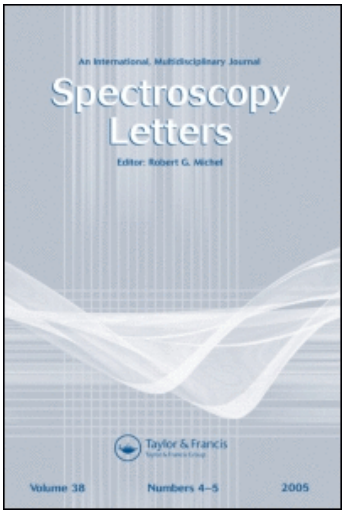

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DERIVATIVE RATIO SPECTROPHOTOMETRY AND DIFFERENTIAL DERIVATIVE SPECTROPHOTOMETRIC DETERMINATION OF ISONIAZID AND PYRIDOXINE HYDROCHLORIDE IN DOSAGE FORMS

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DERIVATIVE RATIO SPECTROPHOTOMETRY AND DIFFERENTIAL DERIVATIVE SPECTROPHOTOMETRIC DETERMINATION OF ISONIAZID AND PYRIDOXINE HYDROCHLORIDE IN DOSAGE FORMS

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ABSTRACT

Derivative ratio spectrophotometric and differential derivative spectrophotometric methods, for the determination of isoniazid and pyridoxine hydrochloride in pharmaceutical dosage forms (tablets) is described. The first method depends on ratio derivative spectra spectrophotometry where isoniazid and pyridoxine hydrochloride were determined by the measurement of the first derivative ratio spectra of amplitudes (Abs.) at 250.7 and 305.8 nm, respectively. The other method, is based on differential derivative spectrophotometry for the simultaneous determination of isoniazid and pyridoxine hydrochloride in binary mixture without any pre-treatment by measuring the ΔD_1 values.

Both methods showed good linearity, precision and reproducibility. The proposed methods were successfully applied to the pharmaceutical dosage forms containing the above-mentioned drug combination without any interference by the excipients.

Key Words: Isoniazid; Pyridoxine hydrochloride; Simultaneous determination; Ratio-spectra derivative spectrophotometric; Differential derivative spectrophotometry; Pharmaceutical dosage formulations

INTRODUCTION

Isoniazid is still the most important drug worldwide for the treatment of all types of tuberculosis. Toxic effects can be minimized by prophylactic therapy with pyridoxine and careful surveillance of the patient. If pyridoxine is not given concurrently, peripheral neuritis is the most common reaction to isoniazid. The drug must be used concurrently with another agent for treatment, although it is used alone for prophylaxis[1]. Some procedures have been described for the assay of either isoniazid or pyridoxine hydrochloride in pharmaceutical preparations and biological fluids, there are only a few methods reported to be able to analyse both drugs in combination[2,3]. These methods use spectrophotometry[4–16], high-pressure liquid chromatography[17], atomic absorption spectroscopy[18] and capillary electrophoresis[19–20]. Several U.V. spectrophotometric methods for simultaneously quantitation of two-component mixtures, without previous chemical separation, have been reported. Recently, a spectrophotometric method based on the use of the first derivative of the ratio spectra was developed by Salinas et al.[21], for resolving binary mixtures. Ratio derivative spectrophotometry as described by Salinas' group, was a simple, fast and useful technique used in the simultaneous determination of binary mixtures of isoniazid and pyridoxine hydrochloride, was also applied in pharmaceutical analysis.

Derivative spectrophotometry represents a successful approach to the problem of resolving spectral overlapping by offering the advantages of increasing selectivity and sensitivity compared with the conventional spectrophotometry[22,23]. Difference spectrophotometry has proved to be a powerful technique for determination of drugs[24–26] as well as detection and determination of decomposition products[27]. The technique involves the reproducible alterations of the spectral proper-

ties of the absorbance (ΔA) between two solutions, provided that the absorbance of the other absorbing substances is not affected by the reagents used to alter the spectral properties. The derivative-difference spectrophotometry will offer further advantages in canceling heavy spectral interferences to drug analysis[28,29] when the irrelevant absorption is pH and solvent dependent.

The primary aim of this present paper was to apply two proposed methods for the determination of isoniazid and pyridoxine hydrochloride in commercial tablets, with resultant coefficient of variation of less than 1%. The methods had sufficiently good accuracy and precision and permitted a simple, rapid, and time-saving assay for isoniazid and pyridoxine hydrochloride in a binary mixture.

EXPERIMENTAL

Apparatus

A double beam, Shimadzu 1601 spectrophotometer model with a fixed slit width (2 nm) connected to an IBM-PC computer loaded with a Lexmark printer was used for all the absorbance signals and treatment of data.

Chemicals Used

Isoniazid and pyridoxine hydrochloride were kindly supplied by DEVA Pharm. Ind. Methanol was of analytical reagent grade (Merck Chem. Ind.)

Pharmaceutical Preparation

A commercial pharmaceutical preparation (ISOVIT tablet DEVA Pharm. Ind. TURKEY, batch no: 4567D1) was assayed. Its declared content was as follows:

Isoniazid	100.0 mg
pyridoxine hydrochloride	25.0 mg/tablet

PROCEDURES

Ratio Spectra Derivative Spectrophotometry

Calibration

Stock solutions of 1 mg ml^{-1} of isoniazid and pyridoxine hydrochloride were prepared in methanol. These solutions were used in the preparation of calibration graphs and for spectra.

Assay Procedure for Dosage Forms

An accurately weighed amount of powdered tablets equivalent to about one tablet was transferred into a 100 ml conical flask in methanol. After 30 min of mechanical shaking, the solution was filtered in a 100 ml calibrated flask through Whatman No. 42 filter paper. The residue was washed three times with 10 ml of solvent and then the volume was completed to 100 ml with the same solvent. Appropriate solutions were prepared by taking suitable aliquots of the clear filtrates and diluting them with methanol.

Differential Derivative Spectrophotometry

Calibration

Reference drug solutions were prepared by weighing accurately 50.0 mg isoniazid and 50.0 mg pyridoxine hydrochloride and dissolving in methanol. Two equal portions of each solution were diluted with 0.1 N NaOH and methanol, respectively, six serial dilutions in the concentration ranges stated in Table 1 were prepared.

Assay Procedure for Dosage Forms

Twenty commercial tablets were accurately weighed, powdered and a weight of the powder equivalent to 100.0 mg of isoniazid (25.0 mg of pyridoxine hydrochloride), was dissolved in 100 ml methanol. After 30 min of mechanical shaking, the solution was filtered in a 100 ml calibrated flask through Whatman No. 42 filter paper. The residue was washed three times with 10 ml of solvent and then the volume was completed to 100 ml with

Table 1. Analytical Data for the Isoniazid and Pyridoxine Hydrochloride Using Ratio Derivative Spectrophotometry and Differential Derivative Spectrophotometry

Drug	Conc. Range ($\mu g\,ml^{-1}$)	Linear Regression					RSD(%)
		Intercept	±S.E.[a]	Slope	±S.E.[b]	Corr. Coeff. (r)	
Ratio Derivative Spectrophotometry							
Isoniazid	10.0–50.0	0.007	0.000089	0.017	0.00004	0.9987	0.78
pyridoxine hydrochloride	6.0–30.0	0.008	0.000059	−0.029	0.00009	−0.9997	0.91
Differential Derivative Spectrophotometry							
Isoniazid	10.0–50.0	0.014	0.000089	0.017	0.00012	0.9990	0.49
pyridoxine hydrochloride	6.0–30.0	0.081	0.000059	0.087	0.00034	0.9995	1.81

RSD., relative standard deviation (n = 5).
[a]Standard error of the intercept (n = 5).
[b]Standard error of the slope (n = 5).

the same solvent. The sample solutions were diluted 1:30 with 0.1 N NaOH and methanol, separately. The difference spectra between the methanolic solution and equimolar 0.1 N NaOH solution of pure drugs and sample were recorded by placing the methanolic solution in the reference compartment and the 0.1 N NaOH solutions in the sample compartment. A first derivative spectrum of each of the differential curves was subsequently recorded.

RESULTS AND DISCUSSION

Ratio Spectra Derivative Spectrophotometry

The absorption spectra of the two components are strongly overlapped (Fig. 1). On the other hand, this spectral overlapping was sufficiently enough to demonstrate the resolving power of the proposed method. For the determination of isoniazid, the stored absorption spectra of standard solutions of isoniazid and pyridoxine hydrochloride and a solution of their binary mixture were divided by the absorption spectrum of a standard solution of 16.0 μ g ml^{-1} of pyridoxine hydrochloride, then the first derivative of the obtained ratio spectra were calculated with $\Delta\lambda = 8$ nm (Fig. 2).

From Fig. 2, isoniazid can be determined in this binary mixture by measuring the analytical signals at 250.7 nm ($^1DD_{250.7}$) where there is no contribution from pyridoxine hydrochloride. The similar procedure was followed for the different concentrations of pyridoxine hydrochloride when isoniazid as 30.0 μg ml^{-1}. In the same way as describe above, the content of pyridoxine hydrochloride was determined by selecting the first derivative of the ratio spectrum in the range 240.0–345.0 nm and measuring the signals at 305.8 nm ($^1DD_{305.8}$). The influence of $\Delta\lambda$ for obtaining the first derivative of the ratio spectra was tested to obtain the optimum wavelength interval; $\Delta\lambda = 8$ nm was considered as suitable. Under the experimental conditions described, standard calibration curves for isoniazid and pyridoxine hydrochloride were constructed by plotting absorbance versus concentration. Conformity with Beer's law was evident in the concentration range of the final dilution cited in Table 1. The correlation coefficients were 0.9987 and 0.9997 indicating good linearity. Five replicate determinations at different concentration levels were carried out to test the precision of the methods. The relative standard deviations were found to be less than 0.9%, indicating reasonable repeatability of the proposed method. The detection limits (LOD) were 0.069 μg ml^{-1} for isoniazid and 0.086 μg ml^{-1} for pyridoxine hydrochloride; while the quantification limits (LOQ) were 0.39 μg ml^{-1} for isoniazid and 0.109 μg ml^{-1} for pyridoxine hydrochloride (Table 2). The

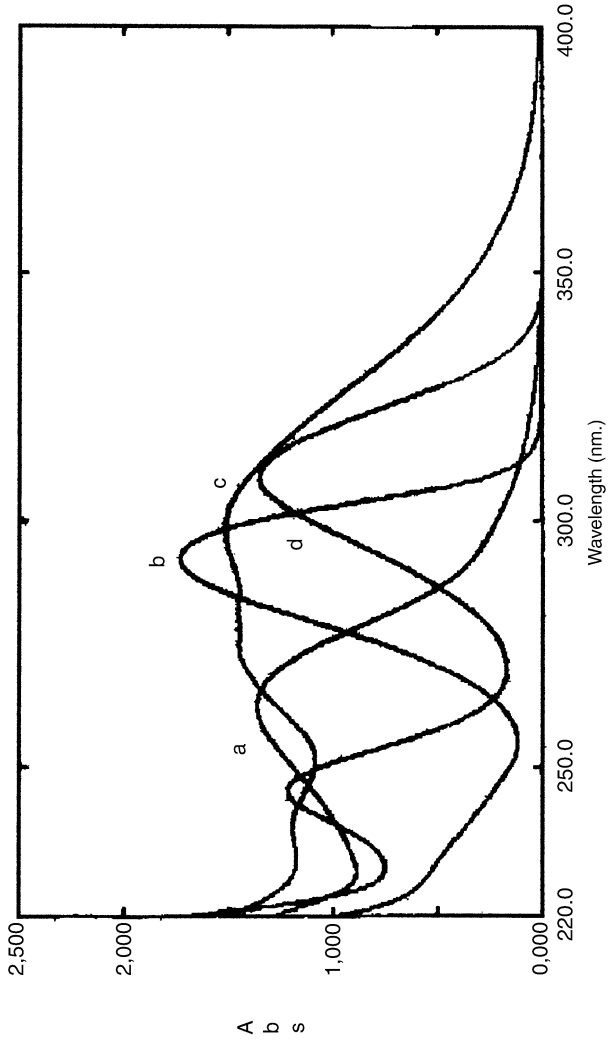

Figure 1. Zero-order absorption spectra of a) 30.0 μg ml^{-1} of isoniazid b) 16.0 μg ml^{-1} of pyridoxine hydrochloride in methanol and, c) 30.0 μg ml^{-1} of isoniazid, d) 16.0 μg ml^{-1} of pyridoxine hydrochloride in 0.1 N NaOH.

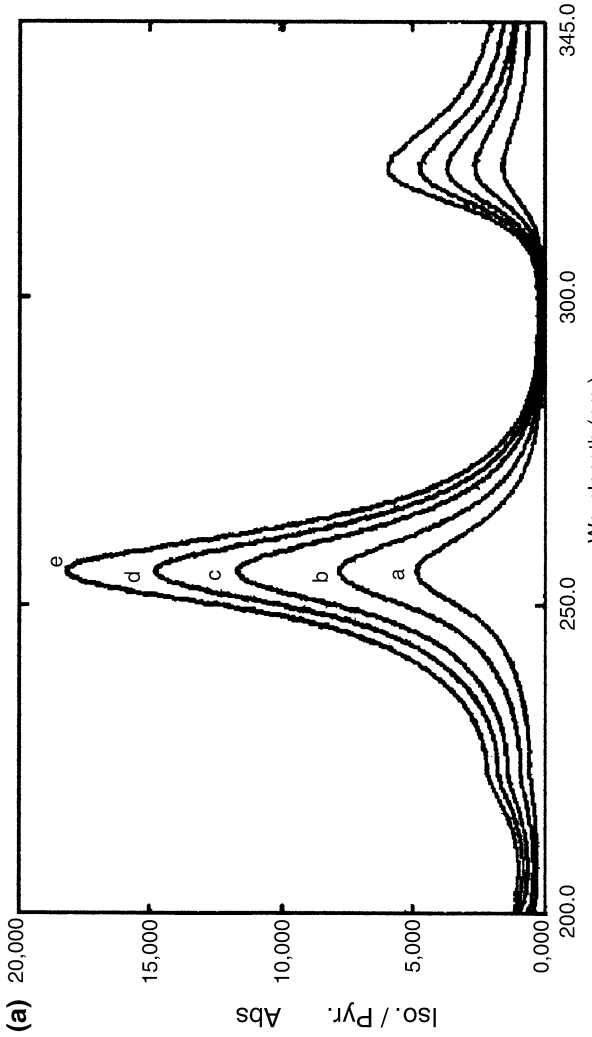

Figure 2a. Ratio spectra of isoniazid of a) 10.0 µg ml^{-1}, b) 20.0 µg ml^{-1}, c) 30.0 µg ml^{-1}, d) 40.0 µg ml^{-1}, e) 50.0 µg ml^{-1}, when 16.0 µg ml^{-1} pyridoxine hydrochloride used as divisor in methanol ($\Delta\lambda$: 8 nm).

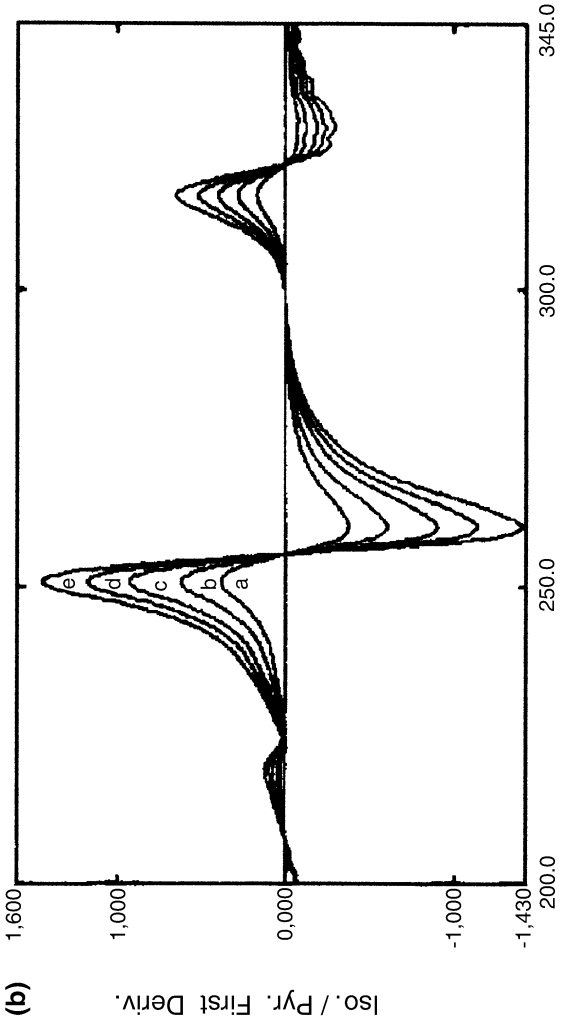

Figure 2b. First derivative of the ratio spectra of isoniazid of a) 10.0 μg ml^{-1}, b) 20.0 μg ml^{-1}, c) 30.0 μg ml^{-1}, d) 40.0 μg ml^{-1}, e) 50.0 μg ml^{-1} when μg ml^{-1} pyridoxine hydrochloride used as divisor in methanol ($\Delta\lambda$: 8 m).

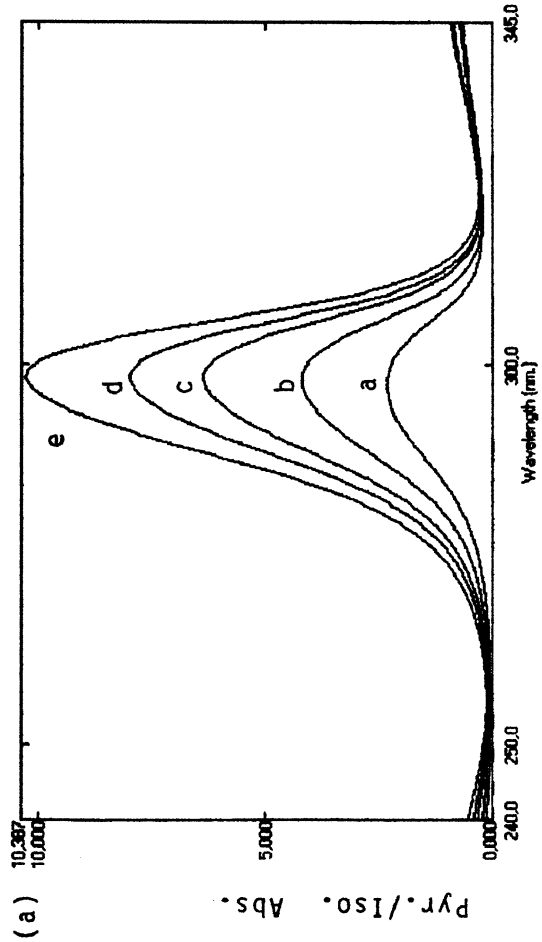

Figure 3a. Ratio spectra of pyridoxine hydrochloride of a) 6.0 g ml^{-1}, b) 12.0 g ml^{-1}, c) 18.0 g ml^{-1}, d) 24.0 g ml^{-1}, e) 30.0 g ml^{-1}, when 30.0 g ml^{-1}, isoniazid used as divisor in methanol ($\Delta\lambda$: 8 nm).

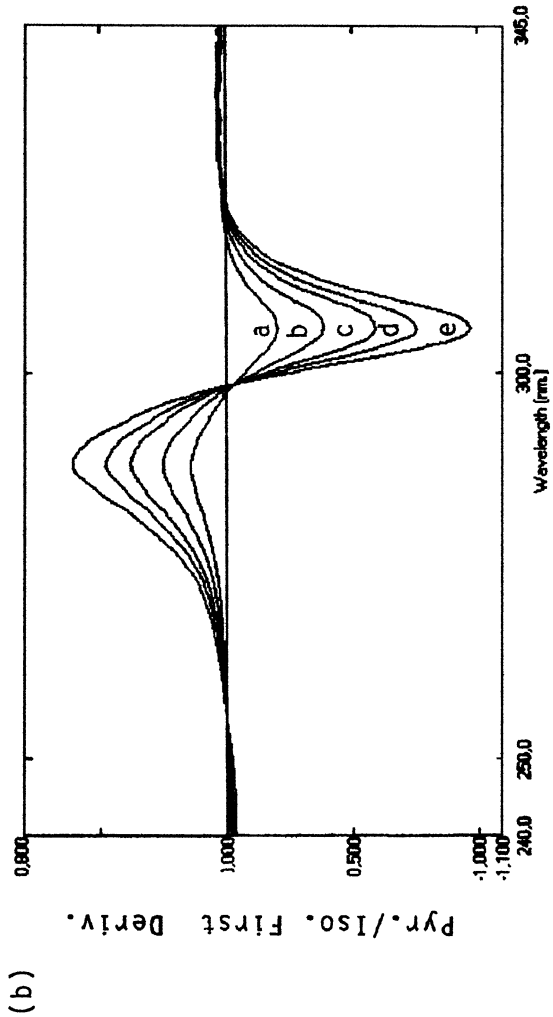

Figure 3b. First derivative of the ratio spectra of pyridoxine hydrochloride of a) 6.0 g ml^{-1}, b) 12.0 g ml^{-1}, c) 18.0 g ml^{-1}, d) 24.0 g ml^{-1}, e) 30.0 g ml^{-1}, when 30.0 g ml^{-1}, isoniazid used as divisor in methanol ($\Delta\lambda$: 8 nm).

Table 2. Assay Results for the Determination of Isoniazid and Pyridoxine Hydrochloride in Laboratory Synthetic Mixture and Commercial Tablets

	Recovery (mean ± sd) %[a]			
	Isoniazid		Pyridoxine Hydrochloride	
Sample	Ratio Derivative Spect.	Differential Derivative Spect.	Ratio Derivative Spect.	Differential Derivative Spect.
Synthetic mixtures	99.7 ± 1.79	99.0 ± 0.79	99.0 ± 0.93	98.8 ± 0.29
	t = 0.198[b]		0.319[b]	
	F = 0.927[b]		0.040[b]	
Commercial tablets	99.7 ± 1.88	99.6 ± 0.81	98.8 ± 1.66	98.8 ± 0.98
	t = 0.836		0.955	
	F = 0.576		0.9157	

[a] Mean and relative standard deviation for five determinations; percentage recovery from the label claim amount.
[b] Values in parentheses are the theoretical values at p = 0.95. Theoretical values at % 95 confidence limits F = 5.19; t = 2.23.
[c] ISOVIT® tablet are the product of Deva Pharm. Ind., Turkey; each one tablet was labeled to contain 100.0 and 25.0 mg of isoniazid and pyridoxine hydrochloride, respectively.

selected methods were successfully applied to the determination of these drugs in laboratory-prepared mixtures and commercial tablets. The results are summarized in Table 2.

Differential Derivative Spectrophotometry

Figure 1 shows the absorption (zero-order) UV spectra of isoniazid and pyridoxine hydrochloride in 0.1 N NaOH and in methanol. The difference absorption spectrum of two drugs and a mixture of isoniazid and pyridoxine hydrochloride showed in Fig. 4a. The first derivative differential curves of both the drugs (Fig. 4b) offered an advantage for their simultaneous determination by having zero crossing points. The ΔD_1 the analytical signals at 304.3 nm (zero-crossing of isoniazid) and at nm (zero- crossing of pyridoxine hydrochloride) were chosen for the simultaneous determination of isoniazid and pyridoxine hydrochloride, respectively, in a binary mixture. The calibration graphs prepared by plotting ΔD_1 signals isoniazid and pyridoxine hydrochloride versus concentrations, respectively, all gave significant linearity with negligible intercepts, confirming the mutual independence of zero-order absorptions signals of the two compounds. In the Table 1, the statistical parameters are given, the regression equations calculated from the calibration graphs. The correlation coefficients were 0.9990 and 0.9995 indicating good linearity. Five replicate determinations at different concentration levels were carried out to test the precision of the methods. The relative standard deviations were found to be less than 1.81%, indicating reasonable repeatability of the proposed method. The detection limits (LOD) were $0.036\,\mu g\,ml^{-1}$ for isoniazid and $0.056\,\mu g\,ml^{-1}$ for pyridoxine hydrochloride; while the quantification limits (LOQ) were estimated $0.78\,\mu g\,ml^{-1}$ for isoniazid and $0.216\,\mu g\,ml^{-1}$ for pyridoxine hydrochloride from the calibration data at the $p = 0.05$ level of significance for $n = 5$ standard specimens (Table 2).

In order to demonstrate the validity and applicability of the proposed methods (ratio derivative spectrophotometry and differential derivative spectrophotometry), recovery studies were performed by analyzing synthetic mixtures that reproduced the composition of the commercial tablets. The results obtained (Table 2) were statistically compared using Student t- and the F-test. As shown from the Table, the calculated t and F-values were less than the theoretical values, indicating no significant difference between the two methods. Commercially available tablets containing a mixture of isoniazid and pyridoxine hydrochloride were analyzed using the developed methods. The results are summarized in Table 2.

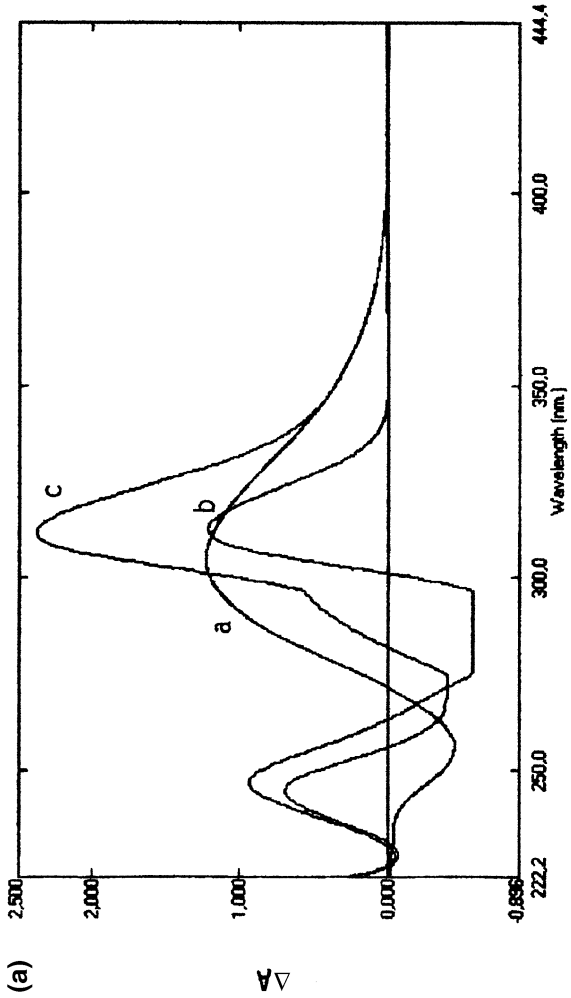

Figure 4a. Differential spectra of a) 30.0 μg ml^{-1} of isoniazid, b) 16.0 μg ml^{-1} of pyridoxine hydrochloride, and c) mixture of (30.0 μg ml^{-1} of isoniazid and 16.0 μg ml^{-1} of pyridoxine hydrochloride) in methanol versus 0.1 N NaOH.

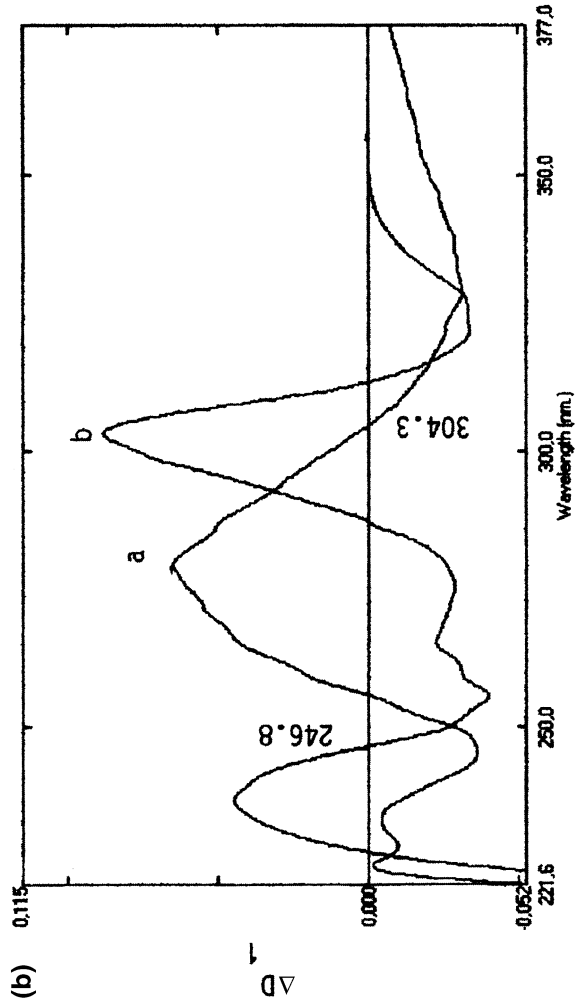

Figure 4b. Differential derivative spectra of a) 30.0 μg ml^{-1} isoniazid, b) 16.0 μg ml^{-1} pyridoxine hydrochloride in methanol versus 0.1 N N NaOH.

CONCLUSIONS

The proposed methods permits rapid, precise, and accurate determination of isoniazid-pyridoxine hydrochloride mixtures in pharmaceutical dosage form without a separation step. The short analysis time and low costs are the main advantages of these methods for routine analysis in quality control.

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