

HPLC METHOD DEVELOPMENT AND VALIDATION: SIMULTANEOUS DETERMINATION OF ACTIVE INGREDIENTS IN COUGH AND COLD PHARMACEUTICALS

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Received: 07 Sep 2014 Revised and Accepted: 03 Oct 2014

ABSTRACT

Objective: This study aimed to develop a simple reversed-phase high performance chromatographic method for simultaneous determination of pseudoephedrine HCl, pheniramine maleate, acetaminophen, guaifenesin, pyrilamine maleate, chlorpheniramine maleate, triprolidine HCl, dextromethorphan HBr, diphenhydramine HCl in cough and cold pharmaceuticals.

Methods: The separation of these compounds was achieved within 37.9 min on a Nucleodur gravity C18 column (250 x 4.0 mm, 5 μ m). The chromatographic separation of these compounds performed in a single run by using isocratic mobile phase consisting of methanol:buffer mixture (38:62, v/v) at room temperature, with flow rate of 0.75 mL.min⁻¹.

An ultraviolet absorption at 210 nm was monitored. 2,4,6-trimethoxybenzaldehyde was used as an internal standard (ISTD). The selectivity, linearity of calibration, accuracy, intraday and interday precision and forced degradation studies were examined as parts of the method validation.

Results: The concentration–response relationship was linear over a concentration range of 0.2-250 μ g.mL⁻¹ for acetaminophen, 0.5–250 μ g.mL⁻¹ for pseudoephedrine HCl and pheniramine maleate, 1–250 μ g.mL⁻¹ for guaifenesin, 2.5-250 μ g.mL⁻¹ for chlorpheniramine maleate and triprolidine HCl, 5-250 μ g.mL⁻¹ for pyrilamine maleate and diphenhydramine HCl, 10-20 μ g.mL⁻¹ for dextromethorphan HBr with correlation coefficients better than 0.9993. The relative standard deviations of the intraday and interday were all less than 4%. Conclusion: The proposed liquid chromatographic method was successfully applied for the routine analysis of these compounds in different cough and cold pharmaceutical preparations such as syrups and tablets.

Keywords: High performance liquid chromatography, Active Ingredients Cough and Cold Pharmaceuticals, Validation.

INTRODUCTION

Cough and cold pharmaceutical preparations are one of the most extended formulations in the world and have got many pharmaceutical forms: syrup, suspension, sachets, capsules and tablets [1]. These preparations represent complex formulations containing several active ingredients and a broad spectrum of excipients such as flavoring agents, saccharose or aspartame, acidulants, natural or artificial colorings and flavoring agents, dyes sweeteners and preservatives [2,3]. These compounds are contained in the pharmaceutical form in very different proportions and present chemical forms of very different nature [4].

Acetaminophen (paracetamol) is analgesic and antipiretic [5]. As pain and fever are common, no home should be without some paracetamol, particularly homes with children. Acetaminophen is available in many different pharmaceutical preparations such as tablets, capsules, and liquid suspensions [6]. Chlorpheniramine maleate inhibits the effects of histamine on capillary permeability and bronchial smooth muscles. It is an anti-allergic drug, widely used in cough-cold preparations. The combination of antihistamine such as pyrilamine maleate and chlorpheniramine maleate is used to overcome the allergic effects and reduce or relieve cold symptoms [3]. Pheniramine maleate, diphenhydramine HCl, triprolidine HCl and pseudoephedrine hydrochloride are widely used in combination with other drugs for the clinical treatment of common cold, sinusitis, bronchitis and respiratory allergies [7]. Two common actives in such products are dextromethorphan HBr and guaifenesin. Dextromethorphan HBr is an antitussive which acts through depression of the medullary centers of the brain to decrease the involuntary urge to cough [8-11]. Guaifenesin is an expectorant believed to stimulate receptors that initiate a reflex secretion of respiratory tract fluid, thereby increasing the volume while decreasing the viscosity of mucus in the lungs. This action facilitates removal of mucus and reduces irritation of the bronchial tissue. Dextromethorphan hydrobromide and guaifenesin were used as cough suppressants antitussive for the relief of nonproductive cough

and cold preparations [12]. All these components have different polarities and exist in very different proportion. Due to these characteristics and because of diverse properties inherent to their formulation, these preparations offer an analytical problem [13].

A variety of methods exist in the literature for the determination of some of these compounds [6,14-23]. Among them Louhaichi et al, have provided maximum separation that six active ingredients were separated, simultaneously [23]. But the presented study has identified the separation of nine active ingredients simultaneously.

The aim of this study was to develop basic, accurate and selective LC method for the simultaneous determination of pseudoephedrine HCl, pheniramine maleate, acetaminophen, guaifenesin, pyrilamine maleate, chlorpheniramine maleate, triprolidine HCl, dextromethorphan HBr, diphenhydramine HCl in cough and cold pharmaceuticals. The method was then subjected to validation. The validation characteristics were evaluated as the selectivity, intraday and interday precision, linearity, accuracy, LOQ and LOD values and stress forced degradation studies.

The proposed liquid chromatographic method was successfully applied for the routine analysis of these compounds in different cough and cold pharmaceutical preparations such as syrups and tablets.

MATERIALS AND METHODS

Instrumentation and Chromatographic Conditions

The integrated high performance liquid chromatography system (LC 1100, Hewlett-Packard, USA) is equipped with a diode-array UV detector, a quaternary pump, a degasser, an autosampler, an injector with 20 μ L loop, and a column oven. Different columns were tested for analysis and pseudoephedrine HCl and acetaminophen peaks was observed to overlap in the other columns. Therefore separation was carried out using Nucleodur gravity C18 column (250 x 4.0 mm, 5 μ m).

The mobile phase was a mixture of 38% methanol, 62% of 80 mM KH_2PO_4 aqueous solution adjusted to pH 3.0, to which was added 10% (v/v) orthophosphoric acid. The mobile phase was vacuum-filtered through a 0.45 μm nylon filter and degassed on-line by micro vacuum degasser. The chromatographic separation of these compounds performed at room temperature. Analysis was run at flow rate of 0.75 $\text{mL}\cdot\text{min}^{-1}$ with 37.9 min run time. The analysis was carried out at 210, 220, 254 and 280 nm and the best separation and high peak area have been monitored at 210 nm. The injection volume was 20 μL .

Reagents and Chemicals

Pheniramine maleate, acetaminophen, guaifenesin, pyrilamine maleate, chlorpheniramine maleate, triprolidine HCl, dextromethorphan HBr, diphenhydramine HCl, HPLC grade methanol, sodium benzoate, 1,2-propylene glycol, citric acid, sorbitol and sodium saccharin were purchased from Sigma-Aldrich. Sodium carboxymethyl cellulose, pseudoephedrine HCl, 2,4,6-trimethoxybenzaldehyde, sunset yellow, and orthophosphoric acid were purchased from Fluka. Potassium dihydrogenphosphate and glycerol were obtained from Riedel-de Haën. Orange and cinnamon flavor were purchased from Eurofragrance.

Water was purified (18 $\text{M}\Omega\cdot\text{cm}^{-1}$ quality) from New Human Power I (Korea).

The commercialized pharmaceutical products used are detailed below:

Actidem syrup (10 mg dextromethorphan HBr, 30 mg pseudoephedrine HCl and 1.25 mg/5 mL triprolidine HCl for 150 mL) was manufactured by GlaxoSmithKline, France.

Actifed syrup (30 mg pseudoephedrine HCl and triprolidine HCl 1.25 mg/5 mL for 150 mL) was manufactured by GlaxoSmithKline, France.

Aferin plus pediatric syrup (160 mg acetaminophen, 1 mg chlorpheniramine maleate, 15 mg/5 mL for 100 mL) was manufactured by Hüsnü Arsan, Turkey.

Benical syrup (10 mg dextromethorphan HBr, 20 mg pseudoephedrine HCl, 2 mg/5 mL chlorpheniramine maleate for 100 mL) was manufactured by Bayer, Germany.

Corsal syrup (120 mg acetaminophen, 2 mg chlorpheniramine maleate, 5 mg/5 mL phenylephrine HCl for 120 mL) was manufactured by İ.E Ulagay, Turkey.

Katarin pediatric syrup (120 mg acetaminophen, 50 mg oxolamine citrate, 1 mg/5 mL chlorpheniramine maleate for 100 mL) was manufactured by Biofarma, Turkey.

Kongest syrup (160 mg acetaminophen, 2.5 mg chlorpheniramine maleate, 1 mg/5 mL phenylephrine HCl for 100 mL) was manufactured by Eczacıbaşı, Turkey.

Peditus syrup (120 mg acetaminophen, 50 mg guaifenesin, 6.25 mg pyrilamine maleate, 5 mg/5 mL phenylephrine HCl for 100 mL) was manufactured by Sandoz, Turkey.

Sudafed syrup (30 mg/5 mL pseudoephedrine HCl for 150 mL) was manufactured by GlaxoSmithKline, France.

Benical cold film tablet (500 mg acetaminophen, 30 mg pseudoephedrine HCl, 20 mg dextromethorphan HBr for one tablet) was manufactured by Bayer, Germany.

Corsal capsule (300 mg acetaminophen, 2 mg chlorpheniramine maleate, 5 mg phenylephrine HCl for one tablet) was manufactured by İ.E Ulagay, Turkey.

Gerakon fort tablet (650 mg acetaminophen, 10 mg phenylephrine HCl, 4 mg chlorpheniramine maleate for one tablet) was manufactured by Münir Şahin, Turkey.

Kongest forte tablet (650 mg acetaminophen, 4 mg chlorpheniramine maleate, 10 mg phenylephrine HCl for one tablet) was manufactured by Eczacıbaşı, Turkey.

Sudafed syrup (60 mg pseudoephedrine HCl for one tablet) was manufactured by GlaxoSmithKline, France.

Theraflu forte film tablet (650 mg acetaminophen, 10 mg phenylephrine HCl, 4 mg chlorpheniramine maleate for one tablet) was manufactured by Novartis, Switzerland.

All these medicines were purchased by local pharmacy.

Standard solutions and sample preparation for quantification

Stock standard solutions of pseudoephedrine HCl, pheniramine maleate, acetaminophen, guaifenesin, pyrilamine maleate, chlorpheniramine maleate, triprolidine HCl, dextromethorphan HBr and diphenhydramine HCl were prepared in ultrapure water. The calibration curves were prepared by diluting the stock solution in the mobile phase to furnish solutions with final concentrations of 0.2-250 $\mu\text{g}\cdot\text{mL}^{-1}$ for acetaminophen, 0.5-250 $\mu\text{g}\cdot\text{mL}^{-1}$ for pseudoephedrine HCl and pheniramine maleate, 1-250 $\mu\text{g}\cdot\text{mL}^{-1}$ for guaifenesin, 2.5-250 $\mu\text{g}\cdot\text{mL}^{-1}$ for chlorpheniramine maleate and triprolidine HCl, 5-250 $\mu\text{g}\cdot\text{mL}^{-1}$ for pyrilamine maleate and diphenhydramine HCl, 10-250 $\mu\text{g}\cdot\text{mL}^{-1}$ for dextromethorphan HBr.

The syrup placebo was prepared wherein: Citric acid was dissolved in glycerin. Sodium benzoate, sorbitol and sodium saccharin were dissolved in ultra pure water. Sodium carboxymethyl cellulose was kept in water for swelling. Then propylene glycol, flavors and colouring agent were added to this mixture and diluted with ultrapure water to 100 mL [24]. The excipients of syrup placebo were shown in Table 1.

Table 1: Excipients of placebo syrup

Excipients	Amount
Citric Acid	0.3 g
Glycerin	10 g
Propylene glycol	10 g
Sodium benzoate	0.02 g
Sodium carboxymethyl cellulose	0.1 g
Sorbitol	20 g
Sodium saccharin	0.04 g
Sunset yellow	enough amount
Orange aroma	1 drop
Cinnamon aroma	1 drop

The amounts of the commercial cough and cold liquid were depending on the drug concentration of various products. The commercial cough and cold syrups were diluted with mobile phase according to linear range of standards. The resulting solutions were vortexed for 15 min and a portion of the sample was filtered through a 0.45 μm filter before injection in the HPLC.

The mean weight of finely powdered tablets were accurately transferred into 50 mL calibrated flask and ultrapure water was added. The mixtures were extracted in the ultrasonic bath for 15 min at room temperature and diluted with ultrapure water to the mark. The solutions were filtered through a 0.45 μm filter. Then the solutions were diluted with mobile phase depending on the drug concentration of various products. All preparations were performed in three replicates.

System Suitability Tests

As system suitability test is an integral part of chromatographic methods development and it is used to verify that the system is adequate for the analysis to be performed, the parameters for pseudoephedrine HCl, pheniramine maleate, acetaminophen, guaifenesin, pyrilamine maleate, chlorpheniramine maleate, triprolidine HCl, dextromethorphan HBr and diphenhydramine HCl were evaluated. Several parameters may be used to demonstrate that the chromatographic system as a whole continues to be fit for the intended purpose. As well as monitoring the column performance, we can monitor the performance of the injector, pumps, and detector and so together provide an overview of system suitability. The user may define the minimum performance values or acceptance criteria according to local needs or business requirements [25].

System suitability test parameters were checked to ensure that the system was working correctly during the analysis [26]. Parameters which are typically used in suitability evaluations are capacity factor (k'), selectivity factor (α), resolution (R), number of theoretical plates (N) and tailing factor (T). For an optimum separation, capacity factor should be in the range of $0.5 < k' < 10$. A value of 1.5 for resolution implies a complete separation of two compounds. The number of theoretical plates must be higher than 2000. The calculated values of tailing factor should be in the range of $0.5 \leq T \leq 2$.

Validation

A full validation of assay consisting of selectivity, linearity, lower limit of detection and quantitation (LOD and LOQ), intraday and interday accuracy and precision of the method was performed according to the ICH description [27].

Selectivity

Selectivity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

Linearity and Range

The linearity of the assay was performed with a six point calibration curve prepared by diluting stock analyte solution in placebo syrup sample for five consecutive days. Six point calibration curves of each analyte were obtained by linear regression analysis.

The lowest concentration that can be quantified (LOQ) with acceptable accuracy and precision was evaluated at a signal-to-noise ratio of 10. Limit of detection (LOD) was evaluated at a signal-to-noise ratio of 3.

Accuracy and Precision

The accuracy of the proposed procedure was evaluated by means of recovery experiments. Recoveries were calculated as peak area ratios of reference standard/analyte (spiked placebo) at different concentration.

The precision was expressed as relative standard deviation of a series of measurements. Three different concentrations of standard solutions were analyzed five consecutive days and five times within the same day.

Forced Degradation Conditions

Forced degradation studies was carried out to demonstrate that the method was stability indicating. Solutions were prepared containing each substance at working standard concentration. They were treated with the following conditions:

- a) Acid conditions: Solutions were acidulated with 37% HCl to reach a final concentration of 0.1 M HCl and heated for 2 h and 8 h at 80°C, respectively.
- b) Basic conditions: Solutions were treated with 1 M NaOH to reach a final concentration of 0.1 M NaOH and heated for 2 h and 8 h at 80°C, respectively.
- c) Oxidation with H₂O₂: Solutions were treated with 3% (v/v) H₂O₂ for 2 h and 8 h, respectively.
- d) UV radiation: Solutions were exposed under a UV light at 254 nm during 35 h.
- e) Thermal conditions: Solutions were heated for 2 h, 8 h and 24 h at 60 °C and 80 °C, respectively.

RESULTS AND DISCUSSION

Method Optimization

The first step of the study was the optimization of the chromatographic conditions. Various mobile phase combinations were tried initially to separate pseudoephedrine HCl, pheniramine maleate, acetaminophen, guaifenesin, pyrillamine maleate, chlorpheniramine maleate, triprolidine HCl, dextromethorphan HBr and diphenhydramine HCl on C18 column. The mixture of methanol

and phosphate buffer (38:62, v/v) was capable of a good separation and sensitivity (Figure1).

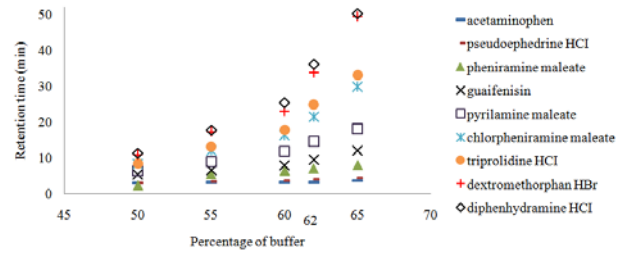


Fig. 1: Effect of methanol and phosphate buffer composition on separation of the mixture

The effect of phosphate buffer concentration (20, 40, 60, 80 ve 100 mM) on the retention time of mixture was investigated in methanol-buffer (38:62) mobile phase. The concentration of the phosphate buffer solution was chosen as 80 mM for optimum separation (Figure 2).

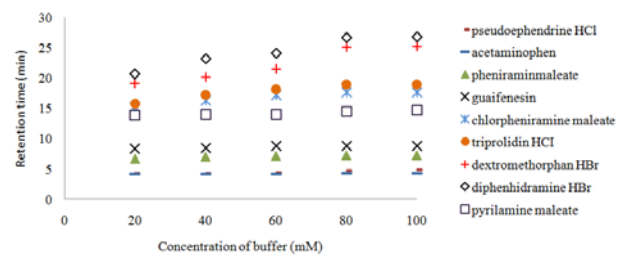


Fig. 2: Effect of phosphate buffer concentration on separation of the mixture.

In order to find suitable buffer pH, the effect of pH on retention and resolution was investigated over the range of 2.0 and 6.0, using 10% (v/v) ortho-phosphoric acid solution. pK_a values of these compounds are higher than 8. The changes in retention time as a function of pH result from changes in the ionization form of these solutes. Therefore, a pH value of 3.0 was selected because of optimum resolution. As shown in Figure 3, the resolution of all active compounds under the optimum conditions was adequate.

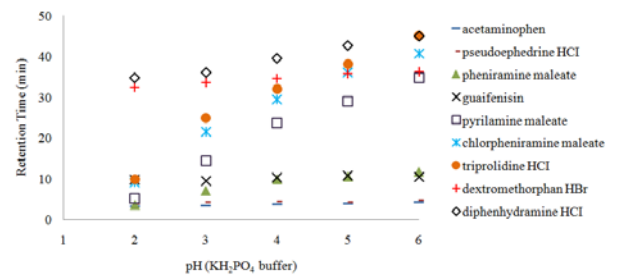


Fig. 3: Effect of phosphate buffer pH on separation of the mixture

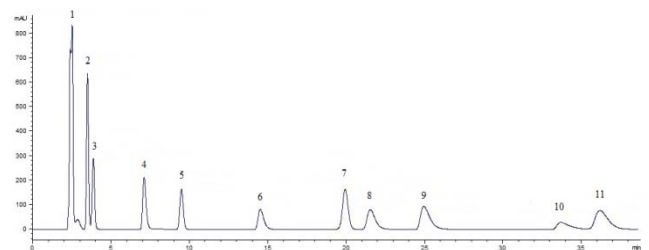


Fig. 4: A typical chromatogram of standard active ingredients. 1, maleic acid; 2, acetaminophen; 3, pseudoephedrine HCl; 4, pheniramine maleate; 5, guaifenesin; 6, pyrillamine maleate; 7, 2,4,6-trimethoxybenzaldehyde; 8, chlorpheniramine maleate; 9, triprolidine HCl; 10, dextromethorphan HBr; 11, diphenhydramine HCl.

Method Validation

System suitability

The important parameter t_0 was 2.319 ± 0.062 min in the analysis. This was the time of KBr peak. The capacity factor (k') values were in the range of $0.5 < k' < 10$ except dextromethorphan HBr and diphenhydramine HCl. The resolution value for separation of acetaminophen and pseudoephedrine HCl was 1.3801 and dextromethorphan HBr and diphenhydramine HCl was 1.4995. The resolution values of

other compounds were higher than 1.5. The theoretical plate numbers of all compounds were higher than 2600 and the calculated tailing factors of them were obtained in the acceptable range of $0.5 \leq T \leq 2$.

Selectivity

The representative chromatogram (Figure 5) of placebo solution constituted by excipient blend showed that there was no interfering peak in the retention times corresponding to the analytes. Therefore, the proposed method was considered to be selective.

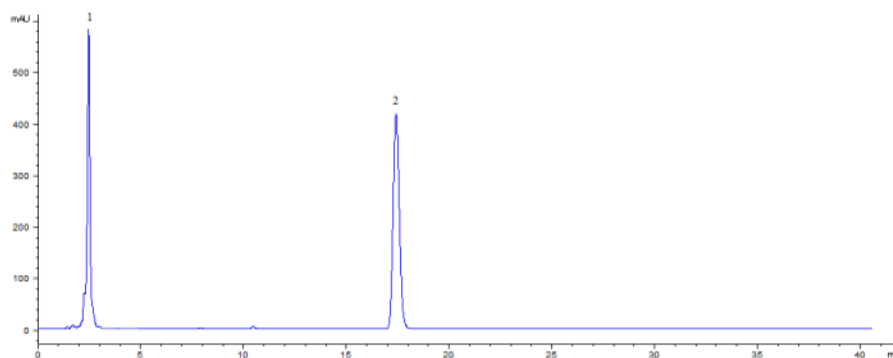


Fig. 5: Chromatogram of placebo solution, 1. citric acid; 2. sodium benzoate.

Linearity and Range

To evaluate linearity of the method, six different concentrations of the nine analytes in the range of 0.2-250 mg mL⁻¹ for acetaminophen, 0.5-250 mg mL⁻¹ for pseudoephedrine HCl and pheniramine maleate, 1-250 mg mL⁻¹ for guaifenesin, 5-250 mg mL⁻¹ for pyrillamine maleate and diphenhydramine HCl, 2.5-250 mg mL⁻¹ for chlorpheniramine maleate and triprolidine HCl, 10-250 mg mL⁻¹ for dextromethorphan HBr were analysed and the calibration curves for nine active compounds constructed under optimum conditions as the ratio of the peak areas of analysed substance to internal standard against the concentration and the results are presented in Table 2.

The limit of detection (LOD) and limit of quantification (LOQ) of the method were determined by injecting progressively low concentrations of the standard solutions.

Accuracy and Precision

Accuracy was evaluated with recoveries obtained in the analysis of synthetic sample prepared in the placebo and compared with the corresponding standards. The results indicate good accuracy of the method for the simultaneous determination of the active compounds as revealed by mean recovery data. The results are given in Table 3. For evaluation of the precision estimates, intra-day and inter-day precision were performed for each active compound. The intra-day precision of the method was determined by preparing the standards of nine active compounds at three different concentration and values for each compounds were determined by five repeated analyses. Inter-day precision was checked with the same concentration as intra-day assay, and the determination of each active compound was repeated day by day during five days. The obtained results are shown in Table 3.

Table 2: Linearity study results (n=5)

	Acetaminophen	Pseudoephedrine HCl	Pheniramine Maleate	Guaifenesin	Pyrillamine Maleate	Chlorpheniramine Maleate	Tripolidine HCl	Dextromethorphan HBr	Diphenhydramine HCl
Regression equation	y=0.3659x+0.0583	y=0.3x+0.0134	y=0.3698x-0.0462	y=0.2798x+0.0225	y=0.2236x-0.0015	y=0.3101x-0.0205	y=0.4438x+0.0116	y=0.2556x-0.0351	y=0.4565x-0.0059
Standard error of intercept	0.0222	0.0047	0.0188	0.0175	0.0011	0.0092	0.0162	0.0046	0.0243
Standard error of slope	0.0244	0.0071	0.0099	0.0162	0.0012	0.0085	0.0058	0.0007	0.0091
Correlation coefficient (r)	0.9993	0.9997	0.9993	0.9991	0.9999	0.9999	0.9998	0.9990	0.9997
Linearity range (mg L ⁻¹)	0.2-250	0.5-250	0.5-250	1-250	5-250	2.5-250	2.5-250	10-250	5-250
LOD (mg L ⁻¹)	0.1	0.1	0.2	0.2	0.2	0.5	0.75	2.5	0.75
LOQ (mg L ⁻¹)	0.3	0.5	0.75	1	5	2.5	2.5	10	5

Forced Degradation

The drug substances were found almost stable in peroxide degradation. No degradation was seen in 3% H₂O₂ at 80 °C up to 2 and 8 hours.

Under the acidic conditional at the end of 2h and 8 h, large amount of fall in active compounds peaks area was observed except guaifenesin.

Under the alkaline conditional at the end of 8 h, same active compounds peaks was disappeared.

For photo degradation studies, all standards solution exposed to UV-light for 35 h: in these condition no a large amount of fall in peaks area.

For thermal degradation studies, all standard solutions were heated for 2 h and 8 h at 60 °C and 80 °C, respectively: in these condition no a large amount of fall in peaks area.

The all results were given Table 4.

Analysis of samples

Chromatograms of the some samples were shown below (Fig.6-12). The amount of each active compound was appointed using calibration curve method. The results demonstrate that the label claims of drugs close with obtained results which confirms the good accuracy of the proposed method (Table 5, Table 6).

Table 3: The intra-assay (intra-day) and between-assay (inter-day) precision and accuracy results (n=5)

Compound	Added (mg L ⁻¹)	Intra-day		Inter-day	
		Precision (RSD%)	Accuracy (Recovery%)	Precision (RSD%)	Accuracy (Recovery%)
Acetaminophen	50	1.4420	101.6520	1.4658	101.9980
	100	0.6876	100.3878	0.6902	101.2141
	250	1.0201	100.5995	1.1025	102.3410
Pseudoephedrine HCl	50	3.3608	98.7927	3.7541	96.3060
	100	1.3449	100.0609	1.3457	100.2120
	250	0.3759	99.9519	0.3854	100.5387
Pheniramine Maleate	50	2.3691	99.1892	2.3499	100.1165
	100	1.2286	101.1458	1.2427	103.1933
	250	1.0122	99.4358	1.0065	100.0570
Guaifenesin	50	0.9491	100.8194	0.9569	101.0235
	100	0.7833	100.2711	0.7854	100.3749
	250	2.2236	101.6682	2.5607	103.1516
Pyrilamine Maleate	50	3.9870	101.2402	4.0364	103.0688
	100	3.3658	100.8789	3.3954	101.0122
	250	3.9282	99.0023	3.8890	100.0521
Chlorpheniramine Maleate	50	2.5739	101.8374	2.6212	102.056
	100	0.5632	100.9189	0.5684	100.9465
	250	1.4962	101.3465	1.5163	102.9471
Triprolidine HCl	50	2.2186	100.0368	2.9564	101.5857
	100	1.6736	102.1042	1.7089	103.2254
	250	2.0286	101.1976	2.6815	103.8761
Dextromethorphan HBr	50	2.0466	99.5917	2.9157	102.0081
	100	2.0346	101.4256	2.0636	101.9875
	250	1.2113	100.8714	1.2218	101.0159
Diphenhydramine HCl	50	1.1565	100.6600	1.1641	101.1572
	100	0.4162	100.2087	0.4170	100.8704
	250	0.4766	99.4162	0.4738	99.9141

Table 4: The results of stress tests

	Recovery(%) ^a												
	HCl ^b		H ₂ O ₂ ^b		NaOH ^b		Photolitic Degradation ^b UV Lamp 35 h	Thermal Degredation ^b					
	2 h 80 °C	8 h 80 °C	2 h 80 °C	8 h 80 °C	2 h 80 °C	8 h 80 °C		2 h 60 °C	8 h 60 °C	24 h 60 °C	2 h 80 °C	8 h 80 °C	24 h 80 °C
Acetaminophen	56.12	0	99.5	86.3	53.8	0	94.5	99.1	98.6	98.7	98.9	96.6	97.3
Pseudoephedrine HCl	73.07	65.8	97.3	95.6	80.5	74.6	91.1	98.9	97.3	96.9	98.3	96.5	95.9
Pheniramine Maleate	Poor peak shape	Poor peak shape	93.1	84.3	70.9	57.2	72.7	96.5	95.7	89.1	94.1	88.2	85.4
Guaifenesin	94.1	91.9	98.2	95.7	83.5	63.8	90.5	99.8	99.2	98.6	99.3	98.9	98.0
Pyrilamine Maleate	76.7	36.3	89.9	86.2	46.8	0	74.4	96.8	95.2	89.7	89.2	85.9	82.9
Chlorpheniramine Maleate	90.4	78.2	94.7	75.9	54.9	20.9	54.3	95.8	94.3	90.1	93.5	90.5	87.3
Tripolidine HCl	81.1	71.7	90.4	79.4	23.3	0	82.0	95.1	93.0	89.4	92.4	88.7	85.8
Dextromethorphan HBr	84.7	63.9	98.8	92.7	93.0	36.4	71.5	99.2	98.5	96.5	98.8	96.1	92.7
Diphenhydramine HCl	18.9	6.6	97.5	85.9	30.3	0	72.5	99.5	97.6	88.7	98.6	90.8	87.2

^aRecovery(%) = [(mg.mL⁻¹) after stres/(mg.mL⁻¹) initial analyze] x 100; ^bStress conditions

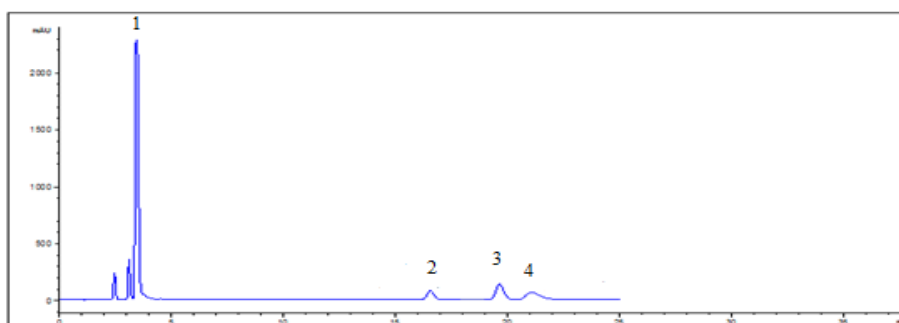


Fig. 6: The chromatogram belongs to corsal syrup, 1, acetaminophen; 2, sodium benzoate; 3, ISTD; 4, chlorpheniramine maleate.

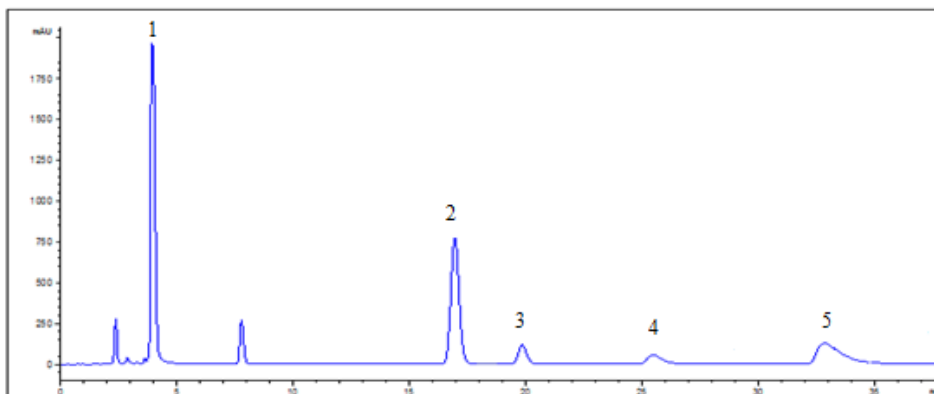


Fig. 7: The chromatogram belongs to actidem syrup, 1, pseudoephedrine HCl; 2, sodium benzoate; 3, ISTD; 4, triprolidine HCl; 5, dextromethorphan HBr.

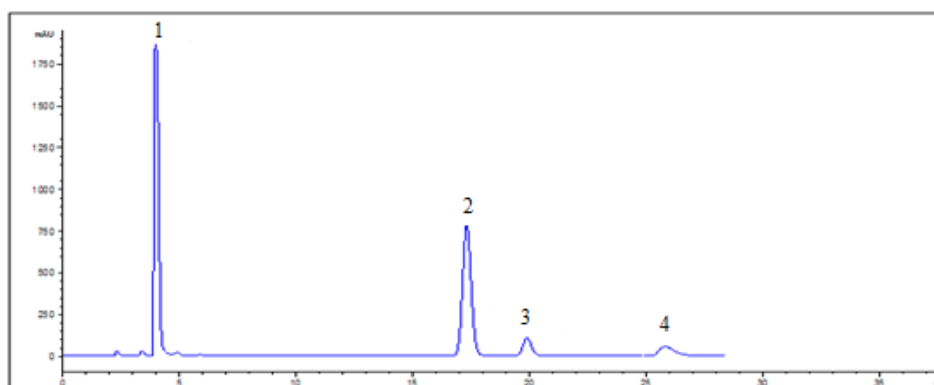


Fig. 8: The chromatogram belongs to actifed syrup, 1, pseudoephedrine HCl; 2, sodium benzoate; 3, ISTD; 4, triprolidine HCl

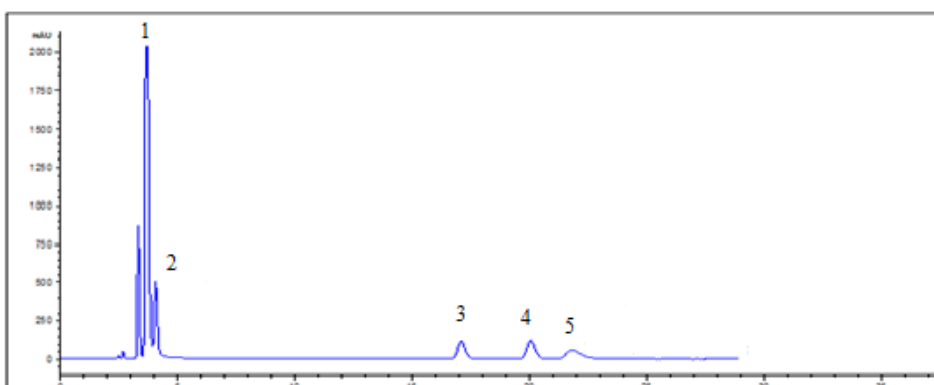


Fig. 9: The chromatogram belongs to aferin syrup, 1, acetaminophen; 2, pseudoephedrine HCl; 3, sodium benzoate; 4, ISTD; 5, chlorpheniramine maleate.

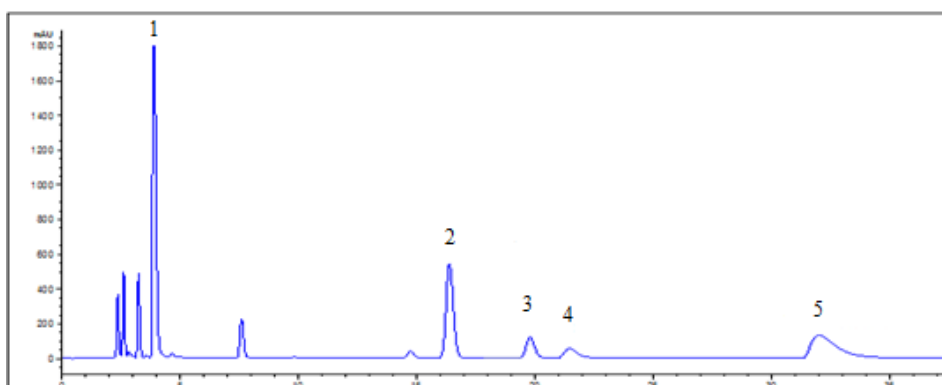


Fig. 10: The chromatogram belongs to benical syrup, 1, pseudoephedrine HCl; 2, sodium benzoate; 3, ISTD; 4, chlorpheniramine maleate; 5, dextromethorphan HBr.

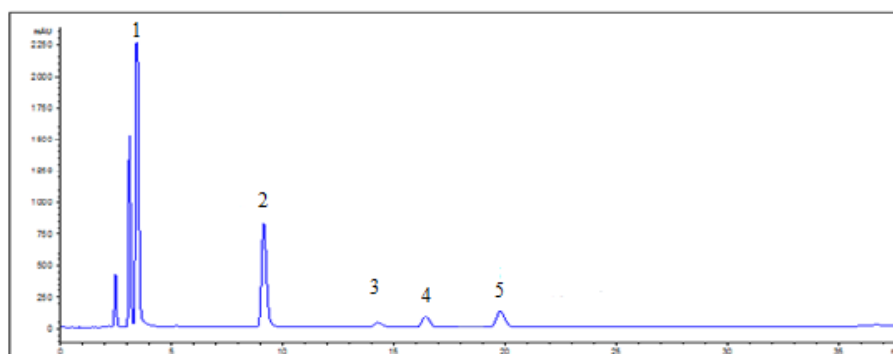


Fig. 11: The chromatogram belong to peditus syrup, 1, acetaminophen; 2, guaifenisin; 3, pyrillamine maleate; 4, sodium benzoate; 5, ISTD.

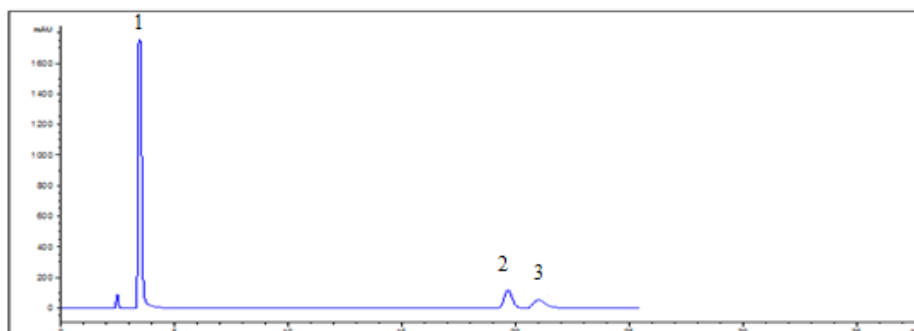


Fig. 12: The chromatogram belongs to kongest tablet, 1, acetaminophen; 2, ISTD; 3, chlorpheniramine maleate.

Table 5: Content of commercial cough syrup with respect to label amount claimed

Samples	Compounds	Labeled Amount (mg.L ⁻¹)	Found Amount±SD (mg.L ⁻¹)	Relative error (%)
Actidem	Pseudoephedrine HCl	6000	5961.2±1.9	-0.65
	Tripolidine HCl	250	250.9±2.6	+0.36
Actifed	Dextrmethorphan HBr	2000	2016.1±3.9	+0.81
	Pseudoephedrine HCl	6000	6275.6±3.5	+4.59
Aferin	Tripolidine HCl	250	251.8±4.3	+0.72
	Acetaminophen	32000	32351.2±3.7	+1.10
Benical	Pseudoephedrine HCl	3000	3072.8 ±1.9	+2.43
	Chlorpheniramine maleate	200	207.41±1.2	+3.71
	Pseudoephedrine HCl	4000	4021.8±1.9	+0.55
Corsal	Chlorpheniramine maleate	400	398.5±4.9	-0.38
	Dextromethorphan HBr	2000	2076.5±3.9	+3.83
Katarin	Acetaminophen	24000	24067.8±0.9	+0.28
	Chlorpheniramine maleate	400	402.6±1.7	+0.65
Kongest	Acetaminophen	24000	24982.4±0.3	+4.09
	Chlorpheniramine maleate	200	216.4±3.4	+8.20
Peditus	Acetaminophen	32000	32842.3±6.1	+2.63
	Chlorpheniramine maleate	500	501.55±0.9	+0.31
Sudafed	Acetaminophen	24000	24412.4±6.1	+1.72
	Guaifenisin	10000	10690.3±3.1	+6.90
	Pyrilamine maleate	1250	1254.4±3.01	+0.35
Sudafed	Pseudoephedrine HCl	6000	6003.1±2.1	+0.05

Table 6: Content of commercial cough tablet with respect to label amount claimed.

Samples	Compounds	Labeled Amount (mg.L ⁻¹)	Found Amount±SD (mg.L ⁻¹)	Relative error (%)
Benical	Acetaminophen	10000	10010.7±1.25	+0.11
	Pseudoephedrine HCl	600	600.2±1.62	+0.33
	Dextromethorphan HBr	400	397.9±1.38	-0.53
Corsal	Acetaminophen	6000	5993.0±0.98	-0.12
	Chlorpheniramine maleate	40	40.1±0.71	+0.25
Gerakon	Acetaminophen	13000	13080.1±2.21	+0.62
	Chlorpheniramine maleate	80	79.8±2.76	-0.25
Kongest	Acetaminophen	6000	6016.6±1.45	+0.28
	Chlorpheniramine maleate	40	40.67±1.92	+1.67
Theraflu	Acetaminophen	13000	13049.7±1.51	+0.38
	Chlorpheniramine maleate	80	80.1±1.27	+0.18
Sudafed	Pseudoephedrine HCl	1200	1205.6±1.78	+0.47

The obtained results were satisfactory for each compound. Because they show that the content of drug corresponds to the drug label. Therefore they confirm the good accuracy of the proposed method.

CONCLUSION

A basic and reliable HPLC method has been developed and validated for the determination of pseudoephedrine HCl, pheniramine maleate, acetaminophen, guaifenesin, pyrilamine maleate, chlorpheniramine maleate, triprolidine HCl, dextromethorphan HBr and diphenhydramine HCl in cough and cold pharmaceuticals. Compared to the other reported ones, the developed method offers separation of a large number of the active ingredients simultaneously. Thus large number of cough and cold pharmaceuticals could be analyzed by only one method. Forced degradation studies led to understand the chemical properties of drug molecules. Stability-indicating nature of the method was demonstrated on the experimental cough and cold syrup preparation under oxidation, acidic and alkaline, UV-light and thermal stress conditions. Although there is no acceptance criteria concerning degradation products, stability study provided to predict usage and storage conditions of the syrup.

ACKNOWLEDGMENT

I thank Dr. M. Sinan Kaynak from Department of Pharmaceutical Technology for preparation of placebo syrup. This research was supported by the İnönü University Research Council (Project no: 2010/131).

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