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Review

Systematic toxicological analysis procedures for acidic drugs and/or metabolites relevant to clinical and forensic toxicology and/or doping control

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Dedicated to Dr Karel Macek, Prague, Czech Republic, on the occasion of his 70th birthday.

Abstract

This paper reviews systematic toxicological analysis (STA) procedures for acidic drugs and/or metabolites relevant to clinical and forensic toxicology or doping control using gas chromatography, gas chromatography–mass spectrometry, liquid chromatography, thin-layer chromatography and capillary electrophoresis. Papers from 1992 to 1998 have been taken into consideration. Screening procedures in biosamples (whole blood, plasma, serum, urine, vitreous humor, brain, liver or hair) of humans or animals (horse, or rat) are included for the following drug classes: angiotensin-converting enzyme (ACE) inhibitors and angiotensin II (AT-II) blockers, anticoagulants of the 4-hydroxy coumarin type, barbiturates, dihydropyridine calcium channel blockers (calcium antagonists), diuretics, hypoglycemic sulfonylureas and non-steroidal anti-inflammatory drugs (NSAIDs). Methods for confirmation of preliminary results obtained by screening procedures using immunoassay or chromatographic techniques are also included. Furthermore, procedures for the simultaneous detection of several drug classes are reviewed. The toxicological question to be answered and the consequences for the choice of an adequate method, the sample preparation and the chromatography itself are discussed. The basic information about the biosample assayed, work-up, separation column, mobile phase or separation buffer, detection mode and validation data of each procedure is summarized in 16 tables. They are arranged according to the drug class and the analytical method. Examples of typical applications are presented. Finally, STA procedures are reviewed and described allowing simultaneous screening for different (acidic) drug classes.

Keywords: Reviews; Toxicological analysis; Doping; Acidic drugs

Contents

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1. Introduction

In clinical toxicology, the diagnosis or the definite exclusion of an acute or chronic intoxication is of great importance. Furthermore, subjects addicted to alcohol, medicaments or illegal drugs have to be monitored. For determination of clinical death as a prerequisite for explantation of organs, the presence of drugs, which may depress the central nervous system, must be analytically excluded. The compliance of patients must be monitored. Finally, monitoring of drugs with a narrow margin of therapeutic safety can be performed by the clinical toxicologist. Similar problems arise in forensic toxicology.

In forensic toxicology, proof of an abuse of illegal drugs or of a murder by poisoning are important tasks. Furthermore, drugs, which may reduce the penal responsibility of a criminal, or which may reduce the fitness to drive a car must be monitored in body fluids or tissues.

In doping control, the use or abuse of drugs, which may stimulate the build-up of muscles, endurance during competition, reduction of body weight, or which may reduce the pain caused by overexertion must be monitored, typically in urine.

1.1. Importance of the reviewed procedures

The basis of a competent toxicological diagnosis and consequent treatment is an efficient toxicological analysis. The choice of the method in analytical toxicology or doping control depends on the problems, which have to be solved. Usually, the compounds, which have to be analyzed are unknown. Therefore, the first step, before quantification, e.g. in plasma, is the identification of the interesting compounds. The analytical strategy often includes a

screening test and a confirmatory analysis. If only a single drug or category has to be monitored, immunoassays can be used for screening in order to differentiate between negative and presumptively positive samples. Positive results must be confirmed by a second independent method that is at least as sensitive as the screening test and that provides the highest level of confidence in the result. Without doubt, GC–MS, especially in the full-scan electron-impact mode, is the reference method for confirmation of positive screening tests [1–8]. This two-step strategy is employed, only if those drugs or poisons have to be determined, which are scheduled, e.g., by law or by international organisations, and for which immunoassays are commercially available (among the acidic drugs covered in this review only for barbiturates!). If these demands are not met, the screening strategy must be more extensive, because several thousands of drugs and toxicants are on the market worldwide [9]. For these reasons, systematic toxicological analysis (STA) procedures are necessary that allow the simultaneous detection of as many toxicants in biosamples as possible. Most often, GC–MS [1,2,8,10–13] or HPLC coupled to diode-array detectors (DAD) [14–21] are used today. However, most of the STA procedures only cover basic (and neutral) drugs, which are the more important toxicants. Nevertheless, some classes of acidic drugs like the cardiovascular drugs ACE inhibitors and AT-II blockers, dihydropyridine calcium channel blockers (metabolites), diuretics, coumarin anticoagulants, hypoglycemic sulfonylureas, barbiturates, or non-steroidal anti-inflammatory drugs (NSAID)s, are relevant to clinical and forensic toxicology or doping control. Therefore, screening procedures for their detection in biosamples are reviewed here. Chromatographic and electrokinetic procedures for determination of other acidic (carboxyl-possessing drugs) compounds have been reviewed in another special issue of the *Journal of Chromatography B* (717 (1998) 1–353).

1.2. Choice of biosamples for systematic toxicological analysis of drugs

Concentrations of drugs are relatively high in urine, so that urine is the sample of choice for a comprehensive screening and identification of unknown drugs or poisons [1]. However, the metabolites of these drugs must be identified in addition or even exclusively. Most of the reviewed papers describe methods for urine analysis (see U in *Sample* column in Tables 1–16). In horse-doping control, urine is also the common sample for screening [22–25]. Blood (plasma, serum) is the sample of choice for quantification. However, if the blood concentration is high enough, screening can also be performed herein (see B, P, S in *Sample* column in Tables 1–16). This may be advantageous, since sometimes only blood samples are available and some procedures allow simultaneous screening and quantification [24,26–33]. The toxicological analysis in hair samples allows the detection of past, chronic drug use [6]. Some acidic drugs like barbiturates or NSAIDs can be screened in hair samples too [34]. However, there still is controversy on how to interpret the results, particularly concerning external contamination, cosmetic treatment or ethnic bias. A few papers describe analysis in tissue samples, like liver or brain [26,29,32,35,36].

1.3. Choice of the references

The reviewed references were selected by searching the databases of Medline, Chemical Abstract Services and Current Contents. English written papers published between 1992 and 1998 were taken into consideration. Papers describing quantification of single drugs have not been reviewed here. Papers concerning diuretics were considered after 1995, since earlier published papers were already reviewed in 1992 [1] and 1996 [37–39].

2. Systematic toxicological analysis procedures for particular classes of acidic drugs and/or metabolites

Procedures for screening, identification and/or quantification of acidic drugs and/or their metabolites are critically reviewed in this chapter. The principal information on each procedure is summarized in Tables 1–16 to simplify the rapid selection of a method suitable for an actual analytical problem. The tables are organized according to the drug class and the method.

Table 1
GC–MS procedure for screening of ACE inhibitors, AT-II blockers and/or their metabolites

Compound	Sample	Work-up	Column	Detection mode	Reference data	Validation data	Ref.
8 ACE inhibitors, 2 AT-II blockers	U	EX-ME	FSC HP-1	EI, scan	RI, MS	REC: 68–88% LOD: 10 ng/ml	[40]

Table 2
GC–MS procedures for screening of coumarin anticoagulants and/or their metabolites

Compound	Sample	Work-up	Column	Detection mode	Reference data	Validation data	Ref.
6 Anticoagulants	U	EX-ME	FSC HP-1	EI, scan	RI, MS	REC: 64–72% LOD: 25 ng/ml	[10]

Table 3
LC procedures for screening of coumarin anticoagulants and/or their metabolites

Compound	Sample	Work-up	Stationary phase	Mobile phase	Detection mode	Validation data	Refs.
6 Coumarin anticoagulants	S, liver	SPE	Ultrasphere C_{18} (250×4.6 mm, ? μm)	Gradient elution: ammonium acetate buffer–MeOH	FL, 318/390	REC: >93% (S); REC: >76% (liver) LOD: 1 ng/ml or g LIN: 5–500 ng/ml or g	[26]
2 Indanedione anticoagulants	S, liver	SPE	Supelco LC18 (150×4.6 mm, ? μm)	Gradient elution: ammonium acetate buffer–MeOH	UV, 285	REC: >74% (S); REC: >68 (liver) LOD: 10 ng/ml or g LIN: 50–1000 ng/ml or g	[26]
5 Anticoagulants	S, Liver (animals)	LLE	Ultrasphere C_{18} (250×4.6 mm, ? μm)	Gradient elution: methanol–ammonium acetate buffer/triethylamine	FL, 318/390	REC: 54–98% LOD: 1–2 mg/g	[35]
6 Anticoagulants	Liver (rat)	LLE-SPE	ODS Spherisorb (250×4.6 mm, 5 μm) guard column: ODS	Gradient elution: aqueous acetic acid (0.25%)–MeOHic acetic acid (0.25%)	FL, 310/390	REC: 77–96% LOD: 2–10 ng/g	[36]
'Superwarfarin rodenticides': 4 Coumarin anticoagulants, 1 Indanedione anticoagulant	S	LLE	Hyperchrome (150×4.6 mm, 5 μm)	20 m*M* aqueous tetrabutylammoniumhydroxide–ACN (25:45, pH 4.7)	UV, 285; FL, 265/400	REC: 55–131% LOD: 20–75 ng/ml (UV) LOD: 3–12 ng/ml (FL) LIN: 100–1000 ng/ml	[27]
13 Anticoagulants	S	LLE	Nucleosil C_{18} (200×4.6 mm, 5 μm)	Gradient elution: ACN/water (9:1)–dihydrogen potassium phosphate buffer (pH 7)	DAD	REC: 36–95% LOD: 25–100 ng/ml LIN: 25/50–5000 ng/ml	[28]
2 Anticoagulants, 3 NSAIDs; a.o.	Hair	SPE	C_8 (250×4.6 mm, 5 μm); guard column: Waters C_{18}	Gradient elution: phosphate buffer (pH 3.8)–ACN	DAD	REC: 62–92% LOD: ?	[34]

Table 4
TLC procedures for screening of coumarin anticoagulants and/or their metabolites

Compound	Sample	Work-up	Stationary phase	Mobile phase	Detection mode	Validation data	Refs.
2 Anticoagulants, 7 NSAIDs; 9 Barbiturates, 3 Diuretics	B, U	SPE, LLE	Silica gel (HPTLC)	1. Isopropanol–chloroform–conc. NH_3 (45:45:10) 2. T~oluene–butylmethylether–acetic acid–MeOH (60:30:9:1)	DCCI, $FeCl_3$	REC: 76–97% LOD: 50–200 ng/ml	[71]
5 Anticoagulants	S, Liver (animals)	LLE	Silica gel	Benzene–ethyl methyl ketone–formic acid (80:6:1)	UV scanner	REC: 54–98% LOD: 1–2 mg/g	[35]
8 Anticoagulants	S, Liver (animals)	LLE	RP18 (HPTLC)	Methanol–phosphoric acid 4.72 *M* (9:1)	UV scanner	REC: 85–95% LOD: 200 ng/ml LIN: 200–2000 ng/ml	[29]

The information concerning quantification, can be found in the Validation column. The type of biosample used is given in the Sample column (B, blood; P, plasma; S, serum; U, urine, etc.). If samples from animals were studied, the species is given in brackets. The sample preparation is concisely summarized in the Work-up column. The principal information on stationary phase, mobile phase, detection mode and published reference data is given. Validation data such as recovery (REC), limit of detection (LOD) or linearity (LIN) are summarized for easy evaluation, whether a procedure can be useful to solve an actual toxicological case. If LIN is given, the procedure is suitable for quantification. The limit of quantification is not given, because it is most often identical with the lowest linearity value. Since precision of all the reviewed procedures was better than 20%, these data were omitted in order to save space. The LOD of parent compounds in urine is of minor value, if these drugs are mainly or even exclusively excreted in metabolized form. In these cases, it should be studied, whether and how long the intake of a therapeutic drug dose can be monitored by the procedure [12,40].

2.1. Sample preparation

Suitable sample preparation is an important prerequisite for chromatography of biosamples. It involves isolation and, if necessary, cleavage of conjugates and/or derivatization of the drugs and their metabolites. Prior to blood, serum or plasma extraction, precipitation of proteins may be useful, which can be achieved using solvents like acetone or acetonitrile [41] or using strong acids like trichloroacetic acid [42]. Deproteinization by adding saturated sodium sulphate solution has further advantages: the organic phase is kept free from water and salting-out effects may improve the extraction rates of LLE [43].

Cleavage of conjugates can be performed by gentle but time-consuming enzymatic hydrolysis (see EN). However, the enzymatic hydrolysis of acyl glucuronides (ester glucuronides of carboxy derivatives like NSAIDs) may be hindered due to ‘acyl migration’ [44]. Acyl migration means intramolecular transesterification at the hydroxy groups of the glucuronic acid, which leads to β-glucuronidase-resistant derivatives. In emergency toxicology, it is preferable to cleave the conjugates by rapid acid hydrolysis (see HY). Only ester conjugates can be cleaved by alkaline hydrolysis. However, the formation of artifacts during chemical hydrolysis must be considered [43]. A compromise between the two cleavage techniques is the use of a column packed with immobilized glucuronidase/arylsulfatase. It combines the advantages of both methods, the speed of acid hydrolysis and the gentle cleavage of enzymatic hydrolysis [45,46]. Hirai et al. [47] studied the influence of enzymatic, acidic and alkaline hydrolysis on the extraction recovery of NSAIDs. As shown in Fig. 1, diclofenac was destroyed during acidic hydrolysis and indometacin during alkaline hydrolysis. The recovery of some of the NSAIDs decreased during hydrolysis. Therefore, it can be advantageous to use extractive alkylation, because

Table 5
GC and GC–MS procedures for screening of barbiturates and/or their metabolites

Compound	Sample	Work-up	Column	Detection mode	Reference data	Validation data	Refs.
9 Barbiturates	P	LLE	FSC SPE-1, SPE-20	NPD, FID	RT on 2 FSC	REC: 83–103% LOD: 500 ng/ml LIN: 1560–25 000 ng/ml	[30]
3 Barbiturates	B	LLE	FSC HP-2 ultra, HP 1701	NPD	RT on 2 FSC	REC: ? LOD: ?	[69]
5 Barbiturates	U	SPE	FSC DB-5	EI, SIM		REC: 82–100% LOD: 10 ng/ml LIN: 20–500 ng/ml	[66]
4 Barbiturates	U	SPE, ME	FSC DB-5	EI, SIM	FI	REC: 80–90% LOD: 20 ng/ml LIN: 50–3200 ng/ml	[60]
10 Barbiturates	S	SPE	FSC HP-1	EI, SIM	FI	REC: 82–172 (!)% LOD: 100 ng/ml	[68]
7 Barbiturates	U	EX-PR	FSC DB-5	EI, ?		REC: ? LOD: 20–60 ng/ml LIN: 20/60–750 ng/ml	[54]
6 Barbiturates; 12 NSAIDs	U (horse)	LLE, ME	FSC HP-5	EI, SIM	FI	LOD: 10 ng/ml	[22]
4 Barbiturates	B	LLE, ME	FSC HP-5	NPD, EI	RT, FI	REC: 87–124% LOD: 250–500 ng/ml	[42]
6 Barbiturates	B, U	LLE, ET	FSC DB-1	EI, scan	RT, MS	REC: 61–90% LOD: 5 ng/ml LIN: 50–10000 ng/ml	[31]
8 Barbiturates	U	SPME	FSC PTE-5	EI, PCI, MS-MS, SIM	FI	REC: 93–104% LOD: 1 ng/ml	[50]
7 Barbiturates	U	Automated SPE	FSC HP-5MS	EI, SIM		REC: 81–104% LOD: 20–200 ng/ml LIN: 20–10 000 ng/ml	[67]
6 Barbiturates	B	Automated SPE, TMS	FSC HP-5	MS, scan	RT, FI	REC: ? LOD: ?	[7]
3 Barbiturates; 4 NSAIDs; a.o.	Hair	SPE	FSC CP SIL 8 CB	EI, scan		REC: 45–100% LOD: ?	[34]

acyl glucuronides were readily cleaved under the conditions of extractive alkylation (alkaline pH, elevated temperature). Therefore, hydrolysis procedures could be renounced when using extractive alkylation [12,40].

Isolation was performed by liquid–liquid extrac-

Table 6
LC procedures for screening of barbiturates and/or their metabolites

Compound	Sample	Work-up	Stationary phase	Mobile phase	Detection mode	Validation data	Refs.
10 Barbiturates	B, U (brain, liver)	LLE	TSK gel Super-ODS (100×4.6 mm, 2 μm)	8 m*M* phosphate buffer–ACN, 30:70	UV, 215	REC: 95–103% (S) LOD: 10–500 ng/ml LIN: 50–5000 ng/ml	[32]
4 Barbiturates	S	SFC	Luna C_{18} (150×2.1 mm, 3 μm) guard column: C_{18} (30×1 μm)	Gradient elution: ACN–10 m*M* ammonium acetate (pH 7.5)	ES-MS-MS	REC: 94–107% LOD: 23–225 ng/ml LIN: 1000–60 000 ng/ml	[83]
6 Barbiturates; 10 NSAIDs; 5 Diuretics; 3 Sulfonylureas; 1 Anticoagulants	B	LLE	ODS-2 (150×3.8 mm, 5 μm) guard column: Novapak C_{18}	Gradient elution: ACN–phosphate buffer (pH 3.1)	DAD	REC: 45–100% LOD: ?	[14]
3 Barbiturates; 7 NSAIDs; 1 Diuretic; 1 Sulfonylurea; 1 Anticoagulant; a.o.	B	LLE	ODS Hypersil (200×2.1 mm, 5 μm)	Gradient elution: ACN–phosphate buffer (pH 3.2)	DAD	REC: 45–105% LOD: ?	[19]

Table 7
TLC procedures for screening of barbiturates and/or their metabolites

Compound	Sample	Work-up	Stationary phase	Mobile phase	Detection mode	Validation data	Ref.
9 Barbiturates; 7 NSAIDs; 3 Diuretics; 2 Anticoagulants	B, U	(EN) SPE, LLE	Silica gel (HPTLC)	1. Isopropanol–chloroform–conc. NH_3 (45:45:10) 2. Toluene–butylmethylether–acetic acid–MeOH (60:30:9:1)	DCCI, $FeCl_3$	REC: 25–102% LOD: 50–200 ng/ml	[71]

tion at a pH at which the analyte is nonionized (see LLE in Work-up column in Tables 1–16) or by solid-phase extraction (see SPE) preceded or followed by clean-up steps. Next, the extract is concentrated. In my experience, SPE is preferable, if particular substances or drug classes have to be

Table 8
CE (MECC) procedures for screening of barbiturates and/or their metabolites

Compound	Sample	Work-up	Capillary	Separation buffer	Detection mode	Validation data	Refs.
6 Barbiturates	B, U, vitreous humor	SPE	FSC (600–400×0.075 mm)	10 m*M* borate buffer–10 m*M* phosphate buffer–100 m*M* SDS–ACN (85:15)	UV, 214	REC: ?% LOD: ?	[73]
10 Barbiturates; 8 Benzoates	S, U	SPME	FSC (750×0.075 mm)	50 m*M* Tris (pH 7.8 with Tapso) for urine, 100 m*M* Tris (pH 7.8 with Tapso) for serum	UV, 230	REC: ? LOD: 100–300 ng/ml	[51]

Table 9
GC–MS procedures for screening of dihydropyridine calcium channel blocker metabolites

Compound	Sample	Work-up	Column	Detection mode	Reference data	Validation data	Ref.
9 Calcium channel blockers	U	EX-ME	FSC HP-1	EI, scan	RI, MS	REC: 67–77% LOD: 10 ng/ml	[12]

Table 10
LC–MS procedures for screening of diuretics

Compound	Sample	Work-up	Stationary phase	Mobile phase	Detection mode	Validation data	Ref.
3 Thiazid diuretics	U (horse)	LLE	Luna C_{18} (150×3.2 mm, 3 μm) Guard column: C_{18} (30×3.2 mm, 3 μm)	Gradient elution: 3% aqueous acetic acid (pH 2.6)–MeOH	APCI-MS, Scan	REC: 48–69% LOD: 131–384 ng/ml	[25]

Table 11
LC procedures for screening of sulfonylureas and/or their metabolites

Compound	Sample	Work-up	Stationary phase	Mobile phase	Detection mode	Validation data	Refs.
4 Sulfonylureas	P	LLE	Partisphere C_{18} (110×4.7 mm, 5 μm) Guard column: C_{18}	50 m*M* phosphate buffer (pH 7)–ACN, 70:30 or 60:40	UV, 200	REC: 69–92% LOD: 10–40 ng/ml	[123]
3 Sulfonylureas; 10 NSAIDs; 6 Barbiturates; 5 Diuretics; 1 Anticoagulants	B	LLE	ODS-2 (150×3.8 mm, 5 μm) Guard column: Novapak C_{18}	Gradient elution: ACN–phosphate buffer (pH 3.1)	DAD	REC: 45–100% LOD: ?	[14]
1 Sulfonylurea, 7 NSAIDs; 3 Barbiturates; 1 Diuretic; 1 Anticoagulant; a.o.	B	LLE	ODS Hypersil (200×2.1 mm, 5 μm)	Gradient elution: ACN–phosphate buffer (pH 3.2)	DAD	REC: 45–105% LOD: ?	[19]
6 Sulfonylureas	S	LLE	Nucleosil C_{18} 7 μm)	Gradient elution: acetic acid (0.05%)–MeOH	APCI-MS, SIM	REC: 95–97% LOD: 10 ng/ml	[122]

Table 12
CE (MECC) procedures for screening of sulfonylureas and/or their metabolites

Compound	Sample	Work-up	Capillary	Separation buffer	Detection mode	Validation data	Refs.
6 Sulfonylureas	U	SPE	FSC polyimide-coated (200–400×0.05 mm)	Borate buffer–phosphate buffer–SDS or sodium cholate (in different concentrations)	UV, 200 nm; DAD, 200–350	REC: ?% LOD: about 50 ng/ml	[74]
6 Sulfonylureas or metabolites	U	SPE	FSC polyimide-coated (400×0.05 mm)	5 m*M* borate buffer–5 m*M* phosphate buffer with 75 m*M* sodium cholate (pH 8.5)	UV, 200 nm; DAD, 190–350	REC: ?% LOD: ?	[75]

Table 13
GC and GC–MS procedures for screening of non-steroidal anti-inflammatory drugs and/or their metabolites

Compound	Sample	Work-up	Column	Detection mode	Reference data data	Validation data	Refs.
26 NSAIDs	S	LLE, TBDMS	FSC HP-1	FID; EI, scan	RT, FI	Validation data only recorded in pure substance solutions	[62]
Ibuprofen+M	U	LLE, ME	FSC HP-1	EI, scan	RI, MS	LOD: 5 ng/ml	[61]
6 NSAIDs	U	EX-ME	FSC Ultra-2C	EI, scan		REC: 30–89% LOD: 10 ng/ml	[55]
20 NSAIDs	U	SPE, TBDMS	FSC Ultra-2	EI, SIM	RT, FI	REC: 76–114% LOD: 0.03–0.9 pg	[63]
17 NSAIDs	P, U (horse)	LLE, ME	FSC HP-1	EI, SIM	RT, FI	REC: 23–100% (P), 37–84% (U) LOD: 5–25 ng/ml	[23]
17 NSAIDs	P, U (horse)	U: SPE, ME P: LLE, SPE, ME	FSC DB-5	EI, Scan	FI	REC: 85–110% LIN: 100–3000 ng/ml	[24]
12 NSAIDs; 6 Barbiturates	U (horse)	LLE, ME	FSC HP-5	EI, SIM	FI	LOD: 10 ng/ml	[22]
4 NSAIDs; 3 Barbiturates; a.o.	Hair	SPE	FSC CP SIL 8 CB	EI, scan		REC: 45–100% LOD: ?	[34]

Table 14
LC procedures for screening of non-steroidal anti-inflammatory drugs and/or their metabolites

Compound	Sample	Work-up	Stationary phase	Mobile phase	Detection mode	Validation data	Refs.
12 NSAIDs	U	SPE	ODS-2 (150×4.6 mm, 5 μm)	ACN–phosphate buffer (pH 5.0), 58:42	UV, 230	REC: 73–95% LOD: 5–50 ng/ml	[47]
10 NSAIDs; 6 Barbiturates; 5 Diuretics; 3 Sulfonylureas; 1 Anticoagulants	B	LLE	ODS-2 (150×3.8 mm, 5 μm) guard column: Novapak C_{18}	Gradient elution: ACN–phosphate buffer (pH 3.1)	DAD	REC: 45–100% LOD: ?	[14]
7 NSAIDs; 3 Barbiturates; 1 Diuretic; 1 Sulfonylurea; 1 Anticoagulant; a.o.	B	LLE	ODS Hypersil (200×2.1 mm, 5 μm)	Gradient elution: ACN–phosphate buffer (pH 3.2)	DAD	REC: 45–105% LOD: ?	[19]
3 NSAIDs; 2 Anticoagulants, a.o.	Hair	SPE	C_8 (250×4.6 mm, 5 μm) Guard column: Waters C_{18}	Gradient elution: ACN–phosphate buffer (pH 3.8)	DAD	REC: 62–92% LOD: ?	[34]

Table 15
TLC procedures for screening of non-steroidal anti-inflammatory drugs and/or their metabolites

Compound	Sample	Work-up	Stationary phase	Mobile phase	Detection mode	Validation data	Refs.
7 NSAIDs; 9 Barbiturates; 3 Diuretics; 2 Anticoagulants	B, U	(EN) SPE, LLE	Silica gel (HPTLC)	1. Isopropanol–chloroform–conc. NH_3 (45:45:10) 2. Toluene–butylmethylether–acetic acid–MeOH (60:30:9:1)	DCCI, $FeCl_3$	REC: 25–102% LOD: 50–200 ng/ml	[71]
3 NSAIDs	U	HY, SPE	Silica gel or diol-bonded silica gel (HPTLC)	Chloroform–MeOH (90:10)	UV, MS-MS	REC: ?% LOD: ? ng/ml	[72]

selectively isolated in series from relatively homogeneous samples like plasma samples in pharmacokinetic studies or urine samples for confirmation of known drugs. Universal LLE procedures are preferable for general screening procedures, because substances with very different physico-chemical properties must be isolated from heterogeneous matrices. Sample pretreatment for SPE depends on the sample type: whole blood and tissue (homogenates) need deproteinization and filtration/centrifugation steps before application to the SPE columns, whereas for urine usually a simple dilution step and/or centrifugation is satisfactory. Whatever SPE column is used, the analyst should keep in mind, that there are large differences from batch-to-batch, and that the same sorbents from different manufacturers also lead to different results [48]. Therefore, use of a suitable internal standard (e.g. deuterated analytes) is recommended. The pros and cons of SPE procedures for STA were discussed by Franke and De Zeeuw [49].

Solid-phase microextraction (SPME) is becoming a modern alternative to SPE and LLE. SPME is a solvent-free and concentrating extraction technique, which was used for fast screening of barbiturates by GC–MS [50] or CE [51].

Extractive alkylation has proved to be a powerful procedure for simultaneous extraction and derivatization of acidic compounds [10–12,40,52–59]. The acidic compounds were extracted at pH 12 as ion pairs with the phase-transfer catalyst into the organic phase. Reaching the organic phase, the phase-transfer catalyst could easily be solvated due to its lipophilic hexyl groups. The poor solvation of the anionic analytes leads to a high reactivity against the alkylation (most often methylation) reagent alkyl iodide. Part of the phase-transfer catalyst could also reach the organic phase as an ion pair with the iodide anion formed during the alkylation reaction or with anions of the urine matrix. Part of the phase-transfer catalyst remained in the organic phase. Therefore, they had to be removed for maintaining the GC column's separation power and to exclude interactions with analytes in the GC injection port. We tested several SPE sorbents and different eluents for efficient separation of the vestige of the phase-transfer catalyst salts from the analytes. The diol sorbent yielded best reproducibility and recovery under the described con-

Table 16
CE procedures for screening of non-steroidal anti-inflammatory drugs and/or their metabolites

Compound	Sample	Work-up	Capillary	Separation buffer	Detection mode	Validation data	Refs.
3 NSAIDs	S, U		FSC (570×0.075 mm)	ACN–phosphate buffer (pH 3, + 2 m*M* β-cyclodexdrin) (10:90)	DAD	REC: 94–103% LOD: 0.3–0.5 ng/ml LIN: 400–40 000 ng/ml	[33]
3 NSAIDs+ glucuronides	U	SPE	FSC (1000×0.075 mm)	20 m*M* ammonium acetate (pH 9)	UV, 200 nm; MS	REC: ?% LOD: ? ng/ml	[130]
6 NSAIDs+ glucuronides	U	SPE	FSC (440×0.05 mm)	50 m*M* ammonium acetate (pH 9.8)	DAD, MS	REC: ?% LOD: ? ng/ml	[76]

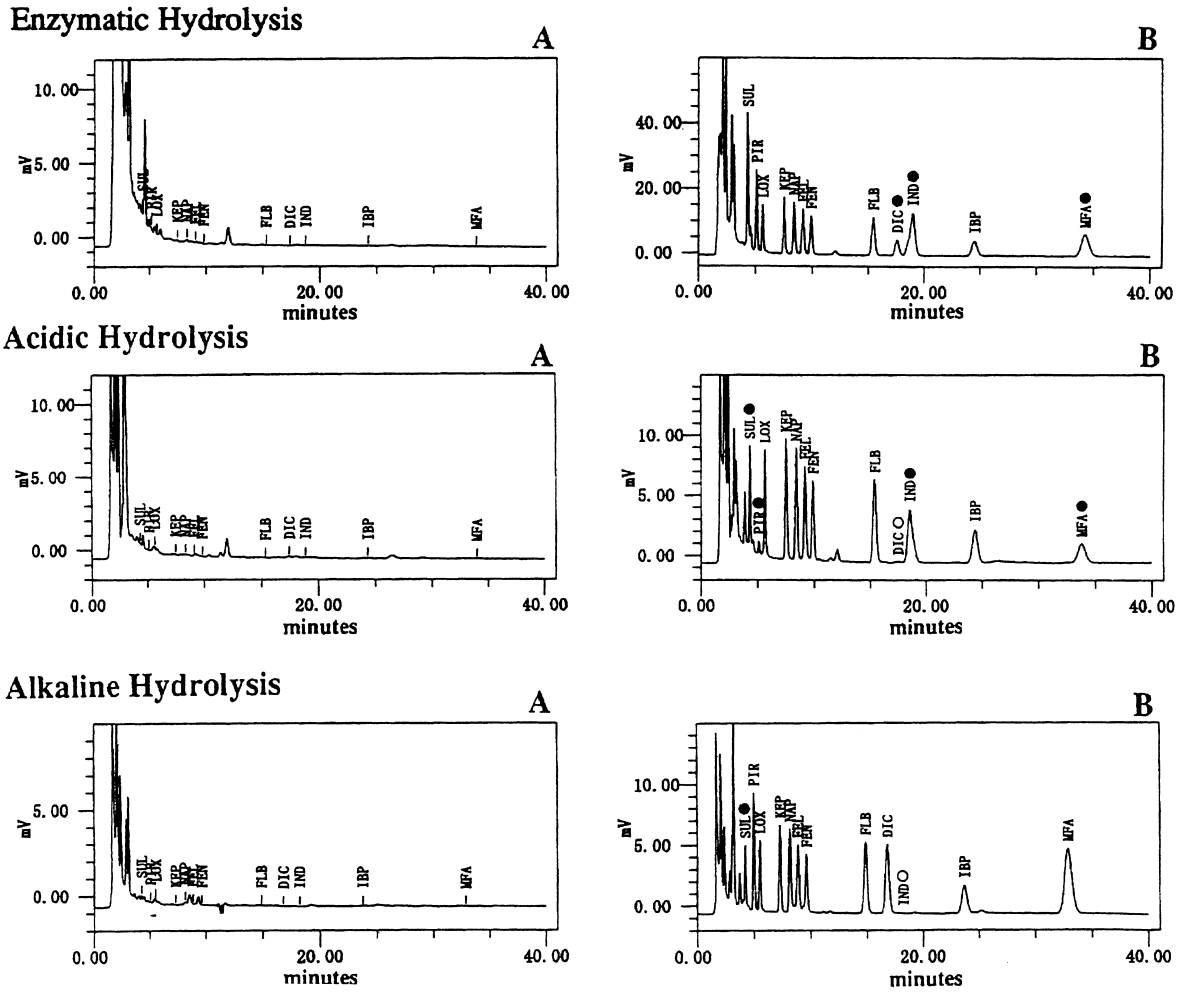

Fig. 1. LC–UV chromatograms of hydrolyzed human urine samples. (A) Drug-free urine; (B) urine spiked with indomethacin (IND), sulindac (SUL), diclofenac (DIC), ketoprofen (KEP), naproxen (NAP), fenbufen (FEN), felbinac (FEL), flurbiprofen (FLB), ibuprofen (IBP), loxoprofen (LOX), piroxicam (PIR) and mefenamic acid (MFA). Diclofenac was completely destroyed during acidic hydrolysis and indomethacin during alkaline hydrolysis. The recovery of some of the NSAIDs decreased during hydrolysis (taken from Ref. [47]).

ditions. Further advantages of such SPE columns are that they can easily be handled, that they are commercially available and that they do not have to be manually prepared as described by Lisi et al. [53].

Derivatization steps are necessary, if relatively polar compounds, like the reviewed acidic drugs, are to be determined by GC or GC–MS. In the reviewed papers, the following procedures were used: methylation (ME) [22–24,42,60,61], extractive methylation (EX-ME) [10–12,40,55,57,58], ethylation (ET) [31], extractive propylation (EX-PR) [54], trimethylsilylation (TMS) [7] or *tert.*-butyldimethylsilylation (TBDMS) [62,63], see Work-up column in Tables 1–16. Methylation has been approved for the identification of numerous classes of acidic drugs. (The power of extractive methylation has already been discussed.). The methyl derivatives are stable and show good gas chromatographic properties. Mass spectra of such derivatives can be found in Ref. [9,64]. The methylation mixture can be evaporated or removed by LLE or SPE before analysis, so that the resolution power of capillary columns does not

decrease. The molecular mass does not increase very much, so that compounds with relatively high molecular mass and several derivatizable groups can be measured with mass selective detectors with a mass range only up to 650 u. Further details on pros and cons of derivatization procedures can be found in a review of Segura et al. [65].

2.2. Chromatographic and electrokinetic procedures

For systematic toxicological analysis of acidic drugs and/or their metabolites, gas chromatographic procedures (GC with MS or other detectors), liquid chromatographic procedures (LC with DAD or other detectors), thin-layer chromatographic (TLC with different detection modes) or capillary electrophoretic (CE) procedures were used. Since in STA a broad range of unknown compounds – even in unknown combinations – must be screened, differentiated and identified, the separation must be as powerful and universal as possible and the detection modes must be of the highest specificity and universality.

For GC separation, most often fused-silica capillaries (FSC) with relatively apolar silicone phases (methylsilicone without or with 2–5% phenylsilicone) have been used for reasons of universality. The most powerful GC detector is without any doubt the MS, especially in the electron-impact (EI) full-scan mode. Most papers cover the GC–MS coupling [7,10,12,22–24,31,34,40,50,54,55,60–63,66–68], while only a few GC procedures were published with common detectors like flame-ionization detectors (FID) [30,62] or nitrogen–phosphorus detectors (NPD) [30,42,69]. The disadvantages for STA of such detectors in contrasts to MS was already discussed by Maurer [1].

For LC separation, most often RP C_{18} columns have been used as stationary phases. The mobile phases consisted of mixtures of buffers with variable pH and different organic solvents like methanol (MeOH) or acetonitrile (ACN). Isocratic or gradient elution was used. For LC–MS volatile buffers or acid solutions are needed [70]. For STA procedures, DAD is most often used [14,19,28,34], since it provides the best compromise between specificity and universality among the LC detectors besides the coming, but not yet generally applied MS. For detection of very low concentrations of fluorescent compounds like coumarins anticoagulants, fluorescence detectors are used [26,27,35,36]. However, the lower specificity must be considered in interpretation of the results.

The application of TLC for STA is still decreasing. Only one TLC procedure for screening of several drug classes, including some acidic anticoagulants, barbiturates, diuretics and NSAIDs, has recently been published [71]. Further procedures using UV scanning were described for anticoagulants [29,35] or using a rather unusual MS–MS detection for NSAIDs [72].

CE procedures are being more and more published for detection of rather high concentrations of drugs in biosamples. Common UV detectors [51,73] or DADs [33,74–76] were used for more or less specific detection. Only one CE–MS procedure for acidic drugs has recently been published by Heitmeier and Blaschke [76]. At present, CE is still limited to rather high analyte concentrations in the biosamples, as already discussed in the review of Tagliaro et al. [77]. To allow CE separation of uncharged analytes, micellar electrokinetic capillary chromatography (MECC) was developed. This technique is based on pseudostationary phases due to micelle-forming surfactants like sodium dodecyl sulfate (SDS) or sodium cholate. These micelles mimic an RP LC column, which means that the analyte can also be separated due to hydrophobic partitioning effects. MECC coupled to DAD has successfully been established for the detection of barbiturates [73] or hypoglycemic sulfonylureas and their metabolites in urine [74,75].

In summary, different chromatographic and electrokinetic procedures have been published in the last years for screening, identification and quantification of acidic drugs, which are described in the following and concisely summarized in the Tables 1–16.

2.2.1. ACE inhibitors and AT-II blockers

Angiotensin-converting enzyme (ACE) inhibitors are widely used in the treatment of hypertension and congestive heart failure. Angiotensin II (AT-II) receptor antagonists, a new drug class, are used for the same indication. In case of intoxication, ACE inhibitors or AT-II antagonists may lead to severe

cardiovascular disorders like hypotension and shock. For diagnosis or for differential diagnostic exclusion of such an intoxication, a screening procedure is necessary for the detection of these drugs in urine. ACE inhibitors have a free carboxylic acid group. A further carboxylic group is formed by hydrolysis of the ethyl esters during metabolism and/or sample preparation. The pharmacologically active dicarboxylic acids, the so-called 'prilates', are used for parenteral application. AT-II antagonists have also acidic properties, resulting from a carboxylic acid function and/or from the electron excess of the tetrazole ring. Only one screening procedure for ACE inhibitors and AT-II blockers was published (cf. Section 2.2.1.1). If necessary, the drugs can be quantified in plasma using GC–MS [78–80], HPLC [81] or using modifications of these procedures.

2.2.1.1. GC–MS procedures. Maurer et al. have recently published a GC–MS procedure, that allows the detection of therapeutic concentrations of most of the ACE inhibitors and the AT II antagonist valsartan in human urine samples after extractive methylation [40]. The principal information is summarized in Table 1. This procedure is part of a comprehensive STA procedure for the detection of various classes of acidic drugs and/or their metabolites (cf. Section 3).

2.2.2. Anticoagulants of the 4-hydroxycoumarins type

Anticoagulants of the 4-hydroxycoumarin type are used as therapeutics or as rodenticides of the so-called first generation. Coumarins of the second generation, the so-called 'superwarfarins', are very potent rodenticides and, therefore, very low-dosed. For the differential diagnosis of unclear coagulopathies, which may occur after ingestion of therapeutic or rodenticide coumarins, screening is needed. Several screening procedures published in the last years are described in Sections 2.2.2.1–2.2.2.3 and concisely summarized in Tables 2–5. If necessary, the identified anticoagulants can be quantified in plasma by HPLC [26,28] or TLC [29].

2.2.2.1. GC–MS procedures. Maurer and Arlt [10] have recently published a GC–MS procedure for the detection of the 4-hydroxycoumarin anticoagulants of the first generation and their metabolites in urine after extractive methylation. Derivatization was essential for sensitive GC–MS detection of these vinylogous carboxylic acids and their metabolites (aniline/anilide derivatives or phenols). Only alcoholic hydroxy groups could not be methylated due to their lower nucleophilicity, but this fact did not markedly influence the sensitivity. Again, this procedure is part of a comprehensive STA procedure for the detection of various classes of acidic drugs and/or their metabolites (cf. Section 3).

2.2.2.2. LC procedures. The relatively polar coumarin derivatives can sufficiently be separated by RP chromatography. DADs were applied for screening of coumarin anticoagulants of the first generation and for indanedione anticoagulants [28,34]. Since coumarins have fluorescent properties, fluorescence detection was used with at least 10 times better sensitivity [26,27,35,36]. For determination of 'superwarfarins', HPLC with fluorescence detection was necessary [27].

2.2.2.3. TLC procedures. TLC procedures using UV scanning were described for identification and quantification of anticoagulants [29,35]. A general TLC procedure also allows the detection of the therapeutic anticoagulants acenocoumarol and phenprocoumon [71]. It is absolutely incomprehensible, that toxicologists still use the cancerogenic benzene and hepatotoxic chloroform in TLC mobile phases or for extraction.

2.2.3. Barbiturates

Barbiturates are still used and misused, but with decreasing frequency. Nevertheless, there are important reasons, why screening for and quantification of barbiturates is necessary in clinical and forensic toxicology. Phenobarbital and its precursor primidone are still used as anticonvulsants. Thiopental is widely used as a short-term intravenous anesthetics. Thiopental and its metabolite pentobarbital are often to be monitored for decision of brain death. The question of penal responsibility of a criminal after ingestion of barbiturates could be raised in the assessment of crimes. Barbiturates may reduce the fitness to drive a car or to work at machines, and they may lead to addiction or to severe intoxications.

Barbiturates can be screened by commercially available immunoassays. Positive results must be confirmed by a second independent method, that is at least as sensitive as the screened test and that provides the highest level of confidence in the result. Since barbiturates have only weakly acidic properties, they can be detected in screening and confirmation procedures for as well basic and neutral drugs [1,82] as acidic drugs [22,57]. Due to relatively high blood levels, barbiturates can be screened also in blood, plasma or serum. Several GC, GC–MS, LC, CE or TLC procedures have been published in the last years for screening, confirmation and/or quantification. They are described in Sections 2.2.3.1–2.2.3.4. and concisely summarized in Tables 5–9. If necessary, the identified barbiturates can be quantified by those procedures, for which the LIN is given in the *Validation* column.

2.2.3.1. GC and GC–MS procedures. Since barbiturate concentrations in blood and urine are relatively high, derivatization prior to GC–(MS) analysis is not mandatory in most cases. Nevertheless, derivatization can improve the separation and the sensitivity. Methylation is most often used [22,42,60]. Ethylation [31] seems not to bring further advantages. The pros and cons of silylation, as recommended by Polettini et al. [7] for a general screening, were already discussed. The latter authors as well as Namera et al. [67] used automated SPE procedures. However, in our experience, such procedures take too long time, especially for application in clinical toxicology. Perhaps, SPME is becoming a fast alternative [50].

NPD or FID are sometimes used for determination [30,69] but the detector of choice is EI-MS, providing good sensitivity and best specificity, especially in the full-scan mode. Thus, most of the procedures in the literature are GC–MS procedures [22,31,50,54,60,66,67]. The LODs ranged between 1 and 500 ng/ml.

2.2.3.2. LC procedures. Barbiturates can sufficiently be separated by reversed-phase (RP) chromatography. DAD was applied for the general screening procedures, which covered barbiturates besides other drugs [14,19]. As already mentioned, the use of UV at single wavelength is critical for toxicological analysis due to low specificity [32]. Determinations of barbiturates after supercritical fluid extraction (SFC) using electrospray LC–MS–MS [83] were described. However, in my experience and opinion, LC–MS–MS is not necessary for barbiturate analysis, when GC–MS is available [70]. Neither the time for sample work-up (35 min!), nor the sensitivity or specificity were better. The only superlative was the price of the apparatus.

2.2.3.3. TLC procedures. The general TLC procedure of Iten and Mueller [71] allows the detection of some barbiturates in blood und urine in addition to several other drugs.

2.2.3.4. CE procedures. For determination of barbiturates, CE was also used in combination with UV detection at single wavelength [51,73]. Ferslew et al. preferred MECC for separation after SPE [73]. Li and Weber used SPME for fast sample preparation followed by classical CE [51].

2.2.4. Calcium channel blockers of the dihydropyridines type

Calcium channel blockers, formerly named calcium antagonists, cover three main types, the phenylalkylamines (e.g. verapamil), the benzothiazepines (e.g. diltiazem) and the dihydropyridines (e.g. nifedipine). They are used in the treatment of cardiac dysrhythmias, angina and/or hypertension. In overdose case, they may lead to severe cardiovascular disorders like hypotension and shock, possibly resulting in life threatening situations. For diagnosis or even more important for differential diagnostic exclusion of such an intoxication, a screening procedure is necessary for the detection of these drugs in urine prior to quantification in plasma. The phenylalkylamines and the benzothiazepines and their metabolites can be detected within the STA procedure of Maurer [1,84] for basic and neutral compounds. The dihydropyridines are excreted only in minor amounts as parent compounds [85–99]. Most of the urinary metabolites are acidic compounds, so that they should be detectable in screening procedures for acidic drugs and/or metabolites, as described in Section 2.2.4.1. If necessary, quantification in plasma can be performed using GC–MS [100–103], GC [94,104–107], HPLC [108–111],

LC–MS–MS [112,113] or modifications of these procedures. Enantioselective determination was reviewed by Tokuma and Noguchi [114].

2.2.4.1. GC–MS procedures. Maurer and Arlt have recently published a GC–MS procedure, that allows the detection of therapeutic concentrations of most of the dihydropyridines calcium channel blockers or their metabolites in human urine after extractive methylation [12]. The principal information is summarized in Table 9. This procedure is part of a comprehensive STA procedure for the detection of various classes of acidic drugs and/or their metabolites (cf. Section 3).

2.2.5. Diuretics

Diuretics are misused mainly in attempt to reduce the body weight. The resulting hypokalemia may lead to severe cardiac disorders. Toxicological screening for diuretics should be performed before extensive diagnostic work is started. Diuretics are also misused for doping reasons and, therefore, they have been banned by the IOC. For both indications, screening is necessary. In 1992, Maurer [1] already reviewed several GC–MS procedures for diuretics, e.g., after extractive methylation [53]. In 1996, Riekkola and Jumppanen [38] and Nguyen and Siegler [39] reviewed CE procedures and finally, Ventura and Segura [37] reviewed different detection procedures for diuretics. Therefore, only procedures published later than 1995 are considered here.

2.2.5.1. LC–MS procedures. Garbis et al. have recently published an atmospheric pressure chemical ionization (APCI) electrospray LC–MS procedure, that allows the detection of three thiazide-based diuretics in equine urine [25]. The principal information is summarized in Table 10. This paper presents a nice LC–MS application, but as the authors themselves stated, the only advantage of LC–MS over GC–MS is, that derivatization is not needed. So at least for practical reasons, a comprehensive GC–MS procedure after (extractive) methylation [53,115–117] should be preferred, that covers simultaneously most of the diuretics with series of other drugs relevant in clinical and forensic toxicology or doping (cf. Section 3 and Refs. [2,9–12,40,56–58,64]).

2.2.6. Hypoglycemic sulfonylureas

Sulfonylurea drugs have been used since the1950s in the treatment of hyperglycemia in diabetes mellitus. Besides this therapeutic use, sulfonylureas are also misused. For differential diagnosis of unclear hypoglycemia, screening is necessary to allow differentiation between a surreptitious misuse of sulfonylureas or pathophysiological reasons like insulinoma. Before exploratory surgery or even subtotal pancreatectomy, misuse of hypoglycemic sulfonylurea drugs should be analytically excluded. Several LC or CE procedures have been published in the last years for screening, confirmation and/or quantification. They are described in Sections 2.2.6.1–2.2.6.2 and concisely summarized in Tables 11 and 12. If necessary, the identified sulfonylureas can be quantified, e.g., by HPLC [118–121]. Susanto and Reinauer [122] stated, that their LC–MS method can be used after screening for ‘prequantification’.

2.2.6.1. LC procedures. Sulfonylureas can sufficiently be separated by RP chromatography. DADs were applied for the general screening procedures, which cover sulfonylureas as well as other drugs [14,19]. As already mentioned, the use of UV at single wavelength [123] is critical for toxicological analysis due to lower specificity. The LC–MS procedure of Susanto and Reinauer [122] shows also a moderate specificity, because they use only one ion per compound for SIM detection. Concerning quantification, they stated, that their LC–MS method can be used for ‘prequantification’. It is incomprehensible, why the referees and editors have accepted, that the title indicates ‘simultaneous quantitative measurement’. As already cited elsewhere [70], this is the only reviewed paper, in which neither the length nor the diameter of the column were reported.

2.2.6.2. CE procedures. MECC was described for detection of sulfonylureas with DAD or UV detection [74]. However, as the authors have seen afterwards, this procedure was not suitable for screening of sulfonylurea drugs of the third generation, since these are excreted in an almost completely metabolized form. Therefore, 2 years later the same

working group published a modified MECC procedure for the detection of the metabolites of sulfonylureas of the third generation [75]. Fig. 2 shows MECC analysis of sulfonylureas and one metabolite with scanning DAD. This example shows, that in urinalysis the authors should always show, that their validated 'pure substance' procedure is really suitable for analysis of authentic urine samples.

2.2.7. Non-steroidal anti-inflammatory drugs (NSAIDs)

Non-opioid analgesics are among the most commonly consumed over-the-counter preparations all over the world. Besides acetylsalicylic acid, paracetamol and pyrrazole derivatives, so-called non-steroidal anti-inflammatory drugs (NSAIDs) are used against acute and chronic pain, inflammation or fever. Although NSAIDs are perceived to be safe drugs, they may lead to severe toxic effects in case of acute overdosage or in case of chronic abuse. They are also misused in doping of humans and horses. Therefore, they may be encountered in clinical and forensic toxicological analysis, as well as in doping control. Analysis of such non-opioid analgesics was recently reviewed by Kraemer and Maurer [124].

In the following, only the non-steroidal anti-inflammatory drugs (NSAIDs) are discussed. NSAIDs are classified in arylacetic acid derivatives like indomethacin or diclofenac, arylpropionic acid derivatives like ibuprofen, naproxen or ketoprofen, or oxicames like piroxicam. All these drugs have acidic properties due to carboxyl groups or keto enol tautomery. Many of the NSAIDs are chiral drugs, but most often marketed as racemates. It is known that the enantiomers have different pharmacodynamic

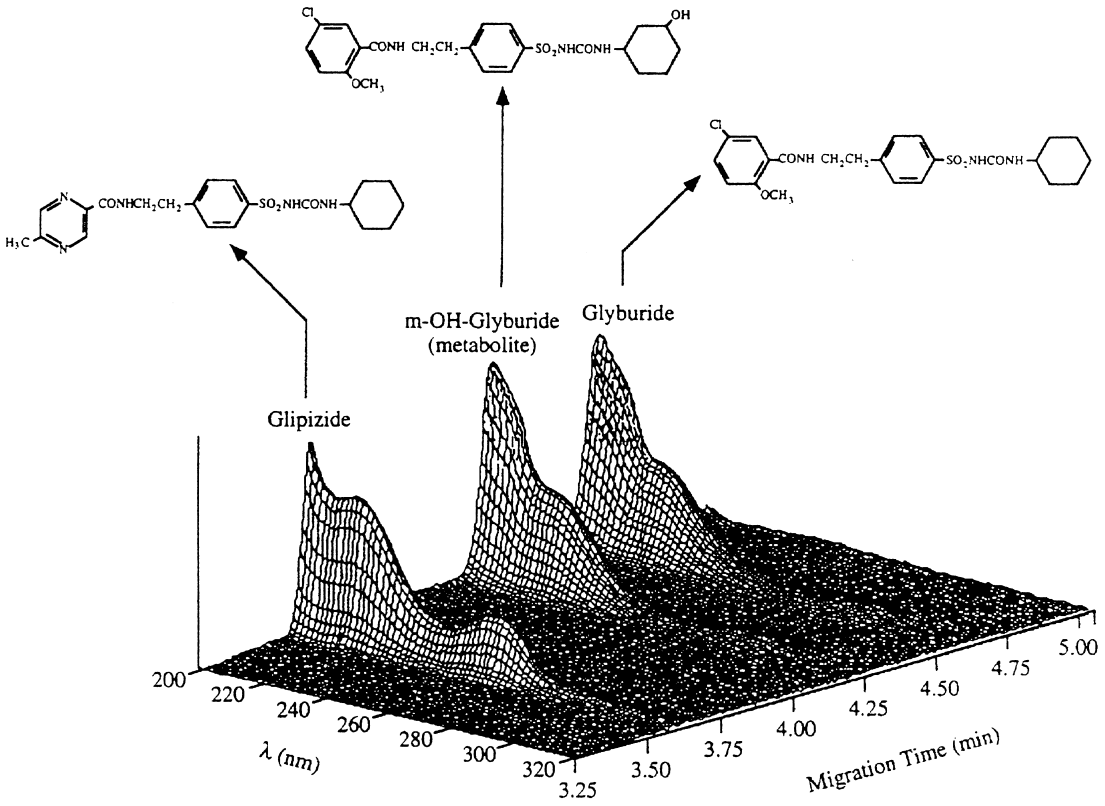

Fig. 2. MECC analysis of the sulfonylureas glipizide, glyburide and hydroxy glyburide in urine with three-dimensional absorbance spectral profile using scanning DAD (taken from Ref. [75]).

and pharmacokinetic properties. The anti-inflammatory activity of NSAIDs has been shown to be largely stereospecific for the *S*-enantiomers [125]. However, this stereoselectivity of action is not manifest in vivo, due to the thus-far-unique unidirectional metabolic inversion of the chiral centre from the inactive *R*(−)-isomers to the *S*(+)-antipodes [126]. Nevertheless, series of enantioselective determination procedures were published, which were reviewed by Davies [127] and Bhushan and Joshi [128]. A review on standards of laboratory practice in analgesics drug monitoring, including colorimetric and immunochemical tests as well as chromatographic procedures, was published by White and Wong [129]. Several GC–MS, LC, CE or TLC procedures have been published in the last years for screening, confirmation and/or quantification. They are described in Sections 2.2.7.1–2.2.7.4 and concisely summarized in Tables 13–16. Further procedures, e.g., for quantification, were recently reviewed by Kraemer and Maurer [124].

2.2.7.1. GC and GC–MS. Derivatization of NSAIDs before GC is recommended to improve chromatographic properties and to avoid thermal decarboxylation in the injection port of the GC. Most often, methylation after extraction is used [22–24,61], but extractive methylation was also applied [55]. Silylation as an alternative for methylation was studied for 26 NSAIDs [62,63]. It is surprising, that Gaillard and Pepin did not describe derivatization of these NSAIDs they want to measure by GC–MS, even in hair samples [34].

In all recent papers, GC is coupled with MS, providing good sensitivity and best specificity. Only Kim et al. still used FID in their earlier paper [62]. The validation of this procedure was measured only using pure substance solutions, but every bioanalyst knows that this is not sufficient. The procedures of Laakkonen et al. [22] and Gaillard and Pepin [34] allow simultaneous detection of other acidic drugs like barbiturates.

2.2.7.2. LC procedures. RP HPLC procedures for screening of NSAIDs have been published. DADs were applied for general screening procedures, which also covered other drugs [14,19]. As already mentioned, the use of UV at single wavelength [47] is critical for toxicological analysis due to low specificity. As already mentioned, enantioselective procedures were reviewed by Davies [127] and Bhushan and Joshi [128].

2.2.7.3. TLC procedures. The general TLC procedure of Iten also includes screening of NSAIDs [71]. A rather singular MS–MS detection of NSAIDs separated by TLC was described by Morden and Wilson [72].

2.2.7.4. CE procedures. For detection of NSAIDs after CE separation, DAD or MS were applied [33,76,130]. As shown in Fig. 3, the glucuronides of naproxen (NG), *O*-demethyl naproxen (ODNG) and ketoprofen (KG), as well as ketoprofen itself, could directly be detected in urine besides biomolecules like creatinine, hippuric acid (HIP) and uric acid (U). Nevertheless, it is questionable, whether CE–MS procedures will routinely be used in the near future, if NSAIDs can easily be analyzed by GC–MS, e.g., after extractive methylation.

3. Systematic toxicological analysis procedures for simultaneous detection of several classes of acidic drugs and/or metabolites

The screening strategy of STA must be very extensive, because several thousands of drugs or pesticides should be considered. Today, only GC–MS and HPLC–DAD provide the needed separation power coupled with high universality and specificity. Therefore, only such procedures are mentioned in this Section. Most of the published STA procedures cover only basic (and neutral) drugs, which are the most important toxicants [1,2,7,8,14,15,34,42,131,132]. As described in Section 2, some classes of acidic drugs like the cardiovascular drugs ACE inhibitors and AT-II blockers, dihydropyridine calcium channel blockers (metabolites), diuretics, coumarin anticoagulants, hypoglycemic sulfonylureas, barbiturates, or non-steroidal anti-inflammatory drugs (NSAIDs) are relevant to clinical and forensic toxicology or doping. Therefore, these acidic drugs should also be monitored, ideally in one procedure.

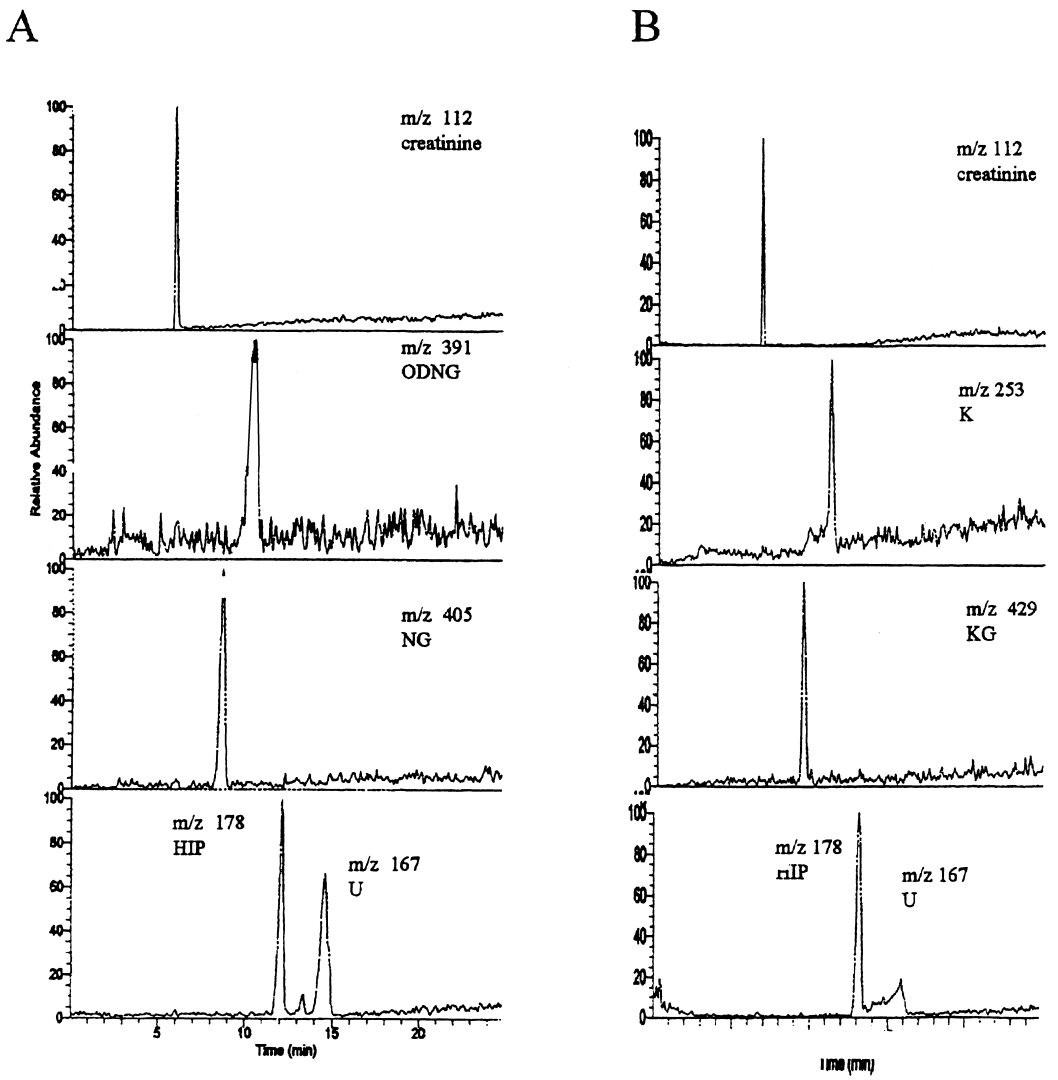

Fig. 3. CE–MS detection of the glucuronides of naproxen (NG), *O*-demethyl naproxen (ODNG) and ketoprofen (KG), as well as ketoprofen itself, could directly be detected in urine besides biomolecules like creatinine, hippuric acid (HIP) and uric acid (U) (taken from Ref. [76]).

3.1. GC–MS procedures

Maurer et al. have developed a comprehensive GC–MS screening procedure for the detection of acidic drugs, poisons and/or their metabolites in urine after extractive methylation. The analytes were separated by capillary GC and identified by computerized MS in the full-scan mode. Using mass chromatography with selective ions, the possible presence of acidic drugs and/or their metabolites could be indicated (cf. Fig. 4). The identity of positive signals in such mass chromatograms was confirmed by comparison of the peaks underlying full-mass spectra with the reference spectra [9,64] recorded during the corresponding study (cf. Fig. 5). This method allowed the detection in urine of most of the ACE inhibitors and AT-II antagonists [40], of coumarin anticoagulants of the first generation [10], dihydropyridine calcium channel blockers [12], barbiturates [57], diuretics [59], hypoglycemic sul-

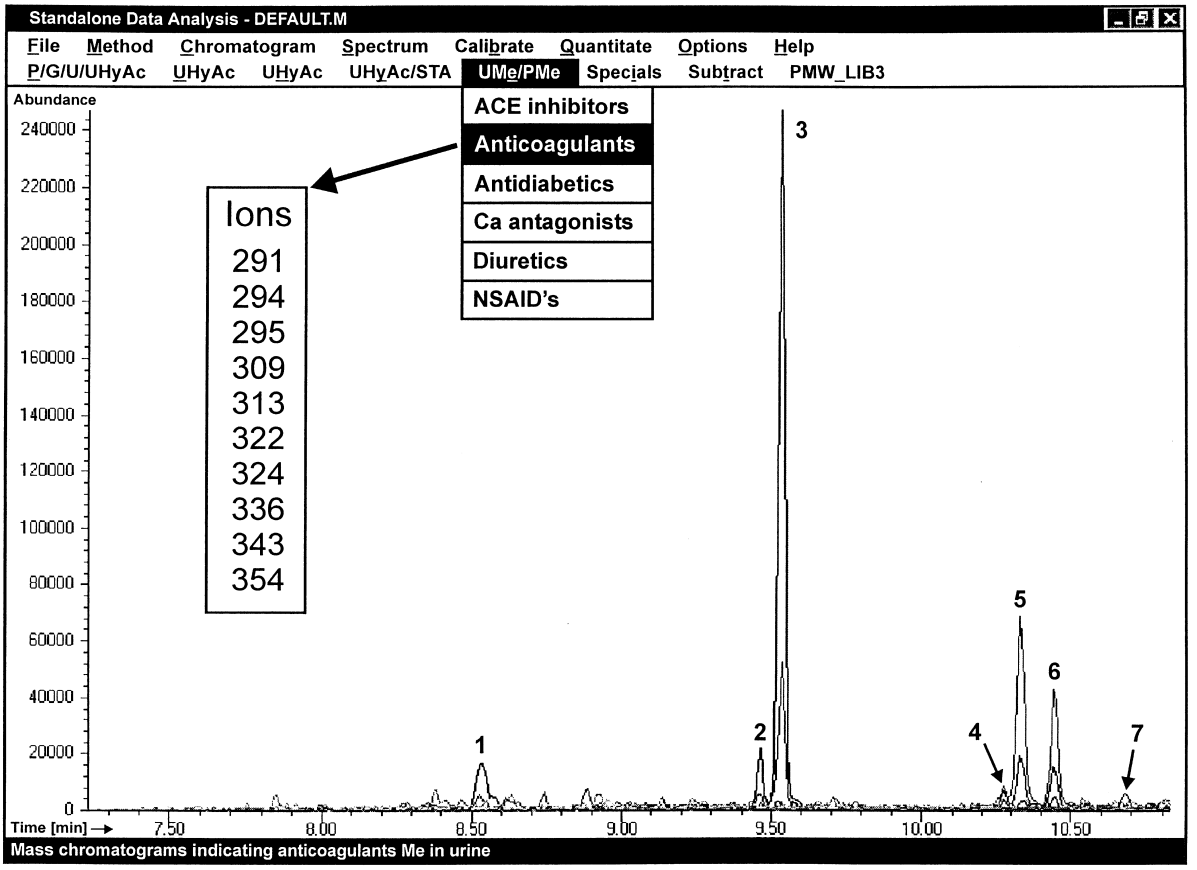

Fig. 4. Typical mass chromatograms with the ions *m*/*z* 291, 294, 295, 309, 313, 322, 324, 336, 343 and 354 (generation of the mass chromatograms can be started by clicking the corresponding pull down menu item, which executes the user defined macros). The shown mass chromatograms indicate the presence of phenprocoumon and its metabolites in a methylated extract of a urine sample taken from a patient after ingestion of a therapeutic dose (3 mg/day) of phenprocoumon. Peak 1 indicates an endogenous biomolecule. Peaks 2–7 indicate the presence of phenprocoumon and its metabolites. The merged chromatograms can be differentiated by their colours on a color screen (taken from Ref. [10]).

fonylureas (sulfonamide part) [58], NSAIDs [11], and various other acidic compounds [57]. Mass spectra of all these drugs and metabolites (methylated and silylated) are included in Ref. [9,64] for specific detection by library search. At least the higher dosed drugs could also be detected in plasma samples after extractive methylation.

3.2. LC–DAD procedures

Drummer et al. [14] as well as Lo et al. [19] have independently developed comprehensive LC–DAD screening procedures for the detection of barbiturates, diuretics, NSAIDs, sulfonylureas, some anticonvulsants and theophylline in blood. The authors stated, that their procedures also allow quantification. However, in cases of doubt, GC–MS confirmation should follow because of its higher confidence.

4. Conclusions and perspectives

Most of the STA procedures only cover basic (and neutral) drugs, which are the more important toxicants. Nevertheless, some classes of acidic drugs like the cardiovascular drugs ACE inhibitors and AT-II blockers, dihydropyridine calcium channel blockers (metabolites), diuretics, coumarin anticoagulants,

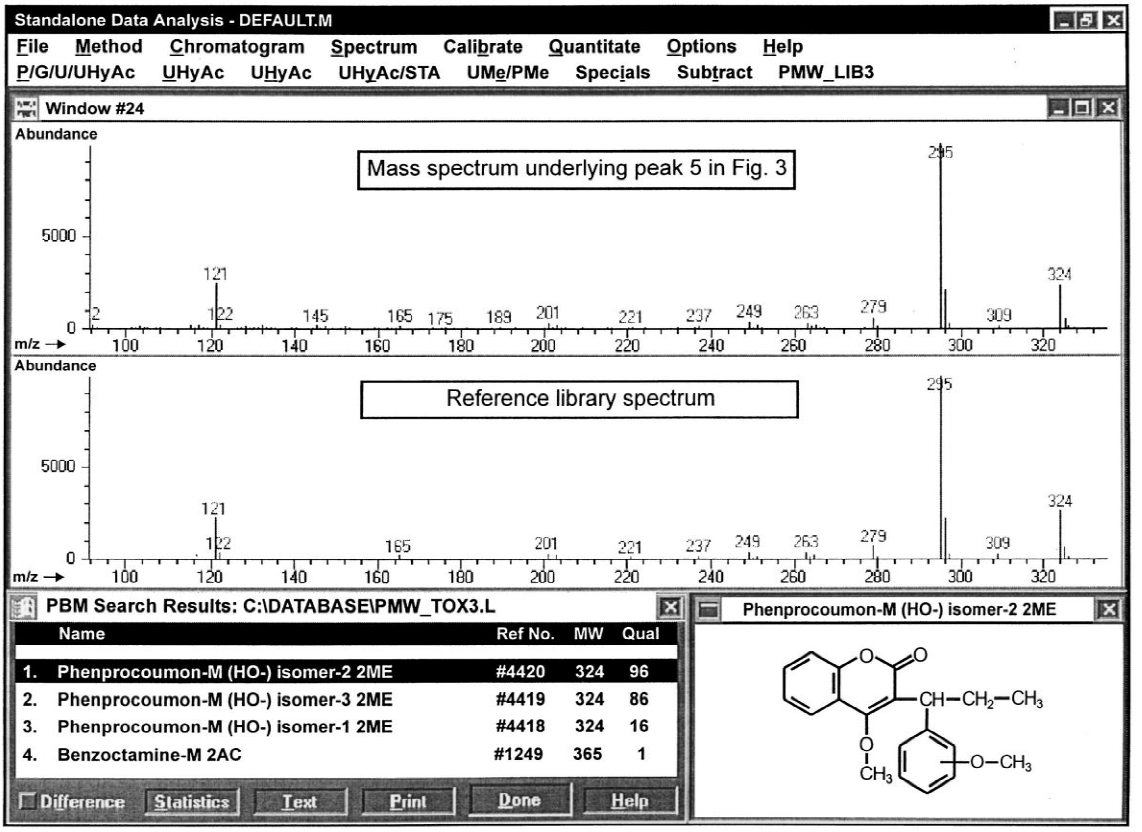

Fig. 5. Mass spectrum underlying the peak 5 in Fig. 4, the reference spectrum, the structure and the hit list found by computer library search (taken from Ref. [10]).

hypoglycemic sulfonylureas, barbiturates, or non-steroidal anti-inflammatory drugs (NSAIDs) have to be screened in clinical and forensic toxicology or doping control.

GC–MS, especially in the electron-impact full-scan mode, is still the method of choice for STA providing best separation power, specificity and universality. Extractive methylation has proved to be an efficient sample preparation procedure for acidic drugs, since both work-up steps, necessary for GC–MS, can be performed simultaneously. Solid phase microextraction may be used in some cases for fast work-up, if derivatization is not necessary.

LC–DAD is also often used for STA, but its separation power and its specificity are still inferior to those of GC–MS, at least in the full-scan EI mode. However, compounds not volatile in GC can be covered. LC–MS coupling has still rarely been used for STA, not only because these apparatus are not yet so widely available, but also because the specificity of the electrospray ionization is inferior to that of classical EI, but LC–MS–MS will overcome these disadvantages.

Today TLC is only rarely used for STA, since TLC results must be confirmed in any case. Procedures doing screening and confirmation in one step like GC–MS are preferred.

CE techniques are not yet widely used in analytical toxicology due to limitations in terms of reproducibility and concentration sensitivity. When these problems will be solved, CE especially coupled with DAD or MS(–MS) may also be useful in STA.

Even if immunoassays are more and more used for screening, STA is still one of the greatest challenges to clinical or forensic toxicologists as well as for doping controllers. If the analytical techniques will

further markedly be improved, STA and simultaneous quantification will be performed in the future even in small amounts of blood.

5. List of abbreviations

ACE	angiotensin-converting enzyme
ACN	acetonitrile
a.o.	and other drugs
APCI	atmospheric pressure chemical ionization
AT-II	angiotensin receptor II blocker
B	blood
CE	capillary electrophoretic
DAD	(photo) diode array detector/detection
DCCI	2,6-dichloroquinone-4-chloroimide
EI	electron impact ionization
EN	enzymatic cleavage of conjugates
ET	ethylation
EX-ME	extractive methylation
EX-PR	extractive propylation
FI	fragment ion
FID	flame-ionization detector/detection
FL	fluorescence detector/detection
FSC	fused silica capillary
GC	gas chromatography
GC–MS	gas chromatography–mass spectrometry
HPLC	high-performance liquid chromatography
HY	acidic or alkaline hydrolysis
LC–MS	liquid chromatography–mass spectrometry
LIN	linearity
LLE	liquid–liquid extraction
LOD	limit of detection
m/z	mass to charge ratio
ME	methylation
MeOH	methanol
MECC	micellar electrokinetic capillary chromatography
MS	mass spectrometry, mass spectral, mass spectrum
NPD	nitrogen–phoshorus detector/detection
NSAID	non-steroidal anti-inflammatory drug
P	plasma
PCI	positive chemical ionization
REC	recovery
RI	retention index
RP	reversed phase
RT	retention time
S	serum
SDS	sodium dodecyl sulfate
SFC	supercritical fluid extraction
SIM	selected ion monitoring
SPE	solid-phase extraction
SPME	solid-phase microextraction
STA	systematic toxicological analysis
TBDMS	*tert*.-butyldimethylsilylated
TLC	thin-layer chromatography
TMS	trimethylsilylated
U	urine

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References

[1] H.H. Maurer, J. Chromatogr. 580 (1992) 3.
[2] H.H. Maurer, J.W. Arlt, T. Kraemer, C.J. Schmitt, A.A. Weber, Arch. Toxicol. 19 (Suppl.) (1997) 189.
[3] T. Kraemer, H.H. Maurer, J. Chromatogr. B 713 (1998) 163.
[4] M.R. Moeller, S. Steinmeyer, T. Kraemer, J. Chromatogr. B 713 (1998) 91.
[5] D.A. Kidwell, J.C. Holland, S. Athanaselis, J. Chromatogr. B 713 (1998) 111.
[6] H. Sachs, P. Kintz, J. Chromatogr. B 713 (1998) 147.
[7] A. Polettini, A. Groppi, C. Vignali, M. Montagna, J. Chromatogr. B 713 (1998) 265.
[8] A. Solans, M. Carnicero, R. de la Torre, J. Segura, J. Anal. Toxicol. 19 (1995) 104.
[9] K. Pfleger, H.H. Maurer, A. Weber, in: Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and their Metabolites, Wiley, Weinheim, 1999.
[10] H.H. Maurer, J.W. Arlt, J. Chromatogr. B 714 (1998) 181.
[11] H.H. Maurer, T. Kraemer, F.X. Tauvel, J. Anal. Toxicol. (in preparation) (1999).
[12] H.H. Maurer, J.W. Arlt, J. Anal. Toxicol. 23 (1999) 73.
[13] A. Polettini, J. Anal. Toxicol. 20 (1996) 579.
[14] O.H. Drummer, A. Kotsos, I.M. McIntyre, J. Anal. Toxicol. 17 (1993) 225.
[15] M. Balikova, Sb. Lek. 95 (1994) 339.
[16] E.M. Koves, J. Chromatogr. A 692 (1995) 103.
[17] R.D. Maier, M. Bogusz, J. Anal. Toxicol. 19 (1995) 79.
[18] Y. Gaillard, G. Pepin, J. Chromatogr. A 763 (1997) 149.

[19] D.S. Lo, T.C. Chao, S.E. Ng Ong, Y.J. Yao, T.H. Koh, Forensic Sci. Int. 90 (1997) 205.
[20] W.E. Lambert, J.F. Van Bocxlaer, A.P. De Leenheer, J. Chromatogr. B 689 (1997) 45.
[21] C.K. Lai, T. Lee, K.M. Au, A.Y. Chan, Clin. Chem. 43 (1997) 312.
[22] U.M. Laakkonen, A. Leinonen, L. Savonen, Analyst 119 (1994) 2695.
[23] G. Gonzalez, R. Ventura, A.K. Smith, R. de la Torre, J. Segura, J. Chromatogr. A 719 (1996) 251.
[24] S. Cardenas, M. Gallego, M. Valcarcel, R. Ventura, J. Segura, Anal. Chem. 68 (1996) 118.
[25] S.D. Garbis, L. Hanley, S. Kalita, J. AOAC Int. 81 (1998) 948.
[26] T. Chalermchaikit, L.J. Felice, M.J. Murphy, J. Anal. Toxicol. 17 (1993) 56.
[27] E.A. Kuijpers, J. den Hartigh, T.J. Savelkoul, F.A. de Wolff, J. Anal. Toxicol. 19 (1995) 557.
[28] H. Lotfi, M.F. Dreyfuss, P. Marquet, J. Debord, L. Merle, G. Lachatre, J. Anal. Toxicol. 20 (1996) 93.
[29] P.J. Berny, T. Buronfosse, G. Lorgue, J. Anal. Toxicol. 19 (1995) 576.
[30] F. Coudore, J.M. Alazard, M. Paire, G. Andraud, J. Lavarenne, J. Anal. Toxicol. 17 (1993) 109.
[31] R. Meatherall, J. Forensic Sci. 42 (1997) 1160.
[32] E. Tanaka, M. Terada, K. Tanno, S. Misawa, C. Wakasugi, Forensic Sci. Int. 85 (1997) 73.
[33] R.T. Perez, L.C. Martinez, A. Sanz, E. Bravo, J. Chromatogr. B 708 (1998) 249.
[34] Y. Gaillard, G. Pepin, J. Chromatogr. A 762 (1997) 251.
[35] I. Rengel, A. Friedrich, Vet. Res. Commun. 17 (1993) 421.
[36] A. Jones, Bull. Environ. Contam. Toxicol. 56 (1996) 8.
[37] R. Ventura, J. Segura, J. Chromatogr. B 687 (1996) 127.
[38] M.L. Riekkola, J.H. Jumppanen, J. Chromatogr. A 735 (1996) 151.
[39] N.T. Nguyen, R.W. Siegler, J. Chromatogr. A 735 (1996) 123.
[40] H.H. Maurer, T. Kraemer, J.W. Arlt, Ther. Drug Monit. 20 (1998) 706.
[41] F. Kees, D. Jehnich, H. Grobecker, J. Chromatogr. B 677 (1996) 172.
[42] P. Lillsunde, L. Michelson, T. Forsstrom, T. Korte, E. Schultz, K. Ariniemi, M. Portman, M.L. Sihvonen, T. Seppala, Forensic Sci. Int. 77 (1996) 191.
[43] H.H. Maurer, in: K. Pfleger, H.H. Maurer, A. Weber (Eds.), Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and their Metabolites, VCH, Weinheim, 1992, p. 3.
[44] L.H. Spahn, L.Z. Benet, Drug Metab. Rev. 24 (1992) 5.
[45] S.W.H. Toennes, H.H. Maurer, in: S.D. Ferrara (Ed.), Proceeding of the XXXVth TIAFT Annual Meeting, Padova, August 1997, Cleup Editrice, Padova, 1997, p. 227.
[46] S.W.H. Toennes, H.H. Maurer, Clin.Chem. (1999) submitted.
[47] T. Hirai, S. Matsumoto, I. Kishi, J. Chromatogr. B 692 (1997) 375.
[48] M.J. Bogusz, R.D. Maier, B.K. Schiwy, U. Kohls, J. Chromatogr. B 683 (1996) 177.
[49] J.P. Franke, R.A. de Zeeuw, J. Chromatogr. B 713 (1998) 51.
[50] B.J. Hall, J.S. Brodbelt, J. Chromatogr. A 777 (1997) 275.
[51] S. Li, S.G. Weber, Anal. Chem. 69 (1997) 1217.
[52] O. Gyllenhaal, U. Tjärnlund, H. Ehrsson, P. Hartvig, J. Chromatogr. 156 (1978) 275.
[53] A.M. Lisi, R. Kazlauskas, G.J. Trout, J. Chromatogr. 581 (1992) 57.
[54] W.A. Joern, J. Anal. Toxicol. 18 (1994) 423.
[55] L. Rivier, C. Grunauer, M. Saugy, in: R.K. Mueller (Ed.), Proceedings of the XXX1st International TIAFT Meeting, Leipzig, August 1993, Molinapress, Leipzig, 1994, p. 419.
[56] H.H. Maurer, T. Kraemer, J. Chromatogr. B (in preparation) (1999).
[57] H.H. Maurer, T. Kraemer, Ther. Drug Monit. (in preparation) (1999).
[58] H.H. Maurer, T. Kraemer, J.W. Arlt, J. Chromatogr. B (in preparation) (1999).
[59] H.H. Maurer, T. Kraemer, Ther. Drug Monit. (in preparation) (1999).
[60] R.H. Liu, A.M. McKeehan, C. Edwards, G. Foster, W.D. Bensley, J.G. Langner, A.S. Walia, J. Forensic Sci. 39 (1994) 1504.
[61] H.H. Maurer, T. Kraemer, A. Weber, Pharmazie 49 (1994) 148.
[62] K.R. Kim, W.H. Shim, Y.J. Shin, J. Park, S. Myung, J. Hong, J. Chromatogr. 641 (1993) 319.
[63] K.R. Kim, H.R. Yoon, J. Chromatogr. B 682 (1996) 55.
[64] K. Pfleger, H.H. Maurer, A. Weber, in: Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants and their Metabolites, Hewlett-Packard, Palo Alto, CA, 1999.
[65] J. Segura, R. Ventura, C. Jurado, J. Chromatogr. B 713 (1998) 61.
[66] R. Pocci, V. Dixit, V.M. Dixit, J. Anal. Toxicol. 16 (1992) 45.
[67] A. Namera, M. Yashiki, K. Okada, Y. Iwasaki, M. Ohtani, T. Kojima, J. Chromatogr. B 706 (1998) 253.
[68] T. Kojima, T. Taniguchi, M. Yashiki, T. Miyazaki, Y. Iwasaki, T. Mikami, M. Ohtani, Int. J. Legal. Med. 107 (1994) 21.
[69] I. Rasanen, I. Ojanpera, E. Vuori, T.A. Hase, J. Chromatogr. A 738 (1996) 233.
[70] H.H. Maurer, J. Chromatogr. B 713 (1998) 3.
[71] P.X. Iten, R.K. Mueller (Eds.), Proceedings of the XXX1st International TIAFT Meeting, Leipzig, August 1993, Molinapress, Leipzig, 1994, p. 299.
[72] W. Morden, I.D. Wilson, Rapid Commun. Mass Spectrom. 10 (1996) 1951.
[73] K.E. Ferslew, A.N. Hagardorn, W.F. McCormick, J. Forensic Sci. 40 (1995) 245.
[74] M. Nunez, J.E. Ferguson, D. Machacek, G. Jacob, R.P. Oda, G.M. Lawson, J.P. Landers, Anal. Chem. 67 (1995) 3668.
[75] M.E. Roche, R.P. Oda, G.M. Lawson, J.P. Landers, Electrophoresis 18 (1997) 1865.
[76] S. Heitmeier, G. Blaschke, J. Chromatogr. B 721 (1999) 109.
[77] F. Tagliaro, S. Turrina, P. Pisi, F.P. Smith, M. Marigo, J. Chromatogr. B 713 (1998) 27.
[78] W. Hammes, B. Hammes, U. Buchsler, F. Paar, H. Bokens, J. Chromatogr. B 670 (1995) 81.
[79] H. Shioya, M. Shimojo, Y. Kawahara, Biomed. Chromatogr. 6 (1992) 59.

[80] N. Goto, T. Kamata, K. Ikegami, J. Chromatogr. 578 (1992) 195.
[81] C.I. Furtek, M.W. Lo, J. Chromatogr. 573 (1992) 295.
[82] H.H. Maurer, J. Chromatogr. 530 (1990) 307.
[83] J.C. Spell, K. Srinivasan, J.T. Stewart, M.G. Bartlett, Rapid Commun. Mass Spectrom. 12 (1998) 890.
[84] H.H. Maurer, Arch. Toxicol. 64 (1990) 218.
[85] H. Meyer, D. Scherling, W. Karl, Arzneimittelforschung 33 (1983) 1528.
[86] H. Meyer, E. Wehinger, F. Bossert, D. Scherling, Arzneimittelforschung 33 (1983) 106.
[87] D. Scherling, K. Buhner, H.P. Krause, W. Karl, C. Wunsche, Arzneimittelforschung 41 (1991) 392.
[88] D. Scherling, W. Karl, G. Ahr, H.J. Ahr, E. Wehinger, Arzneimittelforschung 38 (1988) 1105.
[89] D. Scherling, W. Karl, H.J. Ahr, A. Kern, H.M. Siefert, Arzneimittelforschung 41 (1991) 1009.
[90] D. Scherling, W. Karl, M. Radtke, H.J. Ahr, H.M. Siefert, Arzneimittelforschung 42 (1992) 1292.
[91] J. Kann, G.J. Krol, K.D. Raemsch, D.E. Burkholder, M.J. Levitt, J. Cardiovasc. Pharmacol. 6 (Suppl. 7) (1984) S968.
[92] A.P. Beresford, P.V. Macrae, D. Alker, R.J. Kobylecki, Arzneimittelforschung 39 (1989) 201.
[93] A.P. Beresford, P.V. Macrae, D.A. Stopher, Xenobiotica 18 (1988) 169.
[94] A.P. Beresford, P.V. Macrae, D.A. Stopher, B.A. Wood, J. Chromatogr. 420 (1987) 178.
[95] A.P. Beresford, D. McGibney, M.J. Humphrey, P.V. Macrae, D.A. Stopher, Xenobiotica 18 (1988) 245.
[96] D.A. Stopher, A.P. Beresford, P.V. Macrae, M.J. Humphrey, J. Cardiovasc. Pharmacol. 12 (Suppl. 7) (1988) S55.
[97] C. Baarnhielm, A. Backman, K.J. Hoffmann, L. Weidolf, Drug Metab. Dispos. 14 (1986) 613.
[98] K.D. Raemsch, J. Sommer, Hypertension 5 (1983) II18.
[99] D.R. Abernethy, J.B. Schwartz, Clin. Pharmacokinet. 15 (1988) 1.
[100] C. Jean, R. Laplanche, J. Chromatogr. 428 (1988) 61.
[101] Y. Tokuma, T. Fujiwara, H. Noguchi, J. Chromatogr. 345 (1985) 51.
[102] J.D. Dru, J.Y. Hsieh, B.K. Matuszewski, M.R. Dobrinska, J. Chromatogr. B 666 (1995) 259.
[103] Y. Tokuma, T. Fujiwara, H. Noguchi, Biomed. Environ. Mass. Spectrom. 13 (1986) 251.
[104] M.T. Rosseel, M.G. Bogaert, L. Huyghens, J. Chromatogr. 533 (1990) 224.
[105] J. Van Harten, M.T. Lodewijks, J.W. Guyt Scholten, P. Van Brummelen, D.D. Breimer, J. Chromatogr. 423 (1987) 327.
[106] P.A. Soons, D.D. Breimer, J. Chromatogr. 428 (1988) 362.
[107] G.J. Krol, A.J. Noe, S.C. Yeh, K.D. Raemsch, J. Chromatogr. 305 (1984) 105.
[108] M. Josefsson, A.L. Zackrisson, B. Norlander, J. Chromatogr. B 672 (1995) 310.
[109] K. Shimooka, Y. Sawada, H. Tatematsu, J. Pharm. Biomed. Anal. 7 (1989) 1267.
[110] J. Boutagy, F. Rumble, F. Dunagan, J. Chromatogr. 487 (1989) 483.
[111] T. Uno, T. Ohkubo, K. Sugawara, J. Chromatogr. B 698 (1997) 181.
[112] W.M. Muck, J. Chromatogr. A 712 (1995) 45.
[113] T. Yasuda, M. Tanaka, K. Iba, J. Mass Spectrom. 31 (1996) 879.
[114] Y. Tokuma, H. Noguchi, J. Chromatogr. A 694 (1995) 181.
[115] R. Ventura, D. Fraisse, M. Becchi, O. Paisse, J. Segura, J. Chromatogr. 562 (1991) 723.
[116] A.M. Lisi, G.J. Trout, R. Kazlauskas, J. Chromatogr. 563 (1991) 257.
[117] D. Carreras, C. Imaz, R. Navajas, M.A. Garcia, C. Rodriguez, A.F. Rodriguez, R. Cortes, J. Chromatogr. A 683 (1994) 195.
[118] A. Igaki, K. Kobayashi, M. Kimura, T. Sakoguchi, A. Matsuoka, J. Chromatogr. 493 (1989) 222.
[119] K.H. Lehr, P. Damm, J. Chromatogr. 526 (1990) 497.
[120] R. Huupponen, K.P. Ojala, J. Rouru, M. Koulu, J. Chromatogr. 583 (1992) 270.
[121] J.R. Valdes Santurio, E. Gonzalez Porto, J. Chromatogr. B 682 (1996) 364.
[122] F. Susanto, H. Reinauer, Fresenius' J. Anal. Chem. 357 (1997) 1202.
[123] A. Sener, A.G. Akkan, W.J. Malaisse, Acta Diabetol. 32 (1995) 64.
[124] T. Kraemer, H.H. Maurer, in: M. Bogusz (Ed.), Forensic Toxicology, Handbook of Analytical Separations, vol. 6, Amsterdam, Elsevier, 1999, in press.
[125] J. Caldwell, A.J. Hutt, S. Fournel-Gigleux, Biochem. Pharmacol. 37 (1988) 105.
[126] M. Careri, P. Manini, G. Mori, Anal. Proc. 32 (1995) 129.
[127] N.M. Davies, J. Chromatogr. B 691 (1997) 229.
[128] R. Bhushan, S. Joshi, J. Planar Chromatogr. 9 (1996) 70.
[129] S. White, S.H. Wong, Clin. Chem. 44 (1998) 1110.
[130] A.E. Ashcroft, H.J. Major, S. Lowes, I.D. Wilson, Anal. Proc. 32 (1995) 459.
[131] W.E. Lambert, E. Meyer, A.P. De Leenheer, J. Anal. Toxicol. 19 (1995) 73.
[132] A. Tracqui, P. Kintz, P. Mangin, J. Forensic Sci. 40 (1995) 254.