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Application and validation of chemometrics-assisted spectrophotometry and liquid chromatography for the simultaneous determination of six-component pharmaceuticals

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Abstract

Three methods are developed for the simultaneous determination of theophylline anhydrous (TH), guaiphenesin (GP), diphenhydramine hydrochloride (DP), methylparaben (MP), propylparaben (PP) and sodium benzoate (BZ) in pharmaceutical syrup. The chromatographic method depends on a high performance liquid chromatographic separation on a reversed-phase C_{18} column at ambient temperature with mobile phase consisting of 25 mM KH_2PO_4, pH 3.2—acetonitrile (60:40, v/v). Quantitation was achieved with UV detection at 222 nm based on peak area. The other two chemometric methods applied were partial least squares (PLS-1) and principal component regression (PCR). These approaches were successfully applied to quantify the six components in the studied mixture using information included in the UV absorption spectra of appropriate solutions in the wavelength range of 220–270 nm with $\Delta\lambda = 0.4$ nm. The calibration PLS-1 and PCR models were evaluated by internal validation (prediction of compounds in its own designed training set of calibration), by cross-validation (obtaining statistical parameters that show the efficiency for a calibration fit model) and by external validation over synthetic and pharmaceutical preparation. The results of PLS-1 and PCR methods were compared with the HPLC method and a good agreement was found.

Keywords: Theophylline; Guaiphenesin; Diphenhydramine; Methylparaben; Propylparaben; Sodium benzoate

1. Introduction

Theophylline anhydrous (TH) is a xanthine bronchodilator, which is associated with guaiphenesin (GP), an expectorant and diphenhydramine hydrochloride (DP), an antitussive, antihistaminic and anticholinergic, in addition to methylparaben (MP), propylparaben (PP) and sodium benzoate (BZ), which are used as preservatives. This combination is used for treating acute chronic bronchitis. The UV absorption spectra of TH, GP, DP, MP, PP and BZ display considerable overlap, where the application of the conventional spectrophotometry and its direct derivative and derivative ratio technique failed to resolve it. No analytical method has been reported for the simultaneous determination of TH, GP, DP, MP, PP and BZ in a multicomponent mixture. Several analytical methods have been reported for the determination of TH or GP or DP or MP or PP or BZ in combination with other drugs, including, HPLC [1–21], micellar electrokienetic chromatography (MEKC) [22], spectrophotometry [23], HPLC-densitometry [24], TLC [25] and capillary electrophoresis [26,27].

The utility of chemometrics-assisted spectrophotometry based on PLS for multidetermination of drug combinations has been published for determination of TH with dyphylline and proxyphylline [28]; DP with MP, phenylephrine and naphazoline [29]; DP with phenylpropanolamine and paracetamol [30]. The five-component mixture of GP, acetaminophen, ρ-aminophenol, caffeine and chlorphenamine was determined using PLS [31] and PCR [32]. Application of orthogonal functions was used in determination of GP in presence of sulphadiazine [33] and DP and ephedrine hydrochloride [34].

In this paper, an HPLC method and two chemometric-assisted spectrophotometric methods based on the application of partial least squares and principal component calibrations are proposed for the resolution of the studied six-component mixture.

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2. Experimental

2.1. Instrumentation

A double-beam Shimadzu (Japan) UV–vis spectrophotometer, model UV-1601 PC equipped with 1 cm quartz cells and connected to an IBM compatible computer. HP 600 inkjet printer was used. The bundled software was UVPC personal spectroscopy software version 3.7 (Shimadzu). The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm min^{-1}. PLS and PCR analysis were carried out by using PLS-Toolbox software version 2.1—PC [35] for use with MATLAB5.

The HPLC (Shimadzu, Kyoto, Japan) instrument was equipped with a model series LC-10 ADVP pump, SCL-10 AVP system controller, DGU-12 A Degasser, Rheodyne 7725i injector with a 20 μl loop and a SPD-10AVP UV–vis detector, separation and quantitation were made on a 250 mm × 4.6 mm (i.d.) Shim-pack RP_{18} column (4.6 μm particle size). The detector was set at λ = 222 nm. Data acquisition was performed on class-VP software.

2.2. Materials and reagents

Pharmaceutical grade of TH, GP, DP, MP, PP and BZ were used and certified to contain 99.9, 99.8, 99.9, 99.7, 99.8 and 99.9%, respectively. Acetonitrile and methanol used were HPLC grade (BDH, Poole, UK). Potassium dihydrogen phosphate, hydrochloric and phosphoric acids used were analytical grade.

Tussipect-N® syrup (batch number 412112) (Misr Co. For Pharmaceutical Industries, Mataria, Cairo, Egypt) were used. Each 5 ml contains 46.65 mg of TH, 30 mg of GP, 4.15 mg of DP, 3 mg of MP, 1.5 mg of PP and 5 mg of BZ.

2.3. Procedure

2.3.1. HPLC method

The mobile phase was prepared by mixing 25 mM potassium dihydrogen phosphate (apparent pH was adjusted to 3.2 using phosphoric acid) and acetonitrile in a ratio of 60:40 (v/v). The flow rate was 2 ml min^{-1}. All determinations were performed at ambient temperature.

2.3.1.1. Standard solutions and calibration. Stock standard solutions of TH, GP, DP, MP, PP and BZ were prepared separately by dissolving 50, 60, 40, 60, 40 and 50 mg of TH, GP, DP, MP, PP and BZ, respectively, in 100 ml methanol. Further dilutions were made for HPLC method using the mobile phase to reach the concentration range of 5.0–33.0 μg ml^{-1} for TH, 3–21 μg ml^{-1} for GP, 1.2–4.0 μg ml^{-1} for DP, 0.3–3.0 μg ml^{-1} for MP, 0.4–2.0 μg ml^{-1} for PP and 0.5–4.0 μg ml^{-1} for BZ.

Triplicate 20 μl injections were made for each concentration and chromatographed under the specified conditions described previously. The peak area values were plotted against corresponding concentrations. Linear relationship was obtained.

2.3.2. Multivariate calibration

A calibration set of 25 samples was prepared in 0.1 M hydrochloric acid, applying a multilevel multifactor design [36] in which five levels of concentrations of TH, GP, DP, MP, PP and BZ were introduced. The levels were in the calibration range of 5.0–33.0 μg ml^{-1} for TH, 3–21 μg ml^{-1} for GP, 1.2–4.0 μg ml^{-1} for DP, 0.3–2.1 μg ml^{-1} for MP, 0.4–1.6 μg ml^{-1} for PP and 0.5–3.5 μg ml^{-1} for BZ (Table 1). The electronic UV absorption spectra for these samples were collected each 0.4 nm in the wavelength range of 220–270 nm. The computation was made in PLS-Toolbox software version 2.1.

PCR and PLS-1 models were applied to the UV absorption spectra of these mixtures using six latent variables for TH, GP, DP and BZ and seven latent variables for MP and PP by PLS-1. Seven principal components were used for PCR determination of each compound.

2.3.3. Pharmaceutical sample preparation

Five ml of the syrup equivalent to 46.65 mg of TH, 30.0 mg of GP, 4.15 mg of DP, 3.0 mg of MP, 1.5 mg of PP and 5.0 mg of BZ was diluted to 100 ml with methanol, further dilutions were made using 0.1 M hydrochloric acid (for spectrophotometric methods) or the mobile phase (for HPLC method) to reach the calibration range for each component. The general procedures for PCR, PLS-1 and HPLC methods described under calibration were followed and the concentration of each compound was calculated.

3. Results and discussion

3.1. Spectral features

Fig. 1 shows the UV absorption spectra of TH, GP, DP, MP, PP and BZ at their nominal concentrations in syrup. As can be seen, PP, DP, MP and BZ contribute very little to overall absorption of the sample; also, the absorption band of TH is extensively overlapped with GP, DP, MP, PP and BZ spectra. The simultaneous determination of TH, GP, DP, MP, PP and BZ in syrup by conventional, derivative and derivative ratio spectrophotometric methods is hindered by strong spectral overlap throughout the wavelength range. HPLC or multivariate calibration methods were necessary for such determination due to the presence of interference.

3.2. HPLC method

The developed HPLC method has been applied for simultaneous determination of TH, GP, DP, MP, PP and BZ. The mobile phase composition and pH of 25 mM potassium dihydrogen phosphate were studied and optimized. A satisfactory separation was obtained with a mobile phase composed of 25 mM potassium dihydrogen phosphate (apparent pH was adjusted to 3.2 using phosphoric acid) and acetonitrile (60:40, v/v). Increasing acetonitrile concentration to more than 50% led to overlapping of GP and DP. At lower acetonitrile concentration (<30%) separation occurred but with excessive delay for PP peak. Variation