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HPLC determination of guaifenesin with selected medications on underivatized silica with an aqueous-organic mobile phase

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Abstract

A high performance liquid chromatography procedure has been developed for the simultaneous determination of guaifenesin–pseudoephedrine–dextromethorphan and guaifenesin–pseudoephedrine in commercially available capsule dosage forms and guaifenesin-codeine in a commercial cough syrup dosage form. The separation and quantitation are achieved on a 25-cm underivatized silica column using a mobile phase of 60:40% v/v 6.25 mM phosphate buffer, pH 3.0 — acetonitrile at a flow rate of 1 ml min^{-1} with detection of all analytes at 216 nm. The separation is achieved within 10 min for each drug mixture. The method showed linearity for the guaifenesin–pseudoephedrine–dextromethorphan mixture in the 50–200, 7.5–30 and 2.5–10 μg ml^{-1} ranges, respectively. The intra- and inter-day RSDs ranged from 0.23 to 4.20%, 0.18 to 2.85%, and 0.13 to 5.04% for guaifenesin, pseudoephedrine, and dextromethorphan, respectively. The guaifenesin–pseudoephedrine mixture yielded linear ranges of 25–100 and 3.75–15 μg ml^{-1} and intra- and inter-day RSDs ranged from 0.65 to 4.18% and 0.23 to 3.00% for guaifenesin and pseudoephedrine, respectively. The method showed linearity for the guaifenesin–codeine mixture in the 25–100 and 2.5–10 μg ml^{-1} ranges and RSDs ranged from 0.37 to 4.25% and 0.14 to 2.08% for guaifenesin and codeine, respectively.

Keywords: HPLC; Silica; Guaifenesin; Pseudoephedrine; Dextromethorphan; Codeine

1. Introduction

Several methods describing the simultaneous determination of a wide variety of active compounds in various cough-cold formulations have been reported. This particular study involved the investigation of two capsule formulations and one cough syrup formulation that are generally recommended for the relief of common cough-cold symptoms. One commercial capsule formulation contained guaifenesin (an expectorant), pseudoephedrine (a nasal and bronchial decongestant), and dextromethorphan (an antitussive agent). The

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other commercial capsule formulation contained only guaifenesin and pseudoephedrine. The cough syrup in this study contained guaifenesin and codeine (an analgesic). Previous HPLC methods have measured these compounds either individually or in combination. Simultaneous HPLC assays have been described for pseudoephedrine–dextromethorphan [1–3], guaifenesin–dextromethorphan [4–6], and pseudoephedrine–codeine [7] usually along with other components. The determination of guaifenesin–pseudoephedrine–dextromethorphan [8], guaifenesin–pseudoephedrine [9–12], and guaifenesin–codeine [13,14] was also reported, however, the procedures required the use of more than one column or mobile phase or an increased flow rate which can be time-consuming and uneconomical. For example, in current USP monographs, guaifenesin–pseudoephedrine–dextromethorphan [15] are determined by HPLC in two different mobile phases and at a flow rate of 2 ml min^{-1}. In this paper, an isocratic HPLC assay is presented that will simultaneously analyze for guaifenesin–pseudoephedrine–dextromethorphan, guaifenesin–pseudoephedrine, and guaifenesin–codeine each with a single injection. The compounds are separated on underivatized silica using a buffered aqueous acetonitrile eluent. The separation is achieved within 10 min for all analytes in each drug mixture.

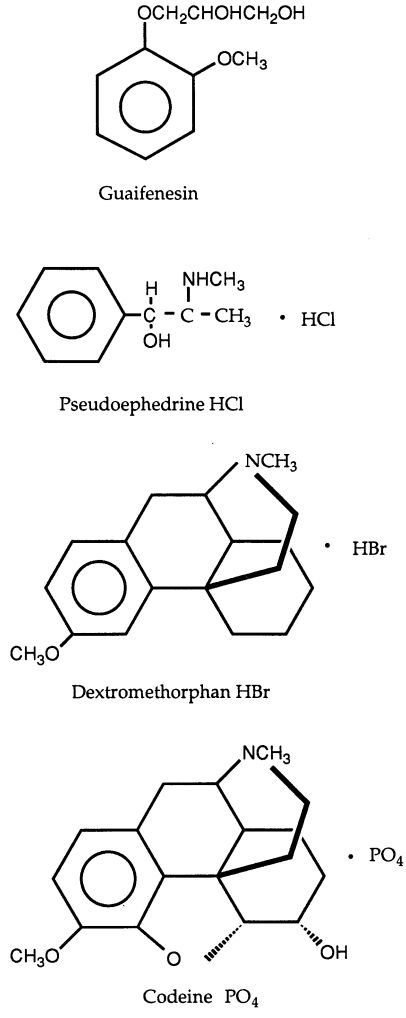

Fig. 1. Chemical structures of compounds studied.

2. Experimental

2.1. Reagents and chemicals

The structure and formulae of the compounds studied are shown in Fig. 1. Codeine phosphate was purchased from the United States Pharmacopoeial Convention, Inc. (Rockville, MD). Guaifenesin, pseudoephedrine HCl and dextromethorphan HBr were purchased from Sigma Chemical Co. (St. Louis, MO). Cheratussin AC™ (Lot: 00789A, Expiration: 01/01), Robitussin Cold and Cough Softgels Tm (Lot: 98207, Expiration: 03/01), and Sudafed™ (Lot: 7E5178, Expiration 10/99) were purchased from a local pharmacy and manufactured by Vintage Pharmaceuticals, Inc., Whitehall-Robins Healthcare, and Warner Lambert Consumer Healthcare, respectively. Acetonitrile (J.T. Baker, Phillipsburg, NJ) was HPLC grade. Monobasic potassium dihydrogen phosphate (KH_2PO_4) and concentrated phosphoric acid were Baker analyzed reagents.

2.2. Instrumentation

An Altex Model 110-A pump (Beckman Coulter, Inc., Fullerton, CA), a Rheodyne Model 7125 injection valve equipped with a 20 μl loop (Rheo-

dyne, Cotati, CA), a Waters 486 UV-VIS detector (Waters Corp., Milford, MA), and a Shimadzu C-R3A chromatopac integrator (Shimadzu Corp., Columbia, MID) constituted the HPLC system used in this study. Separation was accomplished on a 25 cm silica column (4.6 mm i.d., 3 μm particle size, Phenomenex, Torrance, CA). The isocratic mobile phase was composed of a buffer solution [6.25 mM potassium phosphate monobasic in water (pH 3.0) — acetonitrile (60:40% v/v)]. The mobile phase was filtered through a 0.45 μm Nylon-66 filter (Alltech, Deerfield, IL) and degassed by sonication prior to use. The flow rate was set at 1 ml min^{-1}. The UV detector was set at 216 nm.

2.3. Preparation of standard solutions

A combined standard solution containing guaifenesin, pseudoephedrine HCl, and dextromethorphan HBr was prepared by accurately weighing 20, 3, and 1 mg of each powder and transferring to a 10-ml volumetric flask, mixing until dissolved and mobile phase added to volume. Dilutions (1:10, 1:20, and 1:40) were made in the mobile phase from the standard solution to obtain solutions containing 50, 100, and 200 μg ml^{-1} of guaifenesin, 7.5, 15, and 30 μg ml^{-1} of pseudoephedrine HCl, and 2.5, 5, and 10 μg ml^{-1} of dextromethorphan HBr.

A combined standard solution containing guaifenesin and pseudoephedrine HCl was prepared by accurately weighing 10 and 1.5 mg of each powder and transferring to a 10-ml volumetric flask, mixing until dissolved and mobile phase added to volume. Dilutions (1:10, 1:20, and 1:40) were made in the mobile phase from the standard solution to obtain solutions containing 25, 50, and 100 μg ml^{-1} of guaifenesin and 3.75, 7.5, and 15 μg ml^{-1} of pseudoephedrine HCl.

A combined standard solution containing guaifenesin and codeine phosphate was prepared by accurately weighing 10 and 1 mg of each powder and transferring to a 10-ml volumetric flask, mixing until dissolved and mobile phase added to volume. Dilutions (1:10, 1:20, and 1:40) were made in the mobile phase from the standard solution to obtain solutions containing 25, 50, and 100 μg ml^{-1} of guaifenesin and 2.5, 5, and 10 μg ml^{-1} of codeine phosphate.

Three point calibration curves were constructed for each analyte in each drug mixture. Additional dilutions (1:13 and 1:27) of the combined standard solutions were prepared in mobile phase to serve as spiked samples for each analyte in each drug mixture to determine accuracy and precision of the method. Quantitation was based on linear regression analysis of analyte peak height versus analyte concentration in μg ml^{-1}.

2.4. Preparation of analytical samples

2.4.1. Capsules

One commercial gelatin capsule containing 200 mg guaifenesin, 30 mg pseudoephedrine HCl, and 10 mg dextromethorphan HBr was carefully cut using a disposable surgical blade. The capsule was placed in a 100 ml volumetric flask, 25 ml mobile phase added, and heated at 90°C over a steam bath for 10 min. After the gelatin capsule completely dissolved, the solution was allowed to cool for 45 min and mobile phase added to volume. The solution was mixed and sonicated for 10 min. Following sonication, a 1:20 dilution was made for analysis.

The same procedure was followed for a commercial gelatin capsule containing 200 mg guaifenesin and 30 mg pseudoephedrine HCl, however, a 1:40 dilution was made for analysis.

2.4.2. Cough syrup

A volume of cough syrup equivalent to 5 mg guaifenesin and 0.5 mg codeine phosphate (0.25 ml) was transferred to a 100 ml volumetric flask and mobile phase added to volume. The mixture was mixed and sonicated for 10 min.

3. Results and discussion

The goal of this study was to develop a single isocratic HPLC assay for the analysis of three typical cough-cold drug mixtures: guaifenesin–pseudoephedrine–dextromethorphan, guaifenesin–pseudoephedrine, and guaifenesin–codeine. Initial studies to develop a single isocratic HPLC

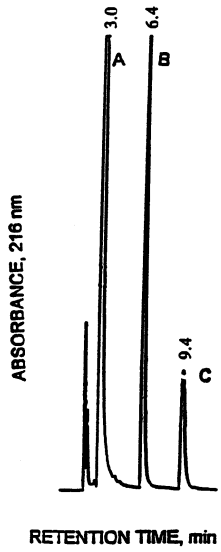

Fig. 2. Typical HPLC chromatogram of guaifenesin (A), pseudoephedrine (B), and dextromethorphan (C) on underivatized silica with acetonitrile–aqueous phosphate buffer pH 3.0 mobile phase. See Section 2 for assay conditions.

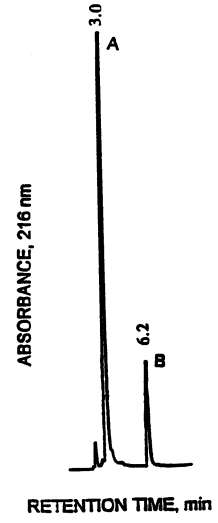

Fig. 3. Typical HPLC chromatogram of guaifenesin (A) and pseudoephedrine (B) on underivatized silica with acetonitrile–aqueous phosphate buffer pH 3.0 mobile phase. See Section 2 for assay conditions.

method for the analytes in each drug mixture involved the use of C_{18} and phenyl columns with various mobile phases containing acetonitrile- or methanol-aqueous phosphate buffers. In almost every system studied, dextromethorphan showed a retention time of greater than 30 min. Furthermore, guaifenesin and codeine were co-eluted.

Thus, our attention turned to the use of an underivatized silica column with a buffered aqueous-organic mobile phase for the separation and quantitation of the analytes in the drug mixtures. This laboratory has previously reported HPLC methods to analyze basic, acidic, and neutral compounds in pharmaceutical dosage forms and biological samples using underivatized silica [16–18]. The separation mechanism for basic drugs with buffered aqueous mobile phases has been ascribed to the interaction of silanols with an amine group to produce a cation exchange mechanism. Since there were no reports describing the separation of our drug mixtures on silica, we investigated chromatographic conditions previously reported by our lab [16]. Despite a pressure drop of 3000 psi, the use of a 25-cm underivatized silica column (3 μm particle size) proved advantageous in the separation of each guaifenesin mixture since guaifenesin behaved as an early eluter with the use of other columns. In addition, dextromethrophan co-eluted with pseudoephedrine with the utilization of other silica columns.

The final HPLC mobile phase consisting of 60:40 v/v phosphate buffer — acetonitrile with pH adjusted to 3.0 and an underivatized silica column, provided chromatograms (Figs. 2 and 3) with a steady base line and the specificity required for the simultaneous quantitation of guaifenesin–pseudoephedrine–dextromethorphan and guaifenesin–pseudoephedrine in capsule dosage forms. The method also afforded the simultaneous quantitation of guaifenesin-codeine (Fig. 4) in a commercially available cough syrup dosage form.

3.1. Linearity

Linearities were demonstrated for the guaifenesin–pseudoephedrine–dextromethorphan combination from 20 μl injections of solutions containing quantities of guaifenesin (50, 100, and 200 μg ml^{-1}), pseudoephedrine (7.5, 15 and 30 μg ml^{-1}), and dextromethorphan (2.5, 5, and 10 μg ml^{-1}). Linearities were demonstrated for the guaifenesin–pseudoephedrine mixture from injec-

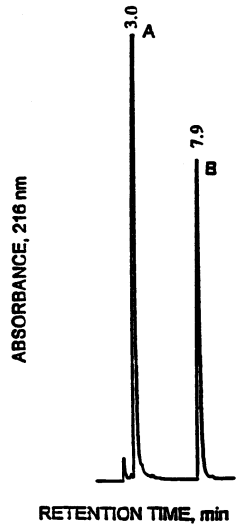

Fig. 4. Typical HPLC chromatogram of guaifenesin (A) and codeine (B) on underivatized silica with acetonitrile–aqueous phosphate buffer pH 3.0 mobile phase. See Section 2 for assay conditions.

tions of 20 μl of solutions containing quantities of guaifenesin (25, 50, and 100 μg ml^{-1}) and pseudoephedrine (3.75, 7.5, and 15 μg ml^{-1}). Linearities were demonstrated from 20 μl injections of solutions containing guaifenesin (25, 50, and 100 μg ml^{-1}) and codeine (2.5, 5, and 10 μg ml^{-1}) for the guaifenesin-codeine combination. The resulting data (Tables 1–3) was plotted as peak height versus concentration and studied by linear regression.

3.2. Precision

To obtain intra- and inter-day precision data for the guaifenesin–pseudoephedrine–dextromethorphan, guaifenesin-pseudoephedrine, and guaifenesin–codeine mixtures, five standard curves for each analyte in each drug mixture was prepared over 3 days. The results of the precision studies are tabulated in Tables 1–3.

Table 1
Intra-day and inter-day data for guaifenesin, pseudoephedrine, and dextromethorphan

Guaifenesin[a,b]	% RSD (50 μg ml^{-1})	% RSD (100 μg ml^{-1})	% RSD (200 μg ml^{-1})
Day			
1	3.05	4.20	3.22
	0.23	0.89	1.02
	1.23	3.32	2.15
2	1.23	2.45	1.82
3	3.55	0.81	0.37
Pseudoephidrine[a,c]			
Day	% RSD (7.5 μg ml^{-1})	% RSD (15 μg ml^{-1})	% RSD (30 μg ml^{-1})
1	1.44	1.11	2.85
	0.35	0.18	0.76
	0.96	0.75	1.30
2	1.84	1.15	0.35
3	1.12	0.38	1.44
Dextromethorphan[a,d]			
Day	% RSD (2.5 μg ml^{-1})	% RSD (5 μg ml^{-1})	% RSD (10 μg ml^{-1})
1	1.34	1.75	5.04
	0.50	0.71	1.41
	0.84	0.66	0.82
2	1.96	2.11	0.13
3	1.13	0.73	0.91

[a] Based on $n = 9$ for each curve constructed.
[b] r^2 ranged from 0.9921–0.9976 ($n = 9$).
[c] r^2 ranged from 0.9892–0.9935 ($n = 9$).
[d] r^2 ranged from 0.9932–0.9990 ($n = 9$).

Table 2
Intra-day and inter-day precision data for guaifenesin and pseudeoephedrine

Guaifenesin[a,b]	% RSD (25 μg ml−1)	% RSD (50 μg ml^{-1})	% RSD (100 μg ml^{-1})
Day			
1	0.65	1.84	0.88
	2.39	0.78	0.79
	4.18	0.36	2.33
2	1.20	1.17	2.30
3	1.29	1.75	1.56
Pseudoepedrine[a,c]			
Day	% RSD (3.75 μg ml−1)	% RSD (7.5 μg ml−1)	% RSD (15 μg ml^{-1})
1	0.68	0.70	3.00
	1.32	2.20	0.23
	0.28	0.92	0.97
2	1.00	1.52	0.45
3	0.90	1.15	0.51

[a] Based on $n = 9$ for each curve constructed.
[b] r^2 ranged from 0.9954 to 0.9999 ($n = 9$).
[c] r^2 ranged from 0.9963 to 0.9996 ($n = 9$).

Table 3
Intra-day and inter-day precision data for guaifenesin and codeine

Guaifenesin[a,b]	% RSD (25 μg ml−1)	% RSD (50 μg ml^{-1})	% RSD (100 μg ml^{-1})
Day			
1	4.25	2.91	1.25
	0.55	0.04	1.90
	1.92	0.37	2.49
2	1.30	3.69	0.76
3	2.30	0.46	2.01
Pseudoepedrine[a,c]			
Day	% RSD (3.75 μg ml−1)	% RSD (7.5 μg ml−1)	% RSD (15 μg ml^{-1})
1	1.59	0.18	2.02
	0.39	1.00	1.45
	2.08	0.58	1.90
2	1.02	1.92	1.02
3	1.26	0.14	1.40

[a] Based on $n = 9$ for each curve constructed.
[b] r^2 ranged from 0.9936 to 0.9999 ($n = 9$).
[c] r^2 ranged from 0.9981 to 0.9997 ($n = 9$).

3.3. Accuracy

Percent error and precision of the method were evaluated using spiked samples containing each analyte. The results shown in Table 4 indicate that the procedure gives acceptable accuracy and precision for all of the analytes in each drug mixture.

3.4. Assay of commercial dosage forms

The three combination standards of guaifenesin–pseudoephedrine–dextromethorphan were injected three times each to obtain a standard curve. The correlation coefficients for the curves were 0.9920, 0.9934, and 0.9942 for guaifenesin,

pseudoephedrine, and dextromethorphan, respectively ($n = 9$ for each curve). The capsule solution was injected three times and the data subjected to linear regression analysis. The percent label claim for the commercial capsule was found to be $100.51 \pm 1.96\%$ ($n = 3$, RSD = 1.95%) or 201.02 mg per capsule for guaifenesin, $103.87 \pm 1.34\%$ ($n = 3$, RSD = 1.29%) or 31.161 mg per capsule for pseudoephedrine, and $104.63 \pm 1.50\%$ ($n = 3$, RSD = 1.43%) or 10.46 mg per capsule for dextromethorphan.

The three combination standards of guaifenesin–pseudoephedrine were injected three times each to obtain a standard curve. The correlation coefficients for the curves were 0.9995 and 0.9990 for guaifenesin and pseudoephedrine, respectively ($n = 9$ for each curve). The capsule solution was injected three times and the data subjected to linear regression analysis. The percent label claim for the commercial capsule was found to be $101.64 \pm 0.58\%$ ($n = 3$, RSD = 0.57%) or 203.28 mg per capsule for quaifenesin and $101.63 \pm 1.29\%$ ($n = 3$, RSD = 1.27%) or 30.49 mg per capsule for pseudoephedrine.

The three combination standards of guaifenesin–codeine were injected three times each to obtain a standard curve. The correlation coefficients for the curves were 0.9936 and 0.9981 for guaifenesin and codeine, respectively ($n = 9$ for each curve). The cough syrup solution was injected three times and the data subjected to linear regression analysis. In quantitation, the percent label claim was found to be $99.38 \pm 0.97\%$ ($n = 3$, RSD = 0.98%) or 99.38 mg per capsule for guaifenesin and $99.93 \pm 0.74\%$ ($n = 3$, RSD = 0.74%) or 9.99 mg per capsule for codeine.

4. Conclusion

The proposed HPLC method in this study has the advantage of simplicity, precision, accuracy, and convenience for the separation and quantitation of guaifenesin–pseudoephedrinedextromethorphan, guaifenesin–pseudoephedrine, and guaifenesin–codeine and can be employed for their assay in dosage forms each with a single injection. Use of the combined method is thus more efficient than analysis of each drug mixture using more than one mobile phase or column. Moreover, the method uses simple reagents, with minimum sample preparation procedures, encouraging its application in routine analysis.

Table 4
Accuracy and precision using spiked drug samples

Analyte	Concn added (μg ml^{-1})	Concn found[a] (μg ml^{-1})	Percent error	% RSD
Guaifenesin–Pseudoephedrine–Dextromethorphan:				
Guaifenesin	153.85	152.35 ± 3.29	0.98	2.16
	74.07	72.36 ± 0.36	2.31	0.50
Pseudoephedrine	23.08	23.61 ± 0.88	2.30	3.72
	11.11	11.45 ± 0.04	3.06	0.35
Dextromethorphan	7.69	7.94 ± 0.14	2.73	1.77
	3.70	3.81 ± 0.04	2.97	1.05
Guaifenesin–Pseudoephedrine				
Guaifenesin	76.92	73.11 ± 0.24	4.95	0.33
	37.04	36.27 ± 0.10	2.08	0.29
Pseudoephedrine	11.54	11.41 ± 0.09	1.13	0.80
	5.56	5.36 ± 0.10	3.60	1.87
Guaifenesin–Codeine				
Guaifenesin	76.92	76.44 ± 1.47	0.62	1.92
	37.04	38.24 ± 0.60	3.24	1.56
Codeine	7.69	8.06 ± 0.13	4.81	1.62
	3.70	3.60 ± 0.03	2.70	0.83

[a] Based on $n = 3$.

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