

## RESEARCH ARTICLE

# Monitoring of the degradation kinetics of diatrizoate sodium to its cytotoxic degradant using a stability-indicating high-performance liquid chromatographic method

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**Abstract**

The X-ray diagnostic agent sodium diatrizoate (DTA) was studied for chemical degradation. The 3,5-diamino derivative was found to be the alkaline and acidic degradation product. The 3,5-diamino degradate is also the synthetic precursor of DTA and it is proved to have cytotoxic and mutagenic effects. A sensitive, selective and precise high-performance liquid chromatographic stability-indicating method for the determination of DTA in the presence of its acidic degradation product and in pharmaceutical formulation was developed and validated. Owing to the high toxicity of the degradation product, the kinetics of the acidic degradation process was monitored by the developed RP-HPLC method. The reaction was found to follow pseudo-first order kinetics. The kinetic parameters such as rate constant ( $K$ ) and half-life ( $t_{1/2}$ ) were calculated under different temperatures and acid concentrations; activation energy was estimated from the Arrhenius plot. The developed RP-HPLC method depends on isocratic elution of a mobile phase composed of methanol–water (25:75 v/v; pH adjusted with phosphoric acid), and UV detection at 238 nm. The method showed good linearity over a concentration range of 2–100  $\mu\text{g/mL}$  with mean percentage recovery of  $100.04 \pm 1.07$ . The selectivity of the proposed method was tested using laboratory-prepared mixtures. The proposed method has been successfully applied to the analysis of DTA in pharmaceutical dosage forms without interference from other dosage form additives and the results were statistically compared with the official USP method. Validation of the proposed method was performed according to International Conference on Harmonization guidelines.

**KEYWORDS**

diatrizoate sodium, kinetic study, RP-HPLC, stability

## 1 | INTRODUCTION

Pharmaceutical product quality is a matter of growing concern for patient safety as the presence of impurities may influence the efficacy and safety of pharmaceuticals. Impurities such as precursors and potential degradation products can cause changes in the chemical, pharmacological and toxicological properties of drugs and thereby have a significant impact on product quality and safety (Katayama et al. 1990). Systematic kinetic studies of the decomposition of drugs using stability-testing techniques are essential for the quality control of such products (Görög, 2006). In the modern analytical laboratory, there is always a need for significant stability-indicating methods of analysis (Samah, Hala, Abdelkawy, & Maha, 2010). An ideal stability-

indicating method quantifies a drug and resolves its degradation products (María, María, Juan, Silvina, & Héctor, 2009). Moreover, kinetic studies and accelerated stability experiments are important to solve problems encountered in quality control and to predict the expiry dates of pharmaceutical products (Mohammad, 2011).

X-ray diagnostic agents (contrast media) are widely used as adjuncts to diagnostic visualization techniques as they help to 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 are iodinated organic compounds, particularly tri-iodinated benzene compounds, and their degree of opacity or

radiodensity is directly proportional to their iodine content (Sean 2010). Iodine-based contrast agents are classified into ionic and non-ionic according to their structures. Sodium diatrizoate (DTA) is one of the most widely used ionic contrast agents in many medical imaging procedures and may be applied either intravenously, such as in angiography, pyelography, computed tomography and myelography, or orally or rectally, as in gastro-intestinal imaging (Barrs, 2005).

A review of the available literature indicated that no stability-indicating HPLC method for the determination of DTA in the presence of its free amino degradant has previously been published in spite of its high toxicity. There are two spectrophotometric methods and a TLC-spectrodensitometric method (Abd El-Rahman, Riad, Gawad, Fawaz, & Shehata 2015). Also, there is one direct UV spectrophotometric method (Tan & Qisu, 1991) and another  $^1\text{H-NMR}$  method (Hanna & Lau-Cam 1996) for the determination of DTA as a single component, DTA was also determined in the presence of its diiodo degradates by capillary electrophoresis technique (Farag & Wells, 1997) and liquid chromatography (Chellquist, Nelson, & Storflor, 1997). DTA is an official drug whose official assay method is a precipitometric titration that depends on the iodide content in the drug (The United States Pharmacopeia and National Formulary, 2011), which makes it of no specificity and limited sensitivity.

The aim of the present work was to develop, optimize and validate the first stability-indicating HPLC method for the determination of DTA with the first kinetic study in presence of its cytotoxic acidic 3,5-diamino degradate (Weiss, Hsu, Wheeler, Norman, & Riley, 1981). The cytotoxicity of this degradate was our ultimate motive to study the kinetics of DTA acidic degradation by HPLC technique, which is more accurate, indicative and specific than the ordinary UV spectroscopy. Moreover, the proposed method is applicable for the quality control and routine analysis of DTA in bulk powder and Gastrografin® solution.

## 2 | EXPERIMENTAL

### 2.1 | Apparatus

The liquid chromatograph consisted of an isocratic pump (Shimadzu LC-10AD) an ultraviolet visible wavelength detector (SPD-10A, Shimadzu) and a Rheodyne injector (Model 7725 I, Rohnert Park, CA, USA) equipped with 20  $\mu\text{L}$  injector loop. Stationary phase was 250  $\times$  4.6 mm  $\text{C}_{18}$ , particle size was 5  $\mu\text{m}$  and the analytical column, ZORBAX.ODS was used. The samples were injected with the aid of a 25  $\mu\text{L}$  Hamilton® analytical syringe. The instrument was connected to an IBM-compatible PC and HP deskjet printer.

### 2.2 | Reference samples

Diatrizoate sodium reference standard was purchased from Sigma-Aldrich Co. Its purity was certified to be  $\geq 99.99\%$ .

### 2.3 | Pharmaceutical formulation

Gastrografin® solution was manufactured by Schering Company (Belimed, Spain). Batch no. 51424 A was labeled to contain 0.6 g/mL of diatrizoate anhydrous base.

### 2.4 | Degraded samples

A 50 mL aliquot of 2 M HCl solution was added to pure DTA (500 mg) in a 250 mL glass-stoppered conical flask and the mixture was boiled under reflux for 6 h. Complete degradation was tested by TLC using chloroform-methanol-ammonium hydroxide (20:10:2 by volume) as the mobile phase. Only one spot was observed not corresponding to DTA. Subsequently, 2 M NaOH solution was added to the degraded solution until pH was adjusted to about 7, the solution was evaporated on a small flame, then the degradate was 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

Orthophosphoric acid and concentrated ammonia (specific gravity 0.91) were of analytical grade purchased from Adwic (El-Nasr Pharmaceutical Chemicals. Co. Cairo, Egypt). Methanol (E. Merck, Darmstadt, Germany) was of HPLC grade.

### 2.6 | Standard solutions

DTA stock standard solution 0.1 mg/mL in deionized water was used for HPLC. The solution was prepared in a 100 mL volumetric flask by dissolving 10 mg of pure DTA in deionized water. Degradation product stock solution (0.1 mg/mL) was also prepared in deionized water.

### 2.7 | Chromatographic conditions

The mobile phase consisted of water-methanol (75:25 v/v) and the pH was adjusted to 3 using orthophosphoric acid. The mobile phase was filtered through a 0.45  $\mu\text{m}$  Millipore membrane filter and was degassed for 15 min in an ultrasonic bath prior to use. The flow rate was 1 mL/min and UV-detection was at 238 nm.

### 2.8 | Procedures

#### 2.8.1 | Construction of calibration graphs

Aliquots from DTA stock solution (0.1 mg/mL) were accurately transferred into a series of 10 mL volumetric flasks and the volume was made up to the mark with deionized water to get a concentration range of 2–100  $\mu\text{g/mL}$ , then 20  $\mu\text{L}$  of each concentration was injected three times successively and chromatographed under the previously mentioned chromatographic conditions. The relative peak area ratios to that of an external standard having a concentration of 14  $\mu\text{g/mL}$  were then plotted against the corresponding concentrations of DTA to get a linear plot from which the regression equation was computed.

#### 2.8.2 | Analysis of artificial mixtures

Laboratory-prepared mixtures containing DTA and different percentages of its degradation product ranging from 10 to 90% were prepared (Tables 1 and 2) and analyzed by the proposed method.

**TABLE 1** System suitability parameters for HPLC method

	Drug	Degradate	Reference value
Retention time ( $t_R$ ), min	5.52	4.04	
Relative $t_R$	1.37		>1
Column efficiency ( $N$ )	3141	3121	$N > 2000$
Tailing factor ( $T$ )	1.01	1.2	$T = 1$ for a symmetric peak
Column resolution ( $R_s$ )	4.33		$R_s > 2$
Capacity factor ( $K'$ )	2.81	1.79	$10 > K' > 1$
Selectivity factor ( $\alpha$ )	1.57		should be > 1
HETP	0.08	0.08	

**TABLE 2** Determination of sodium diatrizoate (DTA) in laboratory prepared mixtures by the proposed HPLC method

Degradation product (%)	Concentration ( $\mu\text{g/mL}$ )		R (%)
10	18	2	101.28
30	14	6	100.61
50	10	10	99.89
70	6	14	98.52
90	2	18	100.58
Mean			100.18
SD			1.05
RSD%			1.05

### 2.8.3 | Application of the proposed method to the analysis of DTA in pharmaceutical preparation

Gastrografin® solution is labeled to contain (0.6 g/mL) of anhydrous DTA. A stock solution with a concentration equal to 600  $\mu\text{g/mL}$  was prepared by transferring 0.1 mL of the solution to a 100 mL volumetric flask and the volume was made up using deionized water. From Gastrografin® solution 1.25 mL was transferred to a 50 mL volumetric flask and the volume was made up with deionized water to get a concentration of 15  $\mu\text{g/mL}$ , which was chromatographed three times using the same procedure as described above.

## 2.9 | Kinetic study

### 2.9.1 | Determination of the kinetic order of the system

Into a 100 mL measuring flask, 0.1 g of DTA in 2 M hydrochloric acid (HCl) were dissolved and made up to the mark with the same solvent. This solution was transferred into another clean and dry stoppered conical flask and refluxed in a thermostatically controlled water bath at 100°C for 4 h; 1 mL sample solutions were taken at 1 h intervals, placed into 10 mL measuring flasks and the volume was made up with deionized water. The solutions (initial concentration  $C_0 = 100 \mu\text{g/mL}$ ) were injected in the liquid chromatograph using the chromatographic conditions described above. The concentration of DTA was calculated from the regression equation. The log percentage of the remaining concentration against time was plotted.

### 2.9.2 | Study of the effect of HCl concentration on the reaction rate

Into a series of 100 mL measuring flasks, 0.1 g of DTA was dissolved in 1.0, 1.5 and 2 M HCl and made up to the mark with the same solvent. These solutions were transferred into other clean and dry stoppered

conical flasks, and then refluxed in a thermostatically controlled water bath at 100°C for 4 h. Sample solutions of 1 mL were taken at 1 h intervals and then made up as described in the previous section. The log-percentage of remaining concentration against time was constructed for different molarities of HCl and the rate constant and half-lives ( $t_{1/2}$ ) were calculated.

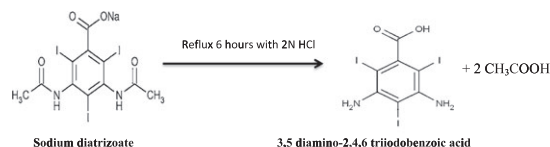
### 2.9.3 | Study of the effect of temperature on the reaction rate

Three portions each of 0.1 g of DTA were dissolved in 100 mL measuring flasks and made up to volume with 1.0, 1.5 and 2 M HCl. These solutions were transferred into other clean and dry stoppered conical flasks and then refluxed in a thermostatically controlled water bath at 80, 90 and 100°C for 4 h. Sample solutions of 1 mL were taken at 1 h intervals and then made up as described above. The log-percentage of remaining concentration against time at different temperatures was plotted. Also the Arrhenius plot for the effect of temperature on the rate of hydrolysis was constructed.

## 3 | RESULTS

DTA is partially deacetylated in liver to the mutagenic and cytotoxic metabolite 3,5-diamino-2,4,6-triiodobenzoate. 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 (Chellquist et al. 1997). This deacetylated degradate was also obtained upon reflux of DTA with acid or alkali (Figure 1); therefore the determination of DTA in the presence of its degradate was essential.

The structure of the degradate was elucidated by mass and IR spectrometry in our recently published work (Abd El-Rahman et al. 2015) The focus of the present study is to develop an accurate, specific, reproducible and sensitive RP-HPLC stability-indicating method for the determination of DTA in pure form and pharmaceutical

**FIGURE 1** The suggested mechanism of degradation

formulation and in the presence of its acidic degradation product, and to monitor the degradation kinetics.

The best resolution with symmetric and sharp peaks was obtained using a mobile phase of water-methanol (75:25 v/v) and pH adjusted using orthophosphoric acid. The flow rate was 1 mL/min and UV detection was at 238 nm. The retention times were  $5.52 \pm 0.1$  min for DTA and  $4.04 \pm 0.1$  min for the degradate (Figure 2).

## 4 | DISCUSSION

### 4.1 | Method development

Several trials were carried out to obtain a good resolution between the drug and its degradation product owing to their structure similarity. These trials involved the use of different mobile phases with different ratios, different pH values and flow rates.

Many parameters were found to critically affect the separation, retention time and the elution order of the two components, such as mobile phase composition and pH. Regarding the composition of the mobile phase, it was found that decreasing the polarity of it by using acetonitrile-methanol (50:50 v/v) resulted in delaying the retention times of both components to 18 and 21 min for DTA and the degradate, respectively. In another trial, the retention times were decreased and the elution order was altered upon increasing the polarity of the mobile phase by using water instead of acetonitrile, but the two components were very close to each other.

Interestingly, optimization of pH played a key role in the whole separation step. We exploited the presence of acidic and basic characters in both the drug and the degradate. DTA has an acidic character with a  $pK_a$  value of 1.13 corresponding to the carboxylic group ( $\text{COO}^-$ ),

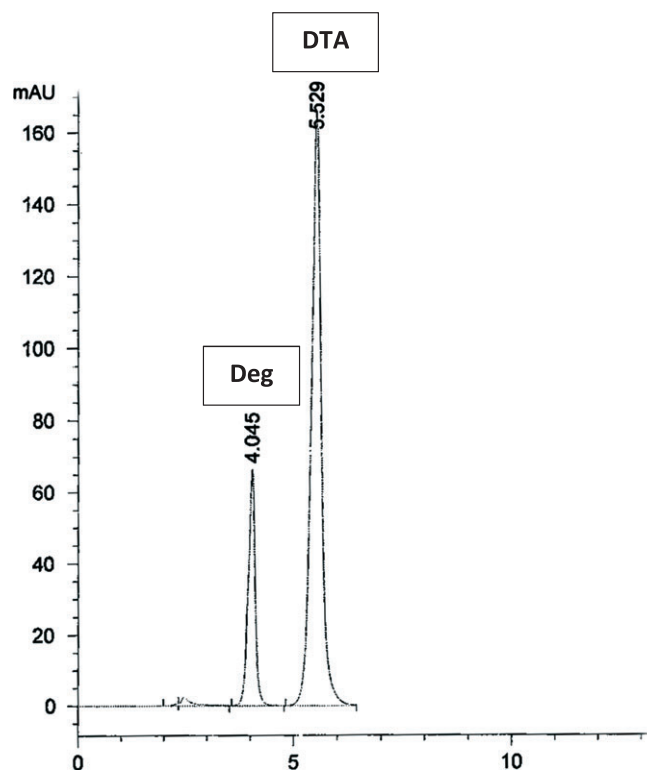
while the degradate has two basic primary amino groups in addition to the carboxylic one. At pH the two components eluted approximately at the same time that the two carboxylic groups of both components are ionized, while pH was found to be the optimum for separation of DTA from its degradate at reasonable retention times. This acidic pH hinders the ionization of carboxylic groups in both components, but strongly favors the ionization of the two primary amino groups in the degradate, which explains its weak interaction with the reversed phase of the  $\text{C}_{18}$ -column; therefore it elutes before the drug.

System suitability parameters of the proposed HPLC method were calculated showing good resolution, selectivity, and symmetrical peaks according to the *US Pharmacopeia* (Table 3). The proposed HPLC method was valid and applicable for the determination of DTA with mean percentage recovery  $100.04 \pm 1.07$  in pure form and  $100.66 \pm 1.07$  in pharmaceutical dosage form.

### 4.2 | Kinetics of the degradation

The linear relationship (Figure 3) between the log-percentage of remaining concentration against time indicated first-order degradation. Since the hydrolysis was performed in a large excess of HCl (2 M), it followed a pseudo-first-order reaction rate (Florence & Attwood 1998), which is the term used when two reactants are involved in the reaction but one of them is in such a large excess (HCl) that any change in its concentration is negligible compared with the change in concentration of the other reactant (drug).

Various parameters that affect the rate of the reaction were studied. The temperature dependence of DTA degradation was studied by conducting the reaction at different temperatures using different concentrations of the acid solution (Figure 4). At each temperature the rate constant and  $t_{1/2}$  were calculated, then the log of the rate



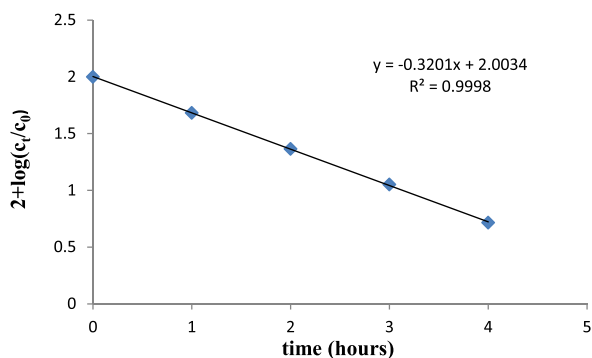
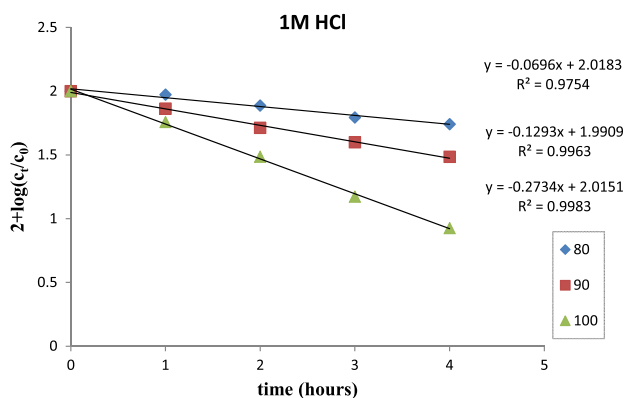
**FIGURE 2** HPLC chromatogram of a resolved mixture [20  $\mu\text{g}/\text{mL}$  sodium diatrizoate (DTA) and 20  $\mu\text{g}/\text{mL}$  degradate]

**TABLE 3** Assay validation sheet of the proposed method for the determination of pure DTA

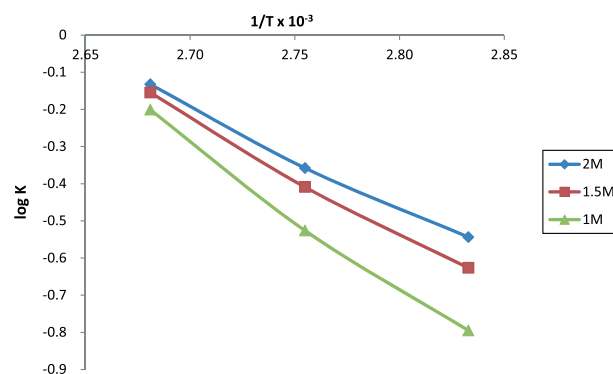
Parameter	HPLC method
Accuracy (mean ± SD)	100.04 ± 1.07
Repeatability <sup>a</sup>	0.11
Intermediate precision <sup>b</sup>	0.49
Specificity	100.18 ± 1.05
Robustness <sup>c</sup>	101.83 ± 0.83
LOD (µg/mL)	0.95
LOQ (µg/mL)	2.87
Linearity	
Slope	0.0696
Intercept	0.04
r	0.9999
Range	2–100 µg/mL

<sup>a,b</sup>Calculated using samples of concentrations equal to 7, 11 and 17 µg/mL of DTA.

<sup>c</sup>The average recoveries of three concentrations of DTA of 40,60 and 100 µg/mL using a mobile phase pH of 4 instead of 3.

**FIGURE 3** First-order plot of the hydrolysis of DTA (100 µg/mL) with 2 M HCl at 100°C**FIGURE 4** First-order plot of the hydrolysis of DTA with 1 M HCl at different temperatures

constant was plotted against the reciprocal of the temperature in Kelvin units (Arrhenius plot, Figure 5) to demonstrate the effect of temperature on the rate constant. It was concluded that, as the temperature increased, the rate of hydrolysis increased with a

**FIGURE 5** Arrhenius plot for the hydrolysis of DTA with 1.0, 1.5 and 2.0 mol L<sup>-1</sup> HCl

decrease in the  $t_{1/2}$  (Table 4). Also, the energy of activation was determined by calculating the rate constant from the following equation (Martin, Swarbrick, & Cammarata, 1983).

$$\log \frac{k_2}{k_1} = \frac{E_a}{2.303R} \left( \frac{T_2 - T_1}{T_1 T_2} \right)$$

where  $E_a$  is the activation energy,  $T_1$  and  $T_2$  are the two temperatures in Kelvin,  $R$  is the gas constant, and 'k1' and 'k2' are the rate constants at the two temperatures used. The average calculated  $E_a$  was found to be 14.866 kcal/mol, which is a comparatively high value for amides (Pilling & Seakins 1995).

Another factor that affects the rate of the reaction is the acid strength of HCl, thus different normalities were used to study the hydrolysis reaction. The rate of hydrolysis increased with an increase in HCl concentration, although the effect was minor compared with the effect of temperature (Figure 4 and Table 4).

In conclusion, the acid hydrolysis of DTA was found to follow a pseudo-first-order reaction rate. Also the reaction rate increased with

**TABLE 4** Kinetic data of DTA acid degradation

HCl molarity	Temperature (°C)	K (h <sup>-1</sup> )	$t_{1/2}$ (h)
2 M	100	0.737	0.94
	90	0.439	1.58
	80	0.286	2.42
1.5 M	100	0.701	0.99
	90	0.390	1.78
	80	0.236	2.94
1 M	100	0.630	1.10
	90	0.298	2.33
	80	0.160	4.33

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

**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	HPLC method	Official method
Mean	100.04	100.85
SD	1.07	0.97
Variance	1.14	0.94
n	12	5
Student's t-test	1.46 (2.13)	

increase in the temperature and the strength of the acid solution.

Table 5.

## 5 | CONCLUSION

The proposed HPLC method provided a simple, sensitive, selective and accurate method for the determination of DTA in bulk powder and in its pharmaceutical formulation, without any interference from the excipients and in the presence of its degradation product. The proposed method was validated and could be used for routine analysis of DTA in quality control laboratories. The method can be used for determination of the rate of the acid degradation of DTA, calculation of the half-life and prediction of the expiration date.

### ABBREVIATIONS USED

DTA sodium diatrizoate

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