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Quantitation of Guaifenesin (Glyceryl Guaiacolate) in Syrup and Spiked Human Plasma using HPLC with Diode Array Detection

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A new, simple and rapid reverse phase-high performance liquid chromatography-diode array detection (RP-HPLC-DAD) method has been developed using sibutramin (S) as internal standard for quantitative determination of guaifenesin (G) and validated both in syrup dosage forms and spiked human plasma samples. The analysis was carried out within 10 min on Inertsil C_{18} column using isocratic mobile phase consists of phosphate buffer and methanol with pH 3 (60:40, v/v) at a flow-rate of 1 mL min^{-1} and detection at 212 nm. The concentration-response relationship was linear over a concentration range of 10-100 μg mL^{-1} for guaifenesin. The developed liquid chromatographic method was successfully applied for the routine analysis of guaifenesin in a cough syrup and human plasma samples without liquid-liquid extraction for quality control purposes. After the method development, bioanalytical method validation was applied. For this purpose, all the validation parameters such as intra-day and inter-day precisions, accuracy, specificity, selectivity and recovery were determined. After the validation procedure, the results were evaluated statistically.

Keywords: Guaifenesin (Glyceryl guaiacolate), HPLC-DAD method, Human plasma, Syrup dosage form.

INTRODUCTION

Guaifenesin (glyceryl guaiacolate), [(RS)-3-(2-methoxy-phenoxy)propane-1,2-diol, $C_{10}H_{14}O_4$, FW = 198.22 g mol^{-1}] (Fig. 1) is an expectorant drug. It is used to relieve chest congestion and may help control symptoms but does not treat the cause of symptoms or speed recovery. It works by thinning the mucus in the air passages to make it easier to cough up the mucus and clear the airways [1-3].

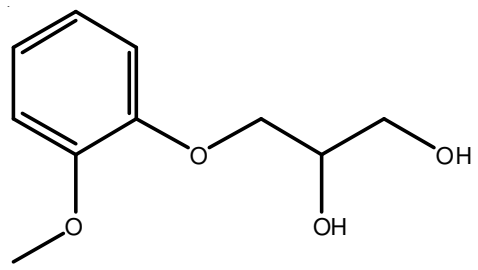

Fig. 1. Chemical structure of guaifenesin

Several liquid chromatographic methods in relation to guaifenesin have been reported in the literature. There are several quantitative HPLC analysis methods for pharmaceutical preparations containing guaifenesin with two, three or five other active ingredients [4-9], cold syrups [10-18] and in human plasma [19-24]. Two HPLC quantitative analysis studies have seen for human and animal plasma containing only guaifenesin [25,26]. In present work, an easy, fast, practical and economical HPLC method with reverse-phase DAD detector was developed for the quantitative analysis of guaifenesin active ingredient alone in a trade preparation (Expectorant syrup containing 200 mg/15 mL guaifenesin) or in human plasma as *in vitro*. This study is the continuation of previous work related to the quantity analysis of guaifenesin mixed with an another active ingredient (Efedrin HCl) in a trade preparation [23]. Furthermore, thanks to the method developed under the same chromatographic conditions with previous study, guaifenesin and sibutramin (internal standard) quantity analysis were carried out using less methanol in very short retention times in an economic and environmentally friendly way and very high recovery values obtained without any need for an extraction process. Therefore, it is the first in literature from this aspect.

EXPERIMENTAL

In this study, certificated pure guaifenesin (glyceryl guaiacolate) (G) standard active ingredient (99.0 % purity), Vicks VapoSyrup expectoran trade syrup containing this active ingredient (200 mg/15 mL) and sibutramin (S), as internal standard were used.

The chemicals were obtained from commercial sources and all of them were analytical grade. Buffer solutions were prepared with deionized water. For the chromatographic analysis, HPLC grade-Merck methanol, KH_2PO_4 (Sigma-Aldrich), H_3PO_4 and deionized water was used. Frozen human plasma for the plasma analysis was obtained from a healthy volunteer subject in Sakarya University, Faculty of Medicine, Practice and Research Hospital (Sakarya, Turkey).

In the analysis, a Samsung Computer, SIL-20A HT Prominence Auto Sampler, SPD-M20A Prominence DAD detector, LC-20 AD prominence liquid chromatography, DGU-20 A5 Prominence degasser, CTO-10AS VP column oven and SHIMADZU brand LC-20 AD prominence liquid chromatography system running LC Solution program were used.

In addition to these, refrigerator (Arcelik), precision scales (AND GR-200), mixer (Vortex 2 GENIE), vacuum pump (Rocker 300), 0,45 μm cellulose nitrate membrane filter (Sartorius Stedim Biotech), pH meter (HANNA), centrifuge (nuve-NF200, ROTINA 420-Hettich), automatic pipet (BIOHIT PROLINE), oven (nuve-FN 120), ultrasonic bath (Bandelin Sonorex), 12 × 32 mm 2 mL silicon septum capped auto-sampler glass vial kit (National Scientific CERT4000), C_{18} reverse-phase column (Inertsil ODS-2 GL Sciences Inc. 5 μm (4.6 × 250 mm)) and plastic vial filters (CHROMACOL) were also used.

Chromatographic conditions and optimization: For the HPLC method developed, 200-400 nm range were scanned with UV-DAD detector using isocratic pump system and separate chromatograms were obtained for 212, 224, 254, 256 and 272 nm wavelengths. The maximum absorbance for guaifenesin was obtained at 212 nm and taken as the most suitable wavelength. All the chromatograms were recorded in this wavelength. As a result of the experiments made in the HPLC with $_{18}C$ inverse phase, 10 μL injection volume and 1 mL min^{-1} flow rate at 25 °C column oven temperature were determined as the most suitable conditions. Among the chromatograms obtained under these conditions, retention times (RT) for guaifenesin and sibutramin were determined as 4.62 and 1.81 min (run time was 10 min) respectively (Fig. 2). For mobile phase selection, different combinations such as methanol-water, methanol-acetonitrile, water-acetonitrile, acetonitrile-phosphate buffer, methanol-phosphate buffer at different concentrations (such as 40:60, 50:50, 20:80, 5:95) were used. As a result of different mobile phase trials, phosphate buffer-methanol mixture at 60:40 rate with pH = 3 was chosen as the most suitable isocratic mobile phase mixture. The column was cleaned by rinsing with dionized water and washed with methanol-deionized water mixture at a rate of 70:30. Prepared mobile phase and all other organic solvents are used after they are filtered by 0.47 μm (nylon-47 mm) membrane filter under vacuum.

System suitability parameters were calculated for guaifenesin. The capacity factor (k_G') was 1.55 in flow rate of 1 mL min^{-1}, the total theoretical plate number (N_G) was 2134, the plate height (H_G) was $1.17.10^{-2}$ cm. The parameters were checked with those of CDER [27].

Preparation of stock and standard solutions: Main stock solution for guaifenesin was prepared in methanol mixture to contain 1000 μg mL^{-1}. To form the calibration line, 8 different

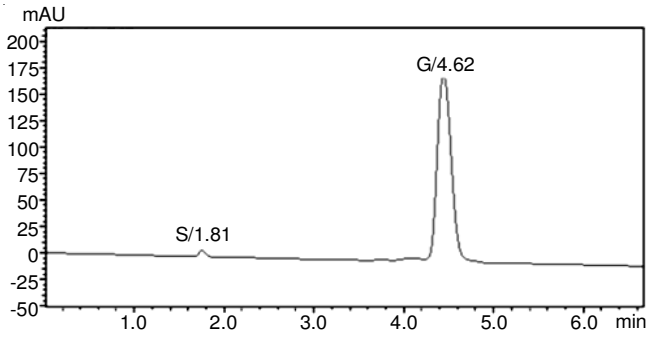

Fig. 2. Chromatogram for 100 μg mL^{-1} guaifenesin (with sibutramin internal standard) in solvent

standard solutions were taken from this main stock solution and prepared daily in 10-100 μg mL^{-1} concentration range for guaifenesin (10, 20, 30, 50, 70, 80, 90 and 100 μg mL^{-1}). 1 mL (50 μg mL^{-1}) internal standard (sibutramin) was added onto these standard solutions and each were diluted to be 25 mL with methanol mixture.

For recovery works, 12 new standard mixtures were prepared daily from the same guaifenesin stock mixture (10, 12,5, 20, 25, 30, 50, 60, 70, 80, 85, 90 and 100 μg mL^{-1}). In the same way 1 mL sibutramin standard were added onto them.

In vitro standard solutions in plasma were prepared by spiking 1 mL sibutramin standard onto the 1 mL of frozen human plasma sample obtained from a healthy volunteer. After that 10, 20, 30, 50, 70, 80, 90 and 100 μg mL^{-1} standards taken from guaifenesin stock solution spiked onto plasma samples and they were diluted to 25 mL with methanol. All the plasma samples were mixed for 5-10 min with vortex mixer and then centrifuged at 6000 rpm for 20-30 min to have the proteins precipitated. In this way, all the plasma samples were prepared easily without any need for liquid-liquid extraction.

For the plasma recovery works, guaifenesin stock solutions between 10-100 μg mL^{-1} were spiked onto plasma samples in the same way. 1 mL sibutramin standard solutions were added onto these standards and deleted with methanol. These solutions were taken into glass capped vials after filtering with 45 μm filters and the chromatograms were recorded at 212 nm with DAD detector (Fig. 3).

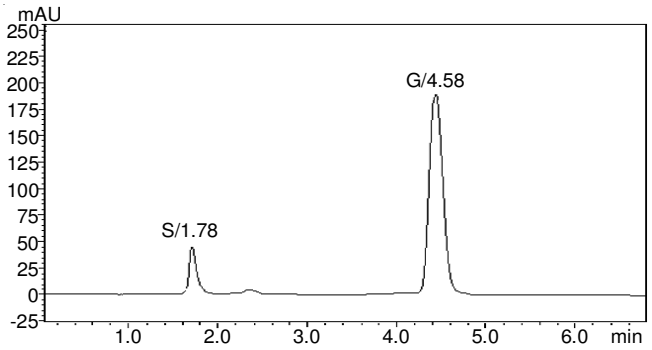

Fig. 3. Chromatogram for 100 μg mL^{-1} guaifenesin (with sibutramin internal standard) in plasma

All the stock and standard solutions were kept at the refrigerator at +4 °C. Before the analysis they were brought to the room temperature and mixed for 5-10 min with vortex mixer.

Preparation of pharmaceutical samples: A stock solution prepared to be 2000 μg mL^{-1} in methanol media from Vicks VapoSyrup expectoran trade syrup (containing 200 mg guaifenesin active ingredient in 15 mL). After that definite amounts were taken from this stock solution and diluted with methanol to be 50 μg mL^{-1} and 6 trade syrup standards were prepared in the same way. 1 mL sibutramin solution were added into each trade sample.

For the sample analysis in plasma, 1 mL of stock solution prepared from trade syrup (containing 200 mg guaifenesin in 15 mL) added into 1 mL plasma samples and internal standard sibutramin solution were added onto each trade sample. After that they are diluted to 25 mL with methanol and 6 plasma standard solutions were prepared in the same way.

Preparation of standard solutions with standard addition method: 15, 20, 25, 40 and 45 μg mL^{-1} guaifenesin stock solutions were put into 5 different balloons. After that 5, 10, 25, 30 and 35 μg mL^{-1} trade syrup solutions and 1 mL sibutramin mixture were added onto them. Finally, they all completed into 25 mL with methanol to have 50 μg mL^{-1} standards.

For the plasma standard solutions, 1 mL plasma sample and 5, 10, 25, 30 and 35 μg mL^{-1} guaifenesin stock solutions were put into 5 different balloons. After that 5, 10, 25, 30 and 35 μg mL^{-1} trade syrup solutions and 1 mL sibutramin mixture added onto them. Each were completed with methanol to to have 50 μg mL^{-1} standards.

RESULTS AND DISCUSSION

Method validation: After method development to determine guaifenesin in human plasma, linearity, the limit of quantitation (LOQ), the limit of detection (LOD), precision, accuracy, selectivity and recovery parameters were determined in accordance with ICH guidelines [28] for validation.

Linearity of calibration curves: In this study, besides direct calibration method, internal standard and standard added methods were used for calibration purposes. Eight different standard solutions were prepared for guaifenesin within 10-100 μg mL^{-1} concentration range and analyzed using HPLC (n = 6). Standard concentration graph obtained from the area under G/S peak curves (y = 0.0645x + 0.0189, R = 0.9999). The standard deviation of the intercept of regression line (S_b) is 0.7211 and the standard deviation of slope (S_m) is 0.0843. LOQ and LOD values obtained were 4.46 and 1.33 μg mL^{-1} respectively.

Furthermore, a new calibration curve was obtained for guaifenesin within 10-100 μg mL^{-1} concentration range by graphing recovery concentrations using the areas under guaifenesin/sibutramin (G/S) peak curves of the recovery solutions prepared from 12 diffe-rent standards (y = 0.0655x + 0.0258, R = 0.9978) (S_b = 0.7460 and S_m = 0.0946). In this method, LOQ and LOD values were 4.43 and 1.33 μg mL^{-1}, respectively.

After that to analyze guaifenesin in human plasma (*in vitro*), 8 different standard solutions within the 10-100 μg mL^{-1} concentration range were used and 1 mL internal standard added onto each (using spike method). Concentration graphs were formed against the areas under G/S peak curves and a calibration curve was obtained (y = 0.0700x-0.0790, R = 0.9970) (S_b = 0.9631 and S_m = 0.272). In this method while LOQ value was 4.21, LOD value was 1.26 μg mL^{-1}.

Recovery calibration curve values were obtained from 12 different standards in plasma (by adding 1 mL internal standard solutions). All plasma recovery concentrations were graphed against the areas under G/S peak curves (y = 0.0609x-0.0689, R = 0.9980) (S_b = 0.9149 and S_m = 0.266). In this method, while LOQ value was 4.25, LOD was 1.28 μg mL^{-1}.

All line equations obtained from the regression analysis and regression coefficients were calculated and very good linearity values obtained.

Precision: Method precision in solvent and plasma medium were calculated at 3 different concentrations (20, 50 and 90 μg mL^{-1}) chosen for guaifenesin (by adding internal standard solution). Intra-day and inter-day precision measurements were made 6 times in a day with certain intervals during 3 consecutive days (for repeatibility purposes). All the intra-day and inter-day precision and repeatibility results, average values, standard deviations and relative standard deviations are given in Table-1. All RSD values for both intra- and inter-day precision were lower than RSD % ≤ 2.05 as given Table-1.

After that, the method was applied to trade syrup preparation containing 100 mg guaifenesin in methanol and plasma medium. The samples obtained from this preparation containing 1 mL internal standard were repeated 6 times. Repeatability values obtained from these samples were summarized in Table-2. Relative standard deviation values are ≤ 3.78 %.

These data indicated that the developed method had a good repeatability for guaifenesin. The precision values for the method were well within the proposed criteria (RSD % < 20).

Accuracy: The method was applied to all standards prepared in solvent and plasma medium by adding internal standard into 12 standards at different concentrations and quite good recovery values were obtained (Table-3). Relative error (accuracy) data were < 10 %.

The results obtained from standard solutions prepared with standard addition method in solvent and plasma medium were

TABLE-1
INTRA-DAY AND INTER-DAY PRECISION VALUES FOR GUAIFENESIN IN PLASMA AND SOLVENT MEDIUM

Standard conc. (μg mL^{-1})	Solvent media						Plasma media					
	Intra-day			Inter-day			Intra-day			Inter-day		
	X*	SD*	RSD* (%)	X*	SD*	RSD* (%)	X*	SD*	RSD* (%)	X*	SD*	RSD* (%)
20	395566	721.02	0.12	395627	4268.42	0.72	391155	862.18	0.14	389096	1711.32	0.26
50	961206	1057.70	0.86	962103	3632.50	0.30	944515	7520.10	0.62	945032	7403.52	0.68
90	1776971	37830.20	2.05	1775838	3790.20	0.24	1802077	37262.15	1.98	1797678	32456.50	1.27

*X: Average area, SD: Standard deviation, RSD (%): Relative standard deviation: Standard deviation.100/Average area (n = 6).

TABLE-2
REPEATABILITY VALUES OBTAINED FOR GUAIFENESIN IN TRADE PREPARATIONS IN PLASMA AND SOLVENT MEDIUM

Added conc. (μg mL^{-1})	Solvent media		Plasma media	
	Found conc. (μg mL^{-1})	Recovery (%)	Found conc. (μg mL^{-1})	Recovery (%)
50	54.62	109.23	56.04	112.07
50	53.45	106.89	56.15	112.31
50	54.75	109.51	55.96	111.92
50	54.52	109.04	55.59	111.20
50	54.25	108.51	55.79	111.58
50	54.52	109.04	56.08	110.12
X	54.35	108.70	55.94	111.53
SD	3.70		2.90	
RSD (%)	3.78		2.60	

*Recovery (%) = (Found/Added) × 100

summarized in Table-4. The percentage recoveries from added guaifenesin standards and commercial samples were calculated by concentrations found. The mean recoveries for guaifenesin were 98.60 % in methanol and 106.70 % in plasma. The RSD values obtained for guaifenesin were quite low both in methanol (2.18 %) and plasma (2.16 %).

Selectivity: Selectivity is the process of correct measurement of the material analyzed while working with matrix. Beside guaifenesin active ingredient, which has the same retention time in this HPLC method, no peaks were observed to interfere the matrix except the peaks of sibutramin internal standard. As it can be seen from the chromatograms of prepared standards, trade preparations and plasma samples, a good separation was obtained under the determined chromatographic conditions (Figs. 2 and 3).

Statistical comparison: The suggested HPLC-DAD method was applied to the analysis of commercial preparations containing guaifenesin (100 μg mL^{-1}) in standard solutions and plasma medium and then it was developed and validated. Furthermore, the results obtained from standard solutions and plasma media were statistically compared by student's t-test (for accuracy) and variance F-test (for precision). At 95 % confidence level, the results showed that the results of both medium were close to each other, since the tabulated t-value was higher than calculated t value and the tabulated F-value was higher than calculated F-value (Table-5).

Conclusion

This new reverse phase-HPLC-DAD method can be applied to trade preparations and human plasma samples containing guaifenesin in a fast and practical manner. To our best of knowledge, this is the first description for guaifenesin determination in both pharmaceutical preparations and human plasma samples (*in vitro*). Quite good recovery values were obtained in a short period of time without any need for liquid-liquid extraction process. The method can be used easily in routine quantitative clinical plasma studies. Furthermore, the

TABLE-3
RECOVERY VALUES OBTAINED FOR GUAIFENESIN STANDARDS IN SOLVENT AND PLASMA MEDIUM

Added concentration (μg mL^{-1})	Solvent media			Plasma media		
	Found concentration (μg mL^{-1})	Recovery (%)	Accuracy (Relative error, %)*	Found concentration (μg mL^{-1})	Recovery (%)	Accuracy (Relative error, %)*
10.0	9.42	94.26	-5.80	10.96	109.60	9.60
12.5	12.64	101.12	1.12	13.60	108.80	8.80
20.0	21.26	106.32	1.30	21.30	106.50	6.50
25.0	25.48	101.94	1.92	26.13	105.52	4.52
30.0	30.93	103.12	3.10	31.83	106.10	6.10
50.0	51.19	102.37	2.38	51.80	103.60	3.60
60.0	62.08	103.48	3.46	62.50	104.16	4.16
70.0	74.89	106.99	6.98	75.10	107.28	7.26
80.0	82.02	102.53	2.53	82.48	103.10	3.10
85.0	89.65	105.47	5.47	89.10	104.83	4.82
90.0	91.72	101.90	1.91	92.45	102.72	2.72
100.0	103.83	103.83	3.83	104.15	104.15	4.15

*Accuracy (Relative error, %): (Found–Added/Added)×100 (n = 6)

TABLE-4
RESULTS OBTAINED FROM STANDARD ADDITION METHOD

Solvent Media			Plasma Media		
Added concentration (μg mL^{-1})		Found concentration (μg mL^{-1})	Added concentration (μg mL^{-1})		Found concentration (μg mL^{-1})
Commercial syrup solution	Standard solution of guaifenesin		Commercial syrup solution	Standard solution of guaifenesin	
35	15	48.27	35	15	53.68
30	20	49.12	30	20	53.21
25	25	48.97	25	25	52.01
10	40	50.80	10	40	53.43
5	45	49.34	5	45	54.11
Average concentration		49.12	Average concentration		53.29
Average recovery (%)		98.60	Average recovery (%)		106.70
SD		2.32	SD		2.30
RSD (%)		2.18	RSD (%)		2.16

TABLE-5
t-TEST AND F-TEST VALUES OBTAINED FOR TRADE PREPARATIONS (α = 0.05, WITH CONFIDENCE LEVEL 95 %)

Statistical data	100 µg mL^{-1} Guaifenesin (with sibutramin internal standard)	
	Solvent media	Plasma Media
Average recovery (%)	108.71	111.62
SD	0.94	0.76
RSD (%)	0.87	0.68
Standard error*	0.39	0.31
t-test $t_{calculated}$ (t_{table})		2.02 (2.57)
F-test $F_{calculated}$ (t_{table})		1.27 (5.05)

*Standard error: Standard deviation/$\sqrt{n}$ (n = 6)

method shall be preferred thanks to its simplicity, economic and sensitivity. This analytical method was validated by applying specificity, sensitivity, precision, repeatability, accuracy and recovery parameters and evaluated statistically. According to the statistical data, there is no significant difference between the quantitative analysis results obtained from solvent and plasma medium (α = 0.05 and 95 % confidence level).

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