Heliyon 5 (2019) e02871

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.heliyon.com

Heliyon

Research article

Method development for simultaneous determination of active ingredients in cough and cold pharmaceuticals by high performance liquid chromatography

Azizollah Nezhadali [a,*], Mahmoud Reza Shapouri [a,b], Mitra Amoli-Diva [c], Amir Hossein Hooshangi [b], Farrokh Khodayari [b]

[a] Department of Chemistry, Payame Noor University, P. B.19395-4697, Tehran 19569, Iran
[b] Research and Development Laboratory, Darou Pakhsh Pharmaceutical. Co., Tehran, Iran
[c] Department of Physics, Sharif University of Technology, P.O. Box 11365-9567, Tehran, Iran

ARTICLE INFO

Keywords:
Pharmaceutical chemistry
Pharmaceutical science
RP-HPLC
Validation
Chlorpheniramine maleate
Phenylephrine hydrochloride
Guaifenesin
Sodium benzoate

ABSTRACT

A rapid, simple and efficient liquid chromatographic method was developed for simultaneous determination of three active ingredients namely, chlorpheniramine maleate, phenylephrine hydrochloride and guaifenesin along with sodium benzoate preservative common cold medications (syrups) and the method was validated based on the International Conference on Harmonization (ICH) and United State Pharmacopeia (USP) guidelines. Separation of the analytes was achieved within 15 min on a nucleosil gravity phenyl column in a single run with a mobile phase consisting of methanol: buffer mixture (15:85 v/v) at room temperature, in isocratic mode with flow rate of 0.8 mL min^{-1}. A comprehensive study on specificity, range, accuracy (recovery), intraday and interday precisions, limit of detection, limit of quantitation, robustness, ruggedness, system suitability and specification was performed as a part of method validation. The linearity was obtained in the range of 7.1–12.2 μg mL^{-1} (r^2 = 0.9984), 17.6–30.1 μg mL^{-1} (r^2 = 0.9995), 39.6–67.8 μg mL^{-1} (r^2 = 0.9995) and 351.1–601.8 μg mL^{-1} (r^2 = 0.9996) for chlorpheniramine maleate, phenylephrine hydrochloride, sodium benzoate and guaifenesin, respectively. The proposed liquid chromatographic method was successfully applied for the routine analysis of these compounds in different commercial cough and cold pharmaceutical preparations including syrups with no interference from the excipients.

1. Introduction

Combinations of decongestant, antihistaminic and analgesic preparations are widely used for cough and cold treatment. Cough and cold segment is one of the major areas of over the counter (OTC). OTC is a fiercely competitive market in which traditional cough and cold remedies exist. There are many reasons why pharmaceutical companies decide to pursue switches from prescription (Rx) to over-the-counter status for their drugs. These reasons include extending revenue generated by a drug (life-cycle management), development of a defense strategy against generic competitors, expansion and growth of an OTC drug portfolio, and broadening consumer access to innovative OTC medications [1, 2].

Medications against the common cold come in different forms (tablets, syrups, etc.) and usually contain a complex mixture of nitrogenous compounds as active ingredient. These are usually present in varying and very different proportions, have diverse properties inherent to their formulation and desired action, and often possess some similar physical and chemical properties, which turn difficult their separation. Moreover, in the case of HPLC analyses, these basic drugs strongly interact with the stationary phases, causing peak asymmetry and lowering separation efficiency [1]. Duo to these characteristics, quality control of preparations against the common cold always offers an interesting analytical challenge [2].

Present days chromatographic methods play a main role in pharmaceutical industry development. Pharmaceutical industry has developed an increasing variety of combination dosage forms (double or multiple actives) for human therapies. Analytical method development for these combination drug products is big challenge to develop the methods for analysis. At this point industry requires chromatographic methods for each active component which acts as an alternative to the commonly

* Corresponding author.
E-mail address: aziz_nezhadali@pnu.ac.ir (A. Nezhadali).

https://doi.org/10.1016/j.heliyon.2019.e02871
Received 21 July 2019; Received in revised form 9 October 2019; Accepted 12 November 2019
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used practice of developing multiple reversed phase (RP) chromatographic methods for analyzing combination pharmaceutical products. In our present study the RP- HPLC method was developed for multiple combination products of cough and analgesic therapeutic drug products. The developed single RP-HPLC method is applicable for three active ingredients combination products i.e. chlorpheniramine maleate (CPM), phenylephrine hydrochloride (PH) and guaifenesin (GU) in pharmaceutical syrup form along with preservative i. e. sodium benzoate (SB). Fig. 1 represents molecular structure of these compounds.

CPM inhibits the effects of histamine on capillary permeability and bronchial smooth muscles. It is an anti-allergic drug, widely used in cough-cold preparations [3]. CPM is chemically known as 2-[p-chloro-(alpha)-[2-(dimethylamino) ethyl] benzyl] pyridine Maleate (1:1). The enantioselective determination of chlorpheniramine and its major metabolites in human plasma using chiral chromatography on a beta cyclodextrin chiral stationary phase and mass spectrometric detection is established [4]. Simultaneous assay of phenylpropanolamine hydrochloride, caffeine, paracetamol and CPM in silabat tablets using HPLC with diod array detection is estimated [5].

PH is a selective α_1-adrenergic receptor agonist used primarily as a decongestant [6, 7]. PH is chemically known as (1R)-1-(3-hydroxyphenyl)-2-(methylamino) ethanol hydrochloride. PH is estimated along with GU, CPM in cough syrup using gradient liquid chromatography [8].

GU is also called as guaiphenesin and an expectorant drug [9]. GU is used for the treatment of cough and primary dysmenorrheal [10]. The common side effects are fine tremor, nervousness, headache, muscle cramps, dry mouth and palpitation, arrhythmias, flushing and myocardial ischemia. GU is chemically known as (2RS)-3-(2-methoxyphenoxy) propane-1, 2-diol. GU is separated in a mixture containing dextromethorphan, phenylpropanolamine in an oral liquid formulation using HPLC [11]. Simultaneous determination of paracetamol, caffeine, GU and preservatives in syrups by micellar LC is also estimated [12].

SB is the sodium salt of benzoic acid, it acts as bacteriostatic and fungistatic under acidic conditions [13, 14]. It is used as preservative for the preparation of acidic foods such as salad dressings, carbonated drinks, jams, fruit juices and cough syrups [15].

The aim of this study was to develop a simple, rapid, specific, precise and accurate reversed-phase (RP) HPLC method for the determination of CPM, PH, GU, and SB in syrup. The determination of these components has also been performed with HPLC in USP 40, but all of them were determined separately and the method does not involve simultaneous determination [16]. The developed single RP-HPLC method has applicable for the above all ingredients and validated the method based on USP 40 and ICH guidelines [17, 18]. The validation was performed as specificity, range, accuracy (recovery), intraday and interday precision, limit of detection (LOD), limit of quantitation (LOQ), robustness, ruggedness and system suitability. The proposed liquid chromatographic method was successfully applied for routine analysis of these compounds in different cough and cold syrups.

2. Materials and methods

2.1. Reagents and chemicals

The reference standards of CPM and GU were obtained from USP and reference standards of PH and SB were obtained from British Pharmacopoeia (BP) and all standards had minimum purity 98.5%. Commercial syrup was supplied from local pharmaceutical company (Darou Pakhsh Mfg. Co., Tehran, Iran). All other chemicals and reagents were of analytical or HPLC grades and purchased from Merck (Darmstadt, Germany). The distilled water was purified using Aqua Max – Basic water purification system and Aqua Max – Ultra ultrapure water purification system (Young Lin, Korea). All glassware was cleaned with distilled water followed by acetic anhydride and then acetone and dried in hot air oven whenever required and solvents were filtered and degassed before use.

2.2. Equipment and chromatographic conditions

The a HPLC system (Young Lin, Korea) was equipped with UV 730 detector, vacuum degasser, SDV 50 A valve module, CTS 30 column oven, YL9150 alias auto-sampler with a variable injection and SP 930 D solvent delivery pump. The output signal was monitored and processed using Young Lin Autochro-3000 software and isocratic elution with flow rate of 0.8 mL min^{-1} was performed on phenyl analytical column (Teknokoroma-nucleosil-100-P-5μm-25×0.46 cm) with injection volume of 10 μL. The run time was set to 15 min and column temperature was 25 °C. The column was equilibrated with mobile phase for 30–40 min prior to sample injection. The UV spectra were measured using Lambda 25 PerkinElmer spectrophotometer at 214 nm.

2.3. Preparation of mobile phase solution

An amount of 4 g of potassium dihydrogen phosphate and 3.5 g of heptan-1-sulfonic acid sodium were dissolve in 800 mL purified water in a 1000 mL volumetric flask. Then, 2 mL triethylamine and 1 mL phosphoric acid were added to the solution and dilute to volume with purified water. The solution was then filtered with filter paper. Final solution has pH about 5.5.

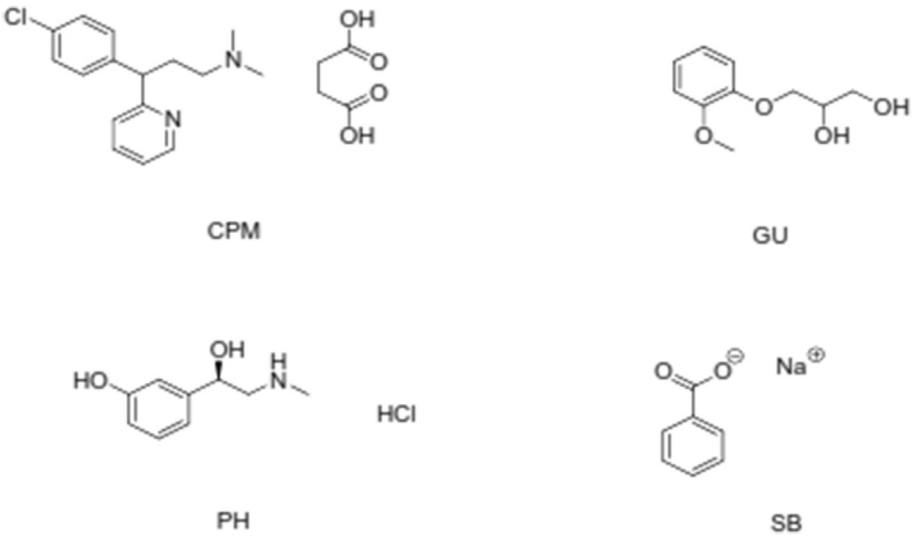

Fig. 1. Represent molecular structure of the proposed compounds.

Finally, an appropriate amount of buffer solution was added to methanol to reach a 85:15 v/v mixture. The pH of this solution was adjusted to 4.5 with phosphoric acid and sonicated for 15 min.

2.4. Preparation of standard solution

To prepare standard solution of all active ingredients, 20 mg of CPM and 50 mg PH were transferred into a 50 mL volumetric flask and dissolved in 20 mL of mobile phase solution. Then it was diluted to the final volume. After that, 5 mL of the above solution was added to 10 mg of SB ' 100 mg of GU in a 200 mL volumetric flask. The mixture was dissolved with mobile phase (20 mL) and diluted to the final volume.

2.5. Preparation of sample solution

To prepare syrup solution, an amount of 400 mg of sucrose was added to a beaker containing 200 mL of boiling purified water. Then, the temperature was decreased to 60 °C and 2 g of SB, 10 mg of trisodium citrate, 4.57 mg of citric acid and 1.5 mg of sodium saccharin were to the solution. After 30 min, the temperature was decreased again to reach 25 °C and 1000 mg of PH, 400 mg of CPM, 200 mg of sorbitol 70%, and 20 g of GU were added to the mixture. Then, appropriate amount of common ethanolic flavors were added and it was diluted with purified water to 1000 mL. The pH was adjusted to 4.5–5.5 and the solution was stirred for another 10 min for further homogeneity [19].

In analysis time, 5 mL of syrup was added to a 200 mL volumetric flask, and diluted with mobile phase to the final volume and vortexed for 15 min. Then, 5 mL of the sample was filtered using a 0.45 μm filter and analyzed with the proposed HPLC method.

The calibration curves for CPM, PH, GU and SB were constructed by analysis of five solutions containing different concentrations of each drug using linear least square regression. All experiments were performed in triplicates and the average was reported.

3. Results and discussion

3.1. Selection of appropriate wavelength

Selection of an appropriate detection wavelength is necessary in order to detect all substances simultaneously and obtaining low LODs. Thus, four CPM, PH, GU and SB solutions with the concentration of 40 ppm was prepared separately in mobile phase and their absorbance was measured over the wavelength range of 190–250 nm using a UV spectrophotometer. An overlaid spectrum of all ingredients is shown in Fig. 2. As can be seen, most of components have high absorbance at 214 nm and it was selected as the optimum wavelength for all analysis [20]. Because all components have maximum absorption in this wavelength.

3.2. Effect of ion pair concentration

Using salt modifiers and ionic liquids to modify analyte retention has been firmly established in reversed phase (RP) HPLC for many years. Since, the retention of ionized analytes in RP-HPLC is low because of high polarity; they have shorter retention time (t_R) and smaller capacity factor (K′). When these analytes form ion-pairs with these modifiers, become electrically neutral and increase in hydrophobic characteristics results in greater affinity to reverse stationary phase and leads better resolution. Hence, the effect heptan-1-sulfonic acid concentration on the capacity factor (K′) of the targeted compounds was investigated in the range of 10–25 mM at pH 4.5. It was found that, increasing ion pair concentration causes an increase in the retention time of targeted compounds. At a concentration of about 17 mM for the ion pair, optimum resolution with reasonable retention time for all compounds was observed [21].

3.3. Effect of methanol amount

Increasing in methanol content of mobile phase decreases the retention time of all analytes in RP-HPLC. This is independent of mobile-phase pH because methanol is a strong proton donor and a strong proton acceptor in hydrogen bonding. The effect of methanol content on the capacity factor (K′) of targeted compounds was investigated in the range of 5–25%v/v. It was found that, the optimum resolution with reasonable retention time for all targeted compounds was obtained using 15%v/v of methanol. In methanol content upper than 15%, the compand's peak starts to overlap and in lower methanol content, the retention time increases and peak tailing leads to loosing peak symmetry [22, 23, 24].

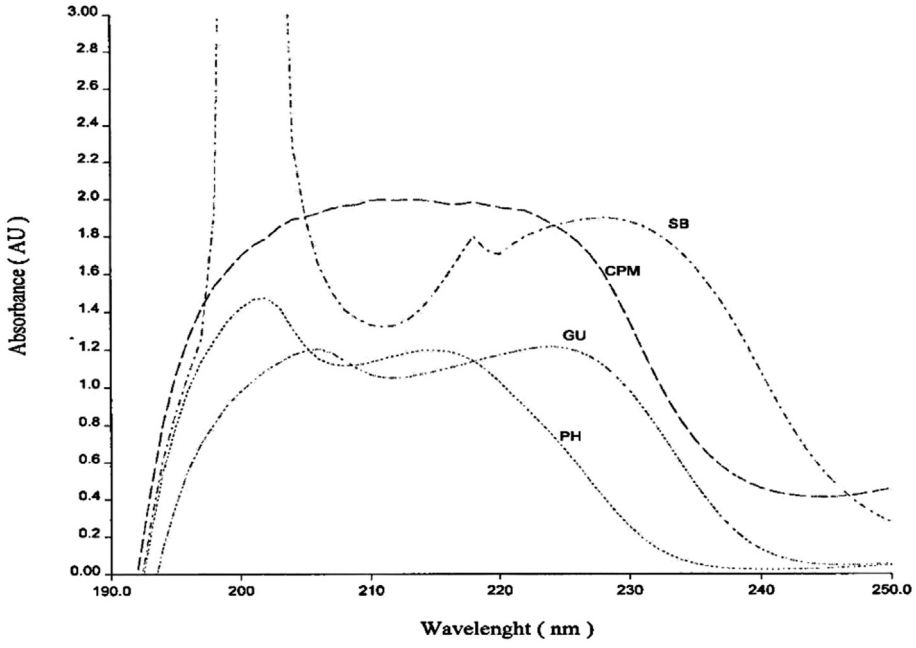

Fig. 2. The overlaid UV absorption spectra of CPM, PH, GU and SB in the 190–250 nm regions. The CPM, PH, SB and GU are in the same proportion as in the studied compounded mixtures.

3.4. Effect of pH of mobile phase

The pH of mobile phase plays a significant role in controlling the selectivity of ionic compounds because they spend shorter time on the stationery phase and elute quickly due to their high polarity. Thus, the effect of pH of mobile phase on the capacity factor (K′) of targeted compounds was investigated in the range 3–6 while the concentration of ion pair was kept at 17 mM. The results revealed that, there is no separation between targeted compounds at pH more than 6 and less than 3. It was found that, the optimum resolution with reasonable retention time for all targeted compounds was obtained using pH: 4.5 for mobile phase [23, 25].

3.5. Effect of flow rate of mobile phase

The effect of flow rate on the chromatographic elution of targeted analytes was investigated in the range of 0.5–2.0 ml/min. Increasing in flow rate decreases the retention time and sensitivity and vis versa. So, the optimum flow rate of mobile phase was observed at 0.8 ml/min [25].

3.6. Method validation

After method development, its validation was performed according to ICH and USP guidelines in terms of range, precision, accuracy, specificity, LOD, LOQ, robustness, ruggedness and system suitability.

The range of an analytical procedure is defined as the interval between upper and lower concentration (amounts) of analyte in the sample. Method range was evaluated using five mixtures of the working standards over the range of 70%–130% of targeted concentration. The solutions were filtered using 0.45 μm membrane filters and each of the dilution was injected in triplicate to the chromatographic system and response area was recorded. Calibration curves were constructed by plotting peak area against the concentration and regression equations were computed (Fig. 3).

Precision is defined as the degree of agreement between individual results when the procedure is applied repeatedly to multiple samplings of a homogenous sample. Inter-day precision of the method was determined using six replicates injection of a prepared sample with concentration of 0.01, 0.025, 0.05 and 0.5 mg mL^{-1} for CPM, PH, SB and GU respectively, and analyzed on the same day. It was found that the relative standard deviation (RSD) for the targeted analytes was obtained 1.2, 0.6, 0.3 and 0.2% respectively. For intra-day precision, the same samples were analyzed in three different days. It was found that the RSD (%) for the targeted analytes was obtained 1.5, 0.9, 0.5 and 0.5% respectively. The results indicate that the method has good precision for detection of targeted compounds and RSD% below 2% confirming that the method is sufficiently precise.

The accuracy of the method was evaluated by carrying out recovery study as per ICH norms. Known concentration of standard solution equivalent to 70, 100 and 130% of label claimed was added to the sample solution. The accuracy was evaluated by six times injection of sample solution with these concentration and the results were summarized in Table 1. As seen, the recoveries were found to be 98.30–100.60% which is within the accepted range of ICH guidelines with RSD (%) $< 2.0\%$. Hence the method is accurate for simultaneous quantitative estimation of

Table 1
The accuracy (recovery) results for CPM, PH, GU and SB.

Compound	Concentration of label claimed (%)	RSD[a] (%)	Means recovery (%)	Average recovery (%)
CPM	70	1.7	100.2	
	100	1.2	99.6	99.97 ± 1.5
	130	1.6	100.1	
PH	70	0.8	98.3	
	100	0.6	100.2	99.43 ± 0.7
	130	0.7	99.8	
GU	70	0.5	100.3	
	100	0.2	100.6	100.33 ± 0.3
	130	0.3	100.1	
SB	70	0.4	99.7	
	100	0.3	100.5	100.20 ± 0.3
	130	0.1	100.4	

[a] n = 3.

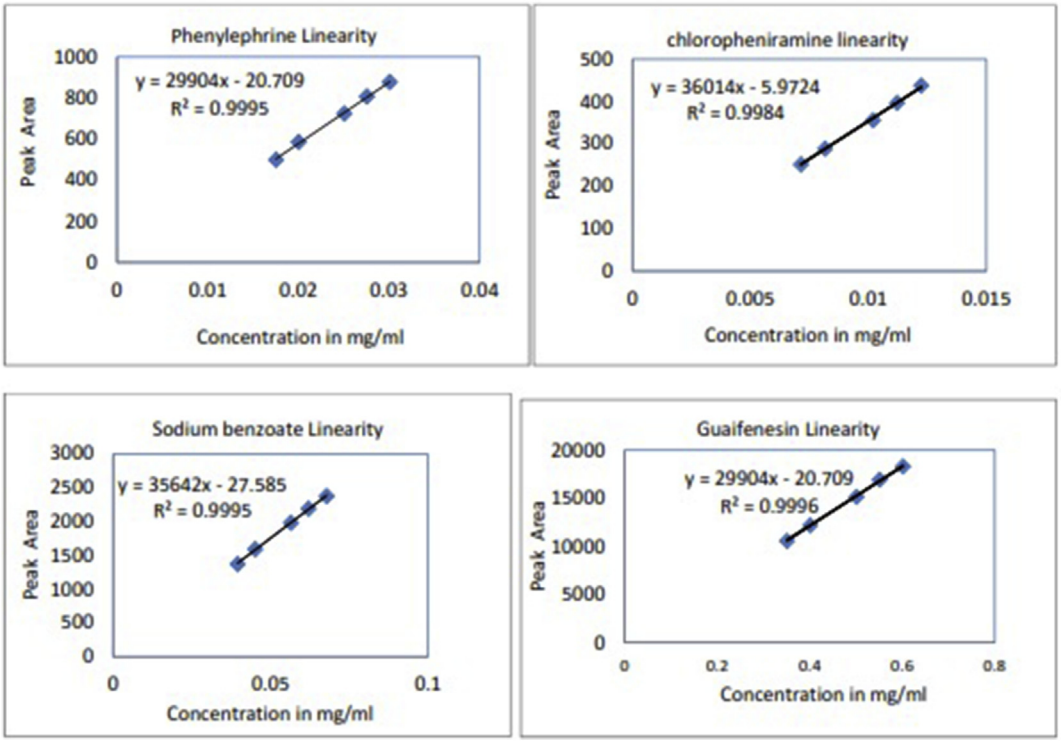

Fig. 3. The calibration curves of (a) PH, (b) CPM, (c) SB and (d) GU compounds.

Table 2
The robustness results of the proposed method.

Parameters	System suitability		
	Variation	Tailing factor	RSD (%)
Standard solution	70–130% of label claimed	1.1–1.3	1.2–1.5
Flow rate (ml.min^{-1})	0.8 ± 0.1	0.8–1.2	0.9–1.5
Column oven temperature (°C)	25 ± 5	1.0–1.4	0.8–1.3

Table 3
The system suitability parameters for CPM, PH, GU and SB.

Parameters	CPM	PH	SB	GU
Retention times (t_r), min	4.42	7.27	10.10	13.35
Theoretical plates (N)	12120	11769	14262	14084
Resolution (R)	6.25	13.30	9.38	8.25
Capacity factor (K')	3.42	6.27	9.10	12.35
Tailing factor (Tf)	1.20	1.14	0.80	1.06
RSD (%) (n = 6)	0.66	0.73	0.62	0.71

targeted components in single dosage form indicating the suitability of the developed method in quantifying the concentration of targeted analytes.

The specificity of the method was evaluated by studying the peak purity index values for the targeted compounds. For specificity study, a typical solution of placebo was analyzed by the proposed method and there was no interfering peak in the retention times corresponding to the analytes. Which can be concluded that the proposed method has good specificity [26].

The minimum level at which the targeted compounds can reliably detect and quantified were determined experimentally as LOD and LOQ respectively. The LOD and LOQ were expressed as the concentration of analyte which generates a response equals to three and ten times of signal to noise (S/N) ratio, respectively [20, 23]. The obtained results revealed that the LOD was obtained 0.8, 1.2, 31.2 and 4.1 μg mL^{-1} and the LOQ was obtained 2.1, 3.9, 102 and 13.21 μg mL^{-1} for CPM, PH, GU and SB respectively.

The robustness is ability to remain unaffected by small but deliberate variations in method. The robustness of the proposed method was validated by changing flow rate and column oven temperature parameters in the range of 70–130% of label claimed. The results were summarized in Table 2 and indicate that the method is robust for all variations because all of the results revealed RSD (%) no more than 2.0% and tailing factor

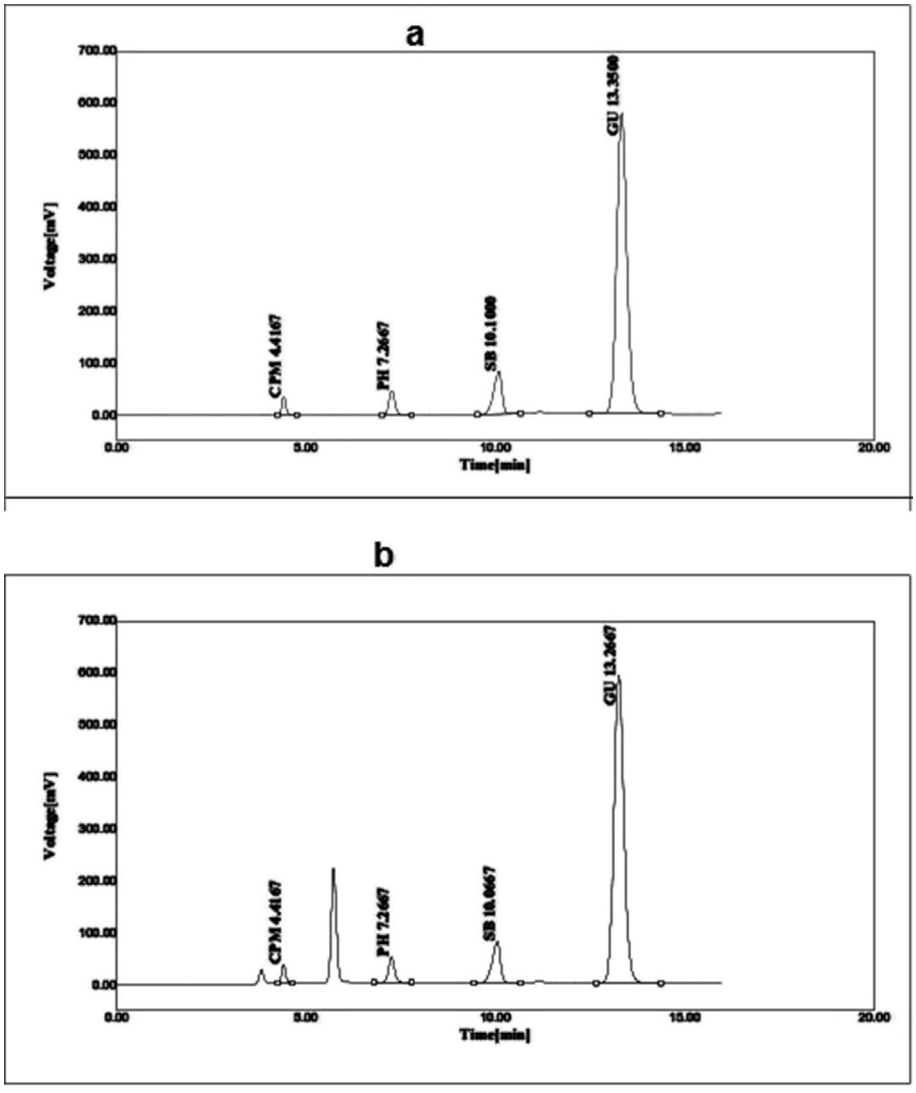

Fig. 4. The obtained chromatograms for (a) standard and (b) sample solutions.

(Tf) < 2. Hence, the method has sufficient robustness with normally expected variations in chromatographic conditions [26].

The ruggedness of the method, which also known as intermediate precision, is reproducibility with the same samples using different laboratories, analysts and days was estimated by carrying out precision study in six preparations of sample by different analysts and columns on different days [26]. All of the results revealed RSD (%) no more than 2.0%. Thus, this HPLC method can be considered to show adequate ruggedness for quantitative analysis of targeted compounds under the experimental conditions described.

Suitability Test is generally performed to evaluate the suitability and effectiveness of the entire chromatographic system during the time of analysis of the targeted components. System suitability was checked by calculating the retention time (RT), theoretical plate (N), resolution (Rs), capacity factor (K′), Tf, and RSD. As shown in Table 3, system suitability parameters were found to be within acceptable limits [26].

Fig. 4a and b represent chromatogram obtained from injection of standard and sample solutions, respectively. Complete separation with reasonable retention time was obtained for each targeted analytes using the optimized HPLC conditions.

4. Conclusion

The developed HPLC method provides simple, accurate, sensitive, specific, precise and direct quantitative analysis for the simultaneous determination of three active ingredients (CPM, PH and GU) in cough and cold syrup along with SB preservative. The advantages of the developed method are low LOD and LOQ, good precision (RSD less than 2.0%) with symmetric for the targeted compounds. This method has been found suitable for the routine analysis of the pharmaceutical dosage forms in quality control and R&D laboratories for products with similar composition.

Declarations

Author contribution statement

Azizollah Nezhadali: Conceived and designed the experiments; Analyzed and interpreted the data.

Mahmoud Reza Shapouri: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Amir Hossein Hooshangi: Performed the experiments; Analyzed and interpreted the data.

Farrokh Khodayari: Performed the experiments.

Mitra Amoli-Diva: Analyzed and interpreted the data.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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