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COMMUNICATION

Simultaneous Determination of Ascorbic Acid, Pyridoxine Hydrochloride, and Tyrosine by Derivative UV Spectrophotometry

Mariana Surmeian

Institute of Chemical and Pharmaceutical Research, 74373-Cal. Vitan 112, Bucharest-3, Romania

ABSTRACT

A new UV spectrophotometric method for resolving a three-component drug mixture of ascorbic acid (I), pyridoxine hydrochloride (II), and tyrosine (III), on the basis of the first-derivative UV spectra and zero-crossing technique, is described. Beer's law was obeyed in the concentration range of 7.0–15.0, 0.25–1.0, and 8.0–40.0 μg/ml for I, II, and III, respectively. Lower limits of detection at the 95% confidence level were 2.88, 0.69, and 5.43 μg/ml, respectively. The advantages of the proposed method (speed, easy sample preparation, and lower cost per analysis) also include its application to the content uniformity and dissolution test of a three-component mixture of drugs.

INTRODUCTION

Ascorbic acid (vitamin C), pyridoxine hydrochloride (vitamin B_6, enzyme cofactor), and tyrosine (amino acid) are used separately or in combination with other drugs in many pharmaceutical preparations. The association of these drugs develops a very efficient result in the therapy of different diseases of the nervous and neuromuscular system.

For the determination of ascorbic acid (1–3), pyridoxine (3–6), and tyrosine (7), from pharmaceutical preparations, singly or in combination with other drugs, derivative UV spectrometry (8) has been applied successfully for qualitative (9) and quantitative (10–13) analysis of multicomponent drugs that present a large overlapping of bands in the UV region. This involves the determination of drugs in the presence of their degradation products (14–16) or matrix interferences (17,18) and from complex mixtures (4–6,19).

The present paper presents the use of derivative UV spectrophotometry and the zero-crossing technique for the simultaneous determination of a three-component

mixture of drugs: ascorbic acid (I), pyridoxine hydrochloride (II), and tyrosine (III). The method is rapid, precise, accurate, and comparable with more time-consuming methods that are used for routine analysis of single drugs and have been used successfully for the determination of I, II, and III from sugar-coated tablets. The method was also applied to the determination of content uniformity and dissolution rate of sugar-coated tablets (20). In order to investigate the physical processes during the dissolution period, the release profiles were numerically simulated by typical equations: zero-order, $mt = f(t)$; first-order, $\log(m_0 - m) = f(t)$; Higuchi matrix (21,22), $mt = f(t)^{1/2}$; and Langenbucher model (23), $\ln(\ln(m_0/(m_0 - m))) - f(\ln t)$, where m is the amount of drug released at time t and m_0 is the initial amount of drug in the sugar-coated tablet.

MATERIALS AND METHODS

Reagents and Materials

Analytical standard grades of ascorbic acid (Takeda, Japan), pyridoxine hydrochloride (Loba, Feinchemie, Austranal Präparate), and tyrosine (Merck, Germany) were used. Fortavit V sugar-coated tablets (Institute of Chemical and Pharmaceutical Research, Bucharest, Romania) contained 75 mg of ascorbic acid, 5 mg of pyridoxine hydrochloride, and 200 mg of tyrosine according to the label. Other reagents used were of analytical or pharmaceutical grade.

Apparatus

UV-VIS spectrophotometer (Specord M-42, Carl Zeiss Jena, Germany) with 1 cm quartz cells were used. The derivative spectra were recorded using the UV-Standard program of the apparatus, modified as follows: spectral slit width 2 nm; integration time 0.5 sec; wavelength range 220–320 nm plotted using a graph editing program. The values of the zero-crossing points and of the corresponded amplitudes were calculated by linear interpolation between two neighboring points ($\Delta\lambda$ = 2 nm).

Preparation of Standard Solutions

Standard solutions were prepared containing separately 75 μg/ml of ascorbic acid, 5 μg/ml pyridoxine hydrochloride, and 200 μg/ml tyrosine. For each drug, 1–5 ml of these solutions was transferred by pipete into 25-ml calibrated flasks and completed with 0.1 N HCl.

Content Uniformity Test

Ten sugar-coated tablets of Fortavit V were dissolved together, by mechanical shaking, with 0.1 N HCl into 100-ml calibrated flasks. One-milliliter aliquots from these solutions were transferred into 50-ml calibrated flasks and completed with the same solvent.

Drug Dissolution Tests

The dissolution test was performed with the basket-stirrer USP-type apparatus (20) operated at 75 rpm in 500 ml of 0.1 N hydrochloric acid (simulated gastric fluid). After an appropriate time interval (2–10 min), fixed volumes of the dissolution medium (1 ml) were withdrawn and subsequently diluted (to 10 ml) with the dissolution medium for spectrophotometric measurement.

RESULTS AND DISCUSSION

The individual UV spectra of ascorbic acid, pyridoxine hydrochloride, and tyrosine in 0.1 N HCl solution, presented in Fig. 1, show an overlapping of bands which makes the rapid simultaneous quantitation by direct UV spectrophotometry impossible to perform. Using the first-derivative spectra and the zero-crossing technique I, II, and III were quantified simultaneously at the zero-crossing points: λ_I = 245.9 nm, λ_{II} = 302.0 nm and λ_{III} = 243.5 nm from the first-derivative spectra of solutions containing 15, 5.0, and 40 μg/ml, respectively.

Calibration Graph

The calibration graph of each compound shows linear relationships obeying Beer's law over the concentration range used: 7–15 μg/ml (I), 0.25–1.0 μg/ml (II), and 8–40 μg/ml (III). Regression equations were made using the linear regression method for intercept, slope, and correlation coefficient (Table 1).

The accuracy and precision were tested by successive determination of six standard mixtures containing 7.25, 10.50, and 14.50 μg/ml of I, 0.25, 0.50, and 1.0 μg/ml of II, and 8.10, 20.25, and 40.50 μg/ml of III. For com-

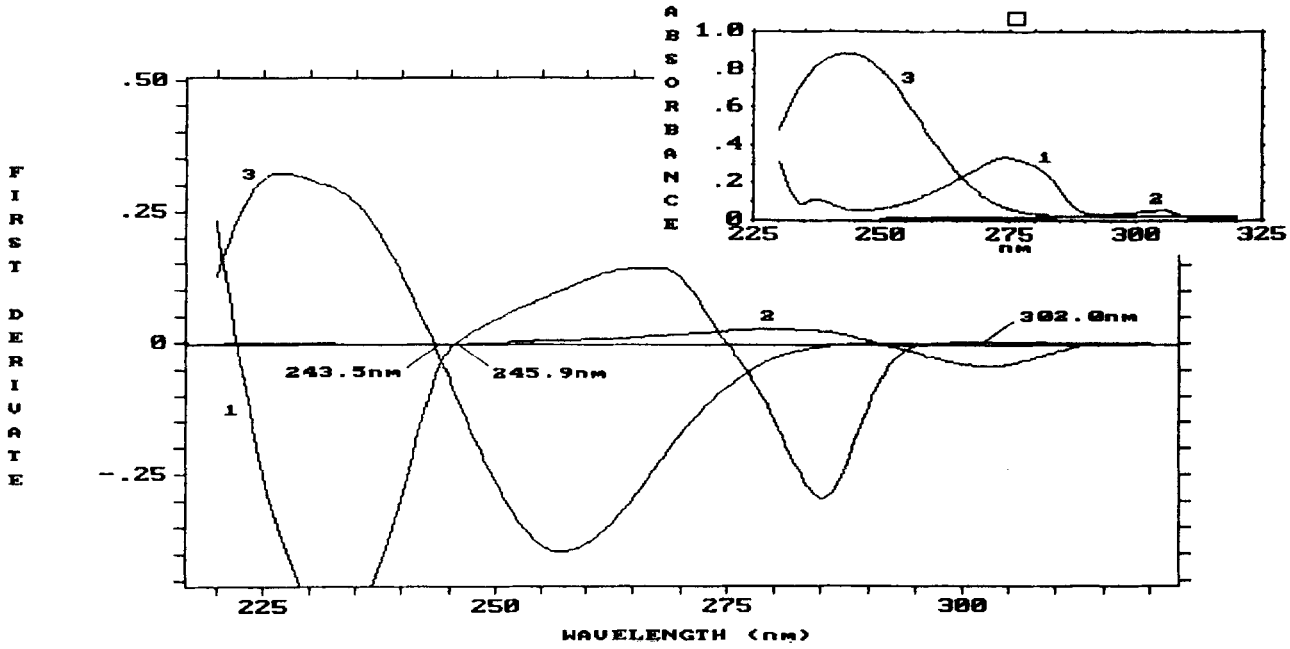

Figure 1. Zero-order and first-derivative spectra of (1) 15 μg/ml ascorbic acid, (2) 5.0 μg/ml pyridoxine hydrochloride, and (3) 40.0 μg/ml tyrosine in 0.1 N hydrochloric acid solution.

parison, routine methods were applied for the determination of each intact drug in the above mixtures: titration with 0.1 N iodine solution, as is indicated for the assay of ascorbic acid by the USP (20), zero-order UV spectrophotometric determination of pyridoxine, at 290 nm, in 0.1 N hydrochloric acid solution, and zero-order UV-VIS spectrophotometric determination of tyrosine–nynhydrine complex at 540 nm (24). Results were acceptable owing to the contribution of the interference of each drug (Table 2). In the absence of spectrally interfering excipients, drug recovery was performed following the content uniformity test (20). Results obtained for the means of the percent label claim are higher than 85% and % RSD ≤ 6.

Drug Dissolution Tests

The dissolution profiles (percentage dissolved versus dissolution time) of all three compounds are shown in

Table 1

Analytical Parameters for the Assay of Ascorbic Acid (I), Pyridoxine (II), and Tyrosine (III)

Drug	Regression Equations[a]	r	Variance S_0^2	Standard Deviation S_a	Standard Deviation S_b	X_i[b] μg/ml
I	$y = 0.003387 - 0.007247x$ (245.9 nm)	0.9993	1.08×10^{-5}	2.80×10^{-3}	2.80×10^{-4}	2.88
II	$y = 0.001475 - 0.030877x$ (302.0 nm)	0.9991	9.40×10^{-7}	8.30×10^{-4}	1.30×10^{-3}	0.69
III	$y = 0.005071 - 0.001749x$ (243.5 nm)	0.9986	2.45×10^{-6}	1.3×10^{-3}	5.20×10^{-5}	5.43

[a] $y = a + bx$, where x is the concentration in μg/ml and y the amplitude of the first-derivative spectra in the zero-crossing point.
[b] X_i limit of detection for $n = 5$ and 95% confidence level.

Table 2

Recovery of Ascorbic Acid, Pyridoxine, and Tyrosine from Synthetic Mixtures

	Ascorbic Acid			Pyridoxine			Tyrosine		
		Recovery			Recovery			Recovery	
No.	Added (μg/ml)	$^1D_{254.9}$ (%)	USP[a] (%)	Added (μg/ml)	$^1D_{302}$ (%)	$^0D_{290}$ (%)	Added (μg/ml)	$^1D_{243.5}$ (%)	$^0D_{570}$ (%)
1	7.25	101.6	100.5	0.25	98.0	99.9	40.50	99.1	100.2
2	7.25	103.1	101.3	0.50	100.5	99.8	20.25	101.5	99.9
3	14.50	102.5	99.9	1.00	101.3	100.1	40.50	100.5	100.5
4	14.50	103.0	100.4	0.50	102.6	100.0	8.10	98.7	99.5
5	10.50	101.1	101.0	0.50	99.8	100.4	20.25	100.7	99.9
6	10.50	102.2	100.2	1.00	100.9	99.8	8.10	99.8	100.4
$\bar{X}$		102.25	100.55		100.5	100.0		100.05	100.06
SD		0.78	0.52		1.54	0.22		1.05	0.37

[a]Assay of ascorbic acid required by USP (20).

Fig. 2. It is evident that the release of drugs increases with their solubility (ascorbic acid and pyridoxine hydrochloride (25) are more soluble in hydrochloric acid solution than is tyrosine (26)).

Taking into account the S-shape of curves, the release of I, II, and III from sugar-coated tablets in simulated gastric fluid was computed to fit the Langenbucher model (23). The dissolution process involves two main steps: an initial step of about 3 min (while the coated layer is removed), followed by a rapid process of active principles dissolution. As can be seen from Table 3, the experimental data fit the Langenbucher model well.

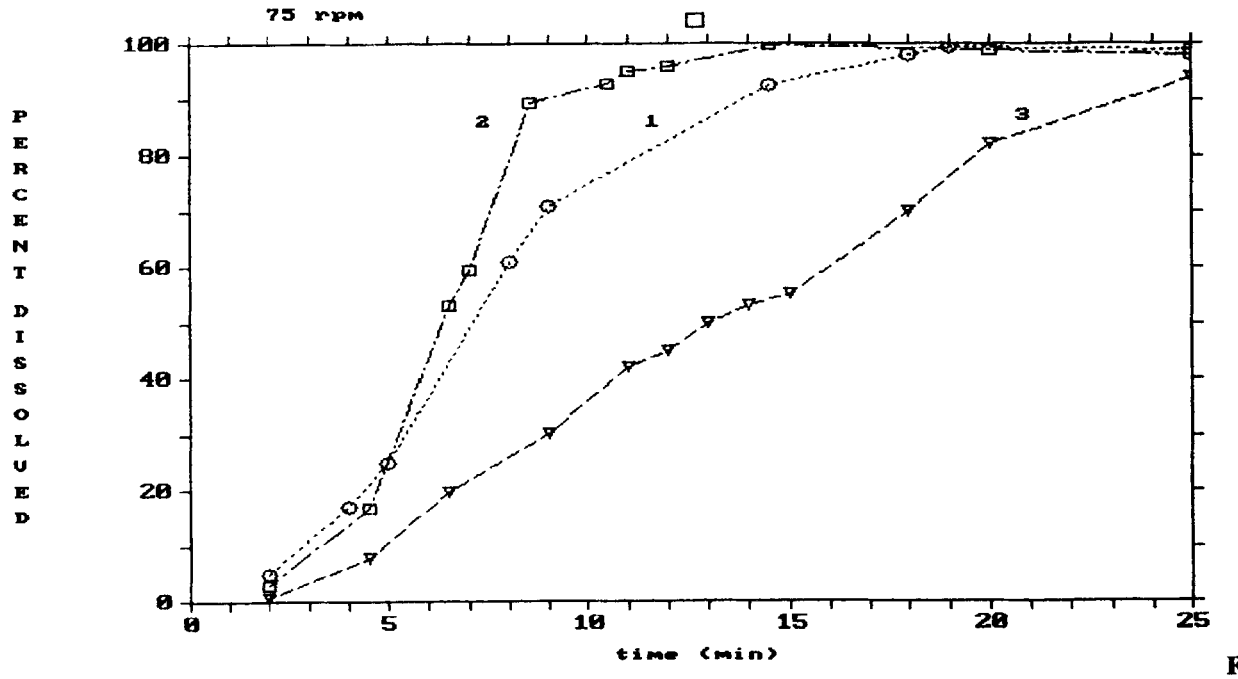

Figure 2. Dissolution profiles of sugar-coated tablets of (1) ascorbic acid, (2) pyridoxine hydrochloride, and (3) tyrosine in 0.1 N hydrochloric acid solution.

Table 3

Content Uniformity of Sugar-Coated Tablets

Sugar-Coated Tablet No.	Percent Label Claim		
	Ascorbic Acid (75 mg)	Pyridoxine (5 mg)	Tyrosine (200 mg)
1	101.9	98.7	92.4
2	100.4	105.3	93.7
3	97.4	96.5	94.3
4	97.9	96.5	90.4
5	98.5	95.4	91.3
6	99.8	95.4	94.6
7	105.0	105.3	100.8
8	96.3	98.7	91.2
9	99.9	96.5	95.6
10	101.7	105.3	100.4
$\overline{X}$	99.8	99.3	94.55
RSD%	2.55	4.27	3.82

The release data, obtained from the rapid process of active principles dissolution (up to 85% of drug relese), were computed to fit the order of kinetic equations (zero-order, first-order, and Higuchi matrix models). The correlation coefficients from the computer output were tabulated and indicate the predominate mechanism of drug release: zero-order for tyrosine, first-order for ascorbic acid, and Higuchi matrix for pyridoxine (Table 4).

CONCLUSIONS

First-derivative UV spectrophotometry was used for the simultaneous determination of a three-component mixture of drugs: ascorbic acid, pyridoxine, and tyrosine. The method can be successfully used for the uniformity content and drug dissolution assay of sugar-coated tablets containing these drugs.

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Table 4

Correlation Coefficient and Slope of Kinetic Equations of Drug Release

Drug	Kinetic Equations			
	Zero-Order	First-Order	Higuchi Matrix	Langenbucher Model
Ascorbic acid	0.93440	0.99519	0.96032	0.99778
	(3.41)	(–0.25)	(24.34)	(2.04)
Pyridoxine	0.87003	0.97319	0.99755	0.9923
	(0.29)	(–0.46)	(–0.16)	(2.72)
Tyrosine	0.99598	0.93919	0.98695	0.98346
	(8.29)	(–0.17)	(–0.16)	(2.39)

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