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Comparative study of the determination of bupivacaine in human plasma by gas chromatography–mass spectrometry and high-performance liquid chromatography

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Abstract

A comparison was made between high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS) as methods for determining bupivacaine in human plasma. Both methods utilized pentycaine as an internal standard, were found to be linear in the range 5–320 ng and had acceptable precision and accuracy. The precision for the HPLC method was better than that for the GC–MS method. The limits of detection of the HPLC and GC–MS methods were ca. 1.0 and 0.1 ng, respectively. Good agreement between the HPLC and GC–MS methods was obtained for the analysis of samples taken from a patient receiving bupivacaine topically. For most purposes the HPLC method would be slightly better. However, for samples containing interfering peaks GC–MS provides a higher degree of resolution from such interferants.

Keywords: High-performance liquid-chromatography; Gas chromatography–mass spectrometry; Bupivacaine; Bioanalysis

1. Introduction

Bupivacaine (1-*n*-butyl-DL-piperidine-2-carboxylic acid-2,6-dimethylanilide hydrochloride) is a local anaesthetic frequently used for lubmar epidural anaesthesia. This long-acting aniline anaesthetic agent was synthesized in 1957 by Ekenstam et al. [1]. The compound has a chiral centre and is administered as the racemate. Both enantiomers are active as nerve blockers but the *R*-(+) form is more toxic than the *S*-(−) form. It has been found that the enantiomers distribute unequally in the body after subcutaneous or intravenous administration with the *S*-(−) isomer inducing a longer duration of anaesthesia [2–4]. Since these effects are directly related to the concentrations of the local anaesthetic in the systemic circulation, their determination in plasma is important [5]. As a result of its favourable proper-

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ties, bupivacaine is one of the most frequently used local anaesthetics, especially in obstetric anaesthesia.

Several methods have been described previously for the determination of bupivacaine and other local anaesthetics in plasma, with detection limits ranging between 1 and 100 ng ml^{-1} [6–19]. Most of the methods for the determination of bupivacaine in plasma or serum have utilized either high-performance liquid chromatography (HPLC) [8–11] or gas chromatography (GC) with [12–14] and without mass spectrometry (MS) [15–19]. In this work, we compared an HPLC procedure with a GC–MS procedure for the determination of bupivacaine in plasma. Apart from determining the accuracy, precision and sensitivity of the two methods, the methods were cross-validated with regard to agreement in the determination of the pharmacokinetic profile of bupivacaine which was administered via a dressing to a patient following a skin graft operation.

2. Experimental

2.1. Reagents and chemicals

All chemicals were of analytical grade and were obtained from the following suppliers: HPLC-grade water, acetonitrile and *n*-hexane from Rathburn Chemical (Walkerburn, UK), sodium hydroxide and potassium monohydrogenphosphate from Aldrich Chemical (Gillingham, Dorset, UK), orthophosphoric acid from BDH (Poole, Dorset, UK) and horse plasma from Sigma Chemical (Poole, Dorset, UK). Pentycaine and bupivacaine as their hydrochloride salts, were kindly supplied by Astra Pain Control (Södertälje, Sweden). Culture tubes (10 ml) with Teflon-lined screw-caps were obtained from Aldrich Chemical.

2.2. High-performance liquid chromatography

The HPLC determination of bupivacaine in human plasma was essentially the same as reported previously [7]. HPLC was carried out using a P100 isocratic high-pressure solvent-delivery system (Thermo Separation Products) and injections were made via a Rheodyne injector fitted with a 20 μl loop. The system was attached to a Hypersil 5 Phenyl column (250 mm × 4.6 mm i.d.) (Phenomenex, UK) and this was coupled to a SpectraSeries UV100 detector (Thermo Separation Products). A Hewlett-Packard model 3395/3396 chromato-integrator was used for recording data. The mobile phase was acetonitrile–potassium phosphate buffer (10 mM, pH 3.3) (50:50, v/v) delivered at 1 ml min^{-1}. The eluent was monitored at 0.002 a.u.f.s. at 210 nm.

2.3. Gas chromatography–mass spectrometry

The GC–MS analyses were performed on a Hewlett-Packard Model 5988A GC–MS system interfaced with an HP RTE-6/VM data system. The gas chromatograph was fitted with a Hewlett-Packard HP-1 fused-silica column (12 m × 0.25 mm i.d., 0.33 μm film thickness) (Crawford Scientific, Strathaven, UK). Helium was used as the carrier gas with a head pressure of 5 p.s.i. The following settings were used for GC–MS system: the GC column temperature programme was 100 °C (held for 1 min) then increased at 20°C min^{-1} to 200°C, followed by 5°C min^{-1} to 220°C and then 20°C min^{-1} to 300°C; the injection temperature was 250°C, the interface temperature was 280°C and the source temperature was 140°C. The following MS conditions were used: the instrument was tuned in the electron impact (EI) ionization mode to the ions at m/z 69, 219 and 502 from the perfluorotributylamine calibrant using an electron energy of 70 eV. Selected-ion monitoring (SIM) was carried out for the ions at m/z 140 for bupivacaine and m/z 154 for pentycaine.

2.4. Treatment of spiked plasma samples

Aliquots of horse plasma (1 ml) were spiked with 200 ng of pentycaine and with amounts of bupivacaine between 5 and 320 ng. To the mixture was added 2 M sodium hydroxide (200 μl), followed by *n*-propanol (200 μl). The spiked aliquots were extracted with hexane (7 ml) by shaking for 15 min. After centrifugation for 15 min, the organic layer was removed and evapo-

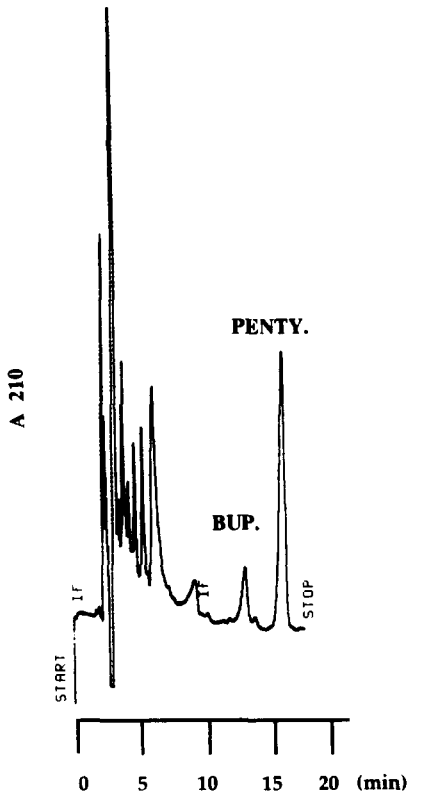

Fig. 1. HPLC of bupivacaine extracted from a human plasma sample withdrawn 60 min after administration of bupivacaine and spiked with pentycaine (200 ng ml^{-1}) before analysis.

rated to dryness under nitrogen at ambient temperature. The residue was dissolved in hexane (100 μl) and then back-extracted into 20 mM phosphoric acid (100 μl) by vortex mixing for 20 s. After centrifuging the mixture, the hexane phase was discarded and duplicate injections of the phosphoric acid phase (20 μl) were made into the HPLC system. Each calibration standard was prepared in duplicate.

After the samples had been analysed by HPLC, they were further subjected to GC–MS. A 100 μl volume of 2 M NaOH was added to the phosphoric acid layer, which was then extracted with hexane (100 μl) by vortex mixing for 20 s. The aqueous phase was discarded and aliquots of the hexane phase (2 μl) were injected into the GC–MS system.

2.5. Determination of precision and accuracy

Precision and accuracy were determined by spiking five aliquots of horse plasma (1 ml) with 20 ng of bupivacaine and 200 ng of pentycaine. The spiked samples were analysed according to the procedure described above and the amount of bupivacaine in the samples was determined from the calibration graph. Five more samples of horse plasma were spiked on the following day and the analysis was repeated in order to determine the between-day precision.

2.6. Patients' samples

Hospital ethical committee approval was obtained for the study. A solution of bupivacaine (20 ml of a 0.75% (w/v) injection) was applied to a Kaltostat dressing (7.5 × 12 cm) laid on a skin graft site and a bandage was applied over the dressing. The samples of blood (ca. 6 ml) were collected in heparinized tubes at 0, 15, 30 and 60 min and 3, 8 and 24 h after the application of the dressing and centrifuged within 1 h of collection. The plasma (ca. 4 ml) was removed and stored

Table 1
Interday and intraday precision and accuracy for HPLC and GC–MS methods for bupivacaine determination in plasma

Day	Method	Concentration spike (ng ml^{-1})	Mean concentration determined ± SD (ng ml^{-1})	RSD (precision)(%)	Accuracy(%)
1	HPLC	20.00	19.95 ± 0.57	2.90	99.75
2	HPLC	20.00	20.55 ± 0.47	2.29	102.75
1	GC–MS	20.00	20.95 ± 1.30	6.33	104.75
2	GC–MS	20.00	20.43 ± 1.22	5.98	102.15

Interday precision: for HPLC, 20.25 ± 0.30 ng ml^{-1}, RSD = 2.09%; for GC–MS, 20.69 ± 0.26 ng ml^{-1}, RSD = 1.76%.

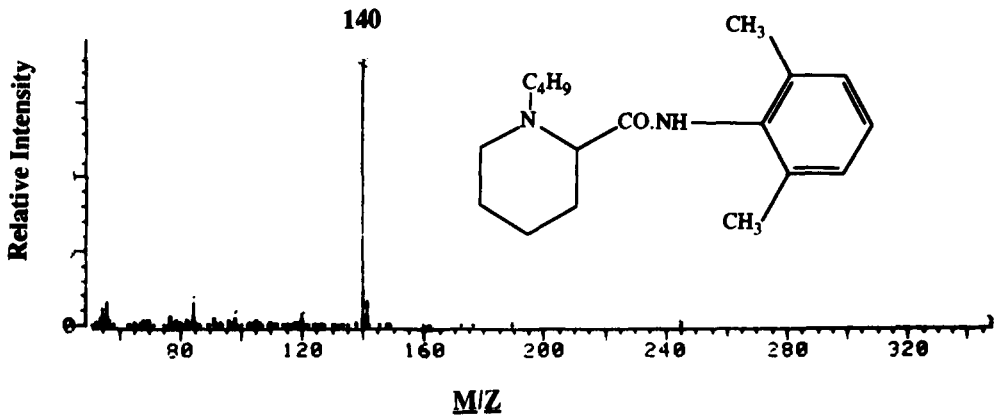

Fig. 2. EI mass spectrum of bupivacaine.

at −20°C in polypropylene tubes until being defrosted for analysis. Duplicate 1 ml aliquots of the plasma were analysed according to the procedure described in Section 2.4.

3. Results and discussion

3.1. Determination of bupivacaine by HPLC

The previously reported HPLC method [7] was essentially unchanged apart from reducing the acetonitrile content in the mobile phase, which improved the resolution of the analytes of interest from some early-eluting interfering compounds. Fig. 1 shows a typical HPLC trace for a sample of plasma obtained from a patient 60 min after administration of bupivacaine spiked with 200 ng of pentycaine (internal standard) and found to contain 33.86 ± 0.88 ng ml^{-1} of bupivacaine. The retention times for bupivacaine and pentycaine were 13.5 and 16.5 min, respectively. The calibration graph obtained by spiking a fixed amount of pentycaine (200 ng) and bupivacaine in the range 5–320 ng into 1 ml of horse plasma was linear ($r = 0.998$, $y = 0.005x - 0.013$). The lower limit of quantitation, obtained by the duplicate analysis of extracted standards in the range 0–5 ng ml^{-1}, was 1 ng ml^{-1}; this is in accordance with the reported method [7]. At low concentrations, com-

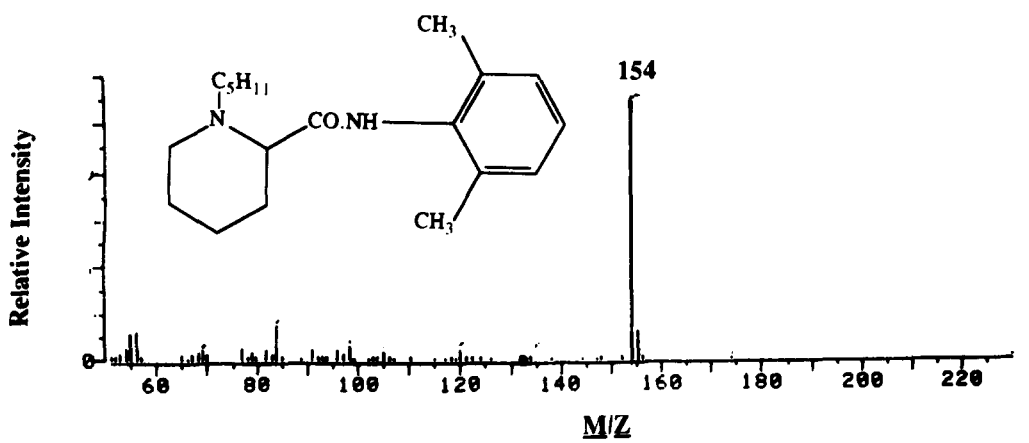

Fig. 3. EI mass spectrum of pentycaine.

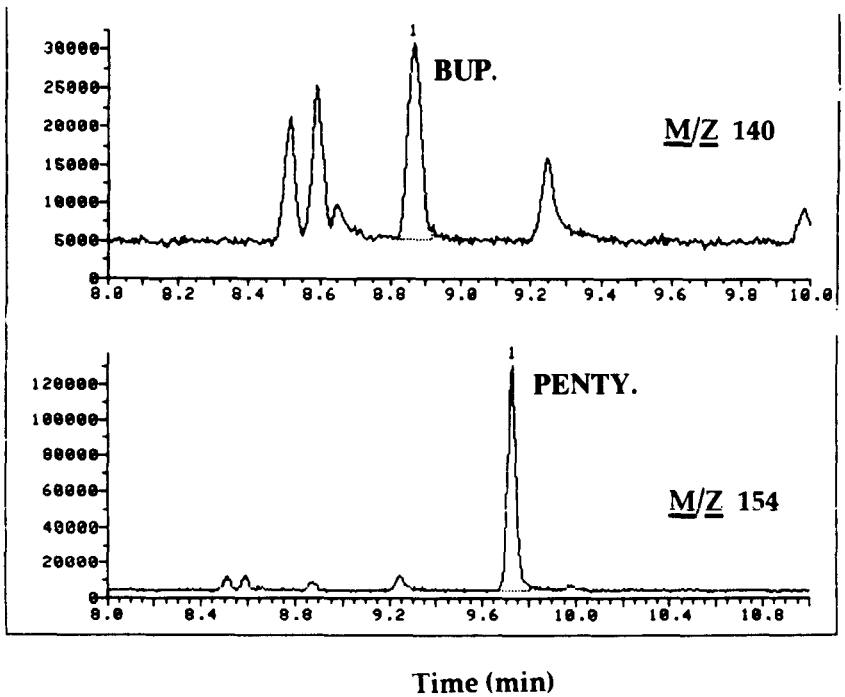

Fig. 4. SIM trace of bupivacaine (m/z 140) and pentycaine (m/z 154) from human plasma. Sample taken 60 min after administration of bupivacaine and spiked with pentycaine (200 ng ml^{-1}) before analysis.

ponents from horse plasma did not interfere with bupivacaine determination. There were, however, potentially more interfering peaks in the samples of plasma from the patient where complex medication led to additional peaks in the chromatograms. The precision, accuracy and between-day precision of the HPLC method in Table 1 show that the method was both precise and accurate for repeated analyses of plasma spiked with 20 ng of bupivacaine.

3.2. Determination of bupivacaine by GC–MS

Figs. 2 and 3 show the EI mass spectra for bupivacaine and pentycaine, respectively; most of the ion current is carried by ions at m/z 140 for bupivacaine (corresponding to the 1-butylpiperidine fragment) and at m/z 154 for pentycaine (corresponding to the 1-pentylpiperidine fragment). Since most of the ion current was carried by these diagnostic ions, this gave the potential for a method of high sensitivity. Fig. 4 shows an extract of plasma taken from a patient 60 min after administration of bupivacaine spiked with 200 ng of pentycaine and found to contain 30.21 ± 0.57 ng of bupivacaine. Although there are additional unidentified peaks in the chromatogram, these are well separated from that of bupivacaine. The calibration graph for bupivacaine with pentycaine as internal standard was linear in the range 5–320 ng ($r = 0.999$, $y = 0.006x + 0.033$). The precision, accuracy and between-day precision of the GC–MS method are given in Table 2 and indicate that the method was both precise and accurate. There was a tenfold increase in sensitivity with a lower limit of quantitation of 0.1 ng ml^{-1} for bupivacaine extracted from horse plasma. In comparison with the HPLC method, any interfering peaks in the patient samples were better separated from the peaks for bupivacaine and pentycaine.

The within-run precision of the GC–MS method, however, was not as good as that of the HPLC method, and this reflects the difficulty of achieving precise sample introduction in capillary GC methods [20], where the sample has to be

Table 2
Comparison of results obtained by HPLC and GC–MS for the determination of bupivacaine in plasma (1 ml) from a patient treated with topical bupivacaine

Time	HPLC		GC–MS	
	Amount found ± SD (ng)	RSD (%)	Amount found ± SD (ng)	RSD (%)
0	0	–	0	–
15 min	42.40 ± 0.95	2.24	42.56 ± 0.89	2.10
30 min	34.72 ± 0.96	2.76	36.28 ± 0.96	2.65
60 min	33.86 ± 0.88	2.60	30.21 ± 0.57	1.89
3 h	22.10 ± 0.84	3.80	22.92 ± 0.10	3.71
8 h	22.2 ± 0.66	2.97	17.87 ± 0.16	3.47
24 h	10.60 ± 0.63	5.94	7.86 ± 0.57	6.11

reproducibly volatilized and cold trapped at the head of the column; even with an internal standard there is still difficulty in obtaining a high injection precision with conventional hot splitless injection. Thus, although both methods are sufficiently precise for bioanalytical work, the HPLC method is better. The between-day precision of the GC–MS method is slightly better than that of the HPLC method and this may reflect the fact the injection errors in splitless GC are not entirely random, although even now they are not fully understood [21], so that the means of two sets of injections can be close. Unfortunately, with the HPLC method, monitoring at 210 nm meant that sometimes it took a long time to obtain a steady baseline. The advantages of the GC–MS method are that there are fewer interfering peaks in the chromatogram owing to the higher detector specificity and a steadier baseline. Both of these factors contribute to a lower limit of detection in comparison with the HPLC method. Attempts to reduce the work-up procedure for the GC–MS method by using a simple direct extraction step rather than using three extraction steps gave GC–MS traces in which there were many large interfering peaks.

This study has confirmed the opinion that for many purposes HPLC methods can provide a precision comparable to or better than that of GC–MS methods at a lower cost. The GC–MS procedure in the case of this assay was more sensitive owing to its higher specificity, and this would allow the analysis of smaller volumes of plasma than are required by the HPLC procedure.

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