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Simultaneous determination of chloramphenicol, dexamethasone and naphazoline in ternary and quaternary mixtures by RP-HPLC, derivative and wavelet transforms of UV ratio spectra

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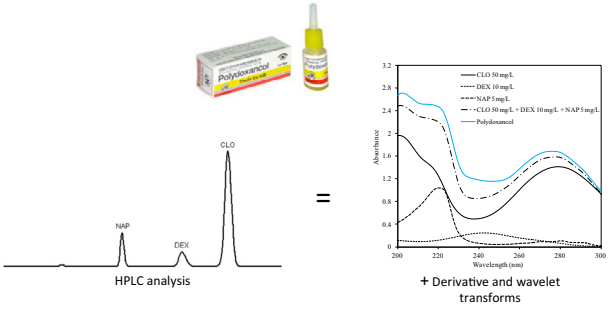
HIGHLIGHTS

- UV spectra of ternary mixtures can be resolved by derivative and wavelet transforms.
- Double signal transform of UV ratio spectra is comparable to RP-HPLC.
- Spectrophotometric methods are preferable to RP-HPLC.

GRAPHICAL ABSTRACT



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ABSTRACT

The application of chemometrics-assisted UV spectrophotometry and RP-HPLC to the simultaneous determination of chloramphenicol, dexamethasone and naphazoline in ternary and quaternary mixtures is presented. The spectrophotometric procedure is based on the first-order derivative and wavelet transforms of ratio spectra using single, double and successive divisors. The ratio spectra were differentiated and smoothed using Savitzky–Golay filter; whereas wavelet transform realized with wavelet functions (i.e. db6, gaus5 and coif3) to obtain highest spectral recoveries. For the RP-HPLC procedure, the separation was achieved on a ZORBAX SB-C18 (150 × 4.6 mm; 5 μm) column at ambient temperature and the total run time was less than 7 min. A mixture of acetonitrile – 25 mM phosphate buffer pH 3 (27:73, v/v) was used as the mobile phase at a flow rate of 1.0 mL/min and the effluent monitored by measuring absorbance at 220 nm. Calibration graphs were established in the range 20–70 mg/L for chloramphenicol, 6–14 mg/L for dexamethasone and 3–8 mg/L for naphazoline (R^2 > 0.990). The RP-HPLC and ratio spectra transformed by a combination of derivative–wavelet algorithms proved to be able to successfully determine all analytes in commercial eye drop formulations without sample matrix interference (mean percent recoveries, 97.4–104.3%).

Introduction

Bacterial eye infections can present in several ways, ranging from mild, self-limiting conditions to visually devastating consequences. But, the proper use of antibiotic-steroid combination

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can protect patients' sight. Chloramphenicol (Fig. 1A) is considered as a prototypical broad-spectrum antibiotic that stops the growth of bacteria by inhibiting protein synthesis. In topical ocular formulations, its bacteriostatic effect for conjunctivitis treatment is further enhanced when being co-formulated with dexamethasone (Fig. 1B), an anti-inflammatory corticosteroid, and naphazoline (Fig. 1C), an alpha receptor agonist used to relieve redness, puffiness, and itchy/watering eyes due to colds, allergies, or eye irritations. Sometimes, vitamin B_2 is also added to this co-formulation because the deficiency of this nutrient can lead to inflamed eyelids and sensitivity to light.

In the analysis of ternary and quaternary mixtures, chromatographic techniques (such as HPLC, GC, CE) coupled with UV–Vis, MS or NMR detection are usually the methods of choice for the quality control and routine analysis of a commercial product in laboratories. Nevertheless, these methods are costly and time consuming due to sophisticated instruments used and a possible prior step required such as derivatization, extraction and other tedious analytical process. Taking into account this, derivative ratio spectrophotometry has been reported in the literature to simultaneously determine active compounds in ternary mixtures without any chemical and/or separation pretreatment [1–9]. These spectrophotometric methods are found to be preferable to conventional UV–Vis spectrophotometry, which uses a discrete number of wavelengths and is not able to extract enough analytical information from a system with severe spectra overlapping.

In the last decade, the development and application of wavelet transform as a promising tool to solve UV spectral overlapping of ternary and quaternary mixtures has been exemplified in the field of analytical chemistry [10–12]. Using this approach, the spectral signal can be decomposed and translated into a time–frequency domain through the translation and dilation operations according to a set of functions called wavelet. Wavelet signals are better than derivative ones in some cases such as (i) the higher order differentiation process diminish peak amplitude as well as signal-to-noise ratio; (ii) the finding of zero-crossing points is very difficult and ratio spectra derivative working wavelength is undetermined.

The aim of this study was to develop derivative- and wavelet-transformed UV ratio spectrophotometric methods for the simultaneous determination of chloramphenicol, dexamethasone and naphazoline in ternary and quaternary mixture formulations using Reversed Phase High Performance Liquid Chromatogram (RP-HPLC) as a reference method. It is noticed that although the determination of chloramphenicol was chromatographically studied [13–15], no analytical procedure has been reported so far in well-known pharmacopoeias as well as in literature for these compounds in multicomponent mixtures by both HPLC and spectrophotometric methods. This study especially stressed on exploiting the advantages of a combination of derivative and wavelet transforms.

Experimental

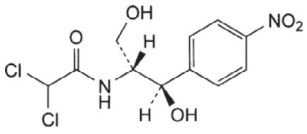
Apparatus and software

Absorption spectra were registered and treated by using a UNICAM UV 300 double beam spectrophotometer (Thermo Spectronic, USA) with a fixed slit width (1.5 nm) connected to an IBM computer loaded with Thermo Spectronic VISION32 software and 1-cm quartz cells. The zero-order spectra were recorded in the wavelength range of 190–325 nm against a blank (water) at Intelliscan mode to enhance the signal-to-noise ratio of absorbance peaks without extended scan duration with a $\Delta\lambda = 0.1$ nm (i.e. 30–120 nm/min). For derivative approach, the spectra were differentiated and smoothed by using Savitzky–Golay filter. For wavelet approach, the data treatment was done using MATLAB R2013a software (The MathWorks, Natick, MA, USA).

RP-HPLC analysis was performed on an Agilent 1100 Series Diode-Array-Detector chromatograph (Agilent Technologies, USA). A ZORBAX SB-C18 (150 × 4.6 mm; 5 μm) column was used. All solutions were filtered through a 0.45 μm membrane filter before injection into the chromatograph. All buffers were filtered through a 0.45 μm Millipore filter and degassed in an ultrasonic bath.

Reagents and standard solutions

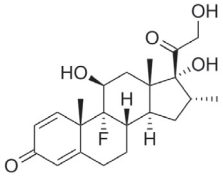
Chloramphenicol (CLO), dexamethasone sodium phosphate (DEX) and naphazoline nitrate (NAP) were kindly provided by the National Institute of Drug Quality Control (Vietnam). De-ionized doubly distilled water was used throughout as spectrophotometric solvent. All reagents were of analytical grade. Stock solutions of CLO, DEX and NAP (1000 mg/L) were freshly made in water. A concentration set of standard solutions were prepared in 50-mL volumetric flasks by using the same stock solutions.



(A) Chloramphenicol:

2,2-Dichloro-N-[1,3-dihydroxy-1-(4-

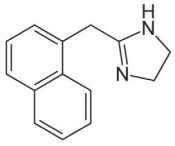
nitrophenyl)-2-propanyl]acetamide



(B) Dexamethasone:

(11β,16α)-9-Fluoro-11,17,21-trihydroxy-16-

methylpregna-1,4-diene-3,20-dione



(C) Naphazoline:

2-(1-Naphthylmethyl)-4,5-dihydro-1H-

imidazole

Fig. 1. Chemical structures and IUPAC names of chloramphenicol (A), dexamethasone (B) and naphazoline (C).