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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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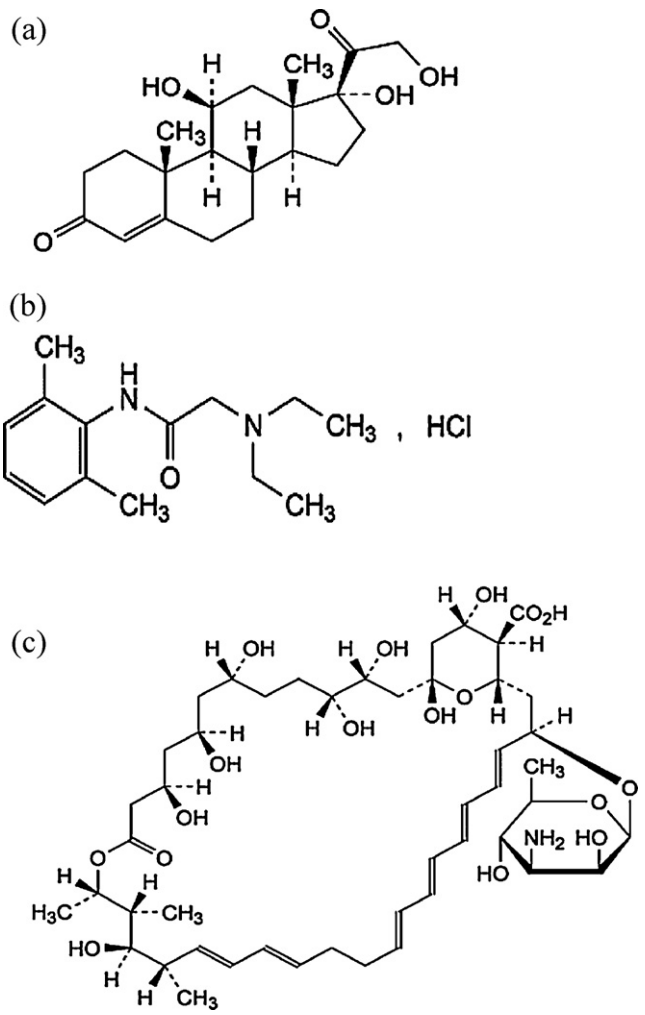

Fig. 1. Chemical structure of (a) hydrocortisone, (b) lidocaine hydrochloride and (c) nystatin.

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