Determination of Patent Blue V and Carmoisine in Gelatine Desserts By Derivative Spectrophotometry

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A first-derivative spectrophotometric method was developed for quantitative determination of two synthetic organic dyes, Carmoisine and Patent Blue V, which are under governmental regulations all over the world because of their toxicity and carcinogenity. In this study, Carmoisine and Patent Blue V were simultaneously determined in their binary mixtures by first-derivative spectrophotometry. The method was applied to different gelatine desserts. The first-derivative derivative method did not eliminate turbid background in the samples. A simple background correction procedure involving a C_{18}-cartridge sample preparation step was developed for determination of dyes in gelatine dessert by first-derivative spectrophotometry. The C_{18}-cartridge sample preparation step improves recovery in determination of Carmoisine and Patent Blue V in gelatine dessert by first-spectrophotometry. Recoveries for Carmoisine and Patent Blue V in different samples were generally higher than 90%. This method also provides rapid, accurate and economical analysis of these dyes.

Introduction

The quality of food, apart from microbiological aspects, is generally based on colour, flavour, texture and nutritive value. One ofthe most important sensory quality attributes of a food is colour. This is because no matter how nutritious, flavourful or well-textured a food may be, it is unlikely to be eaten unless it has the right colour[1].

Synthetic organic food colours are substances that can be added to food in solution or dispersion. They are superior to the natural dye extracts in tinctorial power, consistency of strength, range and brilliance of shade, stability and ease of application[2,3].

In recent years, food additives in general, and colours in particular, have increasingly come under investigation for evaluation of their safety in use. At present, in many developed countries only about ten dyes are permitted for use as food colouring agents, and many others have been banned in the last two decades due to their toxicity and carcinogenity[4,5]. Carmoisine and Patent Blue V are synthetic organic azo dyes that may be present in commmon foods (sweets, drinks, ice-creams, etc.). The analytical control of these compounds is of considerable importance in the food industry.

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Many analytical methods have been developed for the qualitative and quantitative analysis of food colour. These include thin layer chromatography (TLC)[3,4,6], UV/VIS spectrometry[7,8], mass spectrophotometry[9], DC-SPE (dynamic column-solid phase extraction) system[5], capillary electrophoresis, C_{18} cartridge [10], or various combinations of these techniqus Chromatographic techniques have the disadvantage of requiring expensive equipment and expert operators. The other methods require long analysis times and are overly complex. Derivative spectrophotometry is an analytical technique of great utility for resolving mixtures with overlapping spectra. The fundamental principles of derivative spectrophotometry have been described by Talsky et al.[11] and Owen[12].

The aim of this study was to show the ease of derivative spectrophotometry methods in circumventing the problem of overlapping spectral bands, allowing the simultaneous determination of the binary mixtures of Patent Blue V and Carmoisine without prior separation. The method was applied to different gelatine desserts. The first derivative method was not successful in eliminating turbid background in the samples. A simple background correction procedure involving a C_{18} cartridge sample preparation step was developed for determination of dyes in gelatine dessert with first derivative spectrophotometric method. The method yielded accurate results in different gelatine desserts.

Experimental

Apparatus and Chemicals

In this study, a Shimadzu 160 A Spectrophotometer (Japan) was used for all adsorbance measurements. The spectral scan rate was 0.5 nm/min. The derivative spectra were automatically obtanied from the spectrophotometer. The optimum value of $\Delta\lambda$ should be determined by taking into account the noise level, the resolution of the spectrum and the sample concentration. Some values of $\Delta\lambda$ were tested. The optimum $\Delta\lambda$ value was found as 20 nm and 17.5 nm for all the first- and second-derivative absorption spectra, respectively. The C_{18} carridge was obtained from Sep-Pak Cartridge (Millipore).

Patent Blue V (P) and Carmoisine (C) aqueous solutions were prepared from Sigma Chemical Co. P and C solution stocks at a concentration of 1000 μg/mL and the working solutions had a concentration of 50 μg/mL

Standards and Sample Preparation

Standard solutions for C and P of 5.0, 10.0, 15.0 and 20 μg/mL were prepared from working solutions. According to dye contents, 15-40 g of the sample were dissolved in distilled water and the solution was transferred into a 50 mL volumetric flask and diluted to a volume of 50 mL with distilled water.

Procedure

The absorption spectra of sample solutions were recorded between 350 and 750 nm. First, suitable derivative orders with appropriate $\Delta\lambda$ and wavelength, at which each dye can be analysed in the presence of the other, were determined. Their concentrations were then found by measurement of the signal and use of an appropriate calibration graph at the selected derivative order and wavelength. These calibrations were prepared by varying the concentration of one colourant in the absence of the other.

For quantifying the recovery in gelatine dessert samples, two sample preparation steps were carried out. Different volumes of sample solution were transferred into 25 mL calibrated flasks, spiked with different

amounts of C and P and then diluted to a volume of 25 mL. In direct determination by derivative method, the C and P contents were directly determined from the first-derivative spectra by measurement of the peak at their wavelengths, and total amounts of colourant were calculated from calibration graph. The recoveries were calculated by dividing the total dye amount found by the theoretical total amount (present + added).

For the C_{18} cartridge with the first derivative method, 10 mL of solution (sample solution spiked with different amounts of C and P) was percolated through the C_{18} cartridge at a flow rate of about 0.1 mL/s. The cartridge was then washed with 10 mL of 50% isopropanol solution (eluted solution). The recovery was calculated as mentioned in the procedure.

Quantitation was carried out using calibration graphs obtained from the standard solutions which were treated according to the above analytical procedure.

Results and Discussion

Determination of the C and P in the Synthetic Binary Mixtures

Figure 1a shows the zeroth order absorption spectra of C and P in the range of 350-750 nm. C and P can be approximately determinated by direct absorbance measurement at 515 and 640 nm, respectively. However, when either component is in excess, the other can not be determined accurately because of the overlapped spectra. The results are summarised in Table 1. The recovery values were found as 103.8% to 117.6% for C, and 95.2% to 124.0% for P. Therefore, derivative spectra were used for the satisfactory resolution of this problem.

Table 1. Determination of the recovery for Carmoisine and Patent Blue V in synthetic mixture by direct absorbance measurement.

		Recovery, %	
Theoretical, μg/mL		Carmoisine	Patent Blue V
Carmoisine	Patent Blue V	515 nm	640 nm
5	5	103.8	95.2
5	10	112.6	97.9
10	20	117.6	102.1
15	5	104.2	106.0
20	5	106.5	124.0
Average		**108.9∓5.4**	**105.0∓10.2**

The first-derivative spectra of C and P solutions are shown in Figure 1b. It is clear that C can be determined in the presence of P at 465 nm, and also that P can be determin in the presence of T at 655 nm.

The second derivative spectra of C and P solutions are shown in Figure 1c. C was determinated in the presence of P at 460 nm, and P also was determined in the presence of C at 640 nm by second derivative method.

Preliminary experiments showed that the signal of the first derivative at 465 nm and that of the second derivativee at 460 nm (working zero-crossing wavelengths of P) were proportional to the C concentration, whereas the first derivative signal at 655 nm (working zero-crossing wavelengths of C) was proportional to the P concentration. C and P were determined simultaneously in synthetic mixtures by measurement of the signals at the selected wavelength and in the chosen derivative order. The calibration graphs were obtained

by the range of concentrations of C and P (Figure 1 b and 1 c). The regression equations and the correlation coefficients obtained from the calibration graphs are given in Table 2.
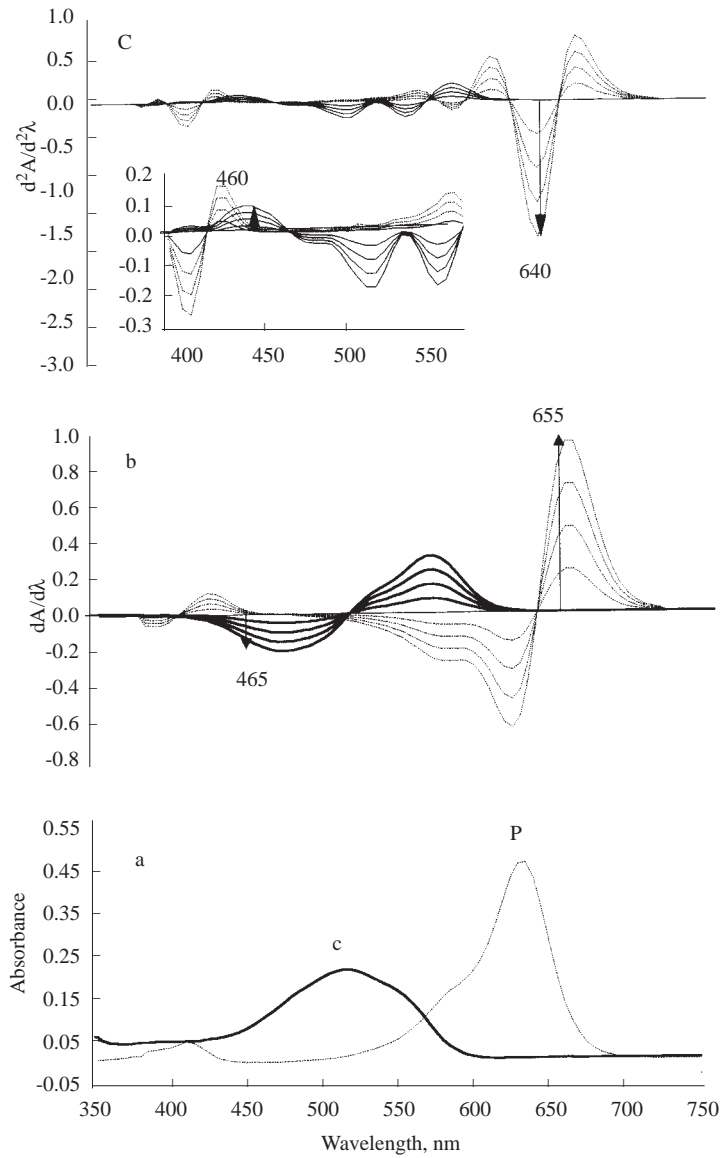

Figure 1. **a)** Fundamantal absorption spectrum of 5 μg/mL of Carmoisine (-) and 5 μg/mL of Patent Blue V ($-----$). **b)** First derivative spectra of 5-20 μg/mL of Carmoisine (-) and 5-20 μg/mL of Patent Blue V ($----------$). **c)** Second derivative spectra of 5-20 μg/mL of Carmoisine (-) and 5-20 μg/mL of Patent Blue V ($----------$).

Table 2. Statistical data for calibration graphs

Regression equation	Correlation coef. r^2(n=5)
$^1D_{465}=0.0095\ C_C+0.001$	0.9998
$^2D_{460}=0.0028\ C_C-0.004$	0.9998
$^1D_{655}=0.0482\ C_P+0.002$	0.9997
$^2D_{640}=0.0784\ C_P+0.008$	0.9994

$^{orders\,derivative}D_{wavelength\,measured}$

C_C : micrograms per milliliter of Carmoisine

C_P : micrograms per milliliter of Patent Blue V

     The results from the simultaneous determination derivative spectrophotometric method of C and P in synthetic binary mixtures are given in Table 3. The recoveries for the first-derivative method determination of C and P were nearly 100%. However, while the recoveries for the second-derivative method determination of P were nearly 100%, the recoveries for C were significantly higher than 100%, and therefore this method was not suitable for C. Possible reasons for such high recovery values can be explained by the overlapping second spectra of P (Figure 1 c) and low sensitivity, which can be up to 3.4 times lower than that of the first derivative data (Figure 2).

     These results indicated that first-derivative spectrophotometry is suitable for determination of C and P in different synthetic binary mixtures.
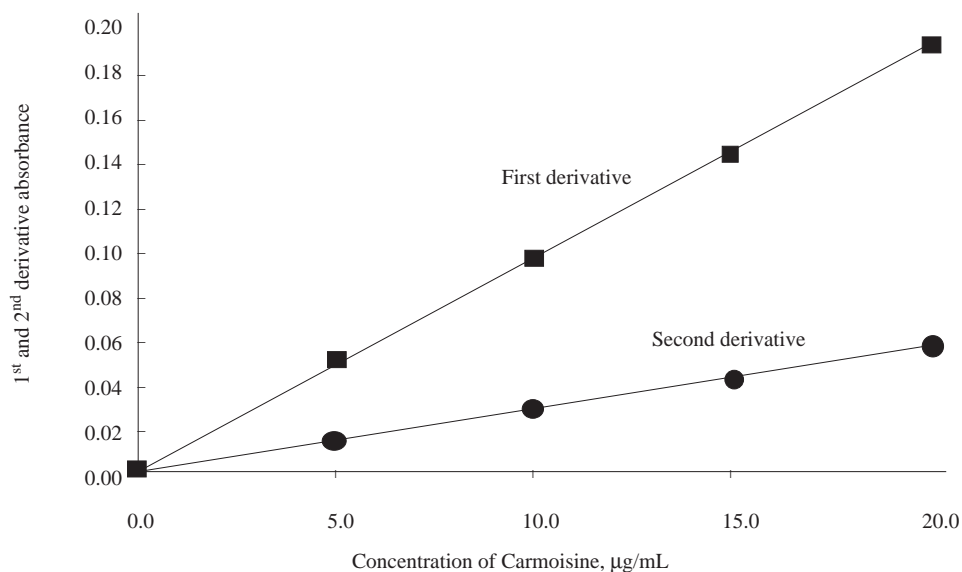

Figure 2. Calibration graphs for Carmoisine by first and second derivative

Table 3. Determination of the recovery for Carmoisine and Patent Blue V in synthetic mixture by first and second order derivative method

		Recoveries, %			
Theoretical, μg/mL		Carmoisine		Patent Blue V	
Carmoisine	Patent Blue V	^{1}D$_{465}$	^{2}D$_{460}$	^{1}D$_{655}$	^{2}D$_{640}$
5	5	99.2	119.6	95.8	96.0
5	10	103.4	112.6	98.3	98.7
10	20	107.6	128.0	102.5	101.5
15	5	102.0	107.3	106.0	106.0
20	5	105.0	107.5	106.0	104.0
	Average	**103.4∓2.8**	**115.0∓7.9**	**101.7∓4.1**	**101.2∓3.6**

Application

The zeroth- and first-order derivative spectra of the mixture (C and P), the gelatine dessert sample solution and he eluted solution are shown in Figures 3 and 4. Figure 3 shows the turbid background of a sample. The turbid background of the sample was not eliminated by the first-derivative spectrum (Figure 4). Thus, if C_{18} cartridges are used, the turbid background is eliminated. It can be seen that the zeroth- and first-order spectra of the mixture and eluted sample solution appeared to be similar to each other (Figure 3 b, c and 4 b, c).

The utility of the direct first and C_{18} cartridge with first derivative method was tested in four different types of gelatine dessert samples spiked with varying amounts of C and P.

The results of the determination of C and P in gelatine dessert samples at the selected wavelengths are shown in Table 4.

The recoveries for C and P determination by direct absorbance measurements were 923.80% and 444.70%, respectively. These high recovery values were due to the turbid background of the sample, which does not contain any food colorants. However, when the first-derivative method was used for determination of C and P in the same sample, the respective values for the recoveries were 76.0 to 78.2% and 82.0 to 87.40%. This shows that the turbid background was not completely eliminated by the first-derivative method. The decrease iun the recovery may be attributed to the first-derivative spectrum of sample 1 (see Figure 5) and the zero point in the first-derivative spectrum are shifted to longer wavelengths (Figure 4 a). On the other hand, when the C_{18} cartridge with first-derivative method was used for determination of C and P in the same sample, the recoveries were in the range of 95.30 to 101.20% and 100.10 to 106.40%, respectively, indicating that the C_{18} cartridge was effective for removing the turbidity. When the synthetic mixture of C and P were treated with C_{18} cartridge by the first-derivative method, the recoveries were 101.60 and 100.60, respectively, with smaller standard deviations.

As indicated again in Table 4, the direct first-derivative method was also used for the determination of C and P in three gelatine desserts, and the recovery values were in the range of 67.70 to 92.20% for C and 69.20 to 86.60% for P. However, the recoveries obtained by C_{18} cartridge by the first-derivative method in all cases were satisfactory in general, being higher than 91% (except in sample 4). These results indicate that the C_{18} cartridge with the first-derivative method is adequate for simultaneous determination of C and P in gelatine desserts.

In conclusion, it may be stated that the proposed C_{18} cartridge sample preparation step improves recovery in determination of C and P in gelatine dessert by first-derivative spectrophometry. This method

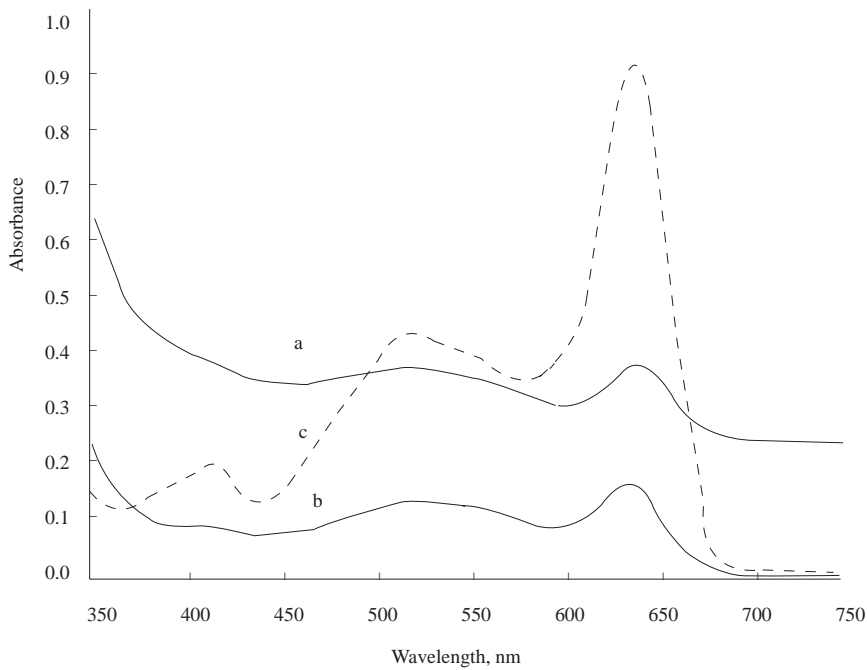also provides, rapid, accurate and economical analysis of these dyes.

Figure 3. Fundamantal Absorption, (a) 25.4 g/100 mL of sample b) synthetic mixture of 10 μg/mL of Carmoisine and Patent Blue V (c) eluted solution
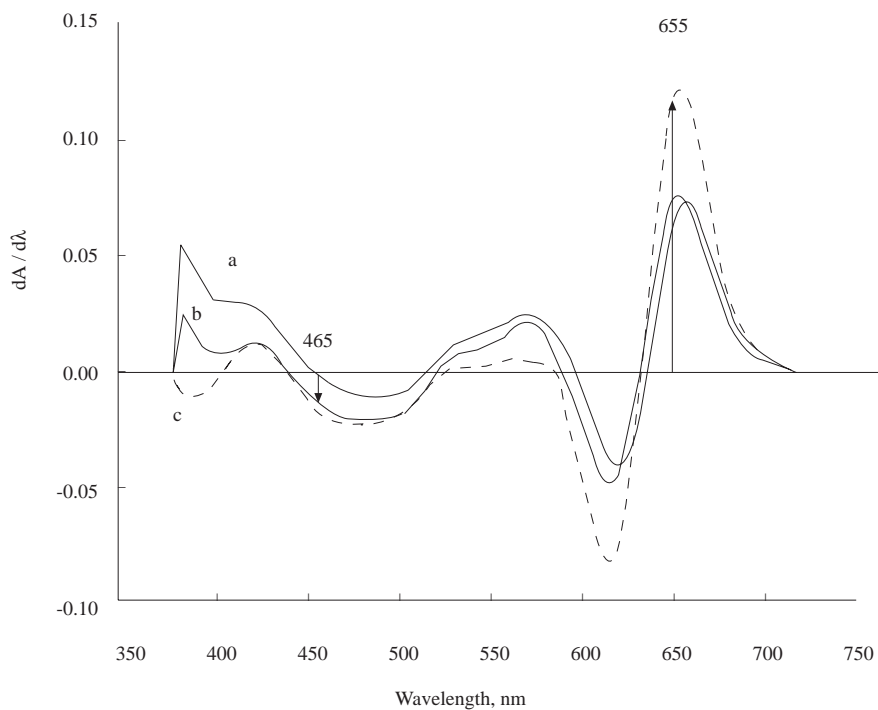

Figure 4. First derivative spectra (a) 25.4 g/100 mL of sample b) synthetic mixture of 10 μg/mL of Carmoisine and Patent Blue V (c) eluted solution

Table 4. First derivative determination of recovery for Carmoisine and Patent Blue V in gelatine dessert sample solutions

Sample		Carmoisine, 465 nm			Patent Blue V, 640 nm			
	Present, μg/mL	Added, μg/mL	Found, μg/mL	Recovery%	Present, μg/mL	Added, μg/mL	Found, μg/mL	Recovery%
Direct-first derivative method								
Sample 1[b]	-	5.00	46.20	923.80[a]	-	5.00	22.20	444.70[a]
	-	5.00	3.91	78.20	-	5.00	4.37	87.40
	-	10.00	7.60	76.00	-	10.00	8.44	84.40
	-	20.00	15.20	76.00	-	20.0	16.43	82.20
Sample 2[c]	1.38	5.00	5.60	87.80	-			
	1.38	10.0	9.39	82.50				
	2.76	5.00	6.86	88.40				
Sample 3[d]	1.27	5.00	4.86	92.20	0.74	5.00	4.83	84.10
	1.59	3.33	4.01	81.50	0.93	3.33	3.69	86.60
Sample 4[e]	0.06	5.00	3.27	64.70	0.33	5.00	3.69	69.20
C$_{18}$-cartridge with first derivative method								
Syntetic mixture	10.00	-	10.16∓0.15	101.60∓1.50	10.00	-	10.06∓0.14	100.60∓1.42
Sample 1	-	5.00	5.06	101.20	-	5.00	5.32	106.40
	- 10.00	9.53	95.30	-	10.00	10.18	101.80	
	- 20.00	19.42	97.10	-	20.00	20.02	100.10	
Sample 2	1.53	5.00	6.12	93.70				
Sample 3	1.18	5.00	5.65	91.40	0.86	5.00	5.48	93.50
	0.78	3.33	4.24	103.10	0.57	3.33	4.03	103.30
1.77	1.00	2.59	93.50	1.27	1.00	2.14	94.30	
Sample 4	0.37	5.00	4.71	87.70	0.36	5.00	4.79	89.40

a : Direct absorbance measuring at 515 and 640 nm for Carmoisine and Patent Blue V, respectively.

b : Containing no food colorants; 18.0 g/100 mL of distilled water

c : 30.53 g/100 mL of distilled water. d: 24.86 g/100 mL of distilled water: e: 37.38 g/250 mL of distilled water
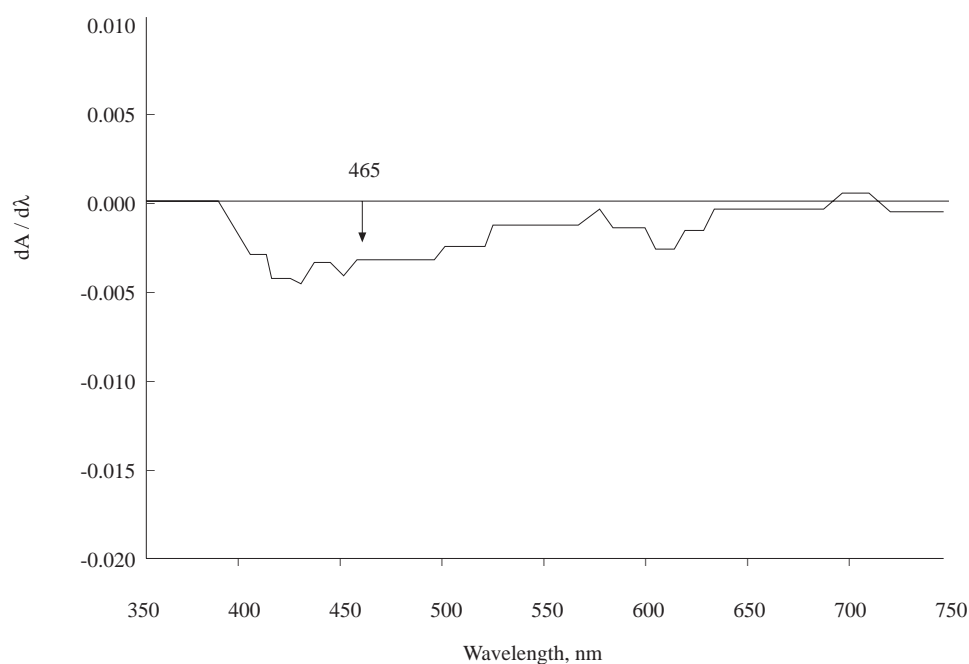
Figure 5. First derivative spectra of 18.0 g/100 mL of sample 1

Acknowledgement

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