Toxichem Krimtech 2011;78(Special Issue):230

Semi-quantitative Determination of Concentrations in Systematic Toxicological Analysis by LC-QTOF-MS

Sebastian Broecker, Fritz Pragst, Gregor Kopf, Max Koch, Sina Wuttig, Sieglinde Herre
Institute of Legal Medicine, University Hospital Charité, Turmstr. 21, 10559 Berlin, Germany

Key words: Accurate mass spectra library, General unknown screening, Liquid chromatography-mass spectrometry, Semi-quantitative concentration, Time-of-flight mass spectrometry.

Abstract

Aim: Besides the unambiguous identification, the concentration is an important prerequisite for toxicological interpretation. Therefore, a procedure for semi-quantitative estimation of concentrations from the LC-QTOF-MS areas of identified peaks was developed and examined by application to spiked and real blood, and hair samples.

Methods: The retention times and peak areas of more than 2,100 toxic substances were measured by LC-QTOF-MS at an Agilent 6530 instrument under standardized conditions by injection of 100 pg substance together with each 100 pg of 33 deuterated standards with retention times evenly spread over the run time. All data are stored in a database LC-TOF-QUANT. In practical application, the sample preparation (protein precipitation, extraction of hair) was performed after addition of all 33 standards and the extracts were measured by LC-QTOF-MS in data dependent acquisition mode under the same standard conditions. For each identified peak, an in-house developed software tool "Estimate Concentration" selects a certain number (e.g. five) nearby eluting deuterated standards, extracts the corresponding standard peak areas of the analyte and selected deuterated standards from LC-TOF-QUANT and calculates the (five) concentrations in usual way. The (five) results are tested for outliers which are omitted and the mean concentration and standard deviation are calculated.

Results and Discussion: The method was tested at spiked and real blood and hair samples. For blood samples which were spiked with 31 illegal and therapeutic drugs the measured and spiked concentrations were in good agreement with a standard deviation between 5 and 30 %. For hair samples spiked with the same drugs the agreement was even better with standard deviations between 1 and 15 %. Furthermore, the results from post-mortem blood and hair samples from real cases agreed well with the quantitative data from HPLC-DAD and GC-MS.

Conclusion: The developed method can successfully be used for a fast approximate estimation of concentrations also if the reference substance is not available. Investigations about the applicability at different instruments are in progress.

1. Introduction

Liquid chromatography-hybrid quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) in data dependent acquisition mode was shown to be a very efficient method for substance identification in systematic toxicological analysis (STA) including metabolites in previous papers of the authors [1-5]. The most important task in STA is the unambiguous identification of the poisonous substances. However, at least an approximate concentration is also necessary for a reasonable toxicological interpretation. In clinical emergency cases these approximate concentrations should be obtained in a short time. Frequently, particularly for seldom occurring poisons, reference substances and validated quantitative methods are not available.

For HPLC-DAD a method for semi-quantitative determination of concentrations from specific peak areas of 1 µg and pre-determined extraction yields under standardized conditions was developed which enabled a fast estimation of concentrations of more than 3,000 substances and proved to be useful in routine application [6,7].

In the present study, a similar method was developed for LC-QTOF-MS with more than 2,100 toxicologically relevant substances. This method is based on the simplified assumption of a linear relationship between peak area and concentration (one-pointcalibration). There are some general difficulties of quantification using LC-MS. The most severe difficulty is that, different from UV absorbance, there is no stable concentration-response relationship because of variation of construction principles of the instruments (e.g. ion sources, ion optics, mass filter, detector), of tuning (algorithm for optimal ion transfer), of measurement parameters (e.g. voltages, vacuum, gas), LC conditions (e.g. mobile phase, pH, gradient, separation), or sample preparation. Further problems are caused by matrix effects in LC-MS, which means ion suppression or ion enhancement, especially for ESI ion sources [8-10].

For these reasons, error compensation by internal standards is always necessary. In targeted analysis, the use of isotopic (deuterated) internal standards is optimal. However, this cannot be realized in systematic toxicological analysis since the substance to be determined is not known before analysis and because of the large number of possible candidates. Instead, a set of internal standards with different chemical character and different retention time is applied in the present study. From this set, a certain number (for instance five) are selected and used to calculate an approximate mean concentration of an identified peak.

2. Material and Methods

2.1. Reference substances

The reference substances were generously donated by a large number of pharmaceutical firms or were purchased from LGC Promochem, Sigma-Aldrich, and other providers. A complete list of all substances is given in the UV spectra library [7]. All following deuterated standards were obtained from LGC (Wesel, Germany) and were used in all measurements for building the database as well as performing the sample measurements: 2-hydroxyethylflurazepam-D4, 6-acetylmorphine (6-AM)-D3, 7-aminoclonazepam-D4, 7-aminoflunitrazepam-D7, α-hydroxyalprazolam-D5, amphetamine-D5, benzoylecgonine-D3, buprenorphine-D4, clonazepam-D4, cocaethylene-D3, cocaine-D3, codeine-D3, desalkylflurazepam-D4, diazepam-D5, EDDP-D3, estazolam-D5, flunitrazepam-D7, lorazepam-D4, MDA-D5, MDE-D6, MDMA-D5, methadone-D9, methamphetamine-D5, methylecgonine-D3, morphine-D3,-nitrazepam-D5, nordiazepam-D5, oxazepam-D5, prazepam-D5, temazepam-D5, THC-D3, THC-COOH-D3, triazolam-D4.

2.2. Instruments and standard procedure for STA by LC-QTOF-MS

A detailed description of all conditions and parameters used for application of LC-QTOF-MS in data dependent acquisition mode was described in previous papers [1-3]. Therefore, only some essential data shall be given. The liquid chromatography was performed using an LC 1200 series (Agilent Technologies) device with a Poroshell 120 EC-18 column (2.1x100 mm 2.7 µm, Agilent Technologies, Waldbronn, Germany) at 50°C. The mobile phases A (10 mM CH_3COONH_4, pH 6.8) and B (methanol) were applied with a flow of 0.4 ml/min and the

following gradient: 0 min 10% B, linear to 50 % in 8 min, linear to 100 % in 20 min hold for 4 min, 3 min conditioning, overall time 24 min.

The MS measurements were performed at a 6530 Accurate-Mass Q-TOF LC-MS device (Agilent Technologies, Santa Clara, USA). The QTOF-MS instrument was operated under the following conditions: Ion source ESI + Agilent Jet Stream Technology in positive or negative ionization mode, the quadrupole was used as an ion guide in MS mode and for selection of precursor ions with $\Delta m/z = 4$ in MS/MS mode, collision cell without CID in MS mode and with CID of precursor ions in MS/MS mode at mass dependent ramped CID energy (offset 4 eV, slope 6 eV/100 m/z), TOF-MS with a mass range of 100-1000 m/z in MS mode and 50-600 m/z in MS/MS mode. The scan rate was 4 Hz in MS and MS/MS experiments. The source parameters were: gas temperature 320 °C, gas flow 8 L/min, nebulizer pressure 35 psi, sheath gas temperature 380 °C, sheath gas flow 11 L/min, VCap voltage 3000 V, nozzle voltage 0 V and fragmentor voltage 150 V.
The LC-QTOF-MS device was operated by the software MassHunter Acquisition B.02.01 with Service Pack 3 for the Agilent TOF and QTOF and MassHunter Qualitative Analysis B.03.01 with Service Pack 3. The Personal Compounds Database and Library Software B.03.01 [1,4] was used for peak identification.

2.3. Development of the database LC-TOF-QUANT

The retention times and peak areas of more than 2,100 substances were measured under the conditions described section 2.2 in groups of 50 substances together with all 33 deuerated standards given in section 2.1. For this purpose, each 100 pg of the 50 substances and of the 33 deuterated standards in 1 µl methanol were injected. Each measurement was performed in three replicates. Each peak was identified by CID spectrum and the retention was recorded. The monoisotopic masses of all detected ion species of a substance (adducts with H^+, Na^+, K^+, NH_4^+), were extracted with a mass window of 60 ppm and the peak areas were registered separately for these ions and for the sum of all four ions. The database contains for every substance the retention time and the standard peak areas of all possible ion species (average of the three measurements).

2.4. Sample preparation

Details of the sample preparation are given in previous papers [1-3]. In this study, protein precipitation of blood samples with acetonitrile and extraction of hair with an acetonitrile/methanol/formic acid mixture were applied.

Blood: 100 µL whole blood were placed in a 1.5 mL Eppendorf vial and 5 µl of a solution containing each 1 ng/µl of all deuterated standards and 400 µL acetonitrile were added. The mixture was vortexed for 1 min and centrifuged for 5 min at 13,200 rpm. Then, 400 µL supernatant were separated and evaporated to dryness in a nitrogen stream at 40 °C. The residue was reconstituted in 80 µL ACN / 0.1 % HCOOH (35:65 v/v). 5 µL were injected for LC-QTOF-MS.

Hair: In case of longer hair samples, the proximal segment 0-6 cm was analyzed. Shorter hair samples were analyzed in full length. The samples were decontaminated by gentle shaking for 1 min in water and two times for 1 min in acetone. After drying on a filter paper the hair was cut to 1-2 mm pieces and about 20 mg were exactly weighed in a 1.5 ml Eppendorf vial. After addition of the mixture of deuterated standards (5 µl of each 1 ng/µl) the hair was incubated for 18 h with 0.5 ml of a mixture of methanol/acetonitrile/2mM ammonium formate

(25:25:50, v/v/v) with gentle shaking at 37 °C. Then the mixture was centrifuged for 5 min at 13,200 rpm. The liquid phase was separated and the incubation of the hair pieces was repeated for 18 h with another 0.5 ml of the solvent mixture. Both extracts were united and evaporated in a nitrogen stream to a residue of 0.5 ml in order to remove the most of the organic solvents. 5 µl of the residue were injected for LC-QTOF-MS measurement without further clean-up procedures.

3. Results and Discussion

3.1. The database LC-TOF-QUANT

The database LC-TOF-QUANT was measured in MS mode of the instrument absence of any matrix and contains for each of the more than 2,100 substances and for the 33 deuterated standards the substance name, the retention time, and the peak areas of 100 pg for all ion species (mean values of three measurements). An excerpt is shown in Table 1. It was observed during the measurement in triplicate of the about 45 solutions of each 50 substances which contained always the 33 deuterated standards that the peak areas of the deuterated standards did not vary to an essential extent over the whole measurement period. Therefore, the peak areas of the toxic substances had not to be corrected in relation to the deuterated standards before entering into the database but could be directly entered.

Tab. 1. Excerpt of the database LC-TOF-QUANT.

Substance name	RT	Paek areas of 100 pg, counts							
	min	M^+	$[M+H]^+$	$[M+Na]^+$	$[M+K]^+$	$[M+NH_4]^+$	Sum 1[1]	Sum 2[1]	$[M-H]^-$
Acetazolamide	1.40	---	19094	98807	15850	---	118733	134684	---
Acetylaminonitro-propoxybenzene	9.33	---	2910232	317561	---	109212	3334814	3392164	---
Acriflavinium	5.65	1202478	---	---	---	---	---	---	---
Ajmaline	7.00	---	51880897	---	---	---	5279504	5261400	---
Allobarbital	5.82	---	---	---	---	---	---	---	5481
Aminoquinuride	10.82	---	---	40637	---	---	40637	40637	---
Aminophenazone	7.32	---	4502281	222378	15240	---	4722476	4742589	---
Amiodarone	20.11	---	5073641	---	---	---	5089228	5088671	---
Amitriptyline	13.11	---	4587099	---	---	---	4587099	4587099	---
Amobarbital	9.52	---	---	---	---	---	---	---	13646
Amrinone	2.75	---	206583	---	---	---	209156	209156	---
Atrazine	10.16	---	2693485	---	---	---	2685126	2684544	---
Atropine	5.01	---	3691602	24966	---	---	3725074	3737983	---
Acebutolol	6.36	---	4201908	42504	---	---	4245109	4265149	5362
Azidamfenicol	4.25	---	---	157687	154033	---	157687	312600	302263
Acetanilide	4.21	---	589364	---	---	---	589364	589364	---
Alprazolam	10.87	---	4833592	1118202	---	---	5951459	6030845	---
Alprenolol	9.23	---	7268153	59076	---	---	7347363	7249966	---
Amantadine	5.29	---	3062036	---	---	---	3057832	3057460	---

[1] Sum 1 = $[M+H]^+ + [M+Na]^+ + [M+NH_4]^+$; Sum 2 = $[M+H]^+ + [M+Na]^+ + [M+K]^+ + [M+NH_4]^+$

The distribution of the retention times of all substances is shown in Fig. 1. It can be seen from the cumulative plot that the substances are well distributed over the run time with a slightly higher frequency between 7 and 15 min. The deuterated standards are also well spread over the retention time range. It is important for compensation of the matrix effect to have a choice several nearby eluting deuterated standards for every compound.

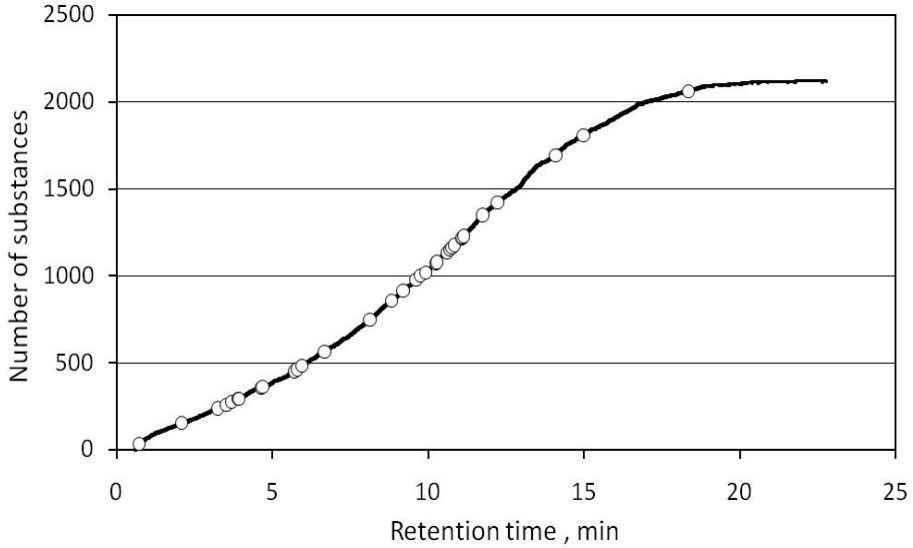

Fig. 1. Cumulative plot of the retention times of more than 2,100 toxicological substances and 33 deuterated standards (circles).

3.2. Software tool "Estimate Concentration"

The software tool "Estimate Concentration" was developed for semi-quantitative calculation of the concentrations of substances which were identified in the LC-QTOF-MS analysis file. The operation scheme is shown in Fig. 2 and starts with the result list of the search in the database and library which is confirmed by visual control and exported to the tool.

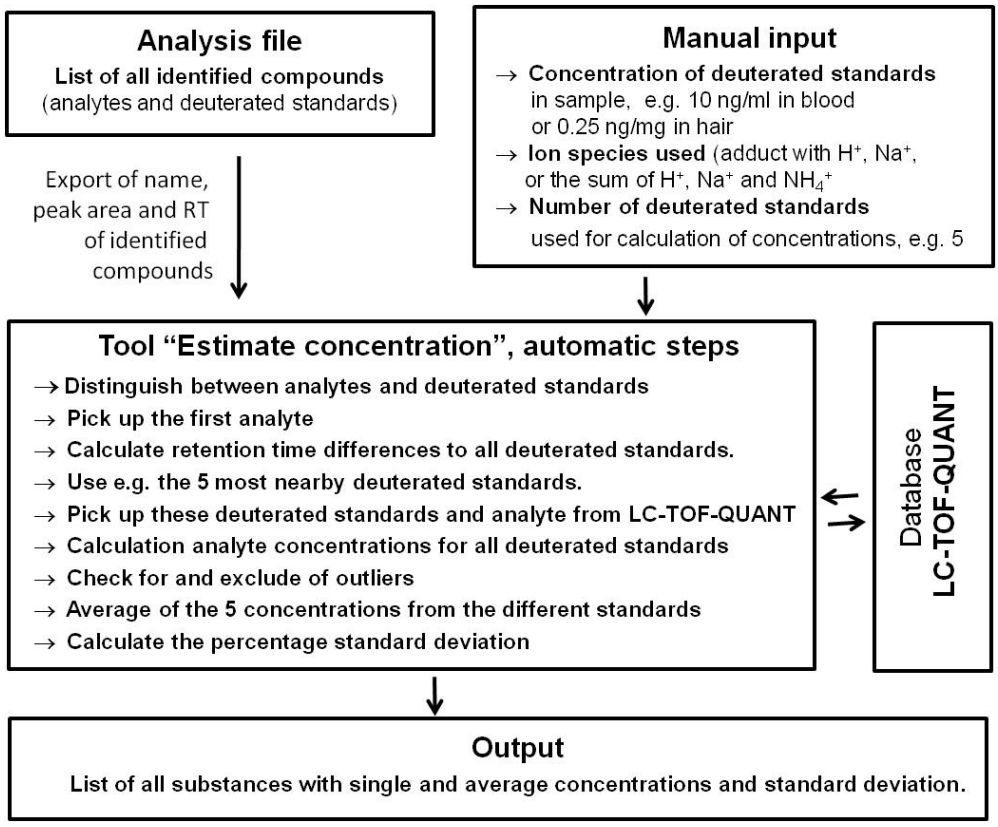

Fig. 2. Operation scheme of the software tool "Estimate Concentration".

As manual input only the concentration of the deuterated standards in the sample, the ion species used (adduct with H^+, Na^+, K^+ and/or NH_4^+), and the number of deuterated standards per analyte used from the 33 for quantification are necessary. The tool separates the analytes from the deuterated standards and calculates the concentration of one analyte after the other by selecting the chosen number of deuterated standard with minimum difference in retention time to the analyte and by use of peak areas measured in the actual analysis file and stored in the database according to the following equation.

$$C_{Analyte} = \frac{C_{deut.\ std.\ in\ sample} \times Area_{Analyte\ in\ sample} \times Area_{deut.\ std.\ in\ database}}{Area_{deut.\ std.\ in\ sample} \times Area_{Analyte\ in\ database}}$$

If, for instance, five deuterated standards per analyte were chosen, each five concentrations are obtained. These are checked for outliers which are excluded. Then, the average concentrations and the standard deviations are calculated. The result list contains name, single concentrations, average concentration and standard deviation of each identified substance, if quantitative data are stored in the in the database.

The working principle is demonstrated in Table 2 for the determination of codeine (spiked concentration 25 ng/ml) from a blood sample. This is one of the rare examples with a strong outlier.

Tab. 2. Estimation of the concentration of codeine from a blood sample using the tool "Estimate Concentration" and the datasbase LC-TOF-QUANT. Spiked concentration 25 ng/ml, Number of deuterated standards: 5.

Nr.	Deuterated Standard	Concentration, ng/ml	Outlier
1	Codeine-D3	25	No
2	7-Aminoflunitrazepam-D7	24	No
3	6-Acetylmorphine-D3	23	No
4	7-Aminoclonazepam-D4	24	No
5	MDE-D6	101	Yes
	Average Concentration	Total 40	
		Outlier excluded: **24**	
	Standard deviation	Total 88%	
		Outlier excluded **2.9%**	

2.3. Application to spiked samples

For test of the applicability, five blood samples were spiked with 31 drugs at the concentrations 5, 25, 100 and 500 ng/ml, analyzed according to the procedure described in section 2.4 and were quantified using tool with each five deuterated standards as described in Fig. 2. In the same way, 5 hair samples were spiked with the same drugs at 0.05, 0.25, 1 and 5 ng/mg, analyzed, and submitted to semi-quantification using the tool. In Table 3, the mean values and standard deviations between the five results are given for one of the blood samples and one of the hair samples. As a criterion, a standard deviation between the five single results of 30 % was accepted.

Tab. 3. Application of the tool "Estimate Concentration" to a blood sample and a hair sample spiked with 31 drugs at different concentrations and analyzed under standardized conditions.

Analyte	**Blood Concentration in ng/ml (Standard deviation in %)**				**Hair Concentration in ng/mg (Standard deviation, %)**			
Spiked Concentration, ng/mg	***5.0***	***25***	***100***	***500***	***0.05***	***0.25***	***1.00***	***5.00***
Alprazolam	4.5 (13)	18 (8.4)	117 (6.5)	480 (6.2)	0.068 (5.7)	0.34 (5.0)	1.2 (5.8)	6.7 (4.1)
Amitriptyline	3.8 (18)	20 (24)	79 (15)	425 (9.8)	0.036 (4.7)	0.25 (2.9)	1.0 (5.3)	7.0 (2.9)
Carbamazepine	5.0 (20)	21 (19)	108 (21)	387 (12)	0.068 (10)	0.32 (14)	1.1 (12)	5.2 (12)
Citalopram	5.6 (20)	21 (19)	118 (21)	496 (12)	0.034 (10)	0.29 (14)	1.1 (10)	7.0 (11)
Clonazepam	3.7 (20)	21 (19)	128 (21)	439 (12)	0.071 (10)	0.34 (14)	1.2 (12)	6.7 (10)
Clozapine	3.9 (18)	*16* (24)	82 (15)	431 (9.8)	0.043 (8.3)	0.30 (2.9)	1.1 (5.3)	6.7 (2.9)
Cocaine	5.2 (20)	22 (19)	141 (20)	508 (10)	0.068 (10)	0.35 (14)	1.2 (11)	7.3 (12)
Codeine	5.2 (20)	23 (26)	123 (21)	558 (18)	0.035 (2.7)	0.28 (2.6)	1.2 (2.6)	6.9 (3.8)
Diazepam	4.2 (11)	23 (11)	111 (11)	481 (8.3)	0.069 (8.9)	0.32 (4.6)	1.1 (6.2)	6.2 (2.3)
Flunitrazepam	6.2 (20)	18 (23)	108 (21)	404 (12)	0.067 (9.1)	0.32 (4.6)	1.0 (12)	5.0 (11)
Hydrocodone	5.2 (18)	28 (25)	148 (20)	473 (13)	0.065 (2.7)	0.27 (2.6)	1.1 (4.8)	6.9 (3.8)
Ketamine	*3.2* (20)	17 (23)	90 (21)	429 (12)	0.061 (9.1)	0.30 (14)	1.1 (12)	5.8 (11)
Lorazepam	*2.4* (10)	20 (8.4)	125 (6.5)	459 (6.2)	---	0.34 (4.6)	1.2 (4.3)	6.9 (4.1)
MDA	4.8 (26)	23 (25)	99 (18)	499 (20)	0.050 (9.9)	0.34 (7.8)	1.1 (5.0)	6.3 (4.8)
MDEA	4.5 (23)	24 (20)	92 (16)	426 (22)	0.065 (4.0)	0.33 (4.7)	1.1 (8.3)	7.2 (6.7)
MDMA	4.8 (26)	22 (25)	91 (18)	472 (20)	0.062 (4.7)	0.33 (6.0)	1.1 (8.3)	7.0 (4.6)
Methadone	3.6 (14)	19 (26)	97 (11)	463 (8.3)	*0.030* (8.9)	0.22 (3.8)	1.2 (6.2)	6.5 (2.3)
Methamphetamine	5.1 (26)	24 (25)	93 (18)	479 (20)	0.052 (4.0)	0.29 (4.7)	1.0 (4.3)	6.6 (4.6)
Metoprolol	4.8 (18)	21 (25)	123 (20)	489 (13)	0.062 (4.6)	0.34 (3.7)	1.2 (4.8)	6.7 (0.8)
Nitrazepam	6.0 (20)	26 (29)	148 (21)	514 (12)	0.067 (10)	0.37 (14)	1.4 (12)	6.5 (12)
Oxazepam	4.0 (13)	*16* (8.6)	119 (6.5)	495 (5.3)	0.069 (6.3)	0.31 (6.5)	1.2 (5.8)	5.5 (3.2)
Oxycodone	5.2 (21)	23 (29)	139 (24)	575 (17)	*0.025* (2.7)	0.27 (2.6)	1.0 (2.6)	7.1 (3.8)
Pethidine	3.7 (20)	18 (19)	86 (20)	453 (10)	0.049 (10)	0.31 (14)	1.2 (11)	7.2 (12)
Phencyclidine	*3.2* (20)	22 (29)	117 (21)	625 (12)	0.043 (10)	0.26 (14)	1.2 (12)	6.7 (12)
Proadifen	3.9 (18)	22 (16)	86 (14)	414 (9.8)	*0.032* (4.3)	0.23 (2.9)	1.2 (5.3)	6.3 (4.6)
Strychnine	*3.2* (18)	21 (13)	93 (20)	437 (13)	*0.015* (7.6)	0.17 (3.7)	1.4 (4.8)	7.0 (0.8)
Temazepam	3.8 (11)	19 (8.6)	121 (11)	517 (6.2)	0.074 (4.3)	0.35 (5.0)	1.2 (5.6)	6.7 (4.1)
Tramadol	3.5 (18)	19 (13)	96 (20)	446 (13)	0.036 (4.6)	0.29 (3.7)	1.1 (4.8)	6.5 (0.8)
Trazodone	4.9 (18)	21 (24)	128 (15)	507 (9.8)	0.057 (8.3)	0.36 (2.9)	1.3 (5.3)	7.1 (2.9)
Verapamil	5.1 (18)	21 (24)	132 (15)	510 (9.8)	*0.026* (8.3)	0.23 (2.9)	1.2 (5.3)	6.4 (2.9)
Zolpidem	4.9 (13)	19 (8.4)	113 (6.5)	487 (6.2)	0.061 (5.7)	0.33 (5.0)	1.3 (5.8)	6.9 (4.1)

As it can be seen from Table 3, the standard deviation was always below 30 % and was lower for hair samples than for blood samples. As a criterion for accuracy, a deviation between of -33 % and + 50 % (recovery between 67 and 150 %) should be acceptable considering the aim of this procedure: a rough estimation of the concentration. This is also fulfilled in the majority of the cases with the exception of some samples spiked with the lowest concentrations 5 ng/ml in blood and 0.05 ng/mg in hair. These deviating results, which always found too low, are printed in italic in Table 3. Furthermore, it is seen from Table 3 that the calculated concentrations from the hair samples spiked with 1.0 ng/mg and with 5.0 ng/mg are always, and from the hair samples spiked with 0.25 ng/mg are in most cases above the spiked values. This one-sided deviation, which is not seen for the blood samples, indicates a systematic error which could not yet been explained.

3.4. Comparison with results previously obtained in real cases by HPLC-DAD and GC-MS

For examination of the procedure under real conditions, blood samples which were previously analysed by HPLC-DAD, and hair samples, which were previously analyzed by GC-MS were re-investigated. Some examples are shown in Table 4. Also for these samples a sufficient agreement between the concentrations was found. Besides methodical errors also differences in sample preparation and decomposition during longer storage time of the samples can be reasons for the differences.

Tab. 4. Comparison of concentrations in blood and hair samples calculated by "Estimate concentration" (LC-QTOF-MS) and previously measured by HPLC-DAD or GC-MS.

Case	Sample	Drug	Concentration, ng/ml or ng/mg	
			LC-QTOF-MS	HPLC-DAD or GC-MS
812/10	Blood	Midazolam	22	20
863/10	Blood	Diazepam	22	15
903/10	Blood	Methadon	465	470
904/10	Blood	Diazepam	46	30
904/10	Blood	Nordazepam	38	170
904/10	Blood	Chlorporothixen	113	50
914/10	Blood	Doxepine	330	700
914/10	Blood	Desmethyldoxepine	214	190
926/10	Blood	Lidocaine	530	640
241/10	Hair	Morphine	3.0	3.9
241/10	Hair	6-Acetylmorphine	4.6	5.9
241/10	Hair	Codeine	0.8	0.86
241/10	Hair	Methadone	4.5	3.2
241/10	Hair	EDDP	0.44	0.47
679/10	Hair	Amphetamine	6.3	7.55
679/10	Hair	MDMA	1.08	0.69
679/10	Hair	Cocaine	0.68	0.38
679/10	Hair	Benzoylecgonine	0.10	0.05

The extracted ion chromatograms obtained from a hair sample of a drug fatality are shown in Fig. 3. Also in this case, a good agreement with previously measured results was found. It can be seen that small peaks (methadone, 0.03 ng/mg) and high peaks (6-acetylmorphine, 2.4 ng/mg) are equally well analyzed by this technique.

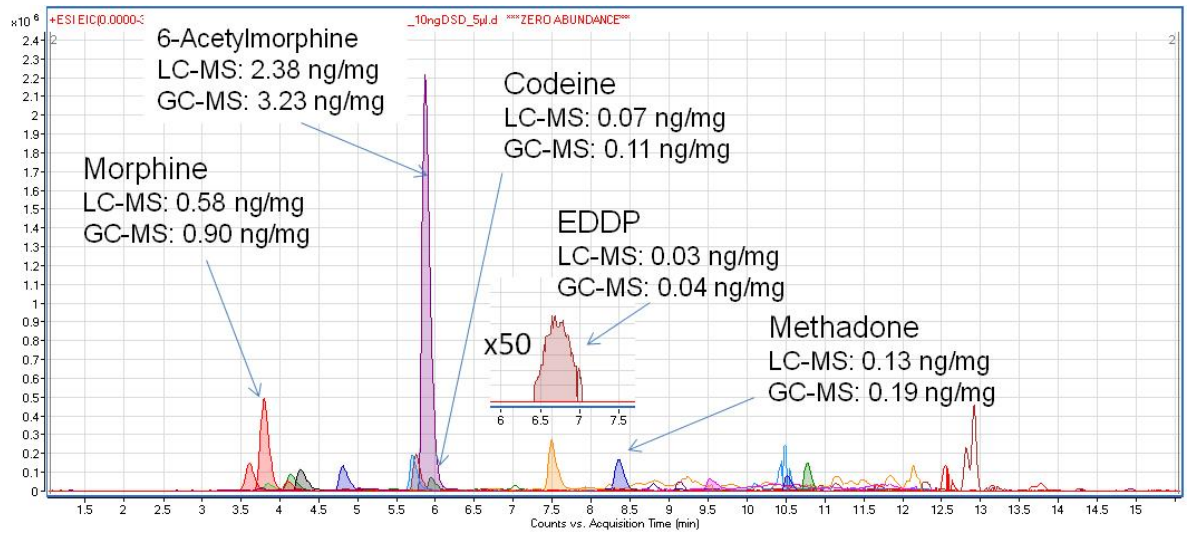

Fig. 3. Extracted ion chromatogram of a hair from a drug fatality sample measured by LC-QTOF-MS. The concentrations were estimated by the tool "Estimate Concentration". For comparison, the concentrations previously measured by GC-MS are given.

4. Conclusion

The systematic toxicological analysis with LC-QTOF-MS described in previous papers by the authors [1-4] was completed by a tool for fast determination of approximate concentrations of the identified substances in a single run. The application to spiked and real blood and hair samples showed a satisfactory agreement with added concentrations or with HPLC-DAD and GC-MS results. The accuracy of the results is sufficient to distinguish between no effect, therapeutic/natural, toxic and comatose-lethal concentrations in blood, e. g. in clinical emergency cases. Further work is in progress to further optimize the procedure and to test its applicability under practical conditions for a larger variety of substances. In particular limitations by chemical structure and matrix effects shall be evaluated more thoroughly. In addition to that, investigations about the applicability of the database LC-TOF-QUANT and the tool "Estimate Concentration" at different instruments are in progress.

5. References

[1] Broecker S, Herre S, Wüst B, Zweigenbaum J, Pragst F. Development and practical application of a CID accurate mass spectra library of more than 2,500 toxic compounds for systematic toxicological analysis by LC-QTOF-MS with data dependent acquisition, Anal. Bioanal. Chem. 400 (2011) 101-117.

[2] Broecker S, Pragst F, Bakdash A, Herre S, Tsokos M. Combined use of liquid chromatography-hybrid quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) and high performance liquid chromatography with photodiode array detector (HPLC-DAD) in systematic toxicological analysis. Forensic Sci. Int. 2011, accepted for publication.

[3] Broecker S, Herre S, Pragst F. General unknown screening in hair by liquid chromatography – hybrid quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). Forensic Sci. Int. 2011, accepted for publication.

[4] Broecker S, Herre S, Pragst F, Kuhlman F, Wüst B, Zweigenbaum J. Toxicological Screening with the Agilent LC/MS-QTOF and the Personal Compound Database and the Broecker, Herre and Pragst Accurate Mass Spectral Library, Agilent Application Note 5990-6419EN Agilent Technologies, Inc., Santa Clara 2010.

[5] Broecker S, Koch M, Pragst F. Detection of metabolites in the general unknown screening of hair by LC-QTOF-MS. Ann. Tox. Anal. 23 (2011) S1-12.

[6] Pragst F, Herzler M, Erxleben B-T. Systematic toxicological analysis by high-performance liquid chromatography with diode array detection (HPLC-DAD), Clin. Chem. Lab. Med. 42 (2004) 1325-1340.

[7] Pragst F, Herzler M, Herre S, Erxleben B-T, Rothe M. UV-Spectra of Toxic Compounds. Database of Photodiode Array UV Spectra of Illegal and Therapeutic Drugs, Pesticides, Ecotoxic Substances and Other Poinsons, Verlag Dieter Helm, Heppenheim 2001; Suppl. Vol. 2007, Edition Toxicological Chemistry, Berlin 2008.

[8] Kruve A, Herodes K, Leito I. Electrospray ionization matrix effect as an uncertainty source in HPLC/ESI-MS pesticide residue analysis. J. AOAC Int. 93 (2010) 306-314..

[9] Souverain S, Rudaz S, Veuthey J.L. Matrix effect in LC-ESI-MS and LC-APCI-MS with off-line and on-line extraction procedures. J. Chromatogr. A 1058 (2004) 61-66.

[10] Wu, J. et al. Study on the matrix effect in the determination of selected pharmaceutical residues in seawater by solid-phase extraction and ultra-high-performance liquid chromatography-electrospray ionization low-energy collision-induced dissociation tandem mass spectrometry. J. Chromatogr. A 1217 (2010) 1471-1475.