Toxicological Screening after the REMEDI™ – Comparison of a GC-MS screening with the REMEDI™

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Abstract

The REMEDI™ will no longer be supported. Therefore, we had to introduce a new procedure for the general unknown screening. We introduced the GC-MS screening procedure published by Maurer et al. (1) and compared its performance with the REMEDI™ for the four different drug classes: antidepressants, antipsychotics, non-opioid analgesics and anticonvulsants.

Half of the urine sample has been hydrolysed by acid hydrolyses and then been combined with the other half. Trimipramine-d3 has been added as internal standard and liquid-liquid extraction was performed with dichloromethane/ isopropanol/ethylacetate. The organic phase was evaporated and the residue derivatized with acetanhydride/pyridine using microwave energy. After evaporation, the residue was dissolved in 50 µl toluene/ethylacetate and injected into a TraceTM GC 2000 coupled to a MD 800 mass spectrometer (ThermoQuest, San José, USA).

With the exception of sertraline, all antidepressants used in Switzerland could be detected with both methods below the concentration usually found in urine after therapeutic use (c_U). The GC-MS procedure had a higher sensitivity for all compounds analysed. Many antipsychotic drugs are only minimally excreted in urine as unchanged drug. Therefore, the detection limit of the parent drug was often much higher than the c_U. The metabolites however could be detected sufficiently. With the exception of amisulpride, sulpiride and tiapride, all antipsychotics had a higher sensitivity with the GC-MS procedure. The non-opioid analgesics and anticonvulsants can only incompletely be detected by the REMEDI™. With the GC-MS procedure all acid drugs of the before mentioned drug classes can only be detected in toxic concentrations. The introduction of a second extraction step using an acidic pH did not improve the sensitivity.

In conclusion, the modified GC-MS screening procedure allows a very complete detection of the antidepressants, antipsychotics, non-opioid analgesics and anticonvulsants. The disadvantage of this new procedure is a turnaround time of about 2 hours.

1. Introduction

Because the REMEDI™ will no longer be supported after 2007, many laboratories including ours had to think about a new procedure for general unknown screening. As the situation today is still in favour for GC-MS for this purpose, we decided to introduce the GC-MS screening procedure published by Maurer et al. [1] in our laboratory and compared its performance in our hands with the REMEDI™ for the drug classes antidepressants, antipsychotics, non-opioid analgesics and anticonvulsants.

2. Methods

The extraction procedure is depicted in Figure 1.

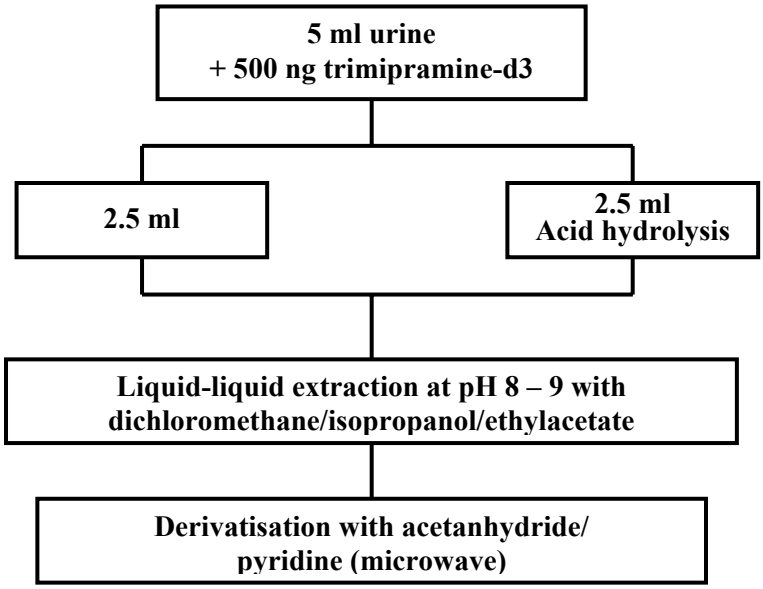

Fig. 1: Extraction method used for GC-MS analysis

The GC-MS conditions were splitless on-column injection on a column having (5% phenyl)-methylpolysiloxane as stationary phase using a TraceTM GC 2000 coupled to a MD 800 mass spectrometer (ThermoQuest, San José, USA). The temperature program started at 90°C with an increase of 30°C/min, the initial time was 3 min, the final time depending on the substances looking for 20 to 60 min.

In order to estimate the concentrations found in urine after the intake of therapeutic doses of the different drugs, the concentration in urine (c_U) was estimated applying the following formula:

$$c_U = \frac{F * D * f_e}{\tau * HMV} \quad [\text{ng/ml}]$$

F: bioavailability; D/t: dose per dose interval (mg/h); f_e: renal fraction; HMV: volume of urine/minute = 2L/24h

3. Results

The results for the different drug classes are depicted in tables 1 to 4.

With the exception of sertraline, all the antidepressants could be detected already in concentrations, which can be encountered in patients who regularly take this drug for therapeutic purposes (Table 1).

Tab. 1: Comparison of the limits of detection for antidepressants (**numbers in bold**: concentration higher than the concentration usually found after therapeutic use)

	c_u (ng/ml)	Limit of detection (ng/ml)	
		GC-MS	REMEDI™
Amitriptyline	600	20	50
Nortriptyline	450	20	> 100
Citalopram	2040	100	> 100
Desmethylcitalopram	-	50	100
Clomipramine	1875	20	> 100
Desmethylclomipramine	-	50	> 100
Dibenzepin	350	20	> 100
Dosulepine	-	50	100
Doxepin	225	100	> 100
Nordoxepin	-	50	> 100
Fluoxetine	150	20	> 100
Norfluoxetine	-	50	> 100
Fluvoxamine	6000	50	> 100
Imipramin	1875	20	> 100
Desipramine	1125	20	> 100
Maprotiline	3188	20	> 100
Melitracen	-	20	> 100
Mianserin	675	20	> 100
Mirtazapine	300	100	> 100
Desmethylmirtazapine	-	20	-
Moclobemide	1500	400	> 400
Nefazodone	400	50	> 100
m-Chlorophenyl-piperazine	-	100	100
Opipramol	1500	400	400
Paroxetin	50	20	> 100
Reboxetine	360	20	> 100
Sertraline	18	**50**	> 100
Desmethylsertralin	-	100	> 100
Tranylcypromine	-	400	400
Trazodone	2000	250	250
Trimipramine	2000	20	200
Venlafaxine	3450	100	> 100
Desmethylvenlafaxine	-	100	100

Of the antipsychotics aripiprazol as well as clozapine, flupenthixol, fluphenazine, fluspirilene, perphenazine, pimozide could not be detected in urine in concentrations which can be expected after therapeutic use of the drug. This is mainly due to the low amount of the drug which is excreted unchanged in urine (Table 2).

Tab. 2: Comparison of the limits of detection for antipsychotics (**numbers in bold**: concentration higher than the concentration usually found after therapeutic use)

	c_u (ng/ml)	Limit of detection (ng/ml)	
		GC-MS	REMEDI™
Amisulpride	6210	> 1000	> 1000
Aripiprazol	87	**>500**	-
Chlorpromazine	825	20	> 250
Chlorprothixene	2000	20	> 250
Clothiapine	-	100	> 250
Clozapine	< 0.1	**250**	> 250
Norclozapine	-	50	> 250
Flupenthixol	< 0.1	**20**	> 250
Fluphenazine	4	**250**	> 250
Fluspirilene	5	**100**	>250
Haloperidol	68	250	**< 250**
N-Desalkyl-haloperidol	-	> 500	> 1000
Reduced haloperidol	-	> 500	> 1000
Levomepromazine	188	20	-
Olanzapine	-	50	> 250
Penfluridol	-	250	-
Perphenazine	20	**250**	> 250
Pimozide	14	**250**	> 250
Pipamperone	-	> 250	< 250
Promazine	-	20	> 250
Quetiapine	203	20	> 250
Risperidone	84	> 500	100
9-Hydroxy-Risperidone	-	> 500	50
Sulpiride	114000	> 500	< 250
Thioridazine	-	20	> 250
Tiapride	153000	> 1000	> 1000
Zuclopenthixol	8	**50**	> 250

The results for the analgesics are depicted in Table 2. Acidic drugs are not so well extracted with the procedure described; therefore the addition of a second extraction step using an acidic pH was tested without success. In addition, they can hardly be separated without derivatization. If the injector of the GC-MS instrument is heated and the solvent for the residue is methanol methylation of the acidic analgesics can take place during the injection process. As our instrument is equipped with a cold-on column injector, this process can not occur. Between the Mosbach meeting and the preparation of this manuscript we changed the sample preparation procedure and split the sample before derivatization. Half of the hydrolysed and extracted urine is acetylated, the other half silylated, respectively. As these acidic analgesic drugs are easily silylated, they can now be detected with a much higher sensitivity (data not shown).

Tab. 3: Comparison of the limits of detection for analgesics (**numbers in bold**: concentration higher than the concentration usually found after therapeutic use)

	c_u(ng/ml)	Limit of detection (ng/ml)	
		GC-MS	REMEDI™
Acetylsalicylic acid	> 1630	**100'000**	-
Aminophenazone	15000	2000	-
Celecoxib	1365	250	-
Diclofenac	2600	100	-
Ibuprofen	> 3000	500	-
Mefenamic acid	> 26'500	**100'000**	-
Meloxicam	50	**500**	-
Metamizol	< 0.1	**100**	-
Nabumetone	< 0.1	**100**	> 250
Nefopam	1013	20	> 250
Oxyphenbutazone	-	100	-
Paracetamol	29250	500	-
Phenazone	45000	250	-
Phenylbutazone	1350	20	-
Piroxicam	338	**> 2000**	-
Propyphenazone	-	20	> 250
Valdecoxib	-	500	-

Tab. 4: Comparison of the limits of detection for antiepileptics (**numbers in bold**: concentration higher than the concentration usually found after therapeutic use)

	c_u (µg/ml)	Limit of detection (ng/ml)	
		GC-MS	REMEDI™
Carbamazepine	> 2.7	0.1	-
Clomethiazole	> 0.96	1.0	-
Clonazepam	> 0.02	**1.0**	2.0
7-Aminoclonazepam	-	0.5	-
Diazepam	> 0.02	**0.1**	1.0
Nordazepam	-	1.0	-
Oxazepam	-	0.5	-
Temazepam	-	0.2	-
Ethosuximide	> 33.8	0.5	-
Gabapentin	> 192	200	-
Lamotrigine	> 2.05	**10.0**	-
Levetiracetam	> 330	10.0	-
Phenobarbital	> 15.8	0.2	-
Phenytoin	> 9.0	0.5	-
Primidone	> 30.0	1.0	2.0
Topiramate	> 6.7	10.0	-
Valproic acid	> 16.9	20.0	-
Vigabatrin	> 246	-	-

With the exception of clonazepam, diazepam and lamotrigine all antiepileptic drugs could be detected in concentrations which can be usually found after therapeutic intake of the drug (Table 4).

4. Conclusions

The GC-MS procedure allows a very complete detection of the antidepressants, antipsychotics, non-opioid analgesics and antiepileptics in urine after intoxication. Much more drugs can be identified with that new procedure as compared with the REMEDI™ method, which was used before. The disadvantages of the new method are the more laborious sample preparation and the longer turn-around time of about 2 hours per sample in our hands.

5. Literature

[1] Maurer, HH & Bickeboeller-Friedrich, J (2000) Screening procedure for detection of antidepressants of the selective serotonin reuptake inhibitor type and their metabolites in urine as part of a modified systematic toxicological analysis procedure using gas chromatography-mass spectrometry. J Anal Toxicol 24: 340-7

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