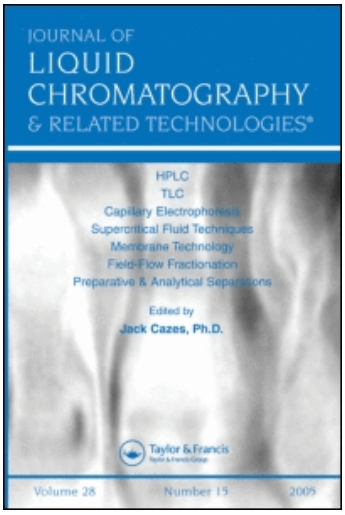

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Drug Screening Using Microextraction in a Packed Syringe (MEPS)/Mass Spectrometry Utilizing Monolithic-, Polymer-, and Silica-Based Sorbents

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Abstract: Micro extraction in packed syringe (MEPS) has been evaluated for drug and metabolites screening online with mass spectrometric detection. In this study, silica based (C_8), polymer based (ENV^+), and a methacrylate based organic monolith were used as sorbents for MEPS. Monolithic material has shown to be an effective chromatographic support for the separation of several classes of compounds. In this study, the focus is subdivided into three parts: 1) Using MEPS for drugs and metabolites screening, 2) Preparation of a monolithic material in situ in a syringe, and 3) Comparison of the monolith, ENV^+, and C_8 as sorbent material. The synthesis of the monolithic material was by radical polymerization of glycidyl methacrylate (GMA), ethylene glycol dimethacrylate (EGDMA), and butyl methacrylate (BMA) in porogenic solvent 1-dodecanol and cyclohexanol. An 8 μL of the synthesized material was drawn into a 250 μL syringe and thermally polymerized at 57°C for 24 h.

Individual syringes containing the monolithic material, ENV^+ (polystyrene) and C_8, were prepared and used for screening ropivacaine, lidocaine in plasma, and lidocaine metabolites (glycylxylidide, monoethylglycylxylidide, and 3-OH-lidocaine) in urine

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samples. Our results showed that all three sorbents could be used for effective and fast screening for analytes in complex matrices, such as plasma and urine. However, for this study, the ENV^+ material performed better than C_8, followed by the monolithic sorbent.

Keywords: Monolithic support, Microextraction in packed syringe, Mass spectrometry, Ropivacaine, Lidocaine, Glycylxylidide, Monothylglycylxylidide, 3-OH-Lidocaine

INTRODUCTION

In the pharmaceutical industry, the measurement of drug and metabolite levels in plasma, answer key questions that are asked during drug discovery and development. The more rapid these measurements, the more quickly drugs progress toward regulatory approval. Liquid chromatography-mass spectrometry (LC-MS) has become a highly developed tool for the determination of drugs and metabolites in plasma and urine samples. Mass spectrometry is presently one of the most powerful detection techniques, particularly in pharmaceutical analysis, where good selectivity and high sensitivity are often needed. The more recent developments in ionization technologies make mass spectrometry an important tool for biological research in general. Recent developments of sample handling techniques are directed toward miniaturization, automatization, and on-line coupling of sample preparation units and detection systems.

Microextraction in packed syringe (MEPS) is a new miniaturized solid-phase extraction method that can be connected on line to GC or LC, without any modifications of the chromatograph.[1–4] In the MEPS technique, approximately 1 mg of the solid packing material is inserted inside a syringe (100-250 μL) as a plug inside the barrel or between the barrel and the needle (Figure 1). Sample preparation takes place on the packed bed. The bed can be coated to provide selective and suitable sampling conditions. This approach to sample analysis is very promising for many reasons: 1) It is easy to use, 2) It is a fully automated on line procedure, 3) It is rapid, and 4) The cost of analysis is minimal when compared to conventional solid phase extraction techniques.

To enhance the MEPS technique and provide an alternative to solid phase extraction techniques, a syringe was packed with methacrylate based monolithic stationary phase and was used in the same format as previously published MEPS applications.[2–4]

As an alternative to porous silica particles, monoliths have attracted considerable attention during recent years.[5–14] Monoliths consist of a continuous piece of support and they are attached to the walls of the chromatographic device, and, therefore, do not need frits. Monolithic materials can be divided into two main categories, silica based and polymer based

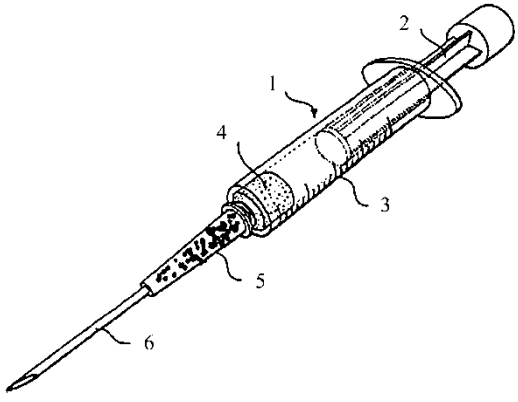

Figure 1. A syringe (1) with a plunger (2) having a syringe barrel (3) slide ably within the barrel and a hollow needle (6) extending from the barrel through which needle the liquid sample is drawn into the syringe barrel characterized in that a solid phase or coating material (4, 5) is provided in the syringe barrel (4) or between the barrel and the needle (5).

monoliths. Generally, monolithic silica columns are prepared by sol-gel technology based on hydrolysis and polycondensation of alkoxisilanes.[8] It was reported, that silica based monolithic columns showed a minimum plate height of 8–10 μm and a lower pressure drop than conventional commercial columns packed with, e.g., 5 μm particles.[15] However, in some cases, narrow pH-stability, shrinkages, and cracking of the monolithic silica rods during the drying process seem to be disadvantages of these monoliths. For organic monoliths, the polymerization reaction mixture consists of monomers or dimers, a cross-linker, a porogenic solvent mixture, and an initiator. Methacrylate based polymer monoliths can easily be fabricated thermally or using ultraviolet light, and can be made from a large variety of different monomers, to obtain monoliths showing different selective interactions such as ion-exchange, hydrophobic, hydrophilic, and affinity.[6,7,9,10] Because monoliths have flow through pores, their back pressure is much lower compared to silica particles. Further advantages include the possibility to adjust the porosity and pore diameter of the material for specific applications, a wide pH-stability (pH 2–12), and a large number of monomers available for fabrication of monoliths showing different selective interactions.

In this work, monolithic packing material was prepared in situ, in a 250 μL syringe for the extraction of ropivacaine, lidocaine, glycylxylidide (GX), monothylglycylxylidide (MEGX), and 3-OH-lidocaine (Figure 2). Likewise, the same extraction scheme was used for syringes containing ENV^+ and C_8 sorbent material. Lidocaine and ropivacaine are amide type local anaesthetics that are widely used in anaesthesiology.

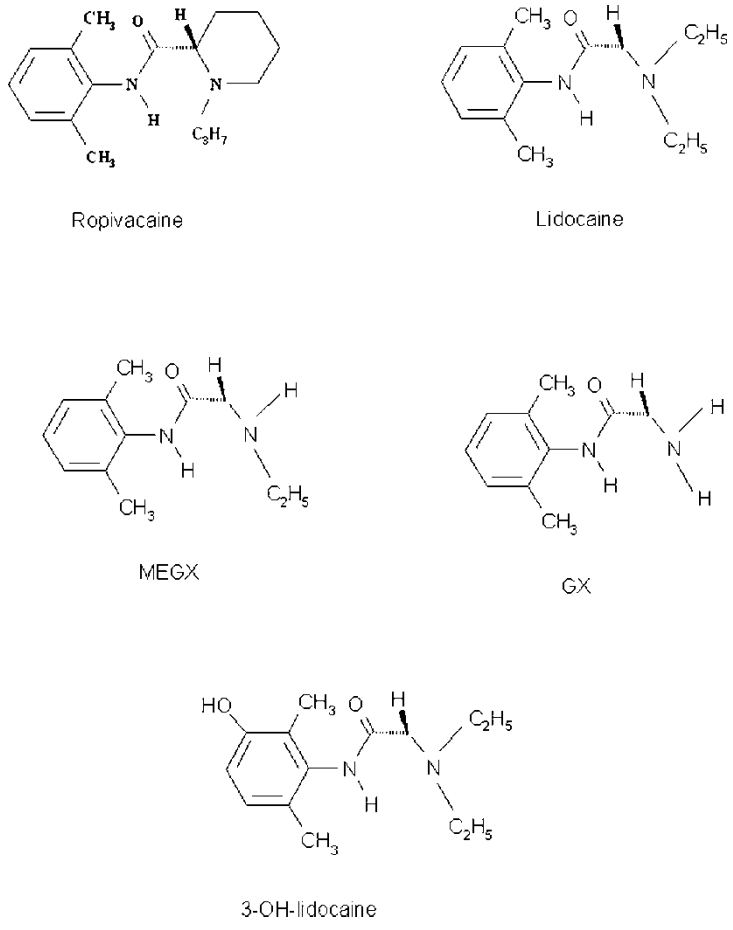

Figure 2. Structures of model substances.

EXPERIMENTAL

Mass spectrometry (MS) was performed with a triple quadrupole mass spectrometer, a Quattro II from Waters, Micromass (Manchester, UK) equipped with a Z-electrospray interface (ESI) and operated in positive ion mode. The electrospray interface was maintained at 150°C. Nitrogen was used as drying and nebulizer gas. Argon was used as collision gases. The settings used were: capillary voltage 3.1 kV, cone voltage at 30–40 V (depending on the compound), extractor at 5 V, RF lens at 0.1 V, and source temperature at 80°C. The data were collected using MassLynx version 3.5. The precursor ions $[M+H]^+$ were m/z 235, 275, 177, 207, and 251 for Lidocaine, Ropivacaine, GX, MEGX, and 3-OH-lidocaine, respectively.

Samples prepared for extractions were made of 4 μM lidocaine, and 1 μM ropivacaine in plasma.

Extraction Procedure

Monolithic, silica (C_8) and polystyrene (ENV^+) sorbents were used. C_8 and ENV^+ were obtained from Argonaut (Mid Glamorgan, UK). These sorbents have irregular particles with an average size of 50 μm and nominal 60 Å porosity. One milligram of the solid material was manually inserted inside the syringe as a plug. The sorbent material was tightened by filters in order to avoid moving inside the syringe. The monolithic sorbent was prepared in our laboratory.

The MEPS syringe was conditioned with 100 μL of methanol, followed by 100 μL of water, twice prior to extraction. For the plasma samples, 125 μL was drawn through the MEPS sorbent, followed by a wash with 100 μL of water. The sample was then eluted directly into the mass spectrometer with 250 μL of acetonitrile/water (1:1) using a syringe pump, a CMA/100, obtained from CMA/Microdialysis (Solna, Sweden). The syringe pump was mounted directly in the front of the orifice of the mass spectrometer, at a distance of 30–50 cm from the orifice, and operated at flow rate of 10 μL/min. For the urine samples, 75 μL was drawn through the MEPS sorbent, followed by a wash with 100 μL of water. The sample was then eluted directly into the mass spectrometer with 250 μL of acetonitrile/water (1:1) using a syringe pump as mentioned above.

Preparation of Poly(GMA-EDGMA-BMA) Monolith

Prior to polymerization, a surface modification of the syringe inner walls was performed using γ-methacryloxypropyltrimethoxysilane.[16] Poly(glycidyl methacrylate-ethylene glycol dimethacrylate-butyl methacrylate) monolith was prepared using a modified method originally suggested by Merhar et al.[12] Briefly, a solution containing GMA (20%), EGDMA (15.5%), BMA (3.5%), AIBN (1 wt% with respect to monomers), 1-dodecanol (20%), and cyclohexanol (40%) was vortexed for 10 min and purged with nitrogen for 10 min, in order to remove oxygen. The mixture was drawn into a syringe and polymerized using thermal polymerization at 57°C for 24 h. After polymerization, the syringe was washed with methanol to remove unreacted compounds. The amount of in situ polymerized monolithic material in the syringe was approximately 2 mg.

RESULTS AND DISCUSSION

The study of drug and metabolite levels in biological samples may improve the understanding of observed pharmacological and toxicological effects.

Therefore, the elucidation of the drug metabolites is of crucial importance for drug discovery and drug development. The matrix and the low concentrations of the metabolites are always challenges in the screening of the metabolites of new drugs. Herein, we describe MEPS as a good tool in drug screening. MEPS can be used for the extraction and preconcentration of the metabolite and the parent drug from plasma and urine samples.

In this study, the objective was to investigate MEPS for screening ropivacaine and lidocaine in plasma samples, and lidocaine and three of its metabolites (GX, MEGX, 3-OH-lidocaine) in patient urine samples. A monolithic based, ENV^+ and C_8 material were used as sorbents and packed in the barrel of a 250 μL syringe for the evaluation of the extraction.

To obtain a monolithic polymer with desired porous properties an optimization of the polymerization mixture is needed. The type and composition of the porogenic mixture is the most frequently used tool for the control over the porous properties. In a porogenic mixture composed of 1-dodecanol and cyclohexanol, the pore sizes of the monolith seems to increase with an increased amount of 1-dodecanol. This was explained as an earlier phase separation when the mixture contains more of the solvent with less solvating capacity.[13] These effects have been extensively studied by Svec et al.[14] Following their procedure, to prepare methacrylate based monolithic material suitable for this application the composition of the porogenic solvent was optimized. Monoliths with properties such as high porosity and, consequently, low backpressure were desirable. Therefore, an important characteristic to be calculated is permeability of the material. Using Darcy's law, the permeability of a poly(GMA-EGDMA-BMA) monolith was calculated as $2.33 \times 10^{-13}\ m^2$, which can be compared to the permeability of a silica column packed with 40 μm particles, which is about $1.89 \times 10^{-13}\ m^2$.[17]

Further, the morphology of the monolithic column was studied by scanning electron microscopy (SEM). Figure 3 shows typical micrographs of the monolith. As can be seen from the micrographs using this polymerization mixture, we obtain monoliths with large pore sizes, which confirms

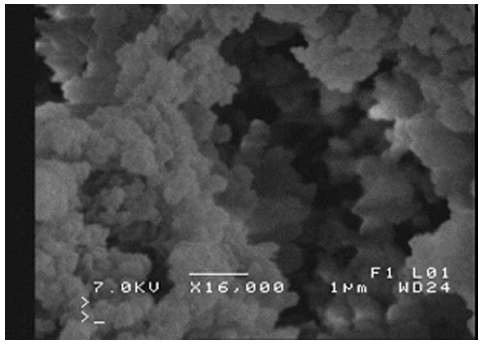

Figure 3. SEM micrographs picture of the poly(GMA-EGDMA-BMA) monolith.

the relatively high permeability of material. This high permeability, which characterizes the monolith, consequently results in low back pressure. The SEM micrographs also show large globules and smaller pores that are critical for a rugged, fast, and effective performance.

To evaluate this new monolithic phase, plasma samples containing lidocaine and ropivacaine were extracted and analyzed (Figure 4). The results were compared to a solvent standard of lidocaine and ropivacaine infused directly into the mass spectrometer (Figure 5). The ions $[M + H]^+$ observed for lidocaine and ropivacaine were m/z 235 and 275, respectively. The extraction of lidocaine and ropivacaine using the monolithic material was compared to extraction using ENV^+ and C_8 sorbents (Figures 6 and 7). The results also show that all three sorbents could be used for fast screening of analytes from complex matrices. In this study, the results showed that ENV^+ performed better than C_8, followed by the monolithic material. Figures 4, 6, and 7 show that the extraction capacities of the three sorbents were about 10:3:1 (ENV^+:C_8:monolithic).

To further evaluate the monolithic material, samples containing lidocaine and its metabolities (GX, MEGX, and 3-OH-lidocaine) were extracted from patients' urine. The ions $[M + H]^+$ observed for lidocaine, GX, MEGX,

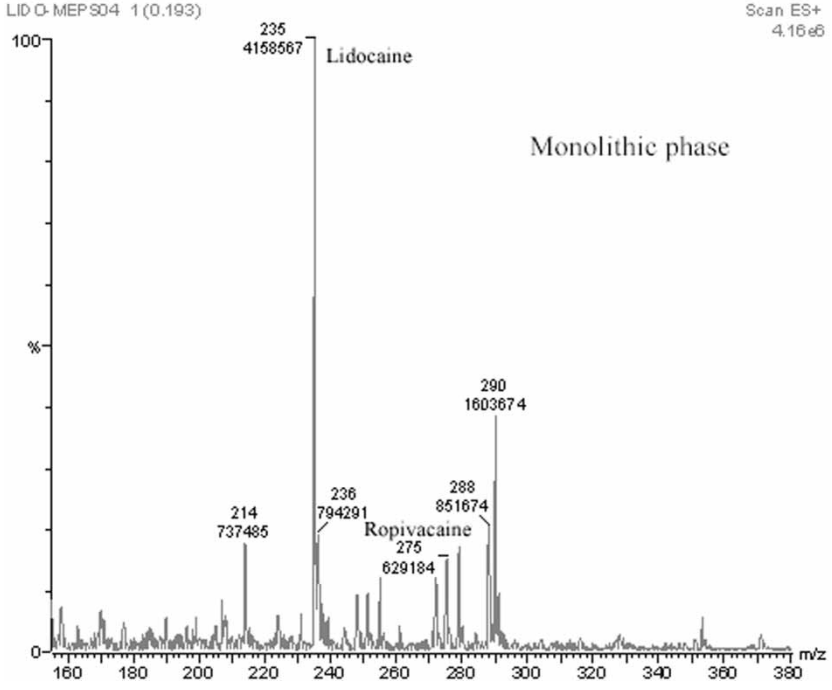

Figure 4. Mass spectrum of 4 μM lidocaine and 1 μM ropivacaine from spiked plasma samples using the monolithic phase.

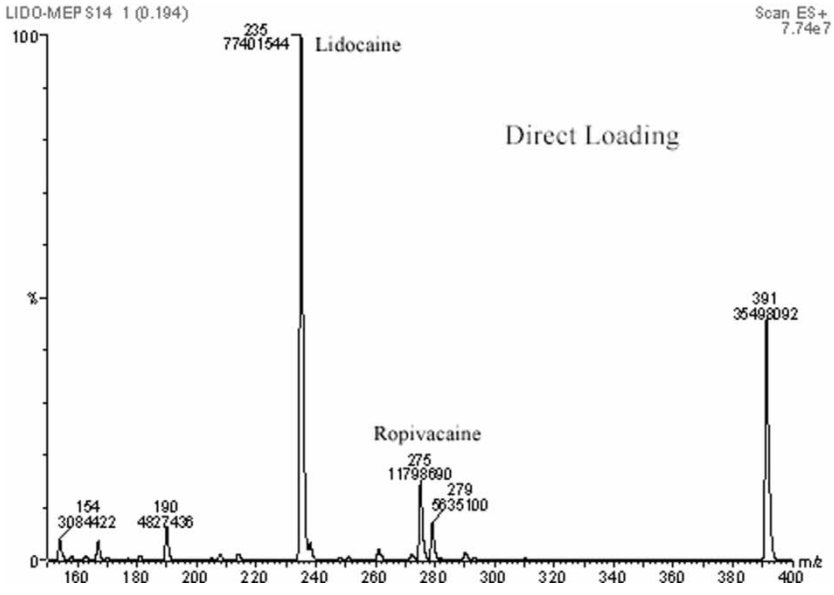

Figure 5. Mass spectrum of a solvent standard of 4 μM lidocaine, and 1 μM ropivacaine from direct infusing into the mass spectrometer.

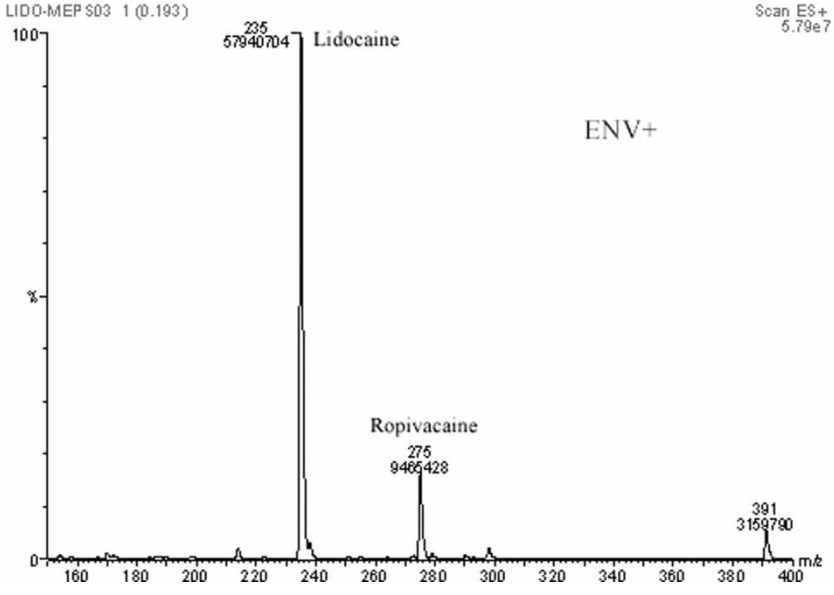

Figure 6. Mass spectrum of 4 μM lidocaine and 1 μM ropivacaine from spiked plasma samples using the ENV^{+} phase.

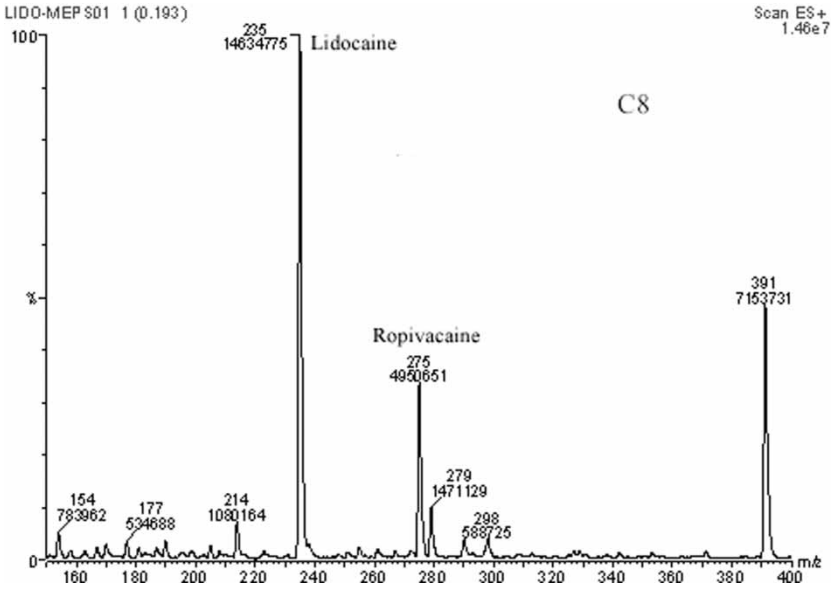

Figure 7. Mass spectrum of 4 μM lidocaine and 1 μM ropivacaine from spiked plasma samples using the C_8 phase.

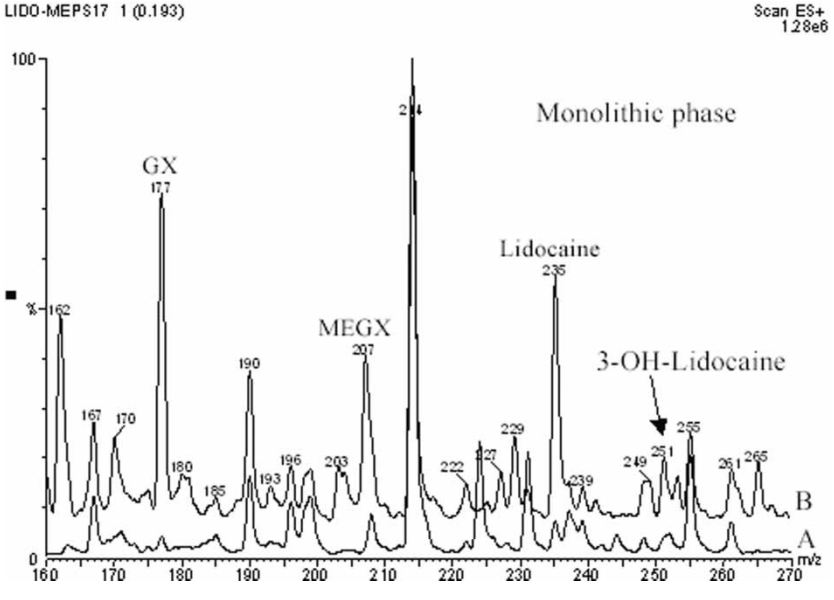

Figure 8. Mass spectra A) Patient urine sample (pre-dose) and B) Lidocaine and its metabolites (GX, MEGX, 3-OH-lidocaine) from patient urine sample (2 h) extracted using the monolithic phase.

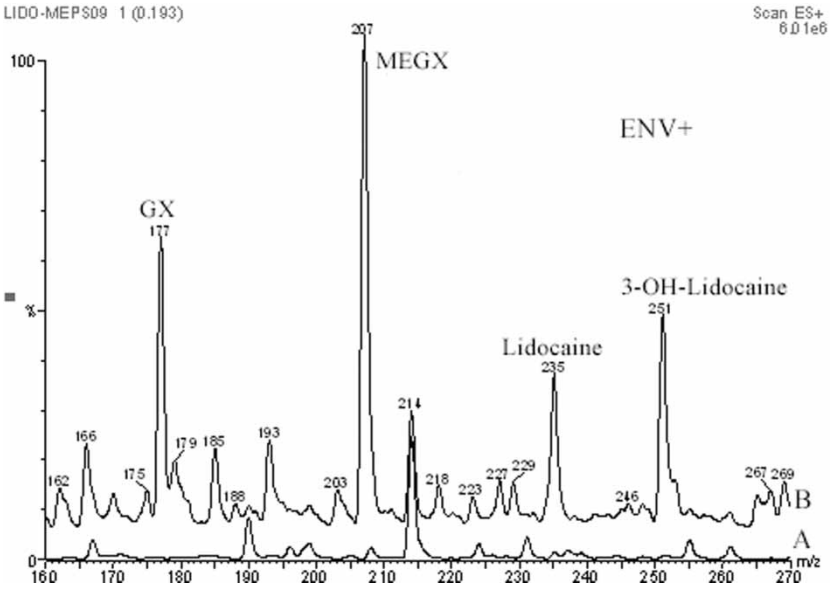

Figure 9. Mass spectra A) Patient urine sample (pre-dose) and B) Lidocaine and its metabolites (GX, MEGX, 3-OH-lidocaine) from patient urine sample (2 h) extracted using the ENV^{+} phase.

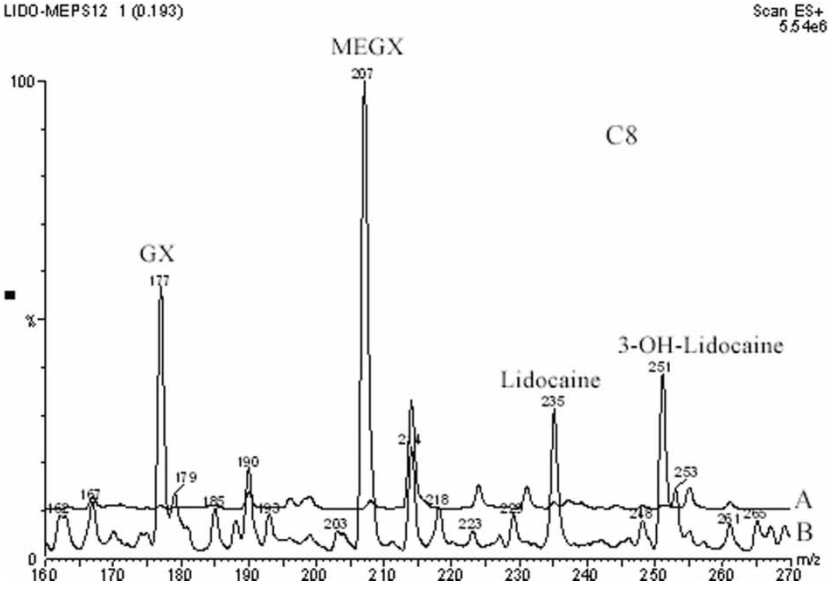

Figure 10. Mass spectra A) Patient urine sample (pre-dose) and B) Lidocaine and its metabolites (GX, MEGX, 3-OH-lidocaine) from patient urine sample (2 h) extracted using the C_8 phase.

and 3-OH-lidocaine were m/z 235, 177, 207, and 251, respectively, and were compared to blank urine extracts (Figures 8, 9, and 10). The extraction of lidocaine, GX, MEGX, and 3-OH-Lidocaine using the monolithic material were compared to extraction done using ENV^+ and C_8 sorbents. Once again, the results showed that ENV^+ performed better for this study than C_8 and the monolithic material. However, this study demonstrated that MEPS could be an effective tool for extraction screening of drugs from complex matrices.

CONCLUSIONS

The data reported here demonstrated that MEPS is a viable technique for fast screening of drugs and metabolites in complex biological matrices. Also, this study showed that both conventional solid phase material (SPE) and monolithic material can be used in the MEPS format. Having the extraction material in a syringe lends itself to automation of the extraction procedures.

Although the data shows that ENV^+ sorbent has high extraction capacity compared with the other sorbents. The monolithic material still holds great promise for low pressure drop (suitable for plasma samples), but provides low binding capacity. We need to develop new monolithic materials with selective functional groups that are advantageous for extracting certain classes of compounds and increase the extraction capacity of the monolithic phase.

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