Revue Roumaine de Chimie, **2006**, *51*(2), 117–122

DETERMINATION OF GUAIFENESIN AND DEXTROMETHORPHAN IN A COUGH SYRUP BY HPLC WITH FLUOROMETRIC DETECTION

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Received May 26, 2005

A practical high-performance liquid chromatographic method with fluorometric detection for the determination of guaifenesin and dextromethorphan in cough syrup is described. The developed method contains simple, accurate and rapid sample preparation steps for the separation and simultaneous determination of active compounds in samples with existence of other excipients. Amilorid (AM) was used as an internal standard (IS) that has a fluorescence character in the working wavelength region. A good chromatographic separation of two drugs and IS was achieved on a Waters Symmetry ® C18 Column 5 µm 4.6 × 250 mm with mobile phase consisting of phosphate buffer (0.2 M, pH=2), acetonitrile and methyl alcohol (v/v, 62:23:15). Eluents were monitored by fluorescence detection at the excitation and emission wavelengths of 277 and 588 nm, respectively. Under the described conditions, the obtained calibration graphs are linear over the concentration range of 0.05–0.2 and 1.2–2.4 mg/mL for dextromethorphan and guaifenesin, respectively and the regression coefficients were greater than 0.999. The validation study was carried out fulfilling the ICH guidelines in order to prove that the new analytical method meets the fundamental criteria including selectivity, linearity, precision, accuracy and sensitivity.

INTRODUCTION

The common cough syrup ingredients are: cough suppressant, expectorant, preservative, sweeteners, acidulates, artificial coloring and flavoring agents. All these excipients are contained in a pharmaceutical form in very different proportions. The analysis of cough syrups is difficult due to their matrix effect coming from inactive ingredients. For this reason the determination of active compounds in syrup samples is very important for the routine quality control without interference from other ingredients in the pharmaceutical formulation.

There are several proposed methods describing the simultaneous determination of GU and DEX in different combinations in various cough-cold formulations.[1-7] This study deals with the investigation of a cough syrup formulation that contains guaifenesin and dextromethorphan generally taken for the relief of common cough-cold symptoms. In the previous studies, the measurement of subjected drugs in different combinations with other substances was done using gas chromatography,[6,7,9] capillary electrophoresis,[4,6] liquid chromatography[1-3,4,8,10] and derivative spectrophotometry.[10]

The main objective of this study is to develop a new, fast, reliable and simple HPLC method for the determination of GU and DEX drugs together with its latter validation study. The method validation was carried out using the parameters proposed by the ICH guidelines.[12-13] The obtained experimental results showed that the developed HPLC method was suitable and reliable for the quantitative analysis of GU and DEX in commercial pharmaceutical preparation.

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EXPERIMENTAL

Instruments

Chromatography was performed with an Agilent 1100 series HPLC system (Agilent Technologies, Inc., California, and USA) provided with a quaternary pump, a thermostatted autosampler, a thermostatted column compartment, and a fluorescence detector. Data were acquired and processed using HP Chem Station for LC (Rev. A0.01 [403]) software from Hawlet-Packard. The column used was a Waters Symmetry ® C18 Column 5 µm 4.6 × 250 mm. The flow rate was maintained at 1.8 mL/min and the injection volume was 25 µL. The mobile phase was prepared daily, filtered through a 0.45 µm membrane filter.

Material and reagents

The commercial pharmaceutical formulation, Robitussin® Cold and congestion syrup (produced by A-H-ROBINS®, USA, Batch no. A42400) containing 10 mg DEX, and 200 mg GU in 5 mL were purchased from a USA pharmacy.

Active compounds were kindly obtained from national industrial firms. All the reagents were HPLC grade quality. Methanol, acetonitrile, phosphoric acid and NaOH were purchased from MERCK and doubly distilled water used in all the solution preparations was provided from the department.

Standard solutions

Stock solutions of 4 mg/100 mL DEX, 200 mg/100 mL GU and 25 mg/100 mL IS were prepared in mixture of phosphate buffer (0.2 M, pH=2), methyl alcohol and acetonitrile (62:23:15,v/v). A standard series of the solutions containing 0.05-0.2 mg/mL DEX and 1.2-2.4 mg/mL GU was obtained from the stock solutions. A validation set consisting of 8 synthetic mixture solutions in the above mentioned working range of 0.05-0.2 mg/mL DEX and 1.2-2.4 mg/mL GU was prepared. For the standard addition technique, six solutions using the stock solutions and tablet solutions were prepared. In all the chromatographic study, 10 µg/mL AM as internal standard were added into each solution. All the solutions were prepared freshly and protected from light.

Sample analysis

An amount equivalent to one dosage was mixed with the mobile phase in a 100 mL calibrated flask. The solution was filtered into a 100 mL calibrated flask by a 0.45 µm membrane filter. Prepared solutions were diluted to the working concentration range of 0.1 mg/mL for DEX and 2.0 mg/mL for GU in a 25 mL-calibrated flask. The developed HPLC method was applied to the final sample solution.

RESULTS

1. Method development

For the chromatographic separation, several mobile phases and flow rates were tested using C18 column (Waters Symmetry ® C18 Column 5 µm 4.6 × 250 mm) at the ambient temperature. The optimal chromatographic separation of GU and DEX in the presence of IS was obtained with a mobile phase consisting of phosphate buffer, methyl alcohol and acetonitrile (62:23:15, v/v) at the flow rate of 1.8 mL/min. We tried different internal standards that give fluorescence at the selected wavelengths. Different suitable compounds were found but for a faster method development, we selected amilorid because of lack of interaction with the sample, its faster elution time (Fig. 1) and its emission at the same wavelength with the active ingredients of the sample. Through the analysis, 25 µL sample were injected into the column. Fig. 1 shows the representative chromatogram of two active ingredients and the internal standard. Retention times were obtained as 1.33 for IS, 2.93 for GU and 5.23 for DEX (Fig. 1). The developed HPLC method is very convenient for fast and reliable determination of the active compounds in the synthetic mixtures and commercial pharmaceutical preparation.

2. Calibration graphs

The chromatograms corresponding to the concentration range of 0.05-0.2 mg/mL DEX and 1.2-2.4 mg/mL GU in the presence of the constant 10 µg/mL IS were plotted using a fluorescence detector at the excitation and emission wavelengths of 277 and 588 nm as shown in Fig. 1. Although the individual active compounds have different emission wavelengths, we found 588 nm as an optimum wavelength for the

emission of three compounds in the same chromatographic conditions. The detector responses were measured in terms of peak area. Although the internal standard chromatogram produced a tailing that is normal for most of the HPLC chromatograms, we control the integration of each chromatogram before getting the peak ratio for the calibration. In this emission wavelength, a straight line for each drug was obtained using the relationship between peak area and concentration. The obtained linear regression equations at their statistical parameters are presented in Table 1. The correlation coefficients of regression equations were found to be higher than 0.999. Calibration graphs were used for the determination of two drugs in samples.

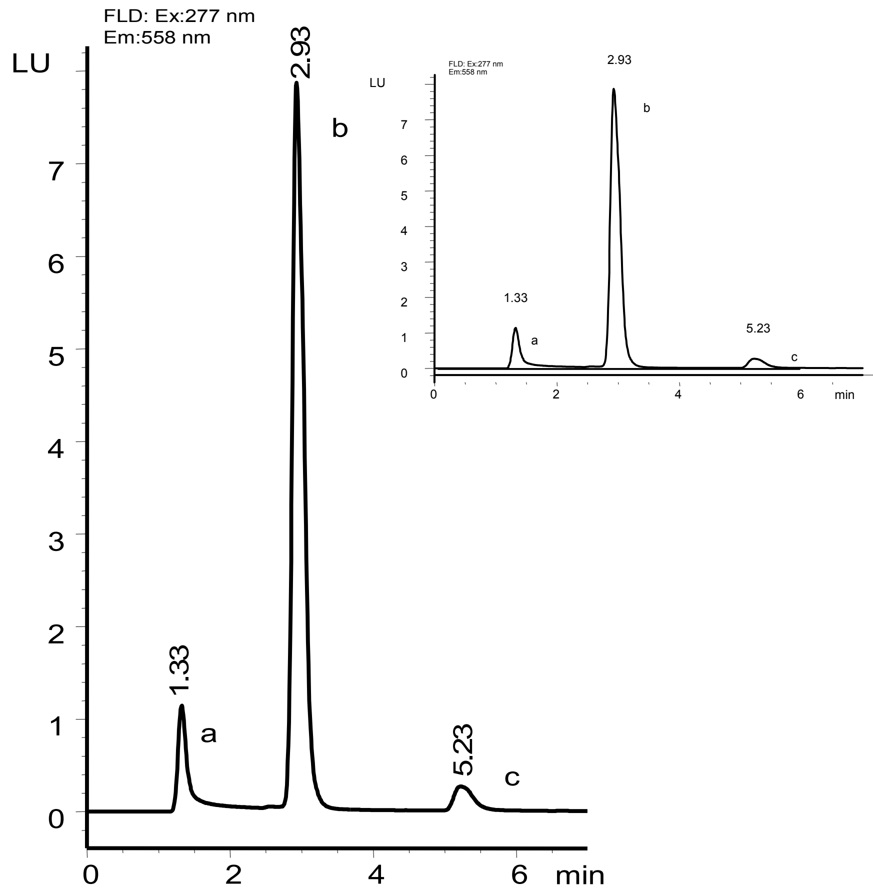

Fig. 1 – HPLC chromatogram of amilorid (IS) (a), guaifenesin (b), and dextromethorphan (c) on RP C18 column with phosphate buffer, acetonitrile and methyl alcohol mobile phase (62:23:15, v/v). Inset figure shows the overlaid chromatogram with the blank sample.

Table 1

Linear regression analysis and its statistical results for GU and DEX

Compound	Equation	r	SE(m)	SE(n)	SE (r)	LOD µg/mL	LOQ µg/mL
GU	A=0.0033×C - 0.0107	0.9999	3.8E-05	0.0701	0.0338	1.1341	3.7816
DEX	A=0.0019×C + 0.1014	1.0000	7.6E-06	0.0010	0.0008	0.0616	0.0855

m : Slope
n : Intercept
r : Correlation coefficient
SE(m) : Standard error of slope
SE(n) : Standard error of intercept
SE(r) : Standard error of correlation coefficient
LOD : Limit of detection
LOQ : Limit of quantitation
C : Concentration (µg/ml)
A : Peak area ratio

3. Method validation

The linearity of the developed HPLC method for the analysis of GU and DEX in the presence of the internal standard was observed in the concentration range of 0.05-0.2 mg/mL DEX and 1.2-2.4 mg/mL GU in the presence of the constant 10 μg/mL IS. Depending on the International Conference on Harmonization (ICH),[12-13] six different concentrations ranging from 0.05 to 0.2 mg/mL DEX and from 1.2 to 2.4 mg/mL GU were used for the construction of two calibration equations. An average of three injections was used for each concentration value. For the optimum detector response, different excitation and emission wavelengths were tested and the highest and linear response was observed at 277 and 588 for excitation and emission, respectively.

The accuracy for the applied HPLC method was confirmed by applying it to the synthetic mixtures in different concentration levels of GU and DEX. The recovery study of the proposed HPLC method was estimated by analyzing the above mentioned mixtures of GU and DEX in the presence of the IS. The obtained average recovery for the synthetic mixtures was 98.9 for GU and 99.0 for DEX.

Repeatability, intra-day and inter-day variability were tested for the evaluation of the precision of the developed HPLC method. The obtained results for inter-day and intra-day are presented in Table 2 and 3, respectively. From the results presented in Tables 2 and 3 inter-day and intra-day precisions of the method were found to be satisfactory. We also applied the one-way ANOVA test to the recovery results. The statistical results with 95% of confidential limit indicate that there is no significant difference between inter and intra-day analysis results in respect of the tabulated F value. The statistical values of the ANOVA test were found as 1.70 for DEX and 0.05 for GU, which is smaller than the F critical value (2.43).

Table 2

The recoveries and their relative standard deviations for inter-day results

No	Added		Day 1 Found μg/mL		Day 2 Found μg/mL		Day 3 Found μg/mL		Day 1 % Recovery		Day 2 % Recovery		Day 3 % Recovery	
	GU	**DEX**	**GU**	**DEX**	**GU**	**DEX**	**GU**	**DEX**	**GU**	**DEX**	**GU**	**DEX**	**GU**	**DEX**
1	2000	50	1994.9	51.3	2050.3	48.9	2032.6	51.1	102.5	97.9	99.7	102.7	101.6	102.3
2	2000	100	1959.9	96.7	2064.5	102.3	2022.2	97.5	103.2	102.3	98.0	96.7	101.1	97.5
3	2000	150	1947.4	146.4	2078.6	146.7	2008.0	148.6	103.9	97.8	97.4	97.6	100.4	99.0
4	2000	200	1974.7	201.3	2036.4	202.5	2025.5	200.9	101.8	101.3	98.7	100.7	101.3	100.5
5	1200	100	1192.4	96.8	1223.8	99.8	1218.1	97.3	102.0	99.8	99.4	96.8	101.5	97.3
6	1600	100	1577.8	98.9	1598.6	96.4	1512.2	98.6	99.9	96.4	98.6	98.9	94.5	98.6
7	2000	100	1973.5	103.5	2005.9	101.9	1980.7	101.7	100.3	101.9	98.7	103.5	99.0	101.7
8	2400	100	2491.0	98.2	2430.4	103.7	2455.7	102.9	101.3	103.7	103.8	98.2	102.3	102.9
								Mean	101.9	100.1	99.3	99.4	100.2	100.0
								SD	1.37	2.57	1.96	2.62	2.51	2.18
								RSD	1.35	2.57	1.98	2.63	2.50	2.18

Another parameter for the validation of the developed HPLC method is the standard addition technique. The standard of two pure drugs equivalent to the pharmaceutical formulation content was added to the pharmaceutical sample solutions in the working concentration range. The prepared solutions were analyzed by the proposed HPLC method. The results and their standard deviations were calculated and presented in Table 4. The recovery values were obtained in an average of three replicate for each active compound. A good agreement was observed for the standard addition assay results by application of the method. During the analysis procedure, the developed HPLC method did not give any interference and systematical error for the determinations. The specificity of the method was examined through observing if

there was any response of the excipients of the syrup. Chromatograms of the synthetic syrup content were compared with those of the commercial syrup content and no significant difference was observed. The peak purity was also tested by comparing the peak area of the active ingredients at different elution times. The resulting eluting peak at various time points revealed exactly the same chromatographic peaks, which indicated peak the purity. In the above evaluation, the main peaks were compared with the impurities and impurity effects were found to be of minimal values under our chromatographic conditions.

Table 3

The recoveries and their relative standard deviations for intra-day results

No	Added		Intra-day 1 Found		Intra-day 2 Found		Intra-day 3 Found		Intra-day 1 % Recovery		Intra-day 2 % Recovery		Intra-day 3 % Recovery	
	GU	**DEX**	**GU**	**DEX**	**GU**	**DEX**	**GU**	**DEX**	**GU**	**DEX**	**GU**	**DEX**	**GU**	**DEX**
1	2000	50	1982.9	47.7	1970.2	49.0	1963.0	48.8	99.1	95.5	98.5	98.1	98.1	97.7
2	2000	100	1962.7	96.4	1960.2	103.8	1956.8	96.5	98.1	96.4	98.0	103.8	97.8	96.5
3	2000	150	1961.3	141.6	1942.3	144.9	1928.6	145.0	98.1	94.4	97.1	96.6	96.4	96.6
4	2000	200	1961.9	201.3	1935.0	196.8	2010.4	194.5	98.1	100.7	96.7	98.4	100.5	97.3
5	1200	100	1172.9	95.8	1201.5	96.3	1165.9	96.3	97.7	95.8	100.1	96.3	97.2	96.3
6	1600	100	1564.2	102.3	1525.5	95.5	1586.3	97.9	97.8	102.3	95.3	95.5	99.1	97.9
7	2000	100	1973.5	104.2	1963.7	97.4	1938.3	97.4	98.7	104.2	98.2	97.4	96.9	97.4
8	2400	100	2454.9	101.2	2306.8	96.9	2315.0	97.8	102.3	101.2	96.1	96.9	96.5	97.8
								Mean	98.7	98.8	97.5	97.9	97.8	97.2
								SD	1.51	3.69	1.51	2.57	1.42	0.63
								RSD	1.53	3.73	1.55	2.62	1.46	0.65

Table 4

Standard addition technique and cough syrup assay results

	Standard addition		Syrup analysis*	
	GU (%)	**DEX** (%)	**GU** (%)	**DEX** (%)
Mean:	101.2	101.2	100.6	100.8
SD	2.90	2.84	2.96	3.51
RSD	2.86	2.81	2.95	3.48
SE	1.30	1.27	1.33	1.57
CL(0.05)	2.54	2.49	2.60	3.07

SD = Standard deviation
RSD = Relative standard deviation
SE = Standard error
CL = Confidential limit
*Assay syrup results were obtained from ten replicated experiments.

Evaluating the limit of detection, the limit of quantitation and the linearity of the detector response performed the validation of the proposed assay. The limit of detection (LOD) defined as the concentration that gives rise to a signal that is three times the noise of the baseline was found as 1.13 μg/mL for GU, 0.06 μg/mL for DEX (see Table 1). The limit of quantitation (LOQ) defined as the concentration that produces a signal that is 10 times the noise in the baseline was 3.78 μg/mL for GU and 0.08 μg/mL for DEX (see Table 1). The obtained results confirmed the high sensitivity of the proposed procedure compared to all the reported methods for the analysis of GU and DEX.

4. Dosage form analysis

The prepared cough syrup solution was injected three times and by introducing the obtained chromatographic data into the linear regression equations. The experimental results were calculated for both active compounds in the commercial samples. The results of the analysis as percentage per label for the developed method were found to be 100.6 for guaifenesin and 100.8 for dextromethorphan (Table 4). From the results, the accuracy of the method can be inferred from the demonstrated lack of interference of the excipients. Also other statistical parameters including standard deviation, relative standard deviation and confidential limit (Table 4) prove the accuracy of the method.

CONCLUSION

In this study a simple HPLC method based on fluorometric detection was proposed for the quantitative determination of GU and DEX in the commercial pharmaceutical preparation. The method has the advantage of fluorometric detection for the subject active compounds and provides shorter elution time than that reported in the literature for these active compounds in the presence of other active compounds. The experimental results gave prices and accurate results and convenience for the separation and quantitation of GU and DEX in the cough-syrup assays. The use of an appropriate column and mobile phase that we already determined in this study, the separation of these active compounds are possible in pharmaceutical preparations. Also the method uses simple reagents, with minimum sample preparation procedures, encouraging its application in routine analysis.

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