Research article

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Separation and simultaneous determination of paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate in a commercial tablet by a rapid isocratic HPLC method

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Abstract

Background and objective: Pharmaceutical formulations for relief of cold signs usually contain high concentration of acetaminophen and small concentration of phenylephrine hydrochloride and chlorpheniramine maleate. The combination makes a problem in simultaneous quantification of the chemicals. Mixture of paracetamol, phenylephrine hydrochloride, chlorpheniramine maleate, and caffeine is commonly used for its analgesic, antipyretic, antihistamine, and antitussive activity. At this study, a simple method based on reversed phase high-performance liquid chromatography was developed and validated for detection of chlorpheniramine maleate, phenylephrine hydrochloride, and paracetamol in pharmaceutical formulations at the same time.

Materials and methods: One ml of stock solutions was diluted with mobile phase to prepare final concentrations of the all three chemicals (20 mg/l of chlorpheniramine maleate, 325 mg/l of phenylephrine hydrochloride, and 50 mg/l of paracetamol). The formulations were prepared by addition of the chemicals to a volumetric flask. Then, they were made up to 100 ml with mobile phase of 0.05 M phosphate buffer:acetonitrile (95:5). pH of the mobile phase was adjusted to 2.5 with 50% orthophosphoric acid. The experiments were done in 250 mm × 4.6 mm × 5 µm C_{18} column.

Results and conclusion: Based on the results, flow rate was 1.5 ml/min during isocratic elution of the samples. The analytes were detected at 210 nm by UV detector. Retention time of the last eluted analyte was 8 min. The method was validated based on ICH guidelines. The results revealed that the proposed method is valid and accurate. Therefore, the validated method can be applied for routine examination of tablets in order to control their quality and stability.

Keywords: Chlorpheniramine maleate, Paracetamol, Phenylephrine hydrochloride, RP-HPLC, Simultaneous determination

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1. Introduction

Drugs that are intended to be used in cold relief contain ingredients at different concentrations with various properties such as acetaminophen, phenylephrine, and chlorpheniramine. A common mixture includes acetaminophen (paracetamol) that is an analgesic and antipyretic as the major component and small concentration of phenylephrine that is sympathomimetic (decongestants) and chlorpheniramine maleate as a H_1-receptor antagonist (antihistaminic) [1]. CPM [(RS)-3-(4-chlorophenyl)-3-(2-pyridyl) propyl methylamine hydrogen maleate] is a histamine H_1 antagonist used in allergic reactions, hay fever, rhinitis, urticaria, and asthma. In addition, PCM [(N-(4-hydroxyphenyl) acetamide)] is a complex with analgesic and anti-pyretic character [2,3], which is considerably healthier than aspirin with regard to gastric irritation, ulceration and gastric bleeding. Furthermore, PE [(3-{(iR)-i-hydroxy-2- methylaminoethyl} phenol hydrochloride)] is α-adrenergic agonist used as a mydriatic, nasal decongestant, and cardio tonic agent [4,5]. Figure 1 shows structure of the three chemicals.

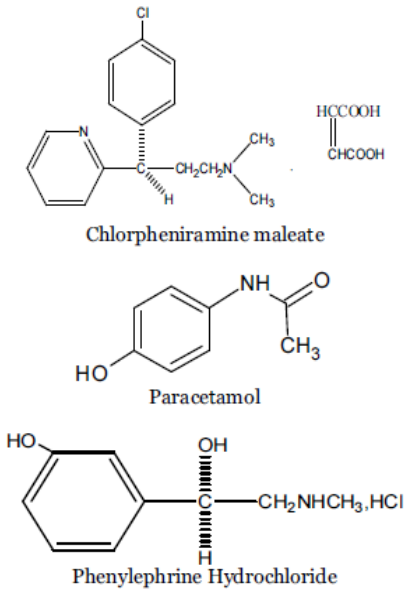

Figure 1- Chemical structures of paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate

Various analytical approaches have been used for detection of these compounds. HPLC, along with UV, fluorimetry or mass spectroscopy (MS) is considered as the most commonly used methods. Other methods include UV-Vis spectroscopy, gas chromatography (GC), GC/MS and capillary electrophoresis. However, no analytical technique has been introduced yet for simultaneous detection of the compounds as it was mentioned for the drugs used for cough and cold [6].

Senyuva and Ozden [7] presented a rapid determination method for three ingredients in a mixed formulation by using Bondapak CN column. However, acetaminophen was not distinguishable from the solvent, which made quantification problems, and no gap time was available for impurities. Kanumula et al. [8] introduced a method developed by internal standard for wavelength programming and determination of pseudoephedrine hydrochloride. Their method was not efficient enough because it was time consuming and several solvents were used through the process. The method developed by Garcia et al. [9] for separation of acetaminophen from analgesic preparation including chlorpheniramine maleate, phenylephrine hydrochloride, and other active components by HPLC equipped with polyethylene glycol column was not appropriate because it was not able to separate the peaks. In this regard, Olmo et al. [10] developed a method by using two cyano columns for separation of same compounds and their impurities but it was time-consuming.

In this work, we tried to develop and validate an analytical method based on HPLC for determination of CPM, PCM, and PE at the same time in tablets. The method was validated by examining the parameters of accuracy, precision, selectivity, linearity, range, and robustness according to ICH guideline.

2. Materials and methods

2.1. Chemical and reagents

Working standards of paracetamol, phenyl-

ephrine hydrochloride, and chlorpheniramine maleate in pharmaceutical grade were obtained as generous gifts from Temad, Behdashtkar and Damavand Darou (Tehran, Iran). They were used without further purification. HPLC grade acetonitrile, methanol, water, orthophosphoric acid, and potassium di-hydrogen orthophosphate were purchased from Amertat (Iran). Tablet of Zagrocold (containing 325 mg paracetamol, 5 mg phenylephrine hydrochloride, and 2 mg chlorpheniramine maleate) was purchased from Zagros Darou (Iran).

2.2. Reversed phase liquid chromatography

HPLC (Younglin, South Korea) was equipped with a 2100 pump series model, vacuum degasser (9101), Rheodyne injector with a 20 µl loop, and UV-Vis detector (9120). Separation and quantitation were done by reversed phase Princeton C_{18} column (250 mm × 4.6 mm × 5 µm) by using a mobile phase consisting of 0.05 M KH_2PO_4 buffer:acetonitrile (95:5). pH of the mobile phase was adjusted to 2.5 by orthophosphoric acid and it was passed through a 0.45 µm membrane filter before injection. Analysis was performed at flow rate of 1.5 ml/min. The system was equilibrated by the mobile phase before injection. The UV detector was set at 210 nm for quantification. Temperature was set at 35 °C.

2.3. Preparation of stock and standard solutions

Stock solutions were prepared by dissolving 2 mg of CPM, 325 mg of PCM, and 5 mg of PE in 100 ml of the mobile phase. Then, 1 ml of the stock solutions were transferred to 10 ml volumetric flasks and the solutions were diluted with the mobile phase to prepare the final concentrations of the all three chemicals.

2.4. Chemicals formulation

Average weights of 20 Zagrocold tablets were determined and they were powdered finely for analysis. Amounts of 325 mg paracetamol, 5 mg phenylephrine hydrochloride, and 2 mg chlorpheniramine maleate were weighted and transferred to 100 ml volumetric flask and then made up to 100 ml with the mobile phase to achieve 200 µg/ml CPM, 3250 µg/ml PCM, and 500 µg/ml PE standard solutions. The solutions were centrifuged at 2500 rpm for 10 min. Further dilutions were prepared by the mobile phase to reach the calibration range of each chemical. Finally, 20 µl of the samples was injected to HPLC.

3. Results and discussion

3.1. Method development and optimization

Condition of chromatography was optimized by pH, mobile phase, wavelength, and flow rate for separation of CPM, PCM, and PE in C_{18} column. In this regard, two organic solvents of methanol and acetonitrile were examined as mobile phase. Concentrations of 3-15% v/v acetonitrile were evaluated through which various retention times of the chemicals and a fast elution rate were observed. The peaks were not well separated out and could not be distinguished at some concentrations. Finally, concentration of 5% v/v acetonitrile was selected as optimum. pH of the mobile phase was adjusted to 2.5- 6.7 by using orthophosphoric acid. Low pH of 2.5 was selected due to its impact on rapid elution, better sharpness of the peaks and decreased analysis time. Final adaptation was included to C_{18} column (250 × 4.6 mm × 5 µm), mobile phase of 0.05 M KH_2PO_4 buffer:acetonitrile (95:5) at pH 2.5, flow rate of 1.5 ml/min and injection volume of 20 µl at ambient temperature. Figure 2 illustrates chromatogram of the standards.

3.2. Linearity

Calibration curves were drawn by standard solutions of CPM, PCM, and PE at seven concentrations in range of 80-120%. Each solution was injected in three replicates and the regression equation was achieved by plotting the mean of peak area against concentration of the chemicals. Correlation coefficient (r^2) of CPM, PCM, and PE standard solutions was calculated

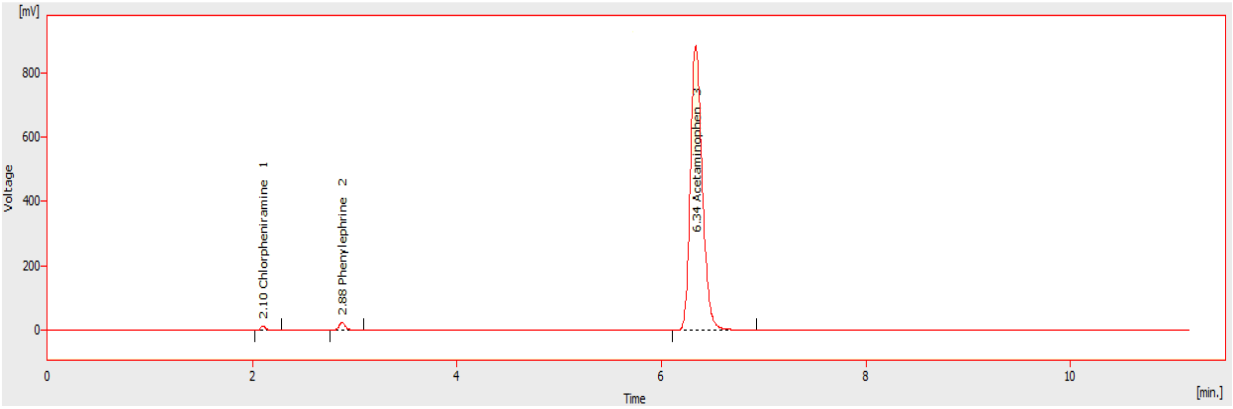

Figure 2- Chromatogram of the standard solutions of CPM, PCM, and PE

as 0.9992, 0.9994, and 0.9990, respectively, indicating high linearity for the all chemicals (Table 1).

Table 1- Characteristics of the calibration curves prepared by the standard solutions of CPM, PCM, and PE

Parameter	PCM	PE	CPM
Calibration range (µg/ml)	16.25-390	0.25-6.0	0.1-2.4
Regression equation	Y=32.205X+251.79	Y=22.933X+0.1088	Y=26.453X+0.1526
Intercept	251.79	0.1088	0.1526
Correlation coefficient	0.9994	0.9990	0.9992

3.3. Specificity

Specificity is defined as ability of a method to detect the analyte among potential impurities and degradation products [11]. Our study revealed the absence of any interfering agent as no peak was observed at the same retention time (Figure 3). Evaluating the possible interactions in the standard solutions was conducted by comparing the peaks with each other.

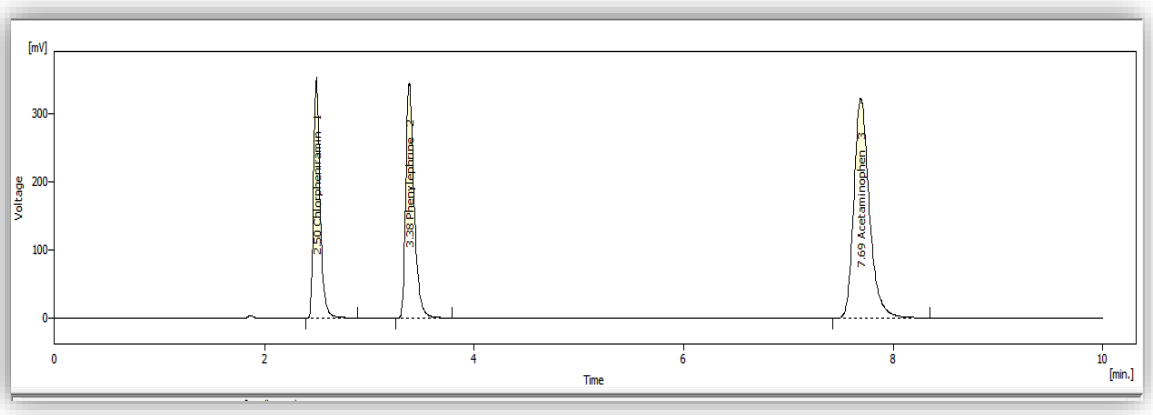

Figure 3- Chromatogram of a mixed standard solution of CPM, PCM, and PE

3.4. Limit of detection and limit of quantitation

Limit of detection (LOD) is considered as the lowest concentration of a chemical in a sample, which can be detected under experimental condition. Limit of quantitation (LOQ) is the

lowest concentration of a chemical in a sample, which can be detected with acceptable precision and accuracy. As recommended, standard deviation (SD) of the intercept and slope (m) of the calibration curve are used for calculation of LOD (Eq. 1) and LOQ (Eq. 2) [12].

$$LOD = 3.3\ (SD/m) \qquad \text{Eq. 1}$$

$$LOQ = 10\ (SD/m) \qquad \text{Eq. 2}$$

In the current study, LOD was calculated as 0.031 µg/ml, 15.157 µg/ml, and 0.048 µg/ml for CPM, PCM, and PE, respectively. Furthermore, LOQ was calculated as 0.093 µg/ml, 45.932 µg/ml, and 0.145 µg/ml for CPM, PCM, and PE, respectively.

3.5. Precision

This parameter is expressed as observation of similar results by the operator when the procedure is repeatedly applied for a sample. Precision is considered as repeatability (intra-day analysis) and intermediate precision (inter-day analysis in three consecutive days). The results are reported as relative standard deviation (RSD) [13]. In our study, RSD of intra- and inter-day precision was accounted 0.054-0.213% and 0.2-0.3%, respectively. The data is presented in Table 2.

Table 2- Results of precision (intra- and inter-day) for the standard solutions of CPM, PCM, and PE

Compound	Intra-day (RSD%)	Inter-day (RSD%)
CPM	0.213	0.26
PCM	0.141	0.2
PE	0.054	0.3

3.6. Accuracy and recovery

To investigate accuracy of the proposed method, recovery tests were done. Accuracy is expressed as closeness of the observed results with a reference value. In the present study, accuracy was calculated by measuring the chemicals' concertation in the mixtures containing known quantities of the chemicals including 110%, 120%, and 130% of the amounts claimed on the label [14]. In practice, the samples were spiked with the chemicals at three mentioned concentrations and were analysed by the method. The experiments were repeated three times.

Relative recovery (RR%) was calculated for the spiked samples according to Eq. 3 and the results are shown in Table 3.

$$RR\% = \frac{(C_t - C_o)}{C_{SP}} \times 100 \qquad \text{Eq. 3}$$

Where, C_t is total concentration of the chemical in the spiked sample (sum of the initial and spiked amount), C_o is initial concentration of the chemical in the sample (before spiking), and C_{sp} is concentration of the spiked chemical. According to the results (Table 3), an acceptable accuracy was achieved.

Table 3- Results of recovery and RSD for the chemicals detected in the solutions of CPM, PCM, and PE

	PCM		PE		CPM	
Label claim	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
110%	98.41	0.092	98.72	0.154	98.99	0.166
120%	98.44	0.119	98.99	0.215	98.70	0.177
130%	98.53	0.105	99.21	0.110	99.32	0.688

3.7. Measurement of the chemicals in the tablets

Amount of CPM, PCM, and PE in the commercial tablets was calculated by using the calibration curves drawn by the standard solutions. According to Table 4, the experimental results were close to the amounts reported on the labels. This observation additionally confirmed the good accuracy of the method.

Table 4- Results of the chemicals detected in the commercial tablets

Chemical	Claimed concentration (mg/tab.)	Mean (µv/sec)	SD	RSD (%)	Calculated concentration (mg/tab.)	Assay (%)
PCM	325	7075.3742	0.1430	0.0439	325.879	99.38
PE	5	112.6420	0.0044	0.0874	4.980	100.30
CPM	2	45.8967	0.0018	0.0915	1.914	96.17

3.8. System appropriateness

This feature should be monitored to make sure that the system is working correctly [15]. At first, the main parameters were evaluated by preparation and analysis of the standard solutions. Then, resolution, theoretical plates, capacity factor, separation factor, asymmetric factor, tailing factor, and retention time were determined (Table 5). It seemed that the column was effective for analysis and the peaks were separated well.

Table 5- Parameters of the system appropriateness

Parameter	PCM	PE	CPM
Resolution	20.18	7.6	-
Theoretical plate	14347	9226	9618
Capacity factor	3.15	0.94	0.42
Separation factor	3.33	2.11	-
Asymmetric factor	1.38	1.35	1.42
Tailing factor	1.25	1.24	1.25
Retention time	6.11	2.86	2.08

4. Conclusion

In the current work, we developed and validated a reliable and rapid isocratic HPLC method for detection of paracetamol, phenylephrine hydrochloride, and chlorpheniramine maleate in commercial tablets. Accordingly, the proposed method was able to diagnose the active ingredients within 10 min in spite of their different characteristics. In addition, the method was simpler and more accurate than the existing analytical methods. Standard deviation (0.0018-0.1430 µg/ml) and RSD (< 2%) were within the limit, indicating high precision of the method. The method was sensitive, accurate, specific, and robust according to ICH guidelines and the adapted model had good linearity. As conclusion, the method is suggested for routine examination of the tablets to evaluate their quality and stability.

5. Acknowledgement

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6. Conflict of interest

The authors have declared that there is no conflict of interest.

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