LC for Analysis of Two Sustained-Release Mixtures Containing Cough Cold Suppressant Drugs

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Abstract

A liquid chromatographic method was applied for the analysis of two sustained-release mixtures containing dextromethorphane hydrobromide, carbinoxamine maleate with either phenylephrine hydrochloride in pharmaceutical capsules (Mix 1) or phenylpropanolamine, methylparaben, and propylparaben, which bonds as a drug base to ion exchange resin in pharmaceutical syrup (Mix 2). The method was used for their simultaneous determination using a CN column with a mobile phase consisting of acetonitrile–12 mM ammonium acetate in the ratio of 60:40 (v/v, pH 6.0) for Mix 1 and 45:55 (v/v, pH 6.0) for Mix 2.

Introduction

Cough, nasal congestion, and sinus inflammations are very common complaints for most patients suffering from viral cold infection (common cold). This gives a great need for medication to relieve these symptoms. These medications are usually a combination of antitussive, antihistaminics, nasal decongestant, and bronchodilators in rapid onset or sustained-release formulations. The common sustained-release formulations may be in the form of microcapsules with the outer shell having different solubilities or the drug bonded to ion exchange resin.

Dextromethorphan hydrobromide (DX) is a cough suppressant found in combination with phenylephrine hydrochloride (PH), a nasal decongestant, and carbinoxamine (CX), an antihistamine, in sustained-release capsules. Also, DX base is found with phenylpropanolamine (PN), another nasal decongestant, CX, methylparaben (MP), and propylparaben (PP), preservatives in sustained-release syrup (1).

No analytical methods have been reported for the simultaneous determination of the two studied sustained-release combinations. Various analytical methods were used for determination of DX with different drugs such as PN (2–15), CX (16), and PH (7,14,17–19) by liquid chromatography (LC) and by capillary electrophoresis with PH (20,21).

CX was determined with PN by LC–tandem mass spectrometry (22,23), LC (24–26), and gas chromatography (27). Also, CX was determined with PH by spectophotometry (28,29) and liquid chromatography (LC) (24,25).

The aim of this work is to investigate the ability of high-performance liquid chromatography (HPLC) method for resolution and assay of the highly overlapping components in sustained-release capsules (Mix 1) or syrup (Mix 2).

The proposed method reduced the duration of the analysis and is simple, sensitive, and suitable for routine determination of the components in the studied mixtures.

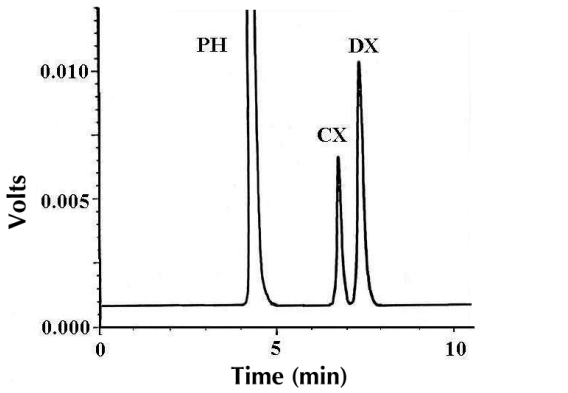

Figure 1. Typical HPLC chromatogram of 20 µL injection of 20 µg/mL of DX, 20 µg/mL PH, and 4 µg/mL of CX.

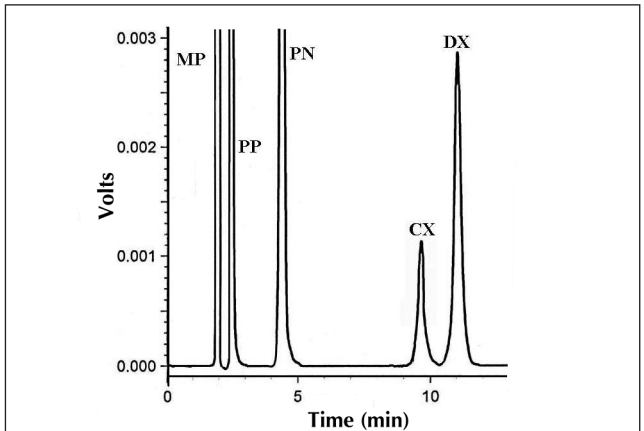

Figure 2. Typical HPLC chromatogram of 20 µL injection of 15 µg/mL of PN, 5.9 µg/mL DX, 1.1 µg/mL CX, 9.5 µg/mL MP, and 4.1 µg/mL PP.

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Table I. Chromatographic Characteristics of DX, PH, and CX in Mix 1

Compound	Retention time (min)	Capacity factor (K')	Selectivity α	Resolution Rs	Tailing factor
PH	4.3	4.73	1.71*	4.59*	1.1
DX	6.8	8.07	1.10†	1.59†	1.06
CX	7.4	8.87			1.15

* α and Rs are calculated for PH–DX. † α and Rs are calculated for DX–CX.

Table II. Chromatographic Characteristics of PN, DX, CX, MP, and PP in Mix 2

Compound	Retention time (min)	Capacity factor (K')	Selectivity α	Resolution Rs	Tailing factor
MP	1.98	1.64	1.41*	1.49*	0.96
PP	2.48	2.31	2.09†	3.15†	1.09
PN	4.37	4.83	2.47‡	4.82‡	1.01
CX	9.68	11.91	1.16§	2.11§	1.05
DX	11.08	13.77			1.08

* α and Rs are calculated for MP–PP. † α and Rs are calculated for PP–PN.
‡ α and Rs are calculated for PN–CX. § α and Rs are calculated for CX–DX.

Table III. Characteristic Parameters of the Calibration Equations for the Proposed HPLC Method for the Determination of DX, PH, and CX in Mix 1

Parameter	DX	PH	CX
Calibration range (µg/mL)	5–20	5–20	2–10
Detection limit (µg/mL)	2.41×10^{-2}	2.43×10^{-2}	5.49×10^{-2}
Quantitation limit (µg/mL)	8.04×10^{-2}	8.13×10^{-2}	1.83×10^{-1}
Regression equation: Slope (b)*	1.35×10^{4}	2.02×10^{4}	3.29×10^{4}
SD† of the slope (Sb)	1.15×10^{2}	1.74×10^{2}	6.42×10^{2}
RSD‡ of the slope (%)	0.86	0.87	1.95
CL of the slope†	$(1.34 \times 10^{4}) - (1.36 \times 10^{4})$	$(2.00 \times 10^{4}) - (2.03 \times 10^{4})$	$(3.23 \times 10^{4}) - (3.36 \times 10^{4})$
Intercept*	-1.20×10^{3}	-3.02×10^{2}	2.62×10^{3}
SD of the intercept (Sa)	1.56×10^{3}	2.35×10^{3}	3.75×10^{3}
CL§ of the intercept†	$(-2.72 \times 10^{3}) - (3.14 \times 10^{2})$	$(-2.59 \times 10^{3}) - (1.98 \times 10^{3})$	$(-1.02 \times 10^{3}) - (6.27 \times 10^{3})$
Correlation coefficient (r)	0.9999	0.9999	0.9998
Standard error of estimation	5.79×10^{2}	8.73×10^{2}	1.60×10^{3}

* Y = a + bC, where C is the conc. of compound in µg/mL, Y is the peak area, and b is the slope.
† SD = Standard deviation ‡ RSD = Relative standard deviation § CL = confidence limit (95%).

Experimental

Instrumentation

The HPLC instrument was equipped with a model series LC-10 ADVP pump, SCL-10 AVP system controller, DGU-12A Degasser, Rheodyne 7725i injector with a 20-µL loop, and a SPD-10AVP UV–Vis detector (Shimadzu, Kyoto, Japan).

Materials and reagents

Pharmaceutical-grade PN, DX, PH, CX, MP, and PP were kindly supplied by October Pharma (Cairo, Egypt) and are certified 99.7, 99.7, 99.9, 99.8, 99.9, and 99.9%, respectively. The acetonitrile and methanol used were HPLC-grade (BDH, Poole, UK). Ammonium acetate and sodium hydroxide (Sigma Aldrich, St. Louis, MO) were used. Triethylamine and acetic acid were analytical-grade.

Rhinotussal capsules and syrup (batch no. B1680400, A1670403, respectively) used were manufactured by October Pharma. Each capsule was labeled to contain 20 mg DX, 20 mg PH, and 4 mg CX. Each 5 mL of the syrup was labeled to contain 16.7 mg PN, 6.5 mg DX, 1.3 mg CX, 10.5 mg MP, and 4.5 mg PP.

HPLC conditions

The HPLC separation and quantitation were made on a Luna 5 µm CN column (250 × 4.6 mm i.d.) (Phenomenex, Macclesfield, U.K.). The mobile phase was prepared by mixing acetonitrile–12 mM ammonium acetate in ratio of 60:40 (v/v) for Mix 1 and 45:55 (v/v) for Mix 2. The pH of the mobile phase was adjusted to the apparent pH 6.0 using acetic acid. The flow rate was 2 mL/min. All determinations were performed at ambient temperature. The injection volume was 20 µL. The mobile phase was filtered using 0.45-µm membrane filter (Millipore, Milford, MA) and degassed by vacuum prior to use. The samples were also filtered using 0.45-µm disposable filters. The detector was set at 214 nm for both mixtures. Data acquisition was performed on a model Schimadzu Class-VP data acquisition system (version 6.1).

Table IV. Characteristic Parameters of the Calibration Equations for the HPLC Method for the Determination of DX, PN, CX, MP, and PP in Mix 2

Parameter	PN	DX	CX	MP	PP
Calibration range (µg/mL)	6–15	2.3–5.9	0.4–1.1	3.8–9.5	1.6–4.1
Detection limit (µg/mL)	4.17×10^{-2}	2.94×10^{-2}	5.86×10^{-2}	5.09×10^{-2}	5.83×10^{-2}
Quantitation limit (µg/mL)	1.39×10^{-1}	9.80×10^{-2}	1.95×10^{-1}	1.70×10^{-1}	1.94×10^{-1}
Regression equation: slope (b)	2.37×10^{5}	1.58×10^{5}	3.43×10^{5}	4.33×10^{5}	3.48×10^{5}
SD of the slope (Sb)	5.69×10^{3}	1.50×10^{3}	6.52×10^{3}	7.15×10^{3}	6.58×10^{3}
RSD of the slope (%)	1.35	0.95	1.90	1.65	1.89
Confidence limit of the slope†	$(2.34 \times 10^{5}) - (2.41 \times 10^{5})$	$(1.56 \times 10^{5}) - (1.59 \times 10^{5})$	$(3.37 \times 10^{5}) - (3.49 \times 10^{5})$	$(4.26 \times 10^{5}) - (4.40 \times 10^{5})$	$(3.41 \times 10^{5}) - (3.54 \times 10^{5})$
Intercept*	-4.18×10^{2}	-6.26×10^{2}	-4.29×10^{2}	4.45×10^{2}	-2.84×10^{2}
SD of the intercept (Sa)	3.15×10^{3}	5.69×10^{2}	4.52×10^{2}	4.44×10^{3}	1.75×10^{3}
CL of the intercept†	$(-3.48 \times 10^{3}) - (2.65 \times 10^{3})$	$(-1.18 \times 10^{3}) - (-7.3 \times 10^{1})$	$(-8.69 \times 10^{2}) - 9.86$	$(-3.87 \times 10^{3}) - 4.76 \times 10^{3}$	$(-1.98 \times 10^{3}) - (1.42 \times 10^{3})$
Correlation coefficient (r)	0.9999	0.9999	0.9998	0.9999	0.9998
Standard error of estimation	8.89×10^{2}	1.65×10^{2}	1.43×10^{2}	1.26×10^{3}	5.08×10^{2}

* Y = a+bC, where C is the concentration of compound in µg/mL and Y is the peak area and b is the slope. † 95% confidence limit.

Standard solutions and calibration

Mix 1

Stock standard solutions were prepared by separately dissolving DX, PH, and CX in methanol to obtain a concentration of 50, 50, and 30 µg/mL for DX, PH, and CX, respectively.

HPLC method

The standard solutions were prepared by diluting the stock standard solutions with mobile phase to reach the concentration range of 5–20 µg/mL for DX and PH or 2–10 µg/mL for CX.

Table V. Determination of DX, PH, and CX in Rhinotussal Capsules Using the Proposed HPLC Method

Sample No.	Compound concentration (µg/mL)			% Recovery (HPLC)		
	DX	PH	CX	DX	PH	CX
1	8.0	8.0	3.2	99.9	100.2	101.2
2	10.0	10.0	4.0	98.7	99.5	100.8
3	12.0	12.0	4.8	100.3	100.4	100.0
4	14.0	14.0	5.6	100.5	101.3	99.6
5	15.0	15.0	6.0	100.2	100.0	99.2
6	17.0	17.0	6.8	99.5	99.6	99.4
7	20.0	20.0	5.0	99.2	98.9	99.2
			Mean*	99.76	99.99	99.91
			SD*	0.65	0.76	0.80

* Mean and SD for seven determinations; % recovery from the label claim amount.

Table VI. Determination of PN, DX, CX, MP and PP in Rhinotussal Syrup Using HPLC

Sample No.	Compound concentration (µg/mL)					% Recovery (HPLC)				
	PN	DX	CX	MP	PP	PN	DX	CX	MP	PP
1	6.0	2.3	0.4	3.8	1.6	99.9	99.8	100.2	99.6	99.6
2	7.0	2.7	0.5	4.4	1.9	99.6	98.7	100.5	99.3	99.5
3	8.0	3.0	0.6	5.0	2.1	99.5	100.3	101.3	100.6	100.4
4	9.0	3.6	0.6	5.7	2.4	101.3	100.5	101.4	100.8	100.6
5	10.0	3.9	0.7	6.3	2.7	100.5	100	99.4	100.9	100
6	11.0	4.0	0.8	7.0	3.0	100.4	99.7	99.5	100	100.8
7	15.0	5.9	1.1	9.5	4.1	100.2	99.6	99.7	99.4	99.4
					Mean*	100.20	99.80	100.29	100.09	100.04
					SD*	0.62	0.58	0.82	0.68	0.57

* Mean and SD for seven determinations; percentage recovery from the label claim amount.

Table VII. Determination of DX, PH, and CX in Mix 1 in Lab-Prepared Mixtures Using the Proposed HPLC Method

Sample No.	Compound concentration (µg/mL)			% Recovery HPLC		
	DX	PH	CX	DX	PH	CX
1	5.0	5.0	2.0	99.9	98.9	99.5
2	7.5	7.5	3.0	99.8	99.3	99.5
3	10.0	10.0	4.0	99.6	99.5	99.1
4	12.5	12.5	5.0	100.2	99.8	100.3
5	15.0	15.0	6.0	100.4	100.5	100.5
6	17.5	17.5	7.0	100.0	100.0	100.7
7	20.0	20.0	10.0	99.5	100.4	100.8
			Mean*	99.91	99.77	100.06
			SD*	0.32	0.58	0.68

* Mean and SD percentage recovery from the added amount.

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 relationship was obtained.

Mix 2

Stock standard solutions were prepared by separately dissolving drug salt equivalent to 50 mg of PN and DX base and 25 mg of CX base in the least amount of distilled water and then rendering alkaline with 2 M sodium hydroxide. The liberated base was extracted three times each with 10 mL chloroform. The combined extracts were evaporated using a rotary evaporator. The free bases were then dissolved in 100 mL methanol.

Further dilutions were made in methanol to obtain a concentration of 50 µg/mL for PN and DX and 25 µg/mL for CX.

Stock standard solutions were prepared for MP and PP by separately dissolving each compound in methanol to obtain a concentration of 25 µg/mL for MP and PP.

HPLC method

The standard solutions were prepared by diluting the stock standard solutions with mobile phase to reach the concentration range of 6–15 µg/mL for PN, 2.3–5.9 µg/mL for DX, 0.4–1.1 µg/mL for CX, 3.8–9.5 µg/mL for MP, and 1.6–4.1 µg/mL for PP.

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 relationship was obtained.

Sample preparation

Mix 1

The content of twenty capsules were weighed and finely powdered. A portion of the powder equivalent to about 20 mg of DX and PH and 4 mg of CX was weighed accurately, extracted, and diluted to 100 mL with methanol. The sample solution was filtered. Further dilution of the filtrate was carried out with mobile phase to reach the calibration range for each compound. The general procedures HPLC method described under calibration were followed, and the concentrations of DX, PH and CX were calculated.

Mix 2

A volume of the syrup equivalent to 16.7 mg PN, 6.5 mg DX, 1.3 mg CX, 10.5 mg MP, and 4.5 mg PP was transferred into a 50-mL volumetric flask containing 5 mL triethylamine and sonicated for 25 min. The volume was completed to 50 mL with methanol and sonicated for 10 min.

The sample solution was filtered. Further dilution of the filtrate was carried out with the mobile phase to reach the calibration range for each compound. The general procedures HPLC method described under calibration were followed, and the concentrations of PN, DX, CX, MP, and PP were calculated.

Results and Discussion

HPLC method

To optimize the HPLC assay parameters for simultaneous determination of DX with PH and CX (Mix 1) and of PN with DX, CX, MP, and PP, the mobile phase composition and pH were studied. A satisfactory separation was obtained with a mobile phase consisting of acetonitrile–12 mM ammonium acetate in a ratio of 60:40 (v/v) for Mix 1 and 45:55 (v/v) for Mix 2. Increasing the acetonitrile concentration to more than 80% led to inadequate separation of the drugs of Mix 1. At lower acetonitrile concentrations (less than 30%), separation occurred but with excessive tailing for DX peak. Variation of apparent pH of the mobile phase of Mix 1 resulted in maximum capacity factor (K′) value at apparent pH 4.0 with loss of peak symmetry for CX. At apparent pH 6.0, optimum resolution with reasonable retention time was observed. For Mix 2, increasing acetonitrile concentrations to more than 60% led to inadequate separation of the drugs. At lower acetonitrile concentrations (less than 30%), separation occurred but with increased retention time for DX and CX associated with excessive broadening for CX. Variation of pH of the mobile phase of Mix 2 resulted in maximum capacity factor (K′) value at pH 4.0 with loss of peak symmetry for DX. However, at apparent pH 6.0, optimum resolution with reasonable retention time was observed. Quantitation was achieved with UV detection at 214 nm for Mix 1 and Mix 2 based on peak area. The specificity of the HPLC method is illustrated in Figure 1–2 where complete separation of the compounds in each mixture was noticed. The HPLC chromatographic characteristics of the studied compounds are given in Table I for Mix 1 and Table II for Mix 2.

To determine the linearity of HPLC detector response, calibration standard solutions for each compound were prepared as described earlier. Linear correlation was obtained between peak area versus concentration of each compound. Characteristic parameters for regression equations of the HPLC method are given in Tables III–IV for Mix 1 and Mix 2, respectively.

Table VIII. Determination of PN, DX, CX, MP, and PP in Mix 2 in Lab-Prepared Mixtures Using the Proposed HPLC Method

Sample No.	Compound concentration (µg/mL)					% Recovery (HPLC)				
	PN	DX	CX	MP	PP	PN	DX	CX	MP	PP
1	6.0	2.3	0.4	3.8	1.6	99.9	98.7	99.6	100.5	100.4
2	7.0	2.7	0.5	4.4	1.9	99.6	100.5	99.8	100.9	100.3
3	8.0	3.0	0.6	5.0	2.1	100.3	100.8	99.6	101.0	99.6
4	9.0	3.6	0.6	5.7	2.4	100.5	100.0	103.0	100.1	99.9
5	10.0	3.9	0.7	6.3	2.7	100.0	100.0	100.9	99.6	98.3
6	11.0	4.0	0.8	7.0	3.0	98.7	99.9	99.5	99.8	100.2
7	15.0	5.9	1.1	9.5	4.1	100.8	99.8	99.2	98.7	99.9
					Mean*	99.97	99.96	100.23	100.09	99.80
					SD*	0.69	0.66	1.34	0.81	0.72

* Mean and SD percentage recovery from the added amount.

Table IX. Application of Standard Addition Technique on Rhinotussal Capsules to the Analysis of DX, PH, and CX Using the Proposed HPLC Method

Sample no. no.	Claimed conc. (µg/mL)			Added conc. (µg/mL)			% Recovery (HPLC)		
	DX	PH	CX	DX	PH	CX	DX	PH	CX
1	5.0	5.0	2.0	2.5	2.5	1.0	99.9	99.5	100.0
2	2.5	2.5	1.0	7.5	7.5	2.0	99.8	100.3	100.1
3	3.0	3.0	1.2	12.0	12.0	4.8	98.8	100.5	98.0
4	4.0	4.0	1.6	13.5	13.5	3.4	100.3	100.0	99.8
5	6.0	6.0	2.4	14.0	14.0	4.6	100.5	99.4	99.0
						Mean	99.86	99.94	99.38
						SD	0.66	0.48	0.88

Analysis of pharmaceutical products

The proposed HPLC method was applied to the simultaneous determination of DX, PH with CX in commercial capsules and DX, PN, CX, MP, with PP in commercial syrup. As the two pharmaceutical preparations are in sustained-release form, this means that the drugs in both formulations are retained either in microcapsule shells (capsules form) or in exchange resin (syrup form).

The drugs in the capsules are found in the form of two-colored microcapsules with different dissolution pattern so that by crushing these microcapsules, the drugs can be extracted from pharmaceutical excipients. But in the case of pharmaceutical syrup, the drugs are bonded to cationic exchanger, so the pH is raised to make the drugs in non-ionized form. This was done with the use of triethylamine; then the drugs extracted by methanol.

Seven replicates determinations were made. Satisfactory results were obtained for compounds of the two mixtures in good agreement with the label claims (Tables V–VI). No pub-

Table X. Application of Standard Addition Technique on Rhinotussal Syrup to the Analysis of PN, DX, CX, MP, and PP Using the HPLC Method

Sample No.	Claimed conc. (µg/mL)					Added conc. (µg/mL)					% recovery (HPLC)				
	PN	DX	CX	MP	PP	PN	DX	CX	MP	PP	PN	DX	CX	MP	PP
1	6.0	2.3	0.4	3.8	1.6	1.0	0.4	0.1	0.6	0.3	99.2	100.0	98.6	99.9	100.2
2	7.0	2.7	4.5	4.4	1.9	2.0	0.9	0.2	1.3	0.5	99.6	100.1	102.0	99.3	100.5
3	9.0	3.6	0.6	5.7	2.4	3.0	0.3	0.2	0.6	1.0	100.5	100.9	99.9	99.1	100.0
4	6.0	2.3	0.4	3.8	1.6	4.0	1.0	0.5	3.0	0.4	100.3	99.3	101.2	100.3	102.0
5	10.0	3.9	0.7	6.3	2.7	5.0	2.0	0.4	3.2	1.4	100.2	99.5	100.5	100.8	98.5
										Mean	99.96	99.96	100.44	99.88	100.24
										SD	0.54	0.62	1.29	0.70	1.25

lished method has been reported for simultaneous determination of the components of two mixtures.

Validation of the methods

Linearity

The linearity of the HPLC for determination of DX, PH with CX; and DX, PN, CX, MP with PP was evaluated by analyzing a series of different concentrations of each drug. According to the International Conference on Harmonization (30), at least five concentrations must be used. In this study, eight concentrations were chosen, ranging between 5–20 µg/mL for DX, 5–20 µg/mL for PH, and 2–10 µg/mL for CX (Mix 1), and 6–15 µg/mL for PN, 2.3–5.9 µg/mL for DX, 0.4–1.1 µg/mL for CX, 3.8–9.5 µg/mL for MP and 1.6–4.1 µg/mL for PP (Mix 2). Each concentration was repeated three times. This approach will provide information on the variation in peak area between samples of same concentration. The high value of the correlation coefficient and the intercept value that was not statistically ($p < 0.05$) different from zero (Tables III–IV) validated the linearity of the calibration graphs. Characteristic parameters for regression equations of the HPLC method obtained by least-squares treatment of the results are given in Tables III–IV.

Precision

For evaluation of the precision estimates, repeatability and intermediate precision were performed at three concentration levels for each compound. The data for each concentration level were evaluated by one-way ANOVA. An eight-day × two-replicates design was performed. Statistical comparison of the results was performed using the P-value of the F-test. Three univariate analyses of variance for each concentration level were made. Because the P-value of the F-test is always greater than 0.05, there is no statistically significant difference between the mean results obtained from one level of day to another at the 95% confidence level.

Range

The calibration range was established through consideration of the practical range necessary, according to each compound concentration present in pharmaceutical product, to give accurate, precise, and linear results. The calibration range of the proposed HPLC method is given in Tables III–IV.

Selectivity

Methods selectivity was achieved by preparing seven mixtures of the studied compounds at various concentrations within the linearity range for HPLC. The prepared laboratory mixtures were analyzed according to the previous procedures described under the proposed method. Satisfactory results were obtained (Tables VII–VIII), indicating the high selectivity of the proposed method for simultaneous determination of DX, PH, and CX (Mix 1) and PN, DX, CX, MP, and PP (Mix 2).

Accuracy

This study was performed by addition of known amounts of the studied compounds to a known concentration of the commercial pharmaceutical products (standard addition method). The resulting mixtures were analyzed, and the results obtained were compared with the expected results. The excellent recoveries of standard addition method (Tables IX–X) suggest good accuracy of the proposed method.

Robustness

Variation of apparent pH of the mobile phase by ± 0.2 and its acetonitrile concentration by ± 2% did not have significant effect on chromatographic resolution in HPLC method.

Analytical solution stability

Analytical solution stability of the studied compounds in the mobile phase, exhibited no chromatographic activity for 4 h when kept at room temperature and for 10 h when stored refrigerated at 5°C.

Conclusion

This HPLC method has been developed for the simultaneous assay of a variety of active ingredients and preservatives found in the most commonly used cough syrups by using one instrument, column, and mobile phase, thus saving time and costs.

The assay results obtained using the proposed HPLC method were convenient, and good coincidence was observed. The proposed HPLC method was found to be suitable for the routine determination of PN, DX, PH, CX, MP, and PP in pharmaceutical capsules and syrups, and they do not require any separation or extraction procedures.

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