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Original article

HPLC and chemometric assisted spectrophotometric methods for simultaneous determination of diprophylline, phenobarbitone and papaverine hydrochloride

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Abstract

Three methods are developed for the simultaneous determination of diprophylline (DP), phenobarbitone (PH) and papaverine hydrochloride (PP). The chromatographic method depends on a high performance liquid chromatographic (HPLC) separation on a reversed-phase C18 column with a mobile phase consisting of 0.02 M potassium dihydrogen phosphate, pH 3.5—acetonitrile (55:45 v/v). Quantitation was achieved with UV detection at 210 nm based on peak area. The other two chemometric methods applied were principal component regression (PCR) and partial least squares (PLS-1). These approaches were successfully applied to quantify the three drugs in the mixture using the information included in the UV absorption spectra of appropriate solutions in the range 215–245 nm with the intervals $\Delta\lambda = 0.2$ nm. The calibration PCR and PLS-1 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 laboratory-prepared mixtures and pharmaceutical preparations. The PCR and PLS-1 methods require neither any separation step, nor any priori graphical treatment of the overlapping spectra of the three drugs in a mixture. The results of PCR and PLS-1 methods were compared with HPLC method obtained in pharmaceutical formulation and a good agreement was found.

Keywords: Diprophylline; Phenobarbitone; Papaverine; Reversed-phase HPLC; Chemometric methods

1. Introduction

Diprophylline (DP) is a xanthine bronchodilator, which is usually associated with phenobarbitone (PH), a anticonvulsant and sedative, and papaverine hydrochloride (PP), a vasodilator. The combination of the three drugs is used for treating of bronchial spasm and chronic bronchitis. The UV absorption spectra of DP, PH, PP display considerable overlap, that the application of the conventional spectrophotometry and its direct derivative and derivative ratio technique failed to resolve the overlapping of DP and PP with PH. No analytical method has been reported for the simultaneous determination of DP, PH and PP in a ternary mixture. DP was determined by several methods including titrimetry using perchloric acid [1,2] and sodium thiosulfate as titrant [3], spectrophotometry [3–5], polarography [6] and high performance liquid chromatography (HPLC) [1,7–9]. Various analytical methods were used for determination of DP with different drugs such as guaifenesin [1]; theophylline [10,11]; doxofylline [12]; proxyphylline and theophylline [13] using HPLC; PP using Vierordt's equation, direct and first-derivative spectrophotometry [14]; proxyphylline using kinetic-spectrophotometry [15]; theophylline using micellar electrokinetic chromatography (MEKC) [16]; theophylline and caffeine using capillary electrophoresis [17]; theophylline and proxyphylline using kinetic-spectrophotometry with PLS [18] and MEKC [19]. Different analytical methods were used for determination of PH with bronchodilators and other drugs such as theophylline, theobromine, aminopyrine, caffeine, ephedrine, phenacetin [20]; theophylline, amylobarbitone, carbamazepine [21]; theobromine, PP, bendazol [22]; theophylline [23] using HPLC; aminophylline, PP, bendazole using thin layer chromatography [24]; theophylline, ephe-

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drine using capillary electrophoresis [25], alkalimetric and acidimetric non-aqueous titration [26]; acepifylline, PP using first-derivative spectrophotometry [27], non-aqueous titration and UV spectrophotometry [28]. Some analytical methods were reported for determination of PP with different drugs such as PH using potentiometric titration [29]; theophylline, ephedrine hydrochloride, hydroxyzine hydrochloride [30]; PH and amidopyrine [31] using HPLC.

Under computer-controlled instrumentation, multivariate calibrations methods are playing a very important role in the multicomponent analysis of mixtures by UV–Vis molecular absorption spectrophotometry. The approach is useful in the resolution of band overlapping in quantitative analysis. The multivariate calibration has been found to be the method of choice for complexed mixtures. The advantage of multicomponent analysis using multivariate calibration is the speed of the determination of the components in a mixture, avoiding a preliminary separation step [32]. Control analyses on pharmaceutical preparations using multivariate calibration method, has been proved to be a valid alternative to HPLC [33].

The application of quantitative chemometric methods, particularly principal component regression (PCR) and PLS to multivariate method needs a calibration step where the relationship between the spectra and the component concentration is deduced from a set of reference samples, followed by prediction step in which the results of the calibration are used to determine the component concentration from the sample spectrum. PLS is a multivariate calibration method based on factor analysis. Partial least squares (PLS-1) perform the optimization of the number of factors for only one component at a time [34]. PCR is simply a principal component analysis followed by a regression step [35]. PLS and PCR, in combination with several techniques, have been widely applied in analytical procedures in recent years. Spectrophotometric [36], spectrofluoremetric [37], differential pulse polarographic [38] and voltammetric [39] signals have been analyzed by these approaches.

This work presents PLS-1 and PCR methods for determination of DP as major component in combination with PH and PP as minor components, whereas the UV absorption spectra of DP and PP are seriously interfere with the UV absorption spectrum of PH. In addition, HPLC method was developed for the assay of the components of the ternary mixture. The fundamental advantages of the investigated methods are the simultaneously analysis of the mixture components without any chemical pre-treatment and during a short period of time, as well as no complex instruments are required. Moreover, the proposed methods are the first publication for the simultaneous determination of DP, PH and PP in pharmaceutical preparation.

2. Experimental

2.1. Instrumentation

The HPLC (Shimadzu) instrument was equipped with a model series LC-10 ADVP pump, SCL-10 AVP system controller, DGU-12A degasser, Rheodyne 7725i injector with a 20 μl loop and a SPD-10AVP UV–Vis detector. Separation and quantitation were made on a 250 × 4.6 mm (i.d.) HAISIL100 C_{18} column (5 μm particle size), USA. The detector was set at λ = 210 nm. Data acquisition was performed on class-VP software.

A double-beam Shimadzu (Kyoto, Japan) UV–Vis spectrophotometer, model UV-1601PC 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-1 and PCR analyses, were carried out by using PLS-Toolbox software version 2.1–PC [40] for use with MATLAB5.

2.2. Materials and reagents

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

Sedocardine tablets, batch number 811062 (Kahira Pharmaceuticals and Chemical Industries Co., Cairo, Egypt) were used. Each tablet was labeled to contain 150 mg DP, 20 mg PH and 30 mg PP.

2.3. HPLC conditions

The mobile phase was prepared by mixing 0.02 M Potassium dihydrogen phosphate, apparent pH was adjusted to 3.5 using phosphoric acid, and acetonitrile in a ratio of 55:45 v/v. The flow rate was 1.5 ml min^{-1}. All determinations were performed at ambient temperature. The injection volume was 20 μl.

2.4. Standard solutions and calibrations

Stock standard solutions of DP, PH and PP were prepared separately by dissolving 50 mg of DP, 20 mg PH and 30 mg PP in 100 ml methanol.

2.4.1. For HPLC method

The standard solutions were prepared by further dilutions of the stock standard solutions with mobile phase to reach the concentration range of 10–25 μg ml^{-1} for DP, 1.5–5 μg ml^{-1} for PH and PP. Triplicate 20 μl injections were made for each concentration and chromatographed under the specified chromatographic conditions described previously. The peak area values were plotted against corresponding concentrations. Linear relationships were obtained.

2.4.2. For PLS-1 and PCR methods

A training set of 23 synthetic mixtures with different concentrations of each compound were prepared by further dilu-