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Review

# Solid-phase extraction procedures in systematic toxicological analysis

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**Abstract**

In systematic toxicological analysis (STA) the substance(s) present is (are) not known at the start of the analysis. In such an undirected search the extraction procedure cannot be directed to a given substance but must be a general procedure where a compromise must be reached in that the substances of interest are isolated at a yield as high as possible and the interfering substances from the biological material are removed. When using solid-phase extraction (SPE) it is desirable to have procedures using just one column. An overview of screening procedures using diatomaceous earth, polystyrene–divinylbenzene copolymer and mixed-mode bonded silica as column material in SPE is given. The latter type of sorbent is most popular at the moment and the critical steps in the procedure are outlined in more detail. Recent developments of SPE disks look very promising for STA. 

*Keywords:* Reviews; Solid-phase extraction; Systematic toxicological analysis; Broad-spectrum drug screening; Extraction methods

## Contents



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## 1. Introduction

The three major tasks in the toxicological analysis of a given specimen are: (1) To detect if the specimen contains any harmful substance(s); (2) to identify the substance(s) involved; and (3) to determine the quantity of the substance(s) involved and to interpret the outcomes in regard to the reason for carrying out the analysis.These three steps are mandatory in all areas of toxicological analysis, such as clinical, forensic, workplace, doping, drugs and driving, etc. The first two steps relate to qualitative analysis and often go hand in hand. Depending on the circumstances or the purpose, two approaches can be distinguished; namely, the directed search, geared to a limited number of substances such as in workplace testing, the analysis of alcohol in traffic; and the undirected search, also called systematic toxicological analysis (STA). STA can be defined as the undirected chemical–analytical search for potentially toxic substances whose presences are uncertain and whose identities are unknown and is obviously required if little or no information is available as to which toxic agent is involved, the so-called General Unknown Cases. It must be noted, however, that even when the toxic agent is known or when there is a strong suspicion, STA remains necessary to check for additional toxic agents hitherto unknown to be present.

In our society we are surrounded by thousands of chemicals, many of which have harmful properties, such as drugs, pesticides, household products, etc. Moreover, the materials available for analysis are a complex biological matrix, in which toxicologically relevant substances are present in trace amounts compared to the endogenous compounds present.

The analytical techniques used for STA of nonvolatile organic substances are either competitive binding assays (i.e. immunoassays or receptor assays) which are specific for a substance or selective for a group of substances or chromatographic techniques coupled to various detector systems.

Especially for the latter techniques, the sample work up – isolation, concentration – is a key step. Even with the most sophisticated instrumentation a substance can not be found when it is not extracted. Sample work up procedures should retain, therefore, all toxicologically relevant substances, at the same time removing all nonrelevant substances and interferences.

For a long time the traditional sample work up technique in analytical toxicology was liquid–liquid extraction (LLE), often combined with sample pretreatment procedures such as conjugate hydrolysis, digestion and protein removal. Although LLE proved to be suitable in a substantial number of cases, the disadvantages of this technique, e.g. matrix interferences, emulsion formation, use of large volumes of hazardous solvents, have troubled the analyst. Solid-phase extraction (SPE) approaches can (partially) overcome these drawbacks of LLE. In recent years the development of suitable materials for SPE has provided a new impetus to extraction approaches. The majority of publications has geared towards the isolation of one substance or a limited number of related substances, i.e. directed analysis. In STA, however, the undirected approach is required, in which a compromise between an acceptable recovery of a great many substances and an adequate removal of matrix compounds within a reasonably short period of time must be reached.

For STA an overview will be given of SPE procedures using basically three different types of solid phases, namely diatomaceous earth, polystyrene–divinylbenzene resin and chemically modified silica.

## 2. Diatomaceous earth (Extrelut®, Chemelut®)

The principle of SPE using diatomaceous earth is closely related to conventional LLE. The aqueous phase is absorbed onto the diatomaceous earth, a porous material which acts as support for the aqueous phase. This provides a large surface area for partition into an elution solvent, which flows through the immobilized specimen on the column under gravity [1]. The elution is a continuous process and theoretically is expected to give superior recoveries compared to LLE. Other advantages are the elimination of centrifugation, aspiration and filtration steps and the prevention of emulsion formation. However, relatively large volumes of (hazardous) organic solvents are still required. A typical procedure with this type of material is as follows:

- The biological sample is slightly diluted with an appropriate buffer. For acidic substances the pH should be below 5 and for basic substances a basic pH is required.
- The diluted sample is poured onto the column. The bed mass of the column and the volume of the aqueous sample must be in agreement with each other in that the whole sample is absorbed onto the column.
- A 10–15 min equilibration period.
- Elution with an organic solvent which is water immiscible. The volume of the solvent is about twice the volume of the diluted aqueous sample.
- Extraction of the substances to an aqueous phase can be carried out, or the organic solvent can be evaporated to dryness.

For screening purposes where acidic, neutral and basic substances may be present, this type of SPE must be carried out with at least two columns: one for the acidic and neutral substances and one for basic and neutral substances.

Interschick et al. [2], however, use one column and determine acidic, neutral and basic drugs in urine and stomach content and start at acidic conditions, eluting the acidic and neutral substances first. Then the absorbed aqueous phase onto the column is made alkaline by flowing ammonium gas through the column. A second elution for the basic substances is then carried out. The elution solvents are evaporated. The residues are dissolved and the extracts are analysed with a series of TLC systems. Lillsunde and Korte [3] describe a general screening procedure for substances in urine where a general common extraction at a slightly alkaline pH is used and where furthermore an extraction of conjugates (benzodiazepines, morphine, 11-nor-$\Delta^9$-tetrahydrocannabinol-9-carboxylic acid, THC-COOH) after acidic or enzymic hydrolysis is carried out. Benzoylecgonine, the main metabolite of cocaine, is determined separately. Ferrara et al. [4] determine drugs of abuse in urine using 6 different SPE procedures for different groups of drugs: morphine, benzoyecgonine, THC-COOH, amphetamines, barbiturates and benzodiazepines.

SPE with diatomaceous earth can also be used to screen for drugs in whole blood. Anderson and Fuller [5] isolate weakly acidic and neutral substances in one step from whole blood. Logan and Stafford [6] investigated the effects of several variables (pH, type of solvent, solvent volume) on extraction yields and cleanliness of the extracts from whole blood samples. They also compared the Extrelut extraction with a liquid/liquid extraction. On average, with Extrelut the chromatograms were cleaner and the recoveries higher (Table 1).

Table 1
Mean recovery and relative standard deviation (RSD; $n=3$) after Extrelut and liquid/liquid extraction

| Compound | Recovery (%) and RSD | | | |
|---|---|---|---|---|
| | Liquid/liquid | | Extrelut | |
| Alprazolam | 73 | 12.3 | 74 | 9.5 |
| Cocaine | 44 | 15.5 | 58 | 8.6 |
| Desipramine | 59 | 12.6 | 54 | 10.1 |
| Diazepam | 60 | 17.1 | 70 | 7.6 |
| Imipramine | 48 | 10.4 | 51 | 12.5 |
| Meprobamate | 0 | | 4 | (40) |
| Pentobarbital | 40 | 6.6 | 41 | 4.8 |
| Pethidine | 21 | 8.4 | 25 | 9.0 |
| Phenobarbital | 0 | | 0 | |
| Promethazine | 42 | 8.4 | 60 | 11.3 |
| Thioridazine | 20 | 19.1 | 41 | 12.6 |
| Mean | 37 | 12.3 | 48 | 9.5 |

Data from Ref. [6]: Concentrations of drugs in blood was 5 μg/ml.
Liquid/liquid extraction conditions were: 0.5 ml blood sample, 0.5 ml borate buffer (pH 9.0) and 12 ml *n*-butyl chloride.
Extrelut extraction conditions were: 0.5 ml blood sample, 0.3 ml borate buffer (0.1 *M*, pH 9.0) and collecting 12 ml *n*-butyl chloride.

## 3. Styrene–divinylbenzene resin (SDB)

Polystyrene–divinylbenzene copolymer (e.g. XAD-2) is a hydrophobic resin that can absorb many water-soluble organic compounds, principally by Van der Waals forces, but hydrophobic bonding and dipole–dipole interactions may also play a role. A typical procedure with this type of material is as follows:

- The biological sample is diluted and the pH is adjusted to the desired value.
- The resin is washed with four column volumes

acetone, three column volumes of methanol and three times with three column volumes of distilled water [7].
- The diluted sample is passed through the column where drugs and metabolites absorb.
- The resin is washed with water.
- The substances are eluted with an organic solvent, e.g. methanol, methanol–chloroform, acetone–ether, ethyl acetate, etc.
- The eluent is dried or filtered through phase-separating filter paper.
- The organic solvent is evaporated to dryness and the residue is reconstituted in a small volume of an organic solvent.

For binding to the resin the substances must be in an hydrophobic state. Therefore, usually 2 columns are needed: one for the acidic and neutral substances and one for the basic and neutral substances. However, a differential elution procedure where acidic/neutral and basic/neutral substances can be eluted in two fractions using a single SDB column has also been described [8–10]. Some typical recovery data regarding acidic and basic compounds extracted from urine are presented in Table 2. Extraction yields are in the order of 80% with a precision of 7–27%, which are on average rather high.

In general, the extracts are clean enough to allow GC and TLC determinations at therapeutic and toxic concentrations [10–13]. SDB resin is especially interesting for analysing urine samples since sulphate and glucuronide conjugates can be isolated. These types of compounds are not amenable for classic solvent extraction. The SDB resin is also used for isolating substances from other biological matrices, such as blood [14–18], serum, bile, gastric content and tissues [19–21].

The extraction yields of substances isolated from different biological samples may vary considerably, however. The resin has to be cleaned very carefully, otherwise interfering substances originating from the resin will appear in the extracts.

The SDB extractions in columns have now been largely replaced by SPE using silica-based column materials. Recently, new SDB-based SPE columns (e.g. Bond Elut ENV, Varian) have become available, with which the above mentioned drawbacks may be overcome. Moreover, SDB material in extraction disks can minimize volumes of elution solvents while still allowing relatively large sample volumes [22,23]. Screening methods for STA using this type of material are not yet available.

Table 2
Recovery data of drugs extracted from urine using encapsulated XAD-2 and sequential elution

| Compound | Recovery (%) | RSD (%) |
|---|---|---|
| *Acidic compounds* | | |
| Amobarbital | 86 | 8 |
| Butabarbital | 82 | 7 |
| Methyprylon | 52 | 10 |
| Pentobarbital | 83 | 10 |
| Phenobarbital | 86 | 7 |
| Secobarbital | 82 | 12 |
| *Basic compounds* | | |
| Amitriptyline | 66 | 13 |
| Cocaine | 72 | 15 |
| Doxepine | 78 | 12 |
| Imipramine | 82 | 14 |
| Lidocaine | 70 | 24 |
| Methadone | 80 | 12 |
| Methapyrilene | 65 | 18 |
| Pethidine | 32 | 16 |
| Phencyclidine | 18 | 27 |
| Propoxyphene | 70 | 13 |

Data from Ref. [10]: Acidic comounds 10 μg/ml urine; basic compounds 4 μg/ml. Two millilitres urine diluted with buffer, internal standard solutions and water. Differential elution with 2.5 ml diethyl ether, wash with pH 9.8 carbonate buffer and elution with 2.5 ml chloroform–2-propanol (4:1). RSDs are based on 6 determinations calculated using the internal standards cyclobarbital for the acidic compounds and SKF-525 for the basic compounds.

## 4. Mixed-mode bonded silica

Chemically modified silica with either hydrophobic groups or with ion exchange groups can only bind one type of substance. Taking into account that substances of toxicological interest can have acidic, neutral and basic properties and that preferably only one SPE column has to be used, a mixture of silica materials in one column has the desired selectivity potential. Such a mixed-mode bonded silica in which the silanol groups are partially derivatized with medium-length alkyl chains and partially with cation exchange substituents can exert at least two types of

interactions. Screening procedures using this type of SPE material have been of increasing interest and SPE columns with mixed-mode phases are now available from a number of manufacturers, e.g. Bond Elut Certify (Varian Sample Preparation Products, Harbor City, CA, USA), Clean Screen DAU (Worldwide Monitoring Corp., Horsham, PA, USA), Isolute HCX (International Sorbent Technology, Hengoed, UK) and TSC (Merck, Darmstadt, Germany).

Mixed-mode bonded silica can retain at a suitable pH acidic and neutral substances by hydrophobic interactions with the alkyl chains and the basic substances by interactions with the cation exchange groups. Differential elution can take place by a proper adjustment of the pH and the choice of solvents. A typical extraction procedure looks as follows:

- Sample preparation. Urine or plasma/serum is diluted with a suitable buffer. Diluted whole blood will clog the usual SPE columns and therefore either deproteinization or ultrasonic treatment followed by centrifugation is recommended. Tissue (liver, brain, etc.) has to be homogenized first. The homogenized tissue sample can then be centrifuged and the supernatant used directly or digestion procedures have to be used to liberate the substances bound to the proteins.
- Column conditioning. The extraction column is conditioned with methanol followed by water or buffer. The column must not become dry before sample application.
- Sample application. The sample is applied under light vacuum at a flow rate not exceeding 2 ml/min.
- Column wash and pH adjustment. The column is washed usually with water and the pH of the extraction system can be adjusted to a suitable pH before elution.
- Column drying. The column is dried by application of 1 ml of hexane or by application of less than a bed volume of methanol. If the presence of water in the eluent can interfere with the final analysis technique (e.g. gas chromatography), this drying procedure is of especial importance.
- Elution of fraction A. The analytes retained by the hydrophobic groups of the sorbent are eluted using a moderately polar solvent or combination of solvents.
- Column wash.
- Elution of fraction B. The basic substances retained by the cation exchange groups of the sorbent in their protonated form are eluted by an organic solvent mixed usually with 2% strong ammonia. A slow elution is critical as ionic interactions are strong and equilibration takes time.
- Evaporation. The fractions A and B are separately evaporated to dryness or until a very small volume of solvent remains.

The critical steps in the general extraction procedure are now described in more detail.

### *4.1. Sample pretreatment*

The pH of the diluted sample is of utmost importance. Dilution with a 0.1 mol/l phosphate buffer pH 6.0 is most widely used [24–33]. At this pH the weakly basic (e.g. diazepam), the neutral and the weakly acidic compounds, such as barbiturates, are in the nonionized form and retained by the hydrophobic substituents of the sorbent. The strongly basic substances are protonated and retained by the cation exchange substituent of the sorbent. It must be realized that strongly acidic compounds such as many nonsteroidal anti-inflammatory drugs (NSAIDs) are deprotonated, ionized and therefore not retained. When blood (plasma/serum) samples are brought to lower pH values coagulation of the proteins occurs, resulting in difficulties in the sample application step: slow or no flow. When a serum or plasma sample is added to 0.1 mol/l phosphoric acid this coagulation can be avoided [34]. The extracts, especially extracts from urine, are however not as clean as when using a pH 6 buffer for dilution.

### *4.2. Column wash and pH adjustment*

Usually the column is washed with 1–2 ml deionized water which is assumed to be adequate to remove interferences from the column [25,26,30]. In the acidic eluate interfering peaks are still present when analysing under very sensitive conditions. To

remove more interferences 20% methanol in water or buffer can be used [24]. It must be noted, however, that more polar acidic and neutral substances can be washed away with such a procedure.

In order to get a reproducible differential elution the pH of the column must be adjusted to about pH 3. At higher pH values a large number of basic compounds will elute in the first fraction (neutral and acidic substances). Lower pH values can deteriorate the extraction column. For the adjustment of the pH, 0.5–1.0 ml diluted acetic acid is sufficient.

### *4.3. Column drying*

Drying of the column is of utmost importance when in the analysing step no water is allowed, for instance in case GC is used. Drying is carried out by applying vacuum to the column for about 5 min and/or by centrifugation of the column. Further drying can be carried out by applying a small volume (e.g. 50 μl) methanol or a larger volume, typically 1 ml, hexane followed by vacuum for about 2 min [24,28,35]. The use of hexane has the advantage that a dry column is easily obtained, but there is a risk of partially eluting hydrophobic substances such as benzodiazepines in this wash process.

### *4.4. Elution of fraction A*

Chen et al. [25,26] have optimized this procedure using acetone–chloroform mixtures and have found that a ratio of 1:1 gave optimum extraction yields for a selection of drugs. More polar elution solvents will result in extracts that are less clean. More hydrophobic elution solvents are used, e.g. dichloromethane [24,36], hexane–diethyl ether (40:60) [36], hexane–ethyl acetate [31,35]. It may be expected that cleaner extracts are obtained, but acidic and neutral substances may be partially retained under these elution conditions. To avoid dirty extracts in fraction B when using a rather hydrophobic eluent for fraction A, an in between polar washing step, for instance with methanol, is needed. Thus, as a compromise, for screening purposes an elution solvent of medium polarity is required. Since chlorinated organic solvents can better be avoided, a solvent mixture of acetone and ethyl acetate will give best results.

Several authors [24,31,34] promote a wash step in between the elution of fraction A and fraction B. Washing with a polar solvent like methanol can elute weakly basic substances still retained on the sorbent. When the polarity of the elution solvent of fraction A is of medium polarity an in between washing step is not needed. The extracts of fraction B will be sufficiently clean.

When a sensitive detection of acidic compounds and a cleaner extract are desired, fraction A can be extracted by LLE using sodium carbonate or sodium hydroxide solutions.

### *4.5. Elution of fraction B*

In order to elute the protonated basic substances from the cation exchange sites of the sorbent the elution solvent for eluting the basic drugs has to contain an amine. In general 2–3% strong ammonia is used for that purpose. Ammoniated ethyl acetate is widely used for elution. However, it appeared that more polar substances did not elute under these circumstances. Ammoniated dichloromethane–2-propanol (80:20) is a better solvent for e.g. the cocaine metabolite benzoylecgonine [27–30,35,36]. Elution of fraction B can take place by using both solvents successively and by combining the extracts.

Table 3 gives an overview of extraction methods using mixed-mode SPE phases for broad spectrum drug screening. The detection method was in nearly all cases gas chromatography with FID, NPD or MS detection. In general, acceptable extraction yields were obtained by all authors independent of the type of SPE column used. Amphetamine and other relatively volatile substances often show lower recoveries, which are probably caused by evaporation in the final step of the SPE procedure. Polar drugs like acids and paracetamol are scarcely retained by the SPE columns under the conditions used and may be washed away. Therefore, Eklund and Wikström [27] applied a liquid–liquid extraction step on the sample coming from the column and column wash.

## 5. Discussion

There is no one single extraction procedure that gives optimum results for all different sample types

Table 3
Overview of mixed-mode SPE methods for drug screening

| Sample type | SPE column type | Sample volume | Drug conc. (μg/ml or μg/g) | Elution method | Detection yield (%) | Extraction (%) | RSD | Ref. |
|---|---|---|---|---|---|---|---|---|
| Urine (U) Plasma (P) | CS DAU | A: 4 ml U B: 5 ml U | 0.5–2 | A: 10 ml DCM B: DCM–2PrOH–25% $NH_3$ (147:49:4) | TLC GC–MS | 61–88[a] | <9 | [37] |
| | BEC | 2 ml U/P | 10 | A: 4 ml Clf–Ac (1:1) B: 2 ml EtAc–33% $NH_3$ (98:2) | GC–FID | 97–104 | <6 | [25] |
| | BEC | 1 ml U | 0.05 | A: 1 ml Hex–EtAc (8:2) B: 2 ml DCM–2PrOH–25% $NH_3$ (80:20:2) | GC–MS | – | – | [35] |
| | 1: BEC 2: Isolute | 5 ml U | 0.4–1 | A: 3 ml Hex–EtAc (75:25) B: 3 ml EtAc–28% $NH_3$ (98:2) | GC–MS | 1: 60–88[b] 2: 48–88[b] | 1: <10 2: <8 | [31] |
| | BEC | 1 ml U/P | 0.1–0.2 | A: 4 ml Clf–Ac (1:1) B: 2 ml EtAc–33% $NH_3$ (98:2) | GC–NPD | U: 82–105 P: 77–103 | U: <8 P: <7 | [38] |
| Whole blood | BEC | 1 ml | 0.05–5 | A: 4 ml DCM B: 4 ml EtAc–25% $NH_3$ (98:2) | GC–FID | 25–104[c] | <14 | [24] |
| | BEC | 1 ml | 2 | A: 4 ml Clf–Ac (1:1) B: 2 ml EtAc–33% $NH_3$ (98:2) | GC–FID | 81–103 | <8 | [26] |
| | BEC | 1 ml | 0.2–4 | A: 2 ml 60% acetone[d] B: 2 ml DCM–2PrOH–25% $NH_3$ (80:20:2) | GC–NPD | 50–100 | <8 | [27] |
| | 1: BEC 2: CS DAU | 1 ml | 1 | A: 3 ml Hex–EtAc (1:1) B: 3 ml DCM–2PrOH–28% $NH_3$ (78:20:2) | GC–NPD | 1: 73–112 2: 59–115 | 1: 9.7[e] 2: 7.8 | [28] |
| | BEC | 1 ml | 0.5 | A: 4 ml Clf–Ac (1:1) B: 2 ml EtAc–25% $NH_3$ (98:2) C: 2 ml DCM–2PrOH–25% $NH_3$ (80:20:2) | GC–MS[f] | – | – | [29] |
| | BEC | 1 ml | 0.05–0.5 | A: 2 ml Clf–Ac (1:1) B: 3 ml EtAc–33% $NH_3$ (98:2) | GC–NPD GC–MS[f] | 58–107[g] 26–117 | <11 <16 | [30] |
| Tissue | XTRACT | 1.25 g | | A: 2 ml DCM; 2 ml Hex–Eth (4:6) B: 4 ml DCM–2PrOH–25% $NH_3$ (80:20:2); 4ml EtAc | GC–MS | – | – | [36] |
| | BEC | 0.1 g | 20 | 4 ml Clf–Ac (1:1) B: 2 ml EtAc, 33% $NH_3$ (98:2) | GC–NPD GC–FID | 45–101 | <9 | [32] |

*SPE column materials*
CS DAU: Clean screen DAU, Worldwide Monitoring, Horsham, PA.
BEC: Bond Elut Certify, Varian Sample Preparation products, Harbor City, CA.
Isolut: Isolute HCX, International Sorbent Technology, Hengoed Mid Glamorgan, UK.
XTRACT: Worldwide Monitoring, Horsham, PA.
*Abbreviations*
Ac=acetone; Clf=chloroform; DCM=dichloromethane; EtAc=ethyl acetate; Eth=diethyl ether; Hex=hexane; $NH_3$=concentrated ammonia; 2PrOH=2-propyl alcohol.
*Notes*
[a]One SPE column is used for acidic and neutral drugs and one for basic drugs. Extraction yield for cannabinol was 0.3%.
[b]Low recoveries for barbital and ephedrine.
[c]Morphine and amphetamine are hardly recovered.
[d]Basic fractions of SPE were cleaned up by liquid–liquid extraction with butyl acetate; recovery of paracetamol is low.
[e]Mean values.
[f]TMS derivatization.
[g]Extraction yields at a spiked concentration of respectively 0.1 and 0.25 μg/ml.

and detection techniques used. Sample pretreatment is very much dependent on the sample type: Whole blood and tissue (homogenates) cannot be applied directly on SPE columns, whereas for urine usually a simple dilution step is satisfactory.

For screening purposes GC with a more or less

specific detection method, FID, NPD, MS, is the method of first choice due to its high identification power, i.e. high separation efficiency, good reproducibility of retention parameters and high sensitivity. Most SPE screening procedures are therefore developed for this detection technique, see Table 3.

Recent developments in HPLC systems for routine use, with detection systems having intrinsically a high identification power, such as diode-array detection (DAD) and MS detection, are very promising for general screening [39]. It must be realised that extraction methods developed for a GC detection cannot be transferred directly, without change, for HPLC detection. For instance, fatty acids from blood (whole blood, serum or plasma) can seriously interfere with GC–FID, GC–MS, whereas these acids are usually not seen in an HPLC–DAD chromatogram due to the low absorptivity of these acids in the usually wavelength range. However, relatively polar compounds from urine are not detected in GC due to their polarity or their low thermal stability but may seriously interfere in HPLC detection systems. Chromatograms of acidics/neutral fraction and a basic fraction of a selection of drugs extracted from plasma are shown in Fig. 1 [40]. The same procedure performed with urine samples resulted in chromatograms were no drugs peaks could be detected any more due to matrix interferences.

Although the same type of SPE material can be obtained from different manufacturers, the results using SPE material from different manufacturers and even results obtained from different batches from the same manufacturer may show significant differences in behaviour, i.e. in particle size distribution, flow velocities [41].

Bogusz et al. [41] also found large differences in extraction yields for morphine: Batch-to-batch and from manufacturer to manufacturer. This is not in agreement with an earlier study [42] in which 4 drugs were measured using different batches of Bond Elut Certify (Varian) over a period of more than a year and columns of Cleanscreen DAU (Worldwide Monitoring) where only small differences in recoveries were seen. Also, with this type of analysis, to check the behaviour of the SPE column materials, internal as well as external quality control is of utmost importance.

Chemically modified silica and SDB sorbents are now also available in extraction disks, e.g. SPEC (ANSYS, Irvine, CA, USA) and Empore (3M, St. Paul, MlV, USA). These materials are very promising since samples can be processed faster using smaller volumes of organic solvents [23]. Degel [31] compared the extraction of some drugs with SPEC Plus AR/MP3 disks (mixed-mode silica) with conventional Bond Elut Certify and Isolute HCX columns. There were no significant differences found in extraction yields and precision. More study, however, is needed before these types of materials can be used routinely for broad drug screening purposes.

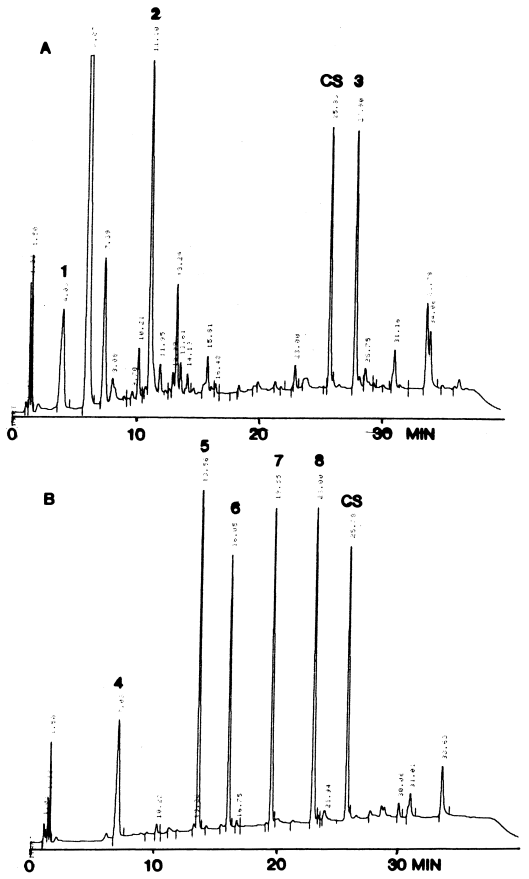


Fig. 1. Liquid chromatograms of plasma extracts using Bond Elut certify columns resulting in an acidics and neutrals fraction (A) and in a fraction containing the basic compounds (B) [40]. Plasma was spiked with paracetamol (1), salicylic acid (2), flufenamic acid (3), mepivacaine (4), papaverine (5), diphenhydramine (6), trimipramine (7) and ketazolam (8) at a level of 1 μg/ml. Diclophenac was used as a chromatographic standard (cs) for the calculations of the extraction yields.

## 6. Conclusions

For STA liquid–liquid extraction procedures are still in use. During the last 20 years alternative SPE procedures have been developed which have distinct advantages compared to LLE. Silica based phases, especially the mixed-bonded phases, still have increasing interest in the toxicological laboratories. Procedures using the latter type of sorbents have been described in detail.

SPE disks allow faster sample processing and smaller volumes of solvents and lend themselves also to automation. Developments in this technology are rising and the few applications published until now in the field of STA look promising.

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