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Research Article

An HPLC Method for Determination of 15 Pharmaceutical Compounds in Anti-Cold Products

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Abstract

A validated HPLC method was developed for determination of paracetamol, phenylephrine hydrochloride, pseudoephedrine hydrochloride, salicylamide, guaifenesin hydrochloride, sodium benzoate, methylparaben , chlorpheniramine maleate, triprolidine hydrochloride, dextromethorphan hydrobromide, diphenhydramine hydrochloride , promethazine hydrochloride , propylparaben and both of para aminophenol and 4-chloroacetanilide as related compounds for paracetamol in different pharmaceutical dosage forms of anti-cold products such as tablet, syrup, suspension and sachet using symmetry C18 column at 25°C with UV detection at 215 nm. A linear gradient elution was employed starting with 92% mobile phase A and 8% mobile phase B for 6 min to reach 73% mobile phase A and 27% mobile phase B at 20 min then 60% mobile phase A and 40% mobile phase B at 35 min. The total run time is 40 min using solution of 30 mM sodium dihydrogen phosphate containing 3 mM hexanesulphonic acid sodium salt and adjusted to apparent pH 3.0 with phosphoric acid as mobile phase A and acetonitrile as mobile phase B. All mentioned compounds have been successfully separated and quantified using the developed method. The developed method was linear with (r =0.9999) for all compounds. The proposed method was applied successfully for determination of the constituent of 22 anti-cold pharmaceutical products.

Keywords: HPLC, anti-cold products, related substances, preservatives

INTRODUCTION

The necessity to ensure the quality of pharmaceutical multi component preparations , and consequently the safety and efficacy of the final marketed product, has led to the development and evaluation of new methods that can reduce the time and cost of analysis of the main active components, preservative and their related compounds in one system. Paracetamol (PA), phenylephrine hydrochloride (PH), pseudoephedrine hydrochloride (PS), salicylamide (SA), guaifenesin hydrochloride(GU), chlorpheniramine maleate (CH), triprolidine hydrochloride (TR), dextromethorphan hydrobromide (DX), diphenhydramine hydrochloride (DI), promethazine hydrochloride (PR) are compounds commonly used as active ingredients in cold medicine formulations due to their analgesic, antipyretic , decongestant and antihistamine effects (Reynolds, 2009). These compounds are formulated in different pharmaceutical dosage forms including syrups; therefore, addition of preservatives such as methylparaben (MP), prorylparaben (PP) or sodium benzoate (SB) is required.

P-aminophenol (PM) and 4-chloroacetanilide (CA) are the related compounds for PA. All of these 15 compounds were simultaneous determined using the developed HPLC method.

Several methods in the literature describing the determination of these active compounds in various formulations have

been reported. UV-Vis spectroscopy (Shija et al., 1992; Jones et al., 1985; Milch and Szabó, 1991; Korany et al., 1982; Goicoechea et al., 2002; Goicoechea et al., 1999; Arama and Georgita, 2002; Hadad et al., 2005; El-Gindy et al., 2006; El-Gindy et al., 2005), high performance liquid chromatography (Hadad et al., 2005; Grosa et al., 2006), thin layer chromatography (Mostafa, 2010; Majlát and Barthos, 1984), gas chromatography (Alberti et al., 1987; Harsano et al., 2005), capillary electrophoresis (Zhang et al., 2000, Azhagvuel and Sekar, 2007) and spectrofluorimetric (Vilchez et al., 1995) methods have been used to determine some of these compounds in their preparations.

There is no common analytical method have been reported in the literature for simultaneous determination of the studied compounds by a single method for different cough and cold dosage forms (Borkar and Sawant, 2011). In many cases the concentration of PA is significantly higher than the other studied compounds in pharmaceutical products. The presence of active pharmaceutical ingredients with different polarity and the disparity in concentration poses an analytical challenge (Heydari, 2008). Pharmacopoeial HPLC methods reported for each compound are inappropriate for their simultaneous determination due to interferences of their corresponding peaks (Borkar and Sawant, 2011).

In this work, an HPLC method was developed to determine the studied compounds in pharmaceutical products using ion pairing gradient HPLC method with C18 column.

The developed HPLC method is simple, accurate, reduced the duration of the analysis with stable base line. Other advantages of the proposed method include low limits of detection and quantitation with good precision (standard deviation less than 1%). The separated peaks of the studied compounds were symmetric with perfect homogeneity. It is suitable for routine determination of the components of the studied pharmaceutical products.

EXPERIMENTAL

Instrumentation

The HPLC WATERS with UV-visible photodiode array detector model 2996, WATERS binary HPLC pump model 1525 and WATERS 717 plus Auto sampler (WATERS Corporation, Milford, Massachusetts, USA). Symmetry C18 (5 µm) 250 x 4.6 mm column (5 µm particle size, WATERS corporation, Bellefonte, USA) was used for separation and quantitation. The temperature of the column is controlled by water oven unit. The detector was set at 215 nm. An EMPOWER software version 2002 was used for data acquisition.

MATERIALS AND REAGENTS

Pharmaceutical grade of PA, PS, PM and CA (Sigma-Aldrich, Inc, St. Louis, USA), TR (Vision Corporation, Mumbai, India), PH (Iwaki Seiyaku Co., LTD, Tokyo, Japan), SA (Alta Laboratories Limited, Maharashtra, India), GU (Granules India Limited, Hyderabad, India), CH (Ipca laboratories Limited, Mumbai, India), DX (F. Hoffmann-La Roche Ltd., Basal, Switzerland), DI (Dolder AG., Basal, Switzerland), PR (Harika Drugs Private Limited, Hyderabad, India), SB (Ganesh Benzoplast LTD, Mumbai, India), MP and PP (Ren Yu Cosmetics Factory , Hangzhou, China) were used and certified to contain 99.8, 99.6, 99.0, 99.7, 99.7, 99.8, 99.6, 99.6, 99.3, 99.7, 100.0, 100.0, 99.8, 99.5 and 99.7 %, respectively. Acetonitrile, Hexanesulphonic acid sodium salt, sodium dihydrogen phosphates and phosphoric acid were HPLC grade (Merck, Darmstadt, Germany).

Rinofed plus syrup was manufactured by Jazeera Pharmaceutical Industries, Riyadh, Saudi Arabia. Each 5 ml of the syrup was labeled to contain 1.25 mg TR, 25 mg PS, 125 mg PA and 20 mg SB.

The following pharmaceutical products were manufactured by Glaxo Smith Kline, Egypt: Actifed tablets; each tablet was labeled to contain 60 mg PS and 2.5 mg TR. Actifed plus tablet; each tablet was labeled to contain 500 mg PA, 60 mg PS and 2.5 mg TR. Panadol Sinus tablet; each tablet was labeled to contain 30 mg PS and 500 mg PA. Panadol cold tablet; each tablet was labeled to contain 30 mg PS, 500 mg PA and 2 mg CH. Panadol Night tablet: each tablet was labeled to contain 500 mg PA and 25 mg DI.

The following pharmaceutical products were manufactured by Gulf Pharmaceutical Industries (JULPHAR), Ras Al Khaimah, United Arab Emirates: Sedofan syrup; each 5 ml of the syrup was labeled to contain 1.25 mg TR, 25 mg PS, 5 mg MP and 1.5 mg PP. Sedofan DM syrup; each 5 ml of the syrup was labeled to contain 1.25 mg TR, 30 mg PS, 10 mg DX, 5 mg MP and 1.5 mg PP. Sedofan-T tablet; each tablet was labeled to contain 2.5 mg TR. Sedofan II tablet; each tablet was labeled to contain 60 mg PS. Adol tablet; each tablet was labeled to contain 500 mg PA. Adol cold caplet; each caplet was labeled to contain 325 mg PA, 30 mg PS and 15 mg DX. Adol Sinus caplet; each caplet was labeled to contain 325 mg PA and 30 mg PS. Adol Allergy Sinus caplet; each caplet was labeled to contain 325 mg PA, 30 mg PS and 2mg CH. Adol PM caplet; each caplet was labeled to contain 325 mg PA and 25mg DI. Adol PM sachet; each sachet was labeled to contain 650 mg PA and 50 mg DI. Adol Allergy Sinus sachet; each sachet was labeled to

contain 650 mg PA, 60 mg PS and 4 mg CH. Adol syrup; each 5 ml of the syrup was labeled to contain 120 mg PA. Adol suspension; each 5 ml of the suspension was labeled to contain 250 mg PA, 5 mg MP and 1.5 mg pp. Flukit tablet; each tablet was labeled to contain 200 mg PA, 250 mg SA, 5 mg PR and 5 mg PH. Flukit syrup; each 5 ml of the syrup was labeled to contain 120 mg PA, 15 mg PS and 2 mg CH. Exedexe tablet; each tablet was labeled to contain 15 mg DX.

HPLC Condition

The HPLC quantitation was performed by using C18 column (5 µm particle size, WATERS Corporation, Bellefonte, USA) at 25°C with UV detection at 215 nm. A linear gradient elution was used starting with 92% mobile phase A and 8% mobile phase B for 6 min to reach 73% mobile phase A and 27% mobile phase B at 20 min then 60% mobile phase A and 40% mobile phase B at 35 min. Total run time is 40 min using solution of 30 mM sodium dihydrogen phosphate containing 3 mM hexanesulphonic acid sodium salt and adjusted to apparent pH 3.0 with phosphoric acid as mobile phase A and acetonitrile as mobile phase B. The injection volume is 20 µL in triplicate. Before injection, samples were filtered through 0.45 µm filter (Millipore Corporation, Bedford, USA).

Preparation of stock standard solution

Stock standard solutions of the studied compounds were prepared separately by dissolving 50 mg of each compound in 50 mL acetonitrile.

Preparation of the calibration curve

The standard solutions were prepared by further dilutions of the stock standard solutions with mobile phase A to obtain concentrations mentioned in Table 1.
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. A very good correlation coefficient and small intercept were obtained (Table 1).

Table 1. Statistical analysis for the calibration graphs (*) of the studied compounds

Compound determined	calibration range (µg/ml)	detection limit (µg/ml)	quantitation limit (µg/ml)	intercept (a)	standard deviation of intercept S(a)	slop (b)	standard deviation of the slop S(b)	correlation coefficient (r)	C.V** (%)
PM	0.01-10	0.003	0.01	9.1×10^2	1.98×10^2	3.82×10^4	8.6	0.9999	0.62
PA	0.1-150	0.01	0.03	1.91×10^2	1.39×10^2	2.26×10^4	5.4	0.9999	0.65
PH	0.1-40	0.03	0.1	-4.4×10^2	1.39×10^2	1.65×10^4	4.1	0.9999	0.55
PS	0.1-40	0.03	0.1	-3.6×10^2	1.65×10^2	1.98×10^4	10.3	0.9999	0.45
SA	0.1-40	0.03	0.1	-3.9×10^2	1.63×10^2	4.06×10^4	15.5	0.9999	0.31
GU	0.3-40	0.1	0.3	-1.6×10^2	1.68×10^2	1.83×10^4	22.6	0.9999	0.65
SB	0.1-30	0.03	0.1	-8.6×10^3	1.64×10^2	2.13×10^4	5.5	0.9999	0.77
MP	0.1-20	0.03	0.1	-3.4×10^3	1.98×10^2	5.59×10^4	8.6	0.9999	0.62
CH	0.3-40	0.1	0.3	4.1×10^2	1.57×10^2	1.53×10^4	9.2	0.9999	0.68
TR	0.1-30	0.03	0.1	-3.5×10^2	1.89×10^2	3.65×10^4	9.2	0.9999	0.77
DX	0.3-40	0.1	0.3	-1.4×10^2	1.31×10^2	1.13×10^4	10.9	0.9999	0.49
DI	0.3-40	0.1	0.3	-4.4×10^2	1.22×10^2	1.65×10^4	15.3	0.9999	0.85
PR	0.3-40	0.1	0.3	-3.1×10^2	1.40×10^2	2.25×10^4	12.2	0.9999	0.61
PP	0.1-20	0.03	0.1	2.1×10^2	1.71×10^2	4.53×10^4	4.7	0.9999	0.48
CA	0.01-10	0.003	0.01	-1.6×10^2	1.22×10^2	2.15×10^4	4.7	0.9999	0.48

(*) Y = a + bC, where C is the concentration in µg/ mL and Y is the peak area
(**) C.V. for the values of peaks area / concentration (µg/mL) for 15 replicates

Analysis of pharmaceutical products

A suitable quantity of each pharmaceutical product was extracted and diluted with mobile phase A to reach concentration within the calibration range of the specified component. The general procedures described under calibration were followed and the concentrations of the determined compounds were calculated.

RESULTS AND DISCUSSION

For simultaneous determination of PA, PH, PS, SA, GU, CH, TR, DX, DI, PR, MP, PP, SB, PM and CA, it is necessary to make adjustments of the HPLC system to avoid overlapping and provide optimum separation of the peaks. Due to the variation of UV absorbance for the analyzed compounds the wavelength used for detection should be selected to give good response for all the tested compounds. The gradient system and the organic ratio of the mobile phase should be adjusted to give good separation for the tested compounds with less base line noise. The experimental variables were optimized to give a simple, sensitive and accurate HPLC method.

METHOD OPTIMIZATION

Effect of ion pair concentration

The influence of concentration of hexanesulphonic acid sodium salt (as ion pair) on the capacity factor (k`) of tested compounds were investigated at apparent pH 3.0 for 30 mM sodium dihydrogen phosphate. It was found that, an increase the concentration of ion pair caused an increase in the retention time of tested compounds. At a concentration of 3 mM for the ion pair, optimum resolution with reasonable retention time for all compounds was observed.

Effect of acetonitrile concentration

The influence of acetonitrile concentration in gradient elution on the capacity factor (k`) of tested compounds was investigated. It was found that, increase or decrease of the acetonitrile concentration cause interference between peaks of the tested compounds. Decreasing the concentration of acetonitrile cause a marked increase in the retention time with loss of the peak symmetry.

Effect of mobile phase pH

The influence of pH of the 30 mM sodium dihydrogen phosphate of the mobile phase A on the capacity factor (k`) of tested compounds was investigated at a concentration of 3 mM ion pair. It was found that, there is no separation between tested compounds at pH more than 6. Decreasing the pH less than 4 cause marked increase in the retention time of tested compounds and good resolution was obtained at pH lower than 3.5. Optimum resolution with reasonable retention was observed at pH 3.0.

Effect of the changing gradient time

The influence of the changing time of the gradient system on the capacity factor (k`) of tested compounds was investigated. It was found that, the time of changing between the two mobile phase have great effect in the capacity factor (k`) of tested compounds and the base line stability. Rapid changing in the ratio between mobile phase A and B cause overlapping between tested compounds and disturbance in the base line. Using 3 stages for changing gradient ratio between mobile phases A and B as described before give optimum resolution and reasonable retention time for the tested compounds peaks with very smooth baseline.

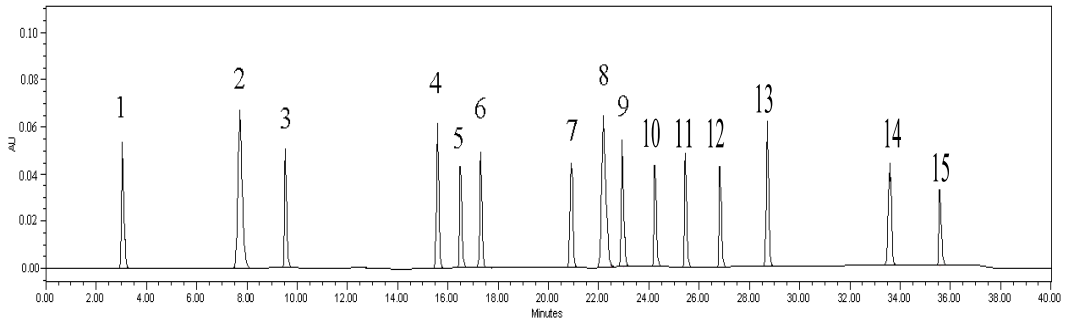

Figure 1. HPLC Chromatogram of synthetic mixture containing: (1) 10 µg/mL of PM (2) 40 µg/mL of PA, (3) 40 µg/mL of PH, (4) 40 µg/mL of PS, (5) 35 µg/mL of SA, (6) 35 µg/mL of GU ,(7) 25µg/mL of SB, (8) 20 µg/mL of MP, (9) 40 µg/mL of CH, (10) 25 µg/mL of TR, (11) 35 µg/mL of DX, (12) 35 µg/mL of DI, (13) 35 µg/mL of PR, (14) 10 µg/mL of PP and (15) 10 µg/mL of CA.

Figure 1 shows chromatogram obtained from injection of synthetic mixture containing PA, PH, PS, SA, GU, CH, TR, DX, DI, PR, MP, PP, SB, PM and CA. Complete separation with reasonable retention time was obtained for each compound using the optimized HPLC conditions. The peaks of all compounds were found to be symmetric, with good peak purity index results. Quantitation was achieved based on peak area.

VALIDATION OF THE METHOD

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range.

The linearity of the calibration graph and the negligible scatter of the experimental points of PA, PH, PS, SA, GU, CH, TR, DX, DI, PR, MP, PP, SB, PM and CA were validated by the value of the correlation coefficient of the regression equation and the value of the intercept, which was close to zero (Table 1). The intercept value was not statistically ($p = 0.05$) different from zero.

Precision and accuracy

To judge the quality of the method, precision and accuracy were determined. The precision of the method, expressed as CV (%), was determined by analysis of three different concentrations within the linearity range for PA, PH, PS, SA, GU, CH, TR, DX, DI, PR, MP, PP and SB in the pharmaceutical products and Intra-day precision was assessed from the results obtained from five replicate analyses of each sample on the same day. Inter-day precision was determined by analysis of the samples on five consecutive days. CV (%) for the studied compounds obtained from determination of intra-day and inter-day precision are listed in Table 2.

Table 2. Results from intra- and inter-day validation of the proposed method determined by recovery of the studied compounds in pharmaceutical products

Compound	Concentration (µg/mL)	Intra -day precision		Inter -day precision	
		Recovery (%)[a]	CV (%)	Recovery (%)[a]	CV (%)
PA	37.5	99.8 ± 0.4	0.41	99.8 ± 0.4	0.41
	75	99.7± 0.3	0.30	100.2 ± 0.5	0.51
	150	100.2± 0.4	0.41	99.9± 0.3	0.3
PH	10	99.8 ± 0.6	0.62	100.2± 0.6	0.61
	20	99.8 ± 0.5	0.52	99.8 ± 0.3	0.32
	40	100.1 ± 0.3	0.3	100.1 ± 0.5	0.52
PS	10	99.8 ± 0.5	0.51	99.6 ± 0.3	0.31
	20	99.9± 0.3	0.32	100.3 ± 0.5	0.52
	40	99.7 ± 0.4	0.42	99.8± 0.4	0.41
SA	10	100.1 ± 0.6	0.61	100.3± 0.4	0.43
	20	99.8 ± 0.4	0.42	99.7± 0.4	0.41
	40	100.2 ± 0.4	0.43	100.2± 0.5	0.52
GU	10	99.9 ± 0.4	0.42	99.8± 0.4	0.42
	20	99.7 ± 0.5	0.51	100.1± 0.5	0.53
	40	100.3 ± 0.4	0.42	99.9± 0.4	0.41
SB	7.5	99.9 ± 0.5	0.51	100.1± 0.5	0.53
	15	99.7 ± 0.4	0.42	99.8 ± 0.3	0.31
	30	100.2 ± 0.4	0.41	100.1 ± 0.5	0.53
MP	5	99.7 ± 0.5	0.51	99.6 ± 0.4	0.41
	10	99.9± 0.4	0.41	100.2 ± 0.5	0.51
	20	99.9 ± 0.5	0.52	99.9 ± 0.3	0.32
CH	10	100.2± 0.5	0.51	100.1± 0.3	0.31
	20	99.9 ± 0.3	0.31	99.7 ± 0.4	0.41
	40	100.1 ± 0.2	0.23	100.2 ± 0.4	0.42
TR	7.5	99.8± 0.3	0.31	99.9 ± 0.4	0.42
	15	99.9± 0.3	0.30	100.1 ± 0.5	0.53
	30	100.2 ± 0.4	0.42	99.9 ± 0.3	0.32
DX	10	99.7 ± 0.4	0.4	100.2± 0.5	0.52
	20	99.9 ± 0.5	0.52	99.7 ± 0.4	0.41
	40	100.2 ± 0.3	0.31	100.2 ± 0.4	0.43

Table 2 continuation

DI	10	99.8 ± 0.4	0.41	99.7 ± 0.4	0.42
	20	99.7± 0.3	0.34	100.3 ± 0.5	0.52
	40	99.9± 0.4	0.42	99.8 ± 0.3	0.31
PR	10	100.1 ± 0.5	0.51	100.2± 0.3	0.33
	20	99.9 ± 0.3	0.32	99.5 ± 0.4	0.41
	40	100.2 ± 0.4	0.42	100.1 ± 0.4	0.41
PP	5	99.9 ± 0.4	0.42	100.0 ± 0.4	0.40
	10	99.8± 0.3	0.31	100.1 ± 0.5	0.51
	20	100.1 ± 0.4	0.42	99.8 ± 0.3	0.31

[a] Mean ±SD for five determinations

The accuracy of the method was examined by standard addition applied to the dosage forms. Mean recovery (%) ±S.D for the proposed method were calculated for six replicate analyses. The results obtained show good precision and accuracy, so excipients in the pharmaceutical formulations don't interfere with analysis of tested compounds in the pharmaceutical products.

Range

The calibration range was established through consideration of the practical range necessary, according to each drug concentration present in the pharmaceutical products, to give accurate, precise and linear results and ensure reach lower concentration as possible for related compounds. The calibration range of each compound is given in table 1.

Detection and Quantitation limits

According to ICH recommendations (The European Agency for the Evaluation of Medical Products, 1996) the approach based on the S.D. of the response and the slope was used for determining the detection and quantitation limits. The theoretical values were assessed practically and given in table 1.

Specificity

The specificity of the developed method was also evaluated by inspection of the three – dimensional chromatograms and studying the peak purity index values for PA, PH, PS, SA, GU, CH, TR, DX, DI, PR, MP, PP, SB, PM and CA. The peak purity index values include purity angle, purity threshold, match angle and match threshold. If the purity angle is smaller than the purity threshold and the match angle is smaller than the match threshold, no significant differences between spectra can be detected. As a result no spectroscopic evidence for co-elution is evident and the peak is considered to be pure [36]. The obtained peak purity index values indicate that the chromatographic peaks of PA, PH, PS, SA, GU, CH, TR, DX, DI, PR, MP, PP, SB, PM and CA in the tested samples, were not attributable to more than one compound.

Robustness

Variation of pH of the 30 mM sodium dihydrogen phosphate of the mobile phase A by ± 0.2, variation of sodium dihydrogen phosphate concentration of the mobile phase A by ± 2 mM, variation of ion pair concentration of mobile phase A by ± 0.2 mM, variation of the percentage of organic solvent by ± 2 % in the 3 gradient steps and variation in column temperature by ±3°C did not have significant effect on chromatographic resolution for the proposed method.

System suitability

In order to determine the adequate resolution and reproducibility of the proposed methodology, system suitability parameters including, selectivity, resolution, tailing factors and plate number were investigated. Chromatograms of Fig.1and 2 show very clear and excellent base line resolution between all tested compounds found either in the synthetic mixture or pharmaceutical product.

Comparative study between the proposed and reported methods.

The proposed method was applied for analysis of active constituent PA, PS, TR and SB in freshly prepared Rinofed plus syrup as a pharmaceutical product example for methods comparative study. Seven replicate determinations were performed. Satisfactory results were obtained for the tested components, in good agreement with the label claim. These obtained results were compared with those obtained from published methods for determination of PA, PS, TR (USP–NF, 2009) and SB (El-Gindy et al., 2005). The accuracy and precision of the proposed method were judged by calculating

Student's t test and F values. At the 95% confidence level the calculated values of t and F did not exceed the theoretical values, indicating there was no significant difference between results obtained from the proposed and compared methods (Table 3).

Expired commercial Rinofed plus syrup stored at ambient temperature under normal conditions were analyzed by the developed method. The mean concentration ±S.D. (n= 7) of the PM and CA in expired Rinofed plus syrup were found to be 19 ±0.42 and 1.6 ±0.31 µg/mL, respectively(Figure 2).

Table 3. Determination of active components in Rinofed plus syrup

		Mean ±SD [a]	
		Proposed method	**Compared Method ([37] for PA, PS, TR and [11]for SB)**
PA		101.1±0.3	101.3± 0.5
	t	0.91	(2.18) [b]
	F	2.77	(4.28) [b]
PS		100.2±0.4	100.1± 0.6
	t	0.37	(2.18) [b]
	F	2.25	(4.28) [b]
TR		100.7±0.3	100.6± 0.4
	t	0.53	(2.18) [b]
	F	1.77	(4.28) [b]
SB		99.9±0.4	100.3± 0.7
	t	1.31	(2.18) [b]
	F	3.06	(4.28) [b]

a Mean ± SD , percentage recovery from the label claim amount
b Theoretical values for t and F at P = 0.05

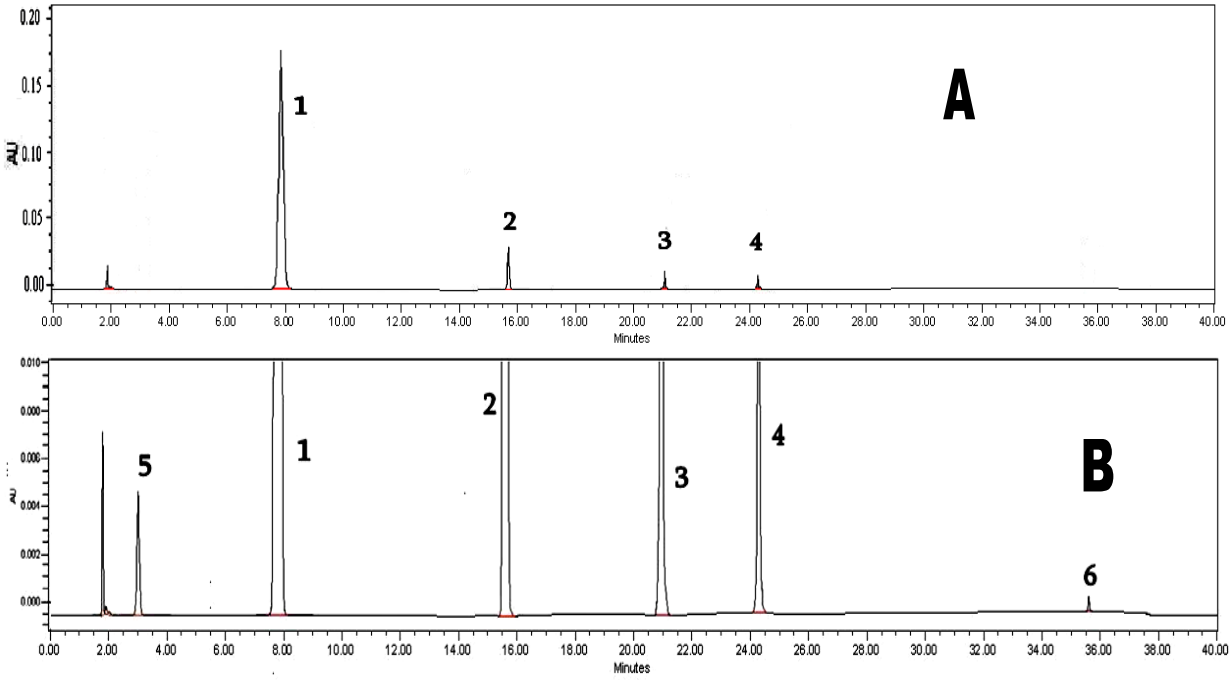

Figure 2. HPLC Chromatograms of (A) freshly prepared and (B) expired Rinofed plus syrup containing peaks of (1) PA, (2) PS, (3) SB, (4) TR, (5) PM, and (6) CA

CONCLUSION

The developed HPLC method provides simple, accurate, sensitive, specific, precise and direct quantitative analysis for the simultaneous determination of PA, PH, PS, SA, GU, CH, TR, DX, DI, PR, MP, PP, SB, PM and CA in pharmaceutical products. The advantages of the developed method include low limit of detection and quantitation, good precision (standard deviation less than 1%) with symmetric, pure and perfect homogeneity for the studied compound peaks. The specificity of the method was evaluated by studying the peak purity index values for the studied compounds.

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