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Stability-indicating methods for the determination of a mixture of almitrine and raubasine by derivative spectrophotometry

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A second-derivative spectrophotometric method (^{2}D) and a derivative ratio spectrum zero crossing (^{1}DD) method were used to determine raubasine and almitrine dismesylate in the presence of raubasine degradation product, using methanol as a solvent. Linear relationships were obtained in the range from 6–20 μg ml^{-1} raubasine for the (^{2}D) method and 12–24 μg ml^{-1} almitrine dismesylate for the (^{1}DD) method.

By applying these methods it was possible to determine raubasine in its pure powdered form with an accuracy of 99.93 ± 1.116 (n = 8) for the (^{2}D) method and almitrine dismesylate with an accuracy of 99.98 ± 0.602 (n = 7) for the (^{1}DD) method.

Laboratory-prepared mixtures containing different ratios of raubasine, almitrine dismesylate and raubasine degradation product were analysed and the proposed methods were valid up to 50% of raubasine degradation product. They were found to be suitable stability-indicating assay methods for raubasine and almitrine dismesylate in pharmaceutical formulations.

Keywords: almitrine dismesylate; derivative ratio; raubasine; zero crossing

Introduction

Raubasine (Figure 1) has the IUPAC name [(19α)-16, 17 Didehydro-19-methyl-oxayohimban-16-carboxylic acid methyl ester].[1] It is an alkaloid obtained from *Rauwolfia serpentina* and has been described as a vasodilator, related chemically to reserpine.[2] The determination of raubasine has been studied using spectrophotometry,[3] electrochemical methods,[4] gas chromatography[5] and high-performance liquid chromatography.[6–13]

Almitrine (Figure 2) has the IUPAC name, 6-[4-[Bis (4-fluorophenyl) methyl] -1-piperazinyl] -N,N′-di-2-propenyl-1,3,5-triazine-2, 4-diamine.[1] It has been used as a respiratory stimulant in acute respiratory failure and in combination with raubasine to treat mental function impairment in the elderly.[2] The determination of almitrine dismesylate has been studied using several gas chromatography[14–16] and high-performance liquid chromatography methods.[9,17–18]

Derivative spectrophotometry is a useful analytical technique that offers background correction and better selectivity than normal spectrophotometry for resolving binary mixtures and some ternary mixtures.

Another method for resolving binary mixtures without previous separation is derivative ratio spectrophotometry, which was developed by Salinas *et al*.[19] In this method the absorption spectrum of the mixture (absorbance at each wavelength) is divided by the absorption spectrum of a standard solution of one of the components, and the first derivative of the ratio spectrum is obtained. The concentration of the other component is then determined from a calibration graph. In this method, overlap of the spectra in a certain region is desirable, because upon dividing one spectrum by another the error increases when one of the absorbencies approaches zero.[19] This method permits the use of the wavelength of greatest sensitivity as the signal of measurements, either a maximum or a minimum amplitude. This method has been applied in the determination of binary mixtures[19–22] and then extended for the determination of ternary mixtures.[23–25]

A derivative ratio spectrum zero crossing method has been developed for the determination of ternary mixtures[25,26] based on the measurement of the amplitude at the zero crossing point in the derivative spectrum of the ratio spectra.

The aim of the present work is to develop feasible, sensitive and specific analytical procedures for the analysis of these drugs in the presence of their degradation products. The adaptation of the proposed procedures to analyse the drugs in the forms in which they are available for use is also important. The available spectrophotometric method for the determination of raubasine is direct and is not stability indicating. There is no spectrophotometric method for the determination of almitrine. For this, we proposed derivative and derivative ratio spectrophotometric methods, which can be used for the analysis of the drugs without preliminary separation. The suggested methods are stability indicating. Comparison of the suggested methods is also investigated against the company HPLC method.

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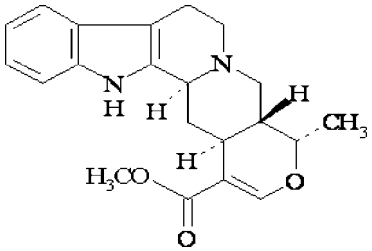

Figure 1. The structure of raubasine.

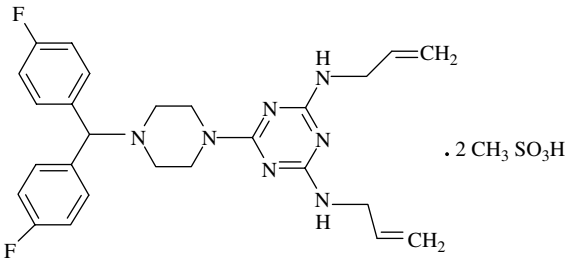

Figure 2. The structure of almitrine dismesylate.

Experimental

Apparatus

Spectrophotometer

A SHIMADZU UV- 1601 PC, dual-beam UV-visible spectrophotometer with two matched 1 cm quartz cells, connected to an IBM compatible personal computer (PC) and a HP-600 inkjet printer. Bundled UV-PC personal spectroscopy software, version 3.7, was used to process the absorption and the derivative spectra. The data resolution was 0.2 nm with a wavelength scanning speed of 2800 nm.min^{-1}.

Gas chromatographer and mass spectrometer (GC/MS)

SHINADZU GC-MS-QP 1000 EX, comprising a GC-14A gas chromatographer and a 70 ev mass spectrometer.

The GC-MS conditions: column; polyethylene glycol (Atwax), mobile phase; helium gas. The temperature program was as follows: the initial temperature was 120 °C; initial time: 1 min; program rate: 10 °C.min^{-1}, final temperature: 210 °C. Injector: 250 µl, Detector temperature: 250 °C.

Pure samples

Raubasine: molecular weight 352.4 (CAS number 483- 04–5). Almitrine dismesylate: molecular weight 669.8 (CAS number 27 469- 53- 0). Servier, an Egyptian pharmaceutical company, kindly supplied these powders. Their purity was checked in our laboratory using the manufacturer's method (an HPLC method using C18 as a stationary phase and methanol: distilled water: sodium heptane sulfonate 5.5% (85: 13: 2 v/v/v) as a mobile phase) and were found to be 100.23 ± 1.247 and 99.89 ± 1.023 for raubasine and almitrine dismesylate respectively.

Market samples

Duxil tablets, batch numbers 9241 and 8720, were purchased from the Egyptian market. Each tablet is claimed to contain 10 mg of raubasine and 30 mg of almitrine dismesylate. Duxil tablets are manufactured by Servier, Egypt.

Standard solutions

Solvents and chemicals used in this work were obtained from Prolabo chemical company, (Egypt). They were:

- ☐ raubasine stock solution (0.25 mg.mL^{-1}) in methanol
- ☐ almitrine dismesylate stock solution (0.25 mg.mL^{-1}) in methanol
- ☐ raubasine degradation product stock solution (0.25 mg.mL^{-1}) in methanol.

Procedures

Preparation of the degradation product for raubasine

Pure raubasine (200 mg) was accurately weighed and dissolved in 100 ml of 2 N hydrochloric acid. The solution was refluxed at 100 °C. Complete degradation was obtained after six-and-a-half hours as confirmed by TLC. The solution was evaporated until the volume reached about 0.5 ml.

The degraded solution was applied as a band to TLC plates. The plates were placed in a chromatographic tank previously saturated for one hour with the mobile phase methanol: chloroform: ethyl acetate (2: 1: 1 v/v/v) and then air dried.

The band was inspected under UV light at 254 nm, then scraped and suspended in the least amount (around 3 mL) of methanol. The suspension was filtered and left to evaporate at room temperature (25 °C) to obtain the degradation product.

Spectral characteristics of raubasine, almitrine dismesylate and the degradation product

Separate aliquots equivalent to 250.0 µg raubasine, 500.0 µg almitrine dismesylate and 250.0 µg degradation product from their stock solutions (0.25 mg mL^{-1}) were transferred into 25 mL volumetric flasks and made up to volume with methanol. The zero-, first- and second-order spectra of the prepared solutions were then recorded from 200–310 nm.

Construction of calibration curve

For (^2D). Accurately aliquots of 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 mL of raubasine stock solution (0.25 mg mL^{-1}) were transferred into 25 mL volumetric flasks, then made up to volume with methanol. Peak amplitudes of the second derivative spectra obtained were measured at 291.2 nm ($\Delta\lambda = 4$ nm). A calibration curve was constructed relating the peak amplitudes of the second derivative curve at 291.2 nm to the corresponding concentrations of raubasine.

For (^1DD). Aliquots of 1.2, 1.4, 1.6, 1.8, 2.0, 2.2 and 2.4 ml of almitrine dismesylate stock solution (0.25 mg mL^{-1}) were transferred into 25 mL volumetric flasks then made up to volume with methanol. Then 1.0 mL of the degradation product stock solution (0.25 mg.mL^{-1}) was transferred accurately into a 25 mL volumetric flask and made up to volume with methanol. The spectra of the prepared standard solutions were scanned from 200–310 nm and stored in the computer.

For the determination of almitrine dismesylate in the presence of raubasine and its degradation product, the stored spectra of almitrine dismesylate and raubasine (absorbance at each wavelength) were divided by the spectrum of 10 µg mL^{-1} of the degradation product, then the first derivatives of the ratio spectra (^{1}DD) with $\Delta\lambda = 4$ nm were obtained. The amplitudes

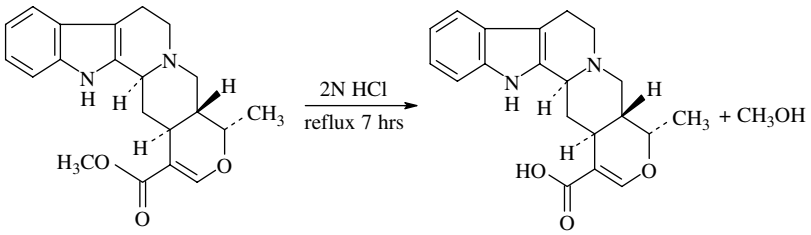

Figure 3. The proposed scheme for the degradation of raubasine.

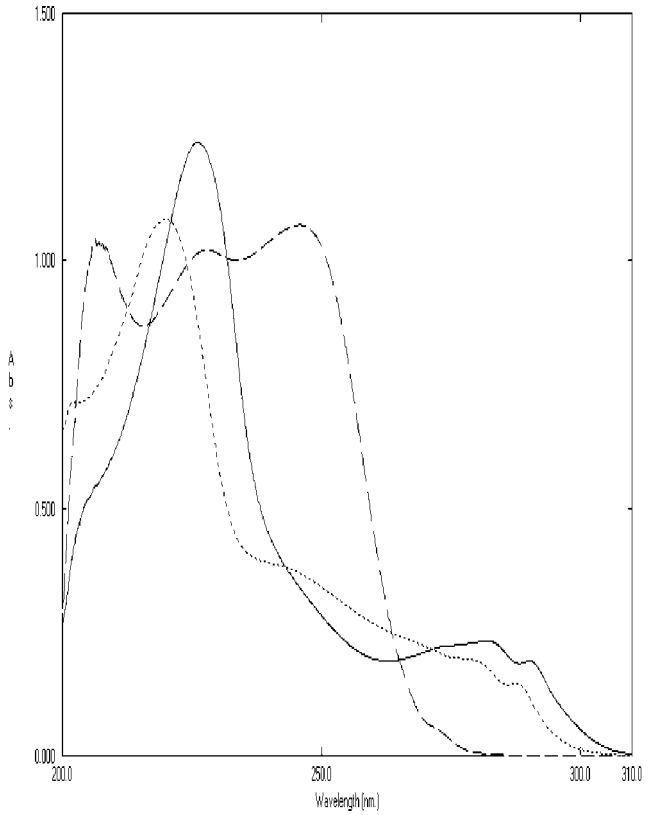

Figure 4. Absorption spectra of raubasine (10 µg mL^{-1}) (– – –), almitrine (20 µg mL^{-1}) (– – –) and degradation product of raubasine (10 µg mL^{-1}) (‐‐‐‐‐‐‐‐).

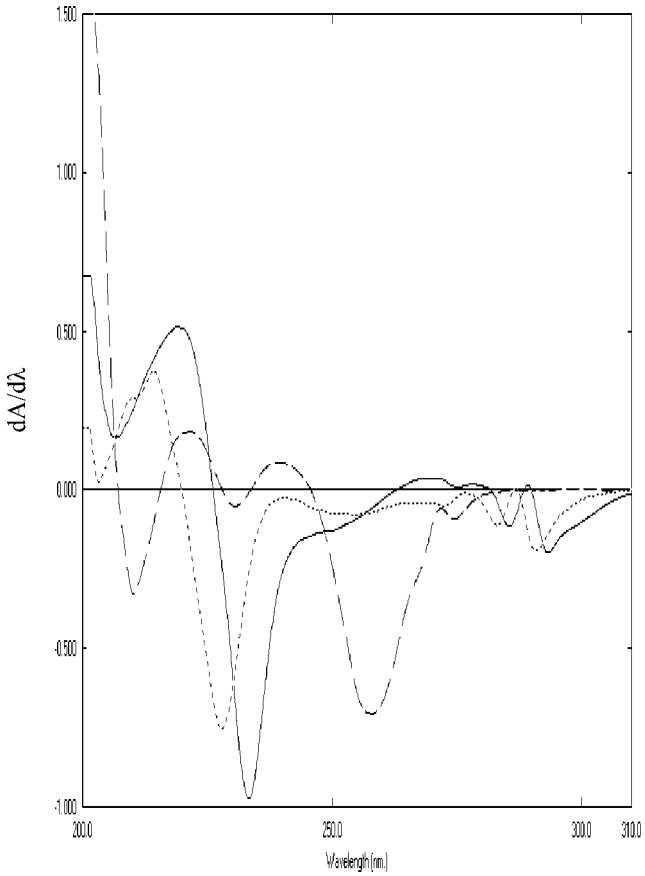

Figure 5. First derivative absorption spectra of raubasine (10 µg mL^{-1}) (– – –), almitrine (20 µg mL^{-1}) (– – –) and degradation product (10 µg mL^{-1}) (‐‐‐‐‐‐‐‐).

of the first derivative peaks of (almitrine dismesylate/degradation product) were measured at 258.5 nm [the λ of zero crossing with the first derivative peak of (raubasine/degradation product)]. A calibration graph was constructed relating the peak amplitude of ($^1DD_{258}$) to the corresponding concentrations in µg.mL^{-1} of almitrine dismesylate.

GC-MS conditions

The degradation product was analysed by the GC-MS adopting the conditions described under 'apparatus'.

Analysis of Duxil tablets

The powder of 10 tablets was accurately weighed. An amount of the powder equivalent to 10 mg raubasine and 30 mg almitrine dismesylate was weighed into a 250 mL beaker and 50 mL methanol was added. The suspension was stirred for 20 minutes using a magnetic stirrer. Then it was filtered into a 100 mL volumetric flask. The residue was washed three times each with 10 mL methanol and made up to volume with methanol. Accurately 2.0 mL of the filtrate was transferred into a 25 mL volumetric flask, then made up to volume with methanol for the determination of raubasine and almitrine dismesylate. The spectra of the prepared solutions were scanned and stored in the computer, then a calibration curve was constructed relating the peak amplitudes of the second derivative curve at 291.2 nm to the corresponding concentrations of raubasine.

Results and Discussion

Degradation occurs through two major pathways: hydrolysis by acids, bases, moisture or via oxidation by light or hydrogen

Table 1. Determination of raubasine and almitrine in laboratory prepared mixtures by the proposed spectrophotometric methods

Concentration (μg mL^{-1})			Ratio	Second derivative method	Derivative ratio zero crossing
Raubasine	*Almitrine*	*Degradation product*	*Raub.:Alm.: deg.product*	*Recovery% raubasine*	*Recovery % almitrine*
8	16	2	4: 8: 1	99.85	98.32
6	15	3	2: 5: 1	102.50	99.24
6	18	0	1: 3: 0	98.82	101.06
6	18	2	3: 9: 1	99.99	100.25
6	18	4	3: 9: 2	98.45	98.89
6	18	6	3: 9: 3	100.40	101.70
6	21	3	2: 7: 1	100.00	98.88
6	24	Zero	1: 4: zero	99.81	100.54
		Mean ± SD		99.97 ± 1.210	99.86 ± 1.201

Table 2. Determination of raubasine and almitrine in Duxil tablets by the proposed methods

Duxil tablets claimed to contain 10 mg raubasine and 30 mg almitrine	Second derivative method	Derivative ratio zero crossing	Reported method[a]	
Batch number	*Percentage ± SD[b] of raubasine found*	*Percentage ± SD[b] of almitrine found*	*Percentage ± SD[b] of raubasine found*	*Percentage ± SD[b] of almitrine found*
9241	97.90 ± 0.980	98.63 ± 1.273	98.95 ± 1.346	98.11 ± 1.445
8720	99.32 ± 0.936	99.21 ± 1.314	98.62 ± 1.477	98.23 ± 1.272

[a] Manufacturer's HPLC method.
[b] Average of four determinations.

peroxide. Being an ester, it was expected that raubasine would be hydrolysed. Almitrine dismesylate contains neither an ester or amide group that can be hydrolysed nor does it had an oxidizable group. So due to bad storage, only raubasine was expected to be degraded due to hydrolysis.

The tablets showed no degradation (as assessed by applying the ICH guidelines) so we decided to force degradation under stressed conditions. The proposed scheme for the degradation of raubasine is shown in Figure 3. The GC-MS can verify the degradation product. The appearance of only one peak in the GC-MS chart confirmed its purity because the presence of impurities would increase the number of peaks in the GC chart. In the MS chart, the parent peak was identified at m/z = 338 indicating the molecular weight of the degradation product as z (the charge) was 1.

Different time periods were tested and thin-layer chromatography was employed using methanol: chloroform: ethyl acetate (2: 1: 1 v/v/v) as a developing system (R_f = 0.6, 0.87 and 0.44 for raubasine, almitrine dismesylate and the degradation product respectively).

The absorption spectra of raubasine, almitrine dismesylate and the degradation product respectively (Figure 4) showed severe overlap, which did not allow the use of direct spectrophotometric analysis for the determination of raubasine and almitrine dismesylate. The first derivative absorption spectra (Figure 5) showed no zero crossing. Upon examining their second derivative spectra (Figure 6) it was noticed that raubasine can be determined at λ = 291.2 nm, whereas both almitrine dismesylate and the degradation product had no contribution (zero crossing). A linear relationship was obtained in the range from 6–20 μg ml^{-1} for raubasine. The spectra were presented in Figure 7. The corresponding regression equation was computed and found to be:

$$A = -0.0557\,C - 0.0252\,r = 0.9997$$

where C is the concentration of raubasine in μg.mL^{-1}, A is the peak amplitude of the second derivative curve at 291.2 nm for raubasine and r is the correlation coefficient.

The derivative ratio method permitted the determination of components, in mixtures, at wavelengths corresponding to a

Table 3. Application of standard addition for the determination of raubasine and almitrine by the proposed methods

Batch number	Standard added (mg)		Second derivative method	Derivative ratio zero crossing
	Raubasine	*Almitrine*	*Recovery of added raubasine (%)*	*Recovery of added almitrine (%)*
	25.00	25.00	99.32	99.21
9241	37.50	37.50	98.23	99.74
	50.00	50.00	100.40	101.21
Mean ± SD[a]			99.31 ± 1.085	100.05 ± 1.036

[a] Average of four determinations.

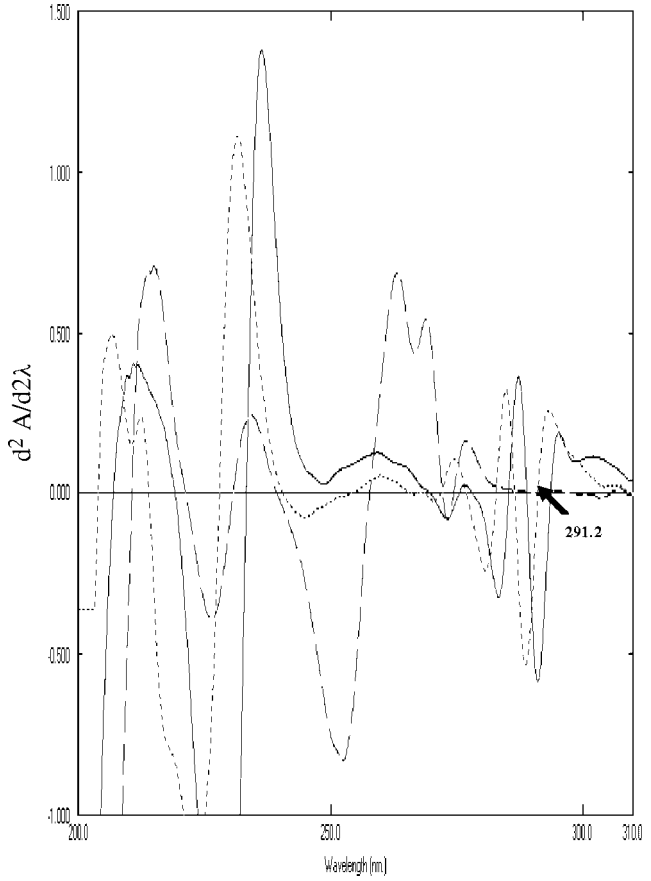

Figure 6. Second derivative absorption spectra of raubasine (10 μg mL^{-1}) (– – –), almitrine (20 μg mL^{-1}) (– – –) and degradation product 10 (μg mL^{-1}) (·····).

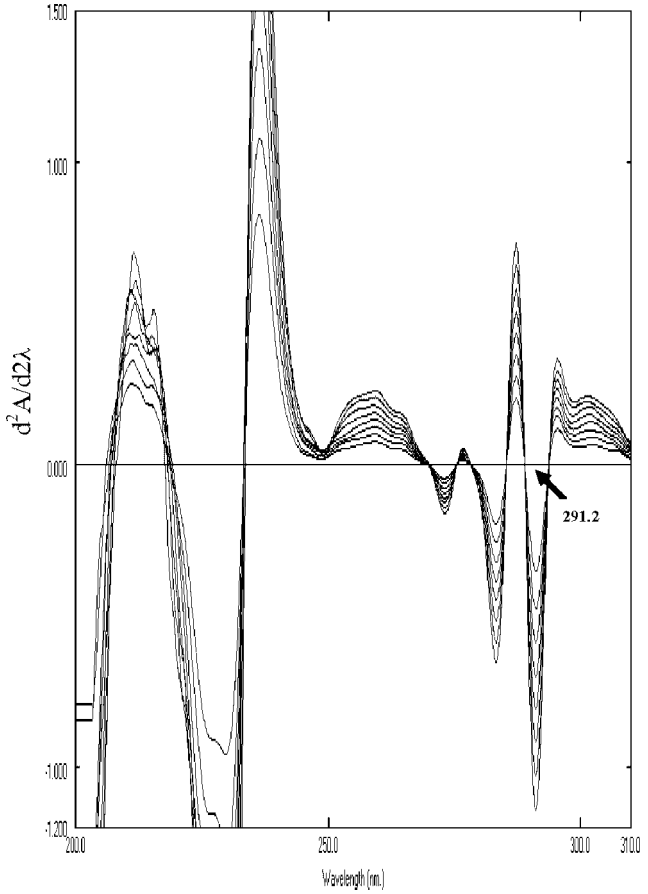

Figure 7. Second derivative absorption spectra of 6–20 μg mL^{-1} raubasine.

Table 4. Statistical comparison for the results obtained by the proposed methods and the reported method for the analysis of raubasine and almitrine in pure powdered form

Item	Second derivative method	Derivative ratio zero crossing	Reported method[a]	
	Raubasine	*Almitrine*	*Raubasine*	*Almitrine*
Mean	99.93	99.98	100.23	99.89
S.D.	1.116	0.602	1.247	1.023
Variance	1.245	0.362	1.555	1.046
n	8	7	6	6
F test	1.248 (3.69)	2.890 (4.39)		
Student's t-test	0.473 (2.179)	0. 197 (2.201)		

The figures in parentheses are the corresponding tabulated values at P = 0.05.[28]
[a] Manufacturer's HPLC method.

Table 5. Assay parameters and method validation[27]

Parameter	Second derivative method	Derivative ratio zero crossing
	Raubasine	*Almitrine*
Range (μg.mL^{-1})	6.0–20.0	12.0–24.0
Slope	−0.0557	−0.0975
Intercept	−0.0252	−0.0474
Mean	99.93	99.98
SD	1.116	0.602
Variance	1.245	0.362
Correlation coefficient (r)	0.9997	0.9996
[a]RSD%[a]	0.267, 0.279	0.323, 0.417
[a]RSD%[b]	0.398, 0.447	0.521, 0.684

[a]RSD%[a], [a]RSD%[b] the intra-day, inter-day respectively (n = 5) relative standard deviation of concentrations (12 and 20 μg mL^{-1} of raubasine and almitrine) for second derivative and derivative ratio zero-crossing methods.

maximum or minimum. The values at these points sometimes permitted better sensitivity and better accuracy.[24] The main parameters that affect the shape of the derivative ratio spectra were wavelength, scanning speed, the concentration of the standard solution used as a devisor, the wavelength increment over which the derivative was obtained (Δ λ) and the smoothing function.[24] The spectra presented in Figures 8 and 9 may be a good proof for this understanding. The effect of wavelength scanning speed was studied. At a high speed, noisy spectra were obtained while at low scanning speed the noise was reduced but a longer time was needed for the measurements, so medium scanning speed was chosen to perform measurements. The concentration of the divisor was also studied and it was found that

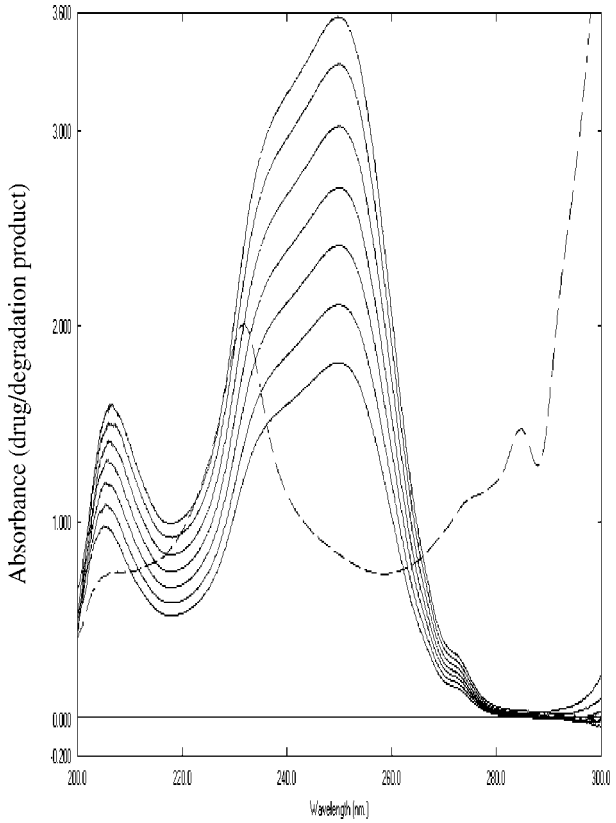

Figure 8. Zero order of ratio spectra of 12–24 μg mL^{-1} almitrine/degradation product (– – –) and 10 μg mL^{-1} raubasine/degradation product (·····) using 10 μg mL^{-1} degradation product as a divisor.

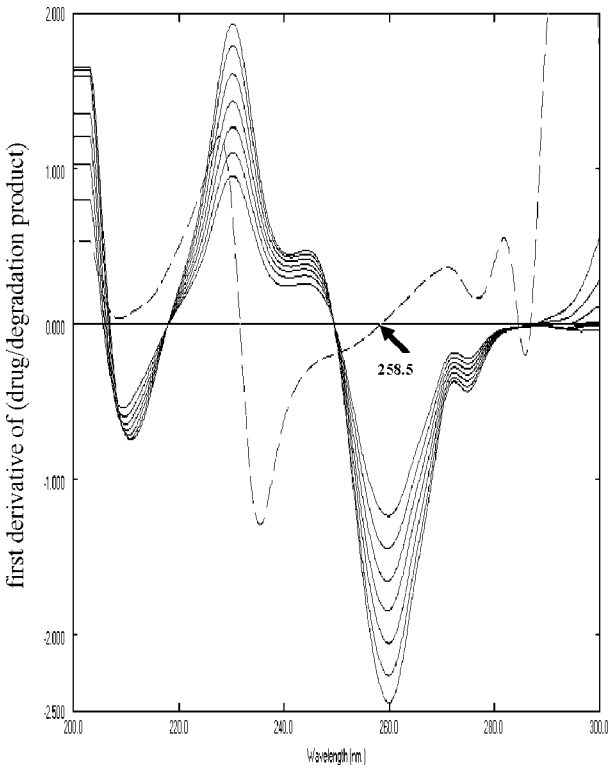

Figure 9. First order of ratio spectra of 12–24 μg mL^{-1} almitrine/degradation product (– – –) and 10 μg mL^{-1} raubasine/degradation product (·····) using 10 μg mL^{-1} degradation product as a divisor.

upon dividing over the normalized spectrum, very high amplitude values were obtained and upon dividing by 30 μg mL^{-1} the sensitivity of the method was reduced. Dividing by the spectrum of 10 μg.mL^{-1} degradation product gave the best compromise in terms of sensitivity, repeatability and signal-to-noise ratio. A linear relationship was obtained in the range from 12–24 μg.ml^{-1} for almitrine dismesylate. The corresponding regression equation was computed and found to be:

$$(^1DD_{258}) = -0.0975\,C - 0.0474\,r = 0.9995$$

where C is the concentration of almitrine dismesylate in μg.mL^{-1}, (^{1} DD_{258}) is the peak amplitude for the first derivative curve of (almitrine dismesylate/degradation product) at 258.5 nm and r is the correlation coefficient.

Method validation

The selectivity and specificity of the proposed methods were assessed by the determination of raubasine and almitrine dismesylate in laboratory prepared mixtures (Table 1). To ascertain the accuracy of the proposed procedures, they were successfully applied in the analysis of Duxil tablets as presented in Table 2. The validity of the proposed procedures was further assessed by the application of the standard addition technique (Table 3).

The results obtained for the analysis of raubasine and almitrine dismesylate in the pure, powdered form by the proposed methods were statistically compared with those obtained using the manufacturer's method of analysis. No significant difference was obtained (Table 4).

The proposed methods were also checked for robustness by making minor changes in assay conditions. The methods proved robust. The intraday and interday relative standard deviation values indicated the robustness of the method. The assay parameters obtained[27] and a validation sheet are presented in Table 5.

Conclusion

From the discussion above it may be concluded that the proposed procedures are simple and do not require sophisticated techniques or instruments. They are also sensitive, selective, stability indicating and could be used for the routine analysis of raubasine and almitrine dismesylate in their available dosage form. The method is also suitable for application in laboratories lacking liquid chromatography instruments.

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