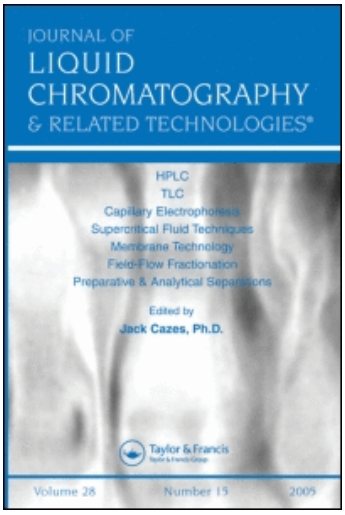

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Abstract: Microextraction in packed syringe (MEPS) is a new technique for miniaturised solid-phase extraction that can be connected online to GC or LC. In this work, a liquid polymer was used as coating polymer on a filter in a 10 μL and 250 μL syringe to handle small sample volumes (≤10 μL plasma). Ropivacaine in plasma samples was used as model substance. The validation of the methodology showed that the accuracy values of quality control samples (QC) were in the range of 103%–114% for GC-MS, and 98–101% for LC-MS-MS. The precisions, given as relative standard deviation (R.S.D.) were in the range 1.9 to11% for Inter- and intra-day precisions. The standard curves were obtained within the concentration ranges 5-2,000 nM in human plasma samples. The regression correlation coefficients (R^2) for plasma samples were 0.99 for all runs using GC-MS and LC-MS-MS.

Keywords: Microextraction in packed syringe (MEPS), Methylcyanopropyl-silarylene, Coating polymer

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INTRODUCTION

Today, separation methods of drugs in biological samples can provide high resolution of complex mixtures and low detection limits, but the most powerful separation method will not give a valid result if the sample preparation is poor or not good enough. The aim of sample preparation is to remove potential interferences from the sample (increasing the selectivity of the method), to concentrate the analyte (increasing of the sensitivity) and to provide a robust and reproducible method that is independent of variations in the sample matrix. Current developments of sample handling techniques are directed toward automation, the ability to use smaller initial sample sizes, and online coupling of sample preparation units and detection systems. In addition, there is a trend toward development of more selective sorbents for sample clean up and enrichment.

Microextraction in packed syringe (MEPS) is a new technique for miniaturised solid-phase extraction that can be connected online to GC or LC without any modifications. In MEPS, approximately 1 mg of the solid packing material is inserted into a syringe (100–250 mL) as a plug. The plasma sample (50–250 μL) is withdrawn through the syringe by an autosampler. When the plasma has passed through the solid support, the analytes are adsorbed to the solid phase. The solid phase is then washed once with water to remove the proteins and other interfering materials. 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. Many different types of adsorbents such as silica based (C_2, C_8, C_{18}), restricted access material (RAM) or molecular imprinted polymers (MIPs) can be used.[1–7]

The primary objective of the present study was to develop a liquid polymer and to make a coating on a filter and on a syringe wall in 10 μL and 250 μL syringes to handle small sample volumes (1–10 μL). The coated syringe was used online with LC-MS-MS and GC-MS for the analysis of plasma samples.

EXPERIMENTAL

Chemicals

Ropivacaine and [$^2H_7$]-ropivacaine (internal standard) (Fig. 1A) were supplied by the Department of Medicinal Chemistry, AstraZeneca (Södertälje). Acetonitrile, methanol, formic acid, sodium hydroxide, hydrochloric acid, and dichloromethane were obtained from Merck (Darmstadt, Germany). The siloxane/silarylene copolymer (Fig. 1B) was synthesized according to the principles outlined in Ref. [8–10].

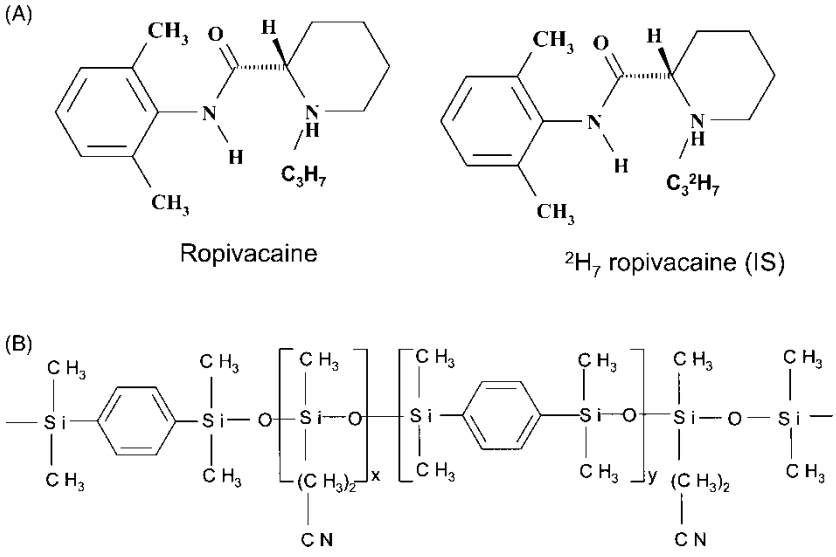

Figure 1. Structure of ropivacaine and internal standard (A) and methylcyanopropyl – silarylene polymer x = y (B).

Apparatus

LC-MS-MS

A high performance liquid chromatography (HPLC) instrument included a Shimadzu LC-10Advp pump, Shimadzu (Kyoto, Japan), an autosampler, CTC-Pal, Crelab (Knivsta, Sweden), and a 20 mL sample loop. A Zorbax (50 × 2.1 mm, SB-C18, 3.5 mm) column, obtained from Agilent (Palo Alto, Calif., USA), was used as analytical column connected to an Optiguard (C8, 10 × 1 mm) as a guard column. A Valco C4W valve, Valco Instruments (Houston, USA) was used as a gate valve between the liquid chromatograph and the mass spectrometer. The water used was purified using a Reagent Grade Milli-Q Plus water purification system from Millipore Corporation (Bedford, Mass., USA). A centrifuge, Hettich Rotanta/AP (Tuttlingen, Germany), was used for plasma centrifugation.

The mobile phase was 0.1% formic acid in acetonitrile/methanol/water (15 : 15 : 70, v/v). The flow rate was 200 μL/min.

All experiments were conducted using a triple quadrupole mass spectrometric instrument, Micromass QII Z-spray (Manchester, UK), equipped with a Z-electrospray interface operated in positive ion mode. Nitrogen was used both as drying (400 L h^{-1}), and nebulizing gases (20 L h^{-1}), the vacuum was 2×10^{-5} mbar in the mass analyzer and 2×10^{-3} mbar in the collision cell (argon was used as collision gas). The gases were from ScanGas (Stockholm,

Sweden). Source block and desolvation temperatures were set to 150°C and 300°C, respectively. The data were collected and processed using MassLynx version 3.4, and all calculations were based on peak area ratios.

The scan mode was multiple reaction monitoring (MRM) using precursor ion at (M + 1) (m/z: 275 and 282), and after collisional dissociation the product ions 126 and 133 were used for quantification of ropivacaine and [$^2H_7$] ropivacaine (IS), respectively. Different parameters such capillary voltage, cone voltage, and collision gas energy were optimized to get a maximum signal in MS and MS-MS. After optimization, the parameter settings were: capillary voltage at 3.1 kV, cone voltage at 38 V, extractor at 5 V, RF lens at 0.2 V. The collision energy was 25 eV.

GC-MS

The GC-MS system consisted of a HP 6890-Plus gas chromatograph and a mass selective detector model 5973 (Palo Alto, CA USA) equipped with a programmed temperature vaporiser (PTV). The software used for data processing, Enhanced Chemstation G1701BA Version B.01.00, was supplied from Hewlett Packard Company, Atlanta, USA. The column used was a fused silica capillary column, CP-Sil8CB, (25 m × 0.32 mm, d_f: 0.25 μm) from Chrompack, The Netherlands. Helium and methane gas were obtained from ScanGas (Stockholm, Sweden).

Method Validation

The plasma and urine used were collected and pooled from different objects. The peak area ratios for ropivacaine and internal standard were measured and a standard curve was constructed. The calibration curves were quadratic and the weight was 1/x. The accuracy and precision were calculated for the QC samples at three different assays. The method was validated under optimized conditions.

RESULTS AND DISCUSSION

MEPS–Preparation and Conditions

MEPS was performed using 10 μL and 250 μL gas-tight syringes. The syringe was filled with hydrochloric acid [0.1M] and left for 30 minutes for washing. After a quick rinse with Milli-Q water, the syringe was left containing sodium hydroxide [1M] for 60 minutes to activate the silanol groups. After an additional rinse with Milli-Q water, the syringe was left to dry. The sorbent polymer used was methylcyanopropyl/silarylene (50/50)

dissolved in dichloromethane to a concentration of about 1.5 mg/mL to facilitate the coating procedure. A small filter was inserted into the syringe and the polymer solution was applied to it. The solution was left for evaporation at room temperature and left over night at 40 degrees. The next day another filter was placed inside the syringe, where the polymer coating ended. This added filter functioned as a protection to prevent the polymer coating from being scraped off. Human plasma was centrifuged at 3500 rpm for 10 minutes and diluted with 0.1% formic acid in Milli-Q water (50/50) before the samples were prepared. For LC-MS-MS, the extracted plasma volume was 10 μL, the washing volume of water 10 μL, and the elution volume of acetonitrile/water (50/50) 20 μL. For GC-MS, the extracted plasma volume was 10 μL, the washing volume of water 10 μL, and the elution volume of acetonitrile 10 μL.

Calibration

For the construction of the calibration curve, 8 levels of the analytes in human plasma were used. The results showed a close relationship between the concentrations and relative peak areas for the analytes studied in the concentration range 5–2000 nM. The correlation coefficient (R^2) values obtained were over 0.99 for GC-MS and LC-MS-MS.

Selectivity, Accuracy, and Precision

The intra-assay precisions (R.S.D.) at two different concentrations for quality control (QC) samples were about 3.4–11% (n = 6) for plasma samples using LC-MS-MS and 9.6–13% for GC-MS. The inter-assay precisions (R.S.D.) were 1.9–4.1% for plasma samples using LC-MS-MS and 10–14% for GC-MS (n = 12). The accuracy varied from 98% to 101% for LC-MS-MS, and 103% to 114% for GC-MS (n = 12). The accuracy and precision data are summarized in Table 1. The accuracy and the precision of the method were within the internationally accepted limits.[11]

Table 1. Accuracy, intra- and inter-day for ropivacaine in plasma samples

Concentration (nM)	Accuracy (n = 12)		Intra-day (RSD%, n = 6)		Inter-day (RSD%, 2-days)	
	GC-MS	LC-MS-MS	GC-MS	LC-MS-MS	GC-MS	LC-MS-MS
700	114	98	13	11	10	4.1
1400	103	101	9.6	3.4	14	1.9

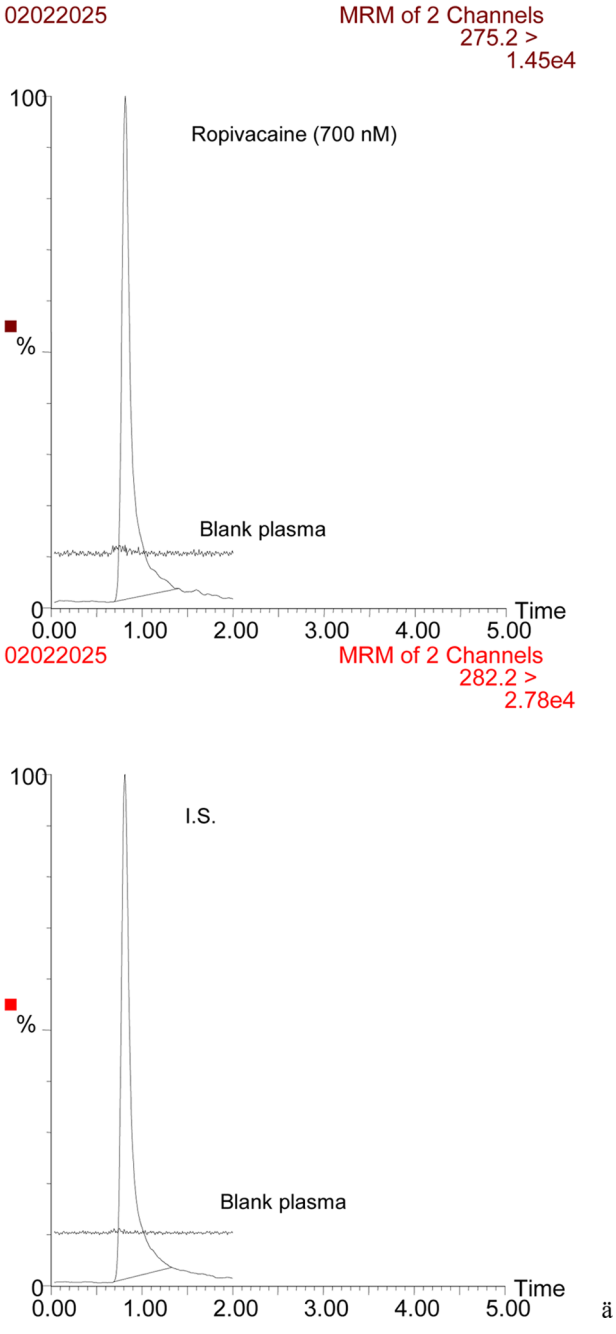

Figure 2. Mass spectrum of spiked plasma sample with ropivacaine and internal standard and blank plasma sample utilizing MEPS-LC-MS-MS.

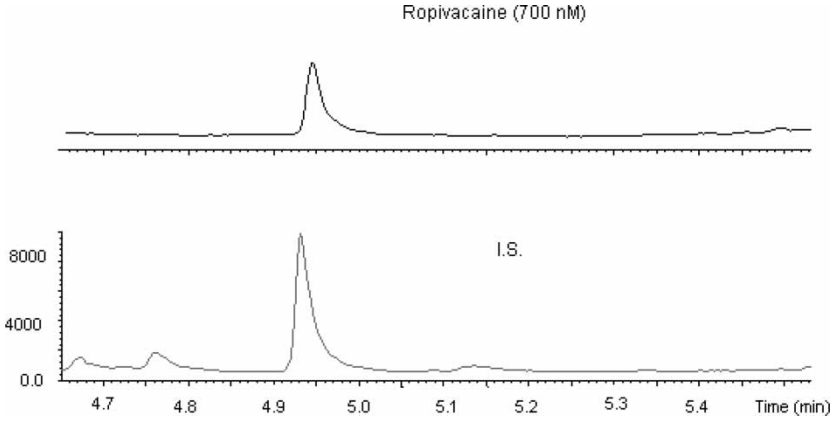

Figure 3. Mass spectrum of spiked plasma sample utilizing MEPS-GC-MS.

When plasma, spiked with ropivacaine, was analysed using LC-MS-MS and compared to blank plasma, no interfering compounds were detected at the same retention times as the studied compounds (Fig. 2). Figure 3 shows mass spectrum of spiked plasma sample (QC sample, 700 nM) utilizing MEPS-GC-MS.

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