BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **21**: 775–779 (2007)
Published online 14 May 2007 in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/bmc.823

SHORT COMMUNICATION

Validation and application of capillary electrophoresis for the analysis of lidocaine in a skin tape stripping study

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Received 3 January 2007; revised 29 January 2007; accepted 30 January 2007

ABSTRACT: A fast and simple capillary zone electrophoresis method was developed and validated for the determination of lidocaine in skin using tape samples. Separation was performed in a 350 mm (265 mm to window) × 50 μm i.d. fused silica capillary using a background electrolyte of phosphoric acid–Tris pH 2.5. The extraction of lidocaine from tape samples was achieved using methanol, which was diluted to 50% with water before injection. Procaine was the internal standard. The migration times for procaine and lidocaine were 2.9 and 3.2 min, respectively. The limit of quantification for lidocaine was 50 μg, with signal to noise ratio greater than 10. The calibration curve was linear from 50 to 1000 μg with r^2 greater than 0.99. The CV for both within- and between-assay imprecision and the percentage of inaccuracy for the quality control samples including lower and upper limits of quantitation were ≤2% and ≤14%, respectively. The absolute recovery of lidocaine was >97%. The accuracy and selectivity of this method allowed the measurement of lidocaine in tape samples obtained from a skin tape stripping study of local anesthetics in healthy subjects.

KEYWORDS: capillary electrophoresis; lidocaine; tape stripping; method validation

INTRODUCTION

Lidocaine is commonly used as local anesthetic and anti-arrhythmic. It is applied onto skin in either cream or patches for local anesthetic effects. Owing to the local activity of lidocaine, its concentration is higher in the local tissue compared with systemic circulation. Some *in vivo* methods, such as microdialysis and tape stripping, are available to sample drugs in the local tissue and outermost layer of the skin, respectively (Benfeldt and Groth, 1998; Kenkel *et al.*, 2004; Kreilgaard, 2001; Kreilgaard *et al.*, 2001; Pershing *et al.*, 2002a; Reddy *et al.*, 2002). Various techniques are available to measure lidocaine in plasma, such as HPLC with UV detection (Lotfi *et al.*, 1997), LC-MS-MS (Bo *et al.*, 1999) and GCMS (Watanabe *et al.*, 1998). Most of the analysis of drugs including lidocaine (Pershing *et al.*, 2002b, 2003; Weigmann *et al.*, 1999) from tape samples has been by HPLC with UV detection since the drug content in tape samples is normally higher than in plasma, especially for local anesthetic studies. Therefore, high-sensitivity techniques such as LC/MS/MS or GC/MS are not required since UV detection is sufficiently sensitive to detect such level of drugs. Moreover, the analysis cost of using UV detection is lower compared with MS detection.

On the other hand, capillary electrophoresis (CE) is an alternative to the chromatographic techniques in drug analysis that can separate a variety of compounds using an electric field. The separation of the compounds by electrophoresis in CE is based on the differences in electrophoretic mobility and the voltage applied. Like HPLC, CE also can be coupled with a UV detector (CE/UV) and MS (CE/MS), forming a powerful analytical method for drug analysis. Capillary electrophoresis with UV and MS detection for the analysis of lidocaine in pharmaceutical formulations has been reported previously (Geiser *et al.*, 2003, 2005; Wang *et al.*, 2001). In this study, we have performed a method validation for the CE analysis of lidocaine in tape samples obtained from a tape stripping study on healthy subjects.

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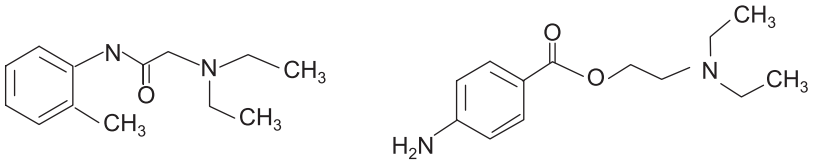
Abbreviations used: ANOVA, analysis of variance.

EXPERIMENTAL

Chemicals

Lidocaine hydrochloride (99.9% purity) and procaine hydrochloride (100% purity; internal standard) were obtained from Sigma-Aldrich Company, Poole, UK. The chemical structures



Lidocaine Procaine

Figure 1. Chemical structures of lidocaine and procaine.

of lidocaine and procaine are shown in Figure 1. All HPLC-grade solvents were obtained from Rathburn Chemicals Limited, Walkerburn, Scotland. All AnalaR grade reagents were obtained from Merck (BDH) Limited, Poole, UK.

Background electrolyte

The aqueous background electrolyte used in this analysis was 100 mm Tris-phosphate buffer at pH 2.5. To prepare this solution, 20 mL phosphoric acid 0.5 M was diluted with 80 mL water, and 15 mL of 0.5 M Tris was added to obtain a solution at pH 2.5. The solution was made up to 100 mL with deionized water.

CE instrumentation and capillaries

Separations were carried out on an Agilent ^{3D}CE G1600AX capillary electropherograph (Agilent, West Lothian, UK) controlled by Chemstation B.02.01 (Agilent, West Lothian, UK). Fused silica capillaries, 350 mm (265 mm to window) × 50 µm i.d. (Composite Metal Services, Ilkley, UK) were conditioned on first use by flushing with 1 M NaOH (BDH, Poole, UK) at >950 mbar, 40°C for 20 min. Pre-conditioning on injection was a 3 min flush with 0.1 M HCl (BDH, Poole, UK), then 2 min flush with background electrolyte (BGE). Separation was at a potential difference of +25.0 kV. The capillary was thermostated at 25.0°C. Detection was by photodiode-array over 195–300 nm, but 200 nm with bandwidth 6 nm was used for quantitation. The run time was 4 min. Both BGE vials were replenished every 12 injections. All samples and standards in the auto-sampler were kept at ambient temperature. The injections of the samples into the system were carried out hydrodynamically for 12 s at 50 mbar.

Assay procedure

Preparation of stock solution. A stock solution of lidocaine and procaine was prepared in 50% methanol. All the sub-stock solutions were stored at –20°C. All the calibrators and QC sample concentrations were prepared by appropriate dilution of sub-stock dilution.

Preparation of tape samples and extraction. For the purpose of validation and assay calibration, a length of self-adhesive polypropylene tape (Tesa 4204 PV5, Beiersdorf, Hamburg Germany) was cut into 10 samples so that each was approximately 3 × 2 cm. A 100 µL volume of standard or quality control solution was spiked onto the adhesive part of the tape. The solution was distributed approximately evenly on all the tapes. The samples were left to dry naturally at room temperature. This required approximately 30 min. The samples were then transferred into 10 mL polypropylene tube and stored frozen at –20°C until analysis. Samples were obtained from patients by applying the tape (3 × 2 cm) onto the skin and then stripping it off using the forceps. The procedure was repeated 10 times to obtain 10 samples at each skin site. The samples were placed in 10 mL polypropylene tube and stored frozen at –20°C until analysis.

Extraction procedure. A 5 mL methanol was dispensed into the tube containing standard/QC tape or samples. The contents were vigorously mixed for 45 min using a vortex mixer. A 5 mL aliquot of a solution of 120 µg/mL procaine (IS) in water was added to the tube to make a 50% methanol solution. The contents were mixed again using a vortex mixer for a further 15 min. A 200 µL aliquot of the solution was transferred into a 250 µL auto-injector vial for CE analysis.

VALIDATION PROCEDURES AND RESULTS

Specificity

Six samples of blank tape and six samples of tape spike with lidocaine were prepared and carried through the extraction. The concentration of lidocaine used was 50 µg (LLOQ) and the internal standard was 600 µg. No significant interfering peaks were found at the migration time of lidocaine or procaine. The signal-to-noise ratio for both drugs were greater than 10. Figure 2 shows the electropherogram obtained from blank tape spiked with 50 µg lidocaine and added IS of 600 µg procaine, while Figs 3 and 4 show the electropherograms of EMLA cream solution and one of the tape samples from the study, respectively.

Calibration curve/linearity

The calibration curve consists of six non-zero calibrators with nominal values of 50, 100, 250, 500, 750 and 1000 µg lidocaine and 600 µg procaine. Five batches of calibration curves were prepared for validation purposes. The calibration curve was plotted using the area ratio of lidocaine to IS vs known concentrations of lidocaine. All the results were calculated using a $y = Ax + B$ linear regression. The regression coefficients for all the calibration curves obtained were

DOI: 10.1002/bmc

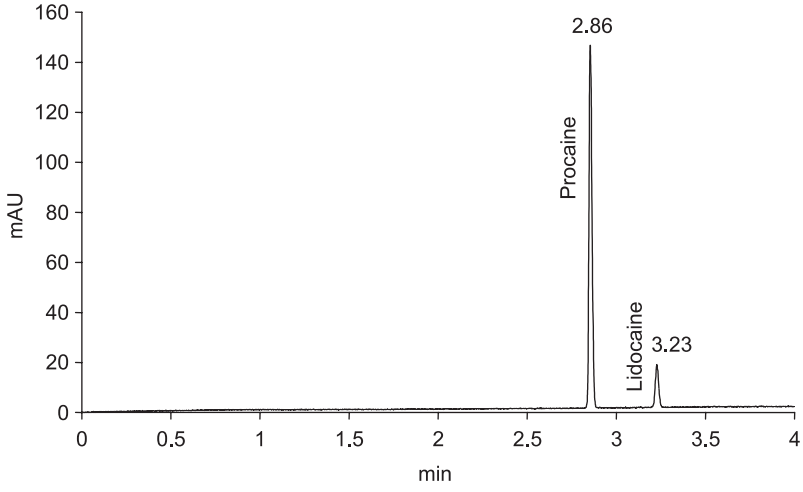


Figure 2. Electropherogram obtained from extracted tape sample spiked with 50 μg lidocaine and added IS of 600 μg procaine. Displayed at 200 nm.

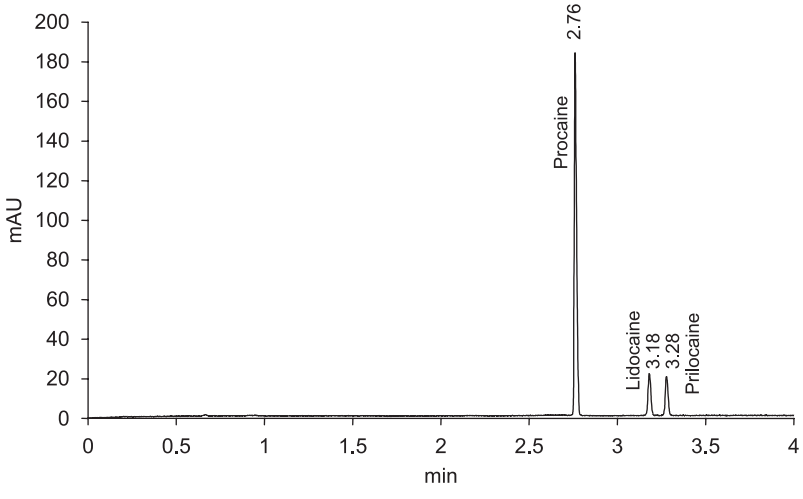


Figure 3. Electropherogram obtained from EMLA cream solution containing 50 μg lidocaine and prilocaine and added IS of 600 μg procaine. Displayed at 200 nm.

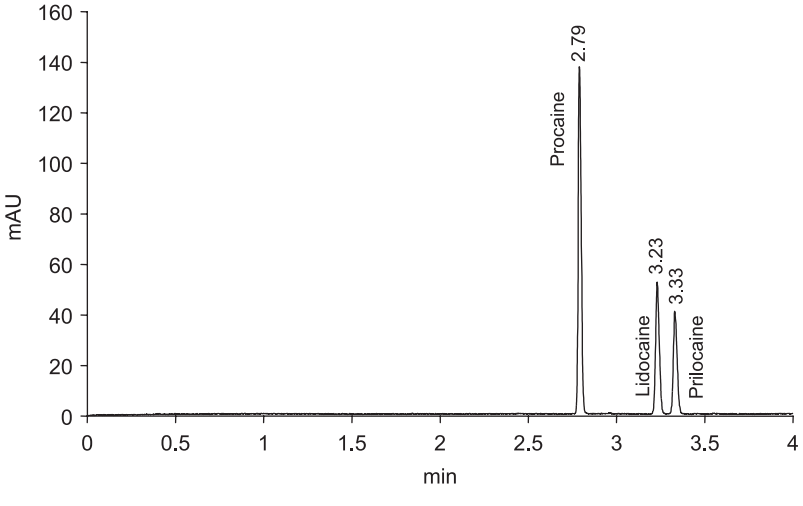


Figure 4. Electropherogram obtained from tape sample at 4 hours post dose with added IS of 600 μg procaine. Displayed at 200 nm.

DOI: 10.1002/bmc

Table 1. Regression parameters for five calibration curves during validation

Batch	Slope (A)	Intercept (B)	r^2
1	0.0257	0.0867	0.9986
2	0.0262	0.0816	0.9974
3	0.0255	0.0995	0.9967
4	0.0257	0.0661	0.9997
5	0.0240	0.1232	0.9914

Linear equation: $y = Ax + B$.

greater than 0.99. Regression parameters obtained from five curves are summarized in Table 1.

Inaccuracy and imprecision

Inaccuracy and imprecision were measured both within-batch and between-batch by repeated analysis of low, medium and high quality control samples, together with the lower (LLOQ) and upper level of quantitation (ULOQ) samples. The nominal values for low, medium and high control samples were 80, 400 and 800 µg, respectively. The nominal values for the ULOQ and LLOQ were the same nominal concentration as the highest and the lowest calibration standards, respectively. All the control samples, LLOQ and ULOQ were each assayed six times in three separate assays. Within-batch and between-batch imprecision and inaccuracy were calculated using the internationally agreed method (FDA, 2001).

Within-assay reproducibility. The three quality control 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 CV for imprecision and the percentage inaccuracy for all the quality control samples including LLOQ and ULOQ were below 2 and 14%, respectively.

Between-assay repeatability. For each of the three assays mentioned above, the mean concentration from each assay was used to calculate the between assay reproducibility. The CV for imprecision and the percentage inaccuracy for all the quality control samples including LLOQ and ULOQ were below 2 and 11%, respectively .

Table 2 summarizes the within- and between-batch, and the total variability obtained from the nested analysis of variance (ANOVA). From the nested ANOVA, the within- and between-batch and the total variability for all the QC samples including ULOQ and LLOQ were all <7%.

Recovery

Absolute recovery of lidocaine was determined using tape samples spiked with lidocaine at the same nominal concentrations as the quality control samples. 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 deviation were calculated from at least three measurements at each level. The absolute recovery of lidocaine ranged from 97 to 103%.

DISCUSSION

Clinical trial of drugs, especially pharmacokinetic studies, normally consist of large numbers of samples. Therefore, the development of rapid and fully validated techniques to analyze the samples in as short a period of time as possible is important. In the development of this CE method we attempted to reduce the migration time even further by short-end injection (Geiser *et al.*, 2005). This resulted in a migration time for procaine of about 1 min and for lidocaine of about 1.2 min. Unfortunately, the prilocaine also found in the EMLA cream could not be fully separated from lidocaine, so the optimized method employed the standard separation mode.

Reducing the capillary length to 35 cm from the original 48.5 cm resulted in lidocaine and prilocaine

Table 2. Within and between batch variability from the nested analysis of variance

	LLOQ	QC1	QC2	QC3	ULOQ
Nominal concentration (µg/mL)	50.0	80.0	400.0	800.0	1000.0
Mean; n = 18 (µg/mL)	45.1	81.9	415.0	822.4	1013.0
SDw	—	0.31	0.11	—	2.78
SDb	0.06	0.39	2.28	2.13	5.73
SDt	0.06	0.50	2.28	2.13	6.37
CVw (%)	—	3.8	0.3	—	2.7
CVb (%)	1.4	4.7	5.5	2.6	5.7
CVt (%)	1.4	6.1	5.5	2.6	6.3

w = Within batch; b = between batches; t = total.

DOI: 10.1002/bmc

being fully separated in only about 2 minutes more than by short-end injection (Figs 3 and 4). The short migration times obtained for procaine (2.7 min) and lidocaine (3.2 min) are an advantage of CE compared with the 5 min retention time for lidocaine using LC/MS/MS in our previous publication (Chik *et al.*, 2006). All the validation results meet the international requirements as outlined by the FDA's 2001 bioanalytical method validation guidelines (FDA, 2001). This method has been used for the analysis of lidocaine in tape samples obtained from tape stripping study in 12 healthy subjects. For the purpose of the study, only lidocaine content was analyzed from the tape samples.

CONCLUSIONS

A fast and reliable method to analyze lidocaine from skin stripping tape samples has been developed and validated using capillary electrophoresis with UV detection. The method has successfully been used to analyze hundreds of tape samples from a tape stripping study.

Acknowledgments

We would like to acknowledge Tesa UK Ltd for provided us with the adhesive tape used in this study.

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DOI: 10.1002/bmc