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A comparative study between three stability indicating spectrophotometric methods for the determination of diatrizoate sodium in presence of its cytotoxic degradation product based on two-wavelength selection

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Abstract

Three sensitive, selective, and precise stability indicating spectrophotometric methods for the determination of the X-ray contrast agent, diatrizoate sodium (DTA) in the presence of its acidic degradation product (highly cytotoxic 3,5-diamino metabolite) and in pharmaceutical formulation, were developed and validated. The first method is ratio difference, the second one is the bivariate method, and the third one is the dual wavelength method. The calibration curves for the three proposed methods are linear over a concentration range of 2–24 µg/mL. The selectivity of the proposed methods was tested using laboratory prepared mixtures. The proposed methods have been

successfully applied to the analysis of DTA in pharmaceutical dosage forms without interference from other dosage form additives. The results were statistically compared with the official US pharmacopeial method. No significant difference for either accuracy or precision was observed.

Key words: Sodium diatrizoate, ratio difference, bivariate, dual wavelength, and stability indicating methods.

1-Introduction

In modern analytical laboratory, there is always a need for significant stability indicating methods of analysis [1,2]. An ideal stability indicating method quantifies a drug and resolves its degradation products [3]. The spectrophotometric methods have the advantages of being the most simple, fast and applicable in all laboratories, as most of the active compounds show absorbance in the UV region. But, usually compounds susceptible to degradation may be present with their degradation products with which they exhibit strongly overlapping spectra that impede the determination of the main compound.

Diatrizoate sodium (DTA) is one of the most widely used ionic contrast agents in many medical imaging purposes as it can enhance the image obtained thanks to its iodine content that can absorb X-rays [4-7]. Reviewing the literature in hand revealed that there are only two spectrophotometric methods

and a TLC-spectrodensitometric method for the determination of DTA in presence of its free amino degradate in spite of its high toxicity [8]. There are only one direct UV spectrophotometric method [9] and another H^1NMR method for the determination of DTA as a single component [10]. DTA was also determined in presence of its diiodo degradates by capillary electrophoresis technique [11] and liquid chromatography [12,13]. DTA is an official drug whose US pharmacopeial assay method is a precipitimetric titration that depends on the iodide content in the drug [14].

The goal of this contribution is the development, validation, and comparison between three different stability indicating spectrophotometric methods for the determination of DTA in presence of its cytotoxic degradation product. The three proposed methods, namely, ratio difference, bivariate, and dual wavelength, are based on careful selection of a pair of wavelengths. They are applicable to the quality control and routine analysis of DTA in pharmaceutical preparations.

2-Experimental

2.1. Apparatus

Spectrophotometer: SHIMADZU dual beam UV-visible spectrophotometer (Kyoto, Japan), model UV-1650 PC connected to IBM compatible and a HP1020 laserjet printer. The bundled software, UV-Probe personal spectroscopy software version 2.21 (SHIMADZU), is used. The spectral band is 2 nm and scanning speed is 2800 nm/min with 0.1 nm interval.

2.2. Reference Samples

Diatrizoate sodium reference standard was purchased from Sigma-Aldrich Co., and its purity was certified to be ≥ 99.99%.

2.3. Pharmaceutical Formulation

Gastrografin® solution manufactured by Schering Company (Belimed, Spain) batch number 51424A is labeled to contain 0.6 g/mL of diatrizoate anhydrous base.

2.4. Degraded Samples

10 mL of 2M HCl solution was added to pure DTA (500 mg) in a 250 mL glass-stoppered conical flask and refluxed for 6 hours. Complete degradation was tested by TLC using chloroform: methanol: ammonium hydroxide (20: 10: 2 by volume) as a mobile phase. Only one spot was observed not corresponding to DTA. Subsequently, 2M NaOH solution was added to the degraded solution till pH was adjusted to about 7, the solution was evaporated on small flame, and then the degradate is dissolved in methanol, filtered, and left to evaporate at room temperature. The structure of the isolated degradation product was elucidated using mass and IR spectrometry.

2.5. Materials and Reagents

All chemicals used throughout this work were of analytical grade, and distilled deionized water was used as a solvent (Adwic, Cairo, Egypt).

2.6. Standard Solutions

DTA stock standard solutions with a concentration of 0.1 mg/mL in distilled water was prepared in 100 mL volumetric flasks by dissolving 10 mg of pure DTA in distilled deionized water. Degradation product stock solution with a concentration of 0.1 mg/mL was prepared in distilled deionized water.

2.7. Procedures

2.7.1. Construction of Calibration Graphs

Aliquots (0.2–2.4 mL) of DTA stock solution (0.1 mg/mL) were transferred into a series of 10 mL volumetric flasks, and the volume was completed with distilled deionized water. The zero-order spectra were recorded using distilled deionized water as a blank.

2.7.1.1. Ratio Difference Method

The scanned spectra of the prepared solutions were divided by the spectrum of 14 μg/mL degradation product. The obtained ratio spectra were recorded. Calibration curve for DTA was constructed by plotting the difference between the peak amplitudes of the obtained ratio spectra at 243 and 253 nm

versus the corresponding concentrations in µg/mL, and the regression equation was computed.

2.7.1.2. Bivariate Method

The absorbance of pure DTA was measured at 238 and 264 nm and plotted against the corresponding concentrations, and then the regression equations were computed at the selected wavelengths.

2.7.1.3. Dual Wavelength Method

Calibration curve for DTA was constructed by plotting the difference between absorbance values at 222.5 and 241.5 nm versus the corresponding concentrations, and then the regression equation was computed.

2.7.2. Analysis of Artificial Mixtures

Laboratory prepared mixtures containing DTA and different percentages of its degradation product were prepared and analyzed using the same procedure described under (Construction of Calibration Graphs).

2.7.3. Application of the Proposed Methods to the Analysis of DTA in Pharmaceutical Preparation

Gastrografin® solution is labeled to contain 0.6 gm/mL of anhydrous DTA. A stock solution with a concentration equal to 900 µg/mL was prepared by transfer of 0.15 mL of the solution to a 100 mL volumetric flask and the volume was completed using distilled water; from this stock solution, 0.1 mL

was transferred into a 10 mL volumetric flask and the volume was completed with distilled water to get a concentration equal to 9 µg/mL. Then the procedure was completed as described under (Construction of Calibration Graphs).

3. Results and Discussion

DTA is partially deacetylated in liver to the deacetylated mutagenic and cytotoxic metabolite 3,5-diamino-2,4,6 triiodobenzoate [15]. This free amino compound can be also detected as an impurity in the final product as it is used as a starting material for the synthesis of DTA which is formed by acetylation of the 3,5-diamino-2,4,6 triiodobenzoate, so manufacturers of DTA should further reduce the level of the aromatic amine [11]. This deacetylated degradate was also obtained upon reflux of DTA with acid (Figure 1); therefore, the determination of DTA in presence of its degradate was essential.

The structure of the degradate was elucidated by mass and IR spectrometry in our previously published work [8].

The zero-order absorption spectra of DTA and its acidic degradate showed that DTA acidic degradate overlaps with that of intact DTA and hinders direct spectrophotometric measurements (Figure 2). The focus of the present work is to develop accurate, specific, reproducible, and sensitive stability indicating spectrophotometric methods for the determination of DTA in pure form or in pharmaceutical formulations in the presence of its acidic degradation product.

3.1. Ratio Difference Method

Ratio difference method was recently developed as a new simple, rapid, and selective method for the determination of components with overlapping spectra, having the advantages of minimal data processing and wide range of applications [16,17,18].

Ratio difference spectrophotometric method was applied to solve the problem of the overlapped absorption spectra of DTA and its degradate as upon dividing the spectrum of DTA by a divisor of a certain concentration of the degradate, a ratio spectrum will result, and a linear relationship between the difference in amplitudes at any two wavelengths and the corresponding concentration of DTA will result, while the ratio spectrum of the degradate will be a straight line of constant amplitude parallel to the *x*-axis and the difference in amplitudes of it at any two wavelengths will be zero [19].

The method comprises two critical steps; the first is the choice of the divisors; the selected divisors should compromise between minimal noise and maximum sensitivity [20]. Different concentrations of the degradate were tried 2, 10, 14, and 20 µg/mL and the divisor concentration of 14 µg/mL was found to be the best regarding sensitivity, repeatability, signal-to-noise ratio, and average recovery percent when used for the prediction of DTA concentration in pure powder form as well as in laboratory prepared mixtures (Figures 3 and 4). The second critical step is the choice of the wavelengths at which the measurements are recorded. Any two wavelengths can be chosen, provided that they exhibit

different amplitudes in the ratio spectrum and a good linearity is present at each wavelength individually. The wavelength pairs 220–238, 220–245, and 220–255 nm could not be used as 220 nm showed poor linearity. 243–250 and 243–253 nm were also tried and 243–253 nm showed the best results.

Linear correlation was obtained between the differences in amplitude at 243–253 nm for DTA in the range of 2–24 μg/mL, and the regression equations were computed (Table 1). The methods were checked by the analysis of laboratory prepared mixtures of DTA and its acidic degradate in different ratios as presented in Table 2. DTA could be determined in presence of up to 90% of its acidic degradate, with mean percentage recovery of 101.40±0.38%

3.2. Bivariate Method

This method is based on the simple mathematic algorithm [21], in which data are used from four linear regression equations, two calibrations for each component at two selected wavelengths using the method of Kaiser [22]. The principle of bivariate calibration is in the measurements of binary mixtures (A and B) at the two selected wavelengths (1 and 2), and then two equations are obtained:

$$A_{AB1} = m_{A1}C_A + m_{B1}C_B + e_{AB1}$$

$$A_{AB2} = m_{A2}C_A + m_{B2}C_B + e_{AB2}$$

where e_{AB1} and e_{AB2} are the sum of the intercepts of the linear calibration at two wavelengths ($e_{AB1} = e_{A1} + e_{B1}$), m_A and m_B are the slopes of the linear regression, and C_A and C_B are the concentrations of the analytes.

The resolution of such equations set allows the evaluation of C_A and C_B values:

$$C_B = m_{A\,(}A_{AB1} - e_{AB1)} + m_{A1}\,(e_{AB2} - A_{AB2}) / m_{A2}m_{B1} - m_{A1}m_{B2}$$

$$C_A = A_{AB1} - e_{AB1} - m_{B1}C_B / m_{A1}$$

This simple mathematic algorithm allows the resolution of the binary mixture by measuring the absorbance of the mixture at the two wavelengths and using the parameters of the linear regression functions evaluated individually for each component at these same wavelengths [23]. The method of Kaiser [22] was used for the selection of optimum wavelength set which assured the best sensitivity for the determination. A series of sensitivity matrices, K, was created for each binary mixture and for every pair of preselected wavelengths:

$$K = \begin{bmatrix} m_{A1} & m_{B1} \\ m_{A2} & m_{B2} \end{bmatrix}$$

where $m_{A1,2}$ and $m_{B1,2}$ are the slopes of the components A and B at the two selected wavelengths (1, 2). The determinants of these matrices were

calculated and the wavelength set selected for which the highest matrix determinant value was obtained.

Six wavelengths were taken and the slope values of the linear regression equations were estimated for DTA and the degradate at the selected wavelengths. Using the obtained data, the sensitivity matrices were created and the respective determinants were calculated (Table 3).

For bivariate determination of DTA, the wavelengths 238 and 264 nm were used. At these selected wavelengths, the calibration curves were obtained in the range of 2–24 µg/mL and the linear regression equations were computed (Table 1). DTA could be determined in presence of up to 90% of its acidic degradate, with mean percentage recovery of 101.11±0.78% (Table 2).

3.3. Dual Wavelength Method

The developed dual wavelength method provides a simple method for selective determination of DTA in the presence of its degradate using the zero-order absorption spectra. The principle of this method is that the absorbance difference at two points on the spectra is directly proportional to the component of interest, independent of the interfering component [24]. The prerequisite for this method is the selection of two wavelengths where the interfering component shows the same absorbance value, while the component of interest shows significant difference in absorbance with concentration [25]. Different pairs of wavelengths were tried such as 242.6–290.3, 251.6–280.4, and 222.5–241.5 nm. Using the absorbance values at 222.5 and 241.5 nm gave the best selectivity.

Linear correlation was obtained between the differences absorbance values at the selected wavelengths in amplitude at 222.5–241.5 nm for DTA in the range of 2–24 μg/mL, and the regression equations were computed (Table 1). The methods were checked by the analysis of laboratory prepared mixtures of DTA and its acidic degradate in different ratios as presented in Table 2. DTA could be determined in presence of up to 90% of its acidic degradate, with mean percentage recovery of 99.77±0.73%.

The usefulness of the proposed methods was successfully applied to the analysis DTA in its pharmaceutical formulation and was studied in presence of additives by assaying Gastrografin® solution. The validity of the methods was assessed by applying the standard addition technique (Table 4).

Results obtained by the proposed procedures for the determination of pure samples of the drug were statistically compared to those obtained by the official US pharmacopeial method and no significant difference was observed (Table 5). The official method depends on using standard 0.05N $AgNO_3$ as a titrant and tetrabromophenolphthalein as an indicator [14]. Method validation was performed according to the USP guidelines [14] for all the proposed methods. Table 1 shows results of accuracy, repeatability, and intermediate precision of the methods.

The three methods described have a common feature of choosing two wavelengths. Unlike the other two methods, the ratio difference method allows the selection of any two wavelengths for determination of the drug, such as

wavelengths of maximum absorbance that can increase the sensitivity of the method. In bivariate method, upon applying Kaiser method [21] for selecting the wavelengths for bivariate method, wavelengths that has low sensitivity may be introduced as shown in the regression equation for DTA at 264 nm. The dual wavelength method has a main disadvantage that the selected wavelengths often meet at peak shoulders, where $\Delta A/\Delta\lambda$ is said to be maximum; therefore the selectivity towards the measured component is extremely affected. Also in high concentrations in the synthetic mixtures, there is slight change in the absorption spectrum of each component which may affect the subtraction step and the obtained recoveries, while in ratio difference method measurements can be done at any two wavelengths throughout the whole ratio spectrum including minima or maxima. These limitations may show the superiority of ratio difference method over the other two methods. The ratio difference method also has advantages over methods manipulating ratio spectra in being very simple (one step method).

Conclusion

The three described spectrophotometric methods have the advantages of being more simple and can analyze DTA in its pure powder and dosage form in the presence of up to 90% of its cytotoxic degradation product without complex calculations or multistep procedures. Unlike the chromatographic methods, these methods do not require expensive solvents, sample preparation, buffers, or

sophisticated techniques and instruments. The methods are suitable and valid for application in laboratories lacking liquid chromatographic instruments. High values of correlation coefficients and small values of intercepts validated the linearity of the calibration graphs and the validity of Beer's law. The new and simple ratio difference method shows some advantages over the bivariate and dual wavelength methods. From the results obtained, we concluded that the suggested methods showed high sensitivity, accuracy, reproducibility, and specificity and can be used as stability indicating methods in quality control laboratories.

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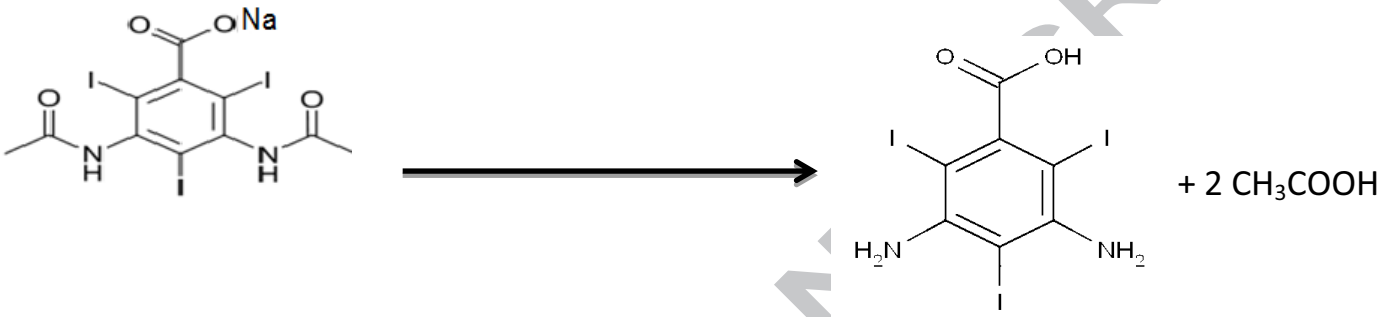


Sodium diatrizoate **3,5 diamino-2,4,6 triiodobenzoic acid**

Figure 1: The suggested mechanism of degradation of DTA.

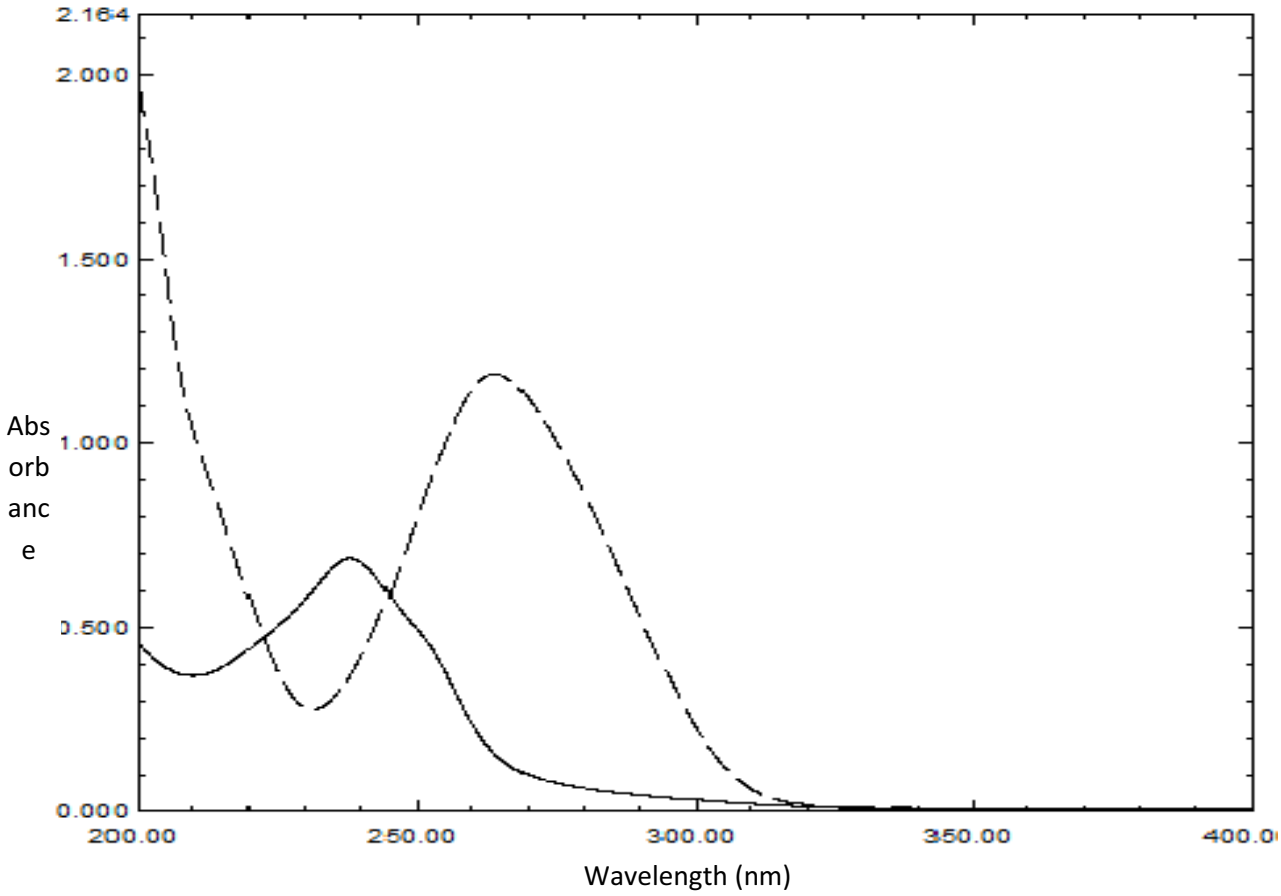


Figure 2: Zero order absorption spectra of DTA 14 µg/mL (——) and the degradate 14 µg/mL(-----).

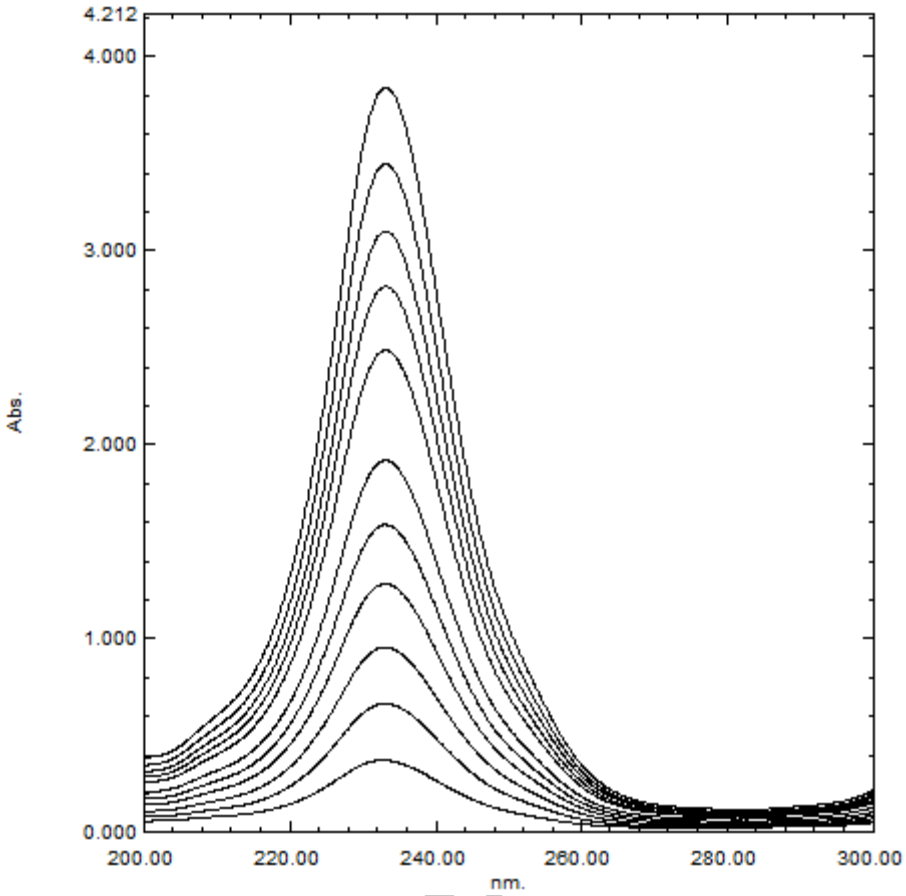


Figure 3: Ratio spectra of DTA (2 - 24 µg/mL) using the spectrum of degradate (14 µg/mL) as a divisor.

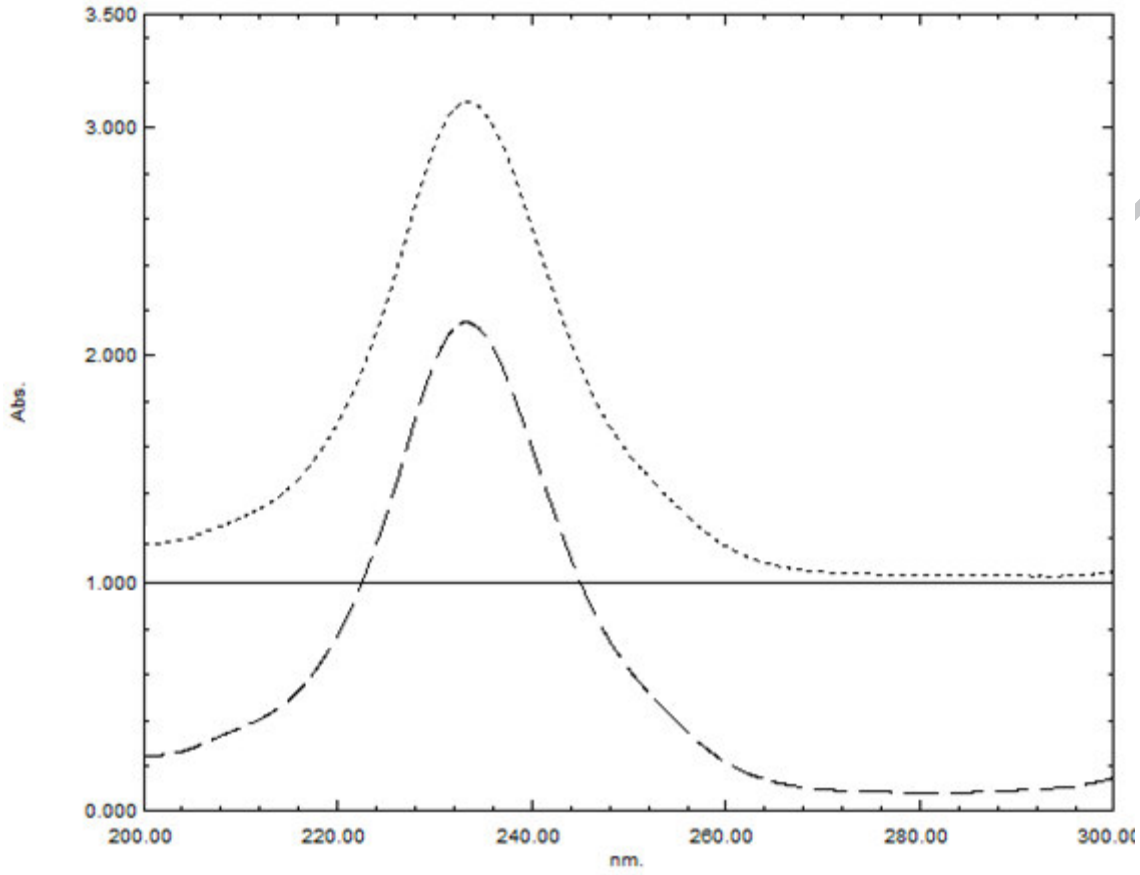


Figure 4 : Ratio spectra of DTA 10 µg/mL (- - -), degradate 10 µg/mL (___) , and a laboratory prepared mixture containing 10 µg/mL of both (…….) using a divisor of 10 µg/mL degradate.

Table 1: Assay validation sheet of the proposed methods for the determination of pure DTA

parameter	Ratio Difference method	Bivariate method	Dual Wavelength method
Accuracy (mean±SD)	100.63 ± 1.06	100.02 ± 0.868	100.06 ± 0.754
Repeatability [a]	0.954	1.050	0.770
Intermediate precision [b]	0.912	0.415	0.680
Specificity	101.40 ± 0.389	101.11 ± 0.787	99.77 ± 0.735
Linearity			
Slope	0.0533	0.0467	0.0130
Intercept	0.0070	0.0116	0.0037
r^2	0.9996	0.9993	0.9999
Range	2 - 24 µg/mL	2 - 24 µg/mL	2 - 24 µg/mL

[a]The intraday and [b] the inter-day RSD values of samples of concentration of 7,11 and 17 µg/mL of DTA.

Table 2 : Determination of DTA in laboratory-prepared mixtures by the proposed methods

% degradation product	Concentration (µg/mL)		Ratio Difference method	Bivariate method	Dual Wavelength method
10	18	2	101.89	101.96	98.85
20	16	4	101.42	100.18	99.42
30	14	6	100.94	101.12	99.62
40	12	8	101.65	99.56	99.71
50	10	10	101.32	101.05	101.31
60	8	12	101.57	101.68	99.81
70	6	14	100.67	101.14	100.38
80	4	16	101.77	101.51	99.86
90	2	18	101.36	101.79	99.02
mean			101.40	101.11	99.77
SD			0.389	0.787	0.735
RSD%			0.383	0.778	0.736

Table 3: Application of Kaiser method for wavelength selection (K* 10^{-6})

λ / λ	230	238	246	252	**264**	270
230	0	959.2	1699	2427	3371	3243
238		0	2032	2903	**4031**	3879
246			0	2391	3320	3194
252				0	2651	2550
264					0	66.56
270						0

Table 4: Quantitative determination of DTA in Gastrografin® solution by the proposed methods and the results of application of standard addition technique

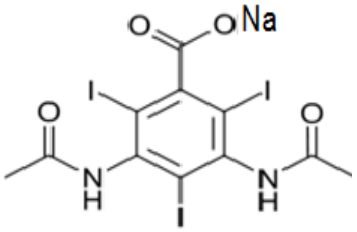
Gastrografin® solution	Ratio Difference method	Bivariate method	Dual Wavelength method
	Recovery ± SD [a]		
Batch no.51424A	100.66 ± 0.44	100.70 ± 1.01	100.60 ± 0.67
Recovery% of standard added	99.49 ± 0.93	100.44 ± 0.96	100.44 ± 0.62

[a]Average of three determinations.

Table 5: Statistical analysis of the results obtained by the proposed methods and the official method for the determination of DTA in pure powder form

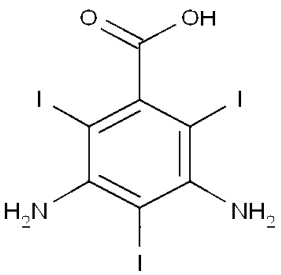
Item	Ratio Difference method	Bivariate method	Dual Wavelength method	Official method [a]
Mean	100.63	100.20	100.06	100.85
SD	1.06	0.87	0.75	0.97
Variance	1.12	0.76	0.56	0.94
n	12	12	12	5
Student's t-test [b]	0.399 (2.13)	1.36 (2.13)	1.82 (2.13)	
F-value [b]	1.19 (5.41)	1.23 (2.98)	1.67 (2.98)	

[a] Precipitimetric titration using standard 0.05N $AgNO_3$ and tetrabromophenolphthalein indicator.
[b] Figures between parentheses represent the corresponding tabulated values of t and F at $P = 0.05$.



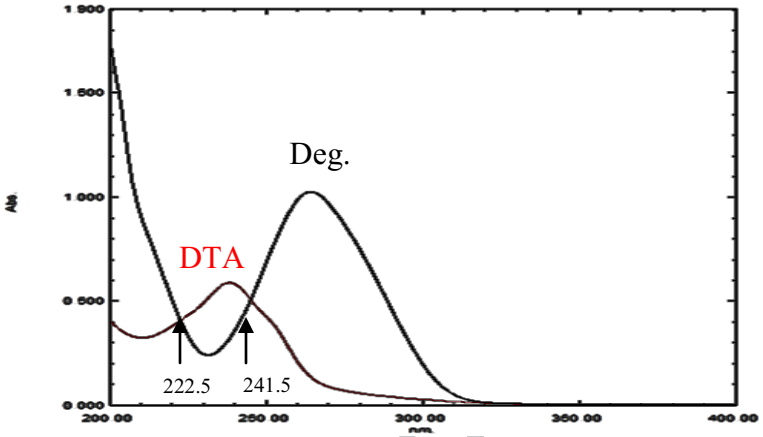
Sodium diatrizoate

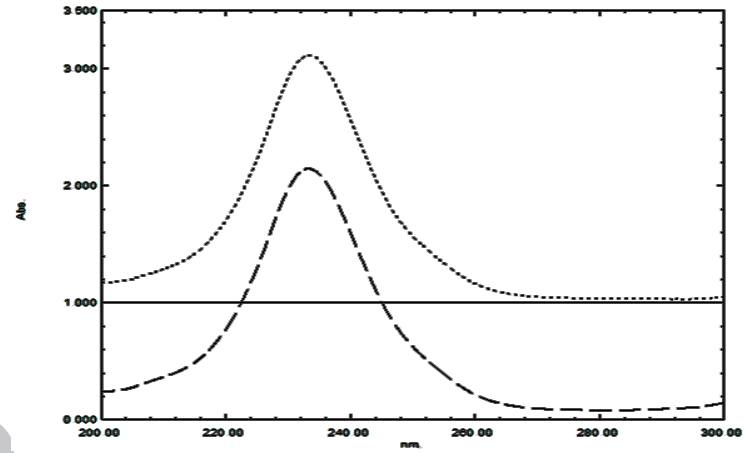
Acidic degradation



+ 2 CH_3COOH

3,5 diamino-2,4,6 triiodobenzoic acid





λ / λ	230	238	246	252	**264**	270
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238		0	2032	2903	**4031**	3879
246			0	2391	3320	3194
252				0	2651	2550
264					0	66.56
270						0

- A comparative study involving three stability indicating spectrophotometric methods.
- The three methods are based on measuring at two wavelengths.
- No need for sophisticated instruments or preliminary separation steps.
- Simple, inexpensive, and applicable methods for quality control laboratories.