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Review

Recent advances in microextraction by packed sorbent for bioanalysis

Mohamed Abdel-Rehim[a,b,*]

[a] Department of Clinical Pharmacology & DMPK, AstraZeneca R&D Södertälje, SE-151 85 Södertälje, Sweden
[b] Department of Chemistry and Biomedical Sciences, Faculty of Technology and Science, Karlstad University, SE-65188 Karlstad, Sweden

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ABSTRACT

Microextraction by packed sorbent (MEPS) is a new format for solid-phase extraction (SPE) that has been miniaturized to work with sample volumes as small as 10 μL. The commercially available presentation of MEPS uses the same sorbents as conventional SPE columns and so is suitable for use with most existing methods by scaling the reagent and sample volumes. Unlike conventional SPE columns, the MEPS sorbent bed is integrated into a liquid handling syringe that allows for low void volume sample manipulations either manually or in combination with laboratory robotics. The key aspect of MEPS is that the solvent volume used for the elution of the analytes is of a suitable order of magnitude to be injected directly into GC or LC systems. This new technique is very promising because it is fast, simple and it requires very small volume of samples to produce comparable results to conventional SPE technique. Furthermore, this technique can be easily interfaced to LC/MS and GC/MS to provide a completely automated MEPS/LC/MS or MEPS/GC/MS system. This extraction technique (MEPS) could be of interest in clinical, forensic toxicology and environmental analysis areas. This review provides a short overview of recent applications of MEPS in clinical and pre-clinical studies for quantification of drugs and metabolites in blood, plasma and urine. The extraction of anti-cancer drugs, β-blockers drugs, local anaesthetics, neurotransmitters and antibiotics from biological samples using MEPS technique will be illustrated.

Contents

* Correspondence address: AstraZeneca R&D Södertälje, SE-151 85 Södertälje, Sweden. Tel.: +46 8 55325604; fax: +46 8 55329026.
E-mail address: mohamed.abdel-rehim@astrazeneca.com.

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1. Introduction

Microextraction by packed sorbent (MEPS) is new development in the field of sample preparation. MEPS are the miniaturization of conventional SPE packed bed devices from millilitre bed volumes to microlitre volumes. MEPS can be connected online to GC or LC without any modifications. In MEPS approximately 1 mg of the solid packing material is packed inside a syringe (100–250 μL) as a plug or between the barrel and the needle as a cartridge. Sample preparation takes place on the packed bed. The bed can be coated to provide selective and suitable sampling conditions. The MEPS differs from commercial solid-phase extraction (SPE) in that the packing is integrated directly into the syringe not in a separate column. Thus, there is no need for a separate robot to apply the sample into the solid phase as with conventional SPE. Also, the packed syringe can be used several times, more than 100 times using plasma or urine samples but conventional SPE column is used only once. MEPS can handle small sample volumes (10 μL plasma, urine or water) as well as large volumes (1000 μL) and can be connected online to GC, LC or capillary electrochromatography (CEC). The MEPS approach to sample preparation is suitable for normal phases, reversed phases, mixed mode and ion exchange chemistries. MEPS can be fully automate—the sample processing, extraction and injection steps as an online sampling device using the same syringe. The great performance of MEPS was recently illustrated by online LC–MS and GC–MS assays of drugs and metabolites in urine, plasma, blood and hair samples [1–28]. The combination of MEPS and liquid chromatography–mass spectrometry (LC–MS) is an excellent tool for screening and determination of drugs and metabolites in blood, plasma and urine samples. This approach for sample preparation is very promising for many reasons: (1) it is easy to use, (2) it is a fully automated online procedure, (3) it is rapid, (4) reduces the solvent and sample volumes, and (5) the cost of analysis is minimal compared to conventional solid-phase extraction.

The MEPS technique has been used to extract a wide range of analytes in different matrices (urine, plasma, blood). Hence, several drugs such as local anaesthetics and their metabolites [1–5,9,10,12], anti-cancer drugs roscovitine, olomoucine, busulphan, cyclophosphamide, and AZD3409 [6–8,18–20], β-blockers acebutolol and metoprolol [15], neurotransmitters dopamine, serotonine [16], methadone [17], cocaine and cocaine metabolites [23] have been extracted from biological samples such as blood, plasma or urine samples using MEPS technique. In addition MEPS online with GC–MS was used for the determination of amphetamine in human hair and monoterpene metabolites in human urine [24,25]. Additionally, MEPS with CE was used for determination of fluoroquinolones in urine and local anaesthetics in human plasma samples. [27,28].

2. Microextraction by packed sorbent (MEPS)

2.1. Description of MEPS

MEPS is a new miniaturized, solid-phase extraction technique that can be connected online to GC or LC without any modifications. MEPS can be fully automated, the sample processing, extraction and injection steps are performed online using the same syringe. MEPS significantly reduces the volume of solvents and sample needed. In MEPS the sorbent, 1–2 mg, is either inserted into the syringe (100–250 μL) barrel as a plug or between the needle and the barrel as a cartridge (Fig. 1). The cartridge bed can be packed or coated to provide selective and suitable sampling conditions. Any sorbent material such silica based (C2, C8, C18), strong cation exchanger (SCX) using sulfonic acid bonded silica, restricted access material (RAM), hilic, carbon, polystyrene–divinylbenzene copolymer (PS-DVB) or molecular imprinted polymers (MIPs) can be used (Fig. 2). The MEPS is invented and developed in our laboratory at AstraZeneca, Södertälje, Sweden [1,2].

2.1.1. How is does it work?

First the plasma sample (10–250 μL) is diluted by water (1:4) and centrifuged for 2 min, for whole blood the dilution is 20 times. The plasma or blood sample is drawn through the sorbent by an autosampler (draw–eject in same vial or draw and eject into waste). The sample can be drawn once or more if the preconcentration of the analytes is required. When the sample has passed through the solid support, the analytes have been adsorbed to the solid phase. The solid phase is then washed once by water (50 μL) to remove the proteins and other interfering material. The analytes are then eluted with an organic solvent such as methanol or the LC mobile phase (20–50 μL) directly into the instrument's injector GC or LC. The process is fully automated. To reuse MEPS cartridge the sorbent was washed 3–4 times with water and 4–5 with solvent (elution solution). This step is to eliminate carry-over. In addition, we observed that conditioning step is not necessary as in SPE. This may due to the smaller amount of the sorbent or may due to the pumping of the sample through the sorbent more than one time. In addition the connection of MEPS to GC was made using large volume injection (30–100 μL). We did not observe any deterioration effect on the GC performance, after injection of many hundreds of samples on the same column, due to the non-drying of the sorbent after washing. This may due to the dead volume is less than 7 μL. Additionally, we added to our CTC-macro that CTC can elute 5 μL from elution solution to the waste (as drying step) before injection but we did not observe any effect on the performance with this step.

MEPS can be regarded as a short LC column in a syringe. The MEPS sorbent could be reused more than 120 extractions without

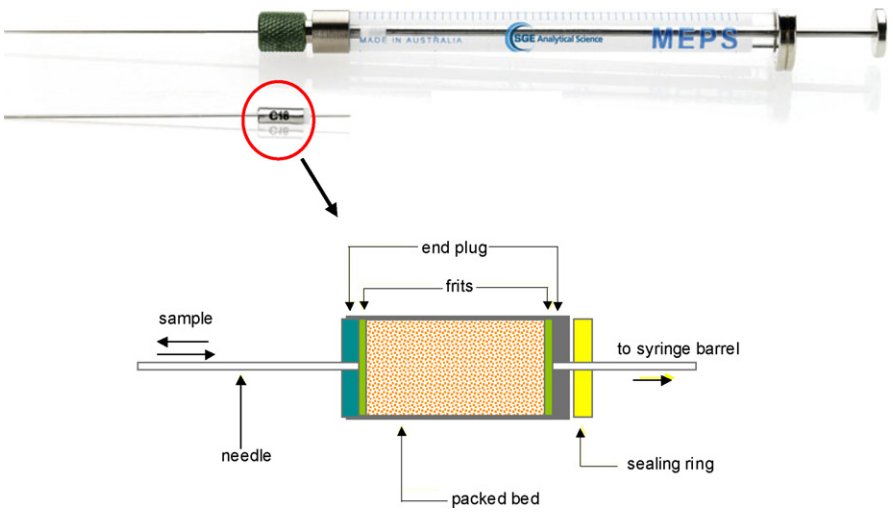


Fig. 1. MEPS product Syringe (250 μL) from SGE with packing bed (the dead volume is about 7 μL).

any loss in its performance for water and urine samples. Concerning plasma samples, the quality of plasma is an important issue for MEPS lifetime. If the plasma sample is centrifuged, MEPS should be reused up to 100 times without any loss in performance. For non-centrifuged plasma we observed that the MEPS performance became worse after 40–50 extractions. In the same way as all packed columns, the MEPS silica bed will be damaged if a sample with extreme pH is loaded.

2.2. Washing and elution solutions

2.2.1. Washing solution

Washing step is to remove unwanted weakly retained interferences. The solvent percentage and the pH are important factors to decrease leaking of analytes under washing process. It was shown that the analyte leakage increased as solvent percentage in washing solution increased [4,24]. Using 10% methanol in water reduced the recovery by about 10% compared with water alone. Leakage increased as well as methanol percentage increased [Fig. 3A]. For basic drugs the optimal washing solution contained 5% methanol or 2-propanol in water.

2.2.2. Elution solution

Pure or high solvent percentage (≥60%) is a typical elution solution. Further, the pH has an important factor (control charged/uncharged analyte) to get high recovery. The best possible elution solvent should elute the analyte using as small volume as possible. The analyte response increases as solvent percentage and elution volume increase [Fig. 3B]. For the extraction of basic drug ropivacaine from plasma samples, it was found that the optimal recovery was obtained when 0.25% ammonium hydroxide (pH >10) was added to elution solution containing 95% methanol and 5% water [3–5].

2.3. Influence of number of extraction cycles (draw–eject) on extraction efficiency

In MEPS the sample can be drawn through the needle into the syringe, once or several times (draw–eject). Fig. 4 shows that the recovery, as peak area, increased linear from one extraction cycle (1× 100 μL) up to eight cycles (8× 100 μL). Thus, sample response increased as applied sample volume increased. The multiply extraction cycles can be made from the same aliquot

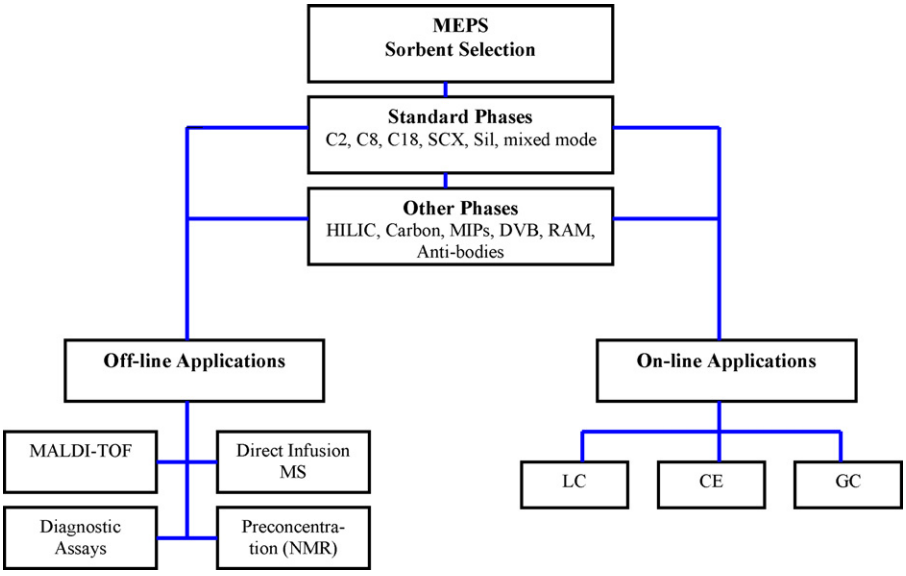


Fig. 2. A scheme of MEPS sorbents and applications.

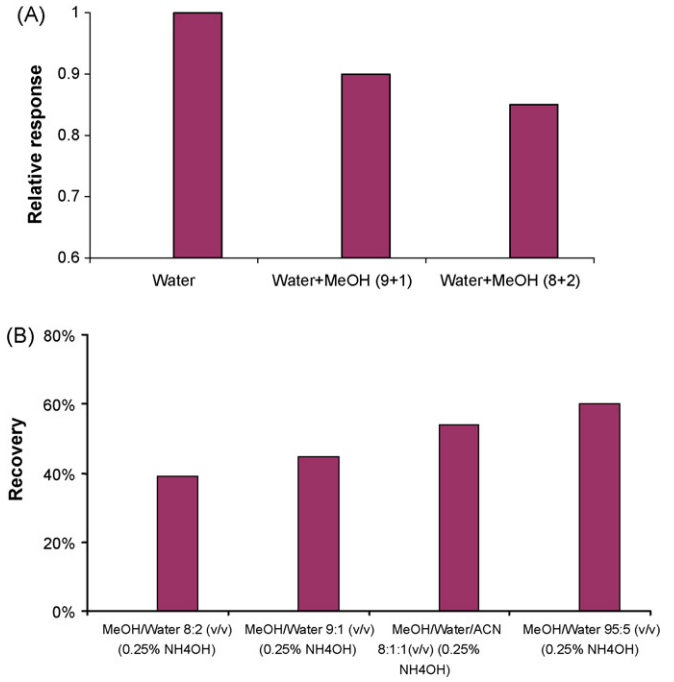


Fig. 3. (A) Effect of washing solution on the ropivacaine response. (B) Effect of elution solution composition on the ropivacaine recovery.

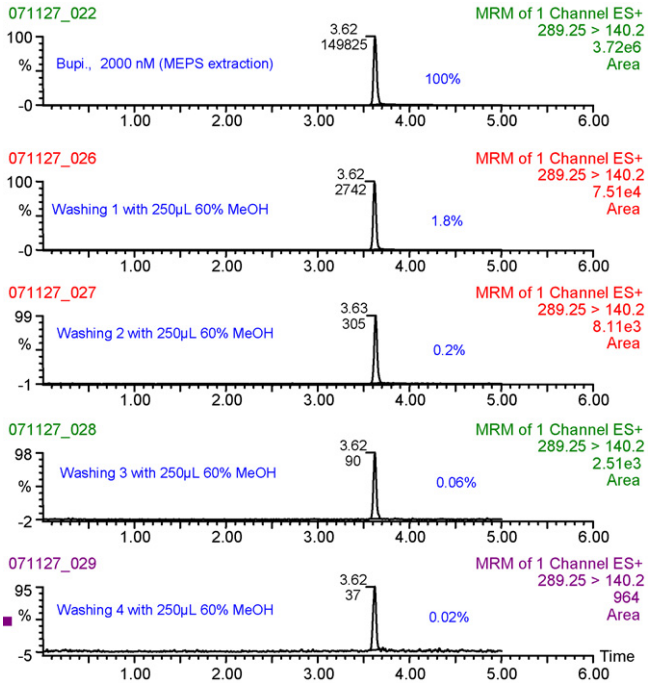


Fig. 5. Carry-over of injected bupivacaine sample (2000 nM) on MEPS-C18 sorbent after various washing using LC–MS/MS.
Source: Ref. [23] with permission.

(draw–eject in the same vial) or by draw up from aliquot and discard in waste (extract–discard). In addition most important thing to get high recovery is the choice of sorbent, C2–C18 phases are suitable for lipophilic analytes (non-polar) and polymeric phases such as polystyrene–divinylbenzene or mixed mod phases (anion–cation exchange mode) are suitable for polar analytes such as acidic and basic compounds. In previous study, we showed that the pumping of sample (draw–eject) 4 times gave a good recovery and can extend the lifetime of the MEPS cartridge [23].

2.4. MEPS and carry-over

The carry-over is one of the common problems in bioanalysis. The carry-over is limiting step for trace analysis giving bad accuracy and precision under method validation. Therefore, it was important to study the carry-over for MEPS. First of all, the carry-over is a compound depending phenomenon and can be caused not only from MEPS but also from analyte adsorption to autosampler or LC system

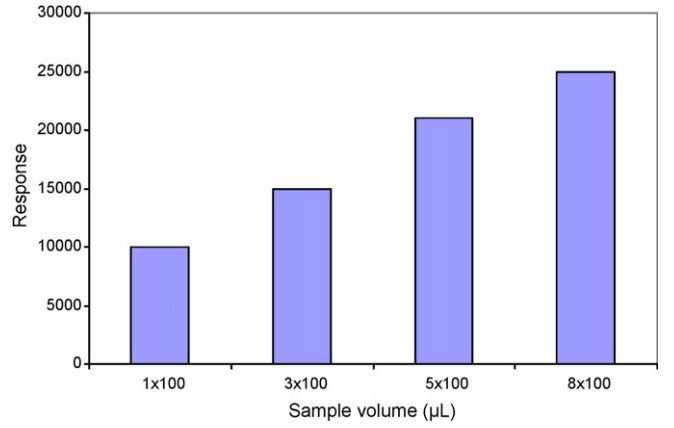


Fig. 4. Influence of the number of extraction cycles (draw–eject) on extraction efficiency.

or MS interface. The small quantity of solid phase in the MEPS can be easily and effectively washed between injections to reduce the possibility of carry-over. This washing process is simply MEPS and not practical with off-line SPE devices. With automation of MEPS washing can occur while the previous sample is running. Carry-over decreased to less than 0.02% when the sorbent was washed at least four times with elution solution and washing solution between extractions. Fig. 5 shows how the carry-over is reduced to 0.02% for bupivacaine (2000 nM) after four washing with the elution solution (60% methanol in water) four times.

2.5. Can MEPS eliminate matrix effects?

Complex matrices such as blood, plasma and urine are potential for ion suppression particularly with electrospray ionization mass spectrometry [30]. MEPS provides flexibility in different parameters such as washing solution, elution solution and type of sorbent materials. In previous publication, MEPS was used to investigate matrices effects for human plasma and compared to protein precipitation. MEPS technique eliminated salts and reduced the phospholipids concentration significantly compared to protein precipitation [21]. Fig. 6 shows full scan ESI-MS of blank human blood that was pre-treated using protein precipitation (PPT), liquid–liquid extraction (LLE) and MEPS. The protein precipitation produced the greatest amounts of phospholipid ions while the LLE reduced it much. MEPS reduced the phospholipids concentration considerably and gave lower noise in MS compared to PPT and LLE. Additionally, if the sample washing solution contains 5–10% isopropanol (IPA), phospholipids could be eliminated and more clean extracted was obtained.

2.6. Syringe-to-syringe variation

The reproducibility measurements of MEPS showed good RSD% values concerning analyte recovery for different analytes and different matrices. The syringe-to-syringe variations were also tested.

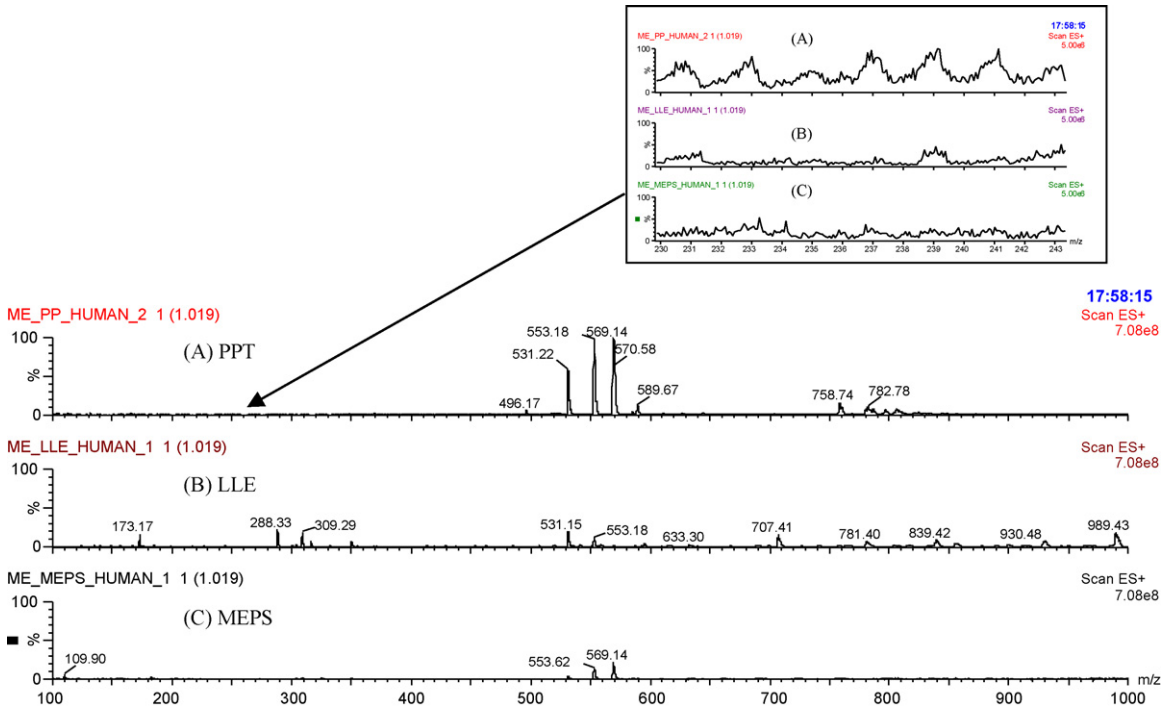


Fig. 6. Full scan ESI-MS resulting from direct infusion of blank human blood pre-treated with (A) protein precipitation (ppt), (B) liquid–liquid extraction (LLE) and (C) MEPS (C18).

Table 1 shows the variations between three different syringes using ropivacaine in human plasma samples. Using different concentrations at two levels (low and high), the accuracy and the precision are similar for the all studied syringes.

2.7. Reusing of MEPS cartridge

The continuous online use of MEPS makes use of the fact that the solid-phase sorbent is simply a large particle—small bed presentation of silica. For off-line use, some SPE users reuse their columns with little or no performance loss while others prefer to start each extraction with a new column. The reason of using new sorbent with each extraction is to eliminate the risk of carry-over, that retentive sites on the sorbent that are important to the extraction's success will not be available or that frits may be fouled by previous samples. In practice, the functional failure of the sorbents during the extraction of biological fluids is almost always associated with blockage, coagulation of sample or deterioration caused by aggressive reagents. As with conventional SPE devices, blockage and coagulation are avoidable with MEPS methods by incorporating into the method appropriate pretreatment of the sample. Typically, pretreatment can include centrifugation to remove suspended materials, pH adjustment, hydrolysis or precipitation. While such steps increase the fluidity of the sample and reduce the chance of physically obstructing the sorbent, they can also reduce competitive interactions between the target analyte and matrix and so increase the effectiveness of analyte–sorbent interactions.

During studies of MEPS, carry-over has been found to be less than 0.1% with 4–5 washes of methanol prior to reuse [23]. Carry-over from the sorbent can be reduced further by adding additional conditioning rinses to the method. The number of reuses possible for a MEPS cartridge is dependent on the sample type. As with conventional SPE, maintaining functional flow during the lifetime of the device is matrix dependent. For samples loaded with extreme pH the MEPS bed will be damaged in the same way as all packed columns. For the extraction of plasma samples and human urine, reversed-phase MEPS devices have been used for in excess of 100 injections.

Table 1
Accuracy and precision at various concentrations of ropivacaine in human plasma using different MEPS syringes.

	Accuracy % ($n = 6$)		Precision (RSD%, $n = 6$)	
	10[a]	800[a]	10[a]	800[a]
Syringe (A)	88	97	9.4	8.3
Syringe (B)	99	97	10.6	8.5
Syringe (C)	89	99	11.0	9.6

[a] Conc. (nmol/L).

3. MEPS applications

MEPS was applied for extraction of many drugs and metabolites from biological samples. The different drugs extracted by MEPS are summarised in Table 2.

3.1. MEPS online with liquid chromatography (LC)

3.1.1. Local anaesthetics from plasma, blood and urine samples by LC–MS/MS

3.1.1.1. Plasma samples. MEPS was used for extraction of amide-type local anaesthetics lidocaine, ropivacaine, mepivacaine, prilocaine, bupivacaine and some of their metabolites from plasma samples [4,5,9,10]. The calibration range 2–2000 nM and the extraction recovery was 60%. The results from calibration curves showed good correlation coefficients ($r^2 > 0.999$, $n = 3$) for all runs. The accuracy, given as a percentage variation from the nominal concentration values, ranged from −6% to 3%. The precision, given as relative standard deviation percentage, at three different concentrations of quality-control samples (QC samples) was consistently between 3% and 10%. The limit of quantification was 2 nM. Also, MIP (bupivacaine imprinted polymer was used) as sorbent for MEPS

Table 2
Summary for MEPS applications of drugs and metabolites from biological samples.

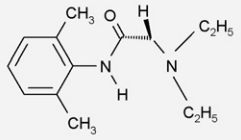
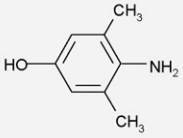
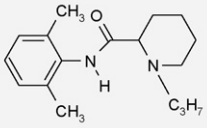
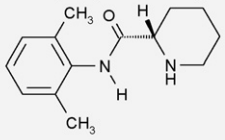
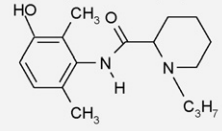
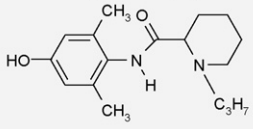
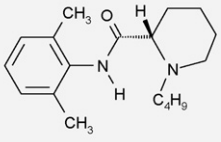
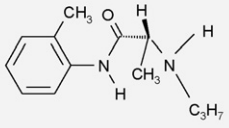
Compound class/Compound	Sample matrix/ Sample volume	MEPS sorbent	Analytical method	Calibration range	Refs.
Local anaesthetics					
- Lidocaine 	Plasma (20–50 μL) Human blood (20 μL)	C2, SCX, C18	GC–MS LC–MS/MS	2–2000 nM 5–2000 nM	[3,5,22]
- 4-OH-xyl (lido. major metabolite) 	Urine (100 μL)	Polystyrene	LC–MS/MS	17–9000 nM	[13]
- Ropivacaine 	Plasma (20–50 μL) Urine (50 μL) Human blood (20 μL)	C2, C8, MIP, SCX Polystyrene C18	GC–MS LC–MS/MS	2–2000 nM 2–2000 nM 5–2000 nM	[3–5,10,11,22]
- PPX (ropi, metabolite) 	Plasma (50 μL) Urine (50 μL)	C2, C8, SCX Polystyrene	LC–MS/MS	2–2000 nM 5–2000 nM	[4,5,14]
- 3-OH-ropivacaine (ropi. metabolite) 	Plasma (50 μL) Urine (50 μL)	C2, C8, SCX, polystyrene (ENV+)	LC–MS/MS	2–2000 nM 5–2000 nM	[1,2,11]
- 4-OH-ropivacine (ropi. metabolite) 	Plasma (50 μL) Urine (50 μL)	C2, C8, SCX Polystyrene	LC–MS/MS	2–2000 nM 5–2000 nM	[4,5,11]
-Bupivacaine 	Plasma (50 μL), Human blood (20 μL)	C2, SCX C18	GC–MS LC–MS/MS	2–2000 nM 5–2000 nM	[3,5,22]
- Prilocaine 	Plasma (50 μL)	C2	GC–MS	2–2000 nM	[3]

Table 2 (*Continued*)

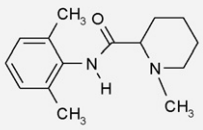
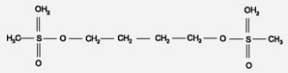
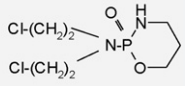
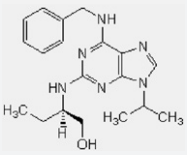
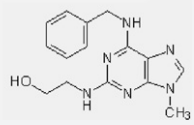
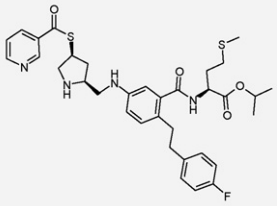
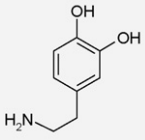
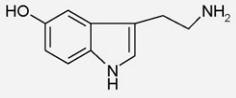
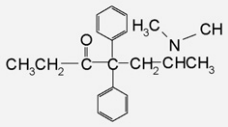
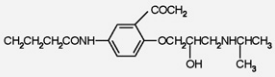
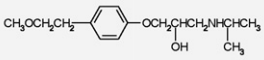
Compound class/Compound	Sample matrix/ Sample volume	MEPS sorbent	Analytical method	Calibration range	Refs.
- Mepivacaine 	Plasma (50 μL)	C2	GC–MS	5–2000 nM	[3]
Anti-cancer drugs					
- Busulfan 	Plasma (25 μL)	polystyrene	LC–MS	0.5–2500 ng mL^{-1}	[18]
- Cyclophosphamide 	Plasma (50 μL), mouse blood (20 μL)	Polystyrene	LC–MS/MS	0.5–150 μg mL^{-1} 0.1–100 μg mL^{-1}	[19,21]
- Roscovitine 	Plasma (50 μL)	Polystyrene	LC–MS/MS	0.5–2000 ng mL^{-1}	[6]
- Olomoucine 	Plasma (50 μL)	Polystyrene	LC–MS/MS	0.5–2000 ng mL^{-1}	[8]
- AZD3409 	Plasma (50 μL)	Polystyrene	LC–MS/MS	20–9000 nM	[20]
Antidepressant					
- Dopamine 	Urine (30 μL)	C8	LC–MS/MS	50–4000 ng mL^{-1}	[16]
- Serotonin 	Urine (30 μL)	C8	GC–MS	50–4000 ng mL^{-1}	[16]
Anti-addictive					
- Methadone 	Urine (50 μL)	C8	LC–MS/MS	2.3–3100 ng mL^{-1}	[17]

Table 2 (*Continued*)

Compound class/Compound	Sample matrix/ Sample volume	MEPS sorbent	Analytical method	Calibration range	Refs.
β-Blockers					
- Acebutolol 	Plasma, urine (50 μL)	Polystyrene		1.0–100 ng mL^{-1}	[15]
- Metoprolol 	Plasma, urine (50 μL)	Polystyrene	LC–MS/MS	1.0–100 ng mL^{-1}	[15]
Monoterpenes metabolites					
cis-Verbenol, transverbenol, α-terpineol, myrtenol and perillyl alcohol	Urine (210 μL)	C18	GC–MS	10–1000 μg L^{-1}	[26]
Antimicrobial					
Fluoroquinolones: ofloxacin, marbofloxacin, enrofloxacin, danofloxacin and difloxacin	Urine (48 μL)	C18	CE-MS	12.5–500 μg L^{-1}	[27]
Drugs of abuse					
Cocaine and its metabolites	Urine (100 μL)	C8, ENV, MCX	DART-TOF	65–1200 ng mL^{-1}	[28]

The extraction time is 1–2 min for all the studied compounds.

with LC–MS/MS for quantification of ropivacaine in plasma was investigated [10].

3.1.1.2. Human blood samples. The extraction of lidocaine, ropivacaine and bupivacaine from human blood samples by MEPS–LC–MS/MS was illustrated [21]. The blood samples were diluted 20 times with 0.1% HCOOH before MEPS handling. The low limit of quantification (LLOQ) was set to 10.0 nM for all the studied analytes. The validation of the method showed that the accuracy of the quality-control (QC) samples ranged from 85% to 97%. The inter-day precision of the studied analytes was within the range 1–5%. The calibration curve in human blood was constructed within the concentration range 10–10000 nM. The regression correlation coefficient (r) was over 0.995 for all runs ($n = 3$). Fig. 7 shows mass chromatogram of blank and spiked blood samples for lidocaine, ropivacaine and bupivacaine (50 nM each).

3.1.1.3. Human urine samples. The extraction of ropivacaine and its metabolites from urine samples using MEPS was reported [11]. The MEPS sorbent utilized was polystyrene polymer and the analysis technique used was LC–MS/MS. The urine sample volume was 50 μL (pumped three times) and washed with 50 μL water. The elution solution was 0.2% ammonium hydroxide in methanol (20 μL). The LLOQ was 5.0 nmol/L. The calibration curves were obtained within the concentration range 5–2000 nmol/L in urine. The regression correlation coefficients for urine samples were 0.999 for all

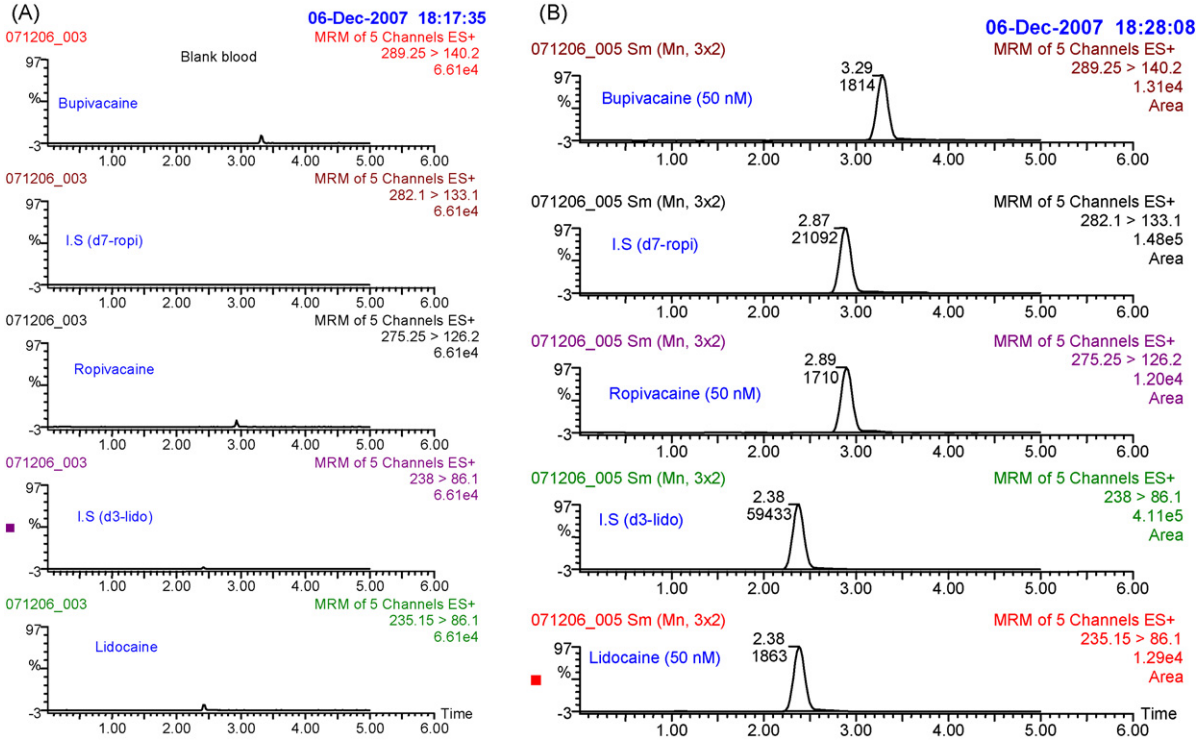


Fig. 7. Total ion chromatogram of the MRM analysis from (A) blank blood and (B) spiked blood samples for lidocaine, ropivacaine and bupivacaine (50 nM each).

Table 3
Comparison between MEPS and other methods for determination of cyclophosphamide in plasma samples.

Analysis method	Extraction method (sample volume)	Extraction time	LOD (ng/mL)	Ref.
LC–MS/MS	MEPS (50 μL)	1.0 min	5.0	[19]
LC–MS/MS	LLE (250 μL)	35 min	3.1	[31]
LC–MS/MS	LLE (100 μL)	20 min	20.0	[32]
μLC–MS/MS	SPE (1000 μL)	20 min	0.04	[33]

runs. The extraction degree was 40–60%. The mean accuracy values for the QC samples, reported were in the range of 99–115% and the precisions were in the range 1.9–11% [11].

3.1.2. Anti-cancer drugs from plasma and blood samples by LC–MS/MS

3.1.2.1. Busulphan in human plasma. The busulphan bioanalytical method using MEPS–LC–MS [18] provides good accuracy and precision within the range of therapeutic relevant levels (5–2500 ng/mL). Furthermore, it reduces the sample preparation time for busulphan (less than 1 min per sample comparing to 40 min using LLE), which is of a great importance in adjusting busulphan dose in clinical settings.

3.1.2.2. Cyclophosphamide in human plasma. MEPS was used as an online, followed by liquid chromatography with tandem mass spectrometry (LC–MS–MS) for the quantification of cyclophosphamide in plasma samples [19]. The new method reduced the sample handling and the analysis time by several folds compared to liquid chromatography and UV-detection. The limit of detection (LOD) was 0.005 μg/mL. The accuracy of the QC samples ranged from 95% to 106%. The inter-day variation was within the range 5–9% while the intra-day variation was 1–5%. The calibration curve in plasma was constructed within the concentration range 0.5–150 μg/mL. The regression correlation coefficient (r^2) was ≥0.99 for all runs. The limit of detection improved by 100 times using MEPS–LC–MS/MS (0.005 μg/mL) compared to LLE-LC–UV (0.5 μg/mL). The method was employed for the quantification of cyclophosphamide in human plasma samples for more than 170 patients samples. A comparison between MEPS and several published methods for determination of cyclophosphamide in plasma samples is presented in Table 3.

3.1.2.3. Cyclophosphamide in mice blood. In this paper illustrates the extraction of the anti-cancer drug cyclophosphamide in whole mice blood directly by MEPS–LC–MS/MS [21]. 20 μL of mice blood was mixed with 80 μL of the anticoagulant agent (EDTA). The blood samples were diluted five times with 0.1% HCOOH before MEPS handling. The LLOQ was set to 0.1 μg/mL. The accuracy of the QC samples ranged from 96% to 114%. The inter-day variation was within the range 2–9% while the intra-day variation was between 5% and 9%. The calibration curve in mice blood was constructed within the concentration range 0.1–100 μg/mL. The regression correlation coefficient (r^2) values were over 0.99 for all runs (n = 3).

3.1.2.4. Roscovitine in human plasma and urine samples. Roscovitine (2-(R)-(1-Ethyl-2-hydroxyethylamino)-6-benzylamino-9-isopropylpurine) has been recently considered as a possible new chemopreventive and chemotherapeutic approach. Roscovitine was extracted from plasma and urine samples by using MEPS. The sampling sorbent was polystyrene (ENV+, 1 mg). The accuracy values of QC samples were between 96% and 115%, and precision (CV%) had a maximum deviation of 11.4% [6].

3.1.2.5. AZD3409 (prodrug) in rat and dog plasma. AZD3409 is a novel oral protein prenyl transferase inhibiting both farnesyl transferase and the geranylgeranyl transferase-1. AZD3409 is extremely unstable; its metabolism involves conversion to a thiol ester intermediate, then, intracellularly to a thiol acid active moiety. Due to the instability of AZD3409, microextraction in packed syringe (MEPS) was used as an online and fast sample-preparation method, followed by liquid chromatography-tandem mass spectrometry (LC–MS–MS) for the quantification of this compound in plasma samples. Good accuracy and precision were obtained and the method was used for the analysis of rat and dog plasma samples [20]. Fig. 8 shows mass chromatogram obtained from MEPS extraction of spiked rat plasma of a quality-control sample (5.4 μM AZD3409) and blank rat plasma sample.

3.1.3. Extraction of β-blocker drugs from human plasma and urine by LC–MS/MS

3.1.3.1. Plasma samples. Acebutolol and metoprolol were extracted from plasma using MEPS online with LC–MS/MS [15]. The LLOQ for acebutolol and metoprolol were set to 1.0 ng/mL. The accuracy for the QC samples of acebutolol and metoprolol varied from 94% to 104% and the precisions (C.V.%) were 9.5–12%.

3.1.3.2. Urine samples. Determination of β-blocker acebutolol and metoprolol in urine was performed using MEPS and LC–MS/MS. The LLOQ for acebutolol and metoprolol were set to 1.0 ng/mL. The accuracy of quality-control samples varied by ±10%, and precision (C.V.%) had a deviation of 1.4–12%.

The calibration curves for acebutolol and metoprolol were obtained within the concentration range 1.0–100 ng/mL in both plasma and urine. The regression correlation coefficients (r^2) for plasma and urine samples were 0.999 for all runs [15].

3.1.4. Dopamine and serotonine in urine samples by LC–MS/MS

Dopamine and serotonine (5-hydroxytryptamine; 5HT) were extracted from human urine samples and preconcentrated using MEPS and determined by LC–MS/MS [16]. The new method is fully automated, of low cost and rapid in comparison with published methods. The calibration range was 50–4000 μg/L. MEPS sorbent was C8 and could be used more than 300 times. The extraction recovery was about 50% and the accuracy of MEPS–LC–MS–MS was 100–101% for dopamine and 99–100% for 5HT. The inter-day precision (RSD%) was 6.0–7.7% for dopamine and 6.1–11% for 5HT. The LLOQ and LOD for dopamine and serotonine were 50 and 1.0 ng/mL, respectively. The carry-over was tested by injecting blank after the highest standard concentration. The carry-over was about 0.1–0.2%. MEPS has comparable values of the accuracy and precision with the published methods. In addition, MEPS improved the limit of detection by two-fold and reduced the extraction time by about 12 times.

3.2. MEPS online with gas chromatography (GC)

3.2.1. Methadone in urine samples by GC–MS

A method for the simultaneous analysis of methadone in urine samples by MEPS–GC–MS is described [17]. The intra-assay precision (RSD%) of the method was about 11–14% (n = 6). The inter-assay precisions were 11–15% for methadone in urine samples (n = 18). The accuracy varied from 89% to 109% for intra-assay (n = 6), and 97% to 107% for inter-assay (n = 18). Fig. 9 shows GC–MS

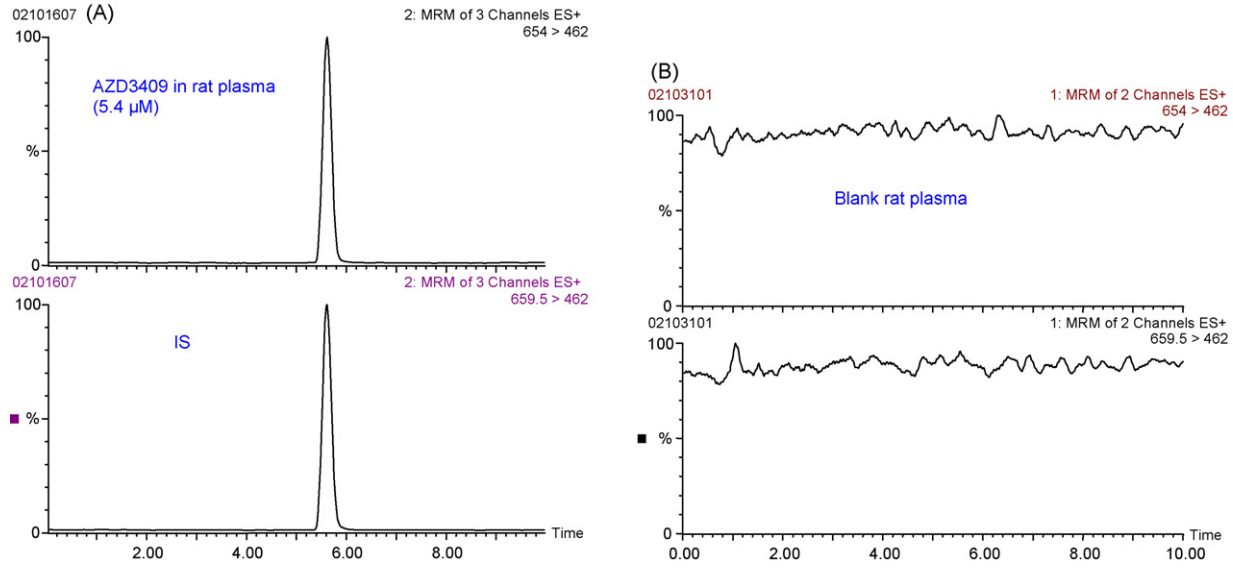


Fig. 8. Total ion chromatogram of the MRM analysis from (A) MEPS extraction of spiked rat plasma of a quality-control sample (5.4 μM AZD3409) and (B) blank rat plasma sample.
Source: Ref. [20] with permission

chromatograms of blank urine sample and spiked urine sample at low quality-control sample (concentration: 62 ng/mL) utilizing MEPS (C8) for the extraction of methadone.

3.2.2. Methamphetamine and amphetamine in human hair by GC–MS

The quantification of methamphetamine and amphetamine in human hair by GC–MS coupled with a novel combination of micropulverized extraction, aqueous acetylation and MEPS [24]. Elution was carried out with 50 μL of methanol into a gas chromatograph using a programmable temperature vaporizing (PTV) technique. The quantification ranges were 0.20–50 ng/mg for methamphetamine and amphetamine using 1 mg of hair and LLOQ was 0.2 ng/mg. The accuracy and precision were in agreement with the FDA guidance (within ±20% at LLOQ and within ±15% at the other concentrations). The MEPS cartridge was used for at least 300 extractions. The carry-over was estimated to be about 1–2%. The use of MEPS as sample preparation showed that the method is fast, robust and labor-saving in comparison with conventional methods [24].

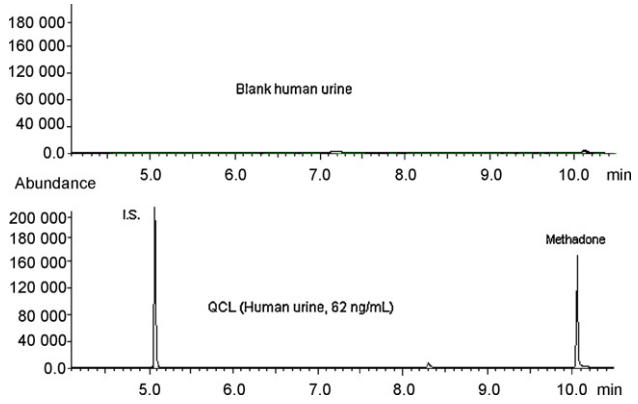


Fig. 9. GC/EI-MS chromatograms of blank urine sample and spiked urine sample at low quality-control sample (concentration: 62 ng/mL) utilizing MEPS (silica-C8) for the extraction of methadone.
Source: Ref. [17] with permission

3.2.3. Local anaesthetics in human plasma by GC–MS

MEPS online with GC–MS was used for quantification of local anaesthetics, mepivacaine, prilocaine, lidocaine and ropivacine, in human plasma samples [3]. The method was validated and the standard curves were evaluated by the means of quadratic regression and weighted by inverse of the concentration: $1/x$ for the calibration range 5–2000 nM. The extraction recovery was between 60% and 90%. The results showed correlation coefficients (r^2) over 0.99 for all analytes in the calibration range studied. The accuracy was between 99% and 115%. The inter-day precision ($n = 3$ days), expressed as the relative standard deviation (RSD%), was ranged from 3% to 10%.

3.2.4. Monoterpenes metabolites in small volumes of human urine by GC–MS

The paper [25] demonstrated the determinations of the monoterpene metabolites cis verbenol, transverbenol, α-terpineol, myrtenol and perillyl alcohol in a urine sample of a volunteer who lived in a dwelling with high indoor air exposure of monoterpenes. A 210 μL of enzymatically hydrolysed urine was used to carry out a complete extraction protocol. The elution volumes were between 1 and 10 μL and can be transferred completely or partly to the GC–MS system. The extraction recovery of the metabolites was between 75% and 90%. The enrichment factor for extractions of monoterpene metabolites was improved by the MEPS compared to SPE. The results showed correlation coefficients (r^2) over 0.996 for all metabolites. The limit of detection was set to 2–5 μg/L.

3.2.5. Opium metabolites from equine urine by GC–MS

Papaver somniferum (opium poppy) is a feed contaminant that can result in positive drug tests for racing horses. In this example, a conventional mixed mode method [26] was scaled down for an off-line MEPS preparation of a urine sample followed my micro-derivatization and GC–MS analysis.

A 300 μL sample of diluted equine urine from an animal receiving contaminated feed was hydrolysed with β-glucuronidase or acid, filtered and extracted on a C8/SCX MEPS cartridge conditioned with methanol (30 μL), and potassium phosphate buffer (0.2 M, pH 6, 30 μL) at a flow rate of 5 μL/s. The exhausted fraction was ejected at the same rate and the sorbent washed with 100 μL phosphate buffer, 50 μL acetic acid (1% v/v) and 100 μL methanol.

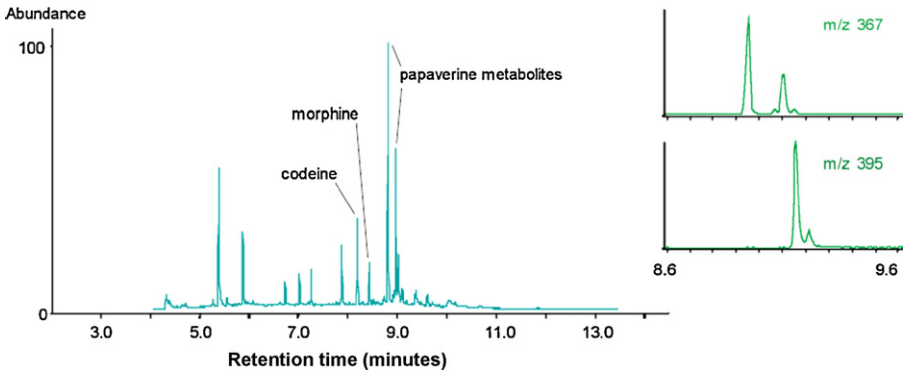


Fig. 10. The analysis of enzyme hydrolysed horse urine by GC–MS following extraction with MEPS (mixed mode C8/SCX) and micro-derivatization (peracetylation) of the recovered basic fraction. (Inset *m*/*z* 367 corresponds to O-desmethylpapaverine metabolites and *m*/*z* 395 is the O,O′-didesmethylmetabolites).

The sorbent was dried with air (3× 80 μL at 50 μL/s) and the sorbent eluted with 20 μL dichloromethane–isopropanol–ammonia (49:49:2). The organic phase was evaporated under nitrogen and derivatized with 10 μL of acetic anhydride–pyridine (1:2) at 80 °C for 30 min before evaporation and reconstitution in 5 μL of ethyl acetate. The extract was analyzed on a GC–MS using a relatively non-polar BPX5 column.

The method allows for the separation of morphine and its metabolites from the metabolites of potential botanical markers that indicate the ingestion of poppy seeds or straw (Fig. 10). The ability to transfer relatively complex SPE methods to MEPS format demonstrates the utility of MEPS in the regulatory testing of equine and human urine samples. When omitting the derivatization step, the method is also suitable for online use with ESI-LC/MS.

3.3. Integration of MEPS into capillary electrophoresis (CE)

3.3.1. Determination of fluoroquinolones in urine by CE-MS

MEPS was integrated into a commercial capillary electrophoresis (CE) equipment to allow the automation of sample treatment and preconcentration using a few microlitres of sample without any additional modifications for the instrument [27]. The analysis of micrograms per litre of the antibiotics fluoroquinolones (ofloxacin, marbofloxacin, enrofloxacin, danofloxacin, and difloxacin) in urine was carried out using 48 μL of urine sample. The obtained LODs were in the range 6.3–10.6 μg/L. The absolute recoveries were in the range of 71–109% while the precision expressed as repetitivity of peak area was lower than 5.9% [27]. The new method (MEPS-CE–MS) offers numerous advantages over the previous micro-SPE-CE-MS couplings, since it is easy to use and reduces the cost of each analysis. The method is suitable to be applied to routine laboratories.

3.3.2. Determination of free and total concentrations of local anaesthetics by CE-MS

The use of MEPS online with CE-MS for determination of free and total concentrations of local anaesthetics drugs (lidocaine, mepivacaine, bupivacaine) in human plasma was described. For the determination of free concentrations, a microdialysis probe was connected into the needle of the MEPS. The system was automated by connecting the MEPS syringe to a syringe pump and interfacing it to a computer. MEPS provided the efficient preconcentration of analytes from a small sample volumes. The method allows the determination of 1 ng/mL of total concentration of studied drugs from 200 μL of sample with an RSD of less than 9% [28].

3.4. MEPS with direct analysis in real time (DART)

3.4.1. Cocaine and its metabolites in urine samples by DART-QTOF

MEPS has been evaluated for fast screening of drugs of abuse with mass spectrometric detection. Several sorbents such as C8, ENV+, Oasis MCX, and Clean Screen DAU were used. In this study the focus was on fast extraction and preconcentration of the drug and metabolites with rapid analysis using a time-of-flight (TOF) mass spectrometer as the detector with direct analysis in real time (DART) source. The analysis time was less than 1 min. This study has demonstrated that the combination of MEPS with DART/QTOF can be a very useful tool for screening of drugs of abuse in a biological matrix. Furthermore, the study made an attempt to demonstrate that it is possible to quantify the analyte of interest using DART source when an internal standard is used [29].

4. Conclusions

MEPS is a new sensitive, selective and accurate online sample-preparation technique. MEPS is more easily automated than SPE and is more rugged than solid-phase microextraction (SPME). MEPS is very promising for many reasons: (1) it is easy to use, (2) it is a fully automated online procedure, (3) it is rapid, (4) it can work with much smaller samples, as small as 10 μL and (5) the cost of analysis is minimal compared to conventional SPE.

MEPS technique provides significant advantages such as the speed and the simplicity of the sample-preparation process. The key aspect of MEPS is that the solvent volume used for the elution of the analytes is of a suitable order of magnitude to be injected directly into LVI-GC or LC systems. Compared with other extraction techniques (SPE, LLE), MEPS significantly reduces the volume of solvents and sample needed. The applied sorbent could be used 50–100 times before it was discarded. The carry-over may be almost eliminated by washing the sorbent 3–4 times first with elution solution and then with washing solution. The use of MEPS can be useful to eliminate matrix effects.

Future work should be focused on extraction of more drugs and metabolites. A broad range of applications in different areas such as environmental and food analysis will be needed. MEPS would be used for on-site environmental analysis. More selective sorbents and the use of antibodies for more selective extraction would be investigated. MEPS is adaptable for other analytical techniques including immunoassay and off-line analysis by NMR, IR and other methods.

MEPS has been commercialised by SGE Analytical Science and is available in a range of common sorbents including C18, C8, C2, C8/SCX, SCX, SAX and Silica. Specialised packing materials for glycopeptide analysis and including carbon, PBA and CMD have also

been used successfully in MEPS format. An extensive range of applications for drugs, metabolites, alkaloids, pollutants and other substances in a range of matrices, developed by SciSEP Limited, are available in a solutions pack that provides all the hardware, software and support needed to add online MEPS capability to a CTC CompiPAL autosampler.

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