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Determination of ropivacaine and bupivacaine in human plasma by programmed temperature vaporiser-fast gas chromatography-mass spectrometry (PTV/fast GC/MS) utilising in-vial liquid-liquid extraction

In-vial liquid-liquid extraction has been investigated as a sample-preparation method in combination with fast gas chromatography and mass spectrometric detection for analysis of ropivacaine and bupivacaine in human plasma. The method is simple, quick, and readily automated. It offers a new possibility for the GC analysis of drugs in biological fluids. Validation showed that the method is accurate and selective. The acceptance criteria for the study validation were well in line with international criteria.

Key Words: LLE; PTV-GC-MS; Local anaesthetics

Received: October 10, 2001; revised: December 31, 2001; accepted: January 31, 2002

1 Introduction

Ropivacaine and bupivacaine are chemical homologues. Ropivacaine is a new amide-type local anaesthetic, mainly used for surgery and for post-operative pain relief. It also has a lower central nervous and cardiotoxic potential than bupivacaine [1].

The aim of the present study was to develop a simple and robust sample-preparation method, and to attain a short separation time and a low quantification limit for the determination of local anaesthetics in human plasma samples.

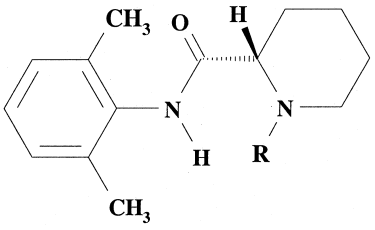
2 Experimental

2.1 Reagents and materials

Ropivacaine, bupivacaine, and [$^2H_7$] ropivacaine (IS) (**Figure 1**), in hydrochloride form, were supplied by the Department of Medicinal Chemistry, AstraZeneca (Södertälje, Sweden). Methanol, sodium hydroxide, methylene chloride, and heptane were obtained from Merck (Darmstadt, Germany). Potassium hydrogencarbonate was obtained from Sigma-Aldrich (Buchs, Switzerland).

2.2 Instrumentation

The GC-MS system consisted of an HP 6890-Plus gas chromatograph and a mass selective detector model 5973 (Palo Alto, Calif., USA) equipped with a programmed temperature vaporiser (PTV) and Combi Pal autosampler (CTC Analytics AG, Zwingen, Switzerland). The PTV system was an OPTIC 2 (ATAS International, Veldhoven, the Netherlands). The injection mode was splitless. The PTV conditions were: vent flow 100 mL/min, vent time 0.5 min (evaporation time), purge flow 2 mL/min (purge pressure 34 kPa), split flow 50 mL/min, and split open time 2 min. The injector temperature was set at 90°C and after the evaporation period the temperature was raised at 10° s^{-1} to 320°C. The initial and transfer pressure was 55 kPa, with a transfer time of 1 min, and the final pressure was set at 414 kPa The GC oven temperature was programmed for an initial hold of 1.0 min at 170°C, the temperature being increased at 100° min^{-1}, to 300°C.



compound	R	Mr
Ropivacaine	C_3H_7	274.4
Bupivacaine	C_4H_9	288.4
[$^2H_7$] Ropivacaine (IS)	$C_3{}^2H_7$	281.4

Figure 1. The structure of ropivacaine and bupivacaine.

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1615-9306/2002/0403–0252$17.50+.50/0

Conditions for MS measurements were: MS transfer line at 280°C, ion source at 230°C, electron impact ionisation at 70 eV, SIM mode with dwell time 70 ms, solvent delay: 0.9 min. The ions corresponding to ropivacaine, bupivacaine, and the internal standard are *m/z*: 126, 140, and 133, respectively. An MSD ChemStation data system (version B.01.00) was used for data processing.

The column used was an HP01 (10 m × 0.1 mm ID, 0.4 μm thickness). The column was obtained from Agilent Technologies (Palo Alto, Calif., USA). Helium was used as carrier gas.

2.3 Procedure

Stock solutions of ropivacaine and bupivacaine were prepared in methanol + water (1 : 1). Spiked plasma samples were prepared by adding a few microlitres of analytes standard to 1.0 mL of plasma. The pH of the plasma sample was adjusted to about 9.5 by adding 50 μL of NaOH (2 M), after which 50 μL of the internal standard was added. The ionic strength of the sample was increased by adding 1 mL 20% $KHCO_3$ in water (*w/v*) to the spiked plasma. 400 μL of spiked plasma was mixed with 800 μL heptane (20%CH_2Cl_2, *v/v*) in a 2 mL vial. CH_2Cl_2 was added to increase the degree of extraction.The vials were shaken for 10 min at a rotation desk followed by centrifugation at 3000 rpm for 5 min to separate the two phases again. 50 μL of the organic phase was injected into the GC.

2.4 Method validation

The concentration range of standard curves was between 0.01 and 5.0 μM. Eight calibration points at different concentrations were used. 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 formula:

$$y = Bx + A$$

where *y* is the peak-area ratio, *x* is the concentration, and *A* and *B* are the intercepts and slopes, respectively. The linear regression equation was 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 three levels of concentrations (QC: low, medium and high), which were 0.1, 0.8, and 2.0 μM ($n = 6$). Selectivity, linearity, accuracy, precision, recovery, and limit of quantification were studied according to Shah et al. [5].

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 of studied compounds. **Figure 2** shows mass chromatograms from spiked plasma.

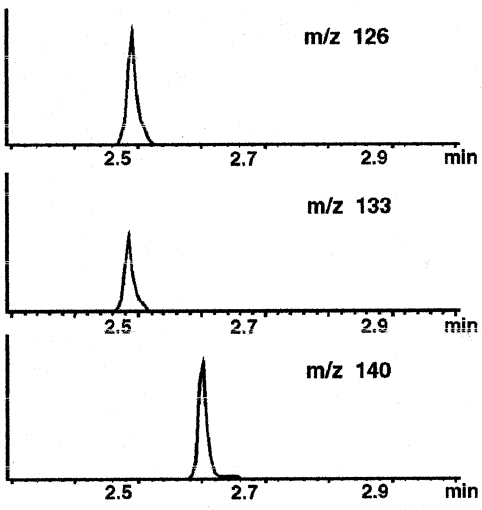

Figure 2. Mass chromatograms obtained from plasma spiked with analytes (*m/z*: 126 and 140, 200 nM each) and I.S. (*m/z*: 133, 400 nM).

3.2 Calibration

In order to plot the calibration curve, eight levels of the analytes in human plasma were used. The method was validated using [$^2H_7$] ropivacaine as internal standard. The results showed a good relationship between the concentrations and relative peak areas for the analytes studied in the concentration range 0.01–5 μM. Regression parameters for all the calibration curves are given in **Table 1**. Correlation coefficient (R^2) values obtained were greater than 0.99. The calibration curves indicated that the method was suitable for quantitative analysis.

Table 1. Regression parameters for calibration curves.

Analyte	Slope	Intercept	R^2
Ropivacaine	3.03E-04	−2.40E-04	0.999
Pubivacaine	3.05E-04	−1.84E-03	0.991

3.3 Accuracy and precision

The accuracy is determined from the ratio of the found and theoretical concentrations for human plasma control

Table 2. Intra- and inter-assay precision and accuracy.

Analyte	Conc. (nM)	Mean accuracy% ($n = 6$)	Precision (RSD%) Intra-assay ($n = 4$)	Inter-assay ($n = 3$ days)
Ropivacaine	112	98	3.5	4.5
	750	106	4.7	4.4
	2100	103	3.0	4.2
Bupivacaine	117	108	9.8	5.0
	784	111	2.1	1.9
	2200	105	2.9	2.5

samples, at three different levels. The precision is expressed as the random error calculated as the percentage coefficient variation of the within- and between-day variations at three levels. The intra- and inter-assays were determined by analysis of quality control samples (QC) at three levels of concentrations, i.e. 0.1 μM, 0.8 μM, and 2.0 μM. The results are shown in **Table 2**. The *CV*% values are between 1.9 and 9.8% for both inter-assay and intra-assay.

3.4 Degree of extraction, limit of quantification (LOQ), and carry-over

The degree of extraction was determined by comparing the peak area after extraction at two different concentration levels (low- and high-quality control samples) with the peak area obtained after adding the concentrations to heptane. The extraction recoveries were about 60% for all analytes (the ratio of plasma volume to organic phase volume was 1:2 (*v/v*)).The limit of quantification for both ropivacaine and bupivacine was 10 nmol/L.The carry-over was tested by injecting heptane after injection of the highest standard concentration. The observed carry-over was less than 1%.

3.4 Method comparison

The results from the present study are compared with the results from the literature (**Table 3**). The results from this study were in close agreement with earlier published data [2–4]. Furthermore, this method reduced the analysis time for the studied analytes at least fourfold compared to earlier studies.

Table 3. Comparison of accuracy and precision between this study and earlier studies.

Analyte	Concentration (μM)	Accuracy %	Precision (RSD%) (Inter-day)	Reference
Ropivacaine	0.112	98	4.5	present study
	12.0	102	4.2	(LC-UV) [2]
	0.04	101	3.8	(GC-MS) [3]
	1.9	101	3.0	(LC-UV) [4]
Bupivacaine	0.117	108	5.0	present study
	12.8	98	2.6	(LC-UV) [2]
	2.9	102	4.1	(LC-UV) [4]

4 Conclusions

A sensitive, selective, and accurate on-line LLE and PTV-GC-MS assay was developed and validated for the determination of ropivacaine and bupivacine. The minimum detectable quantities for ropivacaine and bupivacine were 10 nmol/L. The acceptance criteria for the study validation were well in line with international criteria [5]. The results reported in this paper demonstrate that the method is useful for quantities analysis of drug in plasma.

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