A New Workflow for Fast Elucidation of Drug Metabolites for Screening – A Story of Microsomes and Microchips

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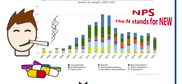
Forensic Toxicology

Introduction

What's the Problem?



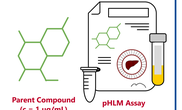
According to data of the EU Early Warning System, there are still 40 to 60 New Psychoactive Substances (NPS) emerging in the European Union each year.

Screening for these compounds in urine can be quite challenging. Cross-reactivities of immunochemical methods can vary from good (e.g. benzodiazepines) to insufficient (e.g. synthetic cannabinoids). Nevertheless, mass spectrometry (MS) is the gold standard for identification of compounds in a standard toxicological analysis. No matter if you use MS for screening right away, or as confirmatory analysis for other screening approaches, these methods need constant updates.

But especially for new emerging substances, reference standards of drug metabolites are usually not commercially available. This is particularly a problem with substances whose parent compound is hardly or not at all detectable in urine. Missing knowledge about metabolism and missing spectral information of metabolites may lead to false negative-findings.

So, What to do About it?

If the parent compound is available, pooled human liver microsome (pHLM) assays are an easy-to-use, animal-free methodology to generate phase I metabolites which show good agreement with the metabolic profile found in human urine samples

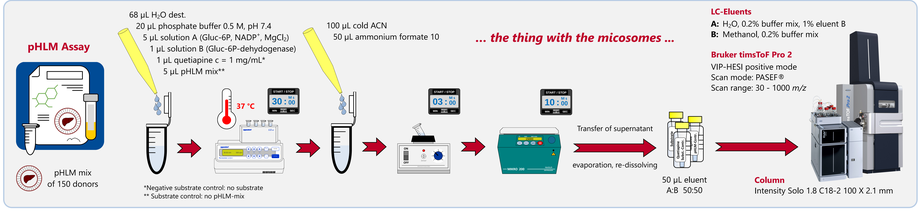


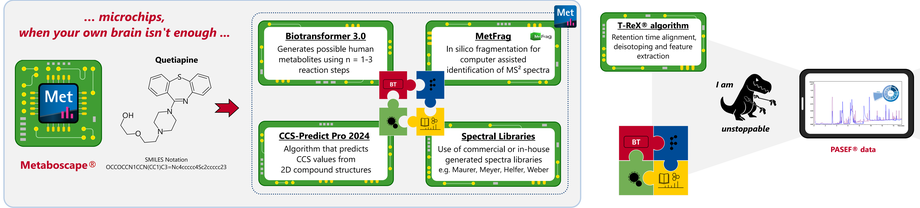
A non-targeted workflow using UPLC-timsTOF-MS combined with sophisticated software tools can be used to identify features of in silico generated metabolites.
Spectral data from pHLM assays can be used for future routine screening with traditional targeted methods, e.g. LC-TQ-MS. Analysis of real urine samples can help to identify the most abundant metabolites in vivo.

Does This Really Work?

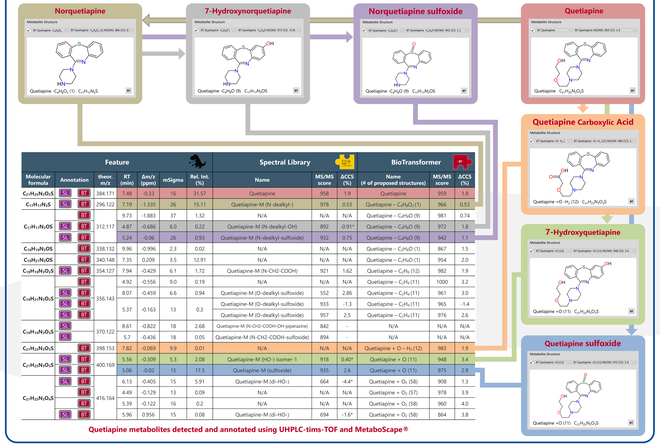
Quetiapine, a neuroleptic yielding multiple well know metabolites, was used to demonstrate the suitability of this workflow using UPLC-timsTOF-MS and MetaboScape® with in silico prediction, in silico fragmentation and collision cross section (CCS) prediction and subsequent analysis of real positive urine samples.

Methods I





Results I: Metabolic Pattern in vitro



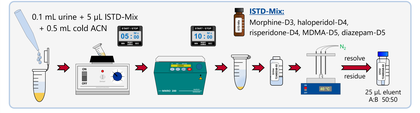
Feature						Spectral Library			BioTransformer		
Molecular formula	Annotation	theor m/z	RT (min)	Δm/z (ppm)	mSigma	Rel. Int. (%)	Name	MS/MS score	ΔCCS (%)	Name (# of proposed structures)	MS/MS score	ΔCCS (%)
$C_{21}H_{25}N_3O_2S$	[M+H]+	384.171	7.48	-0.33	16	31.57	Quetiapine	958	1.9	Quetiapine	999	1.9
$C_{17}H_{17}N_3S$	[M+H]+	296.122	7.19	-1.335	26	15.11	Quetiapine-M (N-dealkyl-)	978	0.53	Quetiapine – $C_4H_8O_2$ (1)	966	0.53
	[M+H]+		9.73	-1.883	37	1.32	N/A	N/A	N/A	Quetiapine – C_4H_8O (3)	981	0.74
$C_{17}H_{17}N_3OS$	[M+H]+	312.117	4.87	-0.686	6.0	0.22	Quetiapine-M (N-dealkyl-OH)	892	-0.91*	Quetiapine – C_4H_8O (9)	972	1.8
	[M+H]+		5.24	-0.96	26	0.93	Quetiapine-M (N-dealkyl-sulfoxide)	932	0.75	Quetiapine – C_4H_8O (9)	942	1.1
$C_{17}H_{17}N_3O_2S$	[M+H]+	328.112	9.96	-0.996	2.3	0.02	N/A	N/A	N/A	Quetiapine – C_4H_8O (1)	867	1.5
$C_{17}H_{17}N_3OS$	[M+H]+	340.148	7.35	0.209	3.5	12.91	N/A	N/A	N/A	Quetiapine – C_4H_8O (1)	954	2.0
$C_{19}H_{19}N_3O_3S$	[M+H]+	354.127	7.94	-0.429	6.1	1.72	Quetiapine-M (N-CH2-COOH)	921	1.62	Quetiapine – C_4H_8 (12)	982	1.9
	[M+H]+		4.92	-0.556	9.0	0.19	N/A	N/A	N/A	Quetiapine – C_4H_8 (11)	1000	3.2
$C_{19}H_{19}N_3O_4S$	[M+H]+	356.148	8.07	-0.459	6.6	0.94	Quetiapine-M (O-dealkyl-sulfoxide)	552	2.86	Quetiapine – C_4H_8 (11)	961	3.0
	[M+H]+		5.37	-0.163	13	0.3	Quetiapine-M (O-dealkyl-sulfoxide)	933	-1.3	Quetiapine – C_4H_8 (11)	965	-1.4
	[M+H]+						Quetiapine-M (O-dealkyl-sulfoxide)	957	2.5	Quetiapine – C_4H_8 (11)	976	2.6
$C_{19}H_{19}N_3O_5S$	[M+H]+	370.122	8.61	-0.822	18	2.68	Quetiapine-M (N-CH2-COOH-OH-piperazine)	842	-	N/A	N/A	N/A
	[M+H]+		5.7	-0.436	18	0.05	Quetiapine-M (N-CH2-COOH-sulfoxide)	894	-	N/A	N/A	N/A
$C_{21}H_{23}N_3O_3S$	[M+H]+	398.153	7.82	-0.069	9.9	0.01	N/A	N/A	N/A	Quetiapine + O – H_2 (12)	983	1.9
$C_{21}H_{23}N_3O_4S$	[M+H]+	400.169	5.56	-0.309	5.3	2.08	Quetiapine-M (HO-) isomer-1	918	0.49*	Quetiapine + O (11)	948	3.4
	[M+H]+		7.06	-0.02	15	17.5	Quetiapine-M (sulfoxide)	935	2.6	Quetiapine + O (11)	975	2.9
$C_{21}H_{23}N_3O_5S$	[M+H]+	416.164	6.13	-0.405	15	5.91	Quetiapine-M (di-HO-)	664	-4.4*	Quetiapine + O, (58)	908	1.3
	[M+H]+		4.49	-0.129	13	0.09	N/A	N/A	N/A	Quetiapine + O, (57)	978	3.9
	[M+H]+		5.39	-0.122	16	0.2	N/A	N/A	N/A	Quetiapine + O, (58)	960	4.0
	[M+H]+		5.96	0.956	15	0.08	Quetiapine-M (di-HO-)	694	-1.9*	Quetiapine + O, (58)	864	3.8

Quetiapine metabolites detected and annotated using UHPLC-tims-TOF and MetaboScape®

Methods II

TargetScreener HR

Retention time, principle ion and fragment ions of detected metabolites can be transferred to existing routine screening methods, e.g. the compound database of the TargetScreener HR approach.



LC-System:	Bruker Elute UHPLC	**MS-System:**	Bruker impact II VIP
Eluent A:	H_2O, 0.2% buffer mix, 1% eluent B	Ion source:	VIP-HESI source, positive mode
Eluent B:	Methanol, 0.2% buffer mix	Scan mode:	bbCID (30eV +/- 6V) @ 2Hz
Gradient:	20 min gradient elution	Scan range:	30 - 1000 m/z
Column:	Intensity Solo 1.8 C18-2 100 x 2.1 mm		

Results II: Analysis of Real Samples

Urine samples from forensic cases with known quetiapine uptake were analyzed using the TargetScreener HR or existing bbCID data was reprocessed retrospectively using the updated TASQ database. For data analysis, the peak areas of the metabolites in each urine sample were normalized to that of the most abundant metabolite.

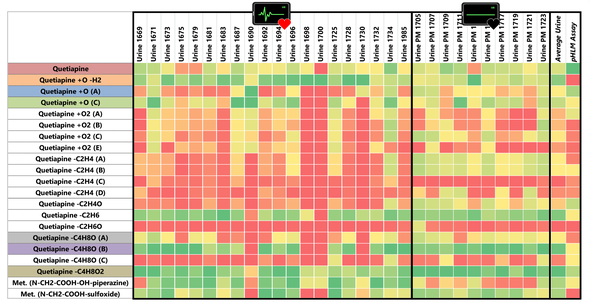
Comparison of the average normalized peak areas of routine samples (***Average Urine***) and the ***pHLM assay*** show some quantitative differences. The analysis of a certrain number of authentic samples is therefore essential for the proper selection of suitable biomarkers.

As expected, norquetiapine (**Quetiapine -$C_4H_8O_2$**) is the most abundant metabolite in almost all samples. Other high abundant metabolites derive from sulfoxidation (**Quetiapine +O**) or hydroxylation (**Quetiapine +O**) and combinations of the latter (**Quetiapine -C_4H_8O**, **Quetiapine -C_4H_8O**).

In contrast to the findings in the pHLM assay, the carboxy metabolite (**Quetiapine +O -H_2**) showed high signal intensities in post- and ante-mortem urine samples.

Quetiapine -C2H4 (A) and **Quetiapine -C2H4 (B)** could only be differentiated due to different CCS values using tims. This metabolite appears to be elevated in post-mortem samples (PM). The reason for this is unclear. However, the data suggests that the same biomarkers can also be used for post-mortem urine samples.

Although human specimens show the complete metabolic pattern with all its variants, there is also a major drawback to using samples from case work: There is usually no information about important details such as the dosage, time of the last intake or, in the case of post-mortem samples, the chronological context between intake, time of death and sampling.



LC-QTOF-MS Findings in Human Urine Samples using TASQ®

(Peak areas normalized to the most intense metabolite of each sample [0 5 10 15 20 25 30 40 50 60 70 80 90 100 %])

Conclusions

So, What did we do?

We successfully demonstrated the use of pooled human liver microsomes, UHPLC-timsToF-MS and a combination of sophisticated software tools to detect metabolites of the model compound quetiapine after biotransformation prediction and in silico fragmentation of potential metabolites. It allowed fast annotation and review of drug metabolites in a single, multi-faceted analysis, with good agreement with known data from the literature and spectral libraries. Obtained metabolic information was used to adapt our LC-QTOF-MS screening approach and screen quetiapine positive urine samples from routine case work.

What is This Workflow for ... and What is it not for?

This easy-to-use, non-targeted workflow allows you to find biomarkers and the necessary feature information to set up an MS-based screening of those metabolites in human urine samples.

Although it can provide valuable support, this workflow is not intended for complete metabolic elucidation of a compound.

What are the Main Limitations?

In vitro assays using pHLMs cannot reproduce the full metabolism of a human body. Therefore, in addition to quantitative differences, qualitative differences between pHLM findings and data from human samples are to be expected.

Some metabolites, e.g. Quetiapine-M (N-CH2-COOH-sulfoxide), could only be annotated due to existing spectra library entries. Therefore, the quality of the used biotransformation prediction appears to be one of the main limitations of this workflow.

How Does This Work in Real Life?

To see how the whole thing works in real life, find the T-Rex on **poster no. ThP 292**

SCAN ME

COI Disclosure: B. Schneider, I. Nordhorn and C. Baessmann are employees of Bruker Daltonics GmbH & Co. KG, Bremen, Germany