DRUG FORMULATIONS AND CLINICAL METHODS

Smart Stability-Indicating Spectrophotometric Methods for Determination of Binary Mixtures Without Prior Separation

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Ratio subtraction and isosbestic point methods are 2 innovating spectrophotometric methods used to determine vincamine in the presence of its acid degradation product and a mixture of cinnarizine (CN) and nicergoline (NIC). Linear correlations were obtained in the concentration range from 8–40 μg/mL for vincamine (I), 6–22 μg/mL for CN (II), and 6–36 μg/mL for NIC (III), with mean accuracies 99.72 ± 0.917% for I, 99.91 ± 0.703% for II, and 99.58 ± 0.847 and 99.83 ± 1.039% for III. The ratio subtraction method was utilized for the analysis of laboratory-prepared mixtures containing different ratios of vincamine and its degradation product, and it was valid in the presence of up to 80% degradation product. CN and NIC in synthetic mixtures were analyzed by the 2 proposed methods with the total content of the mixture determined at their respective isosbestic points of 270.2 and 235.8 nm, and the content of CN was determined by the ratio subtraction method. The proposed method was validated and found to be suitable as a stability-indicating assay method for vincamine in pharmaceutical formulations. The standard addition technique was applied to validate the results and to ensure the specificity of the proposed methods.

Vincamine (Figure 1; CAS No. 1617-90-9) has the International Union of Pure and Applied Chemistry (IUPAC) name (3α,14β,16α)-14,15-dihydro-14-hydroxyeburnamienine-14-carboxylic acid methyl ester (1). It is claimed to have a selective vasoregulatory action on cerebral circulation. It also adapts cerebral blood flow to metabolic needs (2). Several methods have been reported for the determination of vincamine, including spectrophotometry (3), gas chromatography (GC; 4), thin-layer chromatography (TLC; 3), and high-performance liquid chromatography (HPLC; 5–7).

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Nicergoline (NIC; Figure 2; CAS No. 27848-84-6) has the IUPAC name (8β)-10-methoxy-1,6-dimethylergoline-8-methanol 5-bromo-3-pyridine carboxylate (ester) (1). It has been used to treat symptoms of mental deterioration associated with cerebrovascular insufficiency and in peripheral vascular disease (2). NIC was determined by spectrophotometry (8), titrimetry (9), electrochemistry (10, 11), chromatography (12–21), mass spectrometry (MS; 22), immunoassay (23, 24), and X-ray single crystal diffraction (25).

Cinnarizine (CN; Figure 3; CAS No. 298-57-7) has the IUPAC name (E)-1-(diphenylmethyl)-4-(3-phenylprop-2-enyl) piprazine (1). It has an antihistaminic, sedative, and calcium channel-blocking activity. It is used for the prevention and treatment of various peripheral and cerebral vascular disorders (2). Many analytical methods for the assay of CN were described. These methods are based on spectrophotometry (26–30), titrimetry (31, 32), electrochemistry (33, 34), chromatography (35–38), and chemometry (30).

There is only one stability-indicating spectrophotometric method for the determination of vincamine available. Furthermore, CN and NIC, which are formulated together in one tablet, have no spectrophotometric method available for their simultaneous determination. So there was a need to develop a simple and accurate method for the determination of the cited drugs.

The main problem of spectrophotometric binary mixture analysis is the simultaneous determination of the 2 compounds without prior separation. Several spectrophotometric determination methods were used for resolving such mixtures with overlapping spectra, including derivative spectrophotometry, dual wavelength spectrophotometry, pH-induced differential spectrophotometry, and chemometric methods.

The aim of this work is to develop new spectrophotometric methods for resolving those mixtures with spectral overlapping, either drug in the presence of its degradation product or 2 drugs in binary mixture without preliminary separation. The new methods are very simple, accurate, and do not require any sophisticated apparatus and are suitable for computer programs that cannot make derivative spectrophotometry.

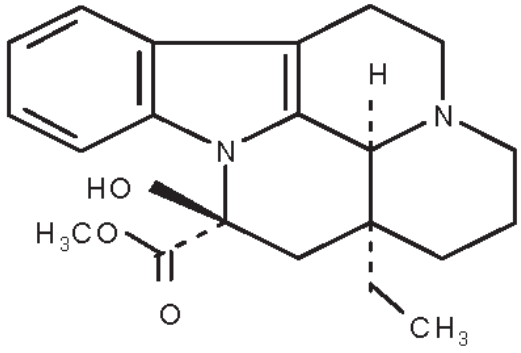

Figure 1. Structure of vincamine.

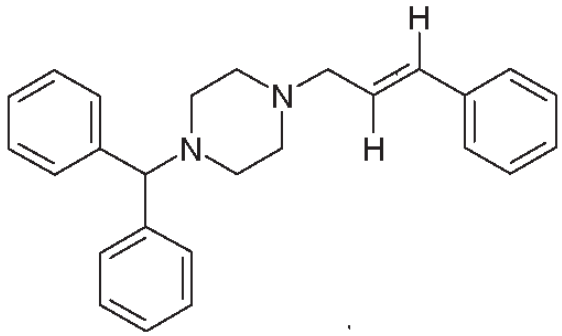

Figure 3. Structure of cinnarizine.

Theory

Ratio Subtraction Method

If you have a mixture of 2 drugs, *X* and *Y* with overlapping spectra, and the spectrum of *Y* is extended more than X, the determination of *X* can be done by dividing the spectrum of the mixture by a certain concentration of *Y* as a divisor (*Y*′). The division will give a new curve that represents

$$\frac{X}{Y'} + \text{constant}$$

If we subtract this constant, then multiply the new curve obtained after subtraction by *Y*′, we obtain the original curve of *X*.

This can be summarized in the following equations:

$$\frac{X+Y}{Y'} = \frac{X}{Y'} + \frac{Y}{Y'} = \frac{X}{Y'} + \text{constant}$$

$$\frac{X}{Y'} + \text{constant} - \text{constant} = \frac{X}{Y'}$$

$$\frac{X}{Y'} \times Y' = X$$

The constant can be determined directly from the curve $\frac{X+Y}{Y'}$ by the straight line that is parallel to the wavelength axis in the region where *Y* is extended.

Isosbestic Point Method

If you have 2 drugs, 1 and 2, the absorbance can be calculated at any wavelength (λ) from the equation:

$$A = A^{1\%}_{1cm}\, b\, C \qquad (1)$$

Therefore, for drug 1:

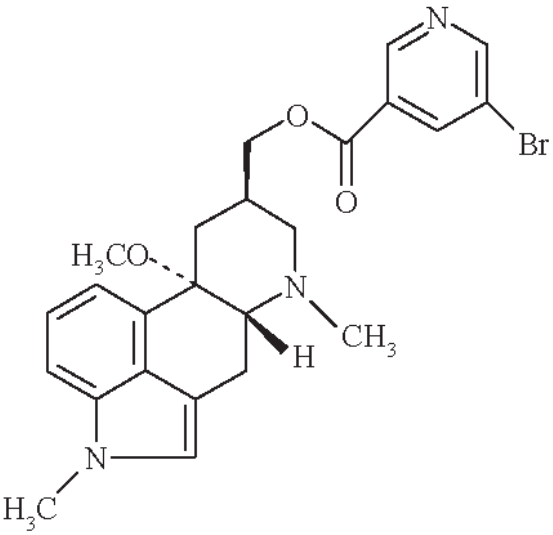

Figure 2. Structure of nicergoline.

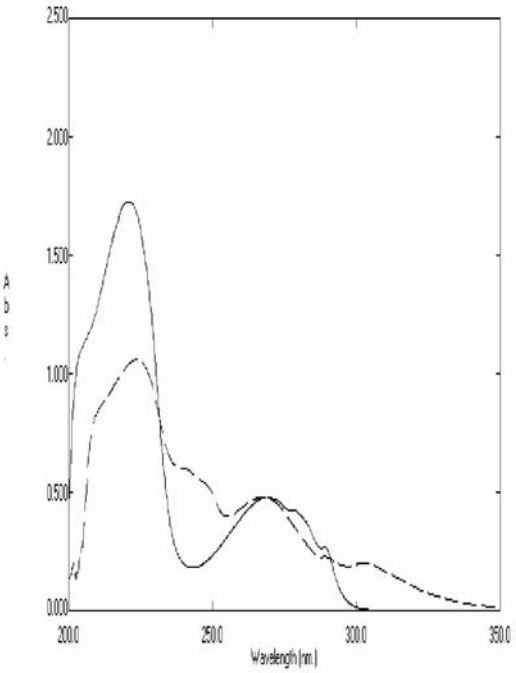

Figure 4. Absorption spectra of vincamine, 20 μg/mL (———) and degradation product, 20 μg/mL (- - - -) using 0.1 M HCl as the solvent.

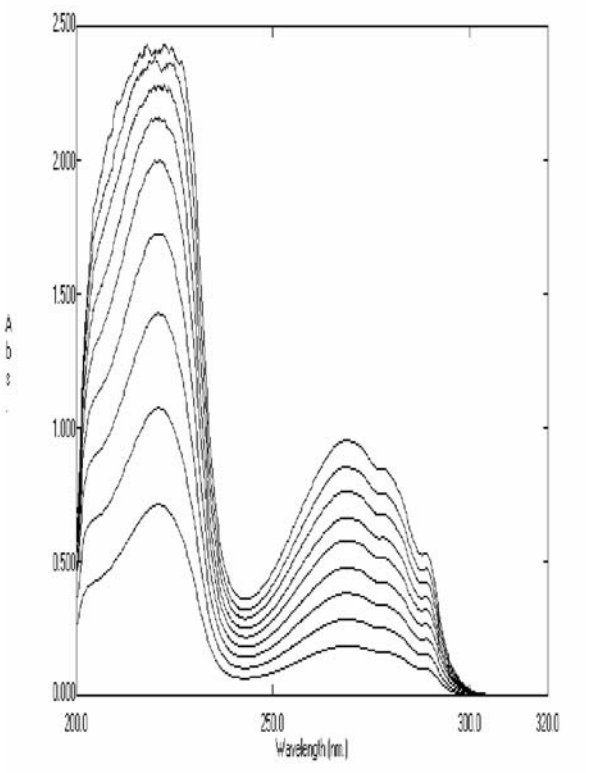

Figure 5. Zero order absorption spectra of vincamine, 8–40 μg/mL.

$$A_1 = A_1^{1\%}{}_{1cm}\, b_1 C_1 \qquad (2)$$

and drug 2:

$$A_2 = A_2^{1\%}{}_{1cm}\, b_2 C_2 \qquad (3)$$

where A_1 and A_2 are the absorbencies of drug 1 and drug 2, respectively; C_1 and C_2 are the concentrations of drug 1 and drug 2, respectively; $A_1^{1\%}{}_{1cm}$ and $A_2^{1\%}{}_{1cm}$ are the absorbtivities when the pathlength is 1 cm and concentration is 1 g/100 mL for drug 1 and drug 2, respectively; and b_1 and b_2 are the path lengths for drug 1 and drug 2, respectively.

If $C_1 = C_2$, $A_1 = A_2$, and $b_1 = b_2$, this λ is called the isosbestic point, and at this λ:

$$A_1^{1\%}{}_{1cm} = A_2^{1\%}{}_{1cm}$$

For a mixture of both drugs, the absorbance at this λ can be calculated from the equation:

$$A_M = A_1^{1\%}{}_{1cm}\, C_{1M} + A_2^{1\%}{}_{1cm}\, C_{2M} \qquad (4)$$

where A_M is the absorbance of their mixture at isosbestic point and C_{1M} and C_{2M} are the concentrations of drug 1 and drug 2 in the mixture, respectively.

Also, $A_1 = A_2 = A_M$, i.e., $A_{1M} + A_{2M} = A_M$ and $A_1^{1\%}{}_{1cm} = A_2^{1\%}{}_{1cm}$

where A_{1M} and A_{2M} are the absorbencies of drug 1 and drug 2 in the mixture, respectively. Therefore, Equation 4 can be written:

$$A_M = A^{1\%}{}_{1cm}\,(C_{1M} + C_{2M}) = A^{1\%}{}_{1cm}\,(C_{TM}) \qquad (5)$$

where C_{1M} and C_{2M} are the concentrations of drug 1 and drug 2 in the mixture, respectively, and C_{TM} is the concentration of their mixture. Therefore, from Equation 5 we can conclude that:

$$(C_{1M} + C_{2M}) = (C_{TM})$$

Table 1. Determination of vincamine in laboratory-prepared mixtures by the proposed procedure

				Ratio subtraction method
Concn, μg/mL		Percentage		Recovery, %
Vincamine	Degradation product	Vincamine	Degradation product	Vincamine
8	32	20	80	98.60
12	28	30	70	98.91
16	24	40	60	98.24
20	20	50	50	99.51
24	16	60	40	100.36
28	12	70	30	99.32
32	8	80	20	99.85
Mean				99.25
SD[a]				0.732

[a] SD = Standard deviation.

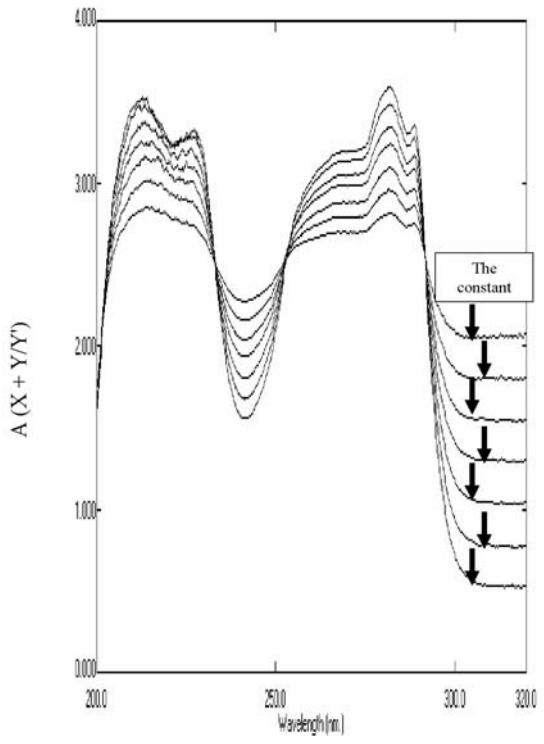

Figure 6. Division spectra of laboratory-prepared mixtures of vincamine (*X*) and its degradation product (*Y*) using 16 μg/mL of degradation product (*Y'*) as the divisor and 0.1 M HCl as the solvent.

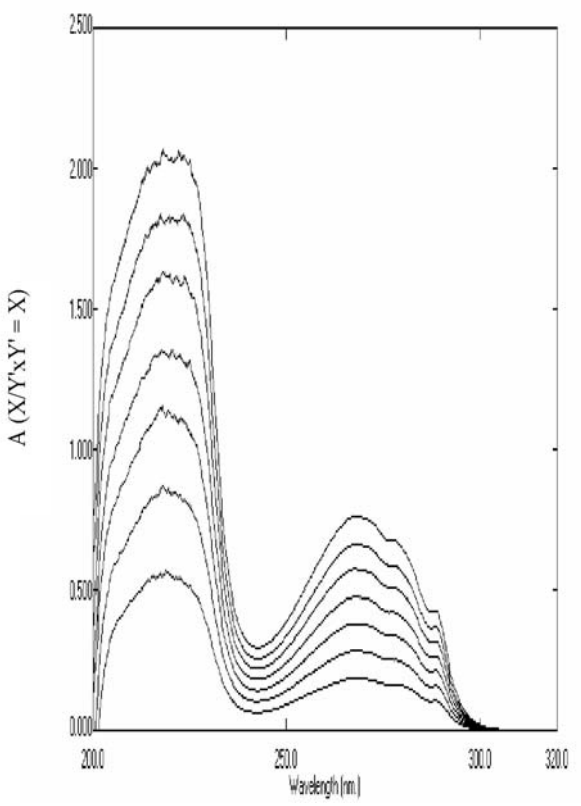

Figure 8. The obtained absorption spectra of vincamine in laboratory-prepared mixtures using the proposed method.

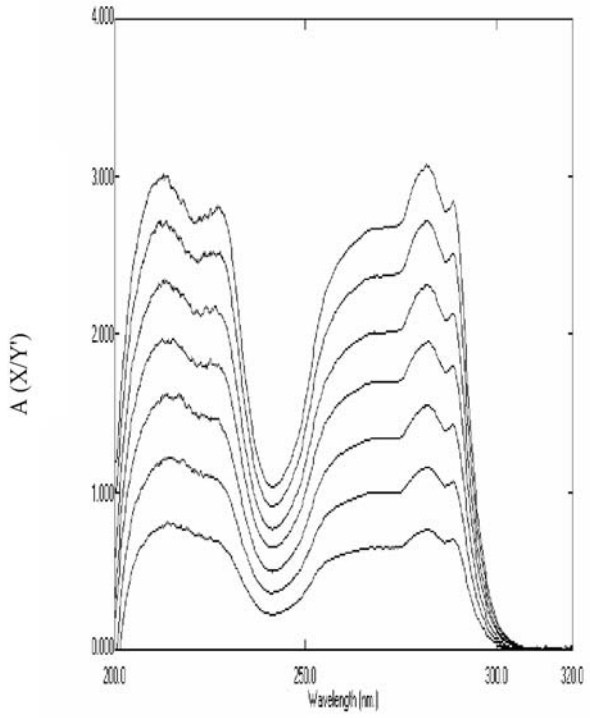

Figure 7. Division spectra of laboratory-prepared mixtures of vincamine (*X*) and its degradation product (*Y*) using 16 μg/mL of degradation product (*Y'*) as the divisor and 0.1 M HCl as the solvent after subtraction of the constant.

Thus, having the total concentration of both drugs, if the concentration of one of them can be determined separately by any other method, such as ratio subtraction, the concentration of the second drug can be calculated by subtraction (39–43).

This theory can be confirmed experimentally by recording the absorbance spectra of a certain concentration of the 2 drugs and the absorbance spectra of a binary mixture containing the same concentration.

Experimental

Apparatus

(**a**) *Spectrophotometer*.—Shimadzu (Columbia, MD) UV-1601 PC, dual-beam UV-visible (UV-Vis) spectrophotometer with 2 matched 1 cm quartz cells, connected to an International Business Machines (IBM) compatible PC and a Hewlett Packard (HP)-600 inkjet printer. Bundled UV-PC personal spectroscopy software Version 3.7 (Boise, ID) was used to process the absorption and derivative spectra. The spectral band width was 0.2 nm with a wavelength scanning speed of 2800 nm/min.

(**b**) *GC/MS instrument.*—Shimadzu GC-MS-QP 1000 EX, composed of gas chromatograph (GC-14A) and mass spectrometer with 70 eV ionization voltage. The GC/MS conditions were as follows: column, polyethylene glycol (At

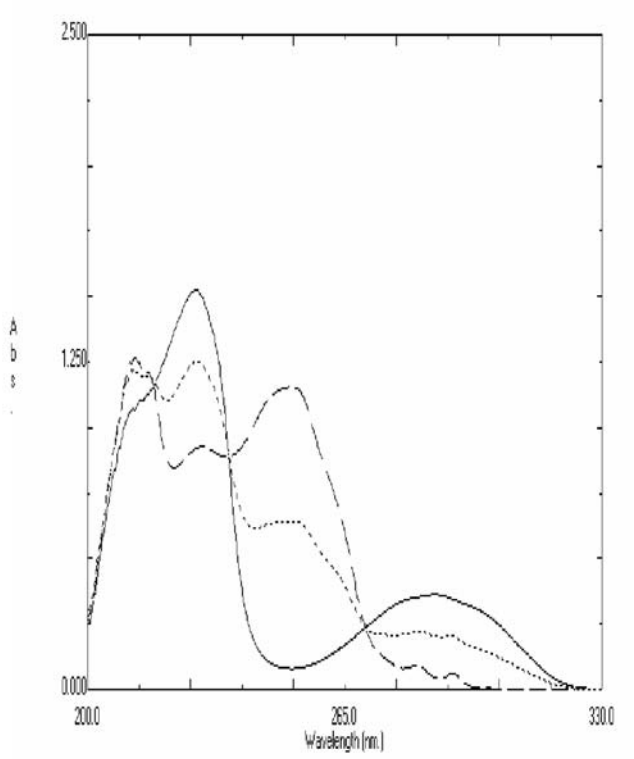

Figure 9. Absorption spectra of NIC, 20 μg/mL (——), and CN, 20 μg/mL (– – – –), and a mixture of 10 μg/mL of each drug (- - - -) using methanol as the solvent.

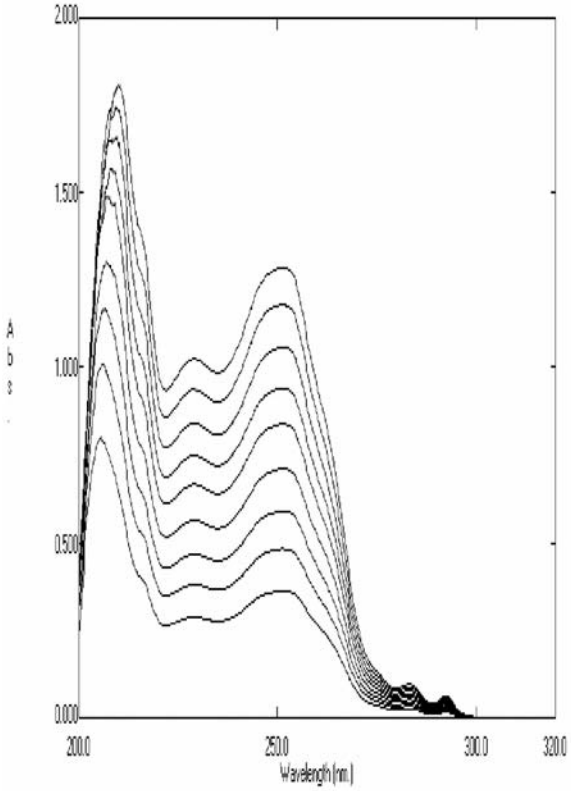

Figure 11. Zero order absorption spectra of CN, 6–22 μg/mL.

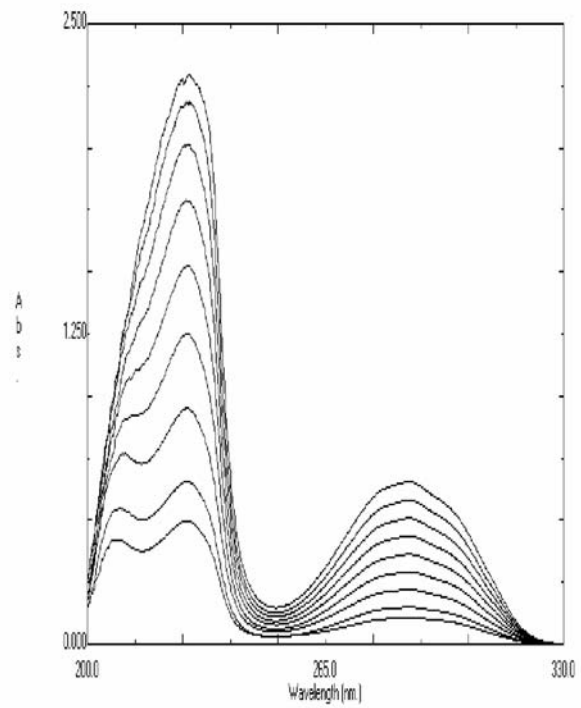

Figure 10. Zero order absorption spectra of NIC, 6–36 μg/mL.

wax); mobile phase, helium gas; temperature program, initial temperature: 120°C, initial time 1 min, program rate 10°C/min, final temperature: 210°C; injector, 250 μL; detector temperature, 250°C.

Solvent and Chemicals

All solvent and chemicals used in this work were of analytical grade and obtained from Prolabo Chemical Co., (Cairo, Egypt) and E. Merck (Darmstadt, Germany).

Pure Samples

Vincamine powder was kindly supplied by Glaxo-Wellcome (Cairo, Egypt). Its purity was checked in our laboratory according to the manufacturer's method (direct spectrophotometric analysis at 268 nm using 0.1 M HCl as the solvent), and was found to be 99.58 ± 1.01%.

NIC and CN powder were kindly supplied by Sigma Pharmaceutical Industrial Co. (Cairo, Egypt). Their purity was checked in our laboratory according to the manufacturer's method [HPLC using a C8 stationary phase and acetonitrile–solution pH 6 (58 + 42, v/v) mobile phase] and were found to be 99.64 ± 0.978 and 99.27 ± 0.714% for NIC and CN, respectively. [pH 6 solution (1.74 g K_2HPO_4 in 900 mL H_20 adjusted to pH 6 with H_3PO_4; 85%, v/v) + 1 mL triethyl amine.]

Table 2. Determination of NIC and CN in laboratory-prepared mixtures by the proposed procedures

Concn, μg/mL		Ratio	Isosbestic point method: Recovery, % NIC		Ratio subtraction method: Recovery, %
NIC	CN	NIC:CN	λ_1 270 nm	λ_2 235 nm	CN
9	13.5	2:3	100.51	100.88	100.97
6	10.5	2:3.5	101.05	101.47	101.45
7	14.0	2:4	99.54	99.77	100.43
6	13.5	2:4.5	98.96	99.17	101.07
6	15.0	2:5	98.68	99.07	99.43
6	16.5	2:5.5	99.75	100.31	100.00
6	18.0	2:6	99.31	99.27	100.14
6	19.5	2:6.5	99.03	98.66	99.96
6	21.0	2:7	100.25	100.00	100.48
Mean ± SD[a]			99.67 ± 0.790	99.84 ± 0.918	100.43 ± 0.635

[a] SD = Standard deviation.

Market Samples

Oxybral capsules (Batch Nos. 052831A and 012261A, expiration May 2004) were manufactured by Glaxo-Wellcome. Each capsule claimed to contain 30 mg vincamine. Cinibral tablets (Batch Nos. 39123 and 40082) were manufactured by Sigma Pharmaceutical Industrial Co. Each capsule claimed to contain 10 mg NIC and 25 mg CN. All were purchased from the Egyptian market.

Standard Solutions

(**a**) *Vincamine stock solution*.—0.25 mg/mL in 0.1 M HCl.

(**b**) *Degradation product (vincaminic acid) stock solution*.—0.25 mg/mL in 0.1 M HCl.

(**c**) *CN stock solution*.—0.25 mg/mL in methanol.

(**d**) *NIC stock solution*.—0.25 mg/mL in methanol.

Procedures for Vincamine

(**a**) *Preparation of degradation product of vincamine*.—200 mg pure vincamine was weighed and dissolved in 100 mL 2 M HCl then refluxed at 100°C. Complete degradation was obtained after 6.5 h as confirmed by TLC. The solution was evaporated to near dryness.

The degraded solution was applied as a band to TLC plates [precoated HPTLC plates, silica gel 60 F_{245} 20 × 20 cm, 0.2 nm thickness (Macheray Nagel)]. The plates were placed in a chromatographic tank previously saturated for 1 h with the mobile phase methanol–chloroform–ethyl acetate (2 + 1 + 1, v/v/v) and then dried in air after development. The band was visualized under UV light at 254 nm, then scraped and suspended in the least possible amount of methanol. The

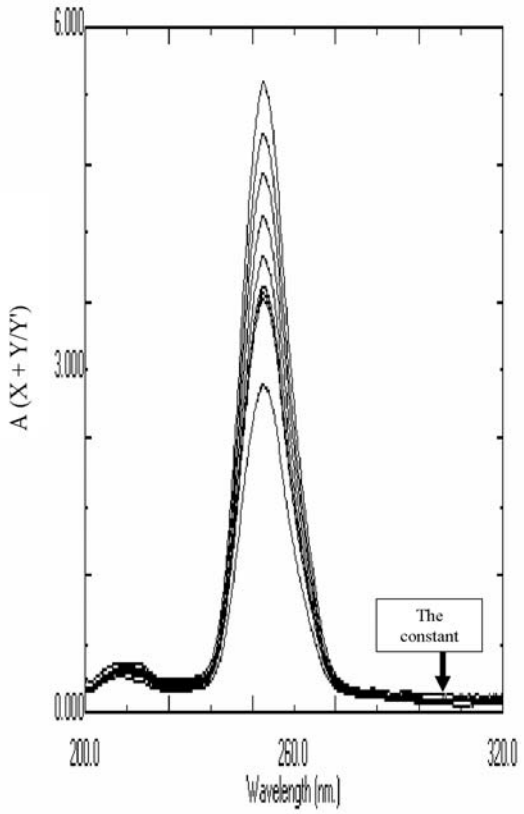

Figure 12. Division spectra of laboratory-prepared mixtures of CN (*X*) and NIC (*Y*) using 6 μg/mL NIC (*Y'*) as the divisor and methanol as the solvent (scale × 0.1).

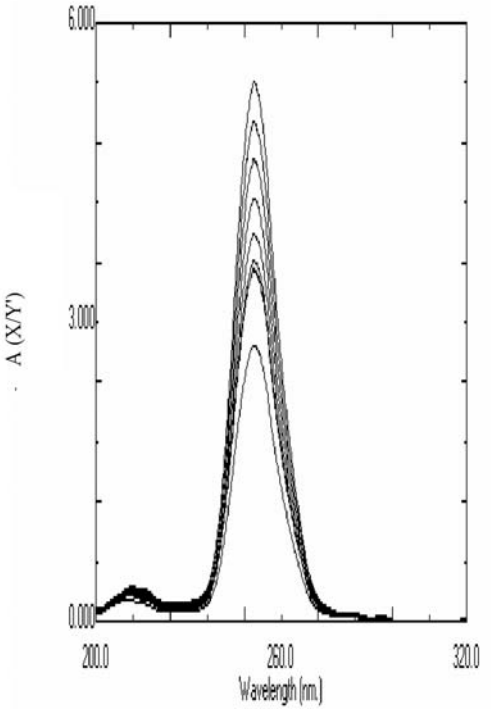

Figure 13. Division spectra of laboratory-prepared mixtures of CN (*X*) and NIC (*Y*) using 6 μg/mL of NIC (*Y'*) as the divisor and methanol as the solvent after subtraction of the constant (scale × 0.1).

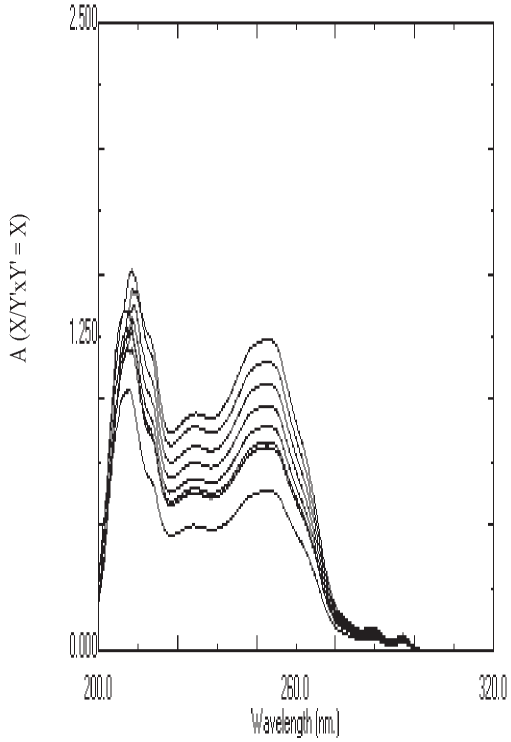

Figure 14. The obtained absorption spectra of CN in laboratory-prepared mixtures using the proposed procedure.

suspension was filtered and left to evaporate at room temperature (25°C) to obtain the degradation product residue used to prepare its stock solution.

(**b**) *Spectral characteristics of vincamine and its degradation product*.—Separate aliquots equivalent to 500 μg vincamine and its degradation product were transferred from their stock solutions (0.25 mg/mL) into two 25 mL volumetric flasks and completed to volume with 0.1 M HCl. The zero order spectra of the prepared solutions were recorded from 200 to 350 nm (Figure 4).

(**c**) *Linearity*.—Into a series of 25 mL volumetric flasks, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, and 4.0 mL aliquots of vincamine stock solution (0.25 mg/mL) were transferred accurately and then completed to volume with 0.1 M HCl. The spectra of the prepared standard solutions were scanned from 200–350 nm and stored in the computer (Figure 5).

A calibration graph relating the absorbance of the zero order spectra of vincamine at 268.2 nm to the corresponding concentrations in μg/mL was constructed, and the regression equation was computed.

(**d**) *Analysis of the laboratory-prepared mixtures*.—Into a series of 25 mL volumetric flasks, aliquots equivalent to 200–800 and 800–200 μg vincamine and its degradation product, respectively, were transferred accurately from their stock solution (0.25 mg/mL) to prepare mixtures containing different ratios of the drug and its degradation product as

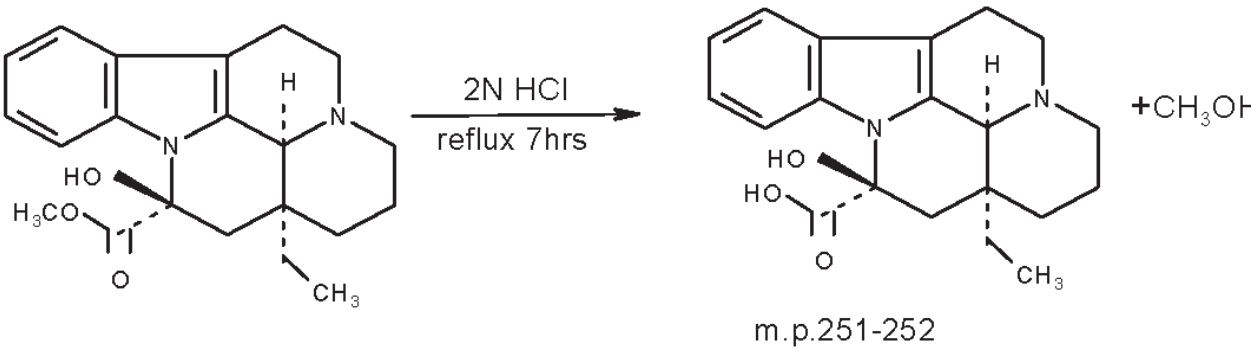

Figure 15. Proposed scheme for the degradation of vincamine.

Table 3. Determination of vincamine in Oxybral capsules by the proposed ratio subtraction method

Oxybral capsules claimed to contain 30 mg vincamine, Batch No.	Found, % ± SD[a]		
	Ratio subtraction method	Reported method[b]	Reported method[c]
052831 A	99.01 ± 0.762	99.07 ± 0.466	99.32 ± 0.956
0012261 A (expired 5/04)	84.63 ± 1.025	84.11 ± 0.501	97.56 ± 0.857

[a] Average of 4 determinations.
[b] Stability-indicating derivative spectrophotometric method (ref. 3).
[c] Manufacturer's spectrophotometric method.

Table 4. Determination of nicergoline and cinnarizine in Cinibral tablets by the proposed methods

Cinibral tablets claimed to contain 10 mg NIC and 25 mg CN Batch No.	Isosbestic point method		Ratio subtraction method	Reported method[b]	
	Found, % ± SD,[a] for NIC				
	270 nm	235 nm	Found, % ± SD,[b] for CN	Found, % ± SD,[b] for NIC	Found, % ± SD,[b] for CN
39123	99.24 ± 0.877	98.61 ± 0.874	99.15 ± 0.212	99.32 ± 0.956	98.37 ± 0.892
40082	100.86 ± 0.619	99.86 ± 0.733	99.58 ± 0.301	98.56 ± 0.857	98.99 ± 0.825

[a] Average of 4 determinations.
[b] Manufacturer's HPLC method.

shown in Table 1, and then were completed to volume with 0.1 M HCl. Into a 25 mL volumetric flask, 1.6 mL from the degradation product stock solution (0.25 mg/mL) was transferred and completed to volume with 0.1 M HCl. The spectra of the prepared standard solutions were scanned from 200–350 nm and stored in the computer. The spectra of the laboratory-prepared mixtures were divided (absorbance at each wavelength) by the spectrum of 16 μg/mL of the degradation product (Figure 6). The absorbance in the plateau region was subtracted at a λ above 305 nm (the constant) as shown in Figure 7. The obtained curves were multiplied (absorbance at each wavelength) by the spectrum of 16 μg/mL of the standard degradation product (Figure 8). The obtained curves (Figure 8) were used for the determination of vincamine from the corresponding regression equation.

(**e**) *GC/MS conditions.*—The degradation product was analyzed by GC/MS using the conditions described under *Apparatus*.

Procedures for Nicergoline and Cinnarizine

(**a**) *Spectral characteristics of NIC and CN.*—Separate aliquots equivalent to 500 μg NIC and CN were transferred from their stock solutions (0.25 mg/mL) into two 25 mL volumetric flasks, then completed to volume with methanol. A binary mixture was prepared by transferring aliquots equivalent to 250 μg of each drug into a 25 mL volumetric flask and then completing to volume with methanol. The zero order spectra of the prepared solutions were recorded from 200 to 330 nm (Figure 9).

(**b**) *Linearity.*—Into a series of 25 mL volumetric flasks, 0.6, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, and 3.6 mL aliquots of NIC stock solution (0.25 mg/mL) were transferred accurately and then completed to volume with methanol (Figure 10). Into a series of 25 mL volumetric flasks, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, and 2.2 mL aliquots of CN stock solution (0.25 mg/mL) were transferred accurately and then completed

Table 5. Application of standard addition for the determination of vincamine by the proposed ratio subtraction method

Batch No.	Vincamine	Ratio subtraction method
	Standard added, mg	Recovery of added, %
052831 A	25	98.56
	37.5	99.01
	50	99.99
Mean ± SD[a]		99.98 ± 0.731

[a] Average of 4 determinations.

Table 6. Application of standard addition for the determination of NIC and CN by the proposed methods

			Isosbestic point method		
	Standard added, mg		Recovery of added NIC, %		Ratio subtraction method
Batch No.	NIC	CN	270 nm	235 nm	Recovery of added CN, %
39123	10.00	25.00	99.26	99.66	98.18
	15.00	37.50	100.03	100.83	98.93
	20.00	50.00	100.32	101.05	98.06
Mean ± SD[a]			99.87 ± 0.547	100.51 ± 0.747	98.39 ± 0.471

[a] Average of 4 determinations.

to volume with methanol (Figure 11). The spectra of the prepared standard solutions were scanned from 200–330 nm and stored in the computer. Two calibration graphs relating the absorbance of the zero order spectra of NIC at the isosbestic points 270.2 nm (λ_1) and 235.8 nm (λ_2) to the corresponding concentrations in μg/mL were constructed, and the regression equations were computed. A calibration graph relating the absorbance of the zero order spectra of CN at 252.0 nm to the corresponding concentrations in μg/mL was constructed, and the regression equation was computed.

(**c**) *Analysis of the laboratory-prepared mixtures.*—Into a series of 25 mL volumetric flasks, aliquots equivalent to 262.5–525 and 150–225 μg CN and NIC, respectively, were accurately transferred from their stock solution (0.25 mg/mL) to prepare mixtures containing different ratios of the 2 drugs, then were completed to volume with methanol as shown in Table 2. For the determination of CN, the spectra of the laboratory-prepared mixtures were divided (absorbance at each wavelength) by the spectrum of 6 μg/mL NIC (Figure 12). The absorbance in the plateau region was subtracted at a λ above 305 (the constant) as shown in Figure 13. The obtained curves were multiplied (absorbance at each wavelength) by the spectrum of 6 μg/mL NIC (Figure 14). The obtained curves (Figure 14) were used for the determination of CN from the corresponding regression equation. For the determination of NIC, the regression equations at either λ_1 or λ_2 were used to obtain the total concentrations of the mixtures. When the concentration of CN was subtracted from them, the concentration of NIC was obtained.

Results and Discussion

The absorption spectra of vincamine and its degradation product and NIC mixed with CN show severe overlapping

Table 7. Statistical comparison for the results obtained by the proposed ratio subtraction method and the reported method for the analysis of vincamine in pure powdered form

	Vincamine			
	Ratio subtraction method		Reported method[a]	Reported method[b]
Mean	99.72	99.72	99.90	99.58
SD	0.917	0.917	1.041	1.011
Variance	0.840	0.840	1.084	1.022
n[c]	9	9	6	6
F-test	1.29 (3.69)[a,d]	1.21 (3.69)[b,d]		
Student's *t*-test	0.35 (2.16)[a,d]	0.27 (2.16)[b,d]		

[a] Stability-indicating derivative spectrophotometric method (ref. 3).
[b] Manufacturer's spectrophotometric method.
[c] *n* = Number of trials.
[d] Values in parentheses are the corresponding tabulated values at *P* = 0.05 (ref. 46).

Table 8. Statistical comparison for the results obtained by the proposed methods and the reported method for the analysis of NIC and CN in pure powdered form

	Isosbestic point method				
	NIC		Ratio subtraction method	Reported method[a]	
	270 nm	235 nm	CN	NIC	CN
Mean	99.58	99.83	99.91	99.64	99.27
SD	0.847	1.039	0.703	0.978	0.714
Variance	0.717	1.079	0.494	0.956	0.509
n	9	9	9	6	6
F-test	1.333 (3.69)[b]	1.128 (4.82)	1.030 (3.69)		
Student's *t*-test	0.126 (2.160)	0.354 (2.160)	1.717 (2.160)		

[a] Manufacturer's HPLC method.

[b] Values in parentheses are the corresponding tabulated values at $P = 0.05$ (ref. 46).

Table 9. Assay parameters and method validation

	Ratio subtraction method	Isosbestic point method		Ratio subtraction method
		NIC		
Parameter	Vincamine	λ1	λ2	CN
Range, μg/mL	8.0–40.0	6–36	6–36	6.0–22.0
Slope	0.0239	0.0119	0.041	0.0578
Intercept	0.0017	–0.0001	0.00349	0.0193
Mean	99.72	99.58	99.83	99.91
SD	0.917	0.847	1.039	0.703
Variance	0.840	0.717	1.079	0.494
Correlation coefficient (r)	0.9998	0.9999	0.9998	0.9997
RSD, %[a]	0.788, 0.903	0.620, 0.805	0.942, 0.641	0.732, 0.897
RSD, %[b]	0.936, 0.984	0.881, 1.173	0.892, 1.274	0.841, 0.875

[a,b] Intraday and interday relative standard deviation, respectively, ($n = 5$) of concentrations of 28 and 32 μg/mL for vincamine, 6 and 8 μg/mL for NIC, and 14 and 16 μg/mL for CN.

that prevents the use of direct spectrophotometry for the analysis of either vincamine or a binary mixture of NIC and CN.

The proposed scheme for degradation of vincamine is shown in Figure 15. MS was used for determination of the degradation product; the parent peak was identified at mass-to-charge ratio (m/z) = 340, indicating that the molecular weight of the degradation product is 340 because $z = 1$. The degradate (vincaminic acid; 44) is one of the metabolites of vincamine in rats (45). It is soluble in 0.1 M HCl as it contains one basic nitrogen in its tertiary amine structure. The absorption spectra of vincamine and its degradation product are shown in Figure 4. The linearity was checked between the peak absorbance at the selected wavelength (268.2 nm) and the corresponding concentrations of vincamine. A linear relationship was obtained in the range of 8.0–40.0 μg/mL for vincamine, as shown in Figure 5. The regression equation was computed and found to be:

$$A = 0.0239\ C - 0.0017 \qquad r = 0.9998$$

where C is the concentration of vincamine in μg/mL, A is the absorbance of vincamine at 268.2 nm, and r is the correlation coefficient.

The regression equation for CN was computed and found to be:

$$A = 0.0578\ C + 0.0193 \qquad r = 0.9997$$

where C is the concentration of CN in μg/mL, A is the absorbance for the zero order curve of CN at 252.0 nm, and r is the correlation coefficient.

The regression equations for NIC were computed and found to be:

$$A_1 = 0.0119\ C - 0.0001 \qquad r = 0.9999$$

$$A_2 = 0.041\ C + 0.0349 \qquad r = 0.9998$$

where C is the concentration of NIC in μg/mL, A_1 and A_2 are the absorbencies for the zero order curve of NIC at their isosbestic points 270.2 and 235.8 nm, respectively, and r is the correlation coefficient.

The specificity of the proposed methods was proved by the analysis of laboratory-prepared mixtures. Results are shown in Tables 1 and 2.

The proposed procedures were successfully applied for the determination of vincamine in Oxybral capsules, including expired ones, and NIC and CN in Cinibral tablets with good recovery. Results are shown in Tables 3 and 4.

The standard addition technique was performed to indicate the accuracy of the proposed methods. Results are shown in Tables 5 and 6.

The results obtained for the analysis of vincamine, NIC, and CN in the pure powder form by the suggested methods were compared by those obtained by the manufacturer's methods, and no significant differences were observed. Also, those for vincamine was compared to a stability-indicating method (3), and no significant difference was obtained. Results are shown in Tables 7 and 8. Validation results are presented in Table 9 (47).

Conclusions

It can be concluded that the proposed procedures are simple and do not require sophisticated techniques or instruments. They are also sensitive and selective and can be used for the routine analysis of the cited drugs in their available dosage forms. The methods are also suitable and valid for application in laboratories lacking liquid chromatographic instruments.

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