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Review

Chromatographic screening techniques in systematic toxicological analysis

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Abstract

A review of techniques used to screen biological specimens for the presence of drugs was conducted with particular reference to systematic toxicological analysis. Extraction systems of both the liquid–liquid and solid-phase type show little apparent difference in their relative ability to extract a range of drugs according to their physio-chemical properties, although mixed-phase SPE extraction is a preferred technique for GC-based applications, and liquid–liquid were preferred for HPLC-based applications. No one chromatographic system has been shown to be capable of detecting a full range of common drugs of abuse, and common ethical drugs, hence two or more assays are required for laboratories wishing to cover a reasonably comprehensive range of drugs of toxicological significance. While immunoassays are invariably used to screen for drugs of abuse, chromatographic systems relying on derivatization and capable of extracting both acidic and basic drugs would be capable of screening a limited range of targeted drugs. Drugs most difficult to detect in systematic toxicological analysis include LSD, psilocin, THC and its metabolites, fentanyl and its designer derivatives, some potent opiates, potent benzodiazepines and some potent neuroleptics, many of the newer anti-convulsants, alkaloids colchicine, amantins, aflatoxins, antineoplastics, coumarin-based anti-coagulants, and a number of cardiovascular drugs. The widespread use of LC–MS and LC–MS–MS for specific drug detection and the emergence of capillary electrophoresis linked to MS and MS–MS provide an exciting possibility for the future to increase the range of drugs detected in any one chromatographic screening system. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Systematic toxicological analysis; Reviews; Drug screening assays

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1. Introduction

The conduct of an efficient and extensive drug screening procedure is essential for clinical and forensic cases to either exclude the involvement of drugs and poisons in a case, or to detect such substances should they be present. Unfortunately, not all substances can be detected with one drug screening method. The presence of acidic, basic or neutral properties in drugs and the overall drug lipophilicity affects the ability to extract substances from biological matrices, while thermal stability, polarity and detector sensitivity affect the detectability of drugs in chromatographic systems.

The ability to perform a comprehensive and systematic analysis of specimens, for the presence of chemicals of toxicological importance, is termed systematic toxicological analysis (STA). A review of GC–MS procedures for STA was published in 1992 [1]. A review of HPLC techniques using photodiode array detection (DAD) was published in 1995 [2]. The advantages of HPLC coupled to DAD are also reviewed by Lambert et al. [3]. Hoja et al. [4] have reviewed the use of HPLC coupled to MS. De Zeeuw has been a fervent proponent of STA to properly examine a specimen for an unknown substance and has briefly reviewed the selectivity of chromatographic processes [5–7] particularly when used in combination with TLC.

For any screening system, there are limitations with respect to the ability to detect drugs (and other poisons). Awareness of the strengths and the limitations is of critical importance in any systematic analysis of specimens for the presence of drugs. This review examines the relevant literature published since 1990 and reviews generally the advantages and limitations associated with specific chromatographic drug screening methods.

2. Methods

2.1. Choice of references

Refereed articles written in English were searched using the NLM PubMed MedLine database on the Internet from January 1990 to December 1998 using >systematic toxicological analysis< as search string. Methods cited from these references or other methods available to the author were also included which discussed or presented methods that presented broad class screening systems. To limit the scope of this review, this paper is restricted to illicit and ethical drugs, unless poisons are related to known drugs.

2.2. Definitions and terms used

Standard abbreviations used by this Journal are used in the review. Abbreviations used are included in the list of non-standard abbreviations (Table 1).

3. Specimen preparation

3.1. Choice of specimen

The choice of specimen is often dictated by the case being investigated, however the most common specimens used for the screening of drugs are serum/plasma, blood, bile and urine. Blood, plasma and serum can often be interchanged in most methods,

Table 1
List of non-standard abbreviations

ACE	Angiotensin converting enzyme	MLL	Mean list length
ACN	Acetonitrile	MECC	Micellar electro kinetic chromatography
AM	Amphetamine	MeOH	Methanol
B	Blood	MeI	Methyl iodide
BuCl	1-Butylchloride	MSA	Methane sulfonic acid
BE	Benzoylecgonine	MS–MS	Tandem mass spectrometry
BSTFA	*N*,*O*-bis-(trimethylsilyl)-trifluoroacetamide)	MTBSTFA	*N*-methyl-*N*-(*t*-butyldimethylsilyl)-trifluoroacetamide
Carboxy–THC	11-nor-Δ^9-carboxy–THC	NLM	National Library of Medicine
CO	Cocaine	MO	Morphine
CI	Chemical ionisation	MSTFA	*N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide
CN	Cyano	NCI	Negative ion chemical ionisation
CNPP	Cyanopropylphenyl	NPD	Nitrogen phosphorous detector
CSF	Cerebro-spinal fluid	NSAID	Non steroidal anti-inflammatory drug
DAD	Diode-array detector	ODS	Octadecylsilane
d.a.u.	Drugs of abuse	P	Plasma or serum
DCM	Dichloromethane	PI	Positive ion (MS)
DMS	Dimethylpolysiloxane	PM	Post mortem
EIA	Enzyme immunoassay	PMS	Diphenyldimethylsiloxane
EMIT	Enzyme multiplied immunoassay	RIA	Radioimmunoassay
ECD	Electron capture detector	SPE	Solid phase extraction
EI	Electron impact (MS)	STA	Systematic Toxicological Analysis
FSC	Fused silica column	TEAP	Triethyl phosphate
FID	Flame ionisation detector	TEA	Triethylamine
HIV	Human immunodeficiency virus	TMCS	Trimethylchlorosilane
IOC	International Olympic Committee	TMS	Trimethylsilane
L	Liver		
LLX	Liquid liquid extraction		

although postmortem blood will represent problems in most methods due to its higher viscosity than plasma and even clinically-derived blood.

Urine is the most frequent specimen used in most hospital situations and may require hydrolysis prior to the isolation procedure to convert drug conjugates to more easily measurable compounds. Solid specimens such as liver will require some form of homogenization prior to analysis. Details for the preparation of liver homogenates can be obtained elsewhere [8–10].

3.2. Hydrolysis conditions

The choice of optimum hydrolysis conditions for glucuronide conjugates depends very much on the drug or drug metabolite. Abused drugs most likely to be excreted as hydrolyzable conjugates include particularly many of the benzodiazepines and morphine (heroin). A review of the conditions required for benzodiazepines has recently been reviewed [11]. Acid hydrolysis (heat with concentrated HCl for 30 min) has been used to liberate conjugates and to improve recovery for highly protein-bound drugs [12].

Variations include slightly lower or higher temperatures, the amount and source of enzyme used, the pH of buffer and time of incubation. When quantitative hydrolysis is required, it is recommended that individual variations be properly validated for each drug or poison.

4. Extraction techniques

With few exceptions, chromatographic techniques require some form of isolation procedure to separate the drugs from a biological matrix. These procedures can be separated into 3 distinct types:

(a) liquid–liquid extraction,
(b) solid-phase extraction, and
(c) other techniques.

Table 2
Selected summary of extractabilities of drugs using liquid–liquid extraction

Reference	Extraction conditions	Recoveries of selected drugs	
[13,14]	Blood treated with saturated NH_4Cl and extracted with ethyl acetate (GC and HPLC)	Paracetamol	73/24
		Phenobarbital	84/118
		Frusemide	60/-
		Naproxen	95/-
		Carbamazepine	78/40
		Warfarin	75/-
		Gliclazide	76/-
		Theophylline	-/17
		Diazepam	-/109
[19]	Blood: acidic drugs extracted from NaH_2PO_4 buffer with toluene/ethyl acetate (4:1) and basic drugs from pH 10.5 with DCM/toluene (1:9)	Codeine	92
		Morphine	66
		Benzoylecgonine	84
		Cocaine	81
[59]	Blood treated with Tris buffer, pH 9.2 and extracted with *n*-butyl chloride	Amphetamine	67
		Ephedrine	69
		Pentobarbital	18
		Methadone	76
		Amitriptyline	67
		Oxazepam	70
		Thioridazine	72
		Promethazine	90
		Cocaine	86
		Doxylamine	96
[62]	Blood is diluted with ammonia solution and extracted with diethyl ether	Amitriptyline	86
		Propoxyphene	81
		Methadone	72
		Methaqualone	102
		Diazepam	92
		Thioridazine	70
		Verapamil	80
[117]	Blood treated with bicarbonate buffer and extracted with butyl acetate, no concentration step	Amitriptyline	73
		Cocaine	74
		Codeine	70
		Diazepam	82
		Lignocaine	75
		Methadone	73
		Methaqualone	76
[89]	Plasma treated with bicarbonate and extracted with hexane. Extracts basic and neutral drugs.	Amitriptyline	90
		Chlorpheniramine	94
		Methadone	86
		Propoxyphene	86
		Thioridazine	90
[16]	Blood treated with NH_3 and extracted with toluene. Basic drugs re-extracted from toluene (fraction) and acidic drugs extracted from aqueous layer with toluene (fraction A).	Codeine	79
		Diazepam	84
		Haloperidol	80
		Propranolol	83
		Trazodone	81
		Verapamil	60

(Cont.)

Table 2. Continued.

Reference	Extraction conditions	Recoveries of selected drugs	
[73]	Blood treated with acetonitrile. Supernatant is chromatographed directly. Most drugs extracted.	Paracetamol	75
		Salicylic acid	100
		Carbamazepine	90
		Chlorpropamide	94
		Naproxen	75
		Theophylline	25
		Phenobarbital	43
		Frusemide	31
		Warfarin	51
		Diazepam	50
[2]	Blood treated with pH 9.5 saturated ammonium chloride solution and chloroform/propan-2-ol/heptane (60:14:26). Solvent evaporated and residue reconstituted. Most drug types detected.	Benzodiazepines, barbiturates, anti-depressants, neuroleptics, beta-blockers and opiates have recoveries >60%. Other drug groups such as cyclopyrrolones, imidazopydridines, anti-histamines and NSAIDS also detected.	

4.1. Liquid–liquid extraction

This has been the traditional method for isolating drugs from biological specimens, however many of the broad or STA screening methods published over the last decade have used solid-phase extraction. This applies especially to those methods using GC as chromatographic technique.

Liquid–liquid extraction schemes for acidic drugs have used ethyl acetate [13,14], acetone–chloroform (1:1) [15], toluene [16,17], dichloromethane–acetone (2:1) [18], toluene–ethyl acetate (4:1) [19], dichloromethane–isopropanol–ethyl acetate (1:1:3) [20] chloroform–isopropanol–heptane (60:14:26) [2], chloroform [21], butyl acetate [22] and diethyl ether [23,24]. There is little to distinguish these solvents in terms of their extraction power, although the polar solvents will often also give a higher background.

Unless pH adjustment is made to under, 5.0 the carboxyl-containing drugs such as the non steroidal anti-inflammatory drugs are often not extracted although use of saturated ammonium chloride solution with a strong solvent such as ethyl acetate [13,14,25] or butyl acetate [22] will detect such drugs (Table 2).

4.2. Solid-phase extraction

Solid-phase extraction techniques have been reviewed [8,15,26,27].

Solid-phase extraction supports include XAD-2 resin [28], diatomaceous earth (Chem Elut) [29,30], Bond Elut Certify [10,15,31,32], Chromabond mixed-mode [33] and Clean Screen DAU columns [34]. The mixed-phase extraction columns (Bond-Elut Certify, Chromabond, Isolute HCX, TSC and CleanScreen DAU) show good recoveries and allow retention of all functional groups and differing polarities.

Solid-phase extraction discs are also a useful and rapid way to extract drugs from liquid specimens [26,35]. Both Bond Elut Certify and Clean Screen SPE columns have been shown to be acceptable for routine drug screening in STA [36]. See also reviews [8,26,27].

For benzodiazepines, solid-phase approaches to the extraction of benzodiazepines are common, particularly mixed phase Bond-Elut Certify® for GC applications. These have been reviewed [11]. Casas et al., 1993 [37] studied the extractability and cleanliness of a number of solid-phase extraction columns. They concluded that C_2 column provided the best combination of high recovery and clean extracts from urine, compared to C_8, C_{18}, phenyl and cyclohexyl phases, whilst CN provided little retention on the cartridge due to its polar nature.

Chen et al. [38] provided a drug screening method using the fully automated Gilson ASPEC® solid-phase extraction method for plasma and whole blood. This method used Bond-Elut Certify columns.

Table 3
Summary of extractabilities of selected drugs using solid-phase extraction

Reference	Solid-phase extraction conditions	Recoveries of selected drugs[a]	
[29]	Chem Elut diatomaceous earth–blood	Alprazolam	63
	Sample diluted, applied to column, washed	Cocaine	64
	and extracted with ethyl acetate	Diazepam	65
		Pentobarbital	69
		Pethidine	65
		Promethazine	76
		Thioridazine	41
[77]	Urine treated with dilute phosphoric acid and	Amphetamine	99
	applied to conditioned Bond Elute SCX	Benzoylecgonine	94
	columns. Elution was with ammoniacal	Cocaine	98
	methanol.	Codeine	99
		Diazepam	78
		MDMA	92
		Morphine	93
		Methadone	96
		Oxazepam	96
		Theophylline	82
		Thioridazine	86
[118]	Plasma or urine was treated with pH 6.0	Pentobarbital	100
	phosphate buffer and applied to prepared	Oxazepam	91
	Bond Elut Certify columns and eluted either	Nitrazepam	94
	with acetone/chloroform (1:1) (Fraction A) or	Amphetamine	99
	ammoniated ethyl acetate (Fraction B).	Trimipramine	105
		Cocaine	96
		Morphine	98
		Promethazine	96
[34]	Bond Elut Certify columns–blood	Amitriptyline	ND 86[a]
	Specimen diluted; acidic drugs eluted with	Amobarbitone	99 ND
	acetone–chloroform (1:1) and basic drugs with	Cocaine	ND 97
	ammoniated ethyl acetate	Codeine	ND 91
		Lorazepam	83 ND
		Mepivacaine	ND 99
		Methadone	ND 83
		Oxazepam	99 ND
[60]	Bond Elut Certify columns–blood	Amphetamine	ND 48[a]
		Phenobarbitone	100 ND
	Blood diluted, sonicated; acid/neutral fraction	Methadone	ND 86
	eluted with acetone–chloroform (1:1); basic	Cocaine	ND 89
	fraction with ammoniated ethyl acetate	Oxazepam	63 16
		Morphine	ND 100
		Haloperidol	ND 80
[32]	Bond-Elut Certify columns–urine	Amphetamine	91
		Methoxyphenamine	96
	Hydrolyzed urine, pH 8–9, elution with	Metoprolol	86
	chloroform–isopropanol (4:1), 2% NH_3	Sotalol	56
		Codeine	93

(Cont.)

Table 3. Continued.

Reference	Solid-phase extraction conditions	Recoveries of selected drugs[a]	
[10]	Bond-Elut Certify columns–liver	Allobarbitone	80 ND[a]
		Codeine	ND 87
	Enzyme digest of liver, eluted with ethyl	Diazepam	49 35
	acetate/NH_3 (basic), or acetone/chloroform	Doxepin	ND 95
	(acidic)	Mepivacaine	ND 91
		Methadone	ND 86
		Methamphetamine	ND 75
		Promethazine	ND 52
[81]	Blood, serum, urine, CSF, vitreous humour	Morphine	98
	or diluted bile (0.5–1.5 ml) were treated with	Morphine glucuronides	>90
	0.01 *M* ammonium carbonate buffer, pH 9.3	Codeine	91
	and applied to a prepared Bond Elut C_{18} SPE	Tramadol	94
	cartridges. Elution was with a methanol/0.5 *M*	Methadone	87
	acetic acid (9:1) solvent	Cocaine	85
		Benzoylecgonine	88
		LSD	80

[a] First column refers to acid fraction and second column to basic fraction.

Recoveries of some benzodiazepines were better than 80% using acetone–chloroform (1:1) as eluant. Similar recoveries were obtained from liver homogenates [10].

Solid phase disc extraction (SPEC®) offers an alternative to SPE [26,39], Table 3.

4.3. Direct injection

HPLC has been used with a direct injection method to detect benzodiazepines. The benzodiazepines were preferentially absorbed onto a pre-column and then back-flushed in to the analytical column using column-switching [40–42], or following a dialysis pretreatment on-line [43]. While these techniques avoid an extraction step, they do require more instrumentation than conventional HPLC. Its main advantage over other reported techniques is potential time savings. These techniques are however restricted to the use of HPLC, although solid-phase micro-extraction (SPME) offers distinct advantages in GC analyses.

SPME is a solvent-free extraction technique relying on the absorption of drugs on to a fused-silica fibre coated with a stationary phase. The most common phases have been polydimethylsiloxane and polyacrylate. This technique avoids the use of solvents and concentration steps. Methods have been published for specific drugs and drug classes, e.g. amphetamines [44–47], anesthetics [48] anti-depressants [49–51], barbiturates [52], benzodiazepines [53], cocaine [54], THC and other cannabinoids [55], and volatile substances [56,57]. For reviews see the following references [26,58]. The application of this method to STA has not yet been described.

5. Chromatographic techniques

5.1. GC techniques

Wide-bore, thick film capillary columns such as the fused-silica columns of internal diameter 0.32 mm or greater and film thicknesses greater than 0.5 μm, are very useful in routine toxicological practice because of their high efficiency and capacity [9,10,15,31,34,59,60]. Column types are often non-polar to low polarity capillary columns (Table 4). Basic and neutral drugs are generally chromatographed underivatized [10,13–15,18,22,34,59,61,62].

Those GC procedures directed at acidic drugs employ derivatization using either acetylation

Table 4
Summary of published GC methods[a]

Reference[b]	Tissue[c]	Drug classes	Extraction method	Conditions	Detection limits	Comments
[22]	0.3 ml B, P	Basic drugs	LLX: specimen treated with ammonia solution and extracted with butyl acetate	3% SP-2250 packed column 2.4 m×2 mm I.D., detection by NPD, isothermal at 270°C; run time 10 min	~0.05 mg/l	Over 40 basic drugs detectable including benzodiazepines, used in clinical toxicology
[63]	10 U	Barbiturates Sedatives Hypnotics	LLX: hydrolysis with acid, extraction from pH 8–9 with dichloromethane-2-propanol-ethylacetate (1:1:3)	BP-1 FSC 12 m×0.2 mm I.D., 0.33 μm film, splitless, detection by MS, T=100–310°C, acetylated with acetic anhydride; Run time 15 min	0.01–0.02 mg/l	Allows identification of therapeutic levels of barbiturates, benzodiazepines and other sedative-hypnotics
[15]	2 ml P, U	Acidic, neutral and basic drugs in 2 schemes	SPE: Specimen diluted and applied to Bond Elute Certify columns; acidic drugs eluted with acetone–chloroform (1:1) and basic drugs with ammionated ethyl acetate	HP-1 FSC 30 m 0.53 mm I.D., 0.88 μm film, detection by FID; T=80–285°C; Run time 25 min	n/a but recoveries generally good	Benzodiazepines eluted in both solvent systems
[61]	2 ml P	Basic, neutral and acidic drugs	LLX: Plasma treated with sat. NaCl, acetone and DCM (1:1), residue acetylated, purified by TLC prior to GC	FSC, splitless, T=50–320°C, detection by MS; run time 28 min	>0.1 mg/l	Shows clean chromatograms, detects morphine, barbiturates and paracetamol with usual basic drugs
[9]	B and other tissues	Basic, neutral and acidic drugs	LLX: conventional Toxi-Lab extraction	SE30 FSC, 12–22 m×0.32 mm I.D., 0.52 μm film, trifluoroacyl derivatives of amines and hydroxyl groups, carboxyls by diazomethane, detection by MS	n/a	Used for detecting drugs in drivers, macro designed to automate process for detection of over 120 compounds including morphine, valproate, captopril, THC, pizotifen, oxazepam
[14]	1.0 B	Neutral and acidic drugs	LLX: Blood is treated with saturated NH_4Cl and extracted with ethyl acetate	Dual NB-54 and NB-1701 FSC 25 m× 0.32 mm I.D., 0.1 μm film, splitless, detection by NPD, T=70–280°C; Run time 22 min	n/a, but therapeutic levels detectable	Over 30 drugs detectable using cubic spline retention indices, some benzodiazepines detected but no carboxylic drugs detected
[62]	0.2 B	Basic and neutral drugs	LLX: Blood was diluted with water, treated with ammonia and extracted with diethyl ether	Various FSC with differing polarities, I.D. and film thicknesses, detection by NPD or MS	n/a, but recovery for 8 drugs acceptable	Over 40 drugs listed including many benzodiazepines
[36]	1.2 P, U	Basic, neutral and acidic drugs	SPE: sample diluted and applied to CleanScreen DAU columns on ASPEC system, eluted with acetone–chloroform (1:1) for acidic/neutral drugs and then ammoniated ethyl acetate for basic drugs	HP-1 FSC 30 m×0.53 mm I.D., 0.88 μm film, detection by FID, T=80–285°C; Run time 25 min	n/a, but recoveries good for 11 drugs of differing polarity	Broad class screening extraction system useful for both HPLC and GC separation techniques, see also [15,36,38,118,119]

[60]	1 ml B	Acidic, neutral and basic drugs	SPE: blood diluted, sonicated and applied to Bond Elut Certify columns; acid/neutral fraction eluted with acetone–chloroform (1:1); basic fraction with ammoniated ethyl acetate	Ultra-1 12 m×0.2 mm I.D., 0.33 μm film, detection by NPD and MS, T=100–280°C, run time 46 min; morphine derivatized with BSTFA	>0.05 mg/l	Only weakly acidic drugs detectable, morphine detectable after silylation
[59]	1.0 B, P	Basic/neutral drugs	LLX: 8 ml butyl chloride after 2*M* Tris, pH 9.2 buffer	BP-5 FSC, 12 m×0.53 mm I.D., 1.0 μm film, splitless mode, T=100–310°C, detection by NPD; run time 40 min	0.02 mg/l up	Benzodiazepines detected Barbiturates detected
[31]	1.0 B, P, U	Basic drugs	SPE: Bond-Elut Certify, elution with ammoniated ethylacetate	HP-1 FSC, 30 m×0.53 mm I.D., 0.88 μm film, splitless mode, T=170–270°C, detection by NPD; run time 21 min	0.1 mg/l up	Recoveries >75% Neutral drugs not detected due to acid wash
[18]	2 ml U	Basic, neutral and acidic drugs	LLX A: Urine acetylated, basified with NaHCO3, NaCl added, and extracted with DCM-acetone (2:1) LLX B: Acidic drugs extracted from pH 2 with same solvent as A and dry extract silylated [96]	SPB-5 FSC 30 m×0.25 mm I.D., 0.25 μm film, T=50–320°C, splitless, detection by MS; run time 36 min	n/a, but recoveries good	forward and reverse search algorithm for automated analysis of screening runs, library of over 300 compounds
[32]	2.5 U	Stimulants, narcotics, β-blockers, β-agonists	SPE: Bond-Elut Certify hydrolysed urine, pH 8–9, elution with chloroform-isopropanol (4:1), 2% NH_3	Ultra-2 HP-5 12.5 m×0.2 mm I.D., 0.33 μm film, T=100–290°C, detection by MS; derivatization by MTBF/MSTFA, run time 14 min	n/a	Over 100 compounds listed and banned by IOC, used in Barcelona Olympic games, including morphine and other narcotics, β-agonists, β-antagonists and stimulants
[10]	0.4 L (0.1 g)	Acidic and basic drugs in 2 extracts	SPE: Bond-Elut Certify, enzymic digest of liver, eluted with ethyl acetate/NH_3 (basic), or acetone/chloroform (acific)	HP-1 FSC, 30 m×0.53 mm I.D., 0.88 μm film, T=80–285°C, detection by FID/NPD; run time 27 min	n/a	Good recoveries obtained for most basic drugs, poorer recoveries for benzodiazepines
[19]	1.0 B	Acidic and basic drugs in separate extracts	LLX: acidic drugs extracted from NaH_2PO_4 buffer with toluene/ethylacetate (4:1) and basic drugs from pH 10.5 with DCM/toluene (1:9)	Basic drugs: HP-5 FSC 25 m×0.32 mm I.D., 0.17 μm film, splitless mode, T=120–320°C; derivatization by HFBA, detector NPD/ECD; run time 15 min; Acidic drugs 0.53 mm I.D. and NPD detector, derivatization with phenyltrimethylammonium hydroxide; run time 11 min	0.02 mg/l up for basic compounds and 0.25 mg/l for barbiturates	Good recoveries for most compounds including benzodiazepines; morphine, benzoylecgonine; used for detecting drugs in drivers in combination with other methods

(continued on next page)

Table 4. Continued.

Reference[b]	Tissue[c]	Drug classes	Extraction method	Conditions	Detection limits	Comments
[65]		Acidic/neutral and basic drugs	SPE: uses Chen 1992 [15]	HP-5 FSC 12 m×0.2 mm I.D., 0.33 μm film, T=100–290°C, splitless, detector by MS, trimethylsilyl derivatives; Run time 30 min	n/a	Over 100 drugs and poisons identifiable, analysis by macro for automated analysis of screening runs
[13]	1.0 ml B	Acidic/neutral drugs	LLX: Blood treated with saturated NH_4Cl and extracted with ethyl acetate	HP-5 FSC 25 m×0.25 mm I.D., 0.33 μm, T=100–300°C, detection by FID; run time 35 min	n/a, but recoveries generally good	Detects analgesics, anti-inflammatories, anti-convulsants, anti-diabetics, barbiturates, theophylline, some diuretics etc.
[64]	2–5 ml U	Acid, neutral and basis drugs in 2 schemes	LLX 1. Urine acid hydrolysed and extracted with DCM-isopropanol-ethyl acetate (1:1:3); LLX 2. Urine treated with THAHS at pH 11.5–12, MeI in toluene	HP-1 FSC 12 m×0.2 mm I.D., 0.33 μm film, T=100–310°C, detection by MS of acetylated derivatives; run time 18 min	n/a	Large range of basic/neutral drugs detected including benzoylecgonine, benzodiazepines, stimulants, morphine and other opioids
[33]	0.1–2 ml P, U	Drugs of abuse (MO, CO, AM, BE)	SPE: Dilution with phosphate buffer, pH 6, application to Chromabond mixed-mode columns, elution with DCM-isopropanol–25% NH_3 (80:20:2)	flow-injection analysis with ion-spray ionization and tandem MS	>1 ng/ml, recoveries >85%	Rapid method for targeted drugs of abuse
[120]	0.5–1.0 ml B, P, U	Acidic, neutral and basic drugs	Uses method of Chen et al., 1992 [121] (Bond-Elut Certify in 2 extraction schemes. Use of laboratory robot	Extracts derivatized with MSTFA–toluene (1:4) containing 5% TMCS. HP-5 Ultra-2 (12 m×0.2 μm I.D., 0.33 μm film), splitless injection, T=100–290°C, detection by MS full scan mode, run time 30 min	See [121]	Analysis by macro for automated analysis of screening runs [65]

[a] See Table 1 for abbreviations.
[b] References are cited in chronological order.
[c] Volume of fluid in ml.

[63,64], silyl ethers [9,32,60,65], or reaction with phenyltrimethylammonium hydroxide [19]. Formation of methyl derivatives by reaction with diazomethane has also been described [9].

In most cases, volumes of blood or serum required for adequate detection are 1-ml or less. A combination of liquid–liquid and solid-phase extraction techniques is used in the publications summarised in Table 4. There is little to distinguish many of these methods from each other.

5.2. HPLC techniques

A relatively large number of HPLC procedures have been published since 1989 describing applications of STA, or capable of detecting a large range of drugs (Table 5). Fourteen utilized blood or plasma specimens and only one was limited to urine. Acidic drugs were targeted in 11 papers, basic drugs in 14, and 12 defined procedures for acidic, neutral and basic drugs.

Liquid–liquid extraction or direct precipitation with a solvent was described by 13 publications, direct injection after filtering by two, and only one described a solid-phase extraction procedure. Liquid extraction was clearly preferred over solid-phase techniques.

Nine procedures utilized gradient elution using a variety of solvent systems. The choice of columns also varied widely: nine choosing octadecylsilane-based phases, two each using a C_8, and CN-bonded phase, one used several columns [17], and three papers described the use of commercially protected multi-column technology [66–68]. Microbore columns were only used by two procedures [13,69]. Semi-micro or microbore columns will reduce chromatography time, and together with column switching [70], or applications with mass spectrometry (LC–MS) can lead to significant improvements in detection limits and throughput [71].

All procedures cited use photodiode array detection or multi-wavelength scanning. Clearly this type of detector enables spectral matches to be made to library entries facilitating the detection of the drugs. The use of commercial library matching routines or algorithms to allow spectral matching is a feature of many of the papers published, and is recommended to optimize the use of HPLC systems in STA. A number of papers described libraries of one hundred or more of drugs detected by the procedures described [2,16,21,67–69,72–75].

It is difficult to recommend any particular HPLC procedures however those referenced later offer comprehensive schema for a broad range of drugs and will complement any GC-based screening technique [2,13,16,17,21,73,74,76]. These methods will allow the detection of many of the common acidic drugs and neutral drugs including barbiturates, many benzodiazepines, theophylline, anti-inflammatory drugs, anti-convulsants, non-narcotic analgesics, sulphonylurea anti-diabetics, many diuretics, and many basic drugs when present in potentially toxic concentrations.

A number of papers provide retention data for a large number of compounds including some of the more difficult to detect drugs, however no details of the detection limits in biological fluids were provided [2,16,17,74,75,77–79]. Detection limits were provided for 65 toxic drugs commonly seen in Japan including potent benzodiazepines, barbiturates and a range of largely basic drugs [80]. However, given the absence of a concentration step, it is likely that many of the potent drugs are not detected unless present in toxic concentrations.

The automated drug-profiling system REMEDi™ was subject to a number of publications during this period relating to drug screening [66–68]. The procedure uses a liquid–liquid extraction at pH 8 and the extract is separated by a series of analytical columns. Urinalysis data shows it ability to detect a range of common basic drugs, as well as benzoylecgonine, colchicine, erythromycin, methylprednisolaone, morphine and ranitidine [67]. Expectedly, blood analysis was less sensitive.

LC–MS is an emerging technique and has shown that the separation power of HPLC can be combined with the sensitivity and specificity of MS [4,71]. Published methods utilizing LC–MS rely on detection of a drug or group of drugs. It is likely however that STA using LC–MS (and LC–MS–MS) will be an important development in drug screening techniques for the future. For example, a recently published method used HPLC linked to atmospheric pressure chemical ionization MS described the measurement of morphine, codeine and their glucuronides, cocaine, benzoylecgonine and other cocaine

Table 5
Summary of published HPLC methods[a]

Reference	Tissue	Drug classes	Extraction method	Conditions	Detection limits	Comments
[72]	1 ml U	Basic drugs	SPE: Urine treated with phosphate buffer, and applied to Bond Elut SCX column and eluted with ammonical methanol	Lichrospher 100 CH-8, 250×4.6 mm, I.D., detection by DAD 190–400 nm, gradient elution 10–50% ACN-potassium phosphate, pH 3.2 buffer; Run time 20 min	<0.05 mg/l	Over 200 basic drugs detectable
[80]	0.1 ml B, P, U, g.c.	All drugs	Specimen treated with 2 volumes ACN, filtered	FineSil C_{18}S 250×4.6 mm I.D. at 50°C, detection by DAD, isocratic elution with 10 m*M* perchloric acid/perchlorate in CAN. Run Time 40 min.	DLs provided, recoveries generally good	Uses MCASYST program to identify over 65 drugs
[69]	0.5 ml P	Basic, neutral and weakly acidic drugs	LLX: Treated with NaOH solution, extracted with DCM	Microbore Hypersil ODS, 100×2.1 mm I.D., gradient elution 15–80% ACN-triethylamine/phosphate buffer; Run time 20 min	<0.1 mg/l	Library of over 300 compounds, drugs identified by AUTOLIB macro (HP) with retention window pre-searches, detects barbiturates and benzodiazepines
[89]	1 ml P	Basic drugs	LLX: Plasma treated with Na_2CO_3 and extracted with hexane	Supelcosil LC–PCN, 5 µm, ACN-MeOH–10 m*M* phosphate, pH 7.0 (180:45:75), detection by DAD, isocratic analyses	>0.02 mg/l	Detects mainly anti-depressants, some opioids but not morphine, and some high dose anti-psychotics, but not benzodiazepines
[16]	2 ml B	Basic, neutral and acidic drugs in 2 schemes	LLX: Blood treated with NH_3 and extracted with toluene. Basic drugs re-extracted from toluene (fraction B) and acidic drugs extracted from aqueous layer with toluene (fraction A)	1: APEX ODS 5 µm, 25 cm×4.6 mm I.D., ACN–0.025% phosphoric acid–triethylamine, pH 3.4 (25:10:5) 2: µPhenyl (Waters), 5 µm, 15 cm×3.9 mm I.D., ACN–0.025% phosphoric acid (50:50). Isocratic analysis, detection by DAD. Run time 15 min	>0.05 mg/l	Broad screening technique using automated library searching for ~120 drugs and metabolites
[73]	0.25 ml B	Broad class-screening	Direct precipitation with acetonitrile	Spherisorb S5 ODS-2, 5 µm, 10 cm×3.8 mm I.D., gradient elution 10–60% acetonitrile in pH 3.1 phosphate buffer, DAD detector 195–650 nm; Run time 46 min	>0.1 mg/l	Detects over 120 drugs and metabolites including analgesics, anti-inflammatories, anti-convulsants, anti-diabetics, barbiturates, theophylline, some benzodiazepines and diuretics, herbicides etc.
[66]	U, g.c.	Basic, neutral and acidic drugs	Specimens filtered and centrifuged	Multi-column REMEDi HS (Bio-Rad), chromatographic conditions not given, multiwavelength UV detection	n/a	Semi-automated drug identification system, broad class screening
[17]	1–2 ml B	Basic, neutral and acidic drugs in 4 schemes	LLX: as per [16] with minor modifications	Several HPLC columns and solvent conditions studied, isocratic analyses		Broad screening technique using automated PolyView-MCA (Varian) library searching
[67]	2 ml B, tissue, U	Basic, neutral and acidic drugs	LLX: Blood or tissue treated with KCl and pH 8 phosphate buffer, extracted with butanol–ethyl acetate (1:5); urine filtered and centrifuged	Multi-column REMEDi HS (Bio-Rad), chromatographic conditions not given, multiwavelength UV detection; Run time 16 min	0.05–1 mg/l (B)	Library of over 500 drugs and metabolites, semi-automated drug identification system, broad class screening

[2]	2 ml B,P	Broad class screening	LLX: Extraction of blood treated with NH_4 Cl buffer with chloroform-2-propanol-*n*-heptane (60:14:26)	NovaPak C_{18}, 4 μm, 30 cm×3.9 mm I.D., 30°C, isocratic MeOH–THF-phosphate buffer pH 2.6 (65:5:30), DAD detector 190–800 nm; Run time 20 min	>0.01 mg/l	Detects over 311 substances including barbiturates, benzodiazepines, basic drugs, analgesics and many others
[74]	1.0 ml B, U, g.c., Tissue	Basic and neutral drugs	LLX: Specimen treated with 1 *M* K_2CO_3 and extracted with 1-hexane–ethyl acetate (7:3)	Aluspher RP-select B column 15×4.0 mm I.D., 5 μm, gradient elution of 10–90% MeOH in 0.0125 *M* NaOH; Run time 25 min	n/a but recoveries generally good	Detects over 130 mainly basic drugs but not morphine
[76]	1 ml P, B, L	Acidic, neutral and basic drugs in 3 schemes	LLX: A. Diethyl ether extraction at pH 4.6, B. DCM-2-propanol (9:1) extraction from pH 8.5 C. Treatment with boiling HCl and extraction as for B	Super sphere RP-18, 125×4 mm.I.D., detection by DAD, gradient elution 0–70% ACN-triethylammonium phosphate buffer, pH 3.0; Run time 35 min	n/a	Uses 1-nitroalkane as retention marker, see also [78,79,122]
[68]	1 ml P, U, g.c.	Basic, neutral and acidic drugs	Specimens filtered and centrifuged	Multi-column REMEDi HS (Bio-Rad), chromatographic conditions not given, multiwavelength UV detection, 205–300 nm; Run time ~20 min	>0.04 mg/l	Library of over 500 drugs and metabolites, semi-automated drug identification system, broad class screening
[123]	P	N/a	Extraction details not provided, only chromatographic conditions	LiChrospher 60 RP Select B, 5 μm, 12.5 cm×4 mm I.D., detection by DAD. Gradient elution 0–70% ACN–TEAP, pH 3.0 and step-wise isocratic; Run time 43 min	n/a	Uses stepwise isocratic systems for target drug groups or detections
[13]	1 ml B	Acidic/neutral drugs	LLX: Blood treated with saturated NH_4Cl and extracted with ethyl acetate	ODS–Hypersil, 5 μm, 20 cm×2.1 mm I.D., gradient elution 5–50% ACN in 2 m*M* pH3.2 phosphate buffer, DAD detector; Run time 35 min	n/a, but recoveries generally good	Detects analgesics, anti-inflammatories, anti-convulsants, anti-diabetics, barbiturates, theophylline, some diuretics etc.
[75]	1 ml B, U	Basic, neutral and weakly acidic drugs	LLX: Dilution with water, extraction with Toxi-Tube A, evaporation and reconstitution	Symmetry C8, 250 mm×4.6 mm I.D., 5 μm, 30°C, gradient elution using 15–80% ACN-phosphate buffer, pH 3.8, detection by DAD; Run time 28 min	>5 ng/ml	Library of over 600 compounds including benzodiazepines, barbiturates and NSAIDs. Searching by retention window and vector mathematics
[21]	0.5 ml B, P, U	Basic, neutral and acidic drugs	LLX: Basic/neutral drugs extracted with butyl chloride after treatment with Na_2CO_3 and back extraction into 0.1 ml sulfuric acid: Acid/neutral drugs specimen treated with dilute sulfuric acid and extracted with chloroform	Spherisorb S5 OD/CN 150×4.6 mm I.D., gradient elution using 0–70% ACN in triethylammonium phosphate buffer, pH 3.0, detection by DAD; Run time 15 min in fast screening mode	n/a	Library of over 250 compounds, rapid HPLC screening technique for large number of classes using dual liquid extraction technique

[a] See Table 1 for abbreviations.

metabolites, LSD, methadone and other substances in biological fluids [81]. The extension of this approach to include other difficult to detect drugs is very likely.

A review of applications of LC–MS to a range of drugs or drug groups including amphetamines, cocaine, LSD, opiates, anabolic steroids, anti-hypertensives, benzodiazepines, cardiac glycosides, corticosteroids, immunosuppressants, neuroleptics, anti-inflammatory drugs, quaternary amines, xanthins, aflatoxins, α and β-amantin and many others is reported [71].

5.3. Capillary electrophoresis

CE is a rapidly growing analytical technique with great promise as a screening technique in forensic toxicology. CE separations include capillary zone electrophoresis (CZE), micellar electrokinetic electrophoresis (MECC), capillary electrochromatography (CEC), capillary isoelectric focusing (CIEF), capillary gel electrophoresis (CGE) and capillary isotachophoresis (CITP). The application of these techniques in forensic toxicology is reviewed [82].

Sample preparation is similar to GC and HPLC separation techniques, although only very small sample volumes are introduced, typically a few nanolitres, consequently detection limits can be limited. Direct injection techniques are also used and have been reviewed [83].

At this time, these techniques have not been used for STA, although some papers have described applications for the screening of several drugs. Drugs-of-abuse have been detected in urine with a detection limit of 100 ng/ml using MECC and a fast scanning UV spectrophotometer [84]. These drugs included cocaine, benzoylecgonine, morphine, 6-monoacetylmorphine, methamphetamine, and benzodiazepines. CZE has been used to detect 17 basic drugs including methamphetamine, amphetamine, diazepam, codeine, and methaqualone in plasma and urine with a detection limit of 0.45 μg/ml [85].

Laser-induced fluorescence substantially improves detection limits for analytes capable of exhibiting flourescence. The combination of CE with MS has been reported for a limited number of specific applications, and offers promise for STA [82,86].

5.4. TLC techniques

TLC is still used, particularly clinical laboratories receiving urine as the preferred specimen. In recent years publications have described its use in STA for a large range of drugs (Table 6) [23,30]. Both liquid–liquid [30] and solid-phase extraction has been utilized [23]. In one schema, nine TLC solvents systems were employed to detect 300 target drugs [30]. In one paper scanning or direct TLC linked to liquid secondary ion MS has been used to detect cephalosporin and its metabolites [87].

The use of more than one TLC system per analysis and appropriate color reactions can provide a degree of certainty approaching conventional confirmation techniques (HPLC–DAD and GC) [88].

TLC is subject to the effects of ambient temperature and humidity [7] and is largely limited to large volumes of urine and positive results must still be confirmed by GC–MS.

Table 6
Summary of published TLC methods[a]

Reference	Tissue	Drug classes	Extraction method	Conditions	Detection limits	Comments
[30]	20 ml U	Drugs of abuse, benzodiazepines, phenothiazines, analgesics, halogenated hydrocarbons etc.	SPE: Chem Elut extraction after adjustment of pH to 8–9 with DCM-propan-2-ol (9:1); prior hydrolysis for benzo-diazepines, cannabinoids, and morphine	Nine TLC systems used with several visualisation systems.	Variable but usually capable of detecting therapeutic use	Over 300 drugs detected, confirmation by GC–MS
[23]	20–50 ml U	Basic, neutral and some acidic drugs	LLX: modification of Toxi-Lab method by use of an initial diethyl ether extraction and back-extraction into dilute acid	as per Toxi-Lab (Analytical Systems) method	n/a	Improved sensitivity of Toxi-Lab method for basic/neutral substances in paediatric toxicology

[a] See Table 1 for abbreviations.

6. Advantages and limitations of assay systems

The use of saturated ammonium chloride and polar solvents such as ethyl acetate or butyl acetate provide an ability to extract both neutral, acidic and basic substances in one analytical liquid–liquid extraction schemes. Similarly, the use of mixed phase or strong cation-exchange solid-phase cartridges can extract a similarly large range of substances.

In some cases filtration and injection, or precipitation of proteins with acetonitrile and injection of the supernatant can provide a more direct means to introduce a sample into a HPLC. This technique reduces bias to those compounds extracted by the solvent system employed, however the lack of concentration step may limit detection of some of the more potent drugs.

These drugs include the common drugs-of-abuse amphetamines, cocaine (and benzoylecgonine), morphine and related opiates, barbiturates and benzodiazepines, and a range of common drugs of toxicological significance including anti-depressants, anti-convulsants, anti-histamines and neuroleptics. Hallucinogens such as ''ecstasy'', phencyclidine, ketamine, plant alkaloids such as nicotine, coniceine (coniine) and scopolamine are readily measurable using GC techniques, whereas LSD is only detected by targeted testing. Of the techniques listed in Table 4, five reported the simultaneous detection of morphine with other common drugs of abuse [19,32,60,61,64]. Since morphine itself is quite polar and chromatographs poorly underivatized, it is not surprising that these techniques all used derivatization procedures.

Unfortunately, few published methods provide an exhaustive validation for all common drugs-of-abuse and a large range of common toxic pharmaceuticals and other poisons. Published procedures of note include those of Bogusz et al., 1998 [81], Lillsunde et al., 1996 [19], Drummer et al. [59,73], Zweipfenning et al., 1994 [60], Logan et al., 1990 [77], Chen et al., 1992 [15] and Tracqui et al., 1995 [2].

Techniques reliant on GC-screening system for basic and neutral drugs (and usually also weakly acidic drugs) will allow a range of other important drugs to be detectable, including antidepressants, many benzodiazepines, barbiturates, most amphetamines, many neuroleptics, and substances such as many cardiovascular drugs, antihistamines, and anesthetics. The methods are summarized in Table 4. There are of course numerous HPLC-based screening techniques developed for basic/neutral substances in blood as well (see Table 5).

In many of these GC-based procedures algorithms and other searching routines have been developed to automate the drug screening approach [9,14,18,65]. Similarly, a number of automated or semi-automated methods are available for HPLC systems [2,16,17,67–69,74,75,80,89]. These procedures clearly show advantages over more manual methods, and are encouraged. Mean list length (MLL) has been advocated as an approach to quantify specificity of a method. In this approach co-eluting substances increase the MLL from the ideal one (only one possibility with a given chromatographic system) [7,90].

A number of important (largely acidic) drugs are not detected by conventional chromatographic systems for basic/neutral drugs. These include theophylline, acetaminophen, salicylate, diuretics, oral anti-diabetic drugs, non steroidal anti-inflammatory drugs, some benzodiazepines, warfarin and other anti-coagulants, and many anti-convulsants. Consequently, for a laboratory to provide STA another chromatographic system is required for at least these classes. This can either be a GC-based system using extraction systems or a HPLC system reviewed earlier. A list of drugs readily detectable by a combination of a chromatographic (GC or HPLC) screen for basic/neutral drugs and a chromatographic system (GC or HPLC) for acidic/neutral drugs is shown in Table 7.

Drugs that are normally not easily measurable in systematic chromatographic screening techniques include the potent triazolo benzodiazepines such as triazolam, plant alkaloids including colchicine, digoxin, some of the potent opiates including buprenorphine, fentanyl and its derivatives, THC and other cannabinoids, antibiotics and potent anticoagulants etc. (Table 7). These drugs possess physio-chemical and pharmacological properties that either exclude their ready chromatographic analysis (HPLC or GC) or are too potent to be measurable using conventional detectors (ECD, NPD, FID detec-

Table 7
Detectability of drugs in HPLC and GC chromatographic screening systems[a]

Drug class	Readily detectable drugs	Difficult to detect drugs[b]
Amphetamines	Most including amphetamine, methamphetamine, MDMA, MDA, PMA, MDE etc.	
Antihistamines	Non-selective antihistamines ()	Non-sedating antihistamines (many)
Anti-convulsants	Many, including phenytoin, carbamazepine, lamotrigine	Vigabatrin, valproate
Anti-depressants	Most including tricyclic derivatives, nomifensine, trazodone, mianserin, moclobemide and SSRIs	Tranylcypromine
Anti-diabetic drugs	Sulfonyluereas including tolbutamide, glibenclamide, chlorpropamide	Insulin
Analgesics and anti-inflammatory drugs	Acetaminophen, salicylate and non-steroidal anti-inflammatory drugs	
Benzodiazepines	Most benzodiazepines	Potent analogues, e.g. triazolam, lorazepam, bromazepam, zopiclone, zolpidem, buspirone
Barbiturates	Most including analogs meprobamate, methaqualone and glutethimide	
Cardiovascular drugs	Many beta-blockers (propanolol, metoprolol etc.) and many Ca-channel inhibitors (verapamil, nidefidine, felopidine, diltiazem), and many anti-arrhythmics quinidine, flecainide, mexiletine, perhexiline, tocainide and lignocaine	ACE inhibitors, amiodarone, digoxin, atenolol, sotalol, α_1-antagonists (prazosin, doxazosin), losartan, indapamide
Hallucinogens	Ketamine, phencyclidine, designer amphetamines, e.g. ecstasy	LSD, psilocin, psilocybin
Narcotics	Many including methadone, codeine, meperidine, propoxyphene etc.	Morphine, buprenorphine, etorphine, alfentanil, sufentanil and other fentanyls
Neuroleptics	Phenothiazines (most), clozapine, olanzapine	Haloperidol, droperidol, pericyazine, remoxipride, lithium, drugs from depot injections
Stimulants	Cocaine, caffeine, cathinone, theophylline, phentermine, methylphenidate and many other stimulants	Benzoylecgonine, Phenylpropanolamine
Miscellaneous Drugs	Amantidine, seligiline Chloroquin, hydroxychloroquin, quinine and quinidine HIV-protease inhibitor ziduvudine Plant-based alkaloids scopolamine, atropine, nicotine, coniceine, strychnine, Warfarin	Alflatoxins and ergots Anabolic steroids Anti-neoplastics (e.g., methotrexate, *cis*-platinium, cyclophosphamide etc.) Baclofen Chloral hydrate, trichloroethanol Coumarin-based anti-coagulants Some as, e.g. colchicine, α and β-amanitins, THC and carboxy–THC

[a] Using drug screening methods designed for acidic, basic and neutral drugs such as those listed as STA, for drugs of toxicological significance.
[b] These drugs are either detected at very high concentrations or not at all using standard toxicological screening tests for broad classes of drug

tors for GC, and UV, F, DAD detectors for HPLC). The use of selected ion monitoring MS will allow some of these drugs to be detectable, e.g. narcotics, THC, benzodiazepines, the more potent neuroleptics, however this limits the ability to detect many other drugs in one chromatographic system due to a shortening dwell time. Derivatization for GC analyses will further improve the detectability, particularly for morphine, benzoylecgonine, LSD and some benzodiazepines. Derivatization is mandatory if GC-based techniques are used for acidic drugs.

For example, Neill et al. [9] described a technique in which drugs were derivatized either with trifluoracylating or a methylating agent. This allowed the detection of polar drugs such as the ACE inhibitor captopril, the anti-convulsant valproic acid, as well as morphine, THC, and the anti-migraine drugs pizotifen etc. No details of its applicability to biological specimens were provided, yet this approach offers a potential improvement over GC techniques not employing derivatization. Dual derivatization to inactivate all functional groups, together with extracts containing both acidic, neutral and basic drugs offer an ability to increase the range of detected substances in one chromatographic system.

Specific techniques are available for the identification and confirmation of specific drug classes includ-

ing: barbiturates [91], cannabinoids [92–95], [beta-blockers [96–98], diuretics [99–101], stimulants [44,102–104], narcotic analgesics [32,104–107], LSD [108,109], antihistamines [110,111] and anticonvulsants [112]. Excellent reviews of procedures for xenobiotics used in doping [113] and for the determination of drugs of abuse in blood [114] are available. Specific techniques for less common toxicological agents include methods for 4-hydroxycoumarin anti-coagulants [115], uncommon tranquilizers and sedatives such as zopiclone, zolpidem, buspirone [116].

7. Conclusions

For toxicologists to provide a comprehensive drug screening service no one chromatographic system can provide a sufficiently exhaustive coverage of toxicological significant chemicals. Systems using hyphenated techniques, particularly GC–MS (and GC–MS–MS) provide a reasonable coverage, although physio-chemical properties of drugs limit the extractability of relevant drugs in one extraction system. This is overcome by using two liquid–liquid or two solid-phase extraction systems; one for acids and neutrals, and one for bases and neutrals; and chromatographing both extracts. Alternatively, both extracts could be combined to reduce chromatography time, although derivatization is required to detect any polar drug (strong acids, morphine and some benzodiazepines etc.).

The common drugs of abuse that are most difficult to detect, without resorting to targeted testing, are THC and its metabolites, benzoylecgonine, morphine and its metabolites, and LSD. To overcome these deficiencies, laboratories typically would use specific immunoassays for screening of opiates, LSD, cannabinoids and cocaine metabolites, and other drug classes. If positive to an immunoassay, targeted confirmation is conducted by GC–MS.

Other drugs of importance that are not easily detected by chromatographic screening techniques include those listed in Table 7, e.g. many of the potent benzodiazepines, opiates, and neuroleptics, fentanyl and its designer variations, a number of cardiovascular drugs, many of the plant-derived substances, peptidic drugs, chloral hydrate and its metabolites etc. Evolving techniques, or techniques currently applied should also be targeted at detecting as many of these additional substances to improve the detectability of screening techniques for general unknown cases. Whatever validated technique is used in laboratories, acknowledgement of drugs capable of being detected should also appear in a laboratory report, in addition to those detected.

Techniques using tandem mass spectrometry offer the prospect of being able to target drugs with high specificity and sensitivity for many, if not all of these additional substances in one procedure. The application of HPLC with mass spectrometry or tandem mass spectrometry offers advantages over GC-based techniques since derivatization of highly polar compounds such as morphine, morphine glucuronides and benzoylecgonine is not required [81].

CE offers the potential to act as an alternative to traditional chromatographic systems for STA, particularly with increased sensitivity due to improved detectors and sample stacking techniques to load extracts on to the column. The ability to separate compounds of widely differing polarity and molecular weight provides added advantages without the need for derivatization. The hyphenation with MS offers further potential yet to be realized in drug screening.

Meanwhile, it is important that techniques used as principal screening methods in laboratories are fully validated with respect to the substances capable of being detected at concentrations likely to present in real cases, and that reports provide an indication of substances that were reasonably excluded in the analyses.

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References

[1] H.H. Maurer, J. Chromatogr. 580 (1992) 3–41.
[2] A. Tracqui, P. Kintz, P. Mangin, J. Forensic Sci. 40 (1995) 254–262.
[3] W.E. Lambert, J.F. Van Bocxlaer, A.P. De Leenheer, J Chromatogr. B 689 (1997) 45–53.

[4] H. Hoja, P. Marquet, B. Verneuil, H. Lotfi, B. Penicaut, G. Lachatre, J. Anal. Toxicol. 21 (1997) 116–126.
[5] R.A. de Zeeuw, J. Chromatogr. 488 (1989) 199–213.
[6] R.A. de Zeeuw, J. Chromatogr. B 689 (1997) 71–79.
[7] R.A. de Zeeuw, J.P. Franke, M. van Halem, S. Schaapman, E. Logawa, C.J. Siregar, J. Anal. Toxicol. 18 (1994) 402–406.
[8] J. Scheurer, C.M. Moore, J. Anal. Toxicol. 16 (1992) 264–269.
[9] G.P. Neill, N.W. Davies, S. McLean, J. Chromatogr. 565 (1991) 207–224.
[10] Z.P. Huang, X.H. Chen, J. Wijsbeek, J.P. Franke, R.A. de Zeeuw, J. Anal. Toxicol. 20 (1996) 248–254.
[11] O.H. Drummer, J. Chromatogr. B 713 (1998) 201–225.
[12] M. Bogusz, J. Gierz, J. Bialka, Forensic Sci. Int. 12 (1978) 73–82.
[13] D. Lo, T. Chao, S. Ng-Ong, Y. Yao, T. Koh, Forensic Sci. Int. 90 (1997) 205–214.
[14] I. Ojanperä, I. Rasanen, E. Vuori, J. Anal. Toxicol. 15 (1991) 204–208.
[15] X.H. Chen, J. Wijsbeek, J.P. Franke, R.A. de Zeeuw, J. Forensic Sci. 37 (1992) 61–71.
[16] E.M. Koves, J. Wells, J. Forensic Sci. 37 (1992) 42–60.
[17] E.M. Koves, J. Chromatogr. A 692 (1995) 103–119.
[18] N.B. Smith, J. Anal. Toxicol. 18 (1994) 16–21.
[19] P. Lillsunde, L. Michelson, T. Forsstrom, T. Korte, E. Schultz, K. Ariniemi, M. Portman, M.L. Sihvonen, T. Seppala, Forensic Sci. Int. 77 (1996) 191–210.
[20] H.H. Maurer, J.W. Arlt, T. Kraemer, C.J. Schmitt, A.A. Weber, Arch. Toxicol. Suppl. 19 (1997) 189–197.
[21] S.P. Elliot, K.A. Hale, J. Anal. Toxicol. 22 (1998) 279–289.
[22] S. Dawling, N. Ward, E.G. Essex, B. Widdop, Ann. Clin. Biochem. 27 (1990) 473–477.
[23] N.R. Badcock, G.D. Zoanetti, Ann. Clin. Biochem. 33 (1996) 75–77.
[24] A. Turcant, A. Premel-Cable, A. Callieux, P. Allain, Clin. Chem. 34 (1988) 1492–1497.
[25] M.E. Sharp, J. Anal. Toxicol. 11 (1987) 8–11.
[26] F. Degel, Clin. Biochem. 29 (1996) 529–540.
[27] J.P. Franke, R.A. de Zeeuw, J. Chromatogr. B 713 (1998) 51–59.
[28] M. Klys, J. Brandys, Forensic Sci. Int. 38 (1988) 185–192.
[29] B.K. Logan, D.T. Stafford, J. Forensic Sci. 34 (1989) 553–564.
[30] P. Lillsunde, T. Korte, J. Anal. Toxicol. 15 (1991) 71–81.
[31] X.H. Chen, J.P. Franke, J. Wijsbeek, R.A. de Zeeuw, J. Anal. Toxicol. 18 (1994) 150–153.
[32] A. Solans, M. Carnicero, R. de la Torre, J. Segura, J. Anal. Toxicol. 19 (1995) 104–114.
[33] W. Weinmann, M. Svoboda, J. Anal. Toxicol. 22 (1998) 319–328.
[34] X.H. Chen, J.P. Franke, K. Ensing, J. Wijsbeek, R.A. de Zeeuw, J. Chromatogr. 613 (1993) 289–294.
[35] K. Ensing, J.P. Franke, A. Temmink, X.H. Chen, R.A. de Zeeuw, J. Forensic Sci. 37 (1992) 460–466.
[36] X.H. Chen, J.P. Franke, J. Wijsbeek, R.A. de Zeeuw, J. Chromatogr. 617 (1993) 147–151.
[37] M. Casas, L.A. Berrueta, B. Gallo, F. Vicente, J. Pharm. Biomed. Anal. 11 (1993) 277–284.
[38] X.H. Chen, J.P. Franke, K. Ensing, J. Wijsbeek, R.A. de Zeeuw, J. Anal. Toxicol. 17 (1993) 421–426.
[39] H.J. Helmlin, K. Bracher, D. Bourquin, D. Vonlanthen, R. Brenneisen, J. Anal. Toxicol. 20 (1996) 432–440.
[40] H. Iwase, K. Gondo, T. Koike, I. Ono, J. Chromatogr. B 655 (1994) 73–81.
[41] C. Lacroix, F. Wojciechowski, P. Danger, J. Chromatogr. B 617 (1993) 285–290.
[42] R. Lauber, M. Mosimann, M. Bührer, A.M. Zbinden, J. Chromatogr. B 654 (1994) 69–75.
[43] N.C.V.d. Merbel, J.M. Teule, H. Lingeman, U.A.T. Brinkman, J. Pharm. Biomed. Anal. 10 (1992) 225–233.
[44] F. Centini, A. Masti, I. Barni Comparini, Forensic Sci. Int. 83 (1996) 161–166.
[45] C. Battu, P. Marquet, A.L. Fauconnet, E. Lacassie, G. Lachatre, J. Chromatogr. Sci. 36 (1998) 1–7.
[46] N. Nagasawa, M. Yashiki, Y. Iwasaki, K. Hara, T. Kojima, Forensic Sci. Int. 78 (1996) 95–102.
[47] A. Benko, A. Dona, A. Kovacs, C. Maravelias, H.Z. Mikone, A. Kerner, Acta Pharm. Hung. 68 (1998) 269–275.
[48] T. Watanabe, A. Namera, M. Yashiki, Y. Iwasaki, T. Kojima, J. Chromatogr. B 709 (1998) 225–232.
[49] A. Namera, T. Watanabe, M. Yashiki, Y. Iwasaki, T. Kojima, J. Anal. Toxicol. 22 (1998) 396–400.
[50] X.P. Lee, T. Kumazawa, K. Sato, O. Suzuki, J. Chromatogr. Sci. 35 (1997) 302–308.
[51] S. Ulrich, J. Martens, J. Chromatogr. B 696 (1997) 217–234.
[52] B.J. Hall, J.S. Brodbelt, J. Chromatogr. A 777 (1997) 275–282.
[53] F. Guan, H. Seno, A. Ishii, K. Watanabe, T. Kumazawa, H. Hattori, O. Suzuki, J. Anal. Toxicol. 23 (1999) 54–61.
[54] S. Strano-Rossi, M. Chiarotti, J. Anal. Toxicol. 23 (1999) 7–10.
[55] S. Strano-Rossi, M. Chiarotti, J. Anal. Toxicol. 23 (1999) 7–10.
[56] W.E. Brewer, R.C. Galipo, S.L. Morgan, K.H. Habben, J. Anal. Toxicol. 21 (1997) 286–290.
[57] B.D. Page, G. Lacroix, J. Chromatogr. 648 (1993) 199–211.
[58] L. Junting, C. Peng, O. Suzuki, Forensic Sci. Int. 97 (1998) 93–100.
[59] O.H. Drummer, S. Horomidis, S. Kourtis, M.L. Syrjanen, P. Tippett, J. Anal. Toxicol. 18 (1994) 134–138.
[60] P.G. Zweipfenning, A.H. Wilderink, P. Horsthuis, J.P. Franke, R.A. de Zeeuw, J. Chromatogr. A 674 (1994) 87–95.
[61] K.E. Brooks, N.B. Smith, Clin. Chem. 37 (1991) 1975–1978.
[62] D.N. Sims, P.D. Felgate, H.E. Felgate, R.J. Lokan, Forensic Sci. Int. 49 (1991) 33–42.
[63] H.H. Maurer, J. Chromatogr. 530 (1990) 307–326.
[64] H.H. Maurer, T. Kraemer, O. Ledvinka, C.J. Schmitt, A.A. Weber, J. Chromatogr. B 689 (1997) 81–89.
[65] A. Polettini, J. Anal. Toxicol. 20 (1996) 579–586.
[66] P. Demedts, A. Wauters, F. Franck, H. Neels, Eur. J. Clin. Chem. Clin. Biochem. 32 (1994) 409–417.
[67] K.S. Kalasinsky, T. Schaefer, S.R. Binder, J. Anal. Toxicol. 19 (1995) 412–418.

[68] M. Ohtsuji, J.S. Lai, S.R. Binder, T. Kondo, T. Takayasu, T. Ohshima, J. Forensic Sci. 41 (1996) 881–886.
[69] A. Turcant, A. Premel-Cabic, A. Cailleux, P. Allain, Clin. Chem. 37 (1991) 1210–1215.
[70] O. Shiroto, A. Suzuki, T. Ogawa, Y. Ohtsu, Analusis Magazine 26 (1998) M33–M35.
[71] H.H. Maurer, J. Chromatogr. B 713 (1998) 3–25.
[72] B.K. Logan, S. Nichols, S. Fernandez, D.T. Stafford, Crime Laboratory Digest 17 (1990) 5–12.
[73] O.H. Drummer, A. Kotsos, I.M. McIntyre, J. Anal. Toxicol. 17 (1993) 225–229.
[74] W.E. Lambert, E. Meyer, A.P. De Leenheer, J. Anal. Toxicol. 19 (1995) 73–78.
[75] Y. Gaillard, G. Pepin, J. Chromatogr. A 763 (1997) 149–163.
[76] M. Bogusz, M. Erkens, J. Anal. Toxicol. 19 (1995) 49–55.
[77] B.K. Logan, D.T. Stafford, I.R. Tebbett, C.M. Moore, J. Anal. Toxicol. 14 (1990) 154–159.
[78] R.D. Maier, M. Bogusz, J. Anal. Toxicol. 19 (1995) 79–83.
[79] M. Bogusz, M. Wu, J. Anal. Toxicol. 15 (1991) 188–197.
[80] M. Hayashida, M. Nihira, T. Watanabe, K. Jinno, J. Chromatogr. 506 (1990) 133–143.
[81] M.J. Bogusz, R.-D. Maier, K.-D. Krueger, U. Kohls, J. Anal. Toxicol. 22 (1998) 549–558.
[82] F. Tagliaro, S. Turrina, P. Pisi, F.P. Smith, M. Marigo, J. Chromatogr. B 713 (1998) 27–49.
[83] D.K. Lloyd, J. Chromatogr. A 735 (1996) 29–42.
[84] P. Wernly, W. Thormann, Anal. Chem. 63 (1991) 2878–2882.
[85] G.L. Chee, T.S.M. Wan, J. Chromatogr. 612 (1993) 172–177.
[86] S. Heitmeier, G. Blashke, J. Chromatogr. B 721 (1999) 109–125.
[87] Y. Nagagawa, K. Iwatani, J. Chromatogr. 562 (1991) 99–110.
[88] R.A. de Zeeuw, J. Hartstra, J.P. Franke, J. Chromatogr. A 674 (1994) 3–13.
[89] P.R. Puopolo, S.A. Volpicelli, D.M. Johnson, J.G. Flood, Clin. Chem. 37 (1991) 2124–2130.
[90] J.P. Franke, R.A. de Zeeuw, P.G. Schepers, J. Forensic Sci. 30 (1985) 1074–1081.
[91] R. Meatherall, J. Forensic Sci. 42 (1997) 1160–1170.
[92] K. Kudo, T. Nagata, K. Kimura, T. Imamura, N. Jitsufuchi, J. Anal. Toxicol. 19 (1995) 87–90.
[93] R. Clouette, M. Jacob, P. Koteel, M. Spain, J. Anal. Toxicol. 17 (1993) 1–4.
[94] M.R. Moeller, G. Doerr, S. Warth, J. Forensic Sci. 37 (1992) 969–983.
[95] M.A. elSohly, T.L. Little Jr., D.F. Stanford, J. Anal. Toxicol. 16 (1992) 188–191.
[96] K.E. Brooks, N.B. Smith, Clin. Chem. 35 (1989) 2100–2103.
[97] H. Maurer, K. Pfleger, J. Chromatogr. 382 (1986) 147–165.
[98] M.S. Leloux, R.A.A. Maes, Biomed. Environ. Mass Spectrom. 19 (1990) 137–142.
[99] D. Carreras, C. Imaz, R. Navajas, M.A. Garcia, C. Rodriguez, A.F. Rodriguez, R. Cortes, J. Chromatogr. A 683 (1994) 195–202.
[100] S.J. Park, H.S. Pyo, Y.J. Kim, M.S. Kim, J. Park, J. Anal. Toxicol. 14 (1990) 84–90.
[101] S.F. Cooper, R. Masse, R. Dugal, J. Chromatogr. 489 (1989) 65–88.
[102] H.H. Maurer, Ther. Drug Monit. 18 (1996) 465–470.
[103] P. Marquet, E. Lacassie, C. Battu, H. Faubert, G. Lachatre, J. Chromatogr. B 700 (1997) 77–82.
[104] D.-S. Lho, H.-S. Shin, B.-K. Kang, J. Park, J. Anal. Toxicol. 14 (1990) 73–76.
[105] M. Cremese, A.H. Wu, G. Cassella, E. O'Connor, K. Rymut, D.W. Hill, J. Forensic Sci. 43 (1998) 1220–1224.
[106] R. Wasels, F. Belleville, J. Chromatogr. A 674 (1994) 225–234.
[107] C.L. O'Neal, A. Poklis, J. Anal. Toxicol. 21 (1997) 427–432.
[108] D. Bergemann, A. Geier, L. von Meyer, J. Forensic Sci. 44 (1999) 372–374.
[109] F. Musshoff, T. Daldrup, Forensic Sci. Int. 88 (1997) 133–140.
[110] H. Maurer, K. Pfleger, J. Chromatogr. 428 (1988) 43–60.
[111] H. Maurer, K. Pfleger, Fresenius' Z. Anal. Chem. 331 (1988) 744–756.
[112] H.H. Maurer, Arch. Toxicol. 64 (1990) 554–561.
[113] J. Segura, R. Ventura, C. Jurado, J. Chromatogr. B 713 (1998) 61–90.
[114] M.R. Moeller, S. Steinmeyer, T. Kraemer, J. Chromatogr. B 713 (1998) 91–109.
[115] H.H. Maurer, J.W. Arlt, J. Chromatogr. B 714 (1998) 181–195.
[116] A. Dona, S. Athanaselis, C. Maravelias, A. Koutselinis, Forensic Sci. Int. 99 (1999) 71–77.
[117] R.A. Cox, J.A. Crifasi, R.E. Dickey, S.C. Ketzler, G.L. Pshak, J. Anal. Toxicol. 13 (1989) 224–228.
[118] X.-H. Chen, J.-P. Franke, R.A. de Zeeuw, Forensic Sci. Review 4 (1992) 147–159.
[119] X.H. Chen, J. Wijsbeek, J. van Veen, J.P. Franke, R.A. de Zeeuw, J. Chromatogr. 529 (1990) 161–166.
[120] A. Polettini, A. Groppi, C. Vignali, M. Montagna, J. Chromatogr. B 713 (1998) 265–279.
[121] X.-H. Chen, J.-P. Franke, J. Wijsbeek, R.A. de Zeeuw, J. Anal. Toxicol. 16 (1992) 351–355.
[122] M. Bogusz, M. Erkens, J. Chromatogr. A 674 (1994) 97–126.
[123] D. Hannak, F. Scharbert, R. Kattermann, J. Chromatogr. A 728 (1996) 307–310.