

## **Derivative Spectrophotometry for Simultaneous Analysis of Chlorpheniramine Maleate, Phenylephrine HCl, and Phenylpropanolamine HCl in Ternary Mixtures and Pharmaceutical Dosage Forms**

Maryam Kazemipour<sup>a</sup> and Mehdi Ansari<sup>b\*</sup>

<sup>a</sup>*Department of Chemistry, Faculty of Sciences, Kerman Azad University, Kerman, Iran.*

<sup>b</sup>*Department of Pharmaceutics, Faculty of Pharmacy, Kerman Medical Sciences University, Kerman, Iran.*

---

### **Abstract**

In this study, different derivative spectrophotometric methods are proposed for the simultaneous determination of chlorpheniramine maleate (CP), phenylephrine HCl (PE) and phenylpropanolamine HCl (PP) in their ternary mixtures and in pharmaceutical dosage forms. Spectra of single component and ternary mixtures of various concentrations and combinations from zero- to fourth-derivation were obtained. Also the spectra of the excipients including lactose, starch, and microcrystalline cellulose were obtained to study the possible interference from matrices. Zero-crossing derivative spectrophotometry based on recording the second-derivative curve for PE at 286.5 nm and fourth-derivative curve for PP at 220 nm were used for determining each component. Third component, CP, was determined by measuring absolute amplitudes at 265.8, 262.2, 269.5, and 273.8 nm in its second derivative spectra. Results showed that the matrices have no interferences. The calibration curves were linear in the range of 1-8 µg/ml for PE; 5-30 µg/ml for PP; and 2-8 µg/ml for CP. The limits of detection were 0.2 µg/ml for PE, 0.1 µg/ml for PP, and 0.3 µg/ml for CP. The mean percentage recoveries obtained for different synthetic mixtures by using this method were 95.3% with coefficient of variation of 4.3% for PE, 101.5% with coefficient of variation of 1.4% for PP, and 99.4% with coefficient of variation of 1.5% for CP. This method has been applied successfully for the determination of PE and PP in its combination with CP in Antihistamine Decongestant tablets with a high percentage of recovery, good accuracy and precision.

**Keywords:** Chlorpheniramine; Phenylpropanolamine; Phenylephrine; Derivative spectrophotometry; Antihistamine Decongestant; Tablet.

---

### **Introduction**

CP is an alkylamine derivative with the actions and uses of the antihistamines. PE and PP are sympathomimetic agents are that frequently associated in pharmaceutical

formulations against the common cold. Usually, tablet dosage forms with generic name of antihistamine decongestant and trade name of Hista-Vadrin<sup>®</sup> contain CP, 2 mg; PE, 5mg; and PP, 20mg (1). Some procedures have been described for the assay of CP, PE, and PP separately or combination of two from three of them in pharmaceutical preparations, such as derivative spectrophotometry (2-9), charge

---

\* Corresponding author:

E-mail: mansari@kmu.ac.ir

transfer complex formation (10), GC (11, 12), micellar liquid chromatography (13-15), ion-pair chromatography (16-19), and HPLC (20-24). The CP-PE-PP mixture is not yet official in any pharmacopoeia. To our knowledge, no analytical methods could be traced for the analysis of CP-PE-PP combination in pharmaceutical dosage forms. Therefore, a simple, rapid and reliable method for simultaneous assay of these three drugs in mixture seemed to be necessary. Derivative spectrophotometry (DS) is a useful means of resolving overlapping spectra and eliminating matrix interferences in the assay of mixtures using the zero-crossing technique. Also this method is simple, rapid and accurate. Such features render it suitable for the routine analysis in quality control laboratories. The aim of the present work was the development and validation, of a simple and reliable derivative spectrophotometry in the assay of CP, PE, and PP in combination in pharmaceutical preparations without the necessity of sample pre-treatment. The method should have sufficient accuracy and precision and permit a simple and time-saving assay of CP, PE, and PP in mixtures.

## Experimental

### Materials

PE, PP, and CP were kindly donated by Pursina Pharmaceutical Co., Iran. A commercial tablet formulation (Antihistamine Decongestant) tablet produced by Pursina, Iran, Batch no. 119, Expiration Date 8/2005, containing of 2 mg CP, 5 mg PE and 20 mg PP per tablet was investigated. In all steps double distilled water was used. All reagents used were of analytical reagent grade.

### Apparatus

A Perkin-Elmer Lambda 25 double beam UV-Vis spectrophotometer in 1 cm quartz cells with a fixed slit width (2 nm) connected to a PC computer loaded with UV-Win Lab Software and equipped with a HP 1200 printer was used for all the absorbance measurements and treatment of data.

## Methods

### Preparation of standard solutions and calibration

PE, PP, and CP stock standards (100 µg/ml) were prepared by dissolving 10.0 mg of each, in 100 ml of water. The working standard solutions were prepared by dilution of the stock standard with water. Working standards of 1, 2, 4, 5, 6, and 8 µg/ml; 2, 4, 6, 8, 10, and 12 µg/ml; and 5, 10, 15, 20, 25 and 30 µg/ml were prepared for CP, PE, and PP, respectively. Validation set consisting of 15 synthetic mixture solutions in the concentration range of 1-8 µg/ml for CP, 2-12 µg/ml for PE and 5-30 µg/ml for PP was prepared by using the same stock solutions. All the solutions were prepared freshly and protected from light. Standard, single component solutions of each analyte, containing of 2 µg/ml CP, 5 µg/ml PE, and 20 µg/ml, were separately tested for stability in solution and during the actual analysis. The content of each analyte solution remained unchanged in ambient temperature up to about 2 weeks since their preparation. Further tests of stability (i.e., over two weeks) were found unnecessary and were not attempted. All measurements were made at room temperature.

### Tablet analysis procedure

Ten tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet was dissolved in 50 ml of water in a 100 ml volumetric flask with the aid of mechanical shaking for 20 min and finally diluted to volume with water. This solution was filtered into a 100 ml volumetric flask through Whatman No. 42 filter paper. The residue was washed three times, each with 10 ml of water. The filtrate was diluted 1:10 with the same solvent by diluting 10 ml of it in a 100 ml volumetric flask. The analysis of the sample solutions was carried out using DS methods.

### Derivative spectrophotometric measurements

#### *Analysis of CP by second order DS*

The second derivative spectra ( $\Delta\lambda=3$  nm) of different concentrations of CP (2, 4, 6, 8, 10, and 12 µg/ml) in single component solutions and multicomponent mixtures containing fixed

concentrations of two the other components (PE, 5  $\mu\text{g/ml}$ ; PP, 20  $\mu\text{g/ml}$ ) in the water were recorded against water as a blank. The absolute values of peak to trough amplitudes of four consecutive wavelengths at 273.8, 269.5, 265.9, and 262.2 nm were measured.

#### *Analysis of PE by second order DS*

Second derivative spectra ( $\Delta\lambda=10\text{ nm}$ ) of single component solutions contained PE and multicomponent solutions containing constant concentrations of CP 2  $\mu\text{g/ml}$  and PP 20  $\mu\text{g/ml}$  with different concentrations of PE (1, 2, 4, 5, 6, and 8  $\mu\text{g/ml}$ ) were plotted. Analysis was accomplished by measuring amplitudes at 286.5 nm.

#### *Analysis of PP by second order DS*

In PP normal spectrum, no peaks without interference with two other components could be observed. Thus, the 4<sup>th</sup> derivative spectra of the drug ( $\Delta\lambda=20\text{ nm}$ ) were selected that showed minimum interferences with the other components for quantitative analysis. Analyses were accomplished by measuring the trough zero crossing amplitude of different concentrations of PP (5, 10, 15, 20, 25, and 30  $\mu\text{g/ml}$ ) in the presence of constant concentration of CP and PE (2 and 5  $\mu\text{g/ml}$ , respectively) at 220 nm.

#### **Possible interferences of tablet excipients**

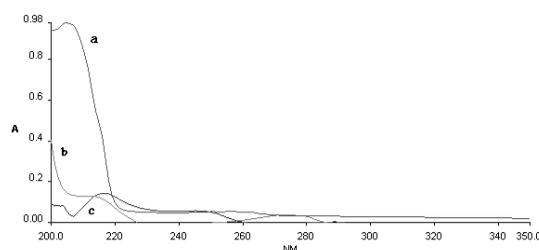
To study possible interfering effects of excipients, those that reported to be used in tablets were analyzed by the spectrophotometric method, separately and in combination, in concentration ranges that are normally used in tablets.

## **Results And Discussion**

#### **Analysis of CP by second order DS**

The direct UV spectra of CP with two other components are shown in Figure 1.

This figure indicates major overlapping between the 3 spectra. In Fig. 2 spectra of different concentrations of CP in absence and presence of fixed concentrations of PE and PP are shown (a and b, respectively). This figure indicates that direct UV method can not be applied in the determination of CP, due to

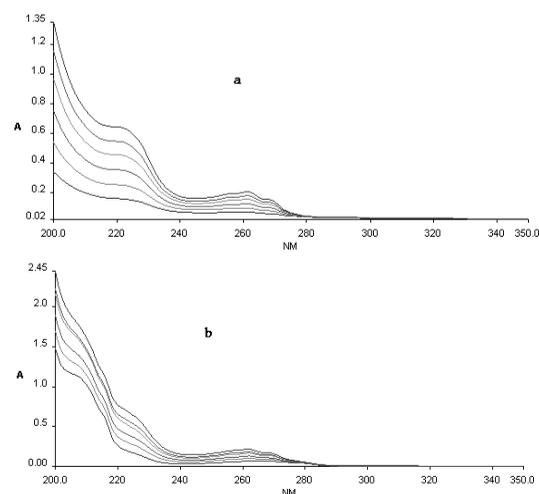


**Figure 1.** Normal spectra of A) PP 20 mg/ml, B) PE 5 mg/ml, and C) CP 2 mg/ml in water.

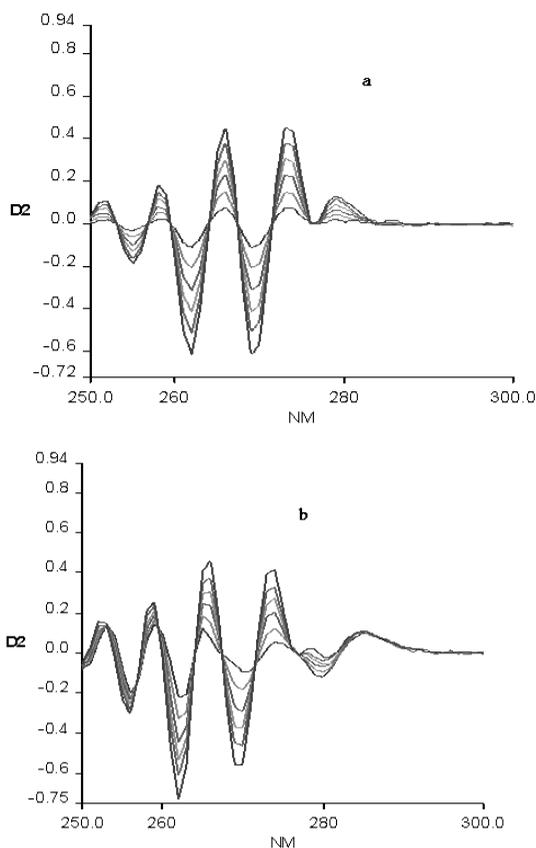
potential interferences from PE and PP.

As shown in fig. 3, 2<sup>nd</sup> order derivative of the spectra of CP showed minimum interferences from other components of the solution at selected wavelengths, and therefore, can be applied to quantitative measurements of CP in single or multi-components solutions.

Thus, a second derivative spectrophotometry was developed for determination of CP in tablet dosage form, based on higher sensitivity, minimum interference simplicity and cost. Analysis was based on the measurement of two peaks to trough amplitudes of four consecutive wavelengths including 273.8, 269.5, 262.2, and 265.9 nm. These four wavelengths were selected to increase sensitivity and selectivity for CP. The result of validation assessment of the method are given in table 1. The linearity of the method was assessed over a concentration range of 2-12  $\mu\text{g/ml}$  of CP. Intra and inter-day variations were measured at 6 replicates and the results are given as RSD values. Limit of detection (LOD) and limit of quantification (LOQ) of the method were determined by at least 25 replicate determinations

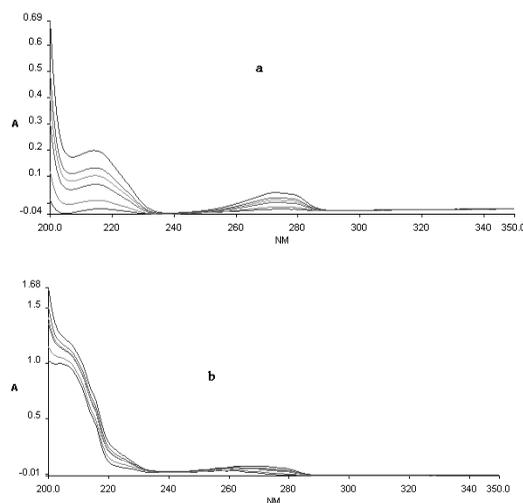


**Figure 2.** Normal spectra of a) different concentrations of CP in absence of PE and PP and b) in the presence of fixed concentrations of PE and PP



**Figure 3.** Second derivative spectra of a) different concentrations of CP in absence of PE and PP and b) in the presence of fixed concentrations of PE and PP.

at the above mentioned wavelengths in second derivated of normal spectrum for blank samples. Recovery of the method was greater than 97.2%. Additive interferences in the presence of constant



**Figure 4.** Normal spectra of a) different concentrations of PE in absence of CP and PP and b) in the presence of fixed concentrations of CP and PP

concentrations of PE (5 µg/ml) and PP (20 µg/ml), were lower than 2.3%. All validation data were summarized in (Table 1).

**Analysis of PE by second order DS**

Fig. 4a and 4b show the absorption (zero-order) spectra of PE alone or in combination with two other components.

The large overlap of the spectral bands of the drugs at 200.0–330.0 nm prevents the formation from the total zero-order spectrum of any spectral feature that could be used for analytical purposes. The second derivative spectra allowed determination of PE in the presence of CP and PP. Fig. 5 shows the second derivative spectra of different concentrations (1, 2, 4, 5, 6, and 8 µg/ml) of PE in absence (a) and presence (b) of constant concentration of CP, and PP.

The D2 spectrum of PE shows a well-defined minimum at 286.5 nm at which CP and PP exhibit no contribution. The result of validation assessment are given in table 1. The method was linear over the concentration range of 1-8 µg/ml. Six replicate determinations at different concentration levels were carried out to test the precision of the method. The relative standard deviation (RSD) values for within day and day-to-day variation indicated repeatability of the proposed method. In order to demonstrate the validity and applicability of the proposed method, recovery studies were performed by analyzing synthetic mixtures of PE, CP, and PP which reproduced different composition ratios. The percentage recoveries of PE were about 101.2%. The lowest detection limit calculated was obtained for second order derivative spectrophotometry (0.2 µg/ml) indicating an acceptable sensitivity. All of the validation data were given in Table 1.

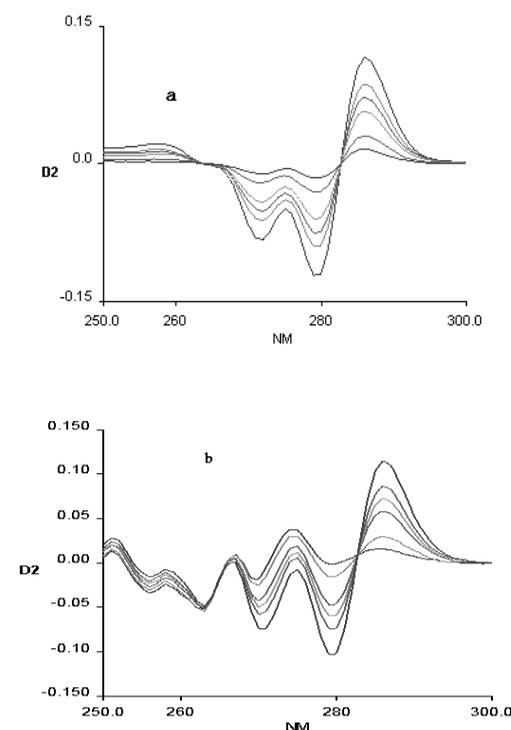
**Analysis of PP by fourth order DS**

As can be seen in normal spectrum of PP (Fig. 1) there is interferences from the two other components of the table. Thus, conventional UV spectrophotometry can not be used for the individual determination of tablet componets in their ternary mixture (Fig 6a and 6b). Therefore derivative spectrophotometry was suggested to overcome the problem and fourth derivative spectra were obtained (Fig.7)

The fourth derivative spectrum of PP exhibits a minimum at 220.1 nm while two other components reads zero. Quantitative investigations using regression analysis have established that the concentration of PP correlates very well with the measured fourth derivative trough in concentration range of 5 to 30  $\mu\text{g/ml}$ . The results are reproducible and precise as RSD values are very low (less than 3.8% for both intra- and inter-day variation). In order to demonstrate the validity and applicability of the proposed method, recovery study was performed by analyzing synthetic mixtures that contained different concentrations of PP in the presence of fixed concentrations of CP and PP. Recovery was calculated to be greater than 96.3% for all concentrations. LOD and LOQ of the method were 0.5 and 1.5  $\mu\text{g/ml}$ , respectively. Validation data of the method are summarized in Table 1.

### Application

Accuracy of the method was determined by analysis of synthetic formulation samples containing 2 mg of CP, 5 mg of PE and 20 mg of PP mixed with 80 mg of excipients (approximately magnesium stearate 1% and a mixture of lactose + microcrystalline cellulose + starch 99%) using the proposed method.

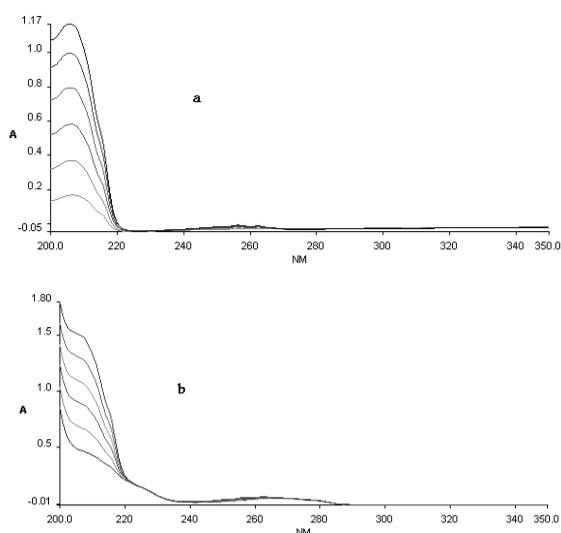


**Figure 5.** Second derivative spectra of a) different concentrations of PE in absence of CP and PP and b) in the presence of fixed concentrations of CP and PP.

The recoveries were found to be 97.6, 102.4, and 97.8% for CP, PE, and PP, respectively. According to the result, it can be concluded that common excipients normally found in tablets do not make interferences in the proposed method.

**Table 1.** Validation parameters of proposed methods for analysis of CP, PE, and PP in synthetic ternary mixtures and results of application of the method for active components analysis of Antihistamine Decongestant tablet

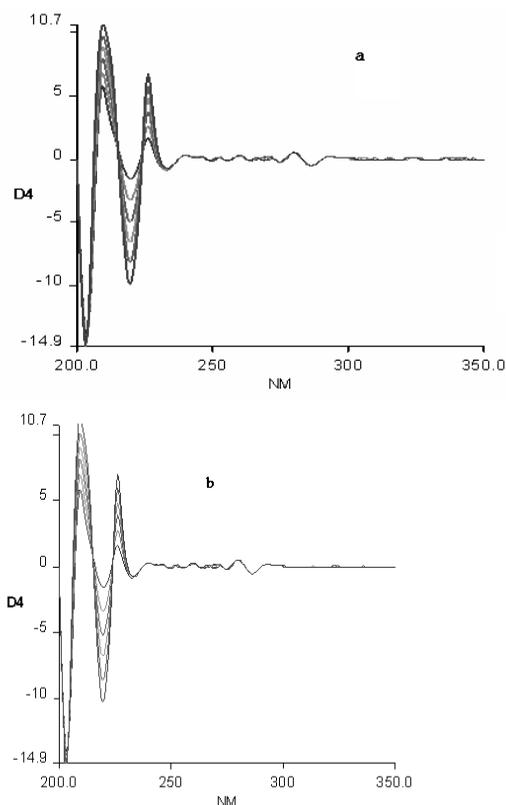
Validation Parameters	Drug		
	CP	PE	PP
Order of derivation	2	2	4
$\Delta\lambda(\text{nm})$	3	10	20
Wavelength (nm)	273.8, 269.5, 262.2, 265.9	286.5	220.0
Linearity (mg/ml)	2-12	1-8	5-30
Calibration curve equation (n=6)	$D2 = 0.16570X + 0.09587$ ( $r^2 = 0.9994$ )	$D2 = 0.01412X + 0.00121$ ( $r^2 = 0.99997$ )	$D4 = 0.3358X + 0.0883$ ( $r^2 = 0.99993$ )
Intra day RSD% (n=6)	3.2	2.8	3.8
Inter day RSD%(n=6)	1.5	1.2	3.8
Interference%	2.3	3.8	0.1
LOD (mg/ml)	0.3	0.2	0.5
LOQ (mg/ml)	1.0	0.8	1.5
Recovery Percent	97.2	101.2	96.3
Real tablet analysis (content %) $\pm$ SD (n=10)	95.5 $\pm$ 2.5	102.0 $\pm$ 2.2	96.0 $\pm$ 1.9



**Figure 6.** Normal spectra of a) different concentrations of PP in absence of CP and PE and b) in the presence of fixed concentrations of CP and PE

The sample preparation was simple, due to high solubility of the sample main component in water. Thus tablets were powdered, dissolved in water, and filtered.

Also, the amount of CP, PE, and PP in a commercial tablet, Antihistamine Decongestant



**Figure 7.** Fourth derivative spectra of a) different concentrations of PE in absence of CP and PE and b) in the presence of fixed concentrations of CP and PE.

(Razak Co, Iran) were determined. This formulation contains nominally 2 mg of CP, 5 mg of PE and 20 mg of PP. The average weight of one lot of this tablet is 100 mg. by applying the method, an average of  $1.91 \pm 0.05$  mg for CP,  $5.1 \pm 0.11$  mg for PE and  $19.2 \pm 0.38$  mg for PP in each tablet Antihistamine Decongestant tablets.

Derivative spectrophotometry, a well established analytical technique which is frequently used in the contemporary analysis of drugs in mixtures when the spectral classic bands of components are overlapped. Although there are many reports for separate analysis of each of PE, CP, and PP or in some binary mixtures of them that present in antihistamine-decongestant product, no simple spectrophotometric method for simultaneous analysis of them in ternary mixtures or tablets have been reported. The results obtained demonstrate that the second and fourth derivative-zero crossing spectrophotometry reported in this paper have given the valid results for the simultaneous determination of three compounds in the ternary mixture, with a simple pretreatment of the sample. The proposed zero-crossing derivative methods are simple, fast and precise, therefore, these procedures can be applied for routine quality control analysis of antihistamine-decongestant tablets.

## References

- (1) Drug Information Online. Available from <http://www.drugs.com/cons/Hista-Vadrin.html>.
- (2) Erk N. Quantitative analysis of chlorpheniramine maleate and phenylephrine hydrochloride in nasal drops by differential-derivative spectrophotometric, zero-crossing first derivative UV spectrophotometric and absorbance ratio methods. *J. Pharm. Biomed. Anal.* (2000) 23: 1023–1031
- (3) Collado MS, Mantovani VE, Goicoechea HC and Olivieri AC. Simultaneous spectrophotometric-multivariate calibration determination of several components of ophthalmic solutions: phenylephrine, chloramphenicol, antipyrine, methylparaben and thimerosal. *Talanta* (2000) 52: 909–920
- (4) Goicoechea HC and Olivieri AC. Simultaneous multivariate spectrophotometric analysis of paracetamol and minor components (diphenhydramine or phenylpropanolamine) in tablet preparations. *J. Pharm. Biomed. Anal.* (1999) 20: 255–261
- (5) Shama SA. Spectrophotometric determination of phenylephrine HCl and orphenadrine citrate in pure and in dosage forms. *J. Pharm. Biomed. Anal.* (2002) 30: 1385-1392

- (6) Enturk ZS, Erk N, Ozkan SA, Akay C and Cevheroglu S. Determination of theophylline and ephedrine HCL in tablets by ratio-spectra derivative spectrophotometry and LC. *J. Pharm. Biomed. Anal.* (2002) 29: 291–298
- (7) Chafetz L. Specificity of spectrophotometric determination of ephedrine and other phenalkanolamine drugs as benzaldehydes after periodate oxidation. *J. Pharm. Sci.* (1971) 60: 291-294
- (8) Gupta DV and de Lara AJ. Colorimetric determinations of chlorpheniramine maleate, ephedrine hydrochloride, and guaiaacolsulfonate potassium in a cough syrup. *J. Pharm. Sci.* (1975) 64: 2001-2002
- (9) Montgomery KO, Jennings PV and Weinswig MH. Ion-exchange separation and ultraviolet determination of phenylephrine, codeine, and selected antihistamines. *J. Pharm. Sci.* (1967) 56: 141-143
- (10) El-Mossalamy EH. Charge-transfer complexes of phenylephrine with nitrobenzene derivatives. *Spectrochimica Acta A* (2004) 60: 1161–1167
- (11) De Fabrizio F. Simultaneous GLC analysis of salicylamide, phenylpropanolamine hydrochloride, caffeine, chlorpheniramine maleate, phenylephrine hydrochloride, and pyrilamine maleate in capsule preparations. *J. Pharm. Sci.* (1980) 69: 854-855
- (12) Hishta C and Lauback RG. Gas chromatographic analysis of amine mixtures in drug formulations. *J. Pharm. Sci.* (1969) 58: 745-746
- (13) Marin A and Barbas C. CE versus HPLC for the dissolution test in a pharmaceutical formulation containing acetaminophen, phenylephrine and chlorpheniramine. *J. Pharm. Biomed. Anal.* (2004) 35: 769-777
- (14) Gil-Agusti M, Monferrer-Pons L, Garcí'a-Alvarez-Coque MC and Esteve-Romero J. Determination of active ingredients in cough-cold preparations by micellar liquid chromatography. *Talanta* (2001) 54: 621–630
- (15) Suntornsuk L. Separation of cold medicine ingredients by capillary electrophoresis. *Electrophoresis* (2001) 22: 139-143
- (16) Halstead GW. Determination of amine ingredients in cough-cold liquids by reversed-phase ion-pair high-performance liquid chromatography. *J. Pharm. Sci.* (1982) 71: 1108-1112
- (17) Koziol TR, Jacob JT and Achari RG, Ion-pair liquid chromatographic assay of decongestants and antihistamines. *J. Pharm. Sci.* (1979) 68: 1135-1138
- (18) Smith DJ. Collaborative study of an ion exchange method for the chromatographic separation of mixtures containing expectorants, sympathomimetic amines, antihistamines, or phenothiazine in pharmaceuticals. *J. Assoc. Off. Anal. Chem.* (1974) 57: 741-746
- (19) Nakashima K, Kanehara S and Kaddoumi A. HPLC determination of phenylpropanolamine in pharmaceutical OTC preparations. *Biomed Chromatogr.* (2002) 16: 463-469
- (20) Marin A, Garcia E, Garcia A and Barbas C. Validation of a HPLC quantification of acetaminophen, phenylephrine and chlorpheniramine in pharmaceutical formulations: capsules and sachets. *J. Pharm. Biomed. Anal.* (2002) 29: 701-714
- (21) Senyuva H and Ozden T. Simultaneous high-performance liquid chromatographic determination of paracetamol, phenylephrine HCl, and chlorpheniramine maleate in pharmaceutical dosage forms. *J. Chromatogr. Sci.* (2002) 40: 97-100
- (22) Gil-Agusti M, Capella-Peiro E, Monferrer-Pons L, Garcia-Alvarez-Coque MC and Esteve-Romero J. Chromatographic analysis of phenethylamine-antihistamine combinations using C8, C18 or cyano columns and micellar sodium dodecyl sulfate-pentanol mixtures. *Analyst* (2001) 126: 457-464
- (23) Garcia A, Ruperez FJ, Marin A, de la Maza A and Barbas C. Poly(ethyleneglycol) column for the determination of acetaminophen, phenylephrine and chlorpheniramine in pharmaceutical formulations. *J. Chromatogr. B* (2003) 785: 237–243
- (24) Ferreyra C and Ortiz C. Analysis of multicomponent formulations containing phenylpropanolamine hydrochloride, caffeine and diazepam by using LC. *J. Pharm. and Biomed. Anal.* (2001) 25: 493–499