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Spectrophotometric determination of two *N*-(4-quinolyl) anthranilic acid derivative (glafenine and floctafenine)

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Abstract

Spectrophotometric methods were developed for the determination of glafenine and floctafenine. The first method depends upon the determination of glafenine in raw material and tablets as well as in the presence of its main degradation product glafenic acid (up to 40%). Differential first derivative spectral response at 245 nm in 0.1 N hydrochloric acid, where the corresponding degradation product exhibits no contribution in 0.1 N sodium hydroxide. The method allows the determination of 2.5–30 μg ml^{-1}. The second method depends upon the reaction of floctafenine with 2,3-dichloro 5,6-dicyano-*p*-benzoquinone (DDQ) in acetonitrile to give highly colored complex that could be measured quantitatively at (about) λ_{max} 538 nm. The method permits the determination of 40–180 μg ml^{-1} or by measuring the first derivative spectral response of the color at 610 nm. The method permits the determination of floctafenine in presence of thiocolchicoside. The methods mentioned both simplicity and sensitivity, having excellent precision and accuracy (100.31 ± 0.63, 100.78 ± 0.77 and $99.9\ 0 \pm 0.56$ for glafenine and floctafenine, respectively). The results were of comparable accuracy and reproducibility with the reported methods.

Keywords: Glafenine; Floctafenine; Spectrophotometry; Stability indicating method; Derivative spectroscopy; 2,3-Dichloro 5,6-dicyano-*p*-benzoquinone

Glafenine 2,3-dihydroxypropyl *N*-(7-chloro-4-quinolyl) anthranilate and floctafenine *N*-[8-(trifluoromethyl)-4-quinolyl] anthranilic acid-2,3-dihydroxypropyl ester are non-narcotic analgesics used to relieve mild to moderate pain, also used for the relief from fever and inflammation [1].

Several analytical methods have been reported for the determination of glafenine. These include, alkalimetric [2]; potentiometric [3]; polarographic [4]; direct ultra violet (UV) [5]; first derivative [6,7]; colorimetric [8,9] and high performance liquid chromatography (HPLC) method [10].

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Floctafenine and its major metabolite were determined using UV, infra red (IR), mass spectrophotometry and gas chromatography (GC) [11], first derivative [6,7] and HPLC [12].

There is proving concern about the level of degradation products in pharmaceuticals and their implication in adverse reaction. Consequently, interest is now focused on the development and validation of sensitive and selective methods for the determination of the intact molecule in the presence of degradation products.

The present work describes a simple sensitive and accurate differential first derivative method for the determination of glafenine in presence of its main degradation product glafenic acid. Also, the use of 2,3-dichloro 5,6-dicyano-*p*-benzoquinone (DDQ) as chromogenic reagents for the colorimetric determination of floctafenine either alone or when admixed with thiocolchicoside. The developed color was subjected to first derivative technique to have a more precise results.

1. Experimental

1.1. Apparatus

Unicam Heλios α UV–vis. Spectrometer with two matched 1 cm cells.

All chemicals and reagents used were of analytical reagent or pharmaceutical grade and solvents are of spectroscopic grade.

Glafenine (El. Naser) certified to contain, 99.08%.

Floctafenine (ROUSSEI UCLAF), certified to contain 99.77%.

1.2. Reagents

2,3-dichloro-5,6-dicyano-*p*-Benzoquinone (DDQ) 0.2% w/v in acetonitrile. The solution was freshly prepared.

Methanol BDH, Pool, UK.

Acetonitrile BDH, HPLC grade.

1.3. Formulations

Gliphanan tablets (Memphis Co. for Pharm., Chemical Ind. Cairo, A.R.E., B.N. 395445) labeled to contain 200 mg of glafenine per tablet; in addition to lactose, potato starch, amijel, talc, rice starch and magnesium stearate.

Analfan tablets (Pharco. Pharmaceuticals, Alexandria B.N. 205) labeled to contain 200 mg of glafenine per tablet; in addition to Mag. stearate, starch and talc.

Idarac 'D' tablets (Hoechst Orient S.A.E. Cairo, R.C.C. 106526 under license of Roussel UCLAF, France, B.N. 164) labeled to contain 200 mg of floctafenine per tablet; in addition to maize starch, dioctylsodium sulfosuccinate, povidone and magnesium stearate.

Idarelax Tablets Roussel, product of Hoechst orient S.A.E., B.N. 023 (200 mg of floctafenine per tablet; 2 mg of thiocolchicoside).

1.4. Stock solutions

1.4.1. Stock solution of glafenine

Accurately weighed 50 mg of glafenine and transfered into 50-ml volumetric flask, dissolved and diluted to volume with ethanol (kept away from light; stabilized for several weeks).

1.4.2. Intact drug solution

Transfered 25.0 ml of stock solution of glafenine into 100-ml volumetric flask and diluted to volume with 0.1 N hydrochloric acid (kept away from light; stabilized for at least 2 weeks).

1.4.3. Degraded drug solution

Transfered 25.0 ml of stock solution of glafenine into 100-ml volumetric flask, added 25.0 ml of 1 N sodium hydroxide, left for 15 min and completed to volume with water (kept away from light; stabilized for at least 2 weeks).

1.4.4. Stock solution of floctafenine

Accurately weighed 50 mg of floctafenine and transfered into 50-ml volumetric flask, dissolved and diluted to volume with methanol (kept away from light; stabbed for several weeks).

1.5. Pharmaceutical preparations

1.5.1. For glifanan and analfan tablets

To a quantity of the mixed content of 20 tablets equivalent to 100 mg of glafenine added 50 ml of ethanol, shook for 10–20 min, filtered, washed the filter paper with ethanol, diluted the filtrate and washing to 100 ml with ethanol. Completed as under 'intact drug solution' and 'degraded drug solution.'

1.5.2. For idarelax and idarac tablets

Twenty tablets were weighed, powdered and an amount of mixed powder equivalent to 100 mg of floctafenine was transferred to a 50-ml volumetric flask, extracted with 25 ml of methanol by shaking 10–20 min, filtered, washed the filter paper with methanol and diluted filtrate and washing to volume with methanol.

1.6. Procedure

1.6.1. Stability indicating method for the determination of glafenine

Into two series of 25-ml volumetric flasks pipette 0.25–3 ml of intact drug solution and degraded drug solution, diluted the first to volume with 0.1 N hydrochloric acid and diluted the second with 0.1 N sodium hydroxide solution.

Using the degraded drug solution as blank, measured the ΔD_1 value of the intact drug solution at 245 nm (peak to zero) at $\Delta\lambda$ 2 nm and normal scan speed, and medium smoothing to the curve.

Calculate the concentration of the drug using the corresponding regression equation (Table 1).

1.6.2. Procedure for determination of floctafenine using 2,3-dichloro-5,6-dicyano-p-benzoquinone

A solution of floctafenine in methanol containing 0.4–1.8 mg ml^{-1} was prepared. This solution (1 ml) was transferred to 10-ml volumetric flask, 5 ml of DDQ solution 0.2% in acetonitrile was added, left for 15 min. Completed to volume with acetonitrile. The absorbance was measured at λ_{max} 538 nm. The concentration of the drug was calculated from corresponding regression equation (Table 1).

1.6.3. Procedure for determination of floctafenine by first-derivative method

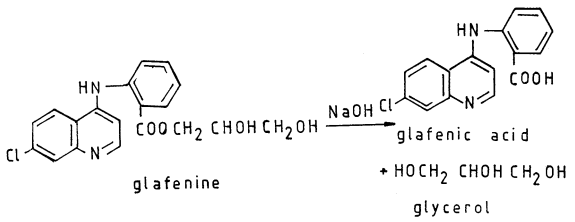
A solution of floctafenine in methanol containing 0.5–4.0 mg ml^{-1} was prepared. This solution (1 ml) was transferred to a 10-ml volumetric flask, 5 ml of DDQ solution was added, left for 15 min. Completed to volume with acetonitrile. Measured the first-derivative value of the solutions at 610 nm (peak to zero) at $\Delta\lambda$ 2 nm, normal scan speed

Table 1
Analytical parameters for the determination of glafenine and floctafenine

Parameters	Glafenine	Floctafenine	
		Colorimetric method	First derivative method
Wavelength (λ_{max} nm)	245	538	610
Beer's law (limits per μg ml^{-1})	2.5–30	40–180	50–400
Number of experiments	7	8	8
Regression equation[a]			
Intercept (a)	0.006	0.009	0.005
R.S.D. (%)	1.81	1.54	1.90
Slope (b)	0.011	0.005	0.0004
R.S.D. (%)	0.63	0.76	0.56
Correlation coefficient (R)	0.9990	0.9986	0.9991
∈ (mol^{-1} cm^{-1})[b]	0.41×10^4	0.21×10^4	0.16×10^3

[a] $A=a+bc$ or $ID=a+bc$ where A is the absorbance, c the concentration in μg ml^{-1} and ID is the amplitude at the first derivative mode.

[b] Molar absorptivity.



Scheme 1.

and medium smoothing. The concentration of the drug was calculated from the corresponding regression equation (Table 1).

2. Results and discussion

2.1. Stability indicating method for the determination of glafenine

Glafenine powder is stable against heat and moisture. The powdered drug is stable when stored at 40°C in the dark for 180 days. Glafenine in the solid form readily undergoes photodegradation when exposed to UV–visible or solar radiation.

In neutral alcoholic solution, glafenine is unstable towards UV–visible radiation. The photodegradation is suggested to occur via intermolecular H-abstraction in the presence of proton donor solvents.

Glafenine, being ester, undergoes alkaline hydrolysis to the corresponding acid (Scheme 1). The structure of the drug and its degradation product show no dramatic changes indicating no interaction of the carboxylate anion group with the electron clouds of the ring.

Due to the significant overlapping of the absorption spectra of glafenine and glafenic acid (Fig. 1), it was only possible to determine glafenine in presence of its degradation product by adopting differential first derivative spectrophotometric technique. As shown in Fig. 2, since the drug in 0.1 N hydrochloric acid has a predominant peak at 245 nm, corresponding to zero-crossing wavelength of the first derivative spectrum of the neutralized degradation product in 0.1 N hydrochloric acid or in 0.1 N sodium hydroxide. Therefore, using the degraded drug solution as blank and measuring the first derivative value of the intact drug solution in 0.1 N hydrochloric acid, glafenine could be determined in the presence of its degradation product.

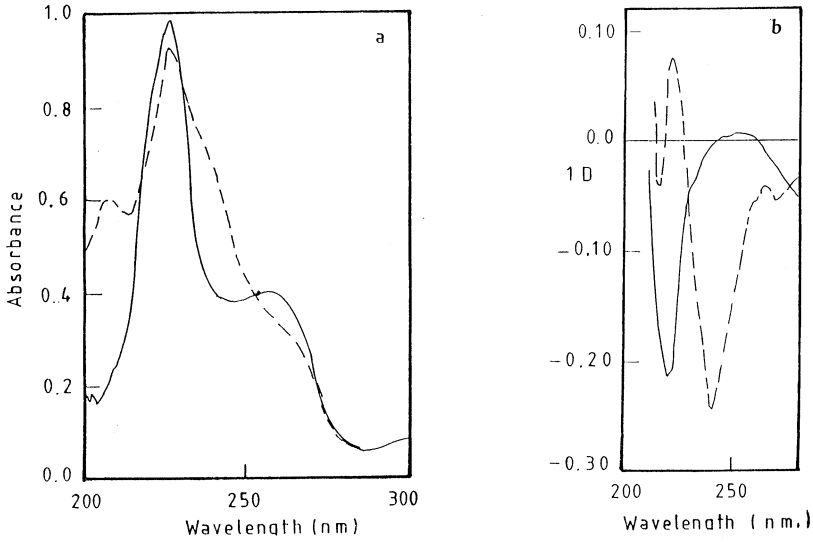


Fig. 1. (a), Zero-order curve of galfenine (10 μg ml^{-1}) in 0.1 N hydrochloric acid (----) degraded glafenine (7.5 μg ml^{-1}) in 0.1 N sodium hydroxide; (b) first derivative absorption spectra of glafenine, 20 μg ml^{-1} (-------) and its degration product, 5 ml^{-1}(-------).

Table 2
Statistical analysis of the results obtained using the proposed method for the determination of glafenine, floctafenine and the reported method

Experiment number	Proposed method for glafenine				Proposed methods for floctafenine					
	Stability indicating method		Reported (16) method		Colorimetric method		First derivative method		Reported method [13]	
	Taken μg	Recovery (%)	Taken (μg)	Recovery (%)	Taken (μg)	Recovery (%)	Taken (μg)	Recovery (%)	Taken (μg)	Recovery (%)
1	5	100.60	10	99.59	80	101.56	100	99.50	10	99.51
2	10	100.70	15	98.50	100	101.16	150	100.33	15	99.85
3	20	100.85	20	99.50	140	99.64	250	100.50	20	99.95
4	25	100.12			160	101.15	300	99.17		
5	30	99.30			180	100.37	400	100.00		
$\bar{X}$		100.31		99.08		100.78		99.90		99.77
S.D.		0.63		0.80		0.77		0.56		0.23
C.V.		0.63		0.81		0.76		0.56		0.23
t		2.44[a]				2.15[a]		0.37[a]		
F		1.61[b]				11.21		5.93[b]		

[a] The theoretical t-value ($P = 0.05$) is 2.447.
[b] Theoretical F (95%-value) is 19.25.

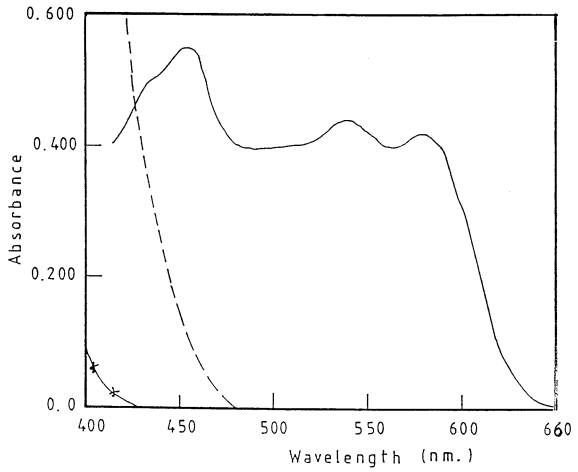


Fig. 2. Absorption spectrum of the colored product of DDO with floctafenine (100 μg ml^{-1}) (—), DDQ solution (---) and μg ml^{-1} of floctafenine (x—x).

Table 3
Analysis of glafenine in presence of its degradation product

Experiment number	Concentration of degradation product added (μg ml^{-1})	Recovery (%)
1	0.5[a]	97.60
2	1.5[a]	98.00
3	2.0[a]	99.40
4	2.5[b]	98.04
5	5[b]	101.62
6	10[b]	102.00
7	12[b]	102.00
8	15[b]	103.23[c]
9	20[b]	109.68[c]
R.S.D. (%)		2.01

[a] Each mixture contains 40 μg ml^{-1} of glafenine.
[b] Each mixture contains 25 μg ml^{-1} of glafenine.
[c] Not taken into consideration.

2.1.1. Preparation of degradation product

Trials were made to know the amount of sodium hydroxide that affects complete hydrolysis done and 1.0 ml of 1 N sodium hydroxide was found to be sufficient for the conversion of 1.0 mg of glafenine to its main degraded product glafenic acid.

Complete disappearance of the main drug spot and appearance of yellow spot of glafenic acid at different R_F values were shown by applying thin layer chromatography (TLC) method, the developing system used was chloroform–methanol (80/20 v/v)or ethyl acetate–methanol–33% ammonia (85/10/5 v/v) and detection done by ultraviolet lamp or naked eye (yellow color spot) ([13]).

2.1.2. Optimization of instrumental parameters

The optimum $\Delta\lambda$ and scan speed were selected by measuring the magnitude of the derivative of spectrum at several $\Delta\lambda$ and scan speeds. Also, the degree of smoothing of the curve. Band width of 2.0 nm, normal speed and medium smoothing were chosen to be optimum and giving the best signal to noise ratio and good resolution.

2.1.3. Quantification, accuracy and precision

Based on the above finding, the calibration curve has been constructed correlating differential first derivative versus the glafenine concentration. Such relationship was linear (2.5–30 μg ml^{-1}) with negligible intercept. The analytical data obtained from calibration measurement are given in Table 1. The accuracy and precision of the method were tested for the analysis of glafenine by replicate determinations at different concentration levels cited in Table 2. The recovery was found to be 100.31 ± 0.63 indicating that good accuracy was achieved. Also, coefficient of variation was less than 2% indicating valuable analytical features. The results obtained were compared with the $A_{1\,cm}^{1\%}$ at 343 nm in 0.1 N hydrochloric acid.

2.1.4. Application to mixture of pure and degraded glafenine

To assess the efficiency of the proposed procedure, as stability-indicating method for the determination of glafenine. The degraded compound in 1 N sodium hydroxide is neutralized with 0.5 N hydrochloric acid and completed with 0.1 N hydrochloric acid. Glafenine and the degraded product were mixed in different ratios and analyzed with the proposed method. The results obtained are given in Table 3.

It is clear that the accuracy of the proposed procedure is not affected by the presence of 1.25–40% of the degradation product. More than 40% degradation gives higher results.

2.1.5. Determination of glafenine in pharmaceutical formulation

Two pharmaceutical formulations containing glafenine (Gliphanan tablets 200 mg per tablet and Analfan tablets 200 mg per tablet) have been assayed by the proposed differential first derivative method and compared with the $A^{1\%}_{1\,cm}$ at 343 nm in 0.1 N hydrochloric acid. Control experiments were performed by adding known concentrations of the working standard of glafenine to a previously analyzed aliquot of tablet powder and the accuracy of the recovery of the added standard is computed. The results obtained are presented in Table 4 with comparison of the results given by the $A^{1\%}_{1\,cm}$ in 0.1 N hydrochloric acid at λ_{max} 343 nm. The accuracy of the suggested procedure were found to be 100.10 ± 0.44 and 101.46 ± 0.76.

2.2. Colorimetric method for the determination of floctafenine

The π acceptor DDQ known to form charge radical anions with a variety of electron donors, is used as the basis to quantify floctafenine.

2.2.1. Identification of wavelength of maximum absorption

The absorption spectrum of DDQ with floctafenine in acetonitrile showed three maxima at 458, 538 and 580. The peak at 538 nm gives the highest absorption intensity (Fig. 2).

2.2.2. Effect of the reaction time

The optimum reaction time was determined by following the color development at ambient temperature (20–25°C). Complete color development

Table 4
Determination of glafenine and floctafenine in their pharmaceutical preparations

Preparations	Recovery ± S.D. (%)*				
	Glafenine		Floctafenine		
	Stability indicating method	Reported (17) method	Colorimetric method	First derivative method	Reported (19) method
Gliphanan tablet					
$\bar{X} \pm$ S.D.	100.10 ± 0.44	100.79 ± 0.67			
C.V.	0.44	0.66			
t	1.49				
F	2.32				
Analfan tablet					
$\bar{X} \pm$ S.D.	101.46 ± 0.76	100.07 ± 0.54			
C.V.	0.75	0.54			
t	2.58				
F	1.98				
Idarac 'D' tablet					
$\bar{X} \pm$ S.D.			100.42 ± 1.18	99.51 ± 0.72	99.56 ± 1.34
C.V.			1.17	0.72	1.35
t			0.83	0.06	
F			1.29	3.40	
Idaralex tablet					
$\bar{X} \pm$ S.D.			101.29 ± 1.29	98.60 ± 0.77	99.03 ± 0.86
C.V.			1.28	0.78	0.86
t			2.52	0.65	
F			2.25	1.25	

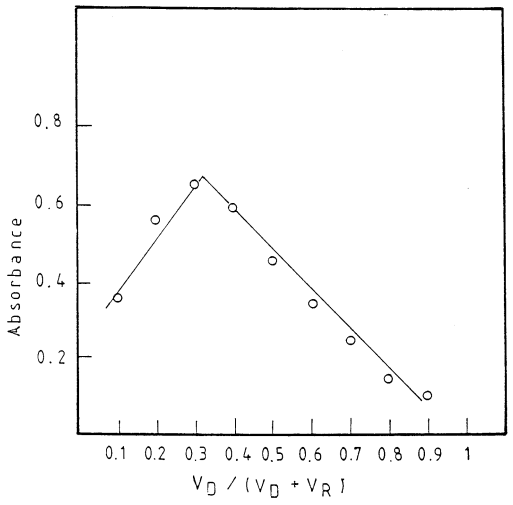


Fig. 3. Job's method of continuous variation of floctafenine–DDQ 2.5×10^{-3} M at λ_{max} 458 nm.

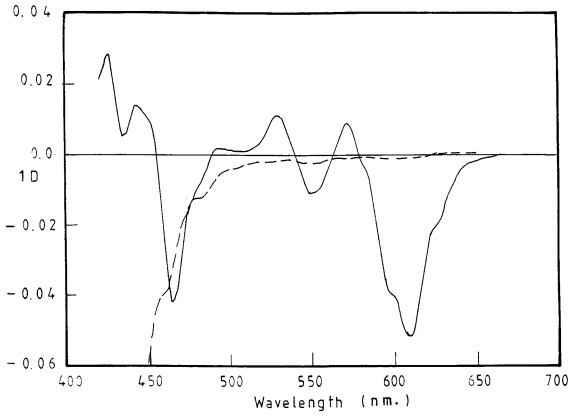


Fig. 4. First derivative absorption spectrum of 100 μg ml^{-1} of floctafenine (—) and DDQ (---).

was attained after 20 min. The color remained stable for further 30 min.

2.2.3. Effect of DDQ concentration

Various volumes of DDQ solution (1–8 ml) were added to constant amount of the drug (80 μg ml^{-1}) and the absorbance was measured at 538 nm. The reagent (4–8 ml) gave constant result and 5 ml of 0.2% DDQ in acetonitrile gave the highest intensity.

2.2.4. Stoichiometric relationship

The molar ratio of (Floctafenine:DDQ) reactants was found to be 1:2 (Fig. 3).

2.2.5. Construction of calibration graph

Under the experimental conditions described calibration graph was constructed for floctafenine over the concentration ranges 40–180 μg ml^{-1}.

The analytical data obtained are given in Table 1. Replicate determination at different concentration levels cited in Table 2 were carried out to test the accuracy and precision of the method. The results obtained were compared with the reported method $A^{1\%}_{1\,cm}$ at 348 nm in 0.1 N hydrochloric acid. The results were found to be in good agreement with the proposed method Table 2. The relative S.D. was found to be 0.76 indicating reasonable repeatability of the selected method.

2.2.6. Determination of floctafenine in its pharmaceutical preparations

The proposed procedure was applied to Idarelax tablets (200 mg of floctafenine, 2 mg of thiocolchicoside per tablet) and Idarac 'D' tablets (200 mg per tablet). The results were assessed by the standard addition technique.

The results obtained are presented in Table 4 with comparison to the results given by the reported method of floctafenine [14]. The results obtained were reproducible with low S.D.

Since the method is applied directly to the commercial preparations without previous treatment, the proposed method can be used as a control method for routine analysis. In addition, the proposed method is suitable for the determination of floctafenine in presence of thiocolchicoside which encounted with in tablets (Idarelax tablet).

Hoping to find more selective and precise procedure, the color formed as a result of the reaction of floctafenine with DDQ was used for the determination of the drug. The method depends upon measuring the first derivative spectral response of the color at 610 nm (peak to zero) (Fig. 4).

2.2.7. Selection of optimum instrumental conditions

In order to obtain a well resolved large peak i.e. selective and highly sensitive peak, the main instrument parameter affecting the shape of deriva-

tive spectra were optimized. A good resolution was obtained at 610 nm. Different $\Delta\lambda$ were used and 2 nm was found satisfactory, also normal scan speed and medium smoothing were chosen to give less noise and good resolution. The response time was automatically selected by the spectrophotometer in accordance with the absorbance and scanning speed. The analytical data obtained are given in Table 1. The accuracy and precision of the method were tested for the analysis by replicate determinations at different concentration levels the results are cited in Table 2 with comparison to reference method. The proposed procedure was applied to Idarac 'D' tablets and Idarelax tablet (containing floctafenine and thiocolchicoside). The results obtained were reproducible with low S.D. (Table 3).

Comparison of the results of the proposed procedures with the reported methods (non-official drugs) showed that the stability indicating procedure for glafenine determination is more sensitive and selective. Since, the comparison method was by $A^{1\%}_{1\,cm}$ at 343 nm, this wavelength does not differentiate between intact and degraded molecule. Moreover, the proposed procedure, derivative spectrophotometry, provides the possibility of measuring with opalescent solutions, since it avoids interference [15]. Also, the two proposed procedures for the determination of floctafenine are simple and accurate and suitable for the determination of the cited drug in presence of thiocolchicoside.

The proposed method for the determination of glafenine is advantageous because it estimates the drug in presence of 1.25–40% of its degradation product glafenic acid; while that of the only published procedure is 4–25% ([7]).

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