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Automated Mass Spectral Deconvolution and Identification System for GC-MS Screening for Drugs, Poisons, and Metabolites in Urine

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BACKGROUND: The challenge in systematic toxicological analysis using gas chromatography and/or liquid chromatography coupled to mass spectrometry is to identify compounds of interest from background noise. The large amount of spectral information collected in one full-scan MS run demands the use of automated evaluation of recorded data files. We evaluated the applicability of the freeware deconvolution software AMDIS (Automated Mass Spectral Deconvolution and Identification System) for GC-MS–based systematic toxicological analysis in urine for increasing the speed of evaluation and automating the daily routine workload.

METHODS: We prepared a set of 111 urine samples for GC-MS analysis by acidic hydrolysis, liquid-liquid extraction, and acetylation. After analysis, the resulting data files were evaluated manually by an experienced toxicologist and automatically using AMDIS with deconvolution and identification settings previously optimized for this type of analysis. The results by manual and AMDIS evaluation were then compared.

RESULTS: The deconvolution settings for the AMDIS evaluation were successfully optimized to obtain the highest possible number of components. Identification settings were evaluated and chosen for a compromise between most identified targets and general number of hits. With the use of these optimized settings, AMDIS-based data analysis was comparable or even superior to manual evaluation and reduced by half the overall analysis time.

CONCLUSIONS: AMDIS proved to be a reliable and powerful tool for daily routine and emergency toxicology. Nevertheless, AMDIS can identify only targets present in the user-defined target library and may therefore not indicate unknown compounds that might be relevant in clinical and forensic toxicology.

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Systematic toxicological analysis (STA)[3] of drugs in biological specimens is important in clinical and forensic toxicology, workplace drug testing, and doping control *(1–5)*. Urine is the most widely used matrix for STA because comparatively large volumes can easily be collected noninvasively and because drugs and metabolites are concentrated in urine *(1, 6)*.

Analytical methods for STA should ideally cover hundreds of relevant drugs, poisons, and metabolites *(1, 2, 7, 8)*. This broad coverage can be achieved by use of GC-MS, HPLC with ultraviolet or diode-array detection, or HPLC coupled with single-stage LC-MS or LC–tandem MS *(1, 3, 5, 9–14)*. Although LC–tandem MS has become increasingly important in recent years *(9, 10)*, in many laboratories routine STA is still performed by use of HPLC with ultraviolet or diode-array detection and GC-MS. GC-MS is still the gold standard for STA in urine samples *(1, 15–17)*, combining the separation power of GC with the high selectivity of electron ionization MS.

A disadvantage of GC-MS–based STA is that the evaluation of full-scan GC-MS data requires a high level of expertise and experience. A major challenge is the detection of analyte peaks in the total ion chromatograms (TIC), where they are often overlapped by more or less intense matrix peaks. One solution is the use of user-defined macros *(2)*, which extract characteristic fragment ions from the total ion current, thus indicating the possible presence of the respective drugs and/or their metabolites. However, macro-based data evaluation is rather time-consuming and analytes not covered by the extracted ions may be overlooked.

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[3] Nonstandard abbreviations: STA, systematic toxicological analysis; TIC, total ion chromatograms; AMDIS, Automated Mass Spectral Deconvolution and Identification System; HP, Hewlett Packard; MMF, minimum match factor; RI, retention index.

Another option is the use of so-called deconvolution algorithms that extract pure compound peaks more or less free of overlapping signals from complex TICs. One of the software solutions based on this principle is the freeware program called AMDIS (Automated Mass Spectral Deconvolution and Identification System; http://chemdata.nist.gov/mass-spc/amdis/). AMDIS first deconvolutes pure component spectra and related information such as peak shape and retention time from complex chromatograms and subsequently matches the obtained spectra with those of a reference library, the so-called target library. AMDIS was originally developed in 1996 for the automated identification of chemical weapons and related compounds, but should be applicable to any method requiring extraction of mass spectra from noisy TIC and the identification of target compounds by full-spectrum matching *(18)*. So far, AMDIS has mainly been used in environmental chemistry *(19–24)*. Only 2 reports have described AMDIS use in the context of clinical or forensic toxicology *(25, 26)*. The methods described in these reports are limited to blood analysis and are focused on a small number of analytes used with small in-house libraries.

Our aim in this study was to evaluate the applicability of AMDIS for automated evaluation data files from routine GC-MS–based STA in urine by using a modified target library version of the Maurer/Pfleger/Weber MPW_2007 *(27)*. The workup used was found to be the method of choice for STA in clinical toxicology owing to its wide analyte spectrum and short workup time *(28)*. The study included optimization of the settings for deconvolution and library search and a systematic comparison of the AMDIS search results with results obtained by an experienced toxicologist using manual macro-assisted data evaluation.

Materials and Methods

CHEMICALS AND REAGENTS

Acetic anhydride, ammonium sulfate, pyridine, and disodium hydrogen phosphate ($Na_2HPO_4 \cdot 2\ H_2O$) were obtained from Fluka. Sodium hydroxide pellets were obtained from Riedel-de Haen. All other chemicals were obtained from Merck. All chemicals were of analytical grade.

URINE SAMPLES

The studied urine samples were submitted to the authors' laboratory for STA. They were collected from a total number of 111 consecutive patients presenting with suspected intoxication/poisoning at the emergency department of the University of Mainz over a 1-year period *(29)*. Any required institutional review board demands for use of the submitted samples were fulfilled.

SAMPLE PREPARATION FOR SYSTEMATIC TOXICOLOGICAL ANALYSIS

Urine samples were prepared as described previously *(29, 30)*. Briefly, the samples (5 mL) were divided into 2 aliquots (2.5 mL each), one of which was subjected to acid hydrolysis. Thereafter, the sample was adjusted to pH 8–9 with 2 mL of 10 mol/L aqueous sodium hydroxide and the other aliquot of untreated urine was added. This mixture was extracted with 5 mL dichloromethane-isopropanol-ethyl acetate (1:1:3 vol/vol/vol), and the organic layer was evaporated to dryness. The residue was acetylated with 100 μL of an acetic anhydride-pyridine mixture (3:2 vol/vol) under microwave irradiation. After evaporation of the derivatization mixture, the residue was dissolved in 100 μL of methanol and 2 μL was injected into the GC-MS system.

GC-MS APPARATUS

The GC-MS settings for the STA analyses were used as described by Maurer et al. *(30)*. We used a Hewlett Packard (HP; Agilent) 5890 series II gas chromatograph combined with an HP 5972A MSD mass spectrometer. An HP MS ChemStation (DOS series) was used with HP G1034C software version C03.00. The GC conditions were as follows: splitless injection mode; column, HP-1 capillary (12 m × 0.2 mm i.d.); cross-linked methyl silicone, film thickness 330 nm; injection port temperature 280 °C; helium carrier gas flow-rate 1 mL/min; column temperature programmed from 100–310 °C at 30°/min, initial time 3 min, final time 8 min. The MS conditions were as follows: electron ionization mode, ionization energy 70 eV, ion source temperature 220 °C, capillary direct interface 280 °C; full-scan mode *m/z* 50–550, 1 scan/s.

DATA ANALYSIS

Manual evaluation. The full-scan data files acquired by the GC-MS system were screened for the presence of peaks and mass spectra of (derivatized) drugs, metabolites, and artifacts by use of the Standalone Data Analysis feature of the HP Chem Station software. A first manual screen of the TIC by an experienced toxicologist was followed by screening for specific drug classes employing previously described user-defined macros *(1, 2, 30)* for the following drug classes: psychotropics, barbiturates, benzodiazepines, stimulants/hallucinogens, opioids, analgesics, anticonvulsants, antidepressants, butyrophenone neuroleptics, cardiovascular drugs, sedative hypnotics, and phenothiazine neuroleptics *(1, 2, 30)*. Identification was achieved by

computer-assisted comparison of the peak underlying mass spectra with those of the Maurer/Pfleger/Weber MPW_2007 mass spectral library *(27)*.

AMDIS evaluation. The full-scan data files acquired by the GC-MS system were analyzed by AMDIS (http://chemdata.nist.gov/mass-spc/amdis/) in simple mode. Library matches were visually inspected and verified by an experienced toxicologist before the respective compounds were reported. The final decision concerning the declaration of a library match to be a "true hit" was always done by a toxicologist on the basis of *m/z* correlations and their respective abundance. We generated an AMDIS-readable library using the Lib2NIST converter software version 1.0.0.13 included in the NIST MS-Search software version 2.0a. This converter can generate AMDIS-readable libraries (*.MSP file format, renamed to *.MSL) from the following database formats: HP (*.L), plain text (*.SDF), NIST, and JCAMP-DX (*.JDX; *.DX; *.HPJ; *.JX; *.JC; *.JCM). The used target library was a modified version of the Maurer/Pfleger/Weber MPW_2007 library *(27)*, from which all mass spectra of silylated and perfluoroacylated compounds had been eliminated by use of the "build one library" option contained in the AMDIS main program. The final settings of the deconvolution and search parameters were derived from the results of a series of optimization experiments. In the first experiment, we investigated the influence of the deconvolution settings on the number of targets proposed by the software. All 111 MS data files were deconvoluted under variation of single parameters while all others were held constant. The parameter settings used were as follows: width, 4, 8, 12, 16, 20, 24, 28, 32; adjacent peak subtraction, 0, 1, 2; resolution, low, medium, high; sensitivity, very low, low, medium, high, very high; and shape requirements, low, medium, high. Settings leading to a maximum number of targets proposed by the software were considered optimal.

In the second experiment, we performed deconvolution with the optimized settings derived from the first experiment, and varied the minimum match factor (MMF) (40, 50, 60, or 70) to find an optimal setting, avoiding false-negative findings, i.e., targets present in urine but not detected by AMDIS, while limiting the number of false-positive hits, i.e., targets proposed by the software but not present in the respective samples.

In the third experiment, we used the analysis type "Use Internal Standard for RI" with various internal standards. The 70 urine samples for which sufficient volumes were left after the first analysis were spiked with 100 μL of a mixture of the internal standard: nomifensine (0.5 g/L), p-tolylpiperazine (0.1 g/L), and cyproheptadiene (0.1 g/L) and extracted again as described above. The following compounds were also used as an internal standard for AMDIS analysis if present in the samples: caffeine, nicotine, and diisooctylphthalate. The setting for retention index (RI)-aided analysis was as follows: RI window 100 + 0, level infinite, and maximum penalty 30.

We performed all statistical evaluations using Graphpad Prism 3.02 software.

AMDIS VS MANUAL EVALUATION

Results obtained by AMDIS with the optimized deconvolution and search settings were compared to the results achieved after manual evaluation. Both data evaluation methods were performed independently of each other. Again, the final decision concerning the declaration of a library match to be a "true hit" was always made by a toxicologist on the basis of *m/z* correlation and respective abundance.

Results

The influence of the deconvolution parameters resolution and sensitivity on the number of targets proposed by the software is shown in Fig. 1. The optimized deconvolution and identification parameters were as follows: width, 32; adjacent peak subtraction, 2; sensitivity, very high; resolution, high; shape requirement, low. These parameters were used in all further experiments.

With respect to the MMF, a setting of 50 was found to be the best compromise between true hits (targets proposed by the software present in urine) and false hits (targets proposed by the software not present in urine). Moreover, at this setting only one false-negative finding was observed. A lower MMF considerably increased the number of false hits, whereas at higher MMF the number of false-negative findings increased. When a higher MMF was used some compounds remained undetected. This can be seen in Table 1, which lists the results obtained with MMF 50 and MMF 60 for the 24 samples in which fewer true hits were detected at MMF 60. Only the names of the parent drugs are listed, although in several cases their metabolites and/or artifacts have been detected. This can be verified via the given entry number in the used MPW_2007 library *(27)*.

The software allowed us to reduce the hits by 16–96 (depending on the amount of identified compounds in the respective sample) with the so-called "Use internal standard for RI" mode. This mode applies penalties on the match factor for potential hits with an RI considerably different from the RI of the respective reference compound. With this procedure, however, overall 36 true hits were also eliminated from the 70 samples from the target list.

Comparison of the results obtained with the optimized AMDIS settings to those obtained by manual

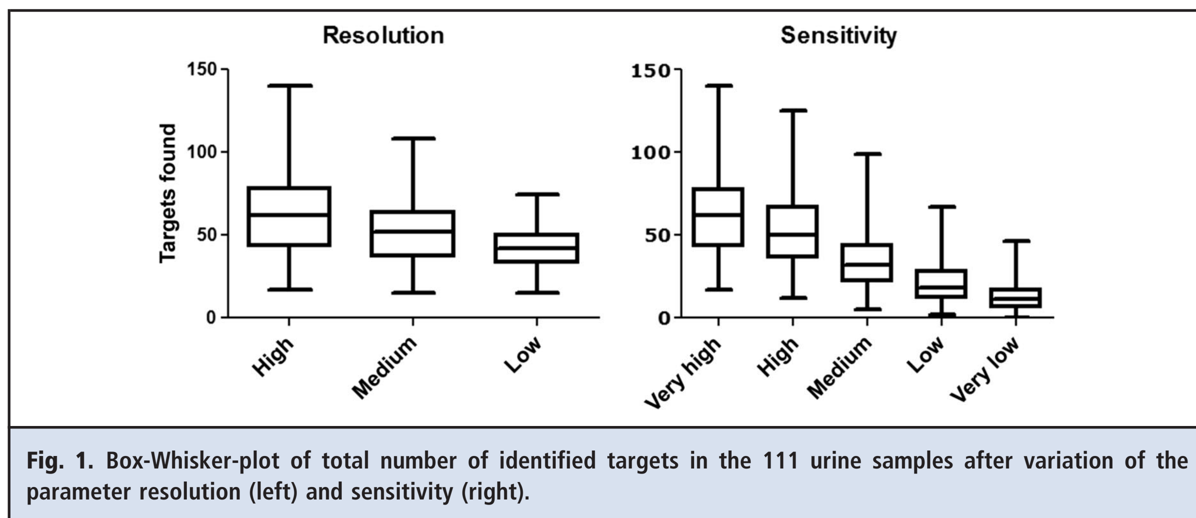

Fig. 1. Box-Whisker-plot of total number of identified targets in the 111 urine samples after variation of the parameter resolution (left) and sensitivity (right).

macroassisted data evaluation by an experienced toxicologist generally showed good agreement between both evaluation procedures. In only 1 sample was a true hit, namely phenobarbital, detected by manual evaluation that was not detected by AMDIS. In 15 samples, additional true targets were identified by AMDIS that had not been detected by manual data evaluation. These findings are listed in Table 2. Most of these results were related to minor (metabolite) peaks, and none of these findings was relevant from an emergency toxicology perspective. Such findings could be relevant in forensic cases, however. The time required for AMDIS-based data evaluation was approximately 5–8 min, less than half the time required for manual macroassisted data evaluation (15–20 min).

Discussion

Our aim was to evaluate the applicability of AMDIS for automated evaluation data files from routine GC-MS–based STA in urine using a modified target library version of the MPW_2007 library *(27)* and, if applicable, to establish AMDIS in routine data evaluation *(29)*. In this library version, silylated and perfluoroacylated compounds were eliminated because they are not expected in acetylated urine extracts, and a smaller number of reference spectra increases the speed of deconvolution/matching while reducing the number of proposed hits.

On the basis of a scan rate of 1 scan per second, compound peaks were formed by 4 to 100 scans depending on the peak form (e.g., very small and sharp peaks for haloperidol or very broad for acetaminophen).

In a first step, the deconvolution settings were optimized. The settings leading to a maximum of number of detected components were considered optimum, because detection of a compound in the deconvolution step is a prerequisite for matching the mass spectrum of the respective component with those of the target library. In other words, this strategy was chosen to avoid false-negative findings. The strongest influence on the number of detected compounds, and therefore on the number of identified targets, is attributable to the parameters sensitivity and shape, whereas the influence of resolution and width is of minor relevance (see Fig. 1). The adjacent peak subtraction has no influence on the number of identified components in the MS data file, because this parameter is responsible only for the purity of the achieved spectrum.

With the above-mentioned optimized deconvolution settings, only a single small peak of phenobarbital found manually by an experienced toxicologist was not detected by AMDIS. This finding demonstrates that the probability of overlooking relevant peaks with these settings is very low. However, a disadvantage of optimizing deconvolution settings for detection of a maximum number of components is that even small matrix peaks are also detected, increasing the number of proposed target hits.

It is therefore essential to choose an appropriate MMF so that false-positive hits, caused by the more or less extensive similarity of their respective mass spectra with a target compound spectrum, are eliminated while none of the true positives are missed because of a too-high MMF. In the present study, an MMF of 50 was found to be the best compromise for limiting the number of proposed target compounds without overlooking true positives.

Lowering the MMF to 40 mainly increased the number of false-positive hits. Experience during routine analysis, however, has shown that evaluation (or

Table 1. Compounds identified in the corresponding urine samples using AMDIS via the given Maurer/Pfleger/Weber (MPW) library entry numbers with MMF of 50 or 60.[a]

Sample no.	Identified compounds with MMF 50	MPW library entry number of compounds identified as target				Identified compounds with MMF 60	MPW library entry number of compounds identified as target			
4	Caffeine	191				Caffeine	191			
	Tilidin	**259**	**260**							
12	Amphetamine	3240	55, 54	5515	1803	Amphetamine	55	5515	1804	
	Nicotine	1150	692			Nicotine	1150	692		
	Caffeine	**191**								
16	Propofol	3305	3521			Propofol	3305	3521		
	Norephedrine	2476				Norephedrine	2476			
	Lidocaine	1064				Lidocaine	1064			
	Methadone	242				Methadone	242			
	Naloxone	**2982**								
19	Nicotine	1150				Nicotine	1150			
	Lidocaine	57	2585	1061		Lidocaine	57	2585	1061	
	Paracetamol	825	188	2383		Paracetamol	825	188	2383	
	Caffeine	191				Caffeine	191			
	Oxazepam	273				Oxazepam	273			
	Diazepam	272				Diazepam	272			
	Morphine	525	225			Morphine	525	225		
	Codeine	224				Codeine	224			
	Papaverin	3688	3685			Papaverin	3688	3685		
	Noscapine	2525				Noscapine	2525			
	Midazolam	**294**								
24	Caffeine	191				Caffeine	191			
	Lidocaine	2585	1061			Lidocaine	2585	1061		
	Propyphenazone	203	205	208	905	Propyphenazone	203	205	208	905
	Promethazine	381	382	383	384	Promethazine	383	384		
	Metamizol	183	220			Metamizol	183	220	184	
	Metoclopramide	**1126**	**1125**			Midazolam	296			
	Midazolam	296				Phenazone	190			
	Phenazone	190								
29	Protriptyline	613								
43	Caffeine	191				Caffeine	191			
	Quinine	**688**	**3745**							
55	Nicotine	1150	692			Nicotine	1150	692		
	Lorazepam	**289**	**290**			Temazepam	5780	418	2099	5779
	Temazepam	5780	418	2099	5779	Morphine	225			
	Morphine	225				Olanzapine	4675	4676	4677	
	Olanzapine	4675	4676	4677						
57	Valproic acid	1019	4670			Valproic acid	1019	4670		
	Propofol	3305				Propofol	3305			
	Nicotine	692				Nicotine	692			
	Lidocaine	6784	1061	1066		Lidocaine	6784	1061	1066	
	Caffeine	191				Caffeine	191			

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Table 1. Compounds identified in the corresponding urine samples using AMDIS via the given Maurer/Pfleger/Weber (MPW) library entry numbers with MMF of 50 or 60.[a] *(Continued from page 579)*

Sample no.	Identified compounds with MMF 50	MPW library entry number of compounds identified as target				Identified compounds with MMF 60	MPW library entry number of compounds identified as target			
	Metoprolol	1183				Metoprolol	1183			
	Midazolam	**296**	**294**	**295**						
58	**Amphetamine**	**55**				Caffeine	191			
	Caffeine	191								
67	Caffeine	191				Caffeine	191			
	Venlafaxine	**5269**				Prothipendyl	385	388		
	Prothipendyl	385	386	388	389					
71	Caffeine	191				Caffeine	191			
	Methadone	242	241			Methadone	242			
	Oxazepam	**273**				Doxepin	332	337		
	Doxepin	64	58	337	31	Sertraline	4685			
	Sertraline	4685								
90	**Paracetamol**	**825**				Lidocaine	1064	57		
	Lidocaine	1064	57	725	1066	Diclofenac	716		2321	1212
	Diclofenac	716	6467	2321	1212					
91	Nicotine	1150	692			Nicotine	1150	692		
	Cocaine	3574	472	465	6252	Cocaine	3574	472	465	2120
	Paracetamol	**825**	**2383**			Diphenhydramine	1241	731	2079	1622
	Diphenhydramine	1241	731	2079	1622	Zopiclone	6556	7801	5317	5314
	Zopiclone	6556	7801	5317	5314	Chlorphenamine	2040			
	Chlorphenamine	2040								
92	Ibuprofen	3380	1941	3382		Ibuprofen	3380	1941	3382	
	Valproic acid	**1019**				Caffeine	191			
	Acebutolol	**1564**	**1568**							
	Caffeine	191								
	Lidocaine	**1065**								
	Flunitrazepam	**284**								
	Propyphenazone	**202**								
94	Acetylsalicylic acid	2637				Acetylsalicylic acid	2637			
	Lidocaine	2585	57			Lidocaine	2585			
	Paracetamol	188	201	2383	825	Paracetamol	188	201	2383	825
	Diphenhydramine	**1626**								
97	**Propofol**	**3305**				Lidocaine	2585	1064	1061	
	Lidocaine	2585	1064	1061		Midazolam	296			
	Midazolam	296				Metronidazole	1831			
	Dobutamine	2981	2484	3531		Dobutamine	2981	2484	3531	
	Metronidazole	1831								
	Atropine	**71**								
100	Salicylic acid	954				Salicylic acid	954			
	Nicotine	1150				Nicotine	1150			
	Paracetamol	**2383**				Caffeine	191			
	Caffeine	191				Lidocaine	1064			

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Table 1. Compounds identified in the corresponding urine samples using AMDIS via the given Maurer/Pfleger/Weber (MPW) library entry numbers with MMF of 50 or 60.[a] *(Continued from page 580)*

Sample no.	Identified compounds with MMF 50	MPW library entry number of compounds identified as target				Identified compounds with MMF 60	MPW library entry number of compounds identified as target			
	Lidocaine	1064								
	Propyphenazone	**1882**								
	Flurpirtine	**1814**								
101	Caffeine	191				Caffeine	191			
	Acebutolol	**1564**	**1568**			Bisacodyl	106	1750	2456	
	Mephenytoin	**4191**								
	Bisacodyl	106	1750	2456						
102	Diphenhydramine	2047	1241	2079	1622	Diphenhydramine	2047	2079	2078	735
	Caffeine	191				Bisoprolol	2791			
	Lidocaine	2585	1061			Etilefrine	768			
	Etilefrine	768				Midazolam	296			
	Trimipramine	410	412	6329	640	Lidocaine	2585	1061		
	Desipramine	**2295**	**1218**	**2292**		Pipamperone	598	5586	179	
	Pipamperone	598	5586	179	599	Trimipramine	410	412	6329	640
	Dobutamine	**2980**				Caffeine	191			
	Bisoprolol	2791				Cafedrine	1739			
	Midazolam	296								
	Mepivacaine	**1085**								
	Cafedrine	1739								
104	Nicotine	1150				Nicotine	1150			
	Lidocaine	57	2585	1061		Lidocaine	57	2585	1061	
	Caffeine	191				Caffeine	191			
	Prilocaine	**1216**				Pipamperone	598	5586	179	599
	Pipamperone	598	5586	179	599	Bisacodyl	106	1750		
	Mepivacaine	**1085**								
	Bisacodyl	2459	106	1750	2456					
	Biperiden	**103**								
109	Nicotine	1150	692			Nicotine	1150	692		
	Carbamazepine	421	309	422	2671	Carbamazepine	421	309	422	2671
	Enalapril	**4736**				Levomepromazine	344	345	6415	347
	Levomepromazine	344	345	346	6415					
	Haloperidol	**182**								
110	Tramadol	4441	4436	4435	4438	Tramadol	4441	4436	4435	4438
	Alprenolol	**1571**				Caffeine	191			
	Caffeine	191				Doxylamine	740	746	2690	
	Doxylamine	740	742	2688	2689	Tilidine	259			
	Tilidine	259								
111	**Paracetamol**	**825**				Caffeine	191			
	Caffeine	191				Lidocaine	2585	1064	1061	
	Lidocaine	2585	1064	1061		Midazolam	294	296		
	Midazolam	294	296							

[a] Compounds in bold identified only with MMF 50.

Table 2. Compounds identified in the corresponding urine samples exclusively using AMDIS via the given Maurer/Pfleger/Weber (MPW) library entry numbers and a minimum match factor of 50.

Sample no.	Substance identified in urine	Entry no.	Name of detected compound
36	Prothipendyl	2275	Prothipendyl-M (HO-ring) AC[a]
		2618	Phenothiazine-M 2AC
41	Fluoxetine	4338	Fluoxetine-M (nor-) AC
		4278	Fluoxetine AC
	Bromazepam	129	Bromazepam HYAC
	Maprotiline	352	Maprotiline-M (HO-ethanediyl-) 2AC
		349	Maprotiline AC
50	Caffeine	191	Caffeine
	Ketamine	1050	Ketamine
	Bisacodyl	106	Bisacodyl
		1750	Bisacodyl-M (methoxy-bis-deacetyl-) 2AC
58	Paracetamol	825	Paracetamol
		188	Paracetamol AC
	Diazepam	272	Diazepam HY
60	Amphetamine	55	Amphetamine AC
68	Venlafaxine	5269	Venlafaxine-M (O-demethyl-) AC
69	Prothipendyl	387	Prothipendyl-M (bis-nor-) AC
		1883	Prothipendyl-M (bis-nor-HO-) 2AC
76	Sertraline	4642	Sertraline-M (nor-) AC
		4640	Sertraline AC
		4685	Sertraline-M (di-HO-ketone)–H2O enol 2AC
81	Metformine	6510	Metformine artifact AC
	Dobutamine	1273	Dobutamine-M (N-dealkyl-O-methyl-) 2AC
		2980	Dobutamine-M (N-dealkyl-O-methyl-) AC
83	Midazolam	296	Midazolam-M (HO-) AC
90	Maprotiline	348	Maprotiline-M (nor-) AC
		352	Maprotiline-M (HO-ethanediyl-) 2AC
		349	Maprotiline AC

[a] AC, acetylated; HY, artifact formed during acid hydrolysis, M, metabolite.

reevaluation) of the entire TIC or parts of with a lower MMF can be reasonable if identification of even small peaks is important, e.g., in drug-facilitated crime cases or abstinence control. Using an MMF of 60 or higher was associated with a considerable risk of overlooking relevant analytes in the sample (Table 1), unless the analytes were present in fairly high concentrations as, for example, in poisoning cases.

Including the RI of the detected components in the search algorithm decreased the overall number of proposed hits, but was associated with a considerable risk of eliminating true-positive hits. This finding might be attributable to variations of the used RIs of the compounds contained in the AMDIS target library *(31)* and by the poor peak shape in some poisoning cases. Because the calculated RI does not depend on the internal standard used but instead the number of internal standards used to calculate the relative retention time, the choice of internal standard does not seem to be critical. In the authors' opinion, the reduction of proposed targets does not justify the risk of overlooking relevant analytes, as observed in the present study.

After we established the final AMDIS settings, we compared the results obtained with these settings to those obtained by manual macro-assisted data evaluation by an experienced toxicologist. The discrepancies are most likely attributable to the fact that the respective peaks had a very low abundance and that only a

1-sided background subtract was possible with the ChemStation software used for manual data evaluation. Hence, it was very difficult to obtain a clean mass spectrum, that is, one not overlaid by matrix spectra. The deconvolution algorithm of AMDIS allows a 2-sided background subtraction, resulting in much cleaner mass spectra that in turn lead to better higher-match factors.

In summary, AMDIS proved to be a reliable and powerful tool for daily routine and emergency toxicology. The major advantages of using AMDIS are the better identification of even low-abundant peaks in the TIC and the reduction of the evaluation time by half. The decreased evaluation time is of particular relevance in clinical emergency toxicology where the speed of analysis is important. Results obtained by AMDIS-based data evaluation are comparable or superior to results obtained by manual data evaluation. Nevertheless, it must be stated that AMDIS can identify only targets present in the user-defined target library and may therefore not indicate unknown compounds that might be relevant in forensic and clinical toxicology. The results obtained by AMDIS-based data evaluation still require visual inspection and verification by a toxicologist with experience in MS-based compound identification.

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References

1. Maurer HH. Position of chromatographic techniques in screening for detection of drugs or poisons in clinical and forensic toxicology and/or doping control [Review]. Clin Chem Lab Med 2004;42:1310–24.
2. Maurer HH. Forensic screening with GC-MS. In: Bogusz M, ed. Handbook of analytical separation sciences: forensic science. 2nd ed. Amsterdam: Elsevier Science; 2007. p 429–49.
3. Maurer HH. Hyphenated mass spectrometric techniques: indispensable tools in clinical and forensic toxicology and in doping control [Review]. J Mass Spectrom 2006;41:1399–413.
4. Maurer HH, Peters FT. Analyte identification using library searching in GC-MS and LC-MS. In: Gross M, Caprioli RM, eds. Encyclopedia of mass spectrometry. Oxford: Elsevier Science, 2006. p 115–21.
5. Maurer HH. Systematic toxicological analysis procedures for acidic drugs and/or metabolites relevant to clinical and forensic toxicology or doping control [Review]. J Chromatogr B Biomed Sci Appl 1999;733:3–25.
6. Hammett-Stabler CA, Pesce AJ, Cannon DJ. Urine drug screening in the medical setting. Clin Chim Acta 2002;315:125–35.
7. Wu AH, McKay C, Broussard LA, Hoffman RS, Kwong TC, Moyer TP, et al. National Academy of Clinical Biochemistry laboratory medicine practice guidelines: recommendations for the use of laboratory tests to support poisoned patients who present to the emergency department. Clin Chem 2003;49:357–79.
8. Skelton H, Dann LM, Ong RT, Hamilton T, Ilett KF. Drug screening of patients who deliberately harm themselves admitted to the emergency department. Ther Drug Monit 1998;20:98–103.
9. Maurer HH. Mass spectrometric approaches in impaired driving toxicology [Review]. Anal Bioanal Chem 2009;393:97–107.
10. Maurer HH. Current role of liquid chromatography-mass spectrometry in clinical and forensic toxicology [Review]. Anal Bioanal Chem 2007;388:1315–25.
11. Pragst F, Herzler M, Erxleben BT. Systematic toxicological analysis by high-performance liquid chromatography with diode array detection (HPLC-DAD). Clin Chem Lab Med 2004;42:1325–40.
12. Sadeg N, Francois G, Petit B, Dutertre-Catella H, Dumontet M. Automated liquid-chromatographic analyzer used for toxicology screening in a general hospital: 12 months' experience. Clin Chem 1997;43:498–504.
13. Fabbri A, Marchesini G, Morselli-Labate AM, Ruggeri S, Fallani M, Melandri R, et al. Comprehensive drug screening in decision making of patients attending the emergency department for suspected drug overdose. Emerg Med J 2003;20:25–8.
14. Fabbri A, Ruggeri S, Marchesini G, Vandelli A. A combined HPLC-immunoenzymatic comprehensive screening for suspected drug poisoning in the emergency department. Emerg Med J 2004;21:317–22.
15. Drummer OH. Chromatographic screening techniques in systematic toxicological analysis [Review]. J Chromatogr B 1999;733:27–45.
16. Maurer HH. Screening procedures for simultaneous detection of several drug classes used in the high throughput toxicological analysis and doping control [Review]. Comb Chem High Throughput Screen 2000;3:461–74.
17. Segura J, Ventura R, Jurado C. Derivatization procedures for gas chromatographic-mass spectrometric determination of xenobiotics in biological samples, with special attention to drugs of abuse and doping agents [Review]. J Chromatogr B 1998;713:61–90.
18. Stein SE. An integrated method for spectrum extraction and compound identification from gas chromatography/mass spectrometry data. J Am Soc Mass Spectrom 1999;10:770–81.
19. Dagan S. Comparison of gas chromatography-pulsed flame photometric detection-mass spectrometry, automated mass spectral deconvolution and identification system and gas chromatography-tandem mass spectrometry as tools for trace level detection and identification. J Chromatogr A 2000;868:229–47.
20. Finck Y, Aydin N, Pellaton C, Gorin G, Gulacar F. Combination of gas chromatography-mass spectrometry and mass spectral deconvolution for structural elucidation of an unusual C29-steroid detected in a complex sedimentary matrix. J Chromatogr A 2004;1049:227–31.
21. Furtula V, Derksen G, Colodey A. Application of automated mass spectrometry deconvolution and identification software for pesticide analysis in surface waters. J Environ Sci Health B 2006;41:1259–71.
22. Gomez MJ, Gomez-Ramos MM, Aguera A, Mezcua M, Herrera S, Fernandez-Alba AR. A new gas chromatography/mass spectrometry method for the simultaneous analysis of target and non-target organic contaminants in waters. J Chromatogr A 2009;1216:4071–82.
23. Iavicoli I, Chiarotti M, Bergamaschi A, Marsili R, Carelli G. Determination of airborne polycyclic aromatic hydrocarbons at an airport by gas chromatography-mass spectrometry and evaluation of occupational exposure. J Chromatogr A 2007;1150:226–35.
24. Zhang W, Wu P, Li C. Study of automated mass spectral deconvolution and identification system (AMDIS) in pesticide residue analysis. Rapid Commun Mass Spectrom 2006;20:1563–8.
25. Rasanen I, Vyyryläinen H, Ojanpera I, Vuori E. Screening for acidic and neutral drugs in whole blood by gas chromatography-full scan mass

spectrometry utilizing automated mass spectrometry deconvolution and identification system (AMDIS). In: Drummer O, ed. Proceedings of the 41st International TIAFT meeting in Melbourne; 2003 Nov 16–20. Melbourne: TIAFT2003; 2004.

26. Allibe-Signorini N, Berard S, Vincent F, Bessard G, Barret L, Eysseric H. Intérêt d'un logiciel de déconvolution (AMDIS) et d'une détection SIM/SCAN pour le screening toxicologique par CPG-SM. Ann Toxicol Anal 2008;20:57–65.
27. Maurer HH, Pfleger K, Weber AA. Mass spectral library of drugs, poisons, pesticides, pollutants and their metabolites. 4th ed. Weinheim (Germany): Wiley-VCH; 2007.
28. Peters FT, Drvarov O, Lottner S, Spellmeier A, Rieger K, Haefeli WE, Maurer HH. A systematic comparison of four different workup procedures for systematic toxicological analysis of urine samples using gas chromatography-mass spectrometry. Anal Bioanal Chem 2009;393:735–45.
29. von Mach M-A, Weber C, Meyer MR, Weilemann LS, Maurer HH, Peters FT. Comparison of urinary on-site immunoassay screening and gas chromatography-mass spectrometry results of 111 patients with suspected poisoning presenting at an emergency department. Ther Drug Monit 2007;29:27–39.
30. Maurer HH, Pfleger K, Weber AA. Mass spectral and GC data of drugs, poisons, pesticides, pollutants and their metabolites. 3rd ed. Weinheim (Germany): Wiley-VCH; 2007.
31. de-Zeeuw RA, Franke JP, Maurer HH, Pfleger K. Gas chromatographic retention indices of toxicologically relevant substances and their metabolites (report of the DFG commission for clinical toxicological analysis, special issue of the TIAFT bulletin). 3rd ed. Weinheim (Germany): VCH Publishers; 1992.