Simultaneous determination of tryptophan and tyrosine in binary mixture by zero-crossing second derivative spectrophotometry.

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Abstract : Rapid and accurate binary mixture resolution of tryptophan and tyrosine was performed. Differential-derivative spectrophotometry with a zero-crossing measurement technique was used for the quantitative determination of tryptophan and tyrosine in laboratory-prepared mixtures. Neither sample pretreatment nor separation were required. Linear calibration graphs of differential second derivative values were (at 222.4 and 217.9 nm for tryptophan and tyrosine , respectively) versus concentration in the ranges 0.1–20.0 and 1.0– 50.0 µg ml_1, and the linearity was satisfactory (r = 0.9987 and r = 0.9997), for tryptophan and tyrosine , respectively) were obtained .The relative standard deviations were found to be less than 1.03%, indicating reasonable repeatibility of method.The results obtained by the proposed method has been statistically compared by means of Student t-test and by the variance ratio F-test where shows a good agreement .

Introduction

Tryptophan [1] is one of the 20 standard amino acids, as well as an essential amino acid in the human diet. $C_{11}H_{12}N_2O_2$, [2-Amino-3-(3-indolyl)-propanoic acid] formed from proteins during digestion by the action of proteolytic enzymes. It is a heterocyclic compound that is found in small amounts in most proteins. It plays an important role in the growth and development of infants and in the biosynthesis of serotonin and niacin. Tyrosine [1] is a non-essential amino acid that is synthesized in the body from phenylalanine. $C_9H_{11}NO_3$ is [2-Amino-3-(4-hydroxyphenyl)-propanoic acid]. As a building block for several important brain chemicals, tyrosine is needed to make epinephrine, norepinephrine, serotonin, and dopamine, all of which work to regulate mood. It is one of the 20 amino acids that are used by cells to synthesize proteins. It is found in large quantities in casein[2].

A survey of the literature revealed that the analysis of tryptophan and tyrosine either in single or multicomponent mixtures has been reported through high performance liquid chromatography [3–6], voltammetry [7], gas chromatography [8], liquid chromatography [9], thin-layer chromatography [10,11], electrochemical [12,13] and differential-derivative [14–17] methods

Derivative spectrophotometry [18,19] is a spectral technique in which a slope of the spectrum i. e the rate of change of absorbance with wavelength is measured as a function of wavelength, an alternative approach to metal analysis, while at the same time showing good sensitivity and specificity [20]. In the derivative spectrum the ability to detect and to measure minor spectral features is considerably enhanced. Thus first derivative is a plot of spectral slope against wavelength. The second derivative spectrum is the derivative of the first derivative spectrum. In principle both peak heights and peak amplitudes measurements are proportional to analyte concentration. Second derivative used has an additional advantage that in its case considerable reduced bandwidth (depending on band shape), lead to improved resolution of overlapping bands with increased sensitivity and offers the possibility of separating two absorption bands which in fact may merge in zero order spectra. Therefore, we have applied derivative spectrophotometry for the simultaneous determination of tryptophan

and tyrosine. The application of derivative spectrophotometry offers a powerful tool for both qualitative and quantitative analysis of mixtures in pharmaceutical analysis [21–24] and biomedical analysis [25]. The aim of this work was to demonstrate the capability of the second derivative (2D) method to resolve and overcome the problem of overlapping spectral bands and allows the simultaneous determination of tryptophan and tyrosine without the need for prior separation.

Experimental

Equipment and reagents:All spectral measurements and treatment of data were carried out in 1-cm quartz cells using a Jenway 6405 double beam spectrophotometer with a fixed slit width (2 nm) connected to an IBM PC computer. The wavelength range of 200.0–300.0 nm was selected for normal spectra and 200.0–284.0 nm for differential-derivative spectra, and ordinate maximum and minimum were adjusted to the magnitude of derivative values.

All experiments were performed with analytical grade chemicals and solvents.

Procedures: A stock solutions of tryptophan and tyrosine (1 g/L) were prepared by dissolving 50 mg from each in 50 ml of distilled water ,separately. Five ml of the above solutions were diluted separately to 50 ml with distilled water to produce 100 µg.ml^{-1} each of tryptophan and tyrosine in distilled water. Appropriate volume aliquots of the stock solution were transferred to 25-ml calibrated flasks in duplicate. Accurate volumes were transferred into two sets of 25-ml calibrated flasks. The first series contained a constant concentration of tryptophan (10.0 µg.ml^{-1}) and a varying concentration of tyrosine (1.0–50.0 µg.ml^{-1}). The second contained a constant concentration of tyrosine (30.0 µg ml^{-1}) and a varying concentration of tryptophan (0.1–20.0 µg.ml^{-1}). Calibration solutions (n = 5) were used to construct the calibration curves in the standardization of cited method.

The second-order derivative spectra (2D) of tryptophan and tyrosine were scanned in the range of 200.0–284.0 nm against distilled water as blank. The values of the 2D amplitudes at 217.9 nm (zero-crossing of tryptophan) were measured for the determination of tyrosine in presence of tryptophan. The 2D amplitudes values, at 222.4 nm (zero-crossing for tyrosine) were used for the determination of tryptophan in presence of tyrosine. Preliminary observations revealed that the best result were obtained from the second derivative with wavelength interval $\Delta\lambda$= 8 nm.

Results and discussion

Fig. 1 shows the zero order absorption spectra of tryptophan (10 µg.ml^{-1}) , tyrosine (40 µg.ml^{-1}) and mixture of (10 µg.ml^{-1}) tryptophan and (40 µg.ml^{-1}) tyrosine in distilled water. The spectra clearly display considerable overlap, hence the traditional Vierodet's method and its modification for assaying binary mixtures seems to be impossible. The second order derivative spectra (2D) present spectra features which can be used for the simultaneous determination of tryptophan and tyrosine in binary mixture (Fig. 2).

The second derivative differential spectra of both amino acids (Fig 2) offered an advantage for their simultaneous determination by having zero crossing points. In particular absorbance at 222.4 nm for tryptophan and at 217.9 nm for tyrosine were considered as the optimum working wavelengths for their determination. The second differential derivative spectrum (Fig.2) of tryptophan shows a well defined maximum at 222.4 nm while tyrosine has a zero (2D) value at the same wavelength. Tyrosine has a 2D value at 217.9 nm at which tryptophan exhibits no contribution. The measurement of the absolute value of the total derivative spectrum taken at the above mentioned wavelengths afforded the best linear response to analyte concentrations. Least-squares regression analysis was carried out on the slope, the intercept and correlation coefficient (r) values are compiled in (Table 1). The relative standard deviation calculated for separate determinations of each amino acid was 1.02–0.74% and the relative error was 1.05-0.20% , indicating good precision and reproducibility (Table 2).

To prove the validity and applicability of the proposed method, five synthetic mixtures in the concentration range stated in Table 3 were assayed. The results obtained using the above method were precise and accurate. The selected method was successfully applied to the determination of these amino acids in laboratory-prepared mixtures. The results are summarized in Table 3. The results obtained show the high reliability and reproducibility of the method. The proposed methods were statistically compared with those of Student's (t)-test and variance ratio (F)-test (Table 3). The calculated (experimental) t- and F-values did not exceed the tabulated (theoretical) values in either test, indicating that there was no significant difference with respect to accuracy and precision for the proposed method. The selected method was successfully applied to the

determination of these amino acids in laboratory-prepared mixtures.

Conclusions

The derivative (2D) procedure was shown to be reproducible and sensitive in the analysis of tryptophan and tyrosine in simple binary mixture. In addition to the advantage of the used derivative procedure as there is no need for solvent extraction, the analytical results confirm that the derivative spectrophotometry offers accuracy and precision with the added advantages of the low cost, speed and simplicity. Therefore, the proposed derivative procedure is likely to be very suitable for the analysis of tryptophan and tyrosine.

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Table 1: Assay parameters for differential-derivative spectrophotometry determination of tryptophan and tyrosine in binary mixture.

Parameters	Tryptophan	Tyrosine
Range (μg.ml^{-1})	0.1−20.0	1.0−50.0
Regression equation (Y)[a]	-----	-----
Slope (b)	0.068	0.026
Intercept (a)	0.014	0.008
Correlation coefficient (r)	0.9987	0.9997

[a] $Y=a+bC$ where C is concentration in μg ml^{-1} and Y is absorbance units.

Table 2 :Precision and accuracy for the determination of tryptophan and tyrosine by the differential-derivative spectrophotometry method.

Parameters	Tryptophan	Tyrosine
Added (μg.ml^{-1})	10.0	40.0
Found (μg.ml^{-1}) Mean $\pm$ S.D.	9.896$\pm$0.10	39.920$\pm$0.29
RSD%[a]	1.02	0.74
E_r(%)[b]	1.05	0.20

[a] Relative Standard Deviation.

[b] Percentage relative error.

Table 3 :Statistical parameters for assay of tryptophan and tyrosine in synthetic mixture by the differential-derivative spectrophotometry method.

Statistical parameters	Tryptophan	Tyrosine
$t_{experimental}$	2.30	0.61
$t_{theoretical}$[a]	2.78	2.78
$F_{experimental}$	3.12	3.07
$F_{theoretical}$[b]	6.39	6.39

[a] Theoretical value of t at the 95% level of confidence.

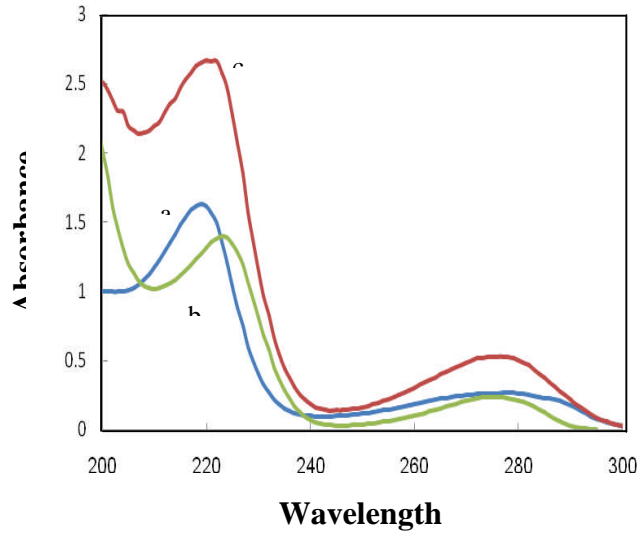[b] Theoretical value of F at the 95% level of confidence.

Fig. 1. Absorption (zero-order) UV spectra of (a) 10.0 µg ml⁻¹ tryptophan, (b) 40.0 µg ml⁻¹ tyrosine,(c) mixture of 10.0 µg ml⁻¹ tryptophan and 40.0 µg ml⁻¹ tyrosine, in distilled water.
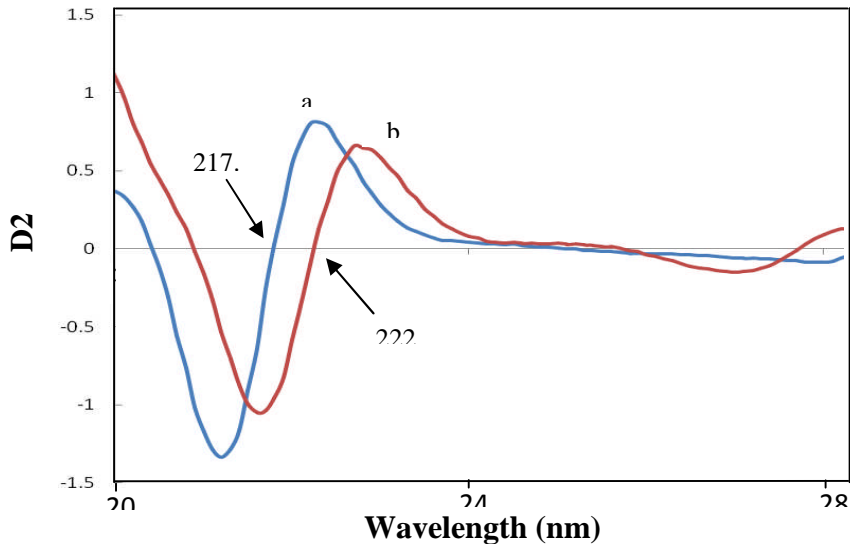
Fig. 2. Differential-derivative spectra of (a) 10.0 µg ml⁻¹ tryptophan, (b) 40.0 µg ml⁻¹ tyrosine in distilled water.

التقدير الآني للتربتوفان والتايروسين في مزيجهما باستخدام المشتقة الطيفية الثانية

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الخلاصة

تم استخدام طريقة دقيقة وسريعة لغرض فصل مركبي التربتوفان و التايروسين في المزيج الثنائي لهما . حيث تم أستخدام تقنية المشتقة الطيفية بالقياس عند نقطة تقاطع الصفر في التقدير الكمي لمركبي التربتوفان و التايروسين في المزيج المحضر مختبريا . اذ لم يلزم الأمر أي معالجة للنموذج المستخدم او أية عملية فصل مسبقة . كانت منحنيات المعايرة الخطية لقيم المشتقة الطيفية الثانية عند الأطوال الموجية   ٢٢٢٫٤ نانوميتر و ٢١٧٫٩ نانوميتر ذات مدى خطي يمتد من ٢٠٫٠-٠٫١ مايكروغرام/مللتر ومن ٥٠٫٠-١٫٠ مايكروغرام/مللتر ومعامل الأرتباط يساوي ٠٫٩٩٨٧ و ٠٫٩٩٩٧ للتربتوفان و التايروسين على التوالي. كما وجد أن الأنحرافات القياسية النسبية أقل من ١٫٠٣% مما يشير الى قابلية معقولة على التكرار في هذه الطريقة. النتائج المستحصلة باستخدام هذه الطريقة تم مقارنتها احصــــائيا بواســـطة اختبـــار الطالـــب(t) ونســبة التبـــاين (f) حيـــث   تبـــين موافقـــة جيـــدة.