

Rapid analysis of tetracaine for a tape stripping pharmacokinetic study using short-end capillary electrophoresis

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ABSTRACT: A rapid and simple short-end (reverse) capillary zone electrophoresis method was developed and validated for the separation and quantification of tetracaine in skin using tape samples. The separation was performed in a 485 mm (400 mm to window) \times 50 μ m internal diameter fused silica capillary using a background electrolyte of phosphoric acid-Tris pH2.5 at -25 kV. The extraction of tetracaine from tape samples was achieved using methanol diluted to 50% with water before injection. Procaine was the internal standard. The migration times for procaine and tetracaine were 1.25 and 1.36 min, respectively. The limit of quantification for tetracaine was 50 μ g, with a signal-to-noise ratio greater than 10. The calibration curve was linear from 50 to 1200 μ g with r^2 greater than 0.99. The CV for both within- and between-assay imprecision and the percentage inaccuracy for the quality control samples including lower and upper limits of quantitation were <12.1% and <11%, respectively. The absolute mean recovery of tetracaine was >97%. The accuracy and selectivity of this method allowed the rapid measurement of tetracaine in tape samples obtained from a skin tape stripping study of local anaesthetics in healthy subjects. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: capillary electrophoresis; short-end injection; tape stripping; local anaesthetics

Introduction

Local anaesthetics can be classified according to their chemical structure into amide or ester groups. Tetracaine (amethocaine, 2-dimethylaminoethyl 4-butylamino-benzoate) was developed in the early 1990s and is a potent local anaesthetic belonging to the amino ester class (Fig. 1). It is used for topical anaesthesia, ophthalmology, antipruritic, spinal anaesthesia and as a nerve block. Also tetracaine has been incorporated into a mucosa adhesive polymer film to relieve the pain of oral lesions resulting from radiation and antineoplastic therapy.

Tetracaine hydrochloride can be found in solutions, creams, gels and as the base in ointments. The topical anaesthetic action of tetracaine is more prolonged than the mixture of lidocaine and prilocaine known as EMLA cream (Martindale, 1996). It also appears to have a more rapid onset of action, i.e. 30–45 min, than EMLA cream, without any risk of methemoglobinaemia. In addition, it causes local vasodilatation, which may be an advantage when used prior to central venous catheter placement, and where obtaining i.v. access or blood is difficult, especially in the newborn.

Most biochemical and pharmacological effects take place in the local tissue and most drugs exert their effect in target tissue or cells, especially local drugs, which are closer to the site of action. Thus assessing tissue concentration is both meaningful and useful for drug pharmacology studies. Ametop is a local anaesthetic applied to skin; its concentration is high in local tissue compared with plasma. At present, microdialysis and tape stripping are available to provide analytical data concerning drug levels in the extracellular space and outermost layer of the skin, respectively.

Tape stripping of the outermost skin layer, the stratum corneum (SC), is a fast and relatively noninvasive technique to measure drug absorbed into the skin (Stinchcomb *et al.*, 1999, Christophe

et al., 2007), usually performed by placing an adhesive tape strip onto the skin surface, after removing the residue of the drugs followed by gentle pressure to ensure a good contact and subsequent removal by a sharp upward movement. The procedure is relatively painless, given that only dead cells (corneocytes) in the outer most layers in the skin are removed.

The determination of tetracaine in biological samples has been performed mainly in plasma. Several methods are available, such as high-performance liquid chromatography (HPLC), (Menon and Norris, 1981; Yang *et al.*, 1984; Mazumdar *et al.*, 1991), nevertheless human plasma contains proteins and endogenous compounds that may interfere with the chromatographic system. There are several reported ways to extract tetracaine from plasma, e.g. organic extraction or solid-phase extraction, but these are time-consuming. Thus the use of coupled column (RAM-columns) or micellar liquid chromatography (MLC) as

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Abbreviations used: BGE, background electrolyte; MLC, micellar liquid chromatography.

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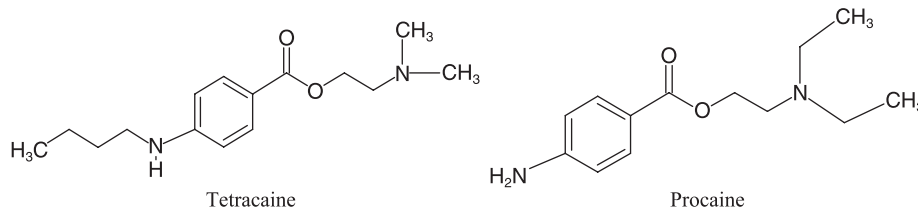


Figure 1. Chemical structures of tetracaine and procaine.

alternative methods has been proposed (Gilbert *et al.*, 2001). In addition gas chromatography–mass spectrometry (GC-MS) (Hino *et al.*, 2000; Yukiko *et al.*, 2002), direct ultraviolet spectrophotometry and colourimetry (Robert and Albert, 2006) can also be used.

Capillary electrophoresis (CE) is an alternative to the chromatographic techniques in drug analysis. It was developed in the 1980s and expanded in the 1990s, and it has become a complementary separation technique that can separate a variety of compounds with separations being based on differences in electrophoretic mobility. CE with UV detection is a simple and fast way to determine local anaesthetics in tape-stripping pharmacokinetic studies since high sensitivity is not usually required to detect the drug levels involved, and it has a cost benefit compared with MS detection.

The purpose of this study was to develop and validate a fast, high-throughput but simple CE method using a short-end injection approach. This method was used to determine tetracaine in skin using tape samples from volunteers given Ametop gel as a part of pharmacokinetic drug delivery study.

Experimental

Chemicals

Tetracaine hydrochloride (HCl; lot no. 065k1501; 99.9% purity) for calibrator and control sample preparation and procaine hydrochloride (HCl; lot no. 114k0569; 100% purity) for use as internal standard were obtained from Sigma Aldrich (Poole, UK).

HPLC-grade solvents were obtained from Rathburn Chemicals Ltd (Walkerburn, UK). All other AnalaR grade reagents were obtained from Merck (BDH) Ltd (Poole, UK).

CE Instrumentation

The analysis and separations were carried out on an Agilent 3DCE G1600AX capillary electropherograph controlled by 3D-CE Chemstation Rev. B.02.01[244] (Agilent, West Lothian, UK). Fused silica capillaries, 485 mm total length with a window at 85 mm from one end \times 50 μ m 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 NaOH (BDH, Poole, UK), then a 3 min flush with background electrolyte (BGE). In the final separation a potential of -25 kV was employed. The capillary was thermostated at 25.0°C. The injections of the samples into the system were carried out hydrodynamically for 10 s at -50 mbar. Detection was by photodiode array over 195–360 nm, but 315 nm with bandwidth 10 nm was used for quantitation since 315 nm is the λ_{max} for tetracaine. The run time was 2 min. Both BGE vials were replenished every 12 injections. All samples and standards in the auto-sampler were kept at ambient temperature.

Experimental Design

Background electrolyte. As the aqueous background electrolyte 0.1 M Tris–phosphate at pH 2.5 was used. The buffer was prepared by diluting 20 mL phosphoric acid (0.5 M) to 80 mL with deionized water followed by adding 15 mL of 0.5 M Tris to obtain a solution at pH 2.5. This solution was made up to 100 mL with deionized water. Then the pH was checked and adjusted if necessary.

Calibrators and quality control samples. Stock solutions for tetracaine (5 mg/mL) were prepared in 50% methanol–water and for procaine 300 μ g/mL in water (internal standard, IS). All stock solutions were stored at -20°C . All calibrators and quality control samples were prepared by appropriate dilution of the stock. Calibrations were nominally 50, 200, 400, 600, 800, 1000 and 1200 μ g tetracaine; 50 and 1200 μ g were the lower limit of quantity (LLOQ) and the upper limit of quantity (ULOQ), respectively, plus 1500 μ g IS, and quality controls were nominally 100, 250, 1000 μ g tetracaine plus 1500 μ g IS.

Preparation of tape sample and extraction. For the purpose of validation and assay calibration, a length of self-adhesive polypropylene tape (Tesa 404 PV5, Beiersdorf, Hamburg Germany) was cut into 10 samples, each approximately 3 \times 2 cm. A 100 μ L volume of standard or quality control solution was placed onto the adhesive part of the tape. The solution was distributed approximately evenly over the tape. The sample was left to dry at room temperature, which required approximately 30 min. The samples then were transferred into a 10 mL polypropylene tube and stored frozen at -20°C until analysis.

Extraction procedure. A 5 mL aliquot of methanol was dispensed into each tube containing standard/QC tape or samples. The contents were mixed for 45 min with a shaker. A 5 mL aliquot of solution of 300 μ g/mL procaine (InternalStandard.) in water was added to the tube to make 50% methanol–water solution. The contents was mixed again using a shaker for further 15 min. A 200 μ L aliquot of the solution was transferred into a 250 μ L auto-injector vial for CE analysis.

Method Development

Optimization of buffer pH. The role of the pH of 0.1 M Tris–phosphate BGE was studied over the range 2–3.5. pH 2.5 was the optimum with respect to the resolution of tetracaine and procaine.

Optimization of temperature. Various temperatures were studied, 15, 20, 25 and 30°C, and 25°C was found to be optimum.

Optimization of buffer concentration. Different buffer concentrations at constant pH in the range 0.05–0.2 were investigated

and 0.1 M was the optimum buffer concentration with respect to peak resolution and current.

Optimization of voltage. A voltage of -25 kV gave the best separation with respect to analysis time and gave only a moderate current, typically 50 – 70 μ A.

Optimization of injection time. Injections time were studied from 1 to 65 s, and 10 s was optimum for both resolution and sensitivity.

Calculating inaccuracy and imprecision. Inaccuracy was tested by the determination of low, medium and high quality control samples, together with the LLOQ and ULOQ samples. Each control sample contained tetracaine. The nominal values for the low, medium and high control samples were 100 , 250 and 1000 μ g, respectively. The nominal values for the ULOQ and LLOQ were the same nominal concentration as the highest and the lowest calibration standards, respectively.

Assay imprecision was measured both within-batch and between-batch by the analysis of three control samples, the LLOQ and the ULOQ. Within-batch and between-batch imprecision were calculated by the nested analysis of variance (ANOVA) using internationally agreed methods (ISO 5725:1994).

Validation Procedures and Results

Specificity. Six samples of blank tape and six samples of tape place with tetracaine were prepared and carried through the extraction. The concentration of tetracaine used was 5 μ g/mL LLOQ and the internal standard was 150 μ g/mL. No significant interfering peaks were found at the migration time of tetracaine or procaine. The signal-to-noise ratios at the LLOQ for both drugs were greater than 10 . Figure 2 shows the electropherogram obtained from blank tape spiked with 50 μ g tetracaine with added internal standard of 1500 μ g procaine while Figures 3 and 4 show the electropherograms of Ametop gel solution and one of the tape samples from the study, respectively.

Calibration curve/linearity. Calibrations were nominally 50 , 200 , 400 , 600 , 800 , 1000 and 1200 μ g tetracaine plus 1500 μ g procaine (internal standard). Seven batches of calibration curve were plotted using the area ratio of tetracaine to internal standard vs known concentration of tetracaine. All the results were calculated using a $y = ax + b$ linear regression (Table 1). The regression coefficients for all the calibration curves obtained were greater than 0.99 .

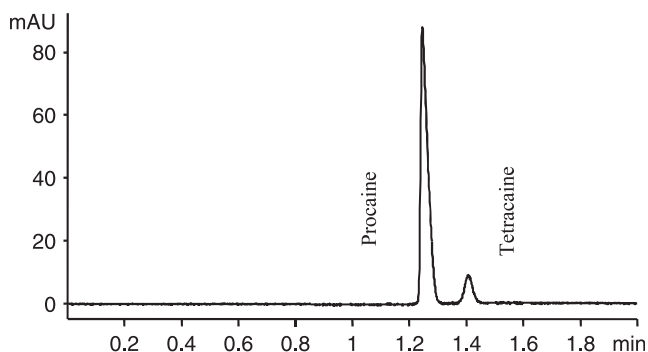


Figure 2. Electropherogram obtained from extracted tape sample spiked with 50 μ g tetracaine and added internal standard of 1500 μ g procaine. Displayed at 315 nm.

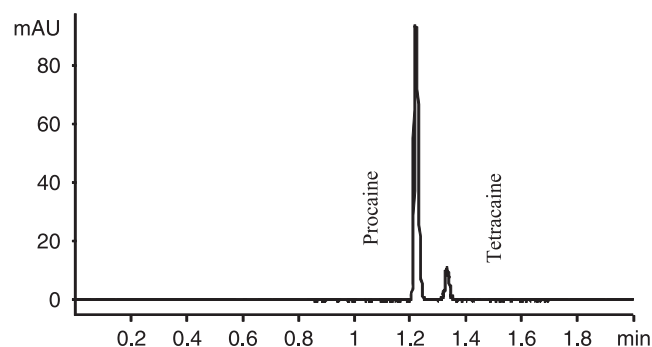


Figure 3. Electropherogram obtained from Ametop gel solution containing 50 μ g tetracaine and added internal standard of 1500 μ g procaine. Displayed at 315 nm.

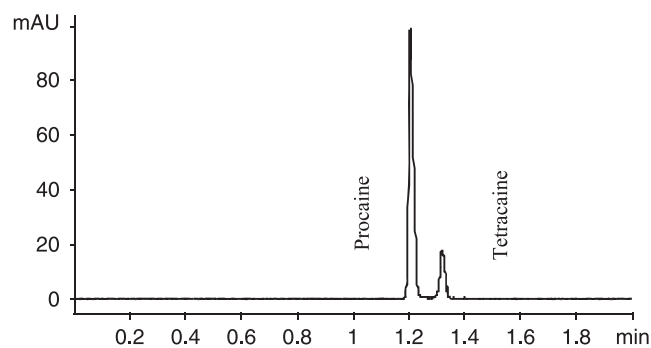


Figure 4. Electropherogram obtained from tape sample at 4 h post dose with added internal standard of 1500 μ g procaine. Displayed at 315 nm.

Table 1. Calibration line parameters for 5 separate runs

Batch	Slope (A)	Intercept (B)	r^2
1	0.0194	-0.0192	0.9992
2	0.0195	0.0099	0.9993
3	0.0194	0.0155	0.9986
4	0.0191	0.0070	0.9998
5	0.0198	0.0041	0.9997

Inaccuracy. The within- and between-assay and the total variability obtained from the ANOVA are summarized in Table 2. The percentage inaccuracy for all the quality control samples including LLOQ and ULOQ was below 11% .

Within-assay reproducibility. For within-batch and between-batch imprecision the LLOQ and ULOQ and the three control samples were each assayed six times in three separate assays. Each assay had an individual calibration curve. The coefficient of variation (CV) for imprecision for all the quality control samples including LLOQ and ULOQ was below 2.5% .

Between assay repeatability. For each of the three assays mentioned above, the mean concentration for each assay was used to calculate the between-assay reproducibility. The CV for imprecision for all the quality control samples including LLOQ and ULOQ was below 12.1% .

Stability. Samples were stable at room temperature for 24 h and for at least 3 weeks when stored at -20°C .

Table 2. The within- and between-batch and the total variability obtained from the nested analysis of variance (ANOVA)

	LLOQ	QC1	QC2	QC3	ULOQ
Nominal concentration ($\mu\text{g/mL}$)	50	10	25	100	120
Mean ($\mu\text{g/mL}$), $n = 18$	55.29	10.27	27.35	106.36	121.00
Inaccuracy (%)	10.58	2.77	9.42	6.36	0.83
SDw	0.052	0.254	0.309	2.063	1.862
SDb	0.560	1.239	1.611	8.996	3.270
SDt	0.563	1.265	1.640	9.230	3.763
CVw (%)	0.94	2.47	1.13	1.94	1.54
CVb (%)	10.13	12.06	5.89	8.46	2.70
CVt (%)	10.18	12.31	6.00	8.68	3.10

W = within batch; b = between batch; t = total.

Recovery. Absolute recovery of tetracaine was determined using tape samples doped with tetracaine at the same nominal concentration as the quality control samples. Peak area measurements from extracted samples were compared with the peak areas from direct solvent injection of the test compounds. Mean and standard deviation were calculated from at least six measurements at each level. Two samples of solution and six samples of tapes loaded with 50, 100, 200, 400, 600, 800, 1000 and 1200 μg of tetracaine were used, and 1500 μg procaine as internal standard. The absolute mean recovery of tetracaine ranged from 97 to 104%.

Discussion

Bioanalysis of biological fluids for the quantitative determination of drugs and their metabolites plays a significant role in the evaluation and interpretation of bioequivalence, pharmacokinetics and toxicokinetics studies. The huge number of samples and the quality of these studies, which is directly reflected by the results obtained, require the development of rapid and simple analytical methods. Thus the validation of the present methods employed the latest guidance and principles of validation approved (FDA, 2001).

In this study we have developed a very short end direction (reverse) CE method. Applying a short end direction (reverse) by negative voltage resulted in tetracaine and procaine being fully separated. The short migration times obtained for procaine (1.25 min) and tetracaine (1.36 min) are an advantage of CE compared with the 5 min retention time for tetracaine using HPLC (Mazumdar *et al.*, 1991). All the validation results meet the international requirements as outlined by the FDA's 2001 bioanalytical method validation guidelines (FDA, 2001). In an attempt to reduce the migration time even further, the capillary length was shortened to 35 cm from the original 48.5 cm, but this resulted in tetracaine and procaine not being fully separated and the run time was only reduced by about 30 s. The purpose of this work was to develop a fast and simple CE method that was fully validated. This method is currently being used to determine tetracaine in skin using tape samples from healthy volunteers given tetracaine as a part of a pharmacokinetic drug delivery study.

Conclusions

A simple and rapid short end direction (reverse) method to determine tetracaine in skin using tape samples has been devel-

oped and validated for the separation and quantification using capillary zone electrophoresis with UV detection. The method was successfully used to analyse hundreds of tape samples from a tape stripping study.

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