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Short communication

Spectrophotometric determination of etidocaine in pharmaceutical (dental) formulation

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Abstract

A spectrophotometric method was developed for the determination of etidocaine hydrochloride (EH) in injectable pharmaceutical preparation. The proposal of this work was to develop a rapid, simple, inexpensive, precise and accurate visible spectrophotometric method. The method is based on the formation of the ion-pair complex by the EH reaction with bromocresol green in the pH 4.6 which after chloroform extraction gives a yellow color that in basic medium change to blue color and exhibits a maximum absorbance at 625 nm. The calibration graph was linear over the range 2.0–6.0 μg ml^{-1} EH calculated on the final yellow solution. The R.S.D. of the slope of the four lines was 0.73%. This method can be applied to injectable pharmaceutical preparation dosage studied.

Keywords: Etidocaine determination; Anesthetic; Quality control; Spectrophotometric method; Dental pharmaceutical formulation; Injectable pharmaceutical preparation

1. Introduction

Etidocaine hydrochloride (EH) is a local anesthetic of the amide type, which is designed as butanamide, *N*-(2,6-dimethylphenyl)-2-(ethylpropylamine)-monohydrochloride, an analogous of lidocaine. It differs from lidocaine by the addition of a propyl for an ethyl group at the amine end, and an addition of an ethyl group on the α carbon in the intermediate chain.

EH has a rapid onset similar to lidocaine, but has a longer duration of action [13].

In vivo animal studies have showed that etidocaine has a rapid onset (3–5 min) and a prolonged duration of action (5–10 h). Based on comparative clinical studies of lidocaine and etidocaine, the anesthetic properties of etidocaine in man may be characterized as follows: initial onset of sensory analgesia and motor blockade is rapid (usually 3–5 min) and similar to that produced by lidocaine [4].

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Duration of sensory analgesia is 1.5–2 times longer than that of lidocaine by the peridural route. Duration of analgesia in excess of 9 h is not infrequent when etidocaine is used for peripheral nerve blocks such as brachial plexus blockaded [12].

Many studies have show the efficacy of etidocaine in reducing postoperative pain following dental procedures, and some have suggested a postanesthetic analgesia and decreased incidence of severe pain in the postoperative period when etidocaine is used rather than an intermediate duration local anesthetic agent [13].

A review showed that there are very few works about its physical and chemical parameters. The methods published for the quantitative determination of EH are gas chromatography [3,5,10,14,15] and high performance liquid chromatography [1,6,8] using biological fluids. No analytical procedures are reported in pharmacopoeias for determination of EH in pharmaceutical formulations.

The purpose of this work was to develop a simple, precise, accurate and inexpensive visible spectrophotometric method for quantitative analysis of etidocaine in injectable solutions for dental use. This extractive spectrophotometric method, already purposed for lidocaine [7,9,11,17] and another pharmaceutical amines [2,16] is based on the formation of ionic associated (ion-pair) of EH cation (EH^+Cl^-) with triphenilmethane dyes anion (bromocresol green (BCG)), showing a maximum absorbance at 625 nm in basic medium. The acid-dye technique is a general procedure for the quantitative analysis of a variety of pharmaceutical amines.

There is no one official method of analysis on pharmaceutical form studied. This proposed method is not rapid but is economic when comparing with a high performance liquid chromatography.

2. Experimental

2.1. Chemical and reagents

Reference EH (assigned purity 99.99%) and pharmaceuticals containing etidocaine injectable 1.5% (Duranest®, Lot 805986) were generously supplied by Astra-USA.

The Duranest® solution composition is to each ml: EH 15 mg, epinephrine (as the bitartrate) 0.005 mg, citric acid 0.2 mg, sodium chloride 6.2 mg, sodium metabissulfite 0.5 mg, pH adjusted to 3.0–4.5 with hydrochloric acid and/or sodium hydroxide. The anesthetic solution is filled under nitrogen atmosphere.

All other chemicals, reagents and solvents were of analytical grade: BCG, anhydrous sodium acetate, sodium hydroxide, chloroform, glacial acetic acid, triethanolamine, ethanol, anhydrous sodium sulfate.

2.2. Apparatus

Shimadzu (Kyoto, Japan) UV-2201 Spectrophotometer, Hanna Instruments Potentiometer Model 8414, Pachane Mechanic Mixing Model TE 244.

3. Method

3.1. Preparation of solutions

3.1.1. Bromocresol green

One hundred milligrams of BCG must be dissolved in 2.8 ml of 0.02 N NaOH then diluted to 100 ml with water. The solution must be filtered and extracted with three 25 ml portions of chloroform. The aqueous extract is used as color reagent.

3.1.2. Sodium acetate buffer

Ten grams of anhydrous sodium acetate must be dissolved in about 60 ml of water; the pH adjusted with glacial acetic acid to 4.6 then transfers to a 100 ml volumetric flask and completed the volume with water.

3.1.3. Standard solution

EH can be accurately weighed and transferred to a 50 ml volumetric flask then dissolved and completed the volume with 0.1 N hydrochloric acid. An aliquot of 10 ml of this solution is transferred to a 50 ml volumetric flask and completed the volume with 0.1 N hydrochloric acid to obtain a 100 μg ml^{-1} EH solution.

Aliquots of 1.0, 1.5, 2.0, 2.5 and 3.0 ml of the above solution are transferred to 50 ml volumetric flasks containing 2.0 ml of bromocresol solution and 2.0 ml of sodium acetate buffer in each one. The flasks are shaken for 3 min. All the solution are transferred to 250 ml funnels and extracted with three 10 ml portions of chloroform. The chloroform extracts are separated and dehydrated with about 0.25 g of anhydrous sodium sulfate then collected in 50 ml volumetric flasks and the volumes completed with the same solvent. The range of concentration will be from 2.0 to 6.0 μg/ml of EH.

To each 2.0 ml of these solutions above are added 5.0 ml of a 30% triethanolamine solution (dilute about 30 ml of triethanolamine to 100 ml with 95% ethanol) and mixed. Absorbances are measured at 625 nm against a blank reagent carry out simultaneously.

The results are used to calculate the line equation by linear regression and the data are evaluated by analysis of variance (ANOVA).

3.2. Preparation of sample

Aliquots of 2 ml are taken from a correspondent volume of 10 units of injectable sample, transferred to five volumetric flasks and the volume completed with 0.1 N hydrochloric acid. Aliquots of 1.0 ml of these solutions are transferred to 50 ml volumetric flasks containing 2.0 ml of bromocresol solution and 2.0 ml of sodium acetate buffer in each one. The flasks are shaken for 3 min. All the solutions are transferred to 250 ml funnels and extracted with three 10 ml portions of chloroform. Chloroform extracts are separated and dehydrated with about a 0.25 g of sodium sulfate anhydrous then collected in 50 ml volumetric flasks and the volumes completed with the same solvent.

To each 2.0 ml of these sample solutions above are added 5.0 ml of a 30% triethanolamine solution (described in Section 3.1.3) and mixed.

The same procedure was carried out to standard solution. The absorbances were measured at 625 nm against a blank reagent carry out simultaneously too.

3.3. Calculation

The drug contents of the injectable was determined by referring to the calibration curve or by sample/equivalent reference substance direct matching.

3.4. Recovery test

To determine the accuracy of the method, recovery test was performed according to the Association of Official Analytical Chemists International—AOAC International [18].

3.5. Method validation

The method was validated by determination of the following parameters: linearity, range, precision, intermediate precision and accuracy, following the ICH and USP 23 recommendation for this applicability of the analysis [19,20].

4. Results and discussion

The BCG color reagent occurs in two acid–base forms ($pK_a = 4.66$) in weakly acidic aqueous solutions with the absorption maximum at 430 nm ($BCGH^-$ form) and 615 nm (BCG^{-2}) [11]. When this triarylmethane dye complexes with etidocaine forms ion-pair in acetate buffer solution at pH 4.6 and develops a yellow color solution that exhibits absorbance maximum at 410 nm and when in basic medium change to blue color solution with maximum absorbance at 625 nm, which are soluble in chloroform.

A stability study of the colored ion-pair complex was developed and showed that yellow color was stable up to 6 h. The stability study of the colored ion-pair complex was conducted based on the variation of absorbance versus time. No one absorbance decrease was showed up to these 6 h studied over the solutions of the calibration curves. The observation did not carry on because no more 2 h were necessary to complete this assay.

The monovalent anion ($BCGH^-$) forms the extractable complex, which in excess, is slightly

extracted from the aqueous phase, then a blank reagent in the same conditions carried out simultaneously is necessary.

The optimum pH was selected through 14 experiments performed at different buffers solutions with the following pH values: 0.53, 1.75, 3.88, 4.50, 5.00, 6.00, 7.03, 8.38, 9.10, 10.86, 11.60, 12.54, 13.03 and 13.45. Sodium acetate buffer solutions at 10 g/100 ml were prepared adjusting the selected pH with 0.5 N NaOH solution or glacial acetic acid.

The better range of pH is presented in the Fig. 1 and the selected pH to the experiments was 4.6, the point where there was the maximum absorption of the ion-pair complex at 625 nm.

Under the established conditions the standard calibration curves were linear and Beer's law was followed for EH in the concentration range 2.0–6.0 μg ml^{-1}, calculated in final solution. The results were used to obtain the regression equations, by the least squares method, and the data evaluated by ANOVA.

The representative linear equation for EH was: $Y = 0.1563X - 0.0567$ ($n = 4$, $r = 0.9986$).

The summary of statistical data for EH is presented in Table 1.

The method was validated by evaluation of the precision (intra-day) and the intermediate precision (inter-day). In the range concentration of 2.0–6.0 μg ml^{-1} the relative standard deviation (R.S.D.) on the absorbances from three replicate solutions were found to be between 1.89 and 2.95%. The intermediate precision was evaluated

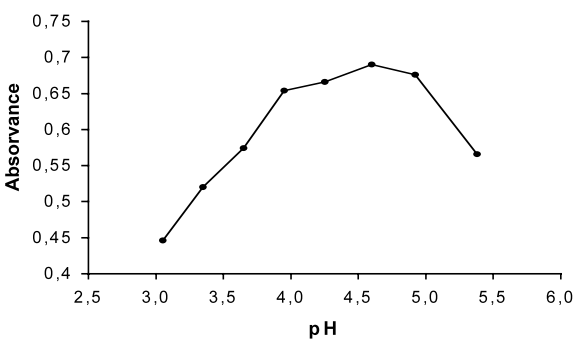

Fig. 1. Influence of the pH on the etidocaine–BCG complex extraction.

Table 1
Statistical data for calibration curves

Statistical parameters	EH (λ_{max} 625 nm)
Range (mg ml^{-1})	2.0–6.0
b	0.1563
a	0.0567
R.S.D. (%)	1.89–2.95
r	0.9986

Average of three determinations. *b*, angular coefficient (slope); *a*, intercept; R.S.D. ($n = 4\%$), relative standard deviation; *r*, Pearson's correlation coefficient.

by comparing the linear regressions of the four standard plots prepared on 4 different days. The R.S.D. of the slopes of the four lines was 0.73%. ANOVA of the date indicated no significant difference in slopes of the four calibrations curves ($P < 0.001$). The applicability and precision (repeatability) of this method were verified by applying to injectable dosage during the same day, at the same concentration and under the same experimental conditions for each one the samples evaluated. The recoveries were determined by adding known amounts of the EH reference substance (25.0, 50.0, 75.0 and 100.0 μg ml^{-1}) to the samples as described in Table 2. A recovery test was then performed.

The good recovery values (96.24–99.61%) showed through the recovery test and the precision presented by this procedure indicate that it was suitable for determination of EH in the formulation examined.

The coefficient of variation obtained to injectable dosage was 2.1% and indicated the precision of the method.

Table 2
Recovery results for EH in dental cartridges

Samples	Amount of standard (μg ml^{-1})		Recovery[a] (%)
	Added	Found	
R_1	25.0	24.45	98.20
R_2	50.0	48.12	96.24
R_3	75.0	74.71	99.61

[a] Average of three determinations.

Table 3
Statistical results obtained from quantitative analysis

Samples	Concentration (μg ml^{-1} of injectable solution)		Purity (%)	Mean ± confidence limits	R.S.D. (%)
	Theoretical	Experimental			
1	15.0	14.75	98.36		
2	15.0	14.61	97.39		
3	15.0	15.18	101.20	14.68 ± 1.40	2.13
4	15.0	14.41	96.10		
5	15.0	14.43	96.22		

Average of three determinations.

Table 3 presents the statistical results obtained from quantitative analysis by visible spectrophotometry by ion-pair with BCG.

This method does not have applicability to the stability pharmaceutical studies once that such study was not evaluated.

The detection limit presented was 0.002 μ ml^{-1} and the quantification limit was 1.0 μ ml^{-1}.

The advantage of the extractive spectrophotometric determination is that the method can be applied in the determination of a single compound in a mixture with possible interferences.

This fact is of major importance in analytical chemistry, since it offers another possibility in the assay of a specific component in a complex dosage formulation.

5. Conclusion

The presented study proposes a simple, inexpensive, precise and accurate method for the determination of EH in dental pharmaceutical preparation. The optimum reaction conditions were found for the determination of etidocaine with BCG. The method demonstrated acceptable linearity and accuracy and the results indicated that the proposed method might be recommended for routine analysis of EH in quality control of medicines.

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