg, Germany

OVERVIEW

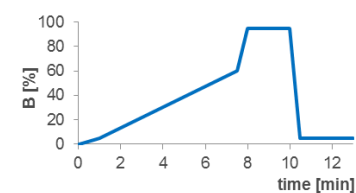
- Dental hard tissue as an alternative matrix in post-mortem toxicology
- Fully retrospective LC-QToF-MS assay
- High sensitivity, semi-automated data evaluation

INTRODUCTION

When dealing with burnt, severely putrefied or skeletonized bodies, traditional sample materials for toxicological analysis (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:

- Dionex UltiMate® 3000RS (Thermo Fischer)
- Acclaim C18 column
- Solvents:
A (H₂O + 5mM NH₄⁺COO⁻ + 0.01% HCOOH + 10% MeOH)
B (MeOH + 5mM NH₄⁺COO⁻ + 0.1% HCOOH)
- 14 min gradient elution
- Injection volume 2 µL

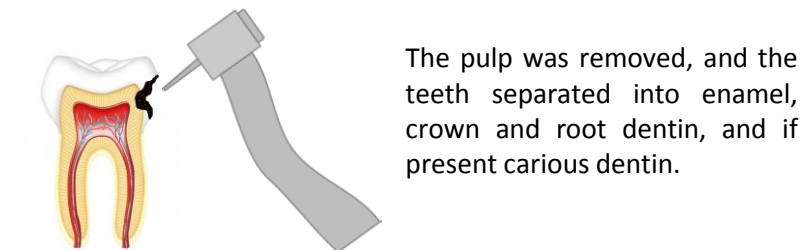


MS conditions:

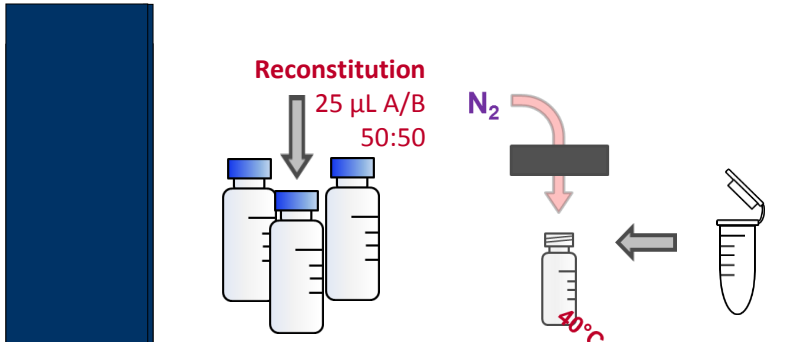
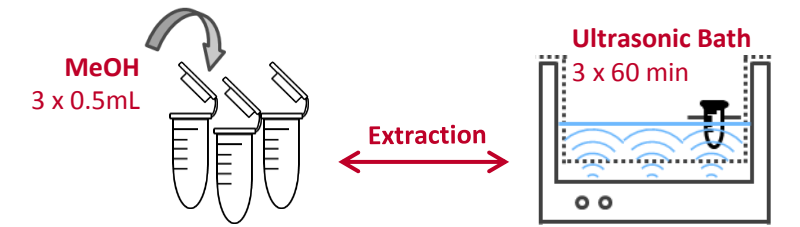
- Impact II™ (Bruker)
- Positive ESI
- Full scan MS / bbCID

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



The extracts were analyzed on an LC-QToF-MS-system to estimate the assay's detection limits in authentic samples. All compounds detected 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	✓	x	✓
	Doxepin	✓	1.8	x
	Lidocaine	✓	20	x
2	Caffeine	✓	x	✓
	6-Acetylmorphine	✓	~ 200	✓
	Morphine	✓	~ 130	✓
	Normorphine	x	34	✓
	Codeine	✓	13	✓
	Diazepam	✓	~ 220	✓
	Nordazepam	✓	10	✓
	Oxazepam	✓	36	✓
	Temazepam	✓	6.1	✓
	Tetrazepam	✓	33	x
	Promethazine	✓	~ 3000	✓
	Chlorprothixene	✓	5.7	x
	Buprenorphine	✓	~ 130	✓
	Norbuprenorphine	✓	x	x
	Phenobarbital	x	x	✓
	Doxepin	✓	1.1	x
	Mirtazapine	✓	0.38	x
	Quetiapine	✓	2.8	x
3	Caffeine	✓	x	✓
	Papaverine	x	x	✓
	Noscapine	✓	x	✓
	Meconine	x	x	✓
	Nicotine	✓	x	✓
	Amphetamine	x	x	✓
	Diazepam	✓	2.8	✓
	Nordazepam	✓	2.8	✓
	Oxazepam	x	x	✓
	Temazepam	x	x	✓
4	Methadone	✓	10	✓
	EDDP	✓	1.3	✓
	Diphenhydramine	x	x	✓
	Acetaminophen	✓	x	✓
	Caffeine	✓	x	✓
	Doxepin	✓	0.89	x
	Methadone	✓	35	✓
	EDDP	✓	x	✓
	Buprenorphine	✓	x	✓
	Norbuprenorphine	x	x	✓
5	Fentanyl	✓	13	✓
	Norfentanyl	x	x	✓
	Mirtazapine	✓	3.8	✓
	Desmethyilmirtazapine	✓	4.7	✓
	Diazepam	✓	20	✓
	Nordazepam	✓	10	✓
	Oxazepam	x	x	✓
	Temazepam	x	x	✓
	THC-COOH	✓	x	✓
	Bisoprolol	x	x	✓
6	Naloxone	x	x	✓
	Nicotine	✓	x	✓
	MDMB-CHMICA	✓	49	✓
	Mirtazapine	✓	1.2	✓
	Desmethyilmirtazapine	✓	1.6	✓
	THC-COOH	x	x	✓
	Cocaine	✓	x	✓
	Caffeine	✓	x	✓
	Nicotine	✓	x	✓
	Nicotine	✓	x	✓

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	x	x	✓
	Diazepam	✓	x	✓
	Nordazepam	x	x	✓
	Lorazepam	✓	180	x
	Risperidone	x	41	x
	9-OH-Risperidone	✓	x	✓
	Citalopram	✓	47	✓
	Melperone	✓	110	✓
	Haloperidol	✓	7.2	✓
	Acetaminophen	✓	x	✓
	Promethazine	✓	14	x
	Caffeine	✓	x	✓
	Lidocaine	✓	4.1	x
7	6-Acetylmorphine	x	x	✓
	Morphine	✓	1.6	✓
	Codeine	x	0.71	✓
	Diazepam	✓	3.5	✓
	Nordazepam	✓	13	✓
	Oxazepam	x	x	✓
	Temazepam	x	x	✓
	THC-COOH	x	x	✓
	Noscapine	✓	x	✓
	Papaverine	x	x	✓
	Nicotine	✓	x	✓
	Buprenorphine	✓	220	x
	Norbuprenorphine	✓	x	x
	Cocaine	✓	x	x
8	Benzoyllecgonine	✓	1.6	x
	Methadone	✓	10	✓
	EDDP	✓	x	✓
	Cocaine	✓	-0.16	x
	Benzoyllecgonine	✓	1.3	x
	Diazepam	✓	4.9	✓
	Nordazepam	✓	1.4	✓
	Oxazepam	x	x	✓
	Temazepam	x	x	✓
	Fentanyl	✓	0.79	x
	Carbamazepine	✓	47	✓
	Promethazine	✓	53	✓
	Metamizole-Met.	✓	12	✓
	Clomethiazole	✓	12	✓
9	Valproic Acid	x	x	✓
	Doxepin	✓	2.1	x
	Mirtazapine	✓	2.9	x
	Pregabalin	x	x	✓
	Morphine	✓	x	✓
	Chlorprothixene	✓	34	x
	Nicotine	✓	x	✓
	Acetaminophen	✓	x	✓
	Morphine	✓	130	✓
	Normorphine	x	10	✓
	Lidocaine	✓	26	✓
	Metamizole-Met.	✓	✓	✓
	Haloperidol	x	17	✓
	Lorazepam	x	x	✓
9	Diazepam	✓	0.66	✓
	Nordazepam	✓	1.7	✓
	Olanzapine	x	x	✓
	Trimipramine	✓	23	✓
	Aripiprazole	✓	69	✓
	Methylphenidate	x	x	✓
	Zuclopenthixole	✓	✓	x
	Chlorprothixene	✓	14	x
	Pregabalin	✓	x	✓
	Pregabalin	✓	x	✓

RESULTS and DISCUSSION

Several 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 ok) were fulfilled.

In dental tissues, several opioids (6-acetylmorphine, buprenorphine, codeine, EDDP, fentanyl, methadone, morphine, and norfentanyl), cocaine, and benzoyllecgonine, numerous benzodiazepines (diazepam, lorazepam, nordazepam, oxazepam, temazepam, and tetrazepam), carbamazepine, chlorprothixene, citalopram, desmethylmirtazapine, 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 supposedly contributed to death could be reliably be identified with the applied assay.

The screening's overall sensitivity seems comparable to targeted MRM methods but in contrast also allows for retrospective data analysis.

The analysis of dental hard tissue may represent a useful alternative matrix for post mortem toxicology, especially if there is no other material available.

Based on an in-house study, the incorporation of medicinal and illicit drugs into dental hard tissue depends on the compound's physico-chemical properties and seems to occur mainly via the blood stream.^[2]

CONCLUSION

The preliminary results of this study are promising so far. Since the window of detection of xenobiotics in dental tissues is somewhere between urine and hair^[2] substances taken up very shortly before death may be missed. However, further investigations especially regarding possible quantitation but also regarding matrix effects will be needed to completely implement the assay in routine post-mortem analysis.

