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Development and validation of a reversed-phase liquid chromatographic method for the assay of lidocaine in aqueous humour samples

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Abstract

A simple, fast and reliable reversed-phase liquid chromatographic method was developed for the assay of lidocaine in human aqueous humour samples. The samples were analysed without any preliminary treatment on a C8 column with UV detection at 225 nm. The mobile phase consisted of methanol/sodium dihydrogen phosphate (30 mM) containing sodium pentansulphonate (10 mM) adjusted to pH 2.5 with phosphoric acid (50:50 v/v). Validation of the method showed it to be precise, accurate and linear over the concentration range of analysis with a limit of detection of 0.2 μg ml^{-1}. The limit of quantitation was 2.5 μg ml^{-1} with a relative standard deviation of 2.5%. Linear regression analysis in the range 2.5–60 μg ml^{-1} gave correlation coefficients higher than 0.999. No interference from three commonly co-administered drugs was observed. The method developed was applied to the analysis of lidocaine in aqueous humour samples in order to evaluate and compare the efficacy of two different forms of administration of lidocaine for topical anaesthesia in cataract surgery.

Keywords: Lidocaine; Topical anaesthesia; Reversed-phase ion-pairing chromatography; Aqueous humour

1. Introduction

Lidocaine hydrochloride is a local anaesthetic agent widely used to alleviate pain associated with medical procedures; in particular, in modern cataract surgery, retrobulbar and peribulbar anaesthetic injections have been recently replaced by less invasive topical administration of lidocaine aqueous solution.

In a preliminary study [1], a gel containing 2% lidocaine was shown to yield better results when compared with lidocaine 4% unpreserved eye-drops in topical anaesthesia for cataract surgery. In order to evaluate whether the efficacy of such a novel form of administration of lidocaine for topical anaesthesia was due to a better penetration of the drug in the anterior chamber, a chromatographic method has been studied in our

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laboratory for rapid quantitation of lidocaine in aqueous humour samples taken during cataract surgery.

A literature survey about determination of lidocaine reveals several liquid [2–11] and gas-chromatographic [12–16] methods for its assay in serum and plasma samples; most of the reported HPLC methods involve extraction procedures for sample preparation and reversed phase elution on C8 or C18 columns with UV detection [3–6,8–10]. In one paper [7] a method is described which uses a silica gel column with normal phase elution.

In order to quantitate lidocaine in humour aqueous samples, two different methods have been previously reported. The first is a non-validated HPLC method [17] that requires a complex and time-consuming extraction procedure before injection onto a C8 column. A gas-chromatographic method [18], which implies extraction of the sample with organic solvents, has also been reported.

The aim of this study was to develop and validate a quick, simple and reliable HPLC method for determination of lidocaine in aqueous humour samples.

2. Experimental

2.1. Chemicals

Lidocaine hydrochloride, phenilephrine hydrochloride, ofloxacin and tropicamide were obtained by Sigma Chemical Company (St.Louis, MO); sodium pentansulphonate and sodium dihydrogen phosphate were supplied by Fluka Chemie AG (Buchs, Switzerland); HPLC-grade methanol was from Carlo Erba (Milan, Italy).

Deionized, double distilled water was used for the mobile phase and the standard solutions preparation. All other reagents were of analytical grade.

2.2. Chromatographic system

The chromatographic apparatus consisted of an Alliance W2690 Separation Module and a W996 Photodiode Array Detector both from Waters (Milford, MA).

For data collection and calculation Waters Millennium 32 Chromatographic Manager software was used.

The chromatographic column was a 5 μm Zorbax SB-C8, 150 × 4.6 mm ID (Agilent Technologies, Palo Alto, CA), thermostated at 35 °C. The column was protected by a Supelguard LC-ABZ guard column, 20 × 4.6 mm ID (Supelco, Bellefonte, PA).

The mobile phase consisted of methanol/sodium dihydrogen phosphate (30 mM) with sodium pentansulphonate (l0 mM) adjusted to pH 2.5 with phosphoric acid (50:50 v/v), delivered at a constant flow rate of 1.0 ml min^{-1}.

The monitoring wavelength was 225 nm and the injection volume was 10 μl.

2.3. Standard and sample solutions

Standard stock solutions of lidocaine hydrochloride at a concentration of about 0.5 mg ml^{-1} were prepared by dissolving the appropriate amount of lidocaine hydrochloride in water and stored at +4 °C for 1 month at most.

The stability of the standard stock solutions was checked over this period by preparing and injecting daily a diluted solution of the analyte.

The standard working solutions at the concentration of the calibration range were prepared daily by appropriate dilution of the stock solutions with water.

Humour aqueous samples of approximately 100 μl were taken by puncturing the anterior chamber of the eye before surgery and stored at +4 °C until analysis. Ten microlitre of the samples were injected directly onto the column without any preliminary treatment.

Weekly injection of a humour aqueous sample showed no change in the concentration of lidocaine for at least 1 month, when the solution was stored at +4 °C.

3. Results and discussion

In order to obtain a good and rapid elution of lidocaine and its separation from three potentially interfering analytes, that may be co-administered to patients being operated on for cataract, different reversed-phase eluents like acetate or phosphate buffers in mixture with acetonitrile or methanol were tested on a C8 column and the effects of mobile phase pH were explored.

It was observed that phosphate buffers at higher pH caused poor peak symmetry and higher retention times for lidocaine with consequent reduction of sensitivity; on the other side lower pH values caused peak narrowing and improved peak shape, but did not permit an adequate separation of lidocaine from the other three analytes. The addition to the mobile phase of a small quantity of a short-chain ion pairing reagent like sodium pentansulphonate at an acid pH, increased lidocaine retention on the stationary phase and allowed the separation of lidocaine from the potentially interfering analytes. Methanol was preferred to acetonitrile in order to avoid any risk of ion pairing reagent precipitation. A representative chromatogram of a mixture of lidocaine with phenylephrine, ofloxacin and tropicamide is shown in Fig. 1. A good separation in a short time of analysis was achieved. Lidocaine peak showed an excellent shape with a symmetry factor of 0.93.

The detection wavelength was set at 225 nm in order to have a good sensitivity for lidocaine that is characterised by low molar absorptivity at higher wavelengths.

The use of a guard column permitted injection of humour aqueous samples directly onto the column without any preliminary treatment.

Fig. 2A shows a representative chromatogram

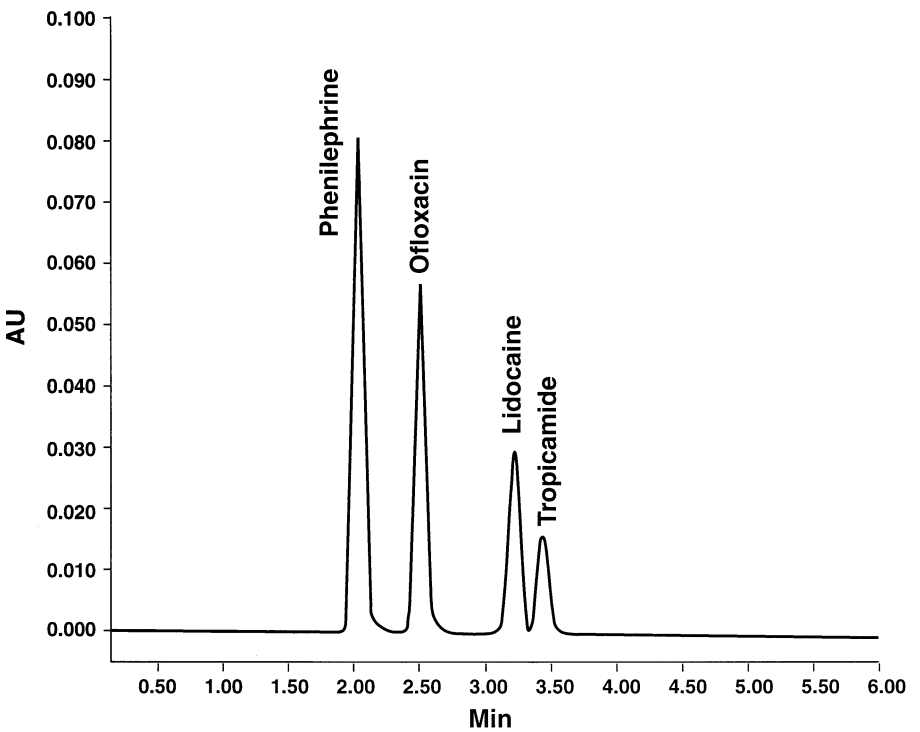

Fig. 1. Representative HPLC separation of a standard mixture of lidocaine (15 μg ml^{-1}) with phenilephrine (30 μg ml^{-1}), ofloxacin (12 μg ml^{-1}) and tropicamide (10 μg ml^{-1}). Column: Zorbax SB-C8 150 × 4.6 mm ID, 5 μm. Mobile phase: methanol–sodium dhydrogenphosphate (30 mM) with sodium pentansulphonate (10 mM) (50:50 v/v) at a flow rate of 1.0 ml min^{-1}. UV detection at 225 nm. Injection volume 10 μl.

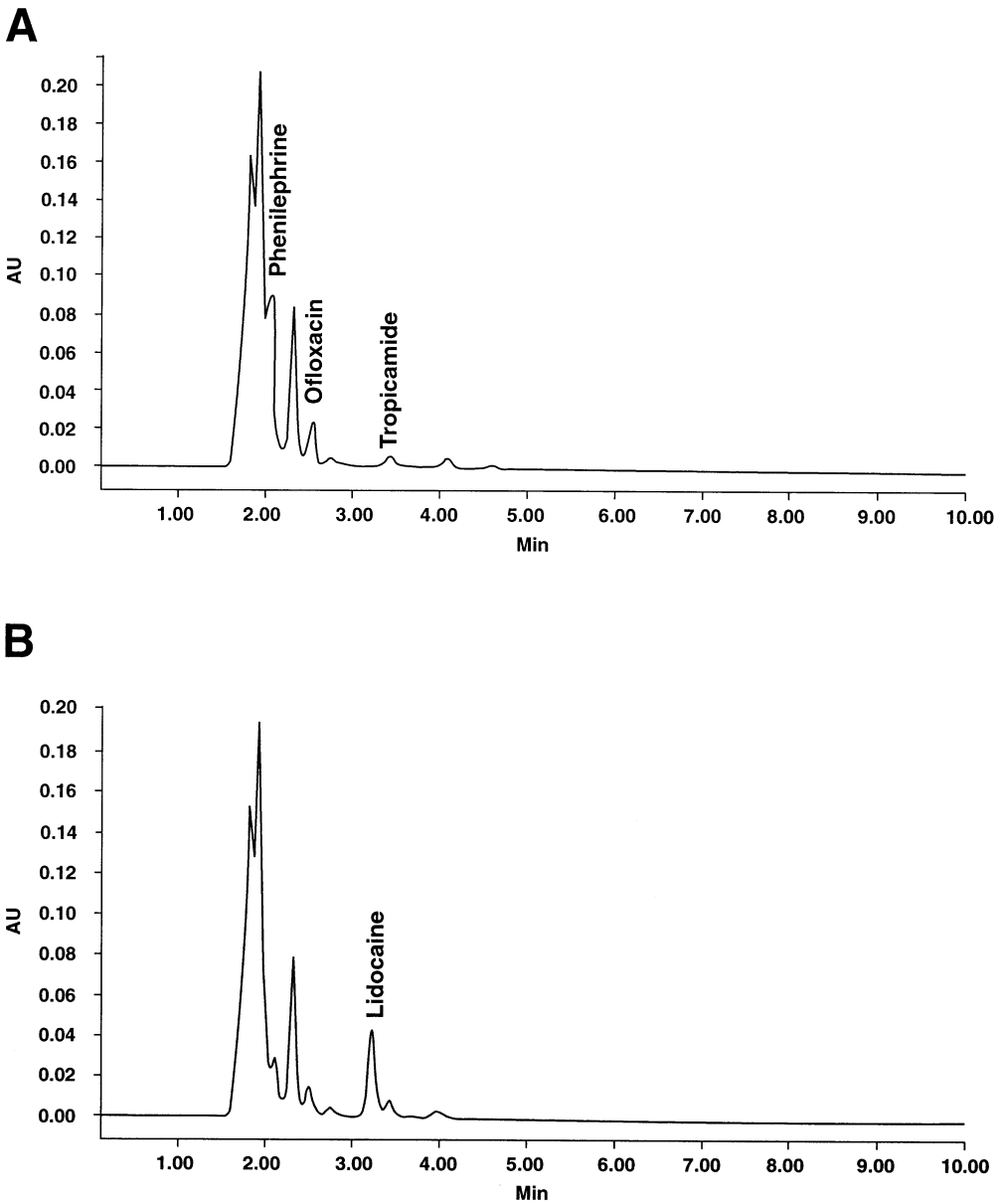

Fig. 2. Typical chromatograms of (A) Blank medium sample. The unlabelled peaks are unknowns. (B) Aqueous humour sample containing lidocaine at a concentration of 21 μg ml^{-1}. Chromatographic conditions are the same as Fig. 1.

of the blank medium obtained by patients who had not received topical lidocaine. As it can be seen there are no interfering peaks at the retention time for lidocaine.

A typical chromatogram of an aqueous humour sample from a patient receiving topical application of lidocaine before surgery is shown in Fig. 2B. Identification of lidocaine was confirmed by comparison of its UV spectrum with a standard of lidocaine.

3.1. Sensitivity and linearity

The limit of detection, based on the 3:1 peak height ratio of lidocaine over noise, was 0.2 μg ml^{-1}, meaning that the lidocaine peak could be reliably detected when 2 ng in 10 μl were injected onto column. The limit of quantitation was 2.5 μg ml^{-1} with a relative standard deviation (RSD) of 2.5%.

Linearity of the assay was examined by injection of lidocaine standards solutions at six concentration levels in the range 2.5–60 μg ml^{-1}. Samples were prepared twice for each concentration. The calibration curve obtained by plotting the lidocaine peak area against the concentration of standard solution was linear in the above mentioned concentration range.

The regression equation was $y = 1.27 \times 10^4 x + 2.4 \times 10^3$ with a correlation coefficient higher than 0.999. The coefficient of variation of the slope was 1.2%.

3.2. Precision and accuracy

Method precision was determined by replicated injections of humour aqueous samples at three different concentration levels of lidocaine. Intra-day repeatability was studied by carrying three measurements for each level on the same day. Inter-day repeatability was obtained by performing three determinations for each concentration on consecutive days.

The RSDs ranged from 0.20 to 0.75% for intra-day repeatability and from 0.52 to 1.0% for inter-day repeatability. Results are shown in Table 1.

Accuracy of the method was determined by analysis of humour aqueous blank samples spiked with 2.5–25–50 μg ml^{-1} of lidocaine. Each level was assayed six times with an overall 100.4% accuracy. Results are shown in Table 2.

Table 1
Intra- and inter-day repeatability for lidocaine

Concentration (μg ml^{-1})	2.5	25	50
Intra-day precision (RSD%)[a]	0.53	0.75	0.20
Inter-day precision (RSD%)[a]	1.0	0.79	0.52

[a] $n = 3$.

Table 2
Accuracy

Expected concentration (μg ml^{-1})	2.5	25	50
Measured concentration ± SD (μg ml^{-1})	2.54 ± 0.06	24.9 ± 0.5	49.9 ± 1.5
No of assays	6	6	5
Recovery (%)	101.6	99.9	99.8

3.3. Ruggedness

During method development it was observed that mobile phases prepared in different days by different analysts caused only slight variations in retention times of lidocaine. On no occasion, however, was the resolution factor between lidocaine and tropicamide significantly affected.

The column-to-column reproducibility was evaluated injecting the same humour aqueous sample on three columns from different manufacturers which contained the same type of packing material. The elution order and the resolution factors of the compounds were not affected and only slight variations in retention times were observed.

3.4. Applicability of the method

The validated method was applied to the determination of lidocaine in humour aqueous samples of patients undergoing cataract surgery. A total of 120 samples were analysed. Concentration levels of lidocaine ranging from 3.1 ± 0.1 μg ml^{-1} to 60.7 ± 0.4 μg ml^{-1} were found.

4. Conclusions

The proposed reversed-phase ion-pairing HPLC method was found to be suitable for lidocaine determination in humour aqueous samples. Validation of the method showed it to be accurate, precise and linear over the range studied. Moreover, since the method does not imply any sample

preparation step, only a small volume of aqueous humour sample is required and a rapid determination of lidocaine can be performed.

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