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Simultaneous HPLC Determination of Lidocaine-Epinephrine-Tetracaine in a topical solution for pediatric anesthesia

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ABSTRACT

Background: A simple and rapid reversed phase-high performance liquid chromatographic method was developed for simultaneous determination of lidocaine, epinephrine and tetracaine in specific pharmaceutical children anaesthetic solution. **Method:** The elution was done with a mobile phase gradient composed of methanol:phosphate buffer(v:v) on Chromosil C18 column (4 × 150 mm, 3 µm particle size). The gradient elution started with 40% (V/V) methanol, ramped up linearly to 55% in 8 min, then kept constant until the end of the run. The mobile phase was pumped at a flow rate of 0.8 mL/min. The wavelength detector was set at 254 nm for lidocaine and tetracaine and 220 nm for epinephrine. **Results and discussion:** Retention times for lidocaine, epinephrine and tetracaine were around 3.058 min, 1.867 min and 6.917 min respectively. The reliability and analytical performance of the proposed HPLC procedure were statistically validated according to the respect of linearity, ranges, precision, accuracy, repeatibility, reproducibility, detection and quantification limits. Linear ranges were establish between 1,2-2,8mg/mL for lidocaine (r^2=0,9973), 15-35µg/mL for epinephrine (r^2= 0,9858) and 150-350µg/mL for tetracaine (r^2=0,9968). **Conclusion:** This is the first time that a study combining simultaneous routinely determination of these three compounds has been performed in lidocaine, epinephrine and tetracaine paediatric anaesthetic solution.

KEYWORDS: Lidocaine, Epinephrine, Tetracaine, RP-HPLC, anaesthetic solution, children, validation method.

1. INTRODUCTION

With the increased interest in the management of pain, many studies have shown the effectiveness of LET (lidocaine-epinephrine-tetracaine)solution for eliminating or reducing the pain involved in skin lacerations[1-2-3].

Anaesthetics are known to be painful when they are injected. That's why a topical anaesthesic solution has been developed. Components are lidocaine(4%), epinephrine(0,05%) and tetracaine(0,5%).The use of LET both in gel or in solution in pediatric patients is a widespread practice since many years both for minor surgery [4].

Lidocaine, 2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide is alocal anaesthetic and used for topical treatment to relieve itching, burning, and pain from skin inflammations (fig.1).

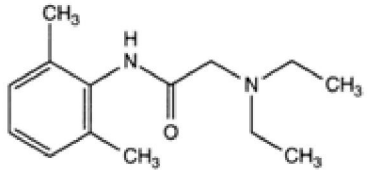

Fig. 1.*Chemical structure of lidocaine*

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Epinephrine, 4-[(1R)-1-hydroxy-2- (methylamino)ethyl]benzene-1,2-diolis used here as a vasoconstrictor to slow the absorption of lidocaine and tetracaine, and extend the action of these anaesthetic agents. The vasoconstricting effect of epinephrineis required to stop bleeding (fig.2).

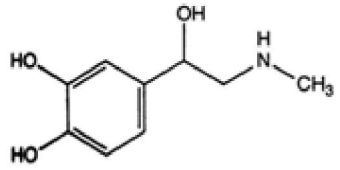

Fig. 2 .*Chemical structure of lidocaine epinephrine*

Tetracaine,2(dimethylamino)-ethyl-4(butylamino)benzoate is a local anaesthetic of the ester-linkage type, used in combination with lidocaine to boost its anaesthetic action (fig.3).

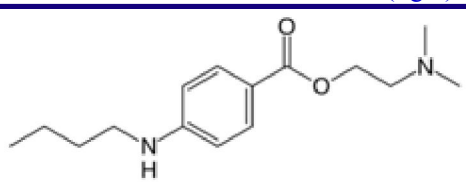

Fig. 3. *Chemical structure of tetracaine*

The presentstudy's purpose is to develop an assay for simultaneous analysis of these three actives drugs. The present method is the first one that determine simultaneousquantitativeanalysis these three anaesthetic agents.

2. MATERIALS AND METHODS

2.1. Instruments

Chromatographic separation was performed using a Thermo Scientific Dionex UltiMate 3000 HPLC system (Germering, Germany) equipped with aDAD-3000(RS) diode array detector and MWD-3000(RS). A METTLER TOLEDO®-Seven multi pH-meter and aXP205 DR/M METTLER TOLEDO® precision scale, were used. A Chromosil C18 column (4 × 150 mm, 3 µm particle size) from Thermo Scientific Inc.,Germering in Germany was used.

2.2. Reagents and materials

All chemicals substances were of analytical reagent grade and solvents were of HPLC grade. Lidocaine hydrochloride, tetracaine hydrochloride and epinephrine tartrate weresupplied by Caelo Co. Acetonitrile and methanol were of HPLC –grade (Sigma-Aldrich Co., Munich, Germany). Potassium dihydrogen phosphate, and di-potassium hydrogen phosphate anhydrous, were obtained from Sigma-Aldrich Co., Munich, Germany. Orthophosphoric acid (85%, v/v) was obtained from Sigma-Aldrich Co., Munich, Germany.

2.2. Chromatographic conditions

Chromatographic separation was performedon a Chromosil C18 column (4 × 150 mm, 3 µm particle size) from Thermo Scientific Inc. A gradient mobile phase involvingmethanol andpotassium dihydrogen phosphate buffer was adjusted to 3.8 pH with orthophosphoric acid 85%. The linear gradient elution started with 40% (V/V) methanol, ramped up linearly to 55% in 8 min.After 8min the gradient program was returned to the initial conditions.

The flow rate was 0,8 mL min^{-1} and the column temperature was maintained at 35°C.The volume of injection was 10 µl. The UV detector was set up at 254 nmfor lidocaine and tetracaine and at 220 nm for epinephrine.

2.3. Preparation of buffer

Potassium dihydrogen phosphate buffer (pH 3.8) was prepared with 2.45 g of potassium dihydrogen phosphate monobasic and 2.10 g of di-potassium hydrogen phosphate anhydrous in a suitable amount of water and diluting to 1000 mL with the same solvent. The pH of the mixture was adjusted to 3.8 with orthophosphoric acid 85%.

2.4 Paediatric LET solution composition

The topical solution consists of 4% lidocaine hydrochloride, 0.09% epinephrine tartrate (0.09%epinephrine tartrate = 0.5% epinephrine base), 0.5% tetracaine and 0.075% sodium metabisulfite was added as preservative agent for prevents epinephrine from oxidation.

2.5. Preparation of calibration solutions

Pipette out 300, 400, 500, 600, 700µL from stock solution into 10 mL volumetric flasks and volume was made up to 10 mL using water as diluent. The stock solution is composed of 4% lidocaine hydrochloride, 0.09% epinephrine tartrate, 0.5% tetracaine and 0.075% sodium metabisulfite.

2.6. Concentration range:

-1,2mg/mL - 2,8mg/mL for lidocaine: 1,2 - 1,6 - 2,0 - 2,4 - 2,8mg/mL-150µg/mL - 350µg/mL for tetracaine: 150 - 200 - 250 - 300 – 350mg/mL-15µg/mL - 35µg/mL for epinephrine: 15 - 20 - 25 - 30 – 35mg/mL. For each concentration range, a zero concentration calibration point was tested.

2.7. Method validation

The proposed method was validated according to the ICH guidelines[5]. The method validation is determined by preparingevery day during three daystwo ranges of solutions and six solution's controls. Each preparation is made with independant weighing. One rangewasprepared with metabisulfite and the other without. At the same time we prepared sixcontrols at 2mg/mL for lidocaine; 250 mg/mL for tetracaine and 25mg/mL for epinephrine.

The peak areas were used for determining the linearity, precision, accuracy, repeatability and specificity. The limit of detection and limit of quantification were determined graphically.

3. RESULTS AND DISCUSSION

3.1. Development and optimization of chromatographic method

The aim of this work was todevelop an easy method to perform and rapid reversed phase-high performance liquid chromatographic assay for simultaneous determination of lidocaine, epinephrine and tetracaine in pharmaceutical children anaesthetic solution.

Different mobile phases consisting ofacetonitrile, methanol and potassium dihydrogen phosphate solution (buffer) mixturewere tested in isocratic condition. However peak resolution, retention time and tailing factor were not satisfying for the three molecules. This is the reason why a gradient consisting of methanol:buffer phosphate potassium(v:v) was chosen.

We also had tested this mobile phase on different Chromosil columns, C18 and C8. The Chomosil C18 column (4 × 150 mm, 3 µm particle size) was chosen to be the most appropriate for separated epinephrine and lidocaine.

We selected 220 nm as the bestwavelenght to for epinephrine because at 254 nm peak areas of epinephrine (0,05%)are nearlyundetectable in contrast to lidocaine (4%) (Fig.4).

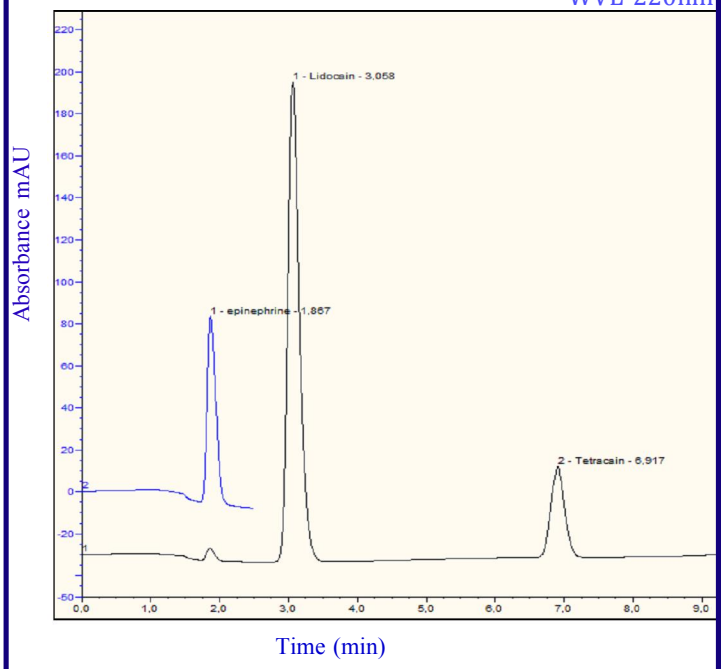

Fig. 4 .Standard chromatogram of lidocaine-epinephrine-tetracaine

3.2. Linearity range

The linearity range was in the interval of 1,2-2,8mg/mL for lidocaine, 15-35µg/mL for epinephrineand 150-350µg/mL for tetracaine. These results were represented by the following linear regression equation: y(lidocaine)=18,88x+0,69 (r2=0,9973), y(epinephrine)=0,44x+0,19 (r^2= 0,9858) and y(tetracaine)=0,034x+0,0094 (r2=0,9968).Linearity was proved (Table 1 and fig.5-6-7).

Table 1. Determination of linearity

Parametrer (with sodiummetabisulfite)	Lidocaine	Epinephrine	Tetracaine
Concentration range µg/mL	1200 - 2800	15 - 35	150 - 350
Regression equation	18.88x+0,69	0.44x+0.19	0.03x+0.01
Correlation coefficient/r	r^2=0.9973	r^2= 0.9858	r^2= 0.9968

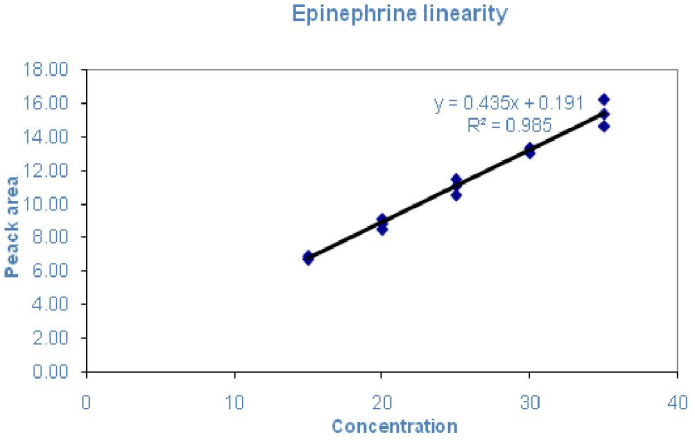

Fig.5. Determination of epinephrine linearity

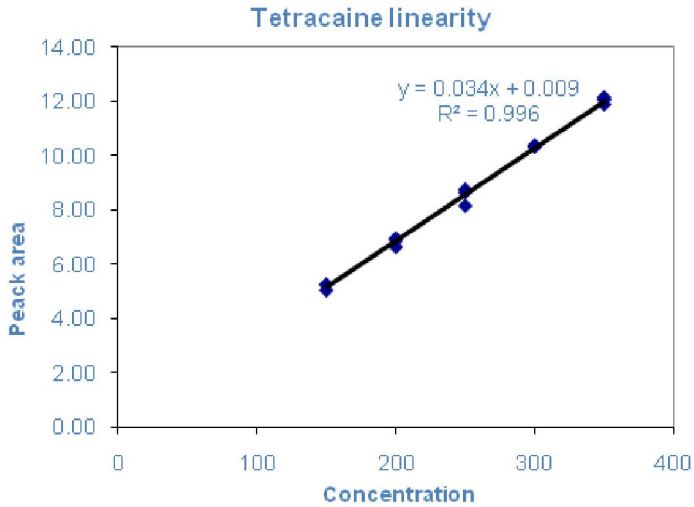

Fig.6. Determination of tetracaine linearity

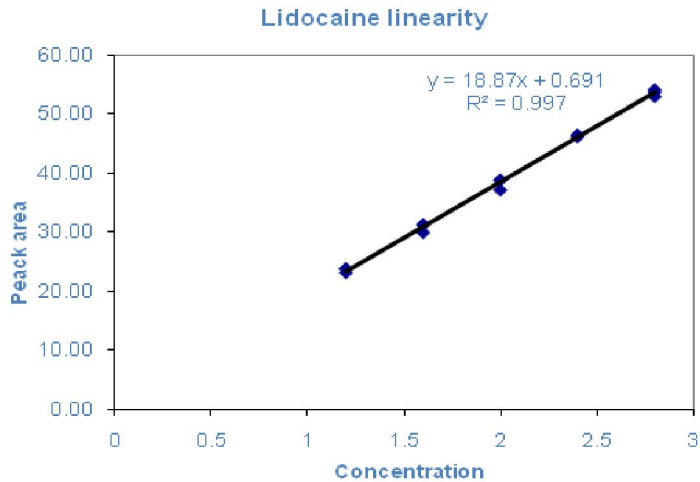

Fig 7. Determination of lidocaine linearity

3.3. Precision

Six controls at the same concentration were analyzed every days during three days. The repeatability and the intermediate precision werecalculated. The variation in the precision and the percentage RSD(relative standard deviation)for lidocaine, epinephrine and tetracaine have an acceptable limit <5%. It can be concluded that the proposed method is precise (Table 2).

Table 2. Determination of repeatibility and intermediate precision

(with sodium metabisulfite)	Repeatability Variance	Standard deviation	% RSD
Lidocaine	0.06	0.25	0.64
Epinephrine	0.12	0.34	3.1
Tetracaine	0.004	0.06	0.71
(with sodium metabisulfite)	**Intermediate precision Variance**	**Standard deviation**	**% RSD**
Lidocaine	0.13	0.36	0.92
Epinephrine	0.23	0.48	4.36
Tetracaine	0.01	0.11	1.29

3.4. Accuracy

The percentage recoveries for lidocaine, epinephrine and tetracaine found were 100,03% - 99,99% - 99,96% respectively. This results demonstrate the accuracy of the proposed method (Table 3).

Table 3. Determination of accuracy

(with sodiummetabisulfite)	Lidocaine	Epinephrine	Tetracaine
Average	100.03	99.99	99.96
Standard deviation	0.8963	1.436	1.1861
%RSD	0.896	1.4361	1.1866

3.5. Specificity

During three days, we realized daily two ranges for each analyte, one with sodium metabisulfite (preservative agent) and the other without. We compared the slopes and the y-intercept from the regression lines of each ranges (Table 4). These results show sodium metabisulfite don't interfere with the method.

Table 4. Determination of specificity (Student with 26 ddl, risk 5%. T.student must be under 2.056 to prove the absence of difference)

	Lidocaine		Epinephrine		Tetracaine	
Sodium metabisulfite	+	-	+	-	+	-
Slope	18.88	19.14	0 .44	0.46	0.03	0.03
y-intercept	0.69	0.52	0.19	0.61	0.01	0.05
T.student (Slope)	0.78		1.38		1.08	
	,8960		,8960		,8960	
T.student (y-intercept)	0.01		0.39		1.07	

4. CONCLUSION

A new, simple, rapid high-performance liquid chromatographic method was developed for simultaneous analysis of lidocaine, epinephrine and tetracaine in pharmaceutical children anaesthetic solution. The present method was sensitive enough to detect the three components and can be applied in routine to control this frequently compounded formulation.

5. DISCLOSURE OF INTEREST

The authors declare no conflicts of interest concerning this article.

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