Validation of High-Performance Liquid Chromatographic–Mass Spectrometric Method for the Analysis of Lidocaine in Human Plasma

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Abstract

A sensitive and simple liquid chromatography–tandem mass spectrometry method is developed and validated for the determination of lidocaine in human plasma. Bupivacaine is used as an internal standard, and the plasma extraction is performed by a simple liquid–liquid extraction. The limit of quantitation (LOQ) is 0.5 ng/mL with a signal-to-noise ratio greater than 5. The calibration curve is linear from 0.5 to 250 ng/mL with an r^2 greater than 0.99. The coefficients of variation for within- and between-assay imprecision, including LOQ, are ≤ 13% and ≤ 8%, respectively. The percentage of inaccuracy for within- and between-assay, including LOQ, are ≤ 9% and ≤ 5%, respectively. The absolute recovery of lidocaine and bupivacaine are greater than 84% and 82%, respectively. The higher sensitivity and accuracy of this method allow the measurement of low concentrations of lidocaine in plasma from a clinical study of topically applied lidocaine in healthy subjects.

Introduction

Lidocaine is a commonly administered drug used both as a local anesthetic and antiarrhythmic. Lidocaine is administered intravenously or parenterally as the oral bioavailability of lidocaine is poor because of the first pass metabolism effect (1,2), and high concentrations of its principal metabolites are neurologically toxic drugs. Procedures involving the insertion of a needle through the skin are painful and may induce great fear and anxiety, especially in children. Bad experiences with a needle may reduce future compliance to the needle procedure. For the described reason, many researchers have searched for a "needle-less" and pain-free local anesthetic method via transdermal delivery.

Absorption from the intact skin for transdermal delivery is poor. Therefore, the systemic absorption is considerably low. To analyze the plasma samples obtained from the clinical study of transdermal delivery of lidocaine, a sensitive and accurate method was needed. In this study, a simple and rapid method for the analysis of lidocaine in human plasma was developed and validated using bupivacaine as an internal standard (IS).

Various analytical methods have been based on high-performance liquid chromatography (HPLC) with UV detection (3,4), liquid chromatography (LC)–tandem mass spectrometry (MS) (5,6), and gas chromatography (GC)–MS (7) for the quantitation of lidocaine. However, the HPLC with UV detection and GC–MS methods are not sensitive enough for dermal absorption studies, as the limit of quantitation (LOQ) of the published methods range from 4 to 100 ng/mL. Only the LC–MS methods are sensitive enough to detect the low concentrations of lidocaine in plasma seen in these studies as the LOQ needs to be of the order of 0.2 ng/mL (5).

Experimental

Chemicals

Lidocaine hydrochloride (99.9% purity) and bupivacaine hydrochloride (100% purity) (IS) were obtained from Sigma-Aldrich (Poole, U.K.). All HPLC-grade solvents were obtained from Rathburn Chemicals (Walkerburn, Scotland). All analytical reagent-grade reagents were obtained from Merck (BDH) (Poole, Dorset, U.K.).

Apparatus

Solvent delivery was achieved using a PerkinElmer series 200 pump (Boston, MA) set at 1 mL/min. Sample injection was carried out using a PerkinElmer series 200 autoinjector. A Shimadzu CTO-10A column oven (Columbia, MD) was used. Detection was by PE SCIEX API2000 MS (Warrington, U.K.). All PE SCIEX software was supplied by Applied Biosystems (Warrington, U.K.). The NM20ZA high-purity nitrogen and air generators were supplied by Peak Scientific Instruments (Reinfrew, Scotland).

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Chromatographic system

The analytical column was a Supelcosil LC-Si (100 × 4.6-mm i.d.), obtained from Merck (BDH) and was maintained at 50°C. The mobile phase was 2% formic acid in acetonitrile–water (50:50). The flow rate was fixed at 1 mL/min. A PE SCIEX API2000 triple quadrupole MS equipped with a turbo-ion spray (heated electrospray) was used to introduce the sample into the MS through off-axis at an angle of 45°. The sample was introduced through 10:1 splitter, which meant only 10 µL of the sample entered the MS. Nitrogen was used as the collision gas. PE SCIEX Analyst software (Version 1.3) was used to control the HPLC–MS, record the output from the detector, perform integration of peak areas, and calculate the lidocaine concentrations.

Bupivacaine was used as an IS. All calculations were based on peak-area ratios of lidocaine and IS. The selection of operating ions is shown in Figure 1. The precursor ions for lidocaine and bupivacaine were m/z 234.99 and 289.09, respectively, and after collisional dissociation, the product ions were 85.98 and 140.09, respectively. The retention times for lidocaine and bupivacaine during the assay were 5.1 and 4.9 min.

Assay procedure

Preparation of stock solution

A stock solution of lidocaine and bupivacaine were prepared in 50% methanol. All the sub stock solutions were stored frozen at approximately –20°C. All of the calibrators and quality control (QC) sample concentrations were prepared by appropriate dilution of sub stock dilution.

Extraction procedure

A 0.5-mL volume of plasma, 0.1-mL IS solution (500 ng/mL), 0.1 mL 1M NaOH, and 3 mL of methyl-tert-butyl ether were placed in a 4.5-mL propylene tube. The contents were mix for a minimum of 5 min by the shaker and then centrifuged at 3000–3500 rpm for 5 min. The top layer was then transferred to a 4.5-mL polypropylene tube containing 0.25 mL of 0.1% formic acid. The tube was mixed again for a minimum of 5 min by the shaker and then centrifuged at 3000–3500 rpm for 5 min. The top solvent layer was discarded by vacuum, and the remaining solution was transferred into an autosampler vial. A 100-µL volume was injected into the analytical column.

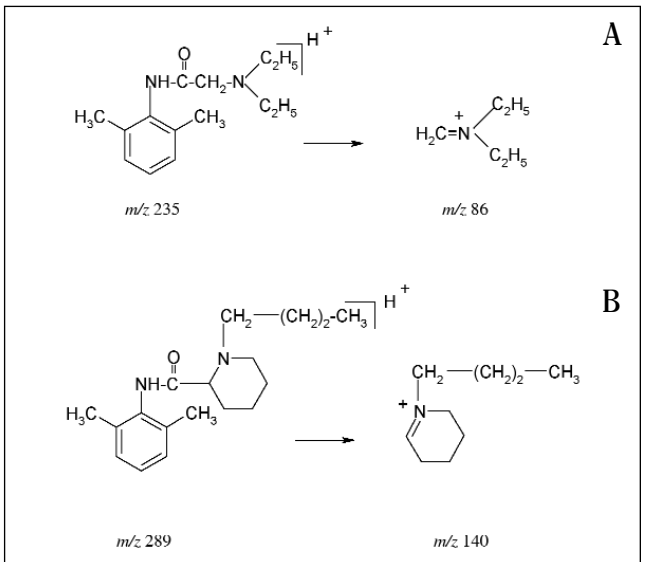

Figure 1. Chemical structure of precursor and product ions of protonated lidocaine (A) and bupivacaine (B).

Results

Selection of operating protonated ions

Figure 1 shows the chemical structure and the protonated ions of lidocaine and bupivacaine used in this study related to their mass. The fragment ions at m/z 86 for lidocaine and m/z 140 for bupivacaine were selected for the assay. The mass spectrum scans for the two analytes are shown in Figure 2.

Specificity

No significant interfering peaks were found at the retention times of lidocaine or bupivacaine. The signal-to-noise ratios for both drugs were greater than 5. Figure 3 shows the chromatogram obtained from blank plasma spiked with 0.5 ng/mL lidocaine and blank plasma spiked with 500 ng/mL bupivacaine.

Calibration curve

The calibration curve covered the range from 0.5 to 250 ng/mL of plasma with seven calibrators. All of the results were calculated using a $1/x^2$ weighted quadratic regression. The peak-area ratio, regression coefficient, and parameters of the calibration line were calculated from the peak area data by the

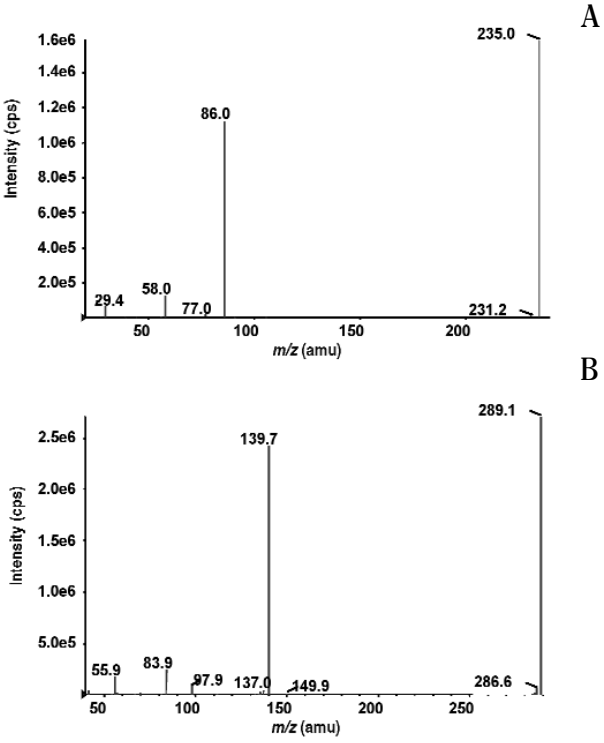

Figure 2. Mass spectra of precursor and product ions scan for lidocaine (A) and bupivacaine (B).

Analyst program. The regression coefficient for all the calibration curves was greater than 0.99. Mean results obtained from five curves are summarized in Table I.

Imprecision and inaccuracy

Table II summarizes the within- and between-batch and total variability obtained from the nested analysis of variance (ANOVA). Precision and accuracy were assessed using three QC samples with nominal lidocaine values of 1.5, 45, and 150 ng/mL and a lower limit of quantitation (LLOQ) of 0.5 ng/mL and upper limit of quantitation (ULOQ) of 250 ng/mL.

Within-assay reproducibility

The three QC samples, LLOQ, and ULOQ were, initially, each extracted six times in one batch. Subsequently, they were extracted six times in two additional batches. On each occasion a separate calibration curve was extracted. The coefficient of variation (CV) and the percentage for imprecision and inaccuracy, including the LLOQ and ULOQ, were all within the accepted range, which was 1.7–13% and 0–9%, respectively.

Between-assay repeatability

For each of the three assays previously mentioned, the mean concentration from each assay was used to calculate the between-assay reproducibility. The CV and the percentage for between-assay precision and accuracy, including LLOQ and ULOQ, were all within the accepted range, which was 1.9–8.3% and 1–5%, respectively.

From the nested ANOVA, the within- and between-batch and total variability for all the QC samples, including ULOQ and LLOQ, were all below than 11%.

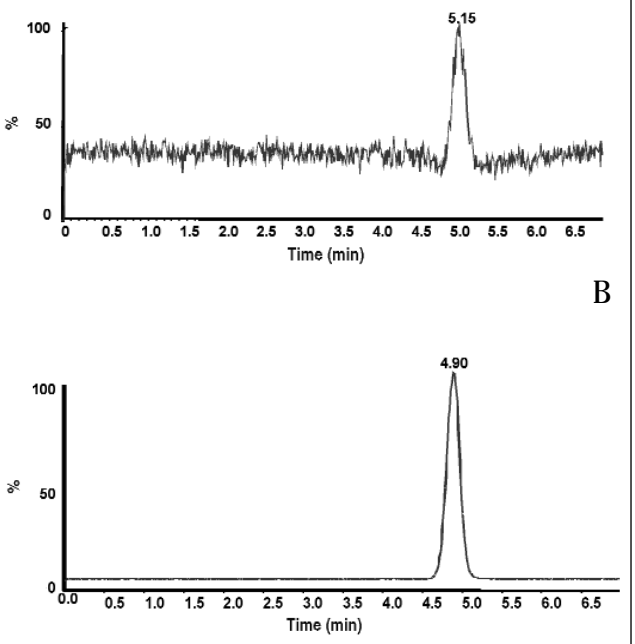

Figure 3. Chromatograms obtained from extracted blank plasma spiked with 0.5 ng/mL lidocaine (A) and 500 ng/mL bupivacaine (B).

Table I. Regression Parameters for Five Calibration Curves During Validation*

Batch	Curvature (C)	Slope (A)	Intercept (B)	r^2
1	6.12 E-6	0.00743	0.000394	0.9995
2	7.75 E-6	0.00772	0.000682	0.9995
3	7.51 E-6	0.00783	0.000210	0.9987
4	7.79 E-6	0.00816	0.000282	0.9994
5	8.52 E-6	0.00830	0.000434	0.9997

* Second-order equation: $y = C \times 2 + Ax + B$.

Table II. Within and Between Batch Variability from the Nested ANOVA*

	LLOQ	QC1	QC2	QC3	ULOQ
Nominal concentration (ng/mL)	0.5	1.47	44.98	149.8	250
Mean (ng/mL); *n* = 18	0.50	1.43	45.72	154.54	263.04
SDw†	0.03	0.09	1.34	5.34	6.99
SDb	0.04	0.08	1.55	2.38	11.18
SDt	0.06	0.12	2.05	5.88	13.18
CVw (%)	6.6	6.1	2.9	3.5	2.7
CVb (%)	8.7	5.9	3.4	1.5	4.2
CVt (%)	10.9	8.5	4.5	3.8	5.0

* Abbreviations: w = within batch; b = between batch; t = total.
† SD = standard deviation

Table III. Results of Stability Tests Carried Out on Lidocaine*

		Concentration (ng/mL)		
		1.47	44.98	149.80
Time 0 stability data	Mean (*n* = 4)	1.34	45.58	151.25
In plasma, after three freeze-thaw cycles	*n*	4.00	4.00	4.00
	Mean	1.52	44.58	141.70
	Difference (%)	14.04	–2.20	–6.31
In plasma after 48 h room temperature	*n*	4.00	4.00	4.00
	Mean	1.47	40.19	134.29
	Difference (%)	10.40	–11.84	–11.21
In plasma after 48 h at 4°C	*n*	4.00	4.00	4.00
	Mean	1.54	44.94	149.24
	Difference (%)	15.46	–1.41	–1.33

* Values express in percentage of the concentration difference between before and after test.

Recovery

The absolute recovery of lidocaine was tested using human plasma spiked with lidocaine at the same nominal concentrations as the QC samples. The absolute recovery of bupivacaine was tested at a nominal concentration of 100 ng/mL. Peak area measurements obtained from the extracted samples were compared with the peak area measurements obtained from direct solvent injection of the test compounds. Mean and standard deviations were calculated from at least three measurements at each level. The absolute recoveries of lidocaine and bupivacaine ranged from 84% to 89% and 82% to 86%, respectively.

Stability

Table III lists the stability data for lidocaine in plasma after three freeze-and-thaw cycles, after 48 h at room temperature, and after 48 h at 4°C. Figure 4 shows the graph of log concentration (ng/mL) versus time (h) for the autosampler stability test. The stability of lidocaine was measured in a sample of analyte-free human plasma spiked with lidocaine at the same nominal concentrations as the QC samples. A minimum of three freeze-and-thaw cycles were tested. The stability of these control samples was further examined at ambient temperature, approximately 20°C, and at approximately 4°C for a period of at least 24 h. For the stability of the sample extracts, the three control samples were each extracted such as to yield a total volume of extract sufficient to allow aliquots to be placed in the autosampler at room temperature and injected over a period of not less than 24 h. The autosampler was operated at ambient temperature, approximately 20°C (maximum temperature 20.5°C, minimum temperature 18.0°C). From all the results obtained, lidocaine was found to be stable in all of the parameters tested.

Discussion

This method validation has been conducted in accordance with the Good Laboratory Practice Regulations, Department of Health, London (U.K.), and the Organization for Economic Cooperation and Development (OECD) Principles on Good Laboratory Practice (Paris, France). All the validation results meet the international requirements as outlined in the Bioanalytical method validation, Food and Drug Administration, 2001 (8). This method has been used for the analysis of human plasma obtained from the clinical studies of transdermal delivery of lidocaine on 100 healthy subjects. Although the procedure of liquid–liquid extraction used in this study was similar to the method published by Bo et al. (5), their procedures have been improved by reducing the amount of plasma used in the extraction and the length of the extraction process. The amount of plasma used in this study is suitable to analyze small amount of blood samples, especially from pediatric patients.

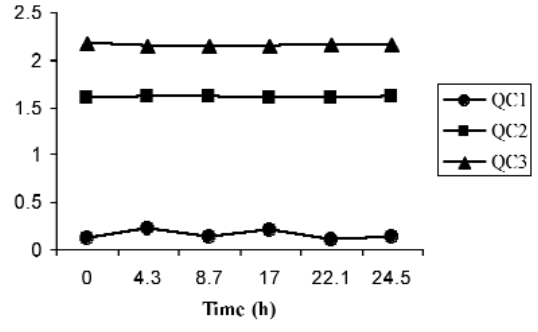

Figure 4. Graph of log concentration (ng/mL) versus time (h) for autosampler stability test.

Conclusion

A simple liquid–liquid extraction and analysis method for lidocaine in plasma has been developed and validated. The sensitivity and accuracy of the assay is suitable for the analysis of low concentrations of lidocaine in plasma for the transdermal delivery study.

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