SIMULTANEOUS DETERMINATION OF ANTHOCYANOSIDE AND BETA-CAROTENE BY THIRD-DERIVATIVE ULTRAVIOLET SPECTROPHOTOMETRY

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ABSTRACT

Derivative spectrophotometry offers a useful approach for the analysis of drugs in multi-component mixtures. In this study a third-derivative spectrophotometry method was used for simultaneous determination of anthocyanoside and beta-carotene using the zero-crossing technique. The measurements were carried out at wavelengths of 625 and 540 nm for anthocyanoside and beta-carotene respectively. The method was found to be linear (r^2>0.999) in the range of 125-750 μg/mL for anthocyanoside in the presence of 25 μg/mL beta-carotene at 625 nm. The same linear correlation was also obtained (r^2>0.997) in the range of 6.25-37.50 μg/mL for beta-carotene in the presence of 500 μg/mL of anthocyanoside at 540 nm. The limit of determination was 125 and 6.25 μg/mL for anthocyanoside and beta-carotene respectively. The method was successfully applied for simultaneous determination of anthocyanoside and beta-carotene in pharmaceutical preparations without any interferences from excipients.

Keywords: Anthocyanoside, Beta-carotene, Third derivative spectrophotometry, Simultaneous

INTRODUCTION

Anthocyanosides of *Vaccinium myrtillus* L. (a well known medicinal plant) has been marketed in combination with beta-carotene mostly as tablet (1). Oral administration of this preparation is commonly used for treatment of ophthalmic and vascular disorders (2). Literature survey showed that in several pharmacoepias such as BP, USP or EP no official procedure is present for simultaneous determination of anthocyanoside and beta-carotene in pharmaceutical preparations. Analysis of this combined drug usually performed through spectrophotometric procedures of each individual compound after separation from the mixture. These procedures are time-consuming and relatively complicated. Derivative spectrophotometry provides a greater selectivity than common spectrophotometry and offers a powerful approach for resolution of band overlapping in quantitative analysis of multi-component mixtures (3, 4). The presence of numerous of maxima and minima is another advantage that provides an opportunity for determination of multi-component mixtures. In the last 20 years, this technique has been rapidly gained its application in the analysis of various pharmaceutical preparations such as simultaneous determination of cephalotin and cefoxitin (5), irbesartane and hydrochlorthiazide (6), dicloxacillin and ampicillin (7), metronidazole and ciprofloxacin (8), fosinopril and hydrochlorthiazide (9), trifluoperazine hydrochloride and isopropamide iodide (10), estradiol and medroxyprogesterone acetate (11), guaiphenesin in anti-tussive preparations (12), amlodipine in the presence of its degradation products (13), stability determination of doxazosin mezylate and celecoxib (14) and also trifluoperazine hydrochloride in the presence of its degradation product (15).

The aim of this study was to develop a simple, fast and sensitive derivative spectrophotometric method for simultaneous determination of anthocyanoside and beta-carotene in pharmaceutical preparations on the basis of zero-crossing measurement. This method could be applied for determination of both drugs in the presence of each other.

MATERIALS AND METHODS

Chemicals and reagents

Anthocyanoside and beta-carotene were from Hellmann Co., Germany and were obtained as a donation by Mehr-Darou Pharmaceutical Company. All chemicals and reagents were of analytical grades and obtained from Merck

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(Darmstadt, Germany). Distilled water was used throughout the experiment wherever it was necessary.

Standard and calibration solutions

Standard stock solutions of anthocyanoside and beta-carotene were prepared by dissolving 125 and 62.5 mg of anthocyanoside and beta-carotene respectively in 100 mL methanolic HCl 1% (prepared by addition of 27 mL 37% HCl to methanol in a 1000 mL calibrated flask and adjusted to the mark with methanol). Accurate volumes were transferred into two sets of 10 mL calibrated flask. The first series contained a constant quantity of anthocyanoside (500 µg/mL) and varying concentrations of beta-carotene (6.25-37.50 µg/mL). The second series contained a constant amount of beta-carotene (25 µg/mL) with varying concentrations of anthocyanoside (125-750 µg/mL). The calibration curves for derivative spectrophotometry were constructed by plotting drug concentration versus the absorbance values of the third-derivative spectrum (D_3) at 625 nm for anthocyanoside and at 540 nm for beta-carotene and the regression equation was computed. All solutions were kept at 4°C and protected from the light.

Instruments

A Schimadzu UV-160 double beam UV-visible spectrophotometer with a fixed bandwidth (2 nm) and data processing capacity was used for all measurements. The zero-order absorption spectra were recorded over the wavelength range of 200-800 nm, against a solvent blank, in quartz cuvettes with 1 cm diameter. For all solutions, the derivative spectra were obtained over 200-800 nm range at different slit width ($\Delta\lambda$). The ordinate maximum and minimum were adjusted to the magnitude of derivative values.

Pharmaceutical tablet formulation

A commercial pharmaceutical formulation of this drug (Anthocyanoside A tablet, produced by Mehr-Darou Pharmaceutical Company, Iran, Batch No: 056) containing 100 mg of anthocyanoside and 5 mg of beta-carotene and excipients (lactose, starch, talk, sodium starch glycolate, magnesium stearate, gelatin and patent blue V) was analyzed by the proposed technique.

Spectrophotometric measurements

The difference between spectra of standard solutions of anthocyanoside and beta-carotene versus their solvent blanks were recorded in the range of 200-800 nm. The third-order derivative spectra of the standard solutions containing varying amounts of each drug and those containing mixtures of both drugs were obtained in the same range of wavelength (200-800 nm) against their blanks. The values of D_3 amplitudes for anthocyanoside in the presence of beta-carotene and vice versa were measured at 625 nm (zero-crossing of beta-carotene) and 540 nm (zero-crossing of anthocyanoside) respectively.

Accuracy and precision

To establish the reliability of the proposed method, two series of solutions containing 125, 250, 500 and 750 µg/mL of anthocyanoside plus 25 µg/mL of beta-carotene and 6.25, 18.75, 25.00 and 37.50 µg/mL of beta-carotene plus 500 µg/mL anthocyanoside were prepared respectively and analyzed as discussed above. Precision of the procedure was calculated by within-day and between-day variations. Accuracy of the method was measured as percentage of deviation between added and measured concentrations.

Analysis of tablets

Ten tablets were weighed and finely powdered. Appropriate amount of material (present in one tablet) was accurately weighed, transferred to a 100.0 ml volumetric flask, dilluted with methanolic HCl 1%, sonicated for 30 min and then adjusted to the mark with the same solvent. After centrifugation at 3000 rpm for 10 min, a clear portion of the centrifugate was used for the analysis. The concentration of anthocyanoside and beta-carotene in tablets were calculated using the corresponding calibrated curve.

RESULTS AND DISCUSSION

Derivative spectrophotometric method

Zero-order absorption spectra of anthocyanoside and beta-carotene showed certain overlapping that interfere with the direct simultaneous determination of this formulation (Fig. 1).

Development of a method for simultaneous determination of two or more compounds in a sample without previous separation is always of interest. Derivative spectrophotometry, based on a mathematical transformation of the spectral zero-order curve into the derivative spectra, allows a fast, sensitive and precise resolution of a multi-component mixture and overcomes the problem of overlapping of a multi-component system (3, 4). Derivative spectro-photometry on the basis of zero-crossing measurements involves measurement of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelength of the derivative spectra of individual components which should be only a function of the concentration of other component (11).

Table 1. Statistical data of calibration curves of anthocyanoside and beta–carotene in mixtures with different concentrations using third-derivative spectra

Parameters	**Anthocyanoside**	**Beta-carotene**
Linearity (μg/mL)	125–750	6.25–37.50
Regression equation[a]	$y = 8.28 \times 10^{-4}x + 2.38 \times 10^{-3}$	$y = 12.33 \times 10^{-3}x - 16.95 \times 10^{-3}$
SD of slope	5×10^{-6}	5×10^{-5}
RSD of slope (%)	0.60	0.41
SD of intercept	7.72×10^{-4}	3.42×10^{-4}
Correlation coefficient	0.9999	0.9979

[a]y = bx + a, where x is the concentration of drug in μg/mL and y is the amplituted at the specified wavelength.

Table 2. Accuracy and precision data of determination of anthocyanoside (125- 750 μg/mL) in the presence of beta–carotene (25 μg/mL) by third-derivative spectrophotometry

Added amount of anthocyanoside (μg/mL)	Within–day (n = 3)			Between–day (n = 9)		
	Found (μg/mL) mean ± sd	CV (%)	Error (%)	Found (μg/mL) mean ± sd	CV (%)	Error (%)
125	126.21 ± 0.72	0.57	0.97	128.15 ± 2.32	1.81	2.52
250	254.54 ± 0.72	0.28	1.82	257.04 ± 3.25	1.26	2.82
500	513.29 ± 0.72	0.14	2.66	516.35 ± 3.11	0.60	3.27
750	773.71 ± 5.05	0.65	3.16	779.82 ± 6.44	0.82	3.97

Table 3. Accuracy and precision data of determination of beta–carotene (6.25- 37.5 μg/mL) in the presence of anthocyanoside (500 μg/mL) by third derivative spectrophotometry

Added amount of beta–carotene (μg/mL)	Within–day (n = 3)			Between–day (n = 9)		
	Found (μg/mL) mean ± sd	CV (%)	Error (%)	Found (μg/mL) mean ± sd	CV (%)	Error (%)
6.25	6.39 ± 0.08	1.23	2.17	6.18 ± 0.19	3.09	-1.46
18.75	19.35 ± 0.12	0.62	3.20	19.30 ± 0.18	0.91	2.93
25.00	25.05 ± 0.14	0.54	0.19	25.61 ± 0.49	1.75	2.46
37.50	36.51 ± 0.24	0.66	-2.62	36.39 ± 0.36	0.98	-2.95

Table 4. Results of the analysis of commercial product containing 100 mg anthocyanoside and 5 mg

Sample	Anthocyanoside (mg)			Beta–carotene (mg)		
	Labelled	Found [a] (mean ± sd)	Error (%)	Labelled	Found [a] (mean ± sd)	Error (%)
Anthocyanoside A	100.00	101.52 ± 0.05	1.52	5.00	5.15 ± 0.06	3.00

beta–carotene per tablet by third-derivative spectrophotometry

[a] mean of ten determinations ± sd

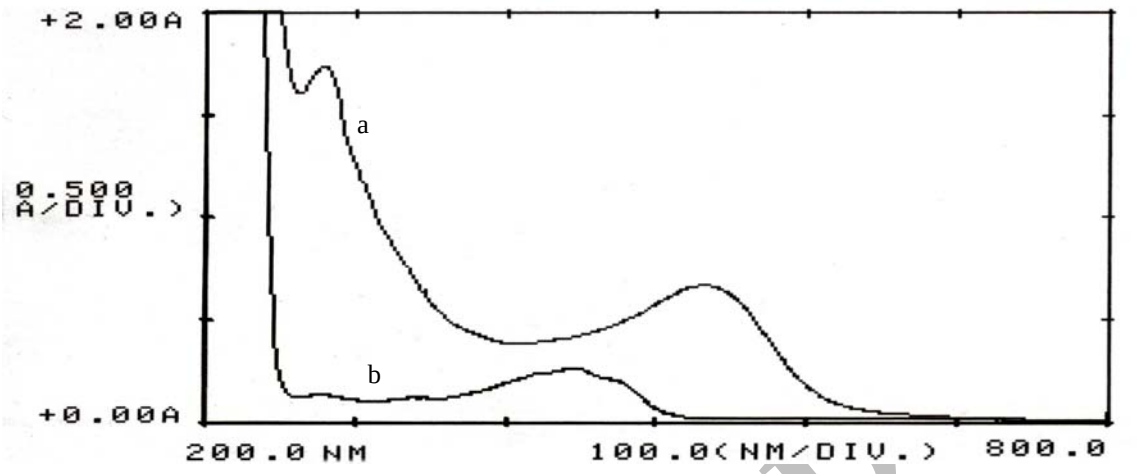

Figure 1. Zero-order spectra of (a) anthocyanoside (500 µg/ mL) and (b) beta-carotene (25 µg/ mL)

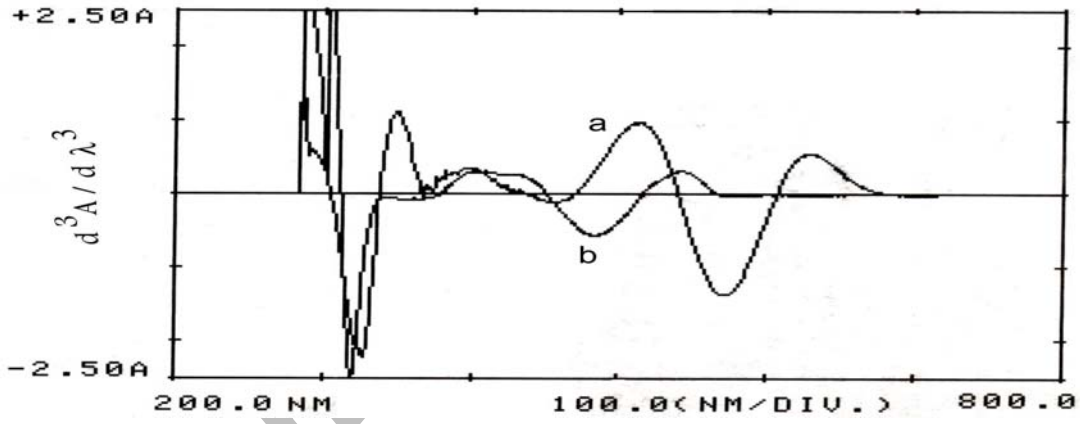

Figure 2. Third-derivative spectra of (a) anthocyanoside (500 µg/mL) and (b) beta-carotene (25 µg/mL)

The spectrophotometric parameters including derivative order, wavelength and Δλ values should be optimized to obtain maximum resolution, sensitivity and reproducibility (13, 15). In this study third-derivative technique (D_3) traced with Δλ=4.9 nm (n=7) was used to resolve the spectral overlapping. Zero-crossing points of antho-cyanoside and beta-carotene in the range of 200-800 nm is presented in figure 2. The optimum D_3 values without interference for anthocyanoside and beta-carotene were at 625 and 540 nm respectively (Fig. 2).

Calibration curves and statistical analysis

The linearity of the method was established from third-derivative spectra by measurement of the absorbance of standard solutions (repeated four times) containing varying concentrations of each compound in the presence of constant concentration of the other one. The calibration curves were constructed by plotting the D_3 value against anthocyanoside or beta-carotene concentration at the zero-crossing wavelength of beta-carotene (625 nm) or anthocyanoside (540 nm) respectively. The obtained results are summarized in Table 1. The linearity of the calibration curves and the adherence of the method to Beer's law are validated by the high value of the correlation coefficient and the value of intercept on ordinate which is close to zero.

Sensitivity

The limit of quantification that can be determined with CV< 5% was found to be 125 µg/mL and 6.25 µg/mL for anthocyanoside and beta-carotene respectively.

The limit of detection that can be reliably detected with a S/N ratio of 3 was found to be 10 μg/mL and 3 μg/mL for anthocyanoside and beta-carotene respectively.

Accuracy and precision

The accuracy and precision were determined by using synthetic mixtures of anthocyanoside and beta-carotene in the laboratory. The mean recoveries and CVs are illustrated in Tables 2 and 3. Data of these tables showed good accuracy and precision over the entire concentration range. The within-day and between-day variations showed coefficient of variation (CV %) values less than 1.81 and 3.09 for anthocyanoside and beta-carotene respectively in all four selected concentrations. The data indicate that the proposed derivative spectrophotometric method is highly precise during one analysis and between different runs.

The percentage of recovery in each case was calculated. The results obtained from the recoveries of both drugs (Tables 2, 3) showed an excellent accuracy.

Specificity

The influence of excipients was studied by addition of different amounts of the excipients to samples containing 500 μg/mL of anthocyanoside and 25 μg/mL of beta-carotene. No interference was observed from the presence of lactose, starch, talk, sodium starch glycolate, magnesium stearate, gelatin and patent blue V in the amounts which are commonly present in tablet dosage forms.

Stability

Study of stability of anthocyanoside and beta-carotene in solutions during analysis showed that analytes were stable for at least 24 h in solutions when protected from light.

Robustness

Variation of the concentration of HCl by ± 5 % did not have any significant effect on D_3 values in spectrophotometric spectra.

Application

The proposed method was successfully applied to the analysis of a pharmaceutical dosage form containing anthocyanoside and beta-carotene. The results are summarized in Table 4. No interference from the sample matrix was observed. The results were in good agreement with the labeled content and the error of determination didn't exceed ±3%.

CONCLUSION

From the results of this study it may be concluded that the proposed third-derivative spectrophotometric method for simultaneous determination of anthocyanoside and beta-carotene is a simple, rapid, practical, reliable and inexpensive method that may be used for routine analysis. Furthermore, no preliminary separation, as well as expensive and unavailable instrument are required.

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