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Stability indicating spectrophotometric and spectrodensitometric methods for the determination of diatrizoate sodium in presence of its degradation product

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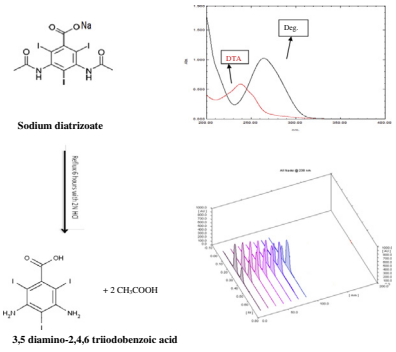
HIGHLIGHTS

- First stability indicating spectrophotometric and TLC methods for DTA.
- No need for a preliminary separation step.
- First degradation study involving the cytotoxic diamino degradate.
- Simple, inexpensive, and applicable methods for quality control laboratories.

GRAPHICAL ABSTRACT



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ABSTRACT

Three sensitive, selective, and precise stability indicating 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 a first derivative (D_1) spectrophotometric one, which allows the determination of DTA in the presence of its degradate at 231.2 nm (corresponding to zero crossing of the degradate) over a concentration range of 2–24 μg/mL with mean percentage recovery 99.95 ± 0.97%. The second method is the first derivative of the ratio spectra (DD_1) by measuring the peak amplitude at 227 nm over the same concentration range as D_1 spectrophotometric method, with mean percentage recovery 99.99 ± 1.15%. The third method is a TLC-densitometric one, where DTA was separated from its degradate on silica gel plates using chloroform:methanol:ammonium hydroxide (20:10:2 by volume) as a developing system. This method depends on quantitative densitometric evaluation of thin layer chromatogram of DTA at 238 nm over a concentration range of 4–20 μg/spot, with mean percentage recovery 99.88 ± 0.89%. 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.

Introduction

X-ray diagnostic agents (contrast media) are widely used as adjuncts to diagnostic visualization techniques as they help to

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illustrate the differences between tissues by introducing them to the area of interest to increase its density and absorption of X-rays; thus, they can enhance the image obtained. Radiographic contrast media are based on elements with high atomic numbers that can absorb X-rays. The most commonly used contrast media are the iodinated organic compounds, particularly tri-iodinated benzene compounds, and their degree of opacity or radiodensity is directly proportional to their iodine content [1–3]. Iodine-based contrast agents are classified according to their structures into ionic and nonionic, and diatrizoate sodium (DTA) is one of the most widely used ionic contrast agents in many medical imaging purposes either intravenously, such as angiography, pyelography, computed tomography, and myelography, or orally or rectally as enema in gastrointestinal imaging [4].

In modern analytical laboratory, there is always a need for significant stability indicating methods of analysis [5,6]. An ideal stability indicating method quantifies a drug and resolves its degradation products [7]. Reviewing the literature in hand revealed that there is no stability indicating method for the determination of DTA in presence of its free amino degradate in spite of its high toxicity. There are only one direct UV spectrophotometric method [8] and another H^1NMR method for the determination of DTA as a single component [9]. DTA was also determined in presence of its di-iodo degradates by capillary electrophoresis technique [10] and liquid chromatography [11,12]. DTA is an official drug whose US pharmacopeial assay method is a precipitimetric titration that depends on the iodide content in the drug [13].

The aim of the present work was to develop and validate stability indicating methods for the determination of DTA in presence of its acidic 3,5-diamino degradate which also can be formed in vivo by deacetylation via liver microsomal enzymes. It is proved that it has a mutagenic and cytotoxic effect on the body [14]. The proposed methods are applicable to the quality control and routine analysis of DTA in pharmaceutical preparations. These methods include first derivative (D_1), first derivative of the ratio spectra (DD_1), and TLC-densitometry.

Experimental

Apparatus

Spectrophotometer: SHIMADZU dual beam (Kyoto/Japan) UV–visible spectrophotometer model UV-1601 PC. TLC plates precoated with silica gel $F_{254,}$ 0.25 mm thickness (Merck, Darmstadt, Germany). TLC scanner 3 densitometer model 3 S/N 130319 (CAMAG, Muttenz, Switzerland). Camag Linomat 5 autosampler with Camag microsyringe (100 μL); (CAMAG, Muttenz, Switzerland). Camag TLC scanner – Model 3 S/N 130319 with winCats software (CAMAG, Muttenz, Switzerland).

Reference samples

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

Pharmaceutical formulation

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

Degraded samples

10 mL of 2 M HCl solution was added to pure DTA (500 mg) in a 250 mL glass-stoppered conical flask and refluxed for 6 h. 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, 2 M NaOH solution was added to the degraded solution till pH was adjusted to about 7, then the solution was evaporated on small flame, and finally 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.

Materials and reagents

All chemicals used throughout this work were of analytical grade, and the solvents were of spectroscopic grade. Methanol and chloroform were purchased from VWR International (West Chester, PA).

Standard solutions

DTA stock standard solutions with a concentration of 0.1 mg/mL in distilled water and 1 mg/mL in methanol for the spectrophotometric and TLC-densitometric methods, respectively, were prepared in 100 mL volumetric flasks by dissolving 10 and 100 mg of pure DTA in distilled water and methanol. Degradation product stock solutions with a concentration of 0.1 mg/mL in distilled water for the spectrophotometric methods and 1 mg/mL in methanol for the TLC-densitometric method were prepared.

Procedures

Construction of calibration graphs

D_1 method. 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 water. The zero-order spectra were recorded using distilled water as a blank. The first derivative of the obtained spectra was recorded using $\Delta\lambda$ = 4 nm and the scaling factor 50. The peak amplitudes of the obtained first derivative spectra were measured at 231.2 nm. Calibration graph relating the peak amplitude to the corresponding concentrations of DTA was constructed, and the corresponding regression equation was computed.

DD_1 method. Aliquots (0.2–2.4 mL) of DTA stock solution (0.1 mg/mL) were transferred into a series of 10 mL volumetric flasks,

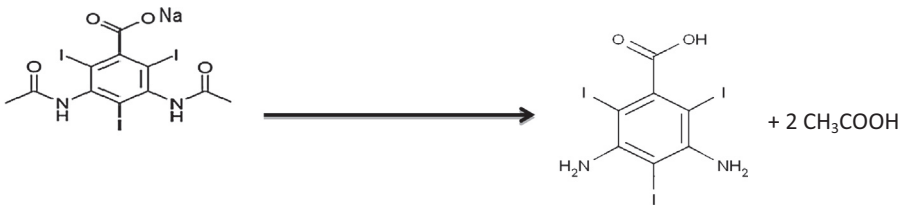

Fig. 1. The suggested mechanism of degradation of DTA.

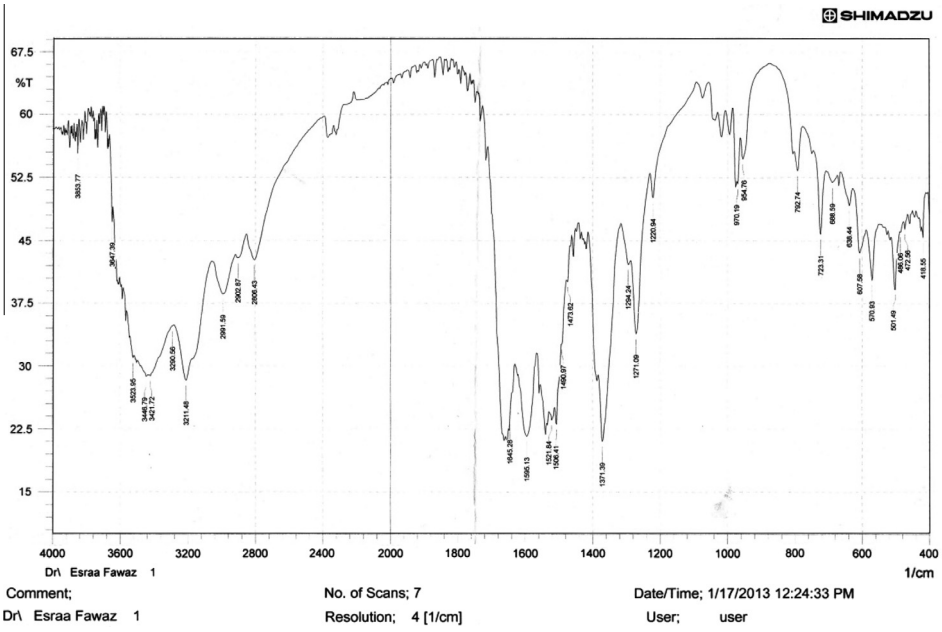

Fig. 2. IR spectrum of intact DTA.

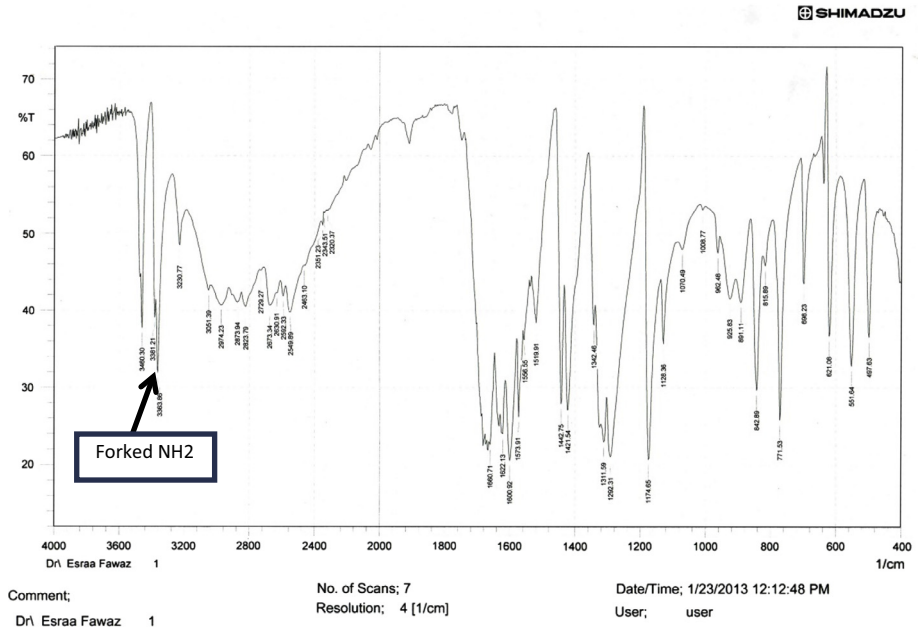

Fig. 3. IR spectrum of DTA degradation product.

and the volume was completed with distilled water. The zero-order spectra of the prepared solutions were divided by the spectrum of 14 μg/mL degradation product, and then the first derivative of the ratio spectra (DD_1) was obtained using scaling factor 50 and $\Delta\lambda = 4$ nm. The peak amplitudes of the first derivative of the ratio spectra were measured at 227 nm. Calibration graph relating the peak amplitudes of ($^1DD_{227}$) to the corresponding concentrations of DTA was constructed, and the corresponding regression equation was computed.

TLC-densitometric method. Aliquots (4–20 μL) from DTA stock solution (1.0 mg/mL) prepared in methanol were spotted onto a TLC plate using Camag Linomat autosampler with Camage microsyringe (100 μL). Spots were spaced 10.5 mm apart from each other and 15 mm from the bottom edge of the plate with a band length of 6 mm. The plates were developed in chromatographic tanks previously saturated with the mobile phase chloroform: methanol: ammonium hydroxide (20:10:2 by volume), by ascending chromatography. The plates were dried, spots were detected under the UV lamp (254 nm), and the plates were scanned at 238 nm. A calibration graph relating the peak to the corresponding concentration of DTA was constructed, and the regression equation was computed.

Analysis of artificial mixtures

Laboratory-prepared mixtures containing DTA and different percentages of its degradation product were prepared and analyzed by the proposed methods.

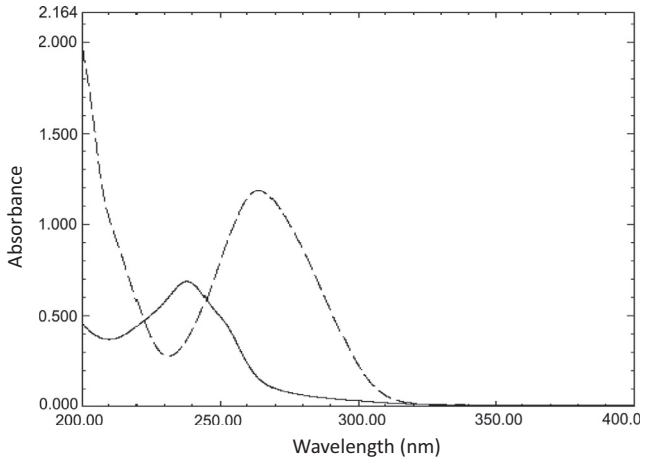

Fig. 4. Zero order absorption spectra of DTA 14 μg/ml (—) and the degradate 14 μg/ml (-----).

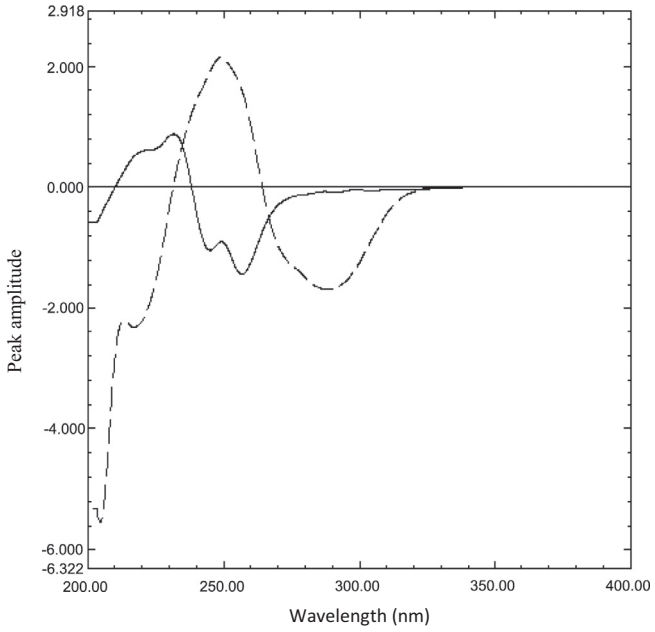

Fig. 5. First derivative absorption spectra of DTA 14 μg/ml (—) and the degradate 14 μg/ml (-----).

Application of the proposed methods to the analysis of DTA in pharmaceutical preparation

D_1 and DD_1 methods. 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 in Sections 'D1 method and DD1 method' of construction of calibration graphs.

TLC-densitometric method. From Gastrografin® solution 0.1 mL was transferred to 100 mL volumetric flask and the volume was completed with methanol. 10 μL of the prepared solution was spotted in triplicate using Linomat applicator onto a TLC plate, and the procedure was completed as described in Section 'TLC-densitometric method' of construction of calibration graphs.

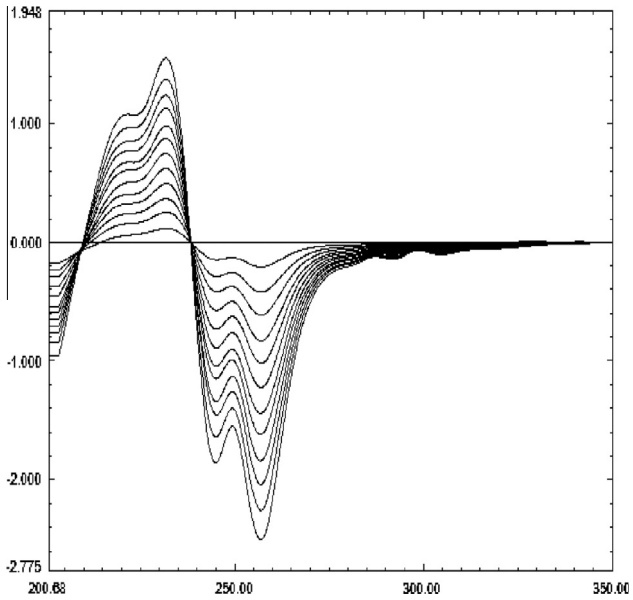

Fig. 6. First derivative absorption spectra of DTA (2–24 μg/ml).

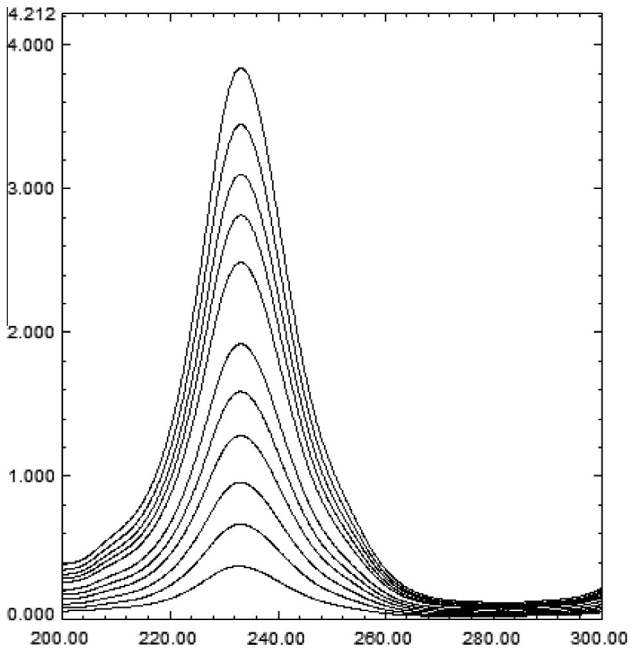

Fig. 7. Ratio spectra of DTA (2–24 μg/ml) using the spectrum of degradate (14 μg/ml) as a divisor.

Results and discussion

DTA is partially deacetylated in liver to the deacetylated mutagenic and cytotoxic metabolite 3,5-diamino-2,4,6 triiodobenzoate [10]. 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; therefore, manufacturers of DTA should further reduce the level of the aromatic amine [10]. This deacetylated degradate was also obtained upon reflux of DTA with acid or alkali (Fig. 1); therefore, the determination of DTA in presence of its degradate was essential.

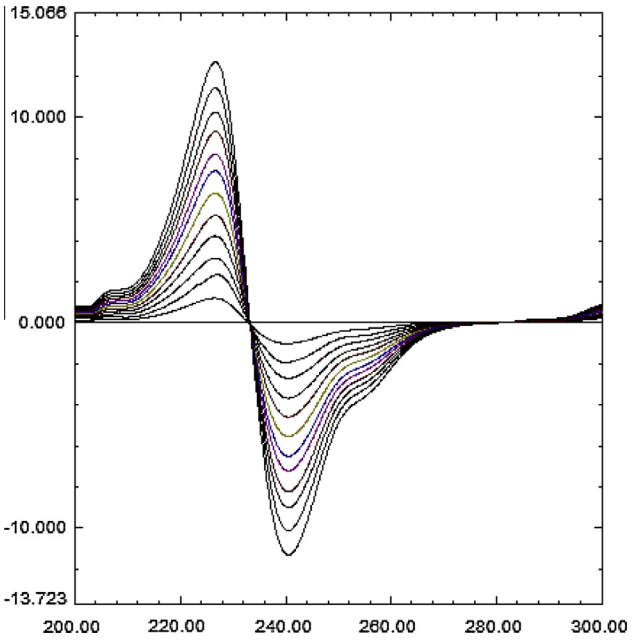

Fig. 8. First derivative of ratio spectra of DTA (2–24 μg/ml) using the spectrum of degradate (14 μg/ml) as a divisor.

The structure of the degradate was elucidated by mass spectrometry, and the electron ionization showed mass ion peak at *m*/*z* 521 corresponding to the acidic 3,5-diamino degradate as shown in Fig. S-1 (Supplementary materials). Moreover, the structure was further confirmed by IR spectrometry which indicated two forked peaks corresponding to the two primary amino groups at 3460 and 3381 cm^{-1} (Figs. 2 and 3).

The focus of the present work was to develop accurate, specific, reproducible, and sensitive stability indicating methods for the determination of DTA in pure form or in pharmaceutical formulations in the presence of its acidic degradation product.

D_1 and DD_1 methods

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 (Fig. 4). Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands and for eliminating the effect of baseline shifts and baseline tilts by using the first or higher derivatives of absorbance with respect to wavelength [15]. A rapid, simple, and low cost spectrophotometric method based on measuring the peak amplitude of D_1 spectrum of DTA at 231.2 nm (corresponding to zero crossing of the degradate) was developed with good selectivity without interference of the degradate as shown in Figs. 5 and 6. In order to optimize D_1 method, different smoothing and scaling factors were tested, where a smoothing factor $\Delta\lambda = 4$ and a scaling factor = 50 showed a suitable signal to noise ratio and the spectra showed good resolution.

In order to improve the selectivity of the analysis of DTA in presence of its acidic degradate, DD_1 spectrophotometric method was also established. The principal advantage of the method is that the whole spectrum of interfering substance is cancelled, so the choice of the wavelength used for calibration is not critical as in the D_1 method [16,17].

In order to optimize DD_1 method, several divisor concentrations 2, 8, and 14 μg/mL of the degradate were tried, and the best result was obtained when using 14 μg/mL of the degradate as a divisor. Different $\Delta\lambda$ and scaling factors were tested, where $\Delta\lambda = 4$ and a scaling factor = 50 were suitable to enlarge the signal of DTA to

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

Parameter	D_1 method	DD_1 method	TLC-densitometric method
Accuracy (mean ± SD)	99.95 ± 0.97	99.99 ± 1.15	99.88 ± 0.89
Repeatability[a]	0.643	0.682	0.371
Intermediate precision[b]	1.290	1.030	0.990
Specificity	100.95 ± 1.37	100.94 ± 0.81	101.02 ± 1.19
Linearity			
Slope	0.0624	0.5169	0.0493
Intercept	0.0072	0.0479	0.3050
r^2	0.9998	0.9993	0.9997
Range	2–24 μg/ml	2–24 μg/ml	4–20 μg/spot

[a] The intraday RSD values of samples of concentration of 7, 11 and 17 μg/ml of DTA for D_1 and DD_1, and 5, 11 and 17 μg/spot for TLC.
[b] The inter-day RSD values of samples of concentration of 7, 11 and 17 μg/ml of DTA for D_1 and DD_1, and 5, 11 and 17 μg/spot for TLC.

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

% Degradation product	Concentration (μg/mL for D_1 and DD_1) and (μg/spot for TLC)		D_1 method	DD_1 method	TLC-densitometry
10	18	2	101.12	99.11	101.00
20	16	4	98.64	99.04	101.64
30	14	6	98.61	101.93	100.70
40	12	8	101.00	100.97	102.07
50	10	10	101.63	99.58	98.53
60	8	12	101.92	100.87	101.59
70	6	14	101.67	101.62	
80	4	16	101.98	101.69	101.63
90	2	18	102.05	101.87	
Mean			100.96	100.74	101.02
SD			1.37	1.19	1.19
RSD%			1.36	1.18	1.18

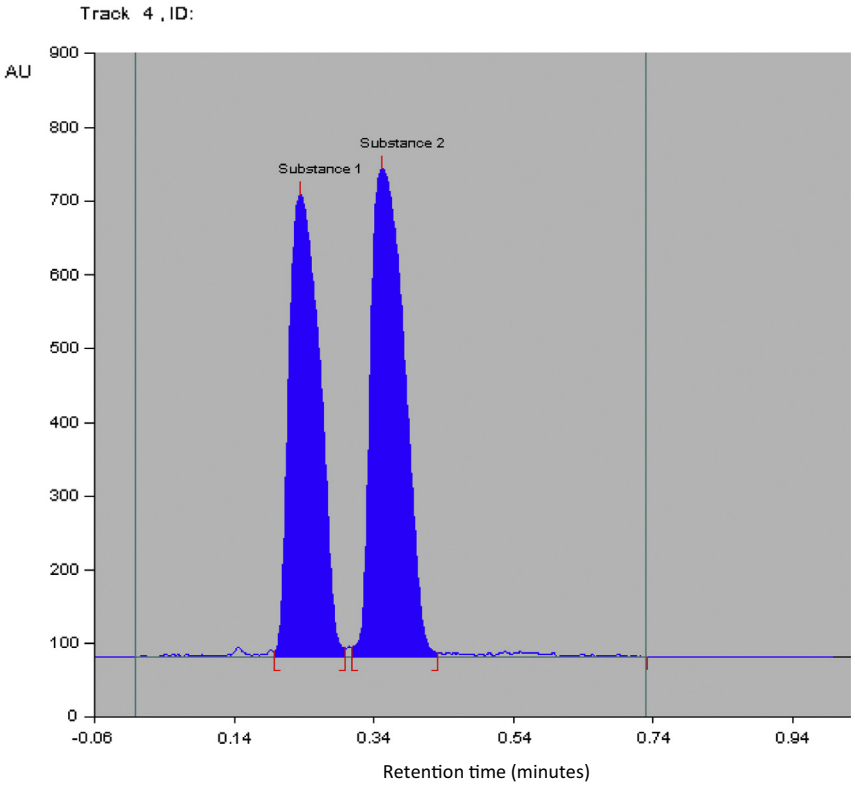

Fig. 9. 2D TLC chromatogram of DTA (R_f = 0.4) and its degradate (R_f = 0.22).

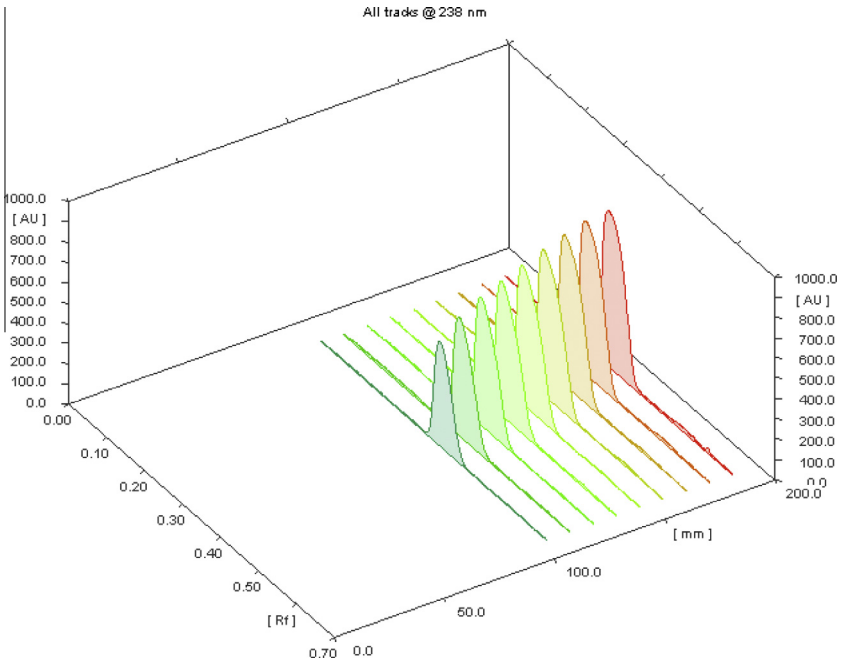

Fig. 10. 3D TLC chromatogram of DTA (4–20 μg/ml).

facilitate its measurement and to diminish error in reading the signal (Figs. 7 and 8). The absorption spectra of DTA in the range of 2–24 μg/mL were divided by the absorption spectrum of 14 μg/mL of the degradate (as a divisor), where the obtained ratio spectra were differentiated with respect to wavelength. DD_1 values showed good linearity and reproducibility at 227 nm.

The linearity of the peak amplitudes of the D_1 curves at 231.2 nm and the peak amplitudes of the DD_1 curves at 227 nm was studied. A linear relationship was obtained in the range of 2–24 μg/mL for the drug, and the regression equations were computed (Table 1). The methods were checked by analysis of laboratory-prepared mixtures of DTA and its acidic degradate in different

Table 3
Quantitative determination of DTA in Gastrografin® solution by the proposed methods and the results of application of standard addition technique.

Gastrografin® solution	D_1 method	DD_1 method Recovery ± SD[a]	TLC-densitometry
Batch no.51424A	101.51 ± 0.98	100.43 ± 1.23	100.54 ± 0.89
Recovery % of standard added	101.11 ± 0.41	100.27 ± 1.08	100.59 ± 0.84

[a] Average of three determinations.

Table 4
Statistical analysis of the results obtained by the proposed methods and the official method for the determination of DTA in pure powder form.

Item	D_1 method	DD_1 method	TLC-densitometric method	Official method[a]
Mean	99.95	99.99	99.88	100.85
SD	0.97	1.15	0.89	0.97
Variance	0.94	1.32	0.79	0.94
n	12	12	9	5
Student's *t*-test[b]	1.74 (2.13)	1.46 (2.13)	1.89 (2.17)	
F-value[b]	1.00 (5.41)	1.43 (5.41)	1.19 (3.84)	

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

ratios as presented in Table 2. DTA could be determined in presence of up to 90% of its acidic degradate in case of D_1 and DD_1 methods, with mean percentage recovery of 100.95 ± 1.37% and 100.78 ± 1.23%, respectively.

Comparing the two proposed spectrophotometric methods, DD_1 method shows more advantages over D_1 method as it avoids critical measurements at zero crossing points where 1 nm shift between different spectrophotometers in different laboratories can seriously affect the results of D_1 method; thus, DD_1 method reveals better accuracy and method transfer.

TLC-densitometric method

A stability indicating TLC-densitometric method for the determination of DTA was also described. Several trials were done to choose a developing system which can separate DTA from its degradation product including methanol: ethyl acetate: ammonium hydroxide (10:5:0.1 by volume), methanol: chloroform: ammonium hydroxide (5:5:0.1 by volume), and chloroform: methanol: ammonium hydroxide (10:5:1 by volume). The first and the second systems were not satisfactory because they did not affect good separation of the drug from its degradation product, the first system showed no separation, while the second one separated the two spots but with R_f close to each other (R_f = 0.18 and 0.15 for intact drug and degradation product, respectively). Satisfactory separation was obtained using the third system, chloroform: methanol: ammonium hydroxide (10:5:1 by volume) as a mobile phase. Respective R_f values were 0.4 ± 0.02, 0.22 ± 0.02 for DTA and its degradate, respectively, as shown in Fig. 9. This separation allowed the determination of DTA at 238 nm without any interference from the degradation product as shown in Figs. 10 and S-4 (Supplementary materials).

Linear relationship was found to exist between the area ratio calculated by division of the peak area of each concentration by the peak area of a certain chosen concentration (14 μg/spot) which is spotted on the same plate and the corresponding concentration of DTA in the range of 4–20 μg/spot at the selected wavelength (238 nm). The regression equation was computed (Table 1). The proposed method was valid for the determination of DTA in different laboratory-prepared mixtures in presence of up to 80% of its acidic degradate (Table 2), with mean percentage recovery of 101.02 ± 1.19%.

The usefulness of the proposed methods was successfully applied to the analysis of 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 3).

The advantage of the TLC-densitometric method is that it is the easiest and fastest way not only for quantitation of the drug but also for visual detection of impurities or trace amounts of the toxic degradate which cannot be easily detected by the UV-spectrophotometric methods. Moreover, it is simple and inexpensive permitting its application in quality control laboratories where spectrophotometric derivatization programs are not applicable, also it avoids further data manipulation and derivatization steps.

Results obtained by the proposed procedures for the determination of pure samples of the drug were statistically compared to those obtained by the US pharmacopeial method and no significant difference was observed (Table 4). The official method depends on using standard 0.05 N $AgNO_3$ as a titrant and tetrabromophenolphthalein as an indicator [13]. Method validation was performed according to USP guidelines [18] for all the proposed methods. Table 1 shows results of accuracy, repeatability, and intermediate precision of the methods.

Conclusion

In the present work, three simple, sensitive, and rapid methods are described for determination of DTA in pure form or in pharmaceutical formulations. The proposed D_1 and DD_1 spectrophotometric methods are simple, more convenient, less time consuming, and more economic stability indicating methods compared to other published methods. The proposed methods have the advantage of being applicable in the presence of up to 90% of the degradate for the spectrophotometric methods and up to 80% for TLC-densitometric method. The advantages of TLC-densitometric method are that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis, and provide high sensitivity and selectivity. High values of correlation coefficients and small values of intercepts validated the linearity of the calibration graphs and the validity of Beer's law. From the results obtained, we concluded that the suggested methods showed high sensitivity, accuracy, reproducibility, and specificity and can be used as stability indicating meth-

ods. Moreover, these methods are simple and inexpensive, permitting their application in quality control laboratories.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2014.10.002.

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