Journal of Chromatographic Science, 2016, Vol. 54, No. 1, 28–35
doi: 10.1093/chromsci/bmv102
Advance Access Publication Date: 22 July 2015
Article

Article

A New UPLC Approach for the Simultaneous Quantitative Estimation of Four Compounds in a Cough Syrup Formulation

Fatma Turak[1], Remziye Güzel[2], and Erdal Dinç[3,*]

[1]Department of Chemistry, Faculty of Science and Art, Yıldız Teknik University, İstanbul, Turkey, [2]Department of Chemistry, Faculty of Education, Dicle University, Diyarbakır, Turkey, and [3]Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, Tandoğan, Ankara, Turkey

*Author to whom correspondence should be addressed. Email: dinc@ankara.edu.tr; dinc@pharmacy.ankara.edu.tr

Received 12 February 2015; Revised 30 May 2015

Abstract

A new ultra-performance liquid chromatographic (UPLC) method was developed for the simultaneous estimation of potassium guaiacolsulfonate (PGS), guaifenesin (GUA), diphenhydramine HCl (DIP) and carbepentane citrate (CAR) in a commercial cough syrup. The chromatographic separation of four compounds PGS, GUA, DIP and CAR was performed on a BEH phenyl column (100 × 2.1 mm, 1.7 µm i.d.) using a mobile phase consisting of acetonitrile and 0.1 M HCl (50 : 50, v/v). In addition, the optimized conditions of the chromatographic analysis were found with the flow rate of 0.38 mL/min, the column temperature of 30°C and the injection volume of 1.2 µL with the photodiode array detection of 220 nm. Calibration curves in the concentration ranges of 10–98 µg/mL for PGS, 5–80 µg/mL for GUA, 5–25 µg/mL for DIP and CAR were computed by the regression of the analyte concentration on the chromatographic peak area. The newly developed UPLC method was validated by analyzing the quaternary mixtures of the related compounds, intraday and interday experiment and standard addition samples. After method validation, the proposed UPLC approach was successfully applied for the analysis of the commercial syrup formulation containing PGS, GUA, DIP and CAR compounds.

Introduction

Potassium guaiacolsulfonate (PGS), 4-hydroxy-3-methoxy-benzene sulfonic acid, is widely used as an expectorant in pharmaceutical preparations for relieving symptoms of cough and mucus in the chest due to respiratory infections, asthma and cold. Guaifenesin (GUA), 3-(2-methoxphenoxy) propane-1,2-diol, has been used as an active compound that reduces the thickness and stickiness of mucus. It is used to treat symptoms of allergy, cold and upper respiratory infections. Diphenhydramine HCl (DIP), which is chemically known as 2-(diphenylmethoxy)-*N*,*N*-dimethylethylamine hydrochloride, is widely used as an antiallergic, antiemetic and antitussive drug found in many pharmaceutical dosage forms. It is usually given orally in a preparation of tablet, capsule or syrup. Carbetapentane or pentoxyverine (CAR), 1-phenylcyclopentane carboxylic acid 2-(2-diethylaminoethoxy) ethyl ester, is an antitussive (cough suppressant) commonly used for cough associated with illnesses like common cold. PGS, GUA, DIP and CAR have been used alone or in the form of binary, ternary and quaternary combinations in pharmaceutical preparations or with other active compounds.

In previous studies, the analysis of compounds in samples containing PGS, GUA, DIP and CAR with different active compounds using various analytical methods including spectrophotometry GUA (1, 2) DIP (3, 4), CAR (5), high-performance liquid chromatography (HPLC) GUA (6–13), CAR (14), thin-layer chromatography GUA (15), DIP (16, 17), liquid chromatography–mass spectrometry GUA (18–20), gas chromatography GUA (21), voltammetry CAR (22), chemiluminescence CAR (23), near infrared and Raman spectroscopy DIP (24), atomic absorption spectrometry DIP (25), HPLC and chemometrics-assisted UV spectrophotometry (26) have been reported for several mixtures and pharmaceutical products. A survey of the

© The Author 2015. Published by Oxford University Press. All rights reserved. For Permissions, please email: journals.permissions@oup.com

Downloaded from https://academic.oup.com/chromsci/article/54/1/28/2754740 by Yildiz Technical University Library user on 20 July 2023

Table I. Composition of the Calibration Set of the Analyzed Compounds

Set no.	PGS (μg/mL)	GUA (μg/mL)	DIP (μg/mL)	CAR (μg/mL)	Set no.	PGS (μg/mL)	GUA (μg/mL)	DIP (μg/mL)	CAR (μg/mL)
1	10	5	5	5	19	54	40	15	15
2	10	5	5	10	20	54	80	15	15
3	10	5	5	15	21	10	40	20	20
4	10	5	5	20	22	32	40	20	20
5	10	5	5	25	23	54	40	20	20
6	32	10	5	5	24	76	40	20	20
7	32	10	10	5	25	98	40	20	20
8	32	10	15	5	26	76	80	5	25
9	32	10	20	5	27	76	80	10	25
10	32	10	25	5	28	76	80	15	25
11	10	20	10	10	29	76	80	20	25
12	32	20	10	10	30	76	80	25	25
13	54	20	10	10	31	98	5	25	5
14	76	20	10	10	32	98	10	25	10
15	98	20	10	10	33	98	20	25	15
16	54	5	15	15	34	98	40	25	20
17	54	10	15	15	35	98	80	25	20
18	54	20	15	15					

Set no. denotes the calibration set of the analyzed compounds.

literature reveals that a ultra-performance liquid chromatographic (UPLC) method has not been reported for the simultaneous quantitative analysis of the quaternary mixture containing titled compounds.

In the chemical and pharmaceutical analysis, HPLC has been frequently used as a powerful technique for the qualitative and quantitative analysis of multicomponent mixtures (27–29). However, some of the main problems of this HPLC method are long period of analysis, tedious studies for finding optimal chromatographic conditions, excess solvent consumption, etc. Recently, in order to overcome the mentioned disadvantages of the conventional HPLC technique, UPLC method has been used to get some new possibilities in liquid chromatography, particularly concerning decrease in analysis time, solvent consumption, etc. For these reasons, this UPLC approach allows to make low-cost analysis for the separation and quantification of compounds in complex mixtures with adequate precise and accurate results.

In this study, the newly UPLC method was developed for the multicomponent determination of PGS, GUA, DIP and CAR in a commercial syrup preparation. This developed UPLC approach was tested and validated using the recovery studies, analysis of interday and intraday experiments and standard addition samples.

Experimental

Instrumentation and reagents

Chromatographic separation and analysis were carried out using Waters ACQUITY UPLC H-Class system, including a quaternary solvent manager photodiode array (PDA) detector, cooling autosampler and oven enabling control of column temperature. Chromatographic data collection and evaluation were made by Waters® Empower2 chromatography software. The elution of the peaks in the chromatogram was performed via a Waters UPLC BEH phenyl column (100 × 1.0 mm, 1.7 μm i.d.).

Acetonitrile, HPLC grade (Sigma–Aldrich), and HCl, analytical grade (Merck, Germany), were of guaranteed reagent grade. Water purified with Milli-Q Gradient A10, Millipore System (Merck Millipore, ABD) was used during chromatographic analysis. All solutions were filtered through a 0.20-μm millipore filter (Minisart hydrophilic syringe filter units, GmBH, Germany).

Chromatographic method conditions

The chromatographic elution of PGS, GUA, DIP and CAR in samples was accomplished using a mobile phase consisting of acetonitrile and 0.1 M HCl (50 : 50, v/v) with the flow rate of 0.38 mL/min and column temperature of 30°C. The chromatograms of the analyzed samples were recorded using a PDA detection at the wavelength 220 nm (see Figure 1A and C). In the sample runs, injection volume was 1.2 μL.

Standard solutions

The stock solutions of PGS (20 mg/100 mL), GUA (20 mg/100 mL), DIP (40 mg/100 mL) and CAR (50 mg/100 mL) were prepared in 0.1 M HCl. A calibration set of 35 mixture solutions containing PGS in the concentration range 10–98 μg/mL, GUA in the concentration range 5–80 μg/mL, DIP in the concentration range 5–25 μg/mL and CAR in the concentration range 5–25 μg/mL was obtained from the above stock solutions. An independent validation set of 20 mixtures containing the analyzed PGS, GUA, DIP and CAR in the above working concentration ranges was prepared using the stock solutions. Intraday and interday samples containing the quaternary combinations of PGS (at three different concentrations consisting of low: 10 μg/mL, medium: 54 μg/mL and high: 98 μg/mL), GUA (at three different concentrations consisting of low: 5 μg/mL, medium: 20 μg/mL and high: 80 μg/mL), DIP (at three different concentrations consisting of low: 5 μg/mL, medium: 15 μg/mL and high: 25 μg/mL) and CAR (at three different concentrations consisting of low: 5 μg/mL, medium: 15 μg/mL and high: 25 μg/mL) were prepared by means of the stock solution of the analyzed compounds. In addition, standard addition samples of the analyzed compounds in the concentration range of 10–76 μg/mL for PGS, 5–40 μg/mL for GUA, 5–20 μg/mL for DIP and 5–20 μg/mL for CAR at four different concentration levels were prepared from the stock solutions of PGS, GUA, DIP and CAR compounds.

Content of commercial preparation

A commercial syrup preparation, Gayaben® syrup (produced by Bilim Pharm. Ind., Istanbul, Turkey) containing 133 mg/5 mL PGS,

Downloaded from https://academic.oup.com/chromsci/article/54/1/28/2754740 by Yildiz Technical University Library user on 20 July 2023

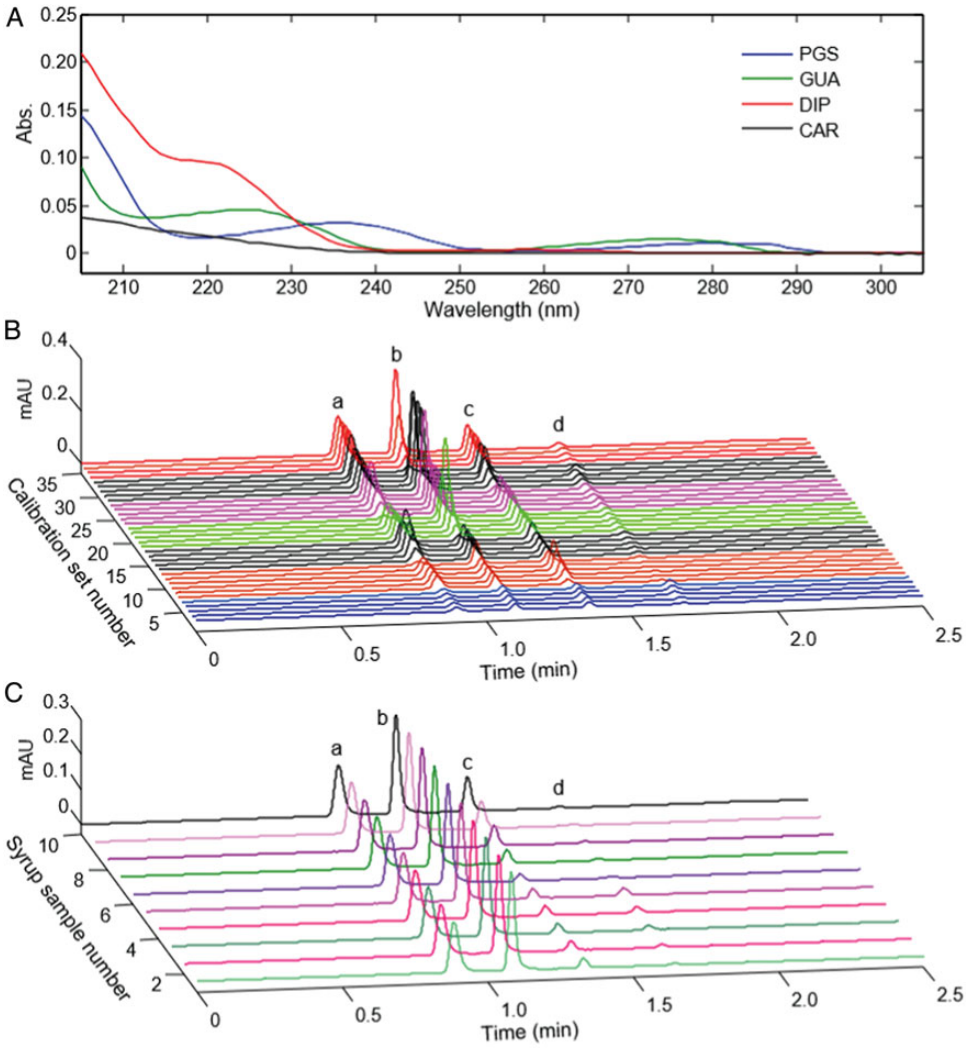


Figure 1. The UV spectra (A), chromatograms of the calibration samples (B) and commercial syrup samples (C) containing PGS (a), GUA (b), DIP (c) and CAR (d) in the quaternary mixture system. This figure is available in black and white in print and in color at *JCS* online.

Table II. Least Squares Regression Analysis and Statistical Results in the Application of the UPLC Method

Parameter	PGS	GUA	DIP	CAR
m	28.09	0.14	59.12	14.64
n	23.85	29.1	13.58	2.38
r	0.9997	0.9996	0.9988	0.9941
SD (*m*)	0.12	0.25	0.51	0.28
SD (*n*)	7.49	10.6	8.44	4.56
SD (*r*)	7.44×10^{-4}	8.03×10^{-4}	1.50×10^{-3}	3.29×10^{-3}
LOD (μg/mL)	0.8	0.57	0.43	0.94
LOQ (μg/mL)	2.67	1.91	1.43	3.12

100 mg/5 mL GUA, 10 mg/5 mL DIP and 8.3 mg/5 mL CAR, respectively, was analyzed by applying the proposed UPLC method. Commercial pharmaceutical preparation was bought from a local regional pharmacy, Ankara, Turkey.

Analysis of commercial sample

In order to analyze the commercial syrup samples, 2 mL of the syrup content containing 133 mg/5 mL of PGS, 100 mg/5 mL of GUA, 10 mg/5 mL of DIP and 8.3 mg/5 mL of CAR was transferred into a 100-mL calibrated flask and then make up to the mark with 0.1 M HCl. This solution was sonicated in an ultrasonic bath for 10 min and then was filtered through a 0.2-μm membrane syringe filter. The filtrate was diluted into the working concentration range. 1.2 μL of the filtrate solution was used for the chromatographic analysis. This sample preparation was repeated 10 times.

Downloaded from https://academic.oup.com/chromsci/article/54/1/28/2754740 by Yildiz Technical University Library user on 20 July 2023

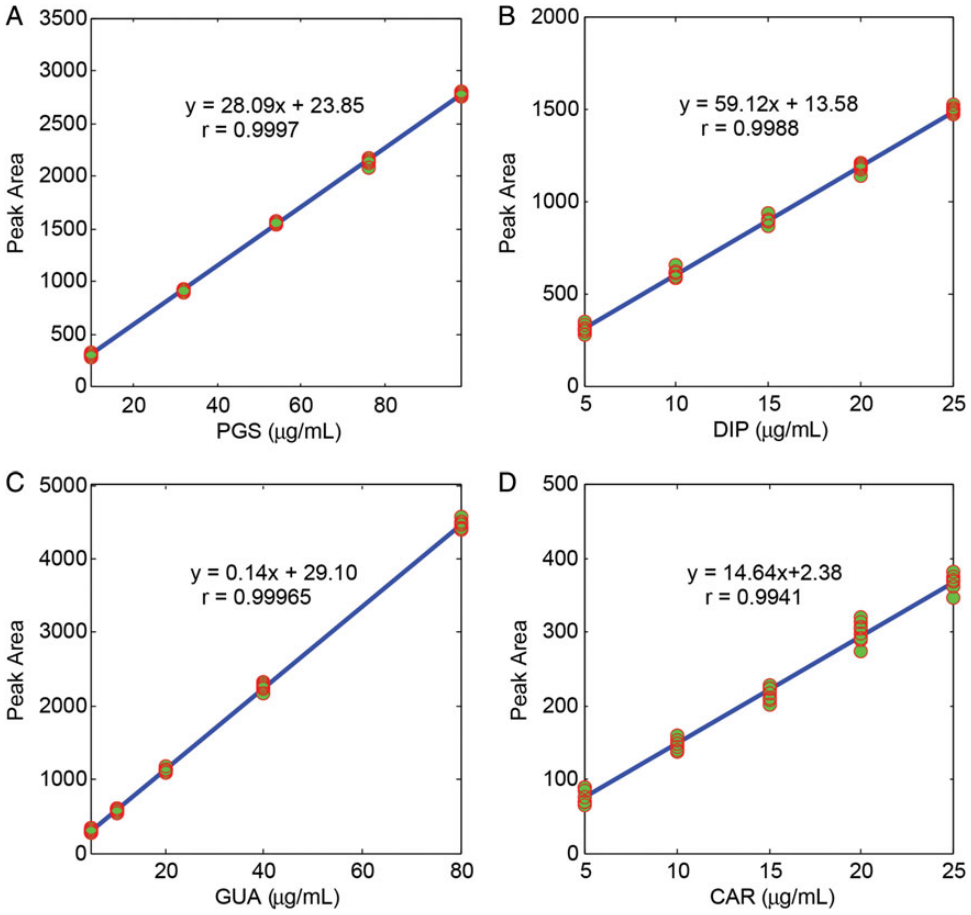


Figure 2. Calibration curves of the concentration of PGS (A), DIP (B), GUA (C) and CAR (D) versus the chromatographic peak area. This figure is available in black and white in print and in color at *JCS* online.

Results

Method development

Several mobile phase systems were tested for developing a new UPLC method for the multicomponent determination of PGS, GUA, DIP and CAR in commercial syrup formulation. The appropriate of the chosen mobile phase and related chromatographic conditions was determined considering the numerical values of chromatographic system suitability, e.g., peak resolution, number of theoretical plates and tailings. Taking into account the system suitability parameters for the analyzed PGS, GUA, DIP and CAR, it was concluded that chromatographic conditions consisting of a mobile phase containing acetonitrile and 0.1 M HCl (50 : 50, v/v) with the flow rate of 0.38 mL/min, column temperature of 30°C and injection volume of 1.2 µL were very suitable to get the optimal UPLC elution of the related drugs. Under the optimized conditions, the chromatographic analysis of the analyzed substances was explained below.

Application of developed method

In the application of the proposed UPLC method, a calibration set of 35 mixture solutions containing PGS in the range of 10–98 µg/mL, GUA in the range of 5–80 µg/mL, DIP in the range of 5–25 µg/mL and CAR in the range of 5–25 µg/mL was prepared as illustrated in Table I. Under optimized conditions, the chromatograms of the calibration set consisting of the analyzed compounds were recorded and presented in Figure 1B. Same procedure was applied for obtaining the chromatograms of all the analyzed samples as well as the chromatogram of syrup as shown in Figure 1C. From the chromatograms of the calibration samples, the calibration curves for PGS, GUA, DIP and CAR were computed by regressing the analyte concentration on the peak area obtained by the detection at 220 nm. The statistical results of the least square regression were illustrated in Table II. The calibration curves of the analyzed compounds PGS, GUA, DIP and CAR were graphically presented in Figures 2A–D. The calibration graphs were used for the quantitative estimation of the amount of PGS, GUA, DIP and CAR in samples.

Discussion

Validity of the developed UPLC method

The validity (or validation) of the developed UPLC method was performed by estimating specificity, selectivity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and system suitability parameters.

As it is shown in Table II, a good linearity with adequate correlation coefficients for the calibration curves of PGS, GUA, DIP and CAR in their working concentration ranges was observed from the relationship between concentration and chromatographic peak area. LOD and limit of quantitation were calculated by using the slopes and the standard deviation (SD) of the intercepts obtained from the linear regression functions (see Table II). Accuracy and precision parameters for the proposed UPLC method were evaluated by the analysis of the

Downloaded from https://academic.oup.com/chromsci/article/54/1/28/2754740 by Yildiz Technical University Library user on 20 July 2023

Table III. Recovery Results of PGS, GUA, DIP and CAR in Their Quaternary Mixtures by the Developed UPLC Method

Mix no.	Added (μg/mL)				Found (μg/mL)				Recovery (%)			
	PGS	GUA	DIP	CAR	PGS	GUA	DIP	CAR	PGS	GUA	DIP	CAR
1	96	70	7	5	95.81	71.27	7.06	5.11	99.8	101.8	100.8	102.1
2	96	70	7	10	95.68	70.41	7.24	9.88	99.7	100.6	103.4	98.8
3	96	70	7	15	96.15	70.59	7.25	15.35	100.2	100.8	103.6	102.3
4	96	70	7	20	94.95	70.40	6.98	19.25	98.9	100.6	99.7	96.2
5	96	70	7	25	95.64	70.47	7.48	24.77	99.6	100.7	106.9	99.1
6	96	70	5	6	96.14	71.24	5.00	5.96	100.1	101.8	99.9	99.3
7	96	70	10	6	97.74	71.81	10.24	6.04	101.8	102.6	102.4	100.7
8	96	70	15	6	96.92	71.95	15.53	5.79	101.0	102.8	103.5	96.4
9	96	70	20	6	95.61	70.49	20.08	5.87	99.6	100.7	100.4	97.9
10	96	70	25	6	95.49	70.77	25.42	6.16	99.5	101.1	101.7	102.6
11	10	70	7	6	9.80	70.23	7.32	6.09	98.0	100.3	104.5	101.4
12	32	70	7	6	31.94	69.76	7.13	5.89	99.8	99.7	101.8	98.2
13	54	70	7	6	54.80	70.10	7.46	6.10	101.5	100.1	106.6	101.7
14	76	70	7	6	78.67	69.90	6.94	5.86	103.5	99.9	99.1	97.7
15	98	70	7	6	98.94	70.46	7.02	5.87	101.0	100.7	100.2	97.8
16	96	5	7	6	96.98	5.20	6.84	5.69	101.0	104.1	97.7	94.8
17	96	10	7	6	96.79	10.16	6.96	6.02	100.8	101.6	99.5	100.4
18	96	20	7	6	96.91	20.57	7.22	6.13	100.9	102.8	103.1	102.2
19	96	40	7	6	97.72	40.29	7.01	6.08	101.8	100.7	100.1	101.3
20	96	80	7	6	95.02	79.98	7.13	6.21	99.0	100.0	101.9	103.5
								Mean	100.4	101.2	101.8	99.7
								SD	1.25	1.16	2.44	2.45
								RSD	1.25	1.15	2.4	2.45

Mix no. denotes quaternary mixtures.

synthetic quaternary mixture intraday and interday experiments and standard addition samples. As described above, the solutions of the synthetic quaternary mixtures containing PGS, GUA, DIP and CAR were proposed starting from the stock solutions of the analytes. Recovery results and relative standard deviations (RSDs) for the analyzed compounds were presented in Table III. These numerical values of recovery results indicated that the proposed UPLC method was very suitable for the quantification of four compounds in quaternary mixture.

In addition, the precision and accuracy of the proposed UPLC approach were verified by analyzing the intraday and interday experiments of the related drugs. The intraday and interday studies for three different concentration levels with six different times within day and six different days for each compound in their quaternary system were carried out to evaluate mean recoveries of the RSD% and percentage of relative errors (RE%) for each analyzed compound. Their results were summarized in Table IV. As it can be seen in Table IV, it was observed that the proposed UPLC methods gave us desirable analytical results for the analyzed substances.

In the application of the proposed UPLC method, the excipient effect of the commercial syrup formulation on the analysis was estimated by using the standard addition samples. Standard addition samples were prepared by adding the standard stock solution of the analyzed compounds into the solution of the commercial syrup. These standard addition samples contain four different concentration levels for each compounds in the presence of a constant content of syrup formulation. The analysis results, which correspond to recovery (%), SD and RSD, were given in Table V. The analysis of the standard addition samples was repeated six times for each concentration level. As it was pointed out in Table V, the effect of syrup excipients in the analysis of related compounds was not reported.

In order to visualize chromatographic conditions, a chromatogram was obtained from the standard mixture solution containing the analyzed compounds as depicted in Figure 3. The chromatogram was used to evaluate the system suitability parameters in the application of the proposed UPLC method to the analysis of the quaternary mixture. The retention times for PGS, GUA, DIP and CAR were observed as 0.902, 1.104, 1.357 and 1.679 min, respectively. From the chromatogram presented in Figure 3, the theoretical plates and tailings were computed as 3,580.2 and 1.55 for PGS, 10,017.7 and 1.57 for GUA, 12,631.4 and 1.34 for DIP and 15,578.6 and 1.36 for CAR, respectively. In addition, the resolution factor for PGS and GUA peaks, GUA and DIP peaks and DIP and CAR peaks was found as 3.88, 5.33 and 6.34, respectively. From these numerical values, it is clear that the proposed UPLC approach is very suitable chromatographic method for the analysis of the related drugs in commercial syrup samples due to the acceptable limits of the chromatographic system suitability.

Analysis of commercial syrup sample

As explained above, the samples of the commercial syrup formulation were prepared and injected into the UPLC system. After recoding the chromatograms as shown in Figure 1C, the areas of the resolved peaks of PGS, GUA, DIP and CAR in syrup samples were computed by means of Waters® Empower2 chromatography software. Replacing the computed peak areas into the least squares regression equations, the analyzed compounds in commercial samples were simultaneously determined. Their experimental results are given in Table VI. The assay results were obtained from the average of 10 experimental replicates. In Table VI, the SD and corresponding RSD for the analyzed PGS, GUA, DIP and CAR were depicted. Each assay result was obtained from three replicate injections during chromatographic analysis. A good agreement was reported for the results obtained by applying UPLC method.

Downloaded from https://academic.oup.com/chromsci/article/54/1/28/2754740 by Yildiz Technical University Library user on 20 July 2023

Table IV. Analysis Results of the Intraday and Interday Experiments by the Developed UPLC Method

	Samples (μg/mL)				Found (μg/mL)			
	PGS	GUA	DIP	CAR	PGS	GUA	DIP	CAR
Intraday	10	5	5	5	9.74	5.14	4.94	4.98
	54	20	15	15	54.01	20.05	15.16	14.79
	98	80	25	25	98.59	80.16	26.00	25.65
Interday	10	5	5	5	9.49	4.86	4.90	4.92
	54	20	15	15	54.21	19.84	15.48	14.78
	98	80	25	25	96.60	79.47	25.37	24.94
					Recovery (%)			
					PGS	GUA	DIP	CAR
Intraday					97.4	102.8	98.8	99.5
					100	100.2	101	98.6
					100.6	100.2	104	102.6
Interday					94.9	97.1	98.1	98.3
					100.4	99.2	103.2	98.5
					98.6	99.3	101.5	99.7
					RSD (%)			
					PGS	GUA	DIP	CAR
Intraday					3.45	1.74	0.98	2.77
					0.41	0.36	0.18	1.9
					0.27	0.23	0.38	1.58
Interday					1.96	2.54	1.77	2.91
					1.05	1.13	0.54	1.9
					0.24	0.08	2.00	1.96
					RE (%)			
					PGS	GUA	DIP	CAR
Intraday					−2.56	2.79	−1.19	−0.48
					0.06	0.25	1.02	−1.78
					0.6	0.2	3.99	2.62
Interday					−5.12	−2.86	−1.91	−1.68
					0.39	−0.79	3.2	−1.45
					−1.42	−0.66	1.49	−0.26

Table V. Analysis Results of the Standard Addition Samples by the Developed UPLC Method

	Added (μg/mL)	Found (μg/mL)	Recovery (%)	SD	RSD (%)
PGS	10	10.12	101.2	2.426	2.398
GUA	5	5.08	101.7	3.175	3.122
DIP	5	4.86	97.1	3.936	4.052
CAR	5	4.98	99.6	1.735	1.742
PGS	32	32.04	100.1	3.033	3.029
GUA	10	9.99	99.9	2.748	2.752
DIP	10	9.8	98	2.475	2.525
CAR	10	10.09	100.9	2.986	2.958
PGS	54	54.47	103.6	1.771	1.709
GUA	20	19.99	103.3	2.152	2.085
DIP	15	14.78	100.9	1.456	1.443
CAR	15	14.71	102.9	5.685	5.523
PGS	76	77.78	102.3	1.029	1.006
GUA	40	40.03	100.1	1.589	1.587
DIP	20	19.84	99.2	1.327	1.338
CAR	20	19.97	99.9	3.352	3.356

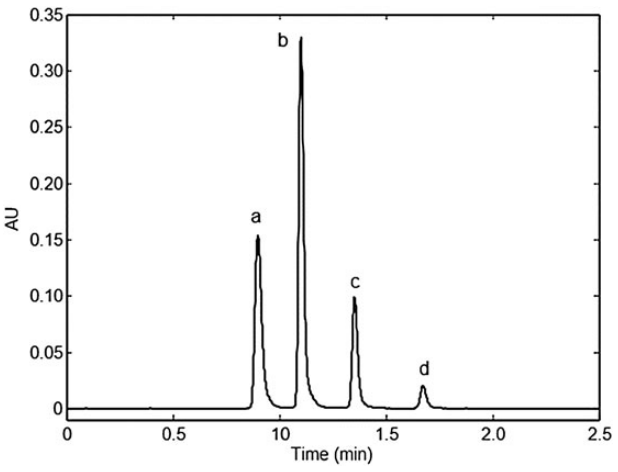


Figure 3. Chromatogram of 98 μg/mL PGS (a), 80 μg/mL GUA (b), 25 μg/mL DIP (c), and 25 μg/mL CAR (d) using a PDA detection at 220 nm.

Conclusion

In the present study, the newly UPLC method was developed for the simultaneous quantitative estimation of PGS, GUA, DIP and CAR in their synthetic quaternary mixtures and commercial syrup samples. It was concluded that the proposed UPLC approach was very applicable to estimate the concentrations of PGS, GUA, DIP and CAR in the commercial syrup because it allows fast and simultaneous analysis of each active compound in the quaternary mixture with reasonable reliable, accuracy and precision with short runtime (2.5 min). Compared with

Downloaded from https://academic.oup.com/chromsci/article/54/1/28/2754740 by Yildiz Technical University Library user on 20 July 2023

Table VI. Analysis Results of PGS, GUA, DIP and CAR in the Commercial Syrup Formulation by the Newly Developed RP-UPLC Method

Sample no.	PGS (mg/5 mL)	GUA (mg/5 mL)	DIP (mg/5 mL)	CAR (mg/5 mL)
1	137.39	97.38	10.22	8.46
2	136.58	96.37	9.89	8.17
3	135.9	96.91	9.71	7.83
4	134.98	95.58	9.79	8.21
5	133.81	96.46	9.86	8.16
6	136.94	96.70	10.20	8.42
7	134.98	97.62	9.75	8.15
8	133.98	98.02	9.74	8.05
9	134.86	99.37	10.08	8.00
10	135.24	95.69	9.86	8.26
Mean	135.5	97	9.9	8.2
SD	1.21	1.14	0.19	0.19
RSD	0.89	1.18	1.93	2.31

Labelled amount of drugs: 133 mg/5 mL PGS, 100 mg/5 mL GUA, 10 mg/5 mL DIP and 8.3 mg/5 mL CAR.

conventional HPLC methods in literature, the proposed UPLC method using the low concentration ranges of the analyzed drugs has many advantages such as a greater peak resolution, higher sensitivity and less solvent consumption and shorter running time and stronger elution ability and low cost of analysis time. As a consequence, the developed UPLC method can be applied to the quantitative estimation and quality control of the syrup formulation containing PGS, GUA, DIP and CAR compounds.

Acknowledgment

The authors would like to thank Ankara University for their support to complete this study.

Funding

This study was performed at the Laboratory of Faculty of Pharmacy, which was supported by the scientific research fund of Ankara University (Project Number 10A3336001).

References

1. Zare-Shahabadi, V., Shamsipur, M., Hemmatenejad, B., Akhond, M.; Simultaneous determination of guaifenesin and theophylline by chemometrics methods; *Analytical Letters*, (2010); 43: 687–700.
2. Hajian, R., Shams, N., Davarpanah, Z.; Combination of first derivative spectrophotometry and H-point standard addition method for simultaneous determination of guaifenesin and theophylline in cough syrup; *E-Journal of Chemistry*, (2011); 8(3): 966–976.
3. Ali, N.W., Abdelkawy, M., Abdelhamid, N.S.; Simultaneous determination of paracetamol and diphenhydramine hydrochloride mixture in the presence of their degradation products; *IOSR Journal of Pharmacy and Biological Science*, (2013); 6(5): 44–52.
4. Tipparat, P., Lapanantnoppakhun, S., Jakmunee, J., Grudpan, K.; Determination of diphenhydramine hydrochloride in some single tertiary alkylamine pharmaceutical preparations by flow injection spectrophotometry; *Journal of Pharmaceutical and Biomedical Analysis*, (2002); 30: 105–112.
5. Yuan, J.B., Hu, Y.A., Nie, L.H.; Non-potentiometric sensing method for the determination of pentoxyverine with PQC sensors based on ionpair complexes; *Analytical Science*, (2001); 17: 1389–1393.
6. Grosa, G., Grosso, E.D., Russo, R., Allegrone, G.; Simultaneous, stability indicating, HPLC-DAD determination of guaifenesin and methyl and propyl-parabens in cough syrup; *Journal of Pharmaceutical and Biomedical Analysis*, (2006); 41: 798–803.
7. Wilcox, L.M., Stewart, J.T.; HPLC determination of guaifenesin with selected medications on underivatized silica with an aqueous-organic mobile phase; *Journal of Pharmaceutical and Biomedical Analysis*, (2000); 23: 909–916.
8. Galli, V., Barbas, C.; High-performance liquid chromatographic analysis of dextromethorphan, guaifenesin and benzoate in a cough syrup for stability testing; *Journal of Chromatography A*, (2004); 1048: 207–211.
9. Stavchansky, S., Demirbaş, S., Reyderman, L., Chai, C.K.; Simultaneous determination of dextrorphan and guaifenesin in human plasma by liquid chromatography with fluorescence detection; *Journal of Pharmaceutical and Biomedical Analysis*, (1995); 13: 919–925.
10. Xu, X., Stewart, J.T.; Mekc determination of guaifenesin, pseudoephedrine, and dextromethorphan in a capsule dosage form; *Journal of Liquid Chromatography & Related Technology*, (2000); 23(1): 1–13.
11. Özdemir, A., Aksoy, H., Dinç, E., Baleanu, D., Dermiş, S.; Determination of guaifenesin and dextromethorphan in a cough syrup HPLC with fluorometric detection; *Revue Roumanie de Chimie*, (2006); 51(2): 117–122.
12. Elkady, E.F.; Simultaneous determination of diclofenac potassium and methocarbamol in ternary mixture with guaifenesin by reversed phase liquid chromatography; *Talanta*, (2010); 82: 1604–1607.
13. Hatami, M., Farhadi, K., Abdollahpour, A.; Using dispersive liquid–liquid microextraction and liquid chromatography for determination of guaifenesin enantiomers in human urine; *Journal of Separation Science*, (2011); 34: 2933–2939.
14. Gad-Kariem, E.A., Abounassif, M.A.; Determination of pentoxyverine in cough preparations by high performance liquid chromatography; *Journal of Liquid Chromatography & Related Technology*, (1997); 20(18): 3049–3059.
15. Abdelkawy, M., Metwaly, F., El Raghy, N., Hegazy, M., Fayek, N.; Simultaneous determination of ambroxol hydrochloride and guaifenesin by HPLC, TLC-spectrodensitometric and multivariate calibration methods in pure form and in cough cold formulations; *Journal of Chromatography Separation Techniques*, (2011); 2(3): 2–9.
16. DiGregorio, D., Sherma, J.; Determination of the sleep aid ingredients diphenhydramine hydrochloride and doxylamine succinate in pharmaceutical products by quantitative HPTLC; *Journal of Liquid Chromatography & Related Technology*, (1999); 22(10): 1599–1606.
17. Muller, E.E., Sherma, J.; Quantitative HPTLC determination diphenhydramine hydrochloride in tablet, gelcap, and capsule antihistamine pharmaceuticals; *Journal of Liquid Chromatography & Related Technology*, (1999); 22(1): 153–159.
18. Chen, X., Huang, J., Kong, Z., Zhong, D.; Sensitive liquid chromatography–tandem mass spectrometry method for the simultaneous determination of paracetamol and guaifenesin in human plasma; *Journal of Chromatography B*, (2005); 817: 263–269.
19. Eichhold, T.H., McCauley-Myers, D.L., Khambe, D.A., Thomson, G.A., Hoke, S.H. II; Simultaneous determination of dextromethorphan, dextrorphan, and guaifenesin in human plasma using semi-automated liquid/liquid extraction and gradient liquid chromatography tandem mass spectrometry; *Journal of Pharmaceutical and Biomedical Analysis*, (2007); 43: 586–600.
20. Wen, J., Zhang, H., Xia, C., Hu, X., Xu, W., Cheng, X., *et al.*; A sensitive liquid chromatography-electrospray ionization–mass spectrometry method for the simultaneous determination of pentoxyverine citrate and guaifenesin in human plasma-application to pharmacokinetic and bioequivalence studies; *Biomedical Chromatography*, (2010); 24(4): 351–357.
21. Sharaf, M.H.M., Stiff, D.D.; Determination of guaifenesin in human serum by capillary gas chromatography and electron capture detection; *Journal of Pharmaceutical and Biomedical Analysis*, (2004); 35: 801–806.
22. Liu, Y., Pan, W., Liu, Q., Yao, S.; Study on the enhancement of Ru(bpy)3(2+) electrochemilumiscence by nanogold and its application for pentoxyverine detection; *Electrophoresis*, (2005); 26(23): 4468–4477.

Downloaded from https://academic.oup.com/chromsci/article/54/1/28/2754740 by Yildiz Technical University Library user on 20 July 2023

23. Zhang, H., Nie, F., Lu, J.; Flow injection chemiluminescence method for the determination of pentoxyverine citrate based on NCS-dichlorofluorescein post-chemiluminescence reaction; *Spectrochimica Acta Part A*, (2009); 72: 858–862.
24. Orkoula, M.G., Kontoyannis, C.G., Markopoulou, C.K., Koundourellis, J.E.; Quantitative analysis of liquid formulations using FT-Raman spectroscopy and HPLC The case of diphenhydramine hydrochloride in Benadryl; *Journal of Pharmaceutical and Biomedical Analysis*, (2006); 41: 1406–1411.
25. El Reis, M.A., Khalil, S.; Indirect atomic absorption determination of atropine, diphenhydramine, tolazoline, and levamisole based on formation of ion-associates with potassium tetraiodometrcurate; *Journal of Pharmaceutical and Biomedical Analysis*, (2001); 25: 3–7.
26. El-Gindy, A., Emara, S., Mesbah, M.K., Hadad, G.M.; New validated methods for the simultaneous determination of two multicomponent mixtures containing guaiphenesin in syrup by HPLC and chemometrics-assisted UV-spectroscopy; *Analytical Letters*, (2006); 39: 2699–2723.
27. Zacharis, C.K.; Accelerating the quality control of pharmaceuticals using monolithic stationary phases: a review of recent HPLC applications; *Journal of Chromatographic Science*, (2009); 47: 443–451.
28. Sultana, N., Shafi, N., Arayne, M.S., Siddiqui, F.A., Hussain, A.; Development and validation of new assay method for the simultaneous analysis of diltiazem, metformin, pioglitazone and rosiglitazone by RP-HPLC and its applications in pharmaceuticals and serum; *Journal of Chromatographic Science*, (2011); 49(10): 774–779.
29. Rezk, M.R., Michael, A.M., Lotfy, H.M., El-Kadi, A.O., Shehata, M.A.; A rapid and sensitive HPLC assay of some concomitant anti-migraine drugs; *Journal of Chromatographic Science*, (2014); 52(7): 704–706.

Downloaded from https://academic.oup.com/chromsci/article/54/1/28/2754740 by Yildiz Technical University Library user on 20 July 2023