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Short communication

Simultaneous determination of lidocaine hydrochloride, hydrocortisone and nystatin in a pharmaceutical preparation by RP-LC

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ABSTRACT

A liquid chromatographic (LC) method was developed to analyze a formulation (mouthwash) containing lidocaine hydrochloride, hydrocortisone and nystatin. A single LC method with UV detection was developed. A Waters Symmetry C18 HPLC column (150 mm × 4.6 mm, 5 μm) was used as stationary phase and the assay was performed with gradient elution using mobile phases containing methanol – 0.1 M NaH_2PO_4 with a pH that was previously adjusted to 4.5 with dilute phosphoric acid. The sample pretreatment was performed by treating the formulation with methanol followed by filtration. After method development, the influence of the different chromatographic parameters on the separation, the interference of other active compounds and excipients, linearity, accuracy, repeatability and intermediate precision were investigated. The method was shown to be selective, linear, accurate, precise and repeatable. Finally, the content of the compounds in the formulation was determined.

1. Introduction

Corticosteroids have been widely used as anti-inflammatory drugs in medicine. Nowadays, pharmaceutical products contain corticosteroids in combination with antibacterials and local anaesthetics since corticosteroids do not cure the fundamental cause. Hydrocortisone (Fig. 1a) is a human glucocorticosteroid which is often associated with nystatin and oxytetracycline [1]. Illnesses related to the respiratory system, such as tonsillitis, pharyngitis and laryngitis are usually treated with hydrocortisone–lidocaine combinations [2].

Lidocaine hydrochloride (Fig. 1b), as a local anesthetic drug, reversibly inhibits nerve impulse transmission. It has a good superficial activity, penetrates in depth through the mucous membranes and reduces the sensation of pain [3].

Nystatin (Fig. 1c) is a macrocyclic lactone consisting of a hydroxylated tetraene diene backbone and a mycosamine residue. It is a polyene antifungal antibiotic that is of particular interest because it exhibits remarkable action against a wide range of pathogenic and non-pathogenic yeasts and fungi [4,5]. Nystatin exerts both a fungistatic and fungicidal action against *Candida albicans*. For the treatment of oral candidiasis, this drug is administered in either suspension or gel dosage forms [6].

Several analytical methods have been described in literature for the analysis of lidocaine, hydrocortisone, nystatin and/or the combination of APIs in pharmaceutical preparations. A micellar electrokinetic chromatographic (MEKC) method was described for quantification of hydrocortisone and its most important associated compounds together with nystatin, oxytetracycline, Zn-bacitracin, polymyxin B, and lidocaine in ocular and cutaneous pharmaceutical products [7]. Sarrafi et al. [8] described the simultaneous spectroscopic determination of lidocaine and hydrocortisone acetate in formulations by multivariate calibration methods. Baratieri et al. [9] reported a multivariate method of analysis of nystatin and metronidazole in a semi-solid matrix, based on diffuse reflectance NIR measurements and partial least squares regression. LC was used to separate a mixture of lidocaine and hydrocortisone acetate in different pharmaceutical preparations [10–13]. In 2002, Lemus Gallego et al. published two relevant articles. One described a LC method for analysis of hydrocortisone and lidocaine in pharmaceutical preparations [4] while another described the simultaneous determination of hydrocortisone, oxytetracycline and nystatin [1]. A LC method for evaluating the stability of nystatin (Nys) in an ointment and a capillary electrophoresis method for its analysis in an oily suspension were developed [14,15]. Moreover, LC methods for the analysis of lidocaine hydrochloride in suppositories, ointment and in injectables have been reported [3,16].

A number of analytical procedures have been described in literature for the determination of nystatin in urine, blood, tissues and saliva [5,6,17,18]. A few LC methods are available to measure nystatin plasma concentrations after parenteral administration in animals [16,19]. Hydrocortisone has been determined in plasma and suppositories by LC [20–23].

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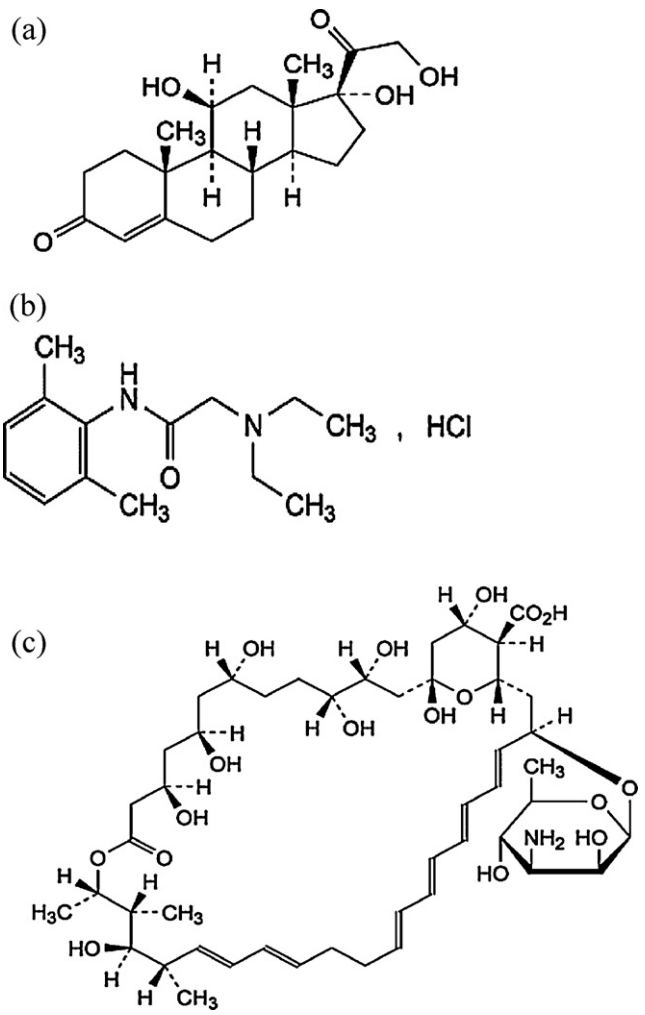

Fig. 1. Chemical structure of (a) hydrocortisone, (b) lidocaine hydrochloride and (c) nystatin.

To our knowledge, no LC method has been reported for the simultaneous determination of lidocaine, hydrocortisone and nystatin in a single formulation. In this study, a reversed phase LC method was optimized and validated for the simultaneous determination of lidocaine hydrochloride, hydrocortisone and nystatin in a pharmaceutical preparation used as mouthwash.

2. Materials and methods

2.1. Reagents and samples

Hipersolv chromanorm methanol for HPLC was purchased from Acros Organics (Geel, Belgium) and disodium hydrogen phosphate and phosphoric acid were obtained from Merck (Darmstadt, Germany). A Milli-Q water purification system from Millipore Bedford (MA, U.S.A.) was used to purify demi-water. The pharmaceutical formulation containing lidocaine hydrochloride, hydrocortisone, nystatin and its excipients (Table 1) was prepared according to the prescriptions of the Therapeutic Magistral Formulary [24]. All active substances were purchased from ABC chemicals (Maiden Newton, U.K.).

2.2. Instrumentation

The LC apparatus consisted of a LC Pump (Waters 600E, Milford, MA, U.S.A.), a LC autosampler (Spectra Physics AS 3000, Santa Clara, CA, U.S.A.) and a UV detector (Thermo Separation Products Spectra 100, U.S.A.). The experiments were performed at room temperature. Data acquisition was supported by a Chromeleon chromatography data system version 6.60 (Dionex Corporation, Sunnyvale, CA, U.S.A.). Chromatographic separations were achieved on a C-18 (150 mm × 4.6 mm, 5 μm) Waters Symmetry column (Waters Corporation, MA, U.S.A.). The pH measurements were performed on a 691 Metrohm pH meter (Herisau, Switzerland).

Table 1
Composition of the mouthwash.

Nystatin	0.346 g
Hydrocortisone	0.200 g
Lidocaine hydrochloride	0.400 g
Hydroxypropylmethylcellulose 4000	5.0 g
Glycerol	7.5 g
Peppermint oil	50 mg
Ethanol (96%)	4.0 g
Aqua conservans q.s. ad	500 g

2.3. Chromatographic conditions

Mobile phase A consisted of methanol – 0.1 M NaH_2PO_4 (60:40) of which the pH was previously adjusted to 4.5 with dilute phosphoric acid. Mobile phase B consisted of methanol – 0.1 M NaH_2PO_4 (70:30) with a pH that was also previously adjusted to 4.5 with dilute phosphoric acid. Both mobile phases were degassed by sparging with helium for 2 min. A gradient program [time (min)/%B] set as 0/0, 6/0, 6.1/100, 10/100, 10.1/0, 15/0 was applied. The Waters Symmetry C18 LC column was kept at room temperature. The flow rate was 1.0 ml/min and the injection volume was 10 μl. The UV detector was set at a wavelength of 230 nm.

2.4. Sample preparation

Test solution. After shaking the suspension well, 2.0 g of the suspension, corresponding to 1.6 mg of lidocaine hydrochloride, 0.8 mg of hydrocortisone and 1.4 mg of nystatin, was mixed with 5 ml of methanol and diluted to 10.0 ml with the same solvent.

Standard solution. 32 mg of lidocaine hydrochloride, 16 mg of hydrocortisone and 28 mg of nystatin were weighed in a 200.0 ml volumetric flask and dissolved in 50 ml of mobile phase A. The solution was made up to 200.0 ml with the same solvent.

3. Results and discussion

3.1. Optimization

As starting point, the methods described by Lemus Gallego et al. were applied [1,4] using a LichroCART-18e C18 (125 mm × 4.6 mm, 5 μm) column. Two problems were observed when the sample solution was injected: (1) interference of the excipient's peak with the lidocaine hydrochloride peak and (2) the nystatin peak could not be well integrated due to poor peak shape. Hence, further optimization was necessary.

In order to avoid interference between the lidocaine hydrochloride and the excipients, another column was applied. Instead of the LichroCART-18e C18 column (125 mm × 4.6 mm, 5 μm) a Waters Symmetry C18-column (150 mm × 4.6 mm, 5 μm) was tested. As all peaks were well separated with this new column, it was used for all further experiments. Using the mobile phase described by Lemus Gallego et al. [1,4], the nystatin peak was eluted after 30 min. Increasing the amount of methanol in the mobile phase caused coelution of lidocaine with the excipients. Consequently, a gradient program as described in Section 2.3 was used. A typical chromatogram obtained using this gradient is shown in Fig. 2. The

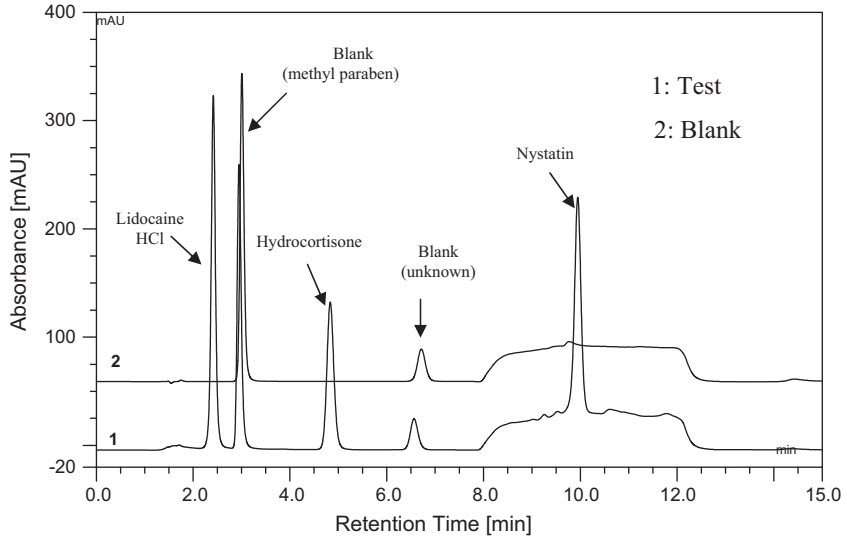

Fig. 2. Typical chromatogram obtained with the final method overlaid with the blank (mobile phase A: methanol – 0.1 M NaH_2PO_4 (60:40) pH 4.5, mobile phase B: methanol – 0.1 M NaH_2PO_4 (70:30) pH 4.5, stationary phase: Waters Symmetry C18-column (4.6 mm × 150 mm, 5 μm).

Table 2
Sensitivity, repeatability and linearity data.

	LOD	LOQ	Repeatability	Linearity			
	(ng)	(ng)	(n = 6, RSD)	Range (%)	R^2	y	$S_{y,x}$
Lidocaine HCl	0.09	0.31	0.05	25–125	>0.999	$0.30x + 0.78$	0.29
Hydrocortisone	0.15	0.46	0.2	25–125	>0.999	$0.22x + 0.43$	0.20
Nystatin	0.27	0.81	0.2	25–125	>0.999	$0.29x + 0.20$	0.41

LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard deviation; range: percentage range studied; n: number of injections; R^2: coefficient of determination; y: peak area; x: concentration (%); $S_{y,x}$: standard error of estimate.

baseline disturbance between 8 and 12 min was also noticed when only solvent was injected and so can be attributed to the change in mobile phase composition due to the gradient. As this formulation consisted of three active compounds, one wavelength at which all compounds have a reasonable absorption had to be found. So, a detector wavelength of 230 nm was selected. No further changes were necessary to obtain a good separation and good peak shapes in a total run time of 15 min. The final conditions are described in detail in Section 2.3.

3.2. Validation

3.2.1. Interference

The developed method was tested to determine lidocaine HCl, hydrocortisone and nystatin without interference of other components in the chromatogram. To see if the excipients have any absorption maxima at 230 nm, the excipients only (=blank) and the excipients with lidocaine HCl, hydrocortisone and nystatin references (=test) were injected and the obtained chromatograms were compared to each other. In Fig. 2, the resulting chromatograms are shown. The excipients gave 2 peaks, the first one corresponding to methyl paraben. As they are well separated from the peaks of the active compounds, there is no interference between the blank and either of the active compounds.

3.2.2. Linearity and repeatability

The linearity of the detector response was examined for the 3 active compounds present in the mouthwash. Five different concentrations in a range of 25–125% were injected in triplicate (100% solution containing 0.16 mg/ml lidocaine HCl, 0.08 mg/ml hydrocortisone and 0.14 mg/ml nystatin). For all the active compounds, the coefficients of determination (R^2 values) were above 0.999 and proved that the method was linear in the specified range.

Repeatability was checked by injecting the 100% test solution six times. The % R.S.D. value was calculated for each active compound. Linearity and repeatability results are shown in Table 3.

Table 3
Results of the intermediate precision study.

	Assay (%)					
	Hydrocortisone	R.S.D. (n = 6)	Lidocaine hydrochloride	R.S.D. (n = 6)	Nystatin	R.S.D. (n = 6)
Day 1	101.5	0.3	97.8	0.5	98.5	1.1
Day 2	101.3	0.1	99.4	1.2	100.0	1.2
Day 3	101.1	0.7	98.1	0.7	100.5	0.4
Mean	101.3		98.4		99.7	
R.S.D. (n = 18)	0.2		0.9		1.0	
Day 4	100.2	0.4	99.0	0.5	101.2	0.6
Days (3–4)	100.7		98.6		100.1	
R.S.D. (n = 12)	0.6		0.8		1.1	

Table 4
Results of recovery test for mouthwash.

Level (%)	% Recovery ($n = 3$, RSD)		
	Lidocaine HCl	Hydrocortisone	Nystatin
80	99.2 (0.1%)	98.0 (0.1%)	100.4 (0.3%)
100	100.7 (0.4%)	98.4 (0.1%)	100.5 (0.4%)
120	98.9 (0.2%)	100.0 (0.05%)	101.0 (0.4%)

3.2.3. Intermediate precision

Intermediate precision of the method was evaluated by assaying the test preparation (100%) on four consecutive days by three analysts using the same experimental conditions. On the fourth day, analysis was made on a different instrument and those results were compared with day 3. On each day, two freshly prepared test solutions and their references were injected in triplicate. The results are shown in Table 3. The coefficients of variation within and between were less than 2.0% suggesting that the results were precise for the experimental variations studied.

3.2.4. Sensitivity and recovery test

The limit of detection and quantification was evaluated on the basis of the signal-to-noise (S/N) ratio. Values found for the limits of detection (S/N = 3) and quantification (S/N = 10) are shown in Table 2.

As shown in Table 4, the recovery test for the mouthwash was performed in a concentration range of 80–120% (100% solution, containing 0.16 mg/ml lidocaine HCl, 0.08 mg/ml hydrocortisone and 0.14 mg/ml nystatin) and each solution was injected three times. The results for the recovered percentages were within the range of 98–102%.

3.2.5. Assay

In order to determine the contents of the active compounds in the mouthwash, samples and references were prepared in duplicate and injected three times. The results were compared to the 100% lidocaine HCl, hydrocortisone and nystatin references. An average content of 101.4% (RSD = 0.3%, $n = 6$) of the label claim was found for lidocaine HCl, 99.6% m/m (RSD = 0.4%, $n = 6$) for hydrocortisone and 100.5% (RSD = 0.3%, $n = 6$) for nystatin.

4. Conclusion

A single LC–UV method was described for the quantitation of 3 active compounds (lidocaine HCl, hydrocortisone and nystatin) in a pharmaceutical formulation. Validation tests were performed and showed that the method is linear, repeatable, precise, accurate, selective and sensitive. Hence, it was possible to quantify lidocaine HCl, hydrocortisone and nystatin simultaneously.

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