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Development and Validation of Stability Indicating UPLC Method for the Determination of Diatrizoic Acid Related Impurities in Bulk and Finished Formulations

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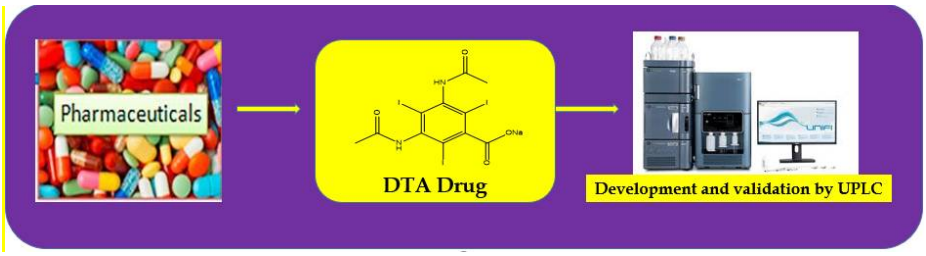
ABSTRACT

*A simple, sensitive and stability indicating RP-UPLC method was developed and validated for the estimation of Diatrizoic acid and its related impurities in bulk and finished dosage forms by using Acquity UPLC CSH C18 (1.7 µm 100*2.1 mm) column with 0.05 % formic acid in milli-Q water and acetonitrile as mobile phase in gradient elution mode with a run time of 12 min at a wavelength of 238 nm. Drug was subjected to forced degradation and the samples were analyzed by the developed method to establish the stability indicating power of the method. The LOD values were 0.01, 0.009, 0.012, 0.011 µg mL^{-1} and the LOQ values were 0.04, 0.027, 0.035, 0.034 µg mL^{-1} respectively for DTA and its 3 related substances. The average recovery values for DTA and impurities were found to be in the range of 97.4-101.9 %. The developed method was linear over a range of 0.03- 0.3 µg mL^{-1} for DTA impurities.*

Graphical Abstract



Keywords: Diatrizoic acid, Amidotrizoate, DTA, Meglumine, Contrast agents, Iodine, UPLC, Method validation, ICH.

INTRODUCTION

Diatrizoic acid (DTA) is a Radiographic Contrast Agent with X-ray contrast activity. It is an organic, iodinated radiopaque X-ray contrast medium used in diagnostic radiography. DTA is an iodine-containing X-ray contrast agent. Iodinated contrast agents were among the first contrast agents developed. Iodine is known to be particular electron-dense and to effectively scatter or stop X-rays. A good contrast agent requires a high density of electron-dense atoms. It's Therefore, more the iodine more the dense of the X-ray effect. Therefore iodine blocks the X-ray film exposure by radiation. This makes it possible to distinguish, on X-ray film, body parts that contain DTA from body parts that do not contain this agent and allows for visualization of different body structures. Iodine based contrast media are water soluble and harmless to the body. These contrast agents are sold as clear colorless water solutions, the concentration is usually expressed as mg I mL^{-1}. Modern iodinated contrast agents can be used almost anywhere in the body. Most often they are used intravenously, but for various purposes they can also be used intra-arterially, intrathecally (the spine) and intra-abdominally. DTA is used as DTA sodium as well as DTA meglumine for gastrointestinal studies, angiography and urography.

Diatrizoic acid may be used as an alternative to barium sulfate for medical imaging of the gastrointestinal tract, such as upper gastrointestinal series and small bowel series. It is indicated for use in patients who are allergic to barium, or in cases where the barium might leak into the abdominal cavity. It does not coat the stomach/bowel lining as well as barium. The osmotic effect of DTA or its combination with Meglumine draws fluid into the intestine, thus helping to dislodge the meconium impaction [**1-9**]. The most common side effects of DTA or its combination with Meglumine include vomiting, diarrhoea and skin redness. Other side effects of DTA administration include itchiness, kidney problems, low blood pressure and allergic reactions. DTA is not recommended in people who have an iodine allergy.

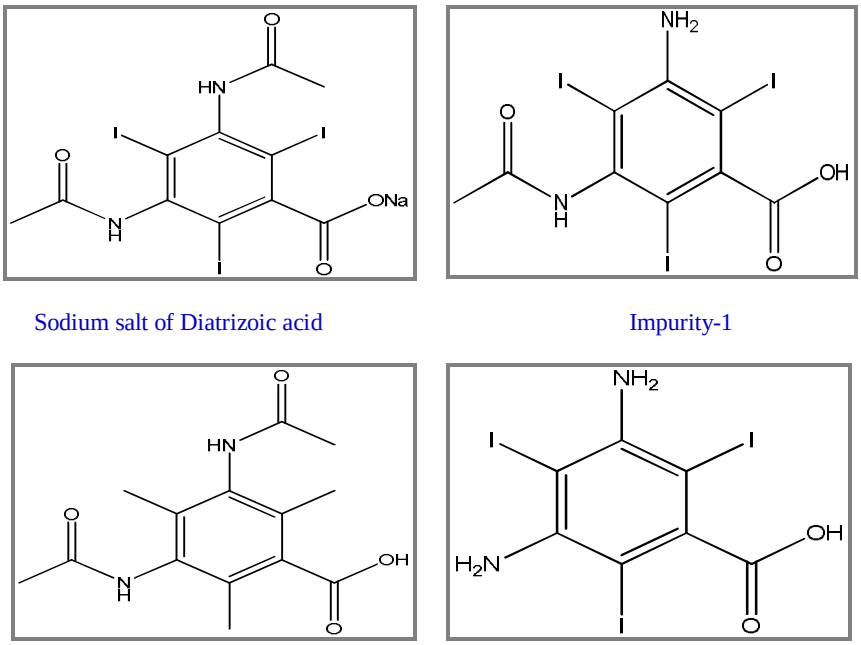
Methods for the determination of the content of DTA is official in both United states pharmacopeia (USP) and British pharmacopeia (BP), DTA related substances were monitored by means of thin layer chromatography and by UV for free aromatic amine (impurity-A). Till date no methods have been reported for the estimation of DTA and its related substances (Figure 1) by chromatographic techniques in bulk and finished dosage forms [**10**]. Hence there is a requirement for the development of stability indicating short run time method with proper peak shapes and resolution for the determination of impurities in drug substance and drug product.

The objective of the current work is to develop a stability indicating UPLC method with short run time. The objective is also to develop a stability indicating method by means of performing forced degradation studies on the bulk as well as finished formulations in order to study the impact of excipient on the developed method. The developed method need to be validated for the parameters as per the current ICH quality guidelines and USP general chapter for Analytical method validations [**11-14**]. Hence the proposed validated method can be used in quality control laboratories for routine as well as stability analysis of bulk and finished formulations.

Physical properties: Diatrizoic acid exists in the form of anhydrous as well as dihydrate form with molecular formula $C_{11}H_9I_3N_2O_4$, chemical name is 3, 5-diacetamido-2, 4, 6-triiodobenzoic acid and in general exists as sodium salt with molecular weight 613.92 g mol^{-1}. It is used in the form of dehydrate/anhydrous due to the hygroscopic nature of the molecule. DTA in general exists as a white powder. Solubility of DTA is determined by UV-Visible spectrophotometer in various solvents at 37°C and was observed as DTA is freely soluble in water shown in figure 2, slightly soluble in ethanol (96 %) and practically insoluble in acetone.DTA melts at about 261°C with decomposition. Partition coefficient in octanol-water system at 25°C is around 3.3. DTA is in general formulated in combination with meglumine as injection with various strengths. It is in general marketed under the

trade names Hypaque, Crystografin, Urovist with label claim as 65% and 76% w/v as DTA. Structures of DTA and its related impurities are shown in figure 1.

Structure of DTA and its related impurities:



Sodium salt of Diatrizoic acid Impurity-1

Impurity-2 Impurity-3

Figure 1. DTA and its related impurities.

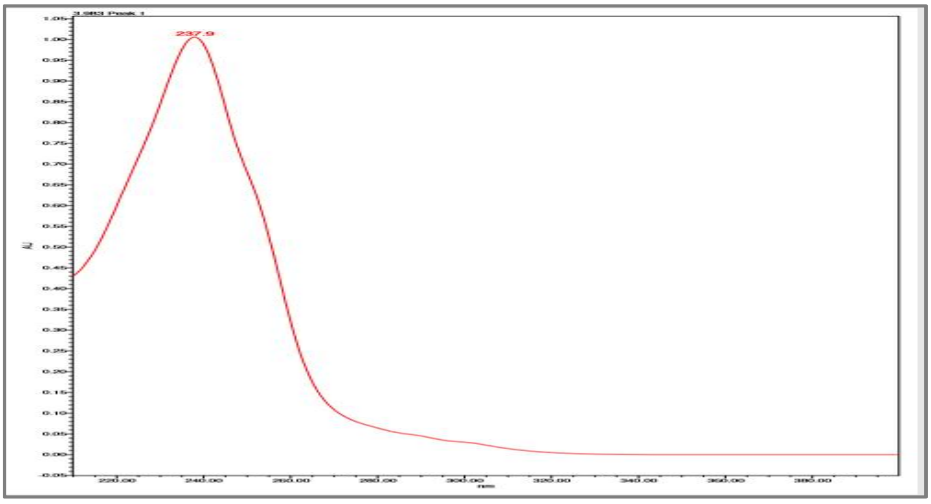


Figure 2. UV spectrum of DTA in water.

MATERIALS AND METHODS

Chemical reagents and sample: Diatrizoic acid drug substance is gifted by synthetic division of Vasudha pharmaceuticals Ltd, Hyderabad, India. Angiografin injection manufactured by German remedies was purchased from the local pharmacy. Acetonitrileof HPLC grade was purchased from Rankem chemicals (Mumbai, India). Formic acid, sodium hydroxide, hydrochloric acid and hydrogen

peroxide were purchased from Merck chemicals (Darmstadt, Germany). HPLC grade water was obtained from milli-Q water purification system (Millipore, Milford, USA).

Equipments: The UPLC system used for the chromatographic method development, forced degradation and validation is Waters UPLC H-Class quaternary pump separation module with a PDA detector. UPLC system consisted of quaternary pump and photodiode array detector. The signal output was monitored and processed using Empower-3 software on a Dell computer. Chromatographic separation was achieved on Acquity UPLC CSH C18 100*2.1 mm, with particle size of 1.7 µm was used. Thermal degradation study was carried out in a hot air oven (Vision lab Equipments), Ultrasonic bath sonicator was purchased from Thermo Analytics and photolytic degradation was carried out on photo stability chamber purchased from Thermo lab scientific instruments.

Chromatographic conditions: The objective of the present study is to develop a rapid stability indicating UPLC method for the estimation of impurities of DTA with proper peak shape and resolution. Chromatographic separation was performed on Waters UPLC with Acquity UPLC CSH C18 100*2.1 mm, 1.7 µm column. Mobile phase A 0.05% formic acid in water and mobile phase B 0.05% formic acid in acetonitrile were used for the chromatographic separation. Diluent was a mixture of water and acetonitrile in the ratio of 50:50 (v/v). Injection volume was 0.5 µL, Flow rate was 0.5 mL min^{-1}, column oven temperature 40°C, analysis was carried out at a wavelength of 238 nm with data acquisition time of 12 min.

Preparation of buffer: Dissolved accurately 0.5 mL of formic acid in 1000 mL of milli-Q water and mixed well.

Preparation of standard solution: A working standard stock solution of DTA was prepared by dissolving standard equivalent to 20 mg of DTA into 100 mL volumetric flask, to this added 60 mL of diluent and sonicated for 5 min and then diluted to the volume with diluent to have a solution concentration of 200 ppm.

Preparation of diluted standard: Diluted 1 mL of the standard stock solution to 100 mL with diluent and mixed well, further diluted 2 mL of the resulting solution to 20 mL with diluent. The obtained solution is of 0.2 ppm.

Preparation of sample solution: Transfer 2 mL of DTA injection into 100 mL volumetric flask and added 60 mL of diluent and sonicated in ultrasonic bath for 20 min with intermediate shaking and diluted to the volume with diluent. Further dilute 0.8 mL of the above sample solution to 50 mL with diluent and mix well. Filter the solution through 0.45 µm nylon membrane filter by discarding 4 mL of filtrate and injected the same solution (0.2 mg mL^{-1}).

Preparation of placebo solution: EDTA and Meglumine sample solutions were used as excipient in the DTA injection. So, in order to establish the specificity, EDTA and Meglumine were prepared at the test concentrations individually (0.2 mg mL^{-1}) in diluent and injected in the proposed method to verify the spectral interference if any at the retention times of DTA and its known impurities.

Method validation:

Specificity: Specificity is the ability of the method to measure the analyte response in presence of its potential impurities. Specificity of the developed method was carried out in the presence of blank, placebo and its known impurities i.e., imp-1, imp-2 and imp-3 for the accurate measurement of amount of impurities present in the sample. As a part of specificity, stress studies were carried out for DTA drug substance, drug product and placebo under stress conditions like oxidation, acid, base, photolytic and thermal (105°C). These stress samples were analysed using the proposed method at a

test concentration of 200 ppm to separate all the three DTA impurities along with its degradation impurities at a quantification level of 0.1 %. In these stress conditions the peak purity test was verified for the DTA peak and its other known impurities by using photo diode array detector.

Precision: Precision of the analytical method is the closeness agreement for a series of measurement from multiple samplings as mentioned in ICH Q2 (R1). As per the quality guidelines, method precision and intermediate precision were analyzed on the homogeneous sample and the % RSD of individual impurity for precision and intermediate precision was calculated and reported.

LOD and LOQ: The detection limit (LOD) and quantification limit (LOQ) for all the three impurities were established by means of linearity method. The impurity solutions from concentration ranging from 0.01 ppm to 0.3 ppm with 5 different levels (LOQ to 150%) were prepared and injected. Based on the impurity response and STEYX value, the least concentration of each impurity up to which it can be identified and quantified were calculated and verified.

Linearity: Linearity of the detector response was established for all the known impurities and DTA with concentration ranging from LOQ to 150 % of the specification level (0.1 %) with respect to test concentration. The samples were analyzed as per the described test method. A linearity graph was plotted between the responses of impurity (Y-axis) against actual concentration in ppm (X-axis) and determined the correlation co-efficient and Y-intercept at 100 % response.

Accuracy: Accuracy of the analytical method is the closeness of agreement between the true value and experimental value. Accuracy of the three impurities was performed at 5 different levels ranging from LOQ to 150 % of the specification level of the impurity with respect to test concentration level. The % recovery was calculated by comparing the impurity level at each level of spiked sample with as such sample.

Robustness: The robustness of the method was evaluated to establish the capability of the method by changing the experimental conditions and studying its impact on the system suitability. Robustness was performed by changing the method parameters like mobile phase flow rate and column temperature.

RESULTS AND DISCUSSION

Method development and Optimization: As there was no stability indicating UPLC method reported for the determination of related substances in DTA bulk and finished product. The intention of the method was to separate all the potential impurity peaks originate during the forced degradation study and stability studies with proper peak shape and resolution. Forced degradation sample was taken as reference, for the optimization of the UPLC method. Trials were taken by varying the pH value of the mobile phase buffer from 5.0 to 2.7. Acetic acid and formic acid buffer were used based on the LC-MS compatibility.

Forced degradation samples were injected and found that all the three acknowledged impurities (Impurity-1, Impurity-2 and Impurity-3) were spectrally pure with longer run time and vast peak shapes in isocratic mode. In order to shorten the run time, gradient separation mode was optimized with adequate separation. Optimal separation was achieved on Acquity UPLC CSH C18 column with dimensions 100*2.1 mm, 1.7 µm accompanied at 40°C. Gradient elution was executed using the combination of 0.05% formic acid in milli-Q water buffer (pH~ 2.7) and acetonitrile as organic modifier at a flow rate of 0.5 mL/min. UPLC detection was carried at wavelength of 238 nm. Sample compartment was maintained at a temperature of 5°C. Gradient programme was cited in table 1.

Mobile phase: Mobile phase A is 0.05 % formic acid in Milli-Q water. Mobile phase B is 0.05 % formic acid in Acetonitrile.

Table 1. Gradient Program for UPLC method.

Time (minutes)	Flow rate (mL min^{-1})	% of mobile phase A	% of mobile phase B
0.0	0.5	95	05
0.3	0.5	95	05
7.0	0.5	05	95
9.0	0.5	05	95
9.2	0.5	95	05
12.0	0.5	95	05

System suitability: System suitability solution was prepared by spiking all the impurities in the sample at the specification level (0.1%) and injected to evaluate the system suitability of the method and found that DTA and its 3 known impurities were separated with good resolution, with typical retention times of 3.97, 3.69, 4.36 and 5.35 respectively. Chromatogram with separation of impurities was mentioned in fig-3. The system suitability results were given in table 2. The developed UPLC method was found to be specific for DTA and its known impurities. Figure 3 shows separation of all the three known impurities from DTA in the proposed method along with blank, placebo and sample.

Table 2. System suitability criteria (Rt-Retention time, RRT-relative retention time)

S.No	Name	Rt (min)	RRT	Theoretical plates	Tailing	Resolution
1	DTA	3.97	1.000	14253	1.05	NA
2	Imp-I	3.69	0.929	--	--	--
3	Imp-II	4.36	1.098	--	--	--
4	Imp-III	5.35	1.347	--	--	--

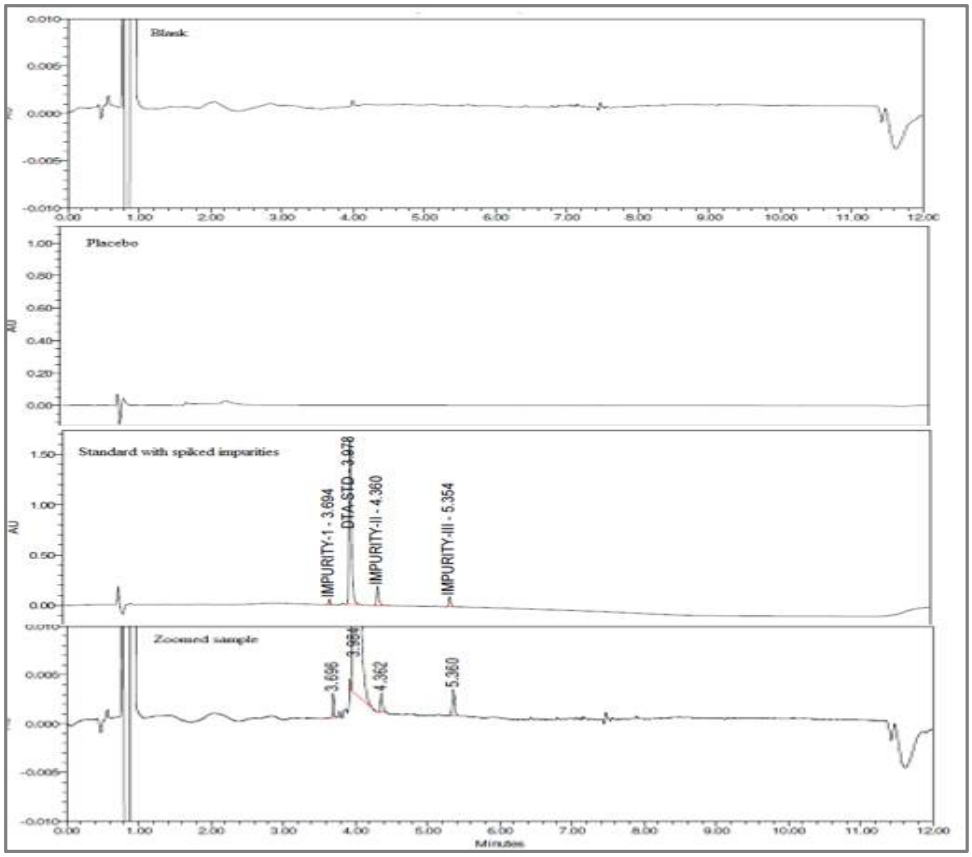


Figure 3. Blank, placebo, DTA spiked with impurities and sample chromatograms.

Precision: Method precision was performed by analyzing the spiked sample with known impurities at the specification level (0.1%) and % impurity was calculated. The % relative standard deviation was found to be 1.02, 0.74 and 1.17 for impurity-1, impurity-2 and impurity-3 respectively. Intermediate precision was performed on a different day, different UPLC system using different UPLC column of the same manufacturer. The relative standard deviation for these spiked samples was also within 2.0% limit. Cumulative RSD for method and intermediate precision for the three impurities were 1.19, 0.78 and 1.10 respectively.

The results were mentioned in table 3. Hence the developed method is precise for its intended use. Representative chromatogram for method precision was mentioned in figure 4.

Table 3. Method precision and intermediate precision data

S. No	Method precision			Intermediate precision		
	% impurity			% impurity		
	Imp-1	Imp-2	Imp-3	Imp-1	Imp-2	Imp-3
1	0.149	0.151	0.148	0.152	0.148	0.152
2	0.148	0.150	0.152	0.151	0.149	0.153
3	0.147	0.152	0.152	0.152	0.149	0.151
4	0.151	0.151	0.149	0.15	0.151	0.151
5	0.147	0.149	0.152	0.149	0.150	0.148
6	0.149	0.150	0.15	0.148	0.151	0.151
Avg	0.149	0.150	0.151	0.150	0.150	0.151
SD	0.002	0.001	0.002	0.002	0.001	0.002
% RSD	1.02	0.74	1.17	1.09	0.81	1.11
Cumulative average				**0.149**	**0.150**	**0.151**
Cumulative SD				0.002	0.001	0.002
Cumulative % RSD				**1.19**	**0.78**	**1.10**

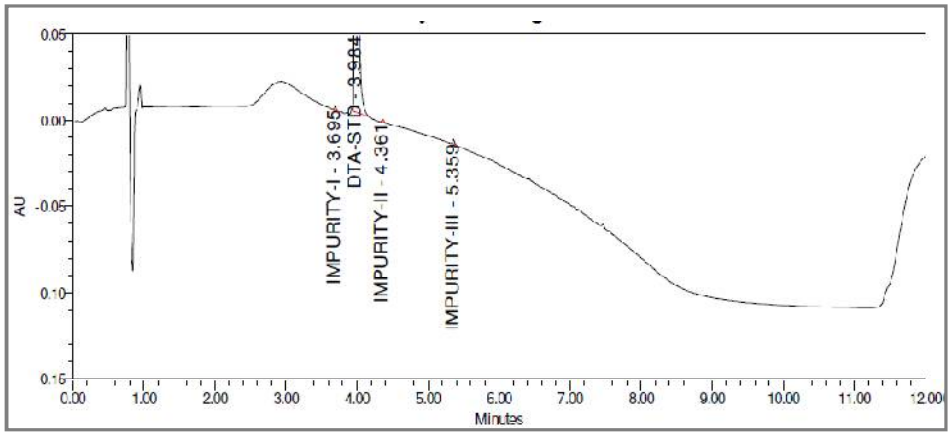


Figure 4. Method precision chromatogram for spiked sample.

LOD and LOQ: Limit of detection and limit of quantification were established by means of slope method. Five different concentration of DTA and its impurity mixture (imp-1, imp-2 and imp-3) below the specification limit of 0.1 % were prepared and injected**.** A calibration curve was plotted between concentration and response of the individual peaks. Slope and STEYX value were calculated for the calibration curve. The results were tabulated in table 4. From these values limit of detection (LOD) and limit of quantification (LOQ) values were calculated. The solution mixture of DTA and its impurities were prepared and injected to confirm the calculated LOQ values. The LOQ mixture chromatogram was mentioned in figure 5.

Table 4. LOD and LOQ establishment of DTA and its related known impurities

S.No	Concentration (ppm)	Area response			
		DTA	Impurity-1	Impurity-2	Impurity-3
1	0.05	125462	50245	76542	90215
2	0.1	252145	105610	151532	188491
3	0.2	509854	204581	311023	365698
4	0.3	767851	305498	451037	552389
5	0.5	1248752	502345	765008	900321
Correlation coefficient		0.9998	0.9999	0.9999	0.9999
Slope		2501992	1000837.3	1525916.7	1797190.9
STEYX		9177.515	2666.5	5318.7	6059.6
Limit of detection (LOD)		0.01	0.009	0.012	0.011
Limit of quantification (LOQ)		0.04	0.027	0.035	0.034
LOQ Precision (% RSD)		1.8	2.1	2.4	1.6

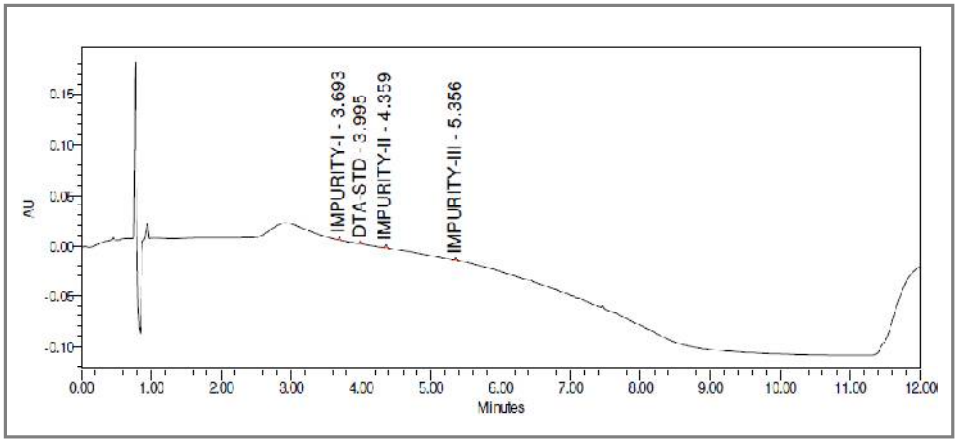


Figure 5. LOQ spiked chromatogram.

Linearity and range: Linearity of the developed method was evaluated for five different levels of DTA and its 3 known impurities. The concentrations ranged from LOQ to 150 % of impurity specification limit (0.1% with respect to test concentration). The respective peak area was recorded and plotted against standard (impurity) concentration and the graph resulted in straight line. The correlation coefficient, slope, intercept and % Y-intercept values were calculated and tabulated for DTA and its known impurities. The compiled results were tabulated below in table 4. Representative chromatogram of Linearity solution at 150% level was shown in figure 6.

Table 5. Linearity results of DTA and its related known impurities

S.No	Concentration (ppm)	Area response			
		DTA	Impurity-1	Impurity-2	Impurity-3
1	0.03	78165	29145	46947	56234
2	0.05	125462	50245	76542	90215
3	0.1	252145	105610	151532	188491
4	0.15	375444	154237	225596	274986
5	0.2	509854	204581	311023	365698
6	0.3	756435	316830	454596	565473
Correlation coefficient		0.9999	0.9997	0.9998	0.9997
Slope		2523242	1056940	1520043	1877152
Intercept		535.75	-2768.77	766.72	-2823.20
% Y-Intercept		0.21	-2.62	0.51	-1.50

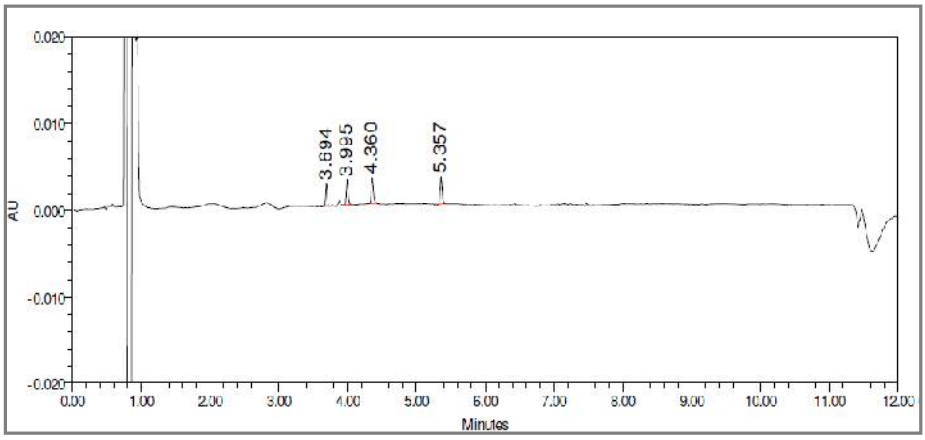


Figure 6. Linearity solution at 150% level.

Accuracy: Recovery studies were performed to judge the accuracy of the test method. The study was evaluated by spiking the known quantity of impurities at various levels on the placebo. From the amount of impurity found the % recovery was calculated. Recovery was performed at five different levels ranging from LOQ to 150 % of the specification level. The % recovery of each impurity was found to be within the acceptance criteria of 95% to 105%. So the method is accurate for the determination of DTA and its impurities. The mean recovery values for the impurities were tabulated in table 6.

Table 6. Accuracy results of DTA and its related impurities

S.No	Conc level	% Mean recovery ± SD		
		Impurity-1	Impurity-2	Impurity-3
1	LOQ	97.42 ± 0.52	99.87 ± 0.33	100.42 ± 0.24
2	50 %	98.63 ± 0.48	98.70 ± 0.29	101.90 ± 0.33
3	100 %	100.02 ± 0.34	99.16 ± 0.15	100.62 ± 0.62
4	150 %	99.26 ± 0.34	100.14 ± 0.05	98.06 ± 0.44

Solution stability: There was no change in the area counts of the DTA and its respective impurities, when both the standard and sample solutions were monitored periodically for a period of 24 h at room temperature (not more than 27°C) and at a temperature of 5 ± 3°C. It was observed that the standard solution is stable for 24 h at 5 ± 3°C and sample solution is stable for 8 hrs at 5 ± 3°C.

Specificity: The specificity of the method was evaluated by verifying the peak purity of the sample. The method was found to be specific as there was no interference from blank and placebo at the retention time of main peak. No degradant peaks were observed at the retention time of DTA during the forced degradation and stability study indicates that the method is stability indicating and also peak purity index for all the impurities and DTA were less than the purity threshold indicates that there was no spectral co-elution for any of the peaks in this method and also the resolution between the neighboring peak was greater than 2.0.

Forced Degradation Studies: Forced degradation studies were performed to establish the stability indicating power of the method. In this study DTA raw material, finished product and placebo were subjected to acidic, basic, peroxide, thermal and photolytic stress studies on sample concentration of 0.2 mg mL^{-1} in diluent. Sample equivalent to 20 mg of DTA was placed into 100 mL volumetric flask added 60 mL of diluent and sonicated for 10 min with intermediate shaking and then added respective degradant (Acid, Alkali, Oxidant) and performed the stress study. Samples were neutralized after degradation and then diluted to the volume with diluent and injected to verify the stability indicating

power of the analytical method. Details of stress conditions and results were tabulated in table 7. The chromatograms for forced degradation study were summarized figure 7.

Table 7. Forced degradation studies of DTA.

S.No	Stress condition	% Drug remained	% imp	Mass Balance
1	2N HCl, 60°C, 8 h	80.02	19.98	97.6
2	1 N NaOH ,60°C, 4 h	88.08	11.92	96.8
3	10 % H2O2, 60°C, 24 h	81.9	18.1	95.2
4	105 °C, 48 h	99.64	0.36	98.9
5	Photolytic stability	99.18	0.82	99.4

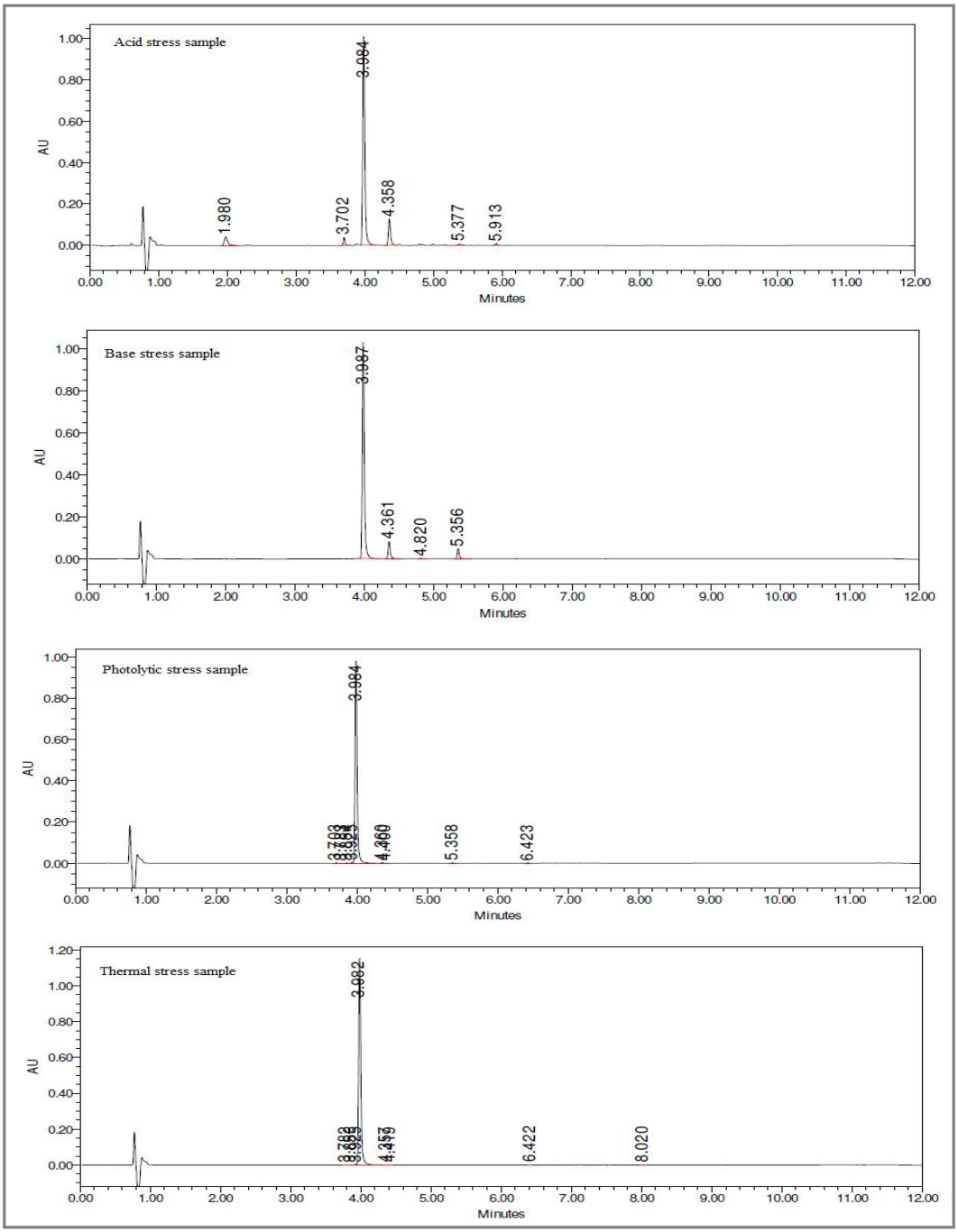


Figure 7. Acid, base, photolytic and thermal stress sample representative chromatograms.

The proposed UPLC method obeys linearity within the concentration range of 0.03-2.0 ppm for impurity-1, impurity-2 and impurity-3 with correlation coefficient of not less than 0.999 for all the three impurities. LOD values are 0.009, 0.012 and 0.011 ppm respectively for the three impurities and LOQ values are 0.027, 0.035 and 0.034 ppm respectively for the three impurities. Inter and intraday precision with cumulative % RSD for the impurities were found to be 1.2, 0.8 and 1.1 respectively for each impurity. % Recovery values for the three impurities were found to be between 97.4 and 101.9 for the concentration range between LOQ and 150% of the test concentration (0.2 mg mL^{-1}).

APPLICATION

There is no chromatographic method available till date for DTA. This method is mass compatible and useful in estimation of DTA and its impurities.

CONCLUSION

A novel, reverse phase liquid chromatographic method has been developed and validated for the estimation of DTA and its known impurities namely impurity-1, impurity-2 and impurity-3 using LC-MS compatible UPLC method. The proposed method is found to be simple, accurate, precise, linear, specific and robust. Hence it can be used for the determination of DTA and its related substances in bulk and in finished formulations.

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REFERENCES

[1]. A. S. Farag, Liquid chromatographic assay of diatrizoic acid and its diiodo degradation products in radio-opaque solutions. *Journal of AOAC International.*, **1995**, 78. 328-33.
[2]. Ens, Waldemar, Senner, Frank, Gygax, Benjamin, Schlotterbeck, Goetz. Development, Validation, and Application of a Novel LC-MS/MS Trace Analysis Method for the Simultaneous Quantification of Seven Iodinated X-ray Contrast Media and Three Artificial Sweeteners in Surface, Ground, and Drinking Water, *Analytical and bioanalytical chemistry*, **2014**, 406.
[3]. D. Rüetschi, Basler Trinkwassergewinnung in den Langen Erlen, Ph.D. dissertation, University of Basel, **2004**.
[4]. M Hanna, G, Lau-Cam, Cesar, A simple method for the identification and assay of iopamidol and iothalamate meglumine in pharmaceutical samples based on proton nuclear magnetic resonance spectroscopy, *Die Pharmazie*, **2001**, 56, 152-5.
[5]. J. Yakatan, Gerald, M. Tuckerman, Murray, Determination of organically-bound iodine in pharmaceuticals, *Journal of Pharmaceutical Sciences*, **1966**, 55, 532-4.
[6]. Alexander T. Yordanov, Adriana L. Lodder,, Eric K. Woller, Mary J. Cloninger, Nicholas Patronas, Diane Milenic, Martin W. Brechbiel, Novel Iodinated Dendritic Nanoparticles for Computed Tomography (CT) Imaging, *Nano Letters*, **2002**, 2(6), 595-599
[7]. B. Abramović, K Horváth, Gaál FAutomatic titrimetric determination of iodide in some pharmaceutical contrasting preparations, *J Pharm Biomed Anal.*, **1993**, 11(6), 447-50.
[8]. G. M. Hanna, C. A. Lau-Cam, Simple ^{1}H NMR spectroscopic method for assay of salts of the contrast agent diatrizoate in commercial solutions, *JAOAC Int.*, **1996**, 79(4), 833-8.
[9]. F Busetti, K. L. Linge, J. W. Blythe, A. Heitz, Rapid analysis of iodinated X-ray contrast media in secondary and tertiary treated wastewater by direct injection liquid chromatography-tandem mass spectrometry, *Journal of Chromatography A*, 2008, 1213, 200–208.

[10]. Development of a Method to Better Quantify Levels of the Persistent Organic Pollutants Iodinated X-Ray Contrast Media and Artifi cial Sweeteners Found in Water . Waldemar Ens. International labmate-August 2014. ICH guidelines for the stability of new drug substances and products, Q1A (R2) ICH, Geneva, **2005**, 1-13.
[11]. Guideline for Industry. Text on Validation of Analytical Procedures, United States, March, **1995**.
[12]. ICH guidelines for validation of analytical procedures: text and methodology,Q2 (R1) ICH, Geneva, **2005**, 1-14.
[13]. M. Blessy, D. P. Ruchi, N. P. Prajesh, Y. K. Agarwal, Development of forced degradation and stability indicating studies of drugs, *J. Pharm. Anal.*, **2014**, 4, 159-65.
[14]. FDA: Guidance for Industry, *Analytical Procedures and Methods Validation*, August, **2000**.