Liquid-Phase Micro Extraction Of Anaesthetics In Urine Combined With Liquid Chromatography – Mass Spectrometry

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ABSTRACT

Liquid-liquid-liquid microextraction was used to preconcentrate three local anaesthetics, Lidocaine, Bupivacaine and Dibucaine, from a sample of human urine. The extraction involved filling a 2.2 cm x 0.6 mm polypropylene hollow fibre membrane with 5 µl of acceptor solution using a conventional microsyringe, followed by impregnating the pore of the fibre with n-hexylether. The fibre was then immersed in 4 ml of donor solution containing spiked human urine in 0.1 M NaOH. With stirring, the neutral anaesthetics in the donor solution were extracted into the organic solvent, and then back extracted into the acceptor solution. Separation and quantitative analysis of the anaesthetics were then carried out using liquid chromatography – mass spectrometry. The mass spectrometry was performed in positive ion mode and selected ion-monitoring (SIM) mode. The analytes were quantified from the chromatograms of Lidocaine at m/z 86, Bupivacaine at m/z 140 and Dibucaine at m/z 271. For spiked deionised water samples, good linearity ($r^2 = 0.9949$) and fair repeatability (RSDs < 10.5 %, n = 6) were obtained. Limits of detection (LODs) were in the range of 2 - 40 ng/ml (S/N = 3). Spiked urine samples also gave good LODs (1 - 40 ng/ml, S/N = 3) and fair repeatability (RSDs < 10.0 %, n = 3). Enrichment factors of up to 625-fold were achieved.

1. INTRODUCTION

Local anaesthetics are widely used in medical practice to reversibly block nerve function. In forensic practices, a simple, accurate and rapid method for analysis of these drugs in human body fluids is required (Watanabe, 1998). Liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are the most commonly used techniques for preconcentration and sample cleanup. However, these methods are tedious, time-consuming, may require a large amount of organic solvent and can be relatively expensive (Ugland, 2000, Shen, 2003).

In this paper, the analysis of three local anaesthetics, Lidocaine, Bupivacaine and Dibucaine is performed using a simple liquid-liquid-liquid microextraction (LLLME) device prior to separation and quantitation by liquid chromatography – mass spectrometry (LC-MS). The coupling of LLLME with LC-MS is novel. LLLME is economical and easy to operate, enables effective sample clean up and gives very good enrichment factors (Zhu, 2001, Shen, 2002). LC-MS (selected ion monitoring mode) provides effective separation, as well as high sensitivity and selectivity of the anaesthetics. All these factors play an important role in lowering their detection limits. The anaesthetics were extracted from spiked deionised water samples to optimise the procedure and evaluate its feasibility before performing the analysis on spiked human urine.
2. MATERIALS AND EXPERIMENTAL

Liquid-Liquid-Liquid Microextraction

The experimental set-up for the offline LLLME is shown in Figure 1. The polypropylene hollow fibre utilised was flame-sealed at one end and had a length of 2.2 cm, an internal diameter of 600 µm, a wall thickness of 200 µm and a pore size of 0.2 µm. Polypropylene was chosen as the material for the fibre because of its excellent compatibility with a broad range of solvents (Watanabe, 1998).

4 ml of the sample solution (donor phase) was placed in a vial, which was placed on a magnetic stirrer plate. A 12 x 4 mm magnetic stirring bar was placed in the solution to ensure efficient stirring during the extraction. Using a conventional 10 µl HPLC syringe, 5 µl of acceptor solution was introduced into the fibre. The fibre was immersed in the organic solvent for 5 s to impregnate the pores of the fibre with the solvent. Then the fibre, which was still attached to the syringe, was placed in the donor solution. After a prescribed time, the syringe-fibre assembly was removed from the solution and 5 µl of acceptor solution was withdrawn from the fibre. This was injected into a 200-µl vial. 15 µl of HPLC-grade methanol was added to provide a sufficient volume for LC-MS analysis. This mixture was subjected to vibration for 30 s to obtain a homogeneous solution. Each piece of porous hollow fibre was used for a single extraction only.

![Figure 1. Schematic diagram of LLLME extraction set-up (not drawn to scale)](image)

Liquid Chromatography – Mass Spectrometry

High performance liquid chromatography was carried out using a C\(_{18}\) column (150 x 3 mm I.D., 4 µm particle size). The sample injection volume was 5 µl. Gradient elution was used for the mobile phase: A linear gradient from 100 % B to 30 % B was performed in 30 minutes at a flow rate of 0.4 ml/min (solvent A was HPLC-grade acetonitrile and solvent B was 30 % 20 mM ammonium acetate (pH 5) and 70 % acetonitrile). After the analytes were separated, the mobile phase was switched to 100 % A for 2 minutes for equilibration. Thus the total run time for each sample was 32 minutes. The LC effluent was directed to the mass spectrometer. Electron spray ionisation was used and the mass spectrometry was performed in positive ion mode and selected ion-monitoring (SIM) mode. The anaesthetics were quantified based on the peak areas from the chromatograms of Lidocaine at m/z 86, Bupivacaine at m/z 140 and Dibucaine at m/z 271.
3. RESULTS AND DISCUSSION

Determination Of Optimum Conditions For Extraction Using Spiked Deionised Water Samples

Organic solvent 1-octanol, n-octane and n-hexylether were evaluated for their suitability as the organic solvent. The extractions involved a donor solution that contained 100 ppb of Lidocaine, Bupivacaine and Dibucaine in 0.1 M NaOH and an acceptor solution of 0.1 M HCl. Each extraction was performed for 20 minutes at a stirring speed of 1000 rpm. From the results in Table 1, n-hexyl ether was selected as the most suitable solvent as it provided the highest enrichment for all three compounds.

Table 1. Enrichment factors of different organic solvents

<table>
<thead>
<tr>
<th>Compound</th>
<th>Enrichment factor (-fold)</th>
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<tr>
<td></td>
<td>n-octanol</td>
<td>n-octane</td>
<td>n-hexyl ether</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>72</td>
<td>68</td>
<td>91</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>69</td>
<td>210</td>
<td>252</td>
</tr>
<tr>
<td>Dibucaine</td>
<td>56</td>
<td>215</td>
<td>338</td>
</tr>
</tbody>
</table>

Compositions of donor and acceptor phase  As the anaesthetics were bases, the donor phase should be alkaline to reduce the solubility of the analytes in it, while the acceptor phase should be acidic to promote mass transfer of the analytes into it. Aqueous NaOH (spiked with anaesthetics) and HCl were chosen as the donor and acceptor phases respectively. Extractions were performed for 20 minutes each at a stirring speed of 1000 rpm to determine the optimum concentrations of the donor and acceptor solutions. 0.1 M NaOH (spiked with anaesthetics) and 0.1 M HCl were selected as the optimum donor and acceptor phases respectively.

Extraction time  As mass transfer is dependent on the extraction time, it was vital to use the extraction time that provided the highest enrichment. From Figure 2, 70 minutes was found to be the optimum time. Although it was relatively long, simultaneous extractions of several samples can be carried out.

Figure 2. Effect of extraction time on analyte enrichment

Stirring speed  Several stirring speeds were considered (results not shown); the optimum stirring speed was found to be 1000 rpm.
Extraction Efficiency  Under these optimum extraction conditions, an enrichment factor of up to 625-fold was achieved for spiked deionised water samples. Good linearity ($r^2 = 0.9949 - 0.9989$) and fair repeatability (RSDs < 10.5 %, $n = 6$) were obtained. Limits of detection (LODs) were in the range of 2 - 40 ng/ml ($S/N = 3$).

Evaluation Of LLLME-LC-MS Using Spiked Urine Samples

The applicability of this method for human urine samples was evaluated using the optimum conditions. From the results in Table 2, the analysis of spiked urine also gave good LODs and fair repeatability.

Table 2. Evaluation of the method

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Spiked water ($n = 6$)</th>
<th>Spiked urine ($n = 3$)</th>
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<tr>
<td></td>
<td>Repeatability (RSD%)</td>
<td>LOD (ng/ml)</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>8.5</td>
<td>40</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>9.4</td>
<td>10</td>
</tr>
<tr>
<td>Dibucaine</td>
<td>10.2</td>
<td>2</td>
</tr>
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</table>

4. CONCLUSION

The coupling of LLLME with LC-MS was successful in giving good limits of detection in the analysis of anaesthetics in urine samples. However, it gave slightly poorer repeatability, possibly due to matrix effects, although this is still acceptable. The procedure is a good alternative to current techniques such as LLE and SPE, and further work will continue to be carried out on its potential for the analysis of biological fluids for a variety of analytes.

5. ACKNOWLEDGEMENTS

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REFERENCES