Journal of Chromatography, 526 (1990) 215–222
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 5111

Note

Determination of prilocaine in human plasma samples using high-performance liquid chromatography with dual-electrode electrochemical detection

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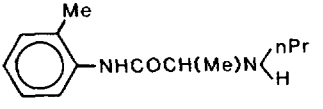
(First received July 24th, 1989; revised manuscript received November 6th, 1989)

Prilocaine (Fig. 1) is an amide local anaesthetic with pharmacological properties similar to lignocaine. To increase the duration of action and delay uptake by the general circulation local anaesthetics may be administered with a vasoconstrictor, usually adrenaline. Prilocaine is the only local anaesthetic avail-



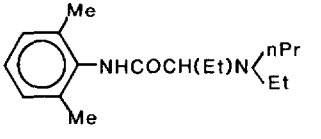


Fig. 1. Structures of prilocaine (upper) and internal standard, etidocaine (lower).

0378-4347/90/$03.50

able with octapressin (felypressin) as vasoconstrictor. This synthetic polypeptide is considered preferable to adrenaline in patients with cardiac problems. After intercostal or epidural injections of 400 mg prilocaine mean maximum plasma concentrations were 3–5 μg/ml [1]. As the dose in the present study was 18 mg an assay capable of quantifying much lower plasma concentrations was required.

A recent review [2] of analytical methods for local anaesthetics in biological materials showed that, generally, gas chromatography (GC) with nitrogen–phosphorus detection (NPD) or high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection in the region of 200 nm appear to be the most commonly used approaches for this class of compound. Maclean et al. [3] assayed prilocaine after brachial plexus blockade. Using HPLC with UV detection their lowest calibration standard was 0.5 μg/ml. Capillary column GC with NPD has been used [4] but for high sensitivity (10 ng/ml) mass fragmentography may be necessary [5]. Previously, we have used packed-column GC with NPD to assay plasma prilocaine after perioral injections of 60 and 80 mg [6]. For lignocaine a 530-μm capillary column was used and the increased sensitivity allowed a single extraction of plasma with an equal volume of toluene followed by injection of an aliquot of the toluene layer into the column (unpublished). As the dose in the present study was only 18 mg, the first method was considered too insensitive and, in the second, prilocaine co-chromatographed with a plasma impurity. A new method, based on combined liquid–liquid and solid-phase extraction with quantification by electrochemical detection (ED) was developed. This method was sensitive enough to determine 5 ng ml^{-1} in as little as 0.2 ml of plasma. The application of the method to samples from healthy volunteers is reported.

EXPERIMENTAL

Materials

HPLC-grade acetonitrile was from Fisons Scientific Apparatus (Loughborough, U.K.). Prilocaine and etidocaine were gifts from Astra Pharmaceuticals (Kings Langley, U.K.). AASP cartridges were purchased from Jones Chromatography (Hengroed, U.K.). Polyethylene centrifuge tubes (1.5 ml volume) were from Alpha Labs. (Eastleigh, U.K.). Prilocaine solutions (3%, w/v) containing octapressin (Citanest) were from Astra. Solutions of prilocaine without vasoconstrictor were prepared and standardised by the Department of Pharmacy, The London Hospital (Whitechapel, London, U.K.).

Biological samples

Plasma samples from four healthy volunteers were obtained from venous blood collected in heparinised tubes. Each volunteer received 18 mg prilocaine on three occasions: (1) as an intravenous injection; (2) as multiple intraliga-

mentary injections of a 3% (w/v) solution; and (3) as multiple intraligamentary injections of 3% (w/v) prilocaine with octapressin as vasoconstrictor. The intraligamentary injections were given with a pressurised syringe into the periodontal membrane. Drug-free plasma was obtained from heparinised blood taken from volunteers in the Department of Pharmacology. The procedure was approved by the Tower Hamlets District Ethics Committee.

Extraction procedure

New diol AASP cartridges were prepared by sequential elution with methanol (1 ml), distilled water (1 ml), HPLC eluent (40%, v/v, acetonitrile in 0.01 *M* H_3PO_4; 1 ml), methanol (0.5 ml) and toluene (0.5 ml) under pressure (ca. 70 KPa). Used cartridges were treated with methanol (0.5 ml) and toluene (0.5 ml). Plasma (0.2 ml) and internal standard solution (1.0 μg ml^{-1} etidocaine in 0.1 *M* K_2HPO_4 solution, 0.1 ml) were pipetted into 1.5-ml polyethylene centrifuge tubes. Toluene (1 ml) was added and the capped tubes mixed for 60 s on a Vortex Genie 2 and centrifuged at 12 000 *g* for 60 s. The toluene extracts (0.75 ml) were transferred to the prepared diol cartridges. Once the toluene had eluted, the cartridges were washed with acetonitrile (0.5 ml). Standard solutions in the range 1000–10 or 500–5 ng ml^{-1} were prepared in plasma and taken through the extraction procedure with every batch of unknown samples. Intra-assay coefficients of variation were determined by replicate ($n=10$) assays of selected unknown samples.

Chromatographic system

The stainless-steel column (150 mm$\times$4.6 mm I.D.) was slurry packed with 5 μm cyanopropyl silica (Spherisorb 5 CN, Phase Separations, Queensferry, U.K.) in methanol. A guard column (20 mm$\times$2 mm I.D.) packed with Co:Pell ODS, 30–40 μm particle size (Whatman, Maidstone, U.K.) was used. The eluent, 0.01 *M* H_3PO_4 acetonitrile–water (40:60, v/v) was pumped at 1.0 ml min^{-1} using an Altex 110A pump. Samples were introduced via the AASP (Varian Assoc., Walton-on-Thames, U.K.). Acetonitrile was used for purge (six strokes) and afterwash (ten strokes). The valve reset time was 2 min. The cycle time was 30 min. The compounds were quantified using an Environmental Science Assoc. Model 5100A Coulochem detector operated in the 'screen' mode. Electrode 1 was set at +0.7 V and electrode 2 at +0.9 V, relative to the palladium reference electrode. The guard cell, placed before the injection valve, was operated at +1.2 V. Peaks were recorded on an analogue recorder or Spectraphysics 4290 integrator (Varian).

RESULTS AND DISCUSSION

Extraction conditions

Although it is usual with solid-phase extraction to apply samples directly to the prepared phases, we are increasingly finding it preferable to combine solid-

phase and liquid–liquid extraction [7]. In attempting to develop a direct method using alkali pre-treated C_8 cartridges, it was not possible to obtain reproducible ratios for the prilocaine-to-internal standard responses when samples were applied in plasma. The recovery of the internal standard was markedly reduced. It was assumed that the problem was due to the differences in plasma protein binding [8]. Use of the higher-capacity C_{18} cartridges, as used for the highly bound drug buprenorphine [9], was considered unsuitable as these produced marked peak broadening on elution from the AASP. These problems were overcome by extracting the compounds into toluene and then adsorbing them onto acid-prepared diol cartridges. This gave clean extracts with high, reproducible recoveries. A further advantage of this procedure was that it avoided clogging of the cartridge inlet frit that sometimes occurred when biological samples were applied directly. The design of the AASP cartridge and work station was such that the blocking of one frit resulted in the loss of ten samples.

The use of the AASP, rather than a standard autosampler, avoided the need to concentrate the sample by evaporation. Furthermore, as all the extracted material was 'injected' it was possible to develop a sensitive assay that only required 0.2 ml of plasma. This in turn allowed the use of small reagent volumes and tubes. Initial experiments, in which the extraction tubes were mixed for only 15 s, gave erratic results. Increasing the time to 60 s overcame the problem. As the Vortex Genie head can hold thirty 1.5-ml tubes it was convenient to process samples in batches of sixty tubes.

The AASP was programmed so that the cartridges were purged with acetonitrile before the injection valve was operated. Removing the air in this way avoided the large pressure drop and injection of air that otherwise had a deleterious effect on the electrochemical detector response. The afterwash was necessary to flush the transfer lines as residual eluent would partially elute the compounds when the next cartridge was purged. A shorter valve reset time had no noticeable effect on the chromatography, so a time of 2 min was chosen to ensure that the cartridges were reactivated by the acidic eluent.

Chromatographic conditions

UV detection of local anaesthetics is usually carried out in the region of 200 nm as their absorption at 254 nm is insufficient for quantification of extracts from biological samples. Jane et al. [10] demonstrated the potential increase in sensitivity that ED could have over UV detection for local anaesthetics. Requiring a simple but sensitive assay, ED was chosen for plasma prilocaine.

Triethylamine has been added to HPLC eluents to reduce adsorption, improve peak shape and hence, improve sensitivity [2,3]. Undoubtedly, this was effective; however, this approach was not considered suitable for ED of prilocaine (also an amine). Chromatography using cyanopropyl columns eluted with acetonitrile in H_3PO_4, a system which has been used successfully with

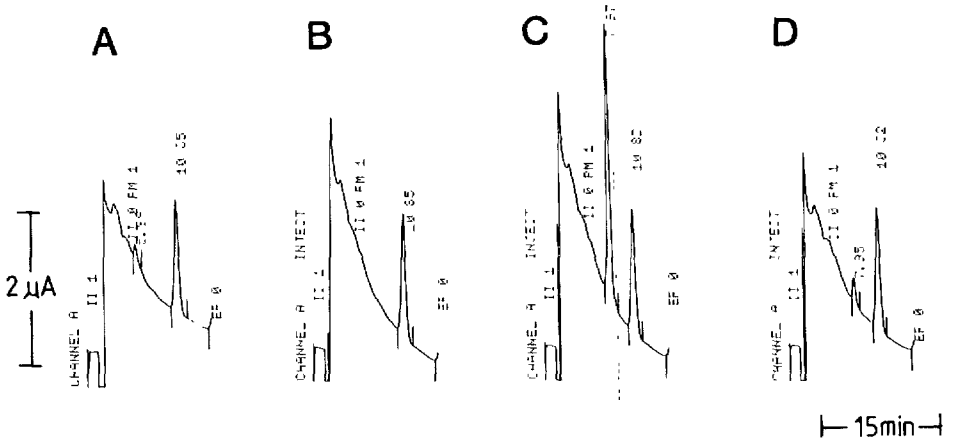


Fig 2. Chromatograms of extracted reference solutions. (A) Reagent blank; (B) drug-free plasma; (C) plasma containing 500 ng ml^{-1} prilocaine; (D) plasma containing 50 ng ml^{-1} prilocaine. The internal standard, etidocaine, chromatographed at 10.8 min.

TABLE I

INTER-ASSAY COEFFICIENTS OF VARIATION FOR PRILOCAINE ASSAY

Concentration added (ng ml^{-1})	Concentration found (ng ml^{-1})	Inaccuracy (%)	Coefficient of variation (%)
500	502	+0.4	0.9
250	256	+2.4	3.5
100	98	−2.0	2.6
50	45	−2.0	4.8
25	25	0	10.4
10	9	−10.0	15.7

other organic bases [11], was considered suitable for this application (Fig. 2). Nation et al. [12] used similar conditions, but with a phenyl rather than a cyanopropyl column. With ED there was no need to use far-UV-grade acetonitrile and it was possible to use toluene as extraction solvent without extensive washing of the AASP cartridge. Traces of toluene would have had to be removed to avoid interference with far-UV detection.

Precision and sensitivity

The mean concentrations (ng ml^{-1}) found and intra-assay coefficients of variation, determined by replicate ($n=10$) assays of three unknown plasma samples, were 680 (1.6%), 200 (3.6%) and 14 (5.7%), respectively. The sam-

ples were chosen to provide representative concentrations. An indication of the inter-assay precision and accuracy of the method can be seen in Table I, which shows expected and found concentrations and coefficients of variation for five assay days.

Calibration curves of prilocaine/etidocaine peak-height ratios versus prilocaine concentrations run between 1000 and 10 or 500 and 5 ng ml^{-1} were linear (e.g. $y=0.009+0.0061x$; $r=0.9998$, $n=7$). The mean recoveries of prilocaine and etidocaine after correction for aliquot losses were 79.8 and 86.2%, respectively. The sensitivity of the method was 5 ng ml^{-1} using a 0.2-ml plasma sample. In chromatograms from plasma spiked at this concentration the prilocaine peak was clearly visible and was detected as a peak by the integrator. Peaks at this retention time were not detected in chromatograms for drug-free plasma. It was difficult to determine a signal-to-noise ratio as the limit to detection was the slope of the solvent front (Fig. 2). However, solutions containing 5 ng ml^{-1} gave responses that were more than three times the intercept of the calibration line.

Application of the assay

Fig. 3 shows the chromatograms obtained for samples taken from a single subject (A.J.) on the three occasions. Each sample was collected at the same time interval after the injection. The results obtained over the 30-min collec-

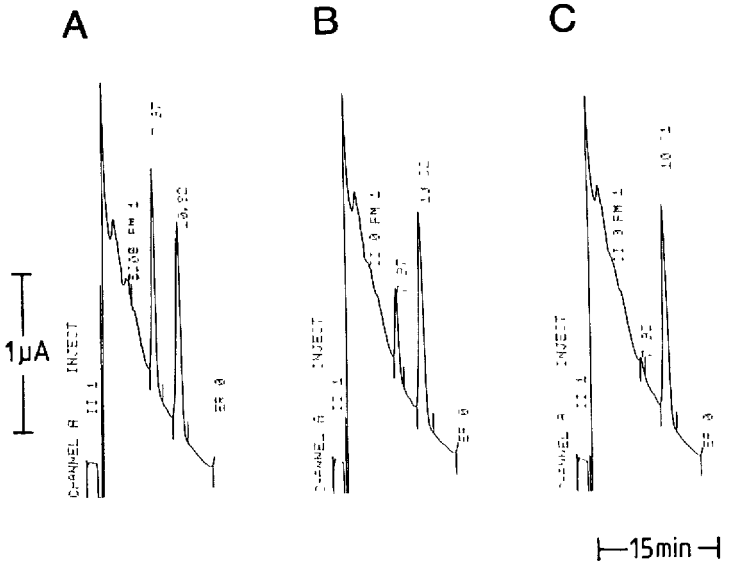


Fig. 3. Chromatograms of samples from subject 1. (A) 18 mg intravenously; (B) 18 mg intraligamentary, (C) 18 mg prilocaine plus octapressin intraligamentary. The internal standard, etidocaine, chromatographed at 10.8 min.

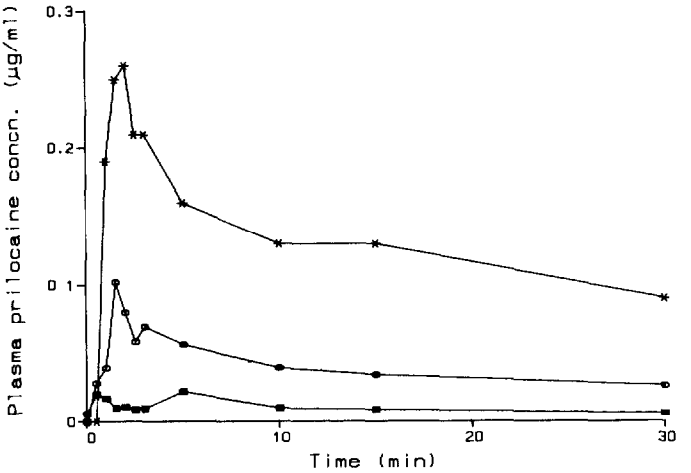


Fig. 4. Time–plasma concentration curves for prilocaine after administration of the three preparations to a single subject. (*) 18 mg prilocaine intravenously; (○) 18 mg prilocaine intraligamentary; (■) 18 mg prilocaine plus octapressin intraligamentary.

tion periods are presented in Fig. 4. The differences between the three occasions were clear. Without octapressin as vasocontrictor, the amounts reaching the systemic circulation following intraligamentary injection were greatly increased and the peak concentration was only approximately 50% less than that measured after the intravenous dose.

CONCLUSION

Liquid extraction followed by concentration on AASP cartridges is a convenient method for sample preparation prior to HPLC that avoids some of the problems associated with the individual approaches. ED of prilocaine offers a sensitive alternative to far-UV detection.

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