Simultaneous HPLC Determination of Thiocolchicoside and Glafenine as well as Thiocolchicoside and Floctafenine in Their Combined Dosage Forms

Mohamed Walash, Fathalla Belal, Manal Eid*, and **Samah Abo El Abass**

Department of Analytical Chemistry, Faculty of Pharmacy,University of Mansoura, Mansoura, 35516, Egypt

Abstract

Sensitive and accurate high-performance liquid chromatographic methods have been developed for the simultaneous determination of thiocolchicoside (TC)–glafenine (GF) (Mix I) and thiocolchicoside–floctafenine (FN) (Mix II) in their pharmaceutical formulations. The analysis for both mixtures was performed using 250 mm × 4.6 mm i.d., 5 µm particle size C18 Waters Symmetry column. The mobile phase consisted of methanol–0.035 M phosphate buffer (50:50, v/v) of pH 4.5 for Mix I and methanol–0.03 M phosphate buffer (70:30, v/v) of pH 4 for Mix II with flow rate of 1 mL/min and UV detection at 400 nm in both cases. The calibration plots were rectilinear over the concentration range of 0.2–2 µg/mL for TC in both mixtures and 20–200 µg/mL for each of GF and FN .The limits of detection for TC and GF were 0.05 µg/mL and 0.62 µg/mL, respectively, and for TC and FN were 0.02 µg/mL and 0.70 µg/mL, respectively. Additionally, the proposed methods were successfully applied to their combined tablets with average percentage recoveries of 100.35 ± 0.61and 100.57 ± 0.72% for TC and GF respectively and for TC and FN the percentage recoveries were 101.2 ± 0.72 and 100.36 ± 0.67%, respectively. The results obtained were favorably compared with those given using the comparison methods.

Introduction

Thiocolchicoside (TC) is a muscle relaxant that has been claimed to possess GABA-mimetic and glycinergic actions. It is used in the symptomatic treatment of painful muscle spasm (1). It is 2-demethoxy-2-glucosidoxythiocolchicine (2) (Figure 1). It is an official drug in the French Pharmacopoeia (3). Thiocolchicoside is coformulated with each of glafenine and floctafenine as nonnarcotic analgesics used to relieve mild to moderate pain, also used for the relief from fever and inflammation (1), glafenine is 2,3-Dihydroxy-propyl N-(7-chloro-4-quinolyl) anthranilate (Figure 1) and floctafenine is 2,3-Dihydroxy-propyl N-(8-trifluoromethyl-4-quinolyl) anthranilate (Figure 1) (1,4).

Several methods were reported for determination of TC individually in its dosage forms including high-performance liquid chromatography (HPLC) (5), radioimmunoassay (6), and LC with tandem mass detection for the determination of primary metabolite in human plasma (7). Various methods have been reported for the determination of GF including spectrophotometry (8–13), H- point standard addition method (14), titrimetric method (15), potentiometry (16), gravimetry (17), polarography (18), thin layer chromatography (19), and HPLC methods (19–21). Similarly FN has been determined adopting spectrphotometric (10,11,13), fluorimetric (22,23), polarographic (24), and HPLC (25,26,27) methods.

Only a TLC method has been reported for the determination of TC (the minor component) in presence of GF and FN (28). This method ignores the determination of GF or FN (the major components) due to their high concentrations, which caused overlapping between the spots. It is evident that there is a need for the simultaneous determination of TC and each of GF and FN in their combined dosage forms, HPLC by virtue of its high versatility could solve this problem.

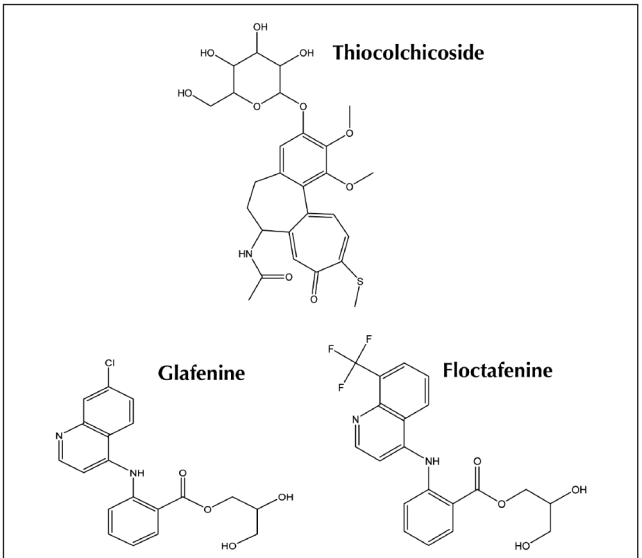

Figure 1. The structural formulae of thiocolchicoside, glafenine, and floctafenine.

*Author to whom correspondence should be addressed: email manal_eid@yahoo.com.

This paper reports two sensitive, accurate, and precise HPLC methods for the simultaneous determination of TC and each of GF or FN in their tablets in a ratio of (1:100).

Experimental

Apparatus

Separations were performed using a Merck Hitachi L-7100 Chromatograph equipped with a Rheodyne injector valve with a 20 µL loop and a L-7400 UV detector (Darmstadt, Germany). Chromatograms were recorded on a Merck Hitachi D-7500 integrator. Mobile phases were degassed using Merck L-7612 solvent degasser.

A Consort NV P901 pH Meter calibrated with standard buffers was used for pH measurements.

Materials and reagents

Thiocolchicoside (with a purity of 100.43%) (5), glafenine (with a purity of 100.20%) (19), and floctafenine (with a purity of 100.83%) (27) pure samples were kindly provided by Memphis Pharm. Co. (Cairo, Egypt) and were used as received. Methanol and acetonitrile (HPLC grade) were obtained from Sigma-Aldrich (Germany). Sodium dihydrogen phosphate was obtained from El-Nasr Chem. Co. (Cairo, Egypt). Phosphoric acid was purchased from Reidel de-Haën (Sleeze, Germany). Glifarelax tablets (Batch #307146) containing 2 mg of thiocolchicoside and 200 mg of glafenine, a product of Memphis Pharm. Co. (Cairo, Egypt), were obtained from commercial sources in the local market.

For prepared tablets containing TC and FN: (TC 2 mg, FN 200mg, talc powder 20 mg, maize starch 15 mg, lactose 15 mg, and magnesium stearate 10 mg per tablet) were prepared.

Chromatographic conditions

The chromatographic separation for both mixtures was achieved on Waters symmetry C18 column (250 mm × 4.6 mm i.d., 5-µm particle size), the mobile phase used for the analysis of Mix I consisted of methanol–0.035 M phosphate buffer (50:50, v/v) of pH 4.5 and that for Mix II consisted of methanol–0.03 M phosphate buffer (70:30, v/v) of pH 4 flow rate of 1 mL/min and UV detection at 400 nm for both mixtures.

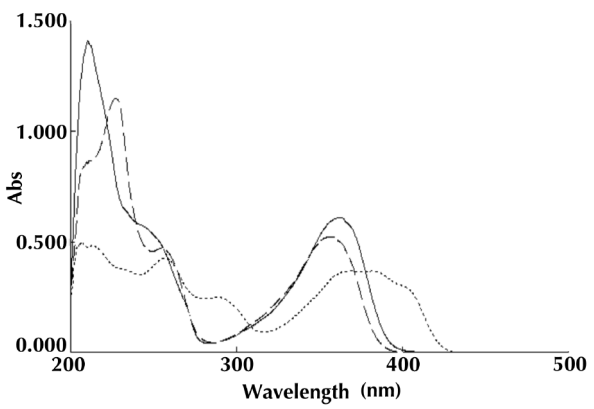

Figure 2. Absorption spectra of TC 10 µg/mL (....), GF 10 µg/mL (—), and FN 10 µg/mL (- - -) in methanol.

Preparation of standard solutions

Stock solutions of 400.0 µg/mL of TC and 2000.0 µg/mL of each GF and FN were prepared in methanol and always protected from light. They were found to be stable for at least one week when kept in the refrigerator. Working standard solutions for the analytical application were prepared by appropriate dilution with the mobile phase.

Construction of calibration graphs

Accurately measured aliquots of the working standard solutions were transferred into a series of 10-mL volumetric flasks so that the final concentrations were in the range of 0.2–2 µg/mL for TC, and in the range of 20–200 µg/mL for both GF and FN. The flasks were completed to the mark with the mobile phase and the resulting solutions were injected (triplicate) and eluted with the mobile phase under the chromatographic conditions described previously. The peak area was plotted Vs the concentration in µg/mL to get the calibration graph. Alternatively, the corresponding regression equations were derived.

Application to pharmaceutical formulations

For tablets containing TC and GF (Glifarelax tablets), an accurately weighed quantity of the mixed contents of 10 pulverized tablets equivalent to 2 mg of TC and 200 mg of GF were transferred into a 100 mL volumetric flask, and ~80 mL of methanol were added. The contents of the flask were sonicated for 15 min, completed to the volume with methanol and filtered.

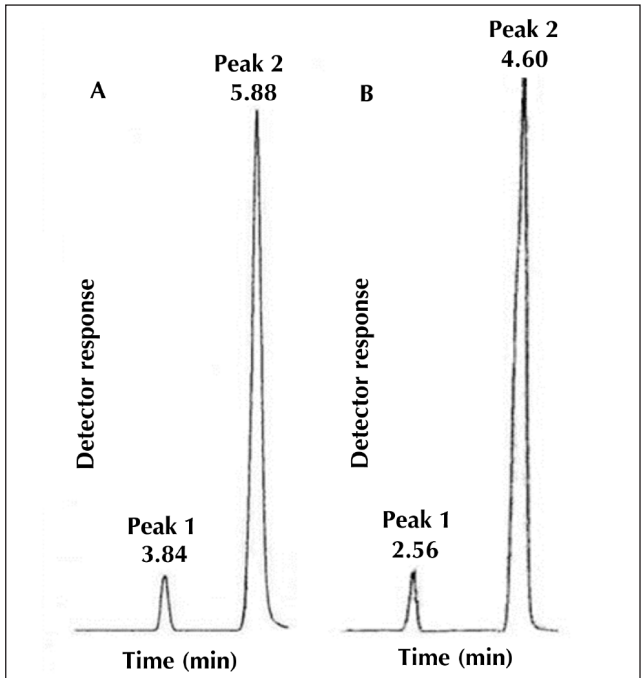

Figure 3. Typical chromatogram of Thiocolchicoside 2 µg/mL (peak 1) and Floctafenine 200 µg/mL (peak 2) in pure form under the optimum chromatographic conditions (A).

Typical chromatogram of Thiocolchicoside 2 µg/mL (peak 1) and Glafenine 200 µg/mL (peak 2) in pure form under the optimum chromatographic conditions (B).

For tablets containing TC and FN, an accurately weighed quantity of the mixed contents of 10 prepared tablets equivalent to 2 mg of TC and 200 mg of FN, then transferred into a 100 mL volumetric flask and complete as under the preparation of Glifarelax tablets.

Suitable aliquots of this solution were successively diluted with the mobile phase and then the general recommended procedure was performed. The nominal content of the tablets were calculated either from the previously plotted calibration graphs or using the corresponding regression equation.

Results and Discussion

Thiocolchicoside is coformulated with each of GF and FN in a ratio of 1:100 rendering the analysis of such mixtures challenging. Moreover, the absorption spectra of TC and each of GF and FN are greatly overlapped (Figure 2) rendering the problem more aggravated. However, adopting HPLC with proper selection of wavelength of detection, the problem could be resolved and quantitation of TC (the minor component) and each of GF and FN could be accomplished accurately. The proposed methods permitted the separation of TC from each of GF and FN in their combined dosage forms with good resolution in a reasonable time as shown in (Figure 3).

Different experimental conditions including mobile phase composition, detection wavelength and flow rate were intensively studied in order to determine the optimum conditions for the assay procedures. Variables were optimized by changing each in turn, while keeping all others constant.

Table II. Performance Data for TCC/GFN and TCC/FFN Mixtures*

	Mixture I		Mixture II	
Parameter	Thiocolchicoside	Glafenine	Thiocolchicoside	Floctafenine
CR (µg/mL) range	0.2–20	20–200	0.2–2	20–200
LOD (µg/mL)	0.05	0.62	0.02	0.70
LOQ (µg/mL)	0.14	1.87	0.07	2.12
r	0.9997	0.9999	0.9999	0.9999
Slope	3.74×10^4	4.60×10^3	3.54×10^4	5×10^4
Intercept	2.50×10^3	-3.58×10^4	2.95×10^3	1.75×10^4
$S_{y/x}$	730	4.7×10^3	3.43×10^2	5.8×10^3
S_a	526	859	247	1060
S_b	465	30	220	37
%RSD	1.10	1.04	0.57	1.23
% Error	0.49	0.46	0.25	0.55

* CR = Concentration range
$S_{y/x}$: Standard deviation of the residuals
% Error = % RSD/√n
r = Correlation coefficient
S_b : Standard deviation of the slope
S_a: Standard deviation of the intercept

Table I. Please Supply Captions

	Mobile phase ratio (Methanol–Phosphate buffer)						pH				
Parameter	70–30	65–35	60–40	55–45	50–50	40–60	3	3.5	4	4.5	5
TC–GF mixture											
TC N	852	950	482	1462	851	651	1117	2203	861	851	1039
GF N	781	686	926	732	2099	3059	857	2328	534	2099	1383
SF (α)*	1.55	1.69	1.89	1.80	2.00	2.08	1.50	1.60	1.54	1.99	3.20
Resolution	0.65	1.20	1.44	1.80	2.50	3.80	1.57	2.40	1.46	2.49	4.99

	Buffer concentration (M)					Flow rate (mL/min)		
Parameter	0.02	0.03	0.035	0.04	0.05	0.7	0.9	1	1.2
TC–GF mixture									
TC N	1150	2110	2053	880	1180	1768	2466	2106	1545
GF N	2243	2760	5530	2410	2460	5301	4672	3955	4531
SF (α)*	2.30	2.20	2.00	2.10	2.00	2.00	2.10	2.20	2.36
Resolution	2.98	2.00	3.80	2.15	2.40	4.61	4.30	4.20	3.90

	Mobile phase ratio Methanol–Phosphate buffer				pH			Buffer concentration (M)				Flow rate (mL/min)			
Parameter	80–20	70–30	65–35	60–40	4	4.5	5	0.02	0.03	0.04	0.05	0.7	0.9	1	1.5
TC–FN mixture															
TC N	587	676	797	838	784	713	701	680	750	680	690	3244	1124	921	762
FN N	1356	1202	1275	1427	1221	1471	1259	1190	3410	1070	1590	2796	1722	2042	1436
SF (α)	3.27	4.56	5.53	5.55	5.50	9.44	9.01	4.44	4.55	4.09	4.07	2.52	2.93	3.24	9.58
Resolution	2.67	4.20	6.38	7.20	5.90	9.98	8.10	4.80	5.26	3.90	5.00	4.70	4.40	4.40	4.80

* SF = Selectivity factor (α)

Detection wavelength

The ratio of TC and each of GF and FN renders the choice of the detection wavelength very critical (Figure 2), because the overlap between the spectra of the drugs should not cause problems after the chromatographic separation. The choice of 400 nm (the λ max of TC, the minor component) allowed the quantitation of TC where GF or FN exhibited very low absorbance values.

Mobile phase composition:

The chromatographic separation was carried out using a 250 mm × 4.6 mm i.d., 5 µm particle size C18 Waters Symmetry column (Milford, MA) which gave good resolution of the peaks.

Several modifications in the mobile phase composition were performed in order to study the possibilities of improving the performance of the chromatographic system. These modifications include the replacement of methanol by acetonitrile, this caused overlap of peaks of TC–GF mixture with consequently low value of resolution (Rs = 0.24). While in Mix II, the peak of TC disappeared. Also the replacement of phosphate buffer by water in both mixtures caused broadness of the peaks and delayed retention times.

Different ratios were tried and the optimum ratios were (50:50) of methanol–phosphate buffer for (Mix I) because increasing the methanol portion more than buffer decreases the resolution value up to the ratio (80:20) of methanol–buffer caused the disappearance of TC peak. While increasing the portion of the buffer more than methanol caused more time consuming (peak of GF14.10 min) and broadness of the peaks so the ratio of (50:50) was used which gave high number of theoretical plates , good resolution and sharp peaks as shown in Table I. The TC–FN mixture using 70:30 of methanol–phosphate buffer gave sharp peaks and good resolution. Using the ratio of (50:50) caused retention of FN on the column. When the methanol portion was increased, the resolution increased up to the ratio of (80:20), then the resolution decreased. So 70:30 of methanol–buffer used with good resolution produced a high number of theoretical plates and symmetrical peaks, as cited in Table I.

Table III. Accuracy and Precision data for Thiocolchicoside Using the Proposed Methods*

	Intra-day precision			Inter-day precision		
Parameter	Conc. taken (µg/mL)	Conc. found (µg/mL)	% Found	Conc. taken (µg/mL)	Conc. found (µg/mL)	% Found
TC–GF method						
Data	0.5	0.50	100.00	0.5	0.50	100.00
	1.0	0.99	99.00	1.0	1.00	100.00
	1.5	1.50	100.00	1.5	1.52	101.33
x ± SD		99.66 ± 0.58			100.44 ± 0.76	
%RSD		0.58			0.75	
%Error		0.33			0.43	
TC–FN method						
Data	0.5	0.50	100.00	0.5	0.51	102.00
	1.0	1.00	100.00	1.0	1.01	101.00
	1.5	1.51	100.66	1.5	1.52	101.33
x ± SD		100.22 ± 0.38			101.44 ± 0.51	
%RSD		0.38			0.50	
%Error		0.22			0.29	

* N.B. Each result is the average of three separate determinations.

Table IV. Accuracy and Precision Data for Glafenine and Floctafenine Using the Proposed Methods*

	Intra-day precision			Inter-day precision		
Parameter	Conc. taken (µg/mL)	Conc. found (µg/mL)	% Found	Conc. taken (µg/mL)	Conc. found (µg/mL)	% Found
Glafenine						
Data	50.0	49.03	98.06	50.0	49.96	99.92
	100.0	99.01	99.01	100.0	100.28	100.28
	150.0	149.03	99.35	150.0	150.86	100.57
x ± SD		98.81 ± 0.66			100.25 ± 0.32	
% RSD		0.66			0.32	
% Error		0.38			0.18	
Floctafenine						
Data	50.0	50.55	101.10	50.0	50.60	101.20
	100.0	98.99	98.99	100.0	99.98	99.98
	150.0	150.32	100.20	150.0	150.93	100.62
x ± SD		100.09 ± 1.06			100.60 ± 0.61	
% RSD		1.06			0.61	
% Error		0.61			0.35	

*N.B. Each result is the average of three separate determinations.

The pH of the mobile phase

The influence of pH on the chromatographic behavior was also studied. For Mix I decreasing the pH of mobile phase down to pH 4 caused broadness of the peaks and increased the retention time. Increasing the pH up to 6, the peak of GF appeared after 30 min, so pH 4.5 was used. For Mix II, decreasing the pH to 3 retained TC. When the pH was increased to 6, FN appeared after 15 min. So pH 4 was used throughout the study, as shown in Table I.

Effect of buffer concentration

Different concentrations of phosphate buffer were studied in the concentration range of 0.02–0.05M. For Mix I and Mix II, 0.035M and 0.03M were the optimum concentrations, respectively, as cited in Table I.

Effect of flow rate

The effect of the flow rate was investigated in the range of 0.7–1 mL/min and a flow rate of 1 mL/min was the optimum for good sep-

aration in a reasonable time for both mixtures, the results obtained are shown in Table I.

Validation

Linearity and range

The calibration graphs for the determination of TC, GFN, and FN by the proposed methods were constructed by plotting the peak area against the concentration of the drug in µg/mL, the calibration graph was rectilinear over the concentration range cited in Table II.

Statistical analysis of the data gave high values of the correlation coefficient of the regression equations, small values of the standard deviation of residuals (S*y*/*x*), of intercept (S*a*), and (S*b*) of slope, and small values of the percentage relative standard deviation and the percentage relative error as shown in Table II. These data proved the linearity of the calibration graph.

Table V. Application of Proposed HPLC Methods for the Simultaneous Determination of TC/GF and TC/FN Mixtures in Tablets*

Preparation	Amount taken (µg/mL)	Proposed method: Amount found (µg/mL)	Proposed method: % Found	Comparison (5): Amount taken (µg/mL)	Comparison (5): % Found
Glifarelax tablets (2 mg of TC + 200 mg of GF)					
Thiocolchicoside	0.5	0.50	100.00	20	101.50
	1.0	1.00	100.00	40	99.70
	1.5	1.51	101.05	80	100.10
		x ± SD = 100.35 ± 0.61			100.43 ± 0.94
t-value = 0.13 (2.77)					
F-value = 2.43 (19)					
		Proposed method		**Comparison (19)**	
Glafenine	50	49.96	99.92	20	101.20
	100	101.35	101.35	40	99.30
	150	150.66	100.44	80	98.90
		x ± SD = 100.57 ± 0.72			99.80 ± 1.23
t-value = 0.93 (2.77)					
F-value = 2.88 (19)					
		Proposed method		**Comparison (5)**	
Prepared tablets of (2 mg of TC + 200 mg of FN)					
Thiocolchicoside	0.5	0.51	102.00	20	101.50
	1.0	1.01	101.00	40	99.00
	1.5	1.510	100.60	80	100.10
		x ± SD = 101.20 ± 0.72			100.20 ± 1.25
t-value = 1.20 (2.77)					
F-value = 3.02 (19)					
		Proposed method		**Comparison (27)**	
Floctafenine	50	50.50	101.00	0.5	100.30
	100	99.66	99.66	0.7	99.00
	150	150.66	100.44	1	99.10
		x ± SD = 100.36 ± 0.67			99.46 ± 0.72
t-value = 1.57 (2.77)					
F-value = 1.15 (19)					

* Composition of Tablet: TC 2 mg, FN 200 mg, talc powder 20 mg, maize starch 15 mg, lactose 15 mg, and magnesium stearate 10 mg per tablet. Each result is the average of three separate determinations. Figures between brackets are the tabulated t- and F-values at (P = 0.05) (30).

Accuracy and precision

To test the validity of the proposed methods, it was applied to the determination of pure samples of TC, GF, and FN over their concentration ranges cited in Table II. The results obtained were in good agreement with those obtained using the comparison methods (5,19,27). Student t-test and the variance ratio F-test (30) revealed no significance difference between the proposed and comparison methods regarding the accuracy and precision.

Intra- and inter-day precisions were assessed using three concentrations and three replicates of each concentration of the studied drugs. The relative standard deviations were found to be very small indicating reasonable repeatability and intermediate precision of the proposed methods cited in Tables III and IV.

Limit of detection and limit of quantitation

The limit of quantitation (LOQ) is the lowest amount of analyte in a sample which can be quantitively determined with suitable precision and accuracy according to ICH Q2 (R1) recommendation (29) mentioned later which the calibration graph is non linear. The limit of detection (LOD) was determined by evaluating the lowest concentration of the analyte that can be readily detected as shown in Table II.

Specificity

The specificity of the methods were investigated by observing any interference encountered from common tablet excipients. It was shown that these compounds did not interfere with the results of the proposed methods as shown in Table V.

Application of the proposed methods to the analysis of the studied drugs in their pharmaceutical formulations

The proposed methods were successfully applied for the simultaneous determination of the combined drugs in their tablets. The average percent recoveries of different concentrations were based on the average of three replicate determinations. The results obtained were in good agreement with those obtained by the comparison method as shown in Table V.

Statistical analysis (30) of the results obtained by the proposed and comparison methods (5,19,27) using Student's t-test and variance ratio test revealed no significant difference between the performance of the two methods regarding the accuracy and precision.

Conclusion

Two simple, accurate, and precise HPLC methods were developed for the simultaneous determination of TC with either GF or FN in their co-formulated tablets without interference from common excipients. The good validation criteria of the proposed methods allow its use in quality control laboratories using a simple chromatographic system. The detection limits of the proposed methods of TC and GF were 0.05 and 0.62 µg/mL, respectively, and the limits of quantitation were 0.14 and 1.87 µg/mL, respectively. The limits of detection of the proposed method for TC and FN were 0.02 and 0.70 µg/mL, respectively, and the limit of quantitation was 0.07 and 2.12 µg/mL, respectively.

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Manuscript received December 19, 2009;
revision received March 8, 2010.