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New trend in sample preparation: on-line microextraction in packed syringe for liquid and gas chromatography applications I. Determination of local anaesthetics in human plasma samples using gas chromatography–mass spectrometry

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Abstract

A new technique for sample preparation on-line with LC and GC–MS assays was developed. Microextraction in a packed syringe (MEPS) is a new miniaturised, solid-phase extraction technique that can be connected on-line to GC or LC without any modifications. In MEPS approximately 1 mg of the solid packing material is inserted into a syringe (100–250 μl) as a plug. Sample preparation takes place on the packed bed. The bed can be coated to provide selective and suitable sampling conditions. The new method is very promising. It is very easy to use, fully automated, of low cost and rapid in comparison with previously used methods. This paper presents the development and validation of a method for microextraction in packed syringe MEPS on-line with GC–MS. Local anaesthetics in plasma samples were used as model substances. 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 applied polymer could be used more than 100 times before the syringe was discarded. The extraction recovery was between 60 and 90%. The results showed close correlation coefficients ($R > 0.99$) for all analytes in the calibration range studied. The accuracy of MEPS–GC–MS was between 99 and 115% and the inter-day precision ($n = 3$ days), expressed as the relative standard deviation (R.S.D.%), was 3–10%.
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Keywords: Microextraction in packed syringe; Anaesthetics

1. Introduction

In cases when the analytes of interest are present in a complex matrix, e.g. plasma or urine, or in samples of environmental origin, the sample preparation is of crucial importance for the analysis. The purpose of sample-preparation is to remove interfering substances and also enrichment of the analytes. The procedure must be highly reproducible, with a high recovery of the target analytes. In addition, an ideal sample preparation method should involve a minimum number of working steps, which should be fully automated.

Nowadays commonly used sample-preparation methods are solid-phase extraction (SPE), liquid–liquid extraction (LLE) and solid-phase microextraction (SPME). With liquid–liquid extraction it is difficult to obtain a high recovery of polar analytes and it is not easy to automate the methods. Solid-phase extraction (SPE) gives both high recovery and good chromatography, but takes longer and more steps are required [1–6]. SPME as sample preparation has become a popular microextraction technique. Today the technique is employed to extract a wide range of analytes in many areas. The extraction is based on partitioning of the analyte between the organic phase on the fused silica fibre and the analyte. Many factors, such as pH, temperature, salt concentration and stirring, affect the equilibrium constant and the equilibration time [7–12]. The major disadvantages of SPME in quantitative analysis are low recovery (low sensitivity), the frequent inability of the fibre to withstand a complete run (standards + blanks + QC samples + patient samples) and the impossibility of treating samples in an organic solvent. SPME showed a higher deviation com-

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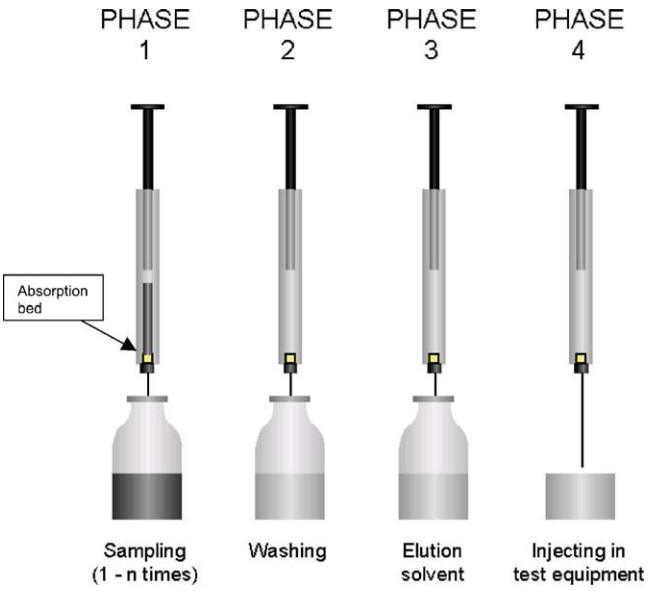

Fig. 1. A scheme of MEPS (the process is fully automated).

pared to LLE and SPE techniques [12]. In addition, the quality of fibre and the fibre length can differ from batch to batch.

Microextraction in packed syringe (MEPS) is a new technique for miniaturised solid-phase extraction that can be connected on-line to GC or LC without any modifications.[1] Included in the patent application. In MEPS approximately 1 mg of the solid packing material is inserted into a syringe (100–250 μl) as a plug (Fig. 1). The plasma sample (50–1000 μl) is drawn through the syringe by an autosampler (which pumps the sample up and down). When the plasma 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. The process is fully automated. Any absorption material such silica based (C2, C8, C18), restricted access material (RAM) or molecular imprinted polymers (MIPs) can be used.

The MEPS technique differs from commercial solid-phase extraction (SPE) in that the packing is inserted directly into the syringe, not into a separate column. Thus, there is no need for a separate robot to apply the sample into the solid phase as with conventional SPE. The packed syringe can also be used several times, more than 100 times with plasma or urine samples and more than 400 times for water samples, whereas a conventional SPE column can only be used once. MEPS can handle small sample volumes (10 μl of plasma, urine or water) as well as large volumes (1000 μl) and can be used for GC, LC and CEC applications. Compared with liquid–liquid extraction (LLE) and solid-phase extraction (SPE), MEPS will reduce sample preparation time and organic solvent consumption.

MEPS is fully automated and takes only about one minute for each sample. Compared with solid-phase microextraction (SPME), the new technique is more robust. In SPME the sampling fibre of SPME is quite sensitive to the nature of the sample matrix. The new technique can be used for complex matrices without problems (such as plasma, urine and organic solvents), which is not the case with SPME. Also, much higher extraction recovery can be obtained (60–90%) compared to SPME (1–10%). Small sample volumes can be treated (10 μl) compared to SPME (>1000 μl).

The aim of the present study was to test and validate MEPS as a new sample preparation technique using local anaesthetics as model compounds.

2. Experimental

2.1. Reagents and materials

Ropivacaine, mepivacaine, lidocaine, prilocaine (Fig. 2) and [$^2H_7$] ropivacaine (IS) in hydrochloride form, were supplied by the Department of Medicinal Chemistry, AstraZeneca (Södertälje, Sweden) and methanol LiChrosolv grade by Merck (Darmstadt, Germany).

2.2. Instrumentation

The GC–MS system consisted of an HP 6890-Plus gas chromatograph and a mass selective detector model 5973 (Agilent, Palo Alto, CA, USA) equipped with a programmed temperature vaporiser (PTV) and a Combi Pal autosampler (CTC Analytics AG, Zwingen, Switzerland). The PTV system was an OPTIC 2 (ATAS International, Veldhoven, the Netherlands).

The PTV conditions were: vent flow 100 ml/min, vent time 3 min (evaporation time), purge flow 2 ml/min (purge pressure 5 psi), split flow 50 ml/min and split open time 2 min. The injector temperature was set at 45 °C and after the evaporation period the temperature was raised by 5 °C s^{-1} to 300 °C. Helium was used as carrier gas and was obtained from AGA (Lidingö, Sweden).

2.2.1. GC conditions

The column used was an HP50 (50% phenyl dimethylpolysiloxane) fused-silica capillary column (25 m × 0.25 mm i.d., 0.31 μm film thickness) obtained from Agilent (Palo Alto, CA, USA). Helium was used as carrier gas and obtained from AGA (Lidingö, Sweden). The gas flow rates were 2 ml/min. The GC oven temperature was programmed for an initial hold of 3 min at 90 °C; the temperature was increased at 50 °C min^{-1} to 280 °C.

Conditions for MS measurements were: MS transfer line at 280 °C, ion source at 230 °C, electron impact ionisation

[1] Included in the patent application.

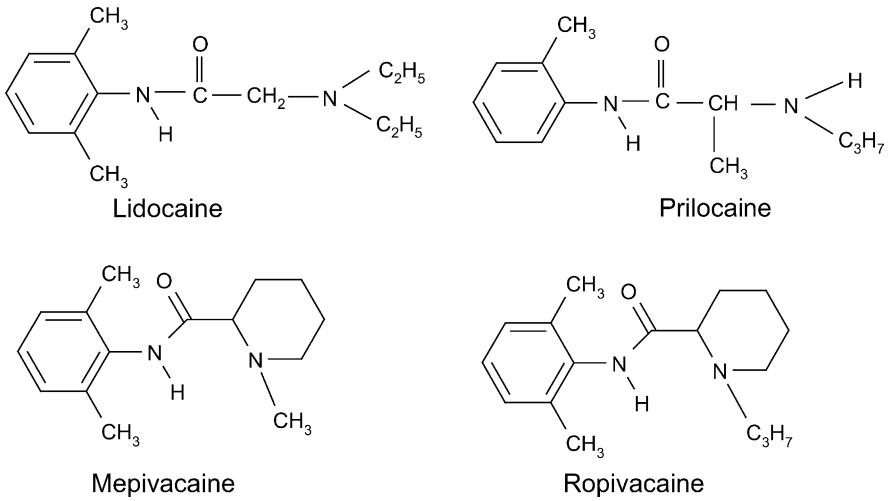

Fig. 2. The structure of lidocaine, prilocaine, ropivacaine and mepivacaine.

at 70 eV, SIM mode with dwell time 50 ms, solvent delay: 4 min. The ions corresponding to ropivacaine, mepivacaine, lidocaine, prilocaine and [$^2H_7$] ropivacaine are *m/z*: 126, 98, 86, 86 and 133, respectively. A MSD ChemStation data system (version B.01.00) was used for data processing.

2.3. Preparation of samples

Stock solutions of the analytes in methanol were prepared. Spiked plasma samples were prepared by adding a few microlitres of analyte standard to 1.0 ml plasma, after which 50 μl of the internal standard (25 μM) was added. The concentration range of standard curves was between 5 and 2000 nM.

2.4. MEPS conditions

One milligram of solid phase material silica-C2 (particle size: 50 μm) was inserted into a 250 μl syringe as a plug with a filter from both sides and fitted manually into the syringe. The plug is fixed to be tightening in the syringe to avoid the moving of the plug inside the syringe. Any absorption material such silica based (C2, C8, C18), restricted access material (RAM) or molecular imprinted polymers (MIPs) can be used. The packed syringe was conditioned first with methanol and then with water (50 μl) before being used for first time. The plasma sample is drawn through the syringe three times (50 μl each) by the autosampler (which pumps the sample up and down three times). The solid phase is then washed once by water (50 μl) to remove the proteins and other source of interference. The analytes are then eluted with 30 μl methanol directly into the GC injector. The multiple puling/pushing of the sample by the syringe increase the extraction recovery. Also, using a small a mount if the adsorbing (1 mg) makes it easy to wash it and use the same syringe many times. In MEPS standard syringe with removable needle was used and no modifications were needed either for autosampler or for GC. In MEPS case the plasma quality is very important issue. If the plasma is thick, you have to dilute it with water at least 1:1 (v/v) otherwise clogging can be encountered.

2.5. Validation

Calibration standard solutions [7–9] with a concentration range of 5–2000 nM in plasma were prepared. Finally, the internal standard was added. A standard curve with at least seven standard concentrations and one zero concentration was prepared. The peak area ratios of solutes and the internal standard were measured and a standard curve without the zero concentration was constructed. Calibration curves were typically described by the equation:

$$y = Ax^2 + Bx + C,$$

where y is the peak-area ratio, x is the concentration and B and C are the slope and intercept, respectively, and A is the curvature. The calibration curves were weighted ($1/x$). The quality-control samples (QC) were treated in the same way as the standards. The intra- and inter-assays were determined by using two levels of concentrations (QC), which were 100 and 800 nM ($n = 6$). Selectivity, linearity, accuracy, precision, recovery and limit of quantification were studied according to Shah et al. [15].

3. Result and discussion

3.1. Selectivity

When plasma spiked with a mixture of analytes and the internal standard was analysed and compared to blank plasma, no interfering compounds were detected at the same retention times as the studied compounds. Figs. 3 and 4 show good selectivity for MEPS as a sample preparation method.

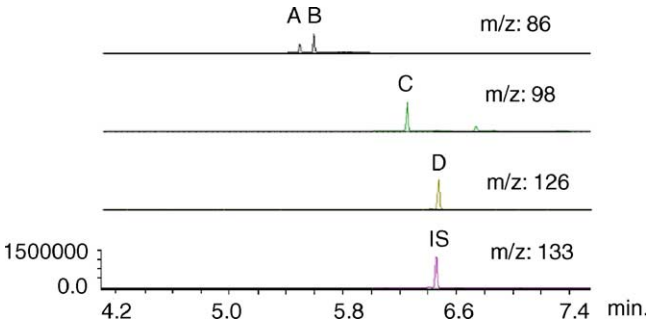

Fig. 3. Mass chromatograms obtained from human plasma spiked with analytes (*m/z*: 86, 86, 98, and 126 for prilocaine, lidocaine, mepivacaine and ropivacaine, respectively, 800 nM each) and I.S. (*m/z*: 133, 1250 nM).

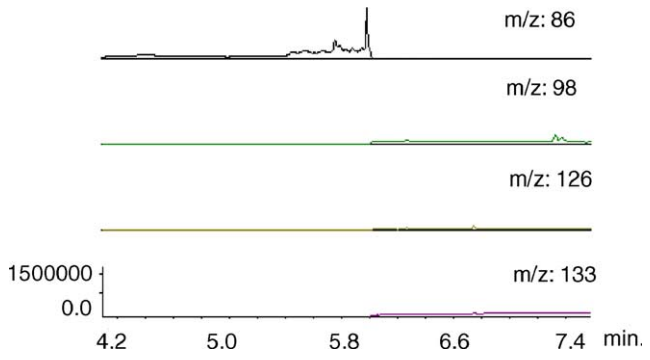

Fig. 4. Mass chromatograms obtained from human blank plasma.

3.2. Calibration

For the construction of the calibration curve, 7–9 levels in human plasma were used for the analytes. The method was validated using [$^2H_7$] ropivacaine as internal standard. The results showed a close relationship between the concentrations and relative peak areas for the analytes studied (Fig. 5) in the concentration range 5–2000 nM. Regression parameters for all the calibration curves are given in Table 1. Correlation coefficient (R^2) values obtained were over 0.996. The

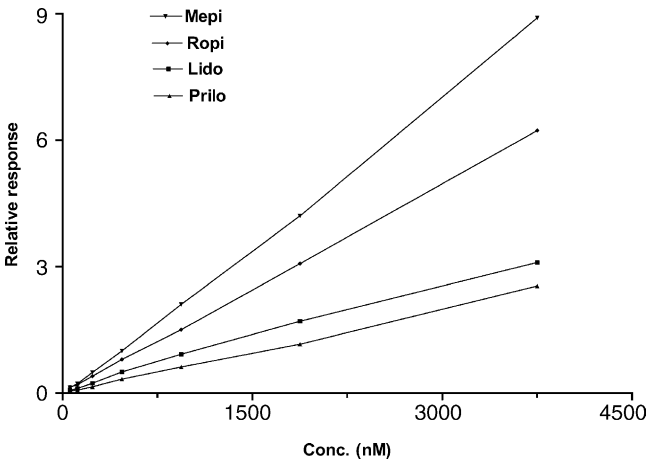

Fig. 5. Typical standard curves of the studied compounds in human plasma.

Table 1
Regression parameters for calibration curves ($n = 4$)

Analyte	Slope	Intercept	R^2
Lidocaine	1.7E−03	5.3E−4	0.999
Prilocaine	1.2E−03	3.7E−3	0.998
Ropivacaine	6.9E−04	9.30E−04	0.999
Mepivacaine	6.6E−04	6.9E−03	0.997

calibration curves indicated that the method is suitable for quantitative analysis.

3.3. Accuracy and precision

The accuracy is determined by the ratio of the found and theoretical concentrations for human plasma control samples, at two different levels. The precision is a measure of the random error and is determined by the percentage coefficient variation of the within- and between-day variations (intra- and inter-assays) at two levels. The intra- and inter-assays were determined by analysis of quality-control samples (QC) at two different concentrations, namely, 100 and 800 nM. The results are shown in Table 2. The CV % values are between 3 and 10% for both inter-assay and intra-assay. Validation of the methodology showed that the method is highly selective for plasma samples. The results showed close correlation coefficients (>0.996) for all analytes in the calibration range studied. The accuracy and precision were well in line with the international criteria [15]. In Table 3, the results of accuracy and precision for ropivacaine from the present study are compared with the results from the literature.

3.4. Extraction degree, limit of quantification (LOQ) and carry-over

The extraction degree was determined by comparing the peak area after extraction at two different concentrations (low- and high-quality control samples) with the peak area obtained after adding the concentrations to heptane. The extraction recoveries were over 60% for all analytes. The LOQ for the analytes studied was 10 nM and in our case the LOQ was satisfactory. The precision of LOQ (given as

Table 2
Intra- and inter-assay precision using C2 as sorbent

Analyte	QC concentration (nM) in plasma	Intra-assay (R.S.D.%) ($n = 6$)	Inter-assay (R.S.D.%) (3 days, $n = 18$)
Lidocaine	100	7	10
	800	6	6
Prilocaine	100	6	10
	800	8	3
Mepivacaine	100	8	9
	800	5	4
Ropivacaine	100	8	7
	800	3	3

Table 3
Comparison of accuracy and precision between present study and earlier studies

Method	Ropivacaine (nM)	Accuracy (%)	Precision (R.S.D.%) (inter-assay)	Reference
Packed syringe/GC–MS	150	105	7.0	Present study
	750	101	3.0	Present study
LLE/GC–NPD	100	96	5.7	[13]
LLE/GC–MS	40	101	3.8	[13]
SPE/LC–UV	1900	101	3.0	[14]
SPME/GC–MS	80	110	6.3	[12]

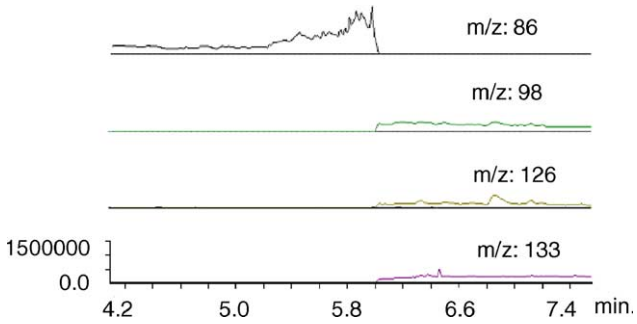

Fig. 6. Plank plasma injected after high concentration plasma standard (2000 nM).

R.S.D.%) was 4.5% ($n = 6$). The carry-over was tested by injecting blank after the highest standard concentration. To eliminate the memory effect, the MEPS is washed four times by methanol and four times by water after every injection. The carry-over was about 0.2% for the IS (Fig. 6).

3.5. Method comparison

The results of the present study were compared with the results from the literature (Table 3). The results from this study were in close agreement with earlier published data [12–14]. Furthermore, this method reduced the time at least fourfold compared to earlier studies.

4. Conclusions

A new sensitive, selective and accurate on-line sample preparation technique was developed and validated for the determination of lidocaine, prilocaine, ropivacaine and mepivacaine in human plasma. Compared to LLE and SPE, the new technique of microextraction in packed syringe (MEPS) reduced sample preparation time and organic solvent consumption. Also, small sample volumes can be treated (10 μl) as well as large volumes. MEPS is more easily automated than SPE and more rugged than SPME. It takes only one minute for each sample compared to 15–20 min with earlier methods (SPE and LLE). Compared to solid-phase microextraction (SPME), the invention is more stable and has a high recovery. In SPME the sampling fibre of SPME is quite sensitive to sample matrix. The new technique can be used for complex matrices without problems (such as plasma, urine and organic solvents), which is not the case with SPME. Also, a much higher extraction recovery can be obtained (60–90%) compared to SPME (1–10%). Small sample volumes can be treated (10 μl) compared to SPME (>1000 μl).

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