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A Sensitive, Stability indicating UPLC method for the identification and characterization of forced degradation products for Drometrizole Trisiloxane through MS^n studies

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ABSTRACT

A rapid, simple and reliable gradient ultra performance liquid chromatography combined with tandem mass spectrometry method was developed and validated for separation, recognition, and characterization of forced degradation products for Drometrizole Trisiloxane. As per International Conference on Harmonization guidelines, the drug was exposed to acidic, basic, photolytic, oxidative and thermal conditions. The main drug shows extensive degradation towards stress conditions such as acid and base hydrolysis. The three degradation products were identified. The chromatographic separation was achieved through the C8 column 2.1 × 100, 1.8 µm) from linear gradient elution and the wavelength detection was set at 305 nm for drug and its forced degradation products. The parameters such as specificity, linearity, accuracy, precision, and robustness were used for validation of the method. The sequential pathway for the fragments was achieved through the acquired mass spectra from drug and its degradation products through LC/MS/MS studies. Accurate masses of drug and its degradation products were confirmed through LC-MS/Q-ToF analysis. Degradation products were characterized by comparing with the pattern of drug molecule fragmentation.

INTRODUCTION

Drometrizole Trisiloxane (DRT) is UV absorber and UV filter. It is a lipophilic benzotriazole derivative and widely used in sunscreen cosmetics which help in the absorption of UV radiation. The drug is soluble in oils and photostable compound. UV sunscreen cosmetic product containing UV filters in its formulation helps in protecting the skin from direct exposure to the UV-light and avoids or minimizes the damage due to the UV radiation which causes human health problems. To prevent the harmful effect on the skin through UV radiation leads to the development of organic chemicals called as UV filters (Hughes *et al*., 2005). The quality of Stability features of active pharmaceutical ingredient (API), explains the drug product quality attributes. The inherent stability of chemical from drug molecule can be found by carrying out various forced degradation studies with different conditions like acidic and basic hydrolysis, photolysis, oxidation and thermal. These studies are especially important for the daily usage of drugs and help in recognizing the proper storage conditions and for drug formulations (Abiramasundari *et al*., 2015).

Literature survey reveals following methods reported viz. the determination studies for sixteen different UV filters in sun care formulations by using high-performance liquid chromatography (Schakel *et al*., 2004); Simultaneous determination of thirteen UV Filters in Sunscreen Products (Application note, Schimadzu, 2006). The drug substance monograph on Drometrizole Trisiloxane in Authorized USP Pending monograph (USP, Authorized pending monograph, 2011) lists six impurities. There were no systematic studies found for the forced degradation behavior of DRT. In recent days, many works were published on characterization studies of probable impurities developed from API and formulation drugs using ICH guidelines with different advanced analytical test methods (Abiramasundari *et al*., 2015; Chinthalpati *et al*., 2018; Gawande *et al*., 2017; Jian *et al*., 2015; Krishnam *et al*., 2017a;

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Krishnam *et al*., 2017b; Prasad *et al*., 2016; Roshan *et al*., 2012).

The recognition of degradation products for DRT helps in further metabolic studies and helps in determination of process related impurity during its bulk synthesis. We have focused our research into three stages in the present study as follows: 1) To develop a suitable validated method by using reversed-phase Ultra performance liquid chromatography (RP-UPLC) for recognition of degradation products formed during application of various forced conditions on drug as per ICH guidelines (International Conference on Harmonization, 2006). 2) Identification and characterization of degradation products using ultra performance liquid chromatography (UPLC) combined with tandem mass spectroscopy (LC/MS^n). 3) The degradation products were subjected through LC-MS/Q-TOF to confirm the proposed structures.

EXPERIMENTAL

Drugs and reagents

DRT and its related impurity samples were used from USP-India, Hyderabad. High pure HPLC grade solvents like Methanol, Acetonitrile, and chemicals like hydrochloric acid, sodium hydroxide, and hydrogen peroxide were used from Merck Corporation, Mumbai, India. The high grade purified water was obtained from a Milli-Q-Water purification system (Millipore Corporation, Billerica, MA, USA).

Instrumentation

The Waters Acquity H-Class UPLC system provided with photodiode array (PDA) detector and Zorbax SB RRHD C8, 100 mm × 2.1 mm column packed with 1.8 µm particles from Agilent was used for the chromatographic separation. The Empower 3 software was used for chromatographic data acquisition. All weights were done on Sartorius balances (CPA225D). The photostability chamber from Atlas SUNTEST XLS+ (Atlas Material Testing, USA) was used for photolytic stress study. The Espec SH-642 Temperature and Humidity Benchtop Chamber (AT Equipment Corporation, CA, USA) was used for humidity stress study. The stress study for thermal conditions was performed in a Memmert hot air oven (100-800 Model, Make: Memmert KG, Germany).

The ion trap mass spectrometer from Thermo Scientific, USA attached to a Dionex Ultimate 3000 UPLC quaternary gradient was used to perform LCMS/MS analysis. LC-MS/MS experimental studies were performed to identify the different patterns of fragmentation of DP1, DP2, DP3, and DRT which creates linkage in a well-ordered manner helps to draw the structures for fragments. The heated electrospray ionization (HESI) source was used for analysis of MS study using both ion modes such as positive and negative. The parameter settings for a mass source were used are 4.0 kV of voltage for the source, 350°C temperature for capillary, 350°C temperature for source heater, 45.0 sheath gas flow, 20.0 of auxiliary gas flow, 60% S-Lens RF level. The data acquisition was done through Xcalibur software (Krishnam *et al*., 2017b).

The SYNAPT G2Q-TOF mass spectrometer from Waters Corporation attached to Waters Acquity H-Class UPLC having a quaternary gradient pump was used to perform the LC-Q-TOF-MS analysis. This study helps to recognize and drive suggested chemical formula for the degradation products by providing mass measurements in accurate from mass accuracy studies. Mass spectrometry analysis was performed with the positive ion mode using electrospray ionization (ESI) source. Parameter settings for a source were kept as follows; 3.25 kV of capillary voltage, 120°C of source temperature, 250°C of desolvation temperature, 40 V of sampling cone, 3.0 V of extract ion cone, and 800 L h^{-1} of desolvation gas flow. The data acquisition was done through Masslynx 4.1 software (Krishnam *et al*., 2017b).

Chromatographic conditions

The column used was Agilent SB RRHD C8 (100 mm × 2.1 mm) with 1.8 µm particles for the studies on chromatographic separation. The water (Solution A) was used as mobile phase A and methanol (Solution B) was used as mobile phase B. Flow rate for the mobile phase was maintained at 0.25 mL min^{-1}. The gradient for UPLC was set as time (min)/% Solution B: 0/80, 6/80, 10/100, 16/100, 16.2/80 and 18/20. The temperature for the column was maintained at 30°C and the wavelength was kept as 305 nm. The volume of 2 µL was used for the injection. The diluent used for the analysis was methanol. The 100 µg mL^{-1} concentration for the sample was used for Assay and 500 µg/mL^{-1} concentration for the sample was used for related substances.

Preparation of sample solution for assay

Accurately weighed and transferred 10 mg of Drometrizole Trisiloxane into 100 ml dry and clean volumetric flask (VF) and 70 ml of methanol was added, dissolve completely and dilute to volume with methanol up to the mark to obtain 100 µg mL^{-1} concentration.

Preparation of organic impurities sample solution

Accurately weighed and transferred 10 mg of Drometrizole Trisiloxane into a 20 mL clean dry volumetric flask, added 7 mL methanol to dissolve completely and made the volume up to the mark with the methanol to obtain a concentration of 500 µg mL^{-1}.

Preparation of impurity standard solutions

A impurities stock solution containing a mixture of impurity (Imp)1, Imp2, Imp 3, Imp4, Imp5 and Imp6 at a concentration of 100 µgmL^{-1} was also prepared in methanol.

Preparation of organic impurities standard preparation

A preparation of a stock solution of DRT and its impurities (100 µg mL^{-1}) was made by taking an appropriate amount in the diluent and dissolves it. The preparation of working solution was done from above stock solution for determination of organic impurities solution containing 1 µg mL^{-1} concentration in the diluent.

Analytical method validation

According to the ICH guidelines (International Conference on Harmonization, 2005), the developed method was validated and used for determination of DRT and six related compounds. The method was tested using parameters such as system suitability, linearity, accuracy, sensitivity, specificity, and robustness.

System suitability

This test was performed by injecting the replicates of a mixture of DRT (100 µg mL^{-L}) and 6 related compounds (100

µg mL^{-1} each impurity). The system suitability criterion was established and confirmed through the parameters using tailing factor, resolution, percentage RSD and theoretical plates. If the criteria were not specified consider the values as follows, not less than 1.5 for resolution, not more than 2.0% value for RSD, the tailing factor range can be 0.7–1.3 and column efficiency in terms of theoretical plates should be greater than 3000.

Linearity

The five different levels of concentration ranges between 0.005 to 1.0% of analyte for impurities were prepared from stock solutions as impurity linearity solutions. The peak area versus concentration data were subjected to least-squares linear regression analysis. The calibration curve was developed by plotting impurity areas against the concentration expressed in µg mL^{-1}. For the Linearity, test solutions were prepared for assay method from stock solutions at five concentration levels from 50 to 150% of analyte concentration (50, 75, 100, 125 and 150 µg mL^{-1}). The calibration curve was drawn by plotting DRT peak areas against the concentrations expressed in µg mL^{-1}.

Accuracy

The accuracy of related substance method was evaluated in triplicate at 0.005% to 1.0%, of analyte concentration (500 µg mL^{-1}). The percentage of recovery for all impurities was calculated. For bulk drugs, assay method accuracy was evaluated in triplicate at three different levels of concentration from 50 to 150 µg mL^{-1}. Prepared and injected three sets of solutions in triplicate at each concentration. The percentage of recovery was derived.

Sensitivity

The Limit of detection (LOD) and Limit of quantification (LOQ) for all six impurities were estimated at a signal-to-noise (S/N) ratio of 3:1 and 10:1 respectively to determine the sensitivity of the developed method. For this study, a series of diluted solutions are injected having known concentration of impurities. Injected six preparations of all six impurities to perform the precision study at the LOQ level and calculated the percentage relative standard deviation for the areas of each impurity (Thomas *et al*., 2012).

Robustness

The experimental conditions were intentionally changed to determine the robustness study of developed method. The system suitability parameter like resolution (Rs) between DRT, Imp1, Imp2, Imp3, Imp4, Imp5 and Imp6 was evaluated. The actual flow rate for mobile phase was 0.25 $mLmin^{-1}$. The flow rate effect on developed method was studied as follows, 0.02 flow units were changed (i.e. 0.23 and 0.27 $mLmin^{-1}$). The effect of column temperature was studied at 20°C and 30°C in place of 25°C for the developed method. The mobile phase components were remained constant in all of the above-varied conditions.

Specificity

The peak response for the analyte in the presence of its potential impurities was measured through specificity study. Drug substance stress testing was carried out to recognize the possible degradation products. This study helps to determine the inherent stability of the molecule and establish its degradation pathways.

Stress degradation studies were performed at an initial concentration of 100 µg mL^{-1} of DRT in active pharmaceutical ingredients to provide the stability-indicating property and specificity of the method as per ICH guidelines (2003). The degradation studies were carried out by attempting intentionally for stress conditions as follows: Acid (0.0001 M HCl for 6 hours at room temperature), Base (0.1 M NaOH for 1 hour at room temperature), Oxidation (6% peroxide for 24 hours at room temperature), Thermal (Exposed at 105°C for 48 h), Humidity (Exposed to 85°C and 85% RH for 3 days) and photolytic stress (1.2 million lux hours followed by 200 watt-hours per square meter).

RESULTS AND DISCUSSION

Method development and optimization

The main aim of this study to develop a new accurate, reproducible, robust and stability indicating, linear method for determination of DRT and its impurities by using RP UPLC, which helps for quality control laboratories routine use and to develop the method which is compatible for LC/MS to perform MS studies both qualitative and quantitatively.

To identify the wavelength maximum absorbance for DRT and its impurities, the individual stock solutions were scanned through photodiode array detector from 200 to 400 nm range and scrutinized the spectrum of each component at different wavelengths. Then identified and confirmed that DRT and its impurities are having the maximum absorbance at about 305 nm wavelength. Based on this study, the wavelength at 305 nm was selected for this research work to identify and characterize the DRT and all impurities.

Table 1: System suitability results.

System suitability from Organic impurities solution					
Compound name	RT*	RRT#	USP Resolution	% RSD	m/z value
Impurity-1	2.26	0.18	-	0.30	226.25
Impurity-2	4.69	0.38	17.74	0.44	280.25
Impurity-3	5.21	0.42	2.85	0.21	280.17
Impurity-4	6.82	0.55	7.30	0.66	282.25
Impurity-5	11.25	0.91	26.71	0.42	522.17
Drometrizole Trisiloxane	12.42	1.00	15.33	0.69	524.26
Impurity-6	12.88	1.04	6.27	0.44	744.26
System suitability from assay standard					
Compound name	RT*	USP Tailing	USP Plate count	% RSD	m/z value
Drometrizole Trisiloxane	12.42	0.94	514799	0.44	524.26

*RT: Retention time; #RRT: Relative Retention time.

Table 1 shows DRT and its impurities were scanned in LC-MS detector and m/z values were identified for known and unknown impurities. The 1000 µg mL^{-1} DRT sample preparation spiked with all the impurities (1 µg mL^{-1}) was used for the separation by RP-UPLC using different buffers, differences in gradient elution by using different columns. The more desirable separation was achieved from C8 column compared to other columns. The chromatographic separation was finally optimized

and identified with UPLC and LC-MS on Agilent SB C8 RRHD (100 × 2.1) mm with 1.8 µm column. The water was used as mobile phase A and methanol was used as mobile phase B. The mobile phase flow rate was kept at 0.25 mL min^{-1}. The program for gradient was set as: time (min)/% solution B: 0/80, 6/80, 10/100, 16/100, 16.2/80 and 18/80. The temperature for the column was kept at 30°C and the wavelength detection was recorded at 305 nm. The 2 µL volume of solution was used for injection and the used diluent was methanol. The 500 µg mL^{-1} concentration for related substances sample and 100 µg mL^{-1} concentration for assay method sample were used. The relative retention times (RRT) for Imp1, Imp2, Imp3, Imp4, Imp5 and Imp6 were 0.18, 0.38, 0.42, 0.55, 0.91 and 1.04 respectively (Table 1).

Method Validation

Precision

For Assay method, the Percentage RSD for the peak area responses from DRT was 0.4 from precision and intermediate precision study. For the related substances precision method, the percentage RSD for peak area responses of Imp1, Imp2, Imp3, Imp4, Imp5, and Imp6 were within 2.0, confirming the good precision of the developed analytical method (Table 1).

Sensitivity

This study represents the drug concentration that achieves a signal-to-noise ratio of 3 for LOD and 10 for LOQ. The drug concentration was found to be 0.03 µg mL^{-1} and 0.10 µg mL^{-1}, respectively for LOD and LOQ. The percentage RSD for all impurities was below 2% from the precision study at LOQ concentration.

To achieve the resolution between Imp-2 and imp-3 peaks was critical. Resulting, the optimization of the method was done to achieve resolution factor (Rs) above 2.0. The tailing factor was attained less than 1.0. The accuracy was calculated by injecting all impurities at three different levels of concentration, i.e., 80%, 100% and 120% with respect to target level with triplicate injections for the developed method. The high recovery percentages averaging between 98.3–101.6% were achieved through this method. The linearity of the assay was tested from 0.05 g mL^{-1} to 0.15 g mL^{-1} for DRT and six impurities. This developed method demonstrated good linearity within tested range and the correlation coefficients (R^2) were greater than 0.999 for DRT and its six impurities.

Stress studies and drug degradation behavior

The drug showed extensive degradation in 0.0001 M HCl for 6 hours at room temperature and one degradation product (DP3) was formed (Figure 1). This drug has shown remarkable degradation towards 0.1 M NaOH treatment at room temperature and after 1 hour three degradation products (DP1, DP, and DP3) were formed (Figure 1).

DRT was stable under oxidation, photolysis, thermal and humidity conditions. No significant degradation was observed until the study period.

The Peak purity test results derived from PDA detector from degradation studies confirm that DRT peak was pure and homogeneous from all analyzed stress samples. The mass balance values were calculated for all stressed samples and observed all values were close to 99.6%. This study confirms the developed method by UPLC was found to be specific in the presence of all impurities and its degradation products and indicates the stability power of this method.

During a study on stress degradation, a total of three DP1, DP2 and DP3 were formed from DRT. Among all the degradation products (DPs), DP1 and DP2 were formed and identified under alkaline conditions; DP3 was formed and identified in both acidic and alkaline conditions. The chromatogram shows clear separation of DRT and all the DPs were shown in Figure 2. The DPs of DRT are marked as DP1 to DP3 in the sequence of peak appeared from left to right in the chromatogram. From this stress studies, the DRT was found to be more susceptible to both acidic and alkaline studies. The forced degradation study and stress composition behavior data were shown in Table 2.

Characterization of DRT and its degradation products

From DRT MS studies, the total of six fragments were formed and identified. The determination of each fragment origin, a multi-stage (MS^n) mass fragmentation study was performed and this helped to propose the fragmentation pathway of DRT. The molecular ion displays as m/z 524.01 was considered as sodium adduct since the mass was shown ~23 Da higher value than the DRT molecular ion peak (m/z 501.24). To make clear about the degradation patterns of DRT, ESI-MS Spectrum of DRT molecular ion ($[M+Na]^+$) at m/z 524 was examined. The MS/MS spectrum displays product ions at m/z 434 (loss of $C_3H_{10}OSi$ from m/z 524), m/z 362 (loss of C_3H_8Si from m/z 434), m/z 346 (loss of CH_4 from m/z 362), 418 (loss of loss of CH_4 from m/z 434), m/z 380 (loss of $C_6H_{16}Si_2$ from m/z 524), m/z 376 (loss of C_4H_{10} from m/z 434). From the data obtained from LC-MS/MS studies sequence spectra for each degradation product, the final proposed fragmentation pathway of DRT was established. The proposed pathway of degradation for DRT is shown in Figure 2. The masses and proposed structures for all the DPs were explained (Ravi *et al*., 2014).

From the LC-ESI-MS spectrum, all three DPs were identified based on $[M+H]^+$ ions observed. Further characterization for all DPs was performed by selecting molecular ions for MS^n studies. The accurate mass measurement was also conducted to confirm the molecular formulae of DRT and its DPs. The spectra from HRMS study for all the DRT and DPs in the positive mode were shown in Figure 3. The accurate mass data for protonated molecules was determined and calculated error in parts per million (ppm) between exact and accurate masses are tabulated in Table 3 and the proposed formulae of fragments are shown in Table 4.

DP1 (m/z 380)

The LC/MS/MS and HRMS data confirm that molecular ions at m/z 380 and 524 are from DP1 and DRT. The significant loss of $C_6H_{16}Si_2$ is due to hydrolysis and loss of two units of trimethylsilanol in DRT. The spectrum from MS/MS specifies product ions at m/z 362 (loss of H_2O from m/z 380), m/z 344 (loss of $2H_2O$ from m/z 380) and m/z 346 (loss of CH_4 from m/z 362). The ESI-QTOF spectrum showed the molecular formulae of $C_{18}H_{23}N_3O_3SiNa^+$ with an error of 5.00 ppm. The molecular ion with m/z 380 was characterized as (3-(3-(2H-benzo[d][1,2,3]triazol-2-yl)-2-hydroxy-5-methylphenyl)-2-methylpropyl)(methyl)silanediol. The fragmentation pathway of DP1 was shown in Figure 4.

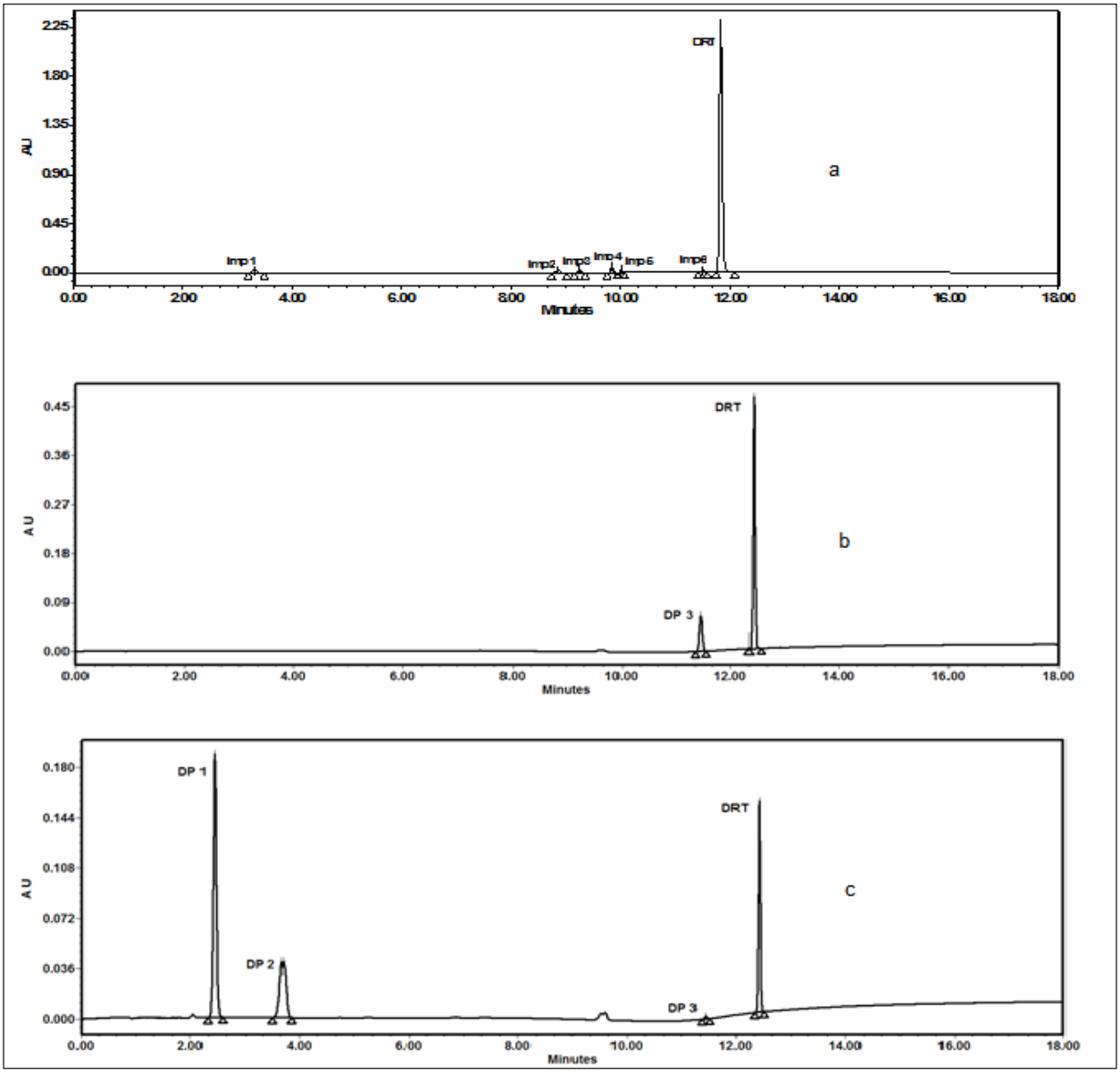

Fig. 1: Full Chromatograms for (a) organic impurities standard preparation, (b) acid degradation and (c) base degradation.

Table 2: Forced Degradation study data.

Degradation conditions	% Degraded, Peak name	Purity angle	Purity Threshold	Mass balance (%)
0.0001 M HCl for 6 hours	18.0, DP 3	0.237	0.341	99.5
0.1 M NaOH bench top at 1 hour at Room temperature	53.0, DP 1	0.215	0.332	99.4
	19.5, DP 2	0.247	0.345	
	1.0, DP 3	0.215	0.327	
Stressed with 6% H_2O_2, 24 hours kept on bench top at Room temperature	Main Peak (No degradation)	0.221	0.315	99.6
Thermal at 105°C for t 48 hours	Main Peak (No degradation)	0.245	0.352	99.9
Exposed to Visible light for about 1.2 Million Lux-hours and UV light for about 200 Watt-hours/meter square	Main Peak (No degradation)	0.218	0.329	99.8
Humidity 85% RH and 85°C for 3 days	Main Peak (No degradation)	0.243	0.342	99.7

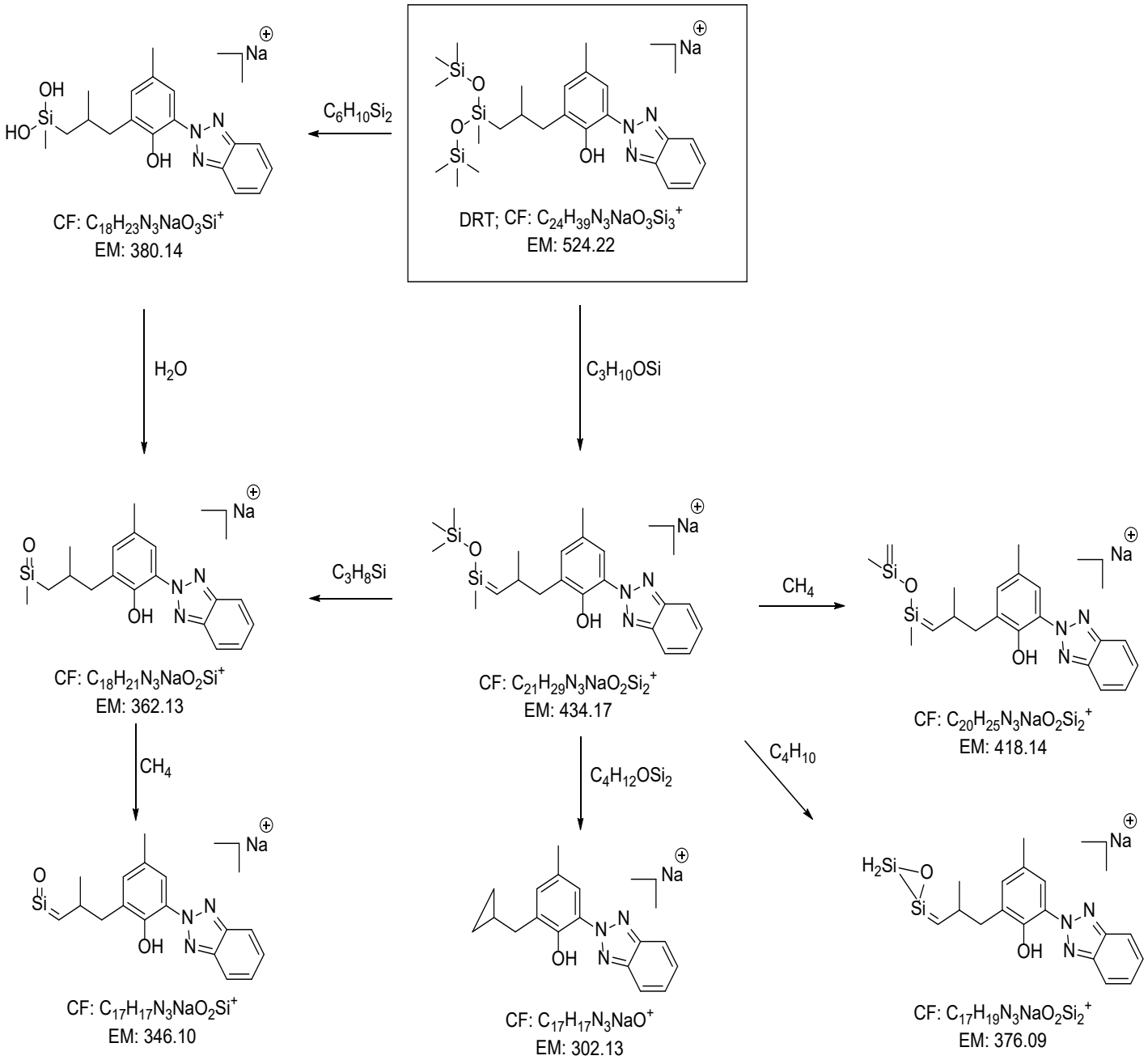

Fig. 2: Mass fragmentation pathway of DRT.

Table 3: Mass accuracy table for DRT and its degradation products (DP 1 to DP 3).

Compound Name	Retention Time (Min)	Molecular formula	Calculated m/z	Observed m/z	Error (mDa)
Drometrizole Trisiloxane	12.61	$C_{24}H_{39}N_3O_3Si_3.Na^+$	524.2191	524.2195	-0.40
DP 1	2.50	$C_{18}H_{23}N_3O_3Si.Na^+$	380.1401	380.1382	1.90
DP 2	3.72	$C_{19}H_{25}N_3O_3Si.Na^+$	394.1563	394.1533	3.00
DP 3	11.57	$C_{22}H_{33}N_3O_3Si_2.Na^+$	466.1953	466.1936	0.17

Table 4: MS/MS data of DRT and Degradation products (DP1 to DP3) in ESI positive mode.

Compound name	Retention Time (Min)	Molecular formula	Observed m/z	Molecular Formula for Fragment ions (m/z)	Fragment Ions (m/z)	RDB (Ring Plus Double Bond)
Drometrizole Trisiloxane	12.61	$C_{24}H_{39}N_3O_3Si_3.Na^+$	524.2195	$C_{21}H_{29}N_3NaO_2Si_2^+$	434	10.5
				$C_{20}H_{25}N_3NaO_2Si_2^+$	418	11.5
				$C_{18}H_{23}N_3NaO_3Si^+$	380	9.5
				$C_{17}H_{19}N_3NaO_2Si_2^+$	376	11.5
				$C_{18}H_{21}N_3NaO_2Si^+$	362	10.5
				$C_{17}H_{17}N_3NaO_2Si^+$	346	11.5
				$C_{17}H_{17}N_3NaO^+$	302	10.5
DP 1	2.50	$C_{18}H_{23}N_3O_3Si.Na^+$	380.1382	$C_{18}H_{21}N_3NaO_2Si^+$	362	10.5
				$C_{17}H_{17}N_3NaO_2Si^+$	346	11.5
				$C_{18}H_{19}N_3NaOSi^+$	343	11.5
DP 2	3.72	$C_{19}H_{25}N_3O_3Si.Na^+$	394.1533	$C_{18}H_{21}N_3NaO_2Si^+$	362	10.5
				$C_{17}H_{17}N_3NaO_2Si^+$	346	11.5
DP 3	11.57	$C_{22}H_{33}N_3O_3Si_2.Na^+$	466.1936	$C_{21}H_{29}N_3NaO_2Si_2^+$	434	10.5
				$C_{20}H_{25}N_3NaO_2Si_2^+$	418	11.5
				$C_{18}H_{23}N_3NaO_3Si^+$	380	9.5
				$C_{17}H_{19}N_3NaO_2Si_2^+$	376	11.5
				$C_{18}H_{21}N_3NaO_2Si^+$	362	10.5
				$C_{17}H_{17}N_3NaO_2Si^+$	346	11.5
				$C_{17}H_{17}N_3NaO^+$	302	10.5

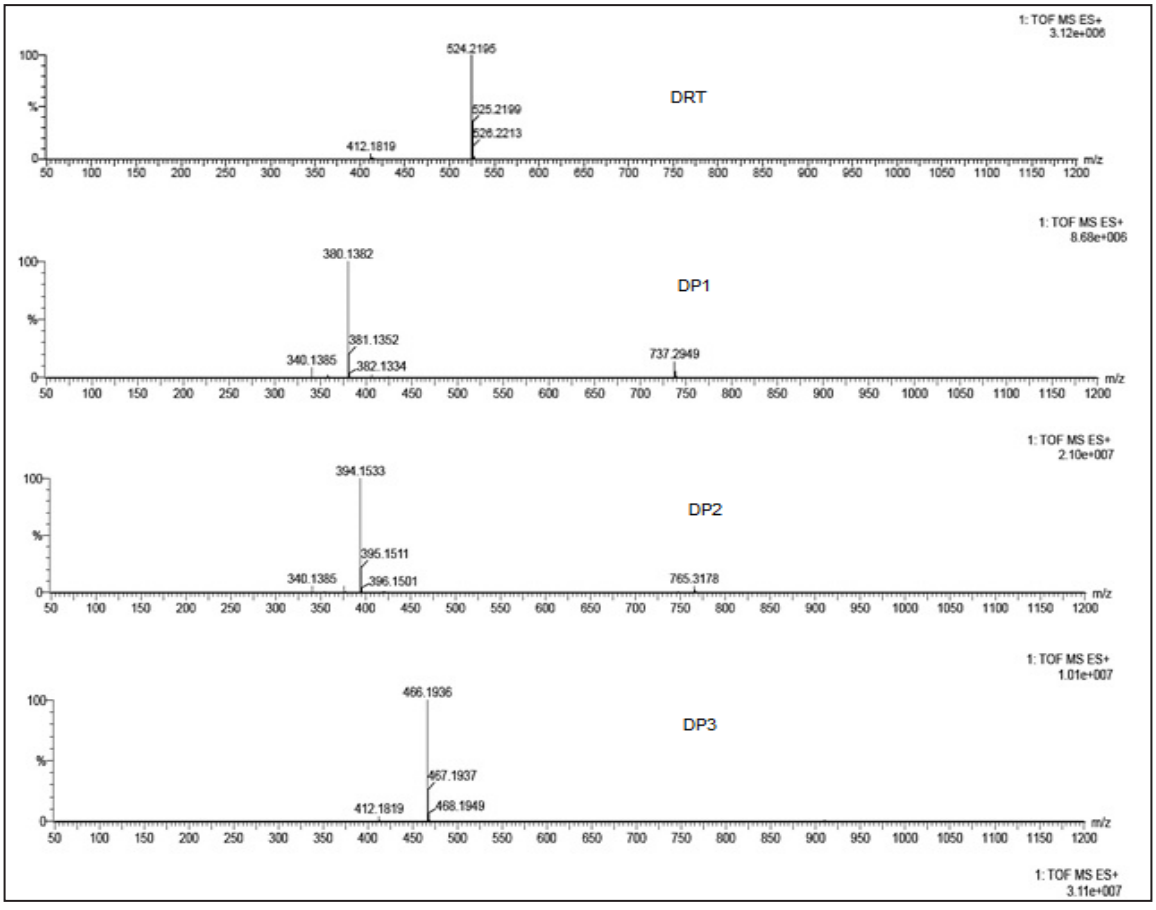

Fig. 3: HRMS Spectra for DRT and its DPs (DP1 to DP3).

DP2 (m/z: 394)

The LC/MS/MS and HRMS data confirm that the molecular ions at m/z 394 and 524 are from DP2 and DRT. The significant loss of $C_5H_{14}Si_2$ is due to water and methanol hydrolysis at two units of trimethylsilanol in DRT. The MS/MS spectrum specifies product ions at m/z 362 (loss of CH_3OH from m/z 394) and m/z 346 (loss of CH_4 from m/z 362). The ESI-QTOF spectrum showed the molecular formulae of $C_{19}H_{25}N_3O_3SiNa^+$ with an error of 7.61 ppm. The molecular ion with m/z 394 was characterized as (3-(3-(2H-benzo[d][1,2,3]triazol-2-yl)-2-hydroxy-5-methylphenyl)-2-methylpropyl)(methoxy)(methyl)silanol. The fragmentation pathway of DP2 was shown in Figure 4.

DP3 (m/z: 466)

The LC/MS/MS and HRMS data confirm that the molecular ions at m/z 466 and 524 are from DP3 and DRT. The significant loss of C_2H_6Si is due to methanol hydrolysis and loss of one unit of trimethylsilanol in DRT. The MS/MS Spectrum specifies product ions at m/z 434 (loss of CH_4O), m/z 376 (loss of C_4H_{10} from m/z 434), m/z 362 (loss of C_3H_8Si from m/z 434). The ESI-QTOF spectrum showed the molecular formulae of $C_{22}H_{33}N_3O_3Si_2Na^+$ with an error of 3.65 ppm. The molecular ion with m/z 394 was characterized as 2-(2H-benzo[d][1,2,3] triazol-2-yl)-6-(3-(1-methoxy-1,3,3,3-tetramethyldiziloxanyl)-2-methylpropyl)-4-methylphenol. The fragmentation pathway of DP3 was shown in Figure 5.

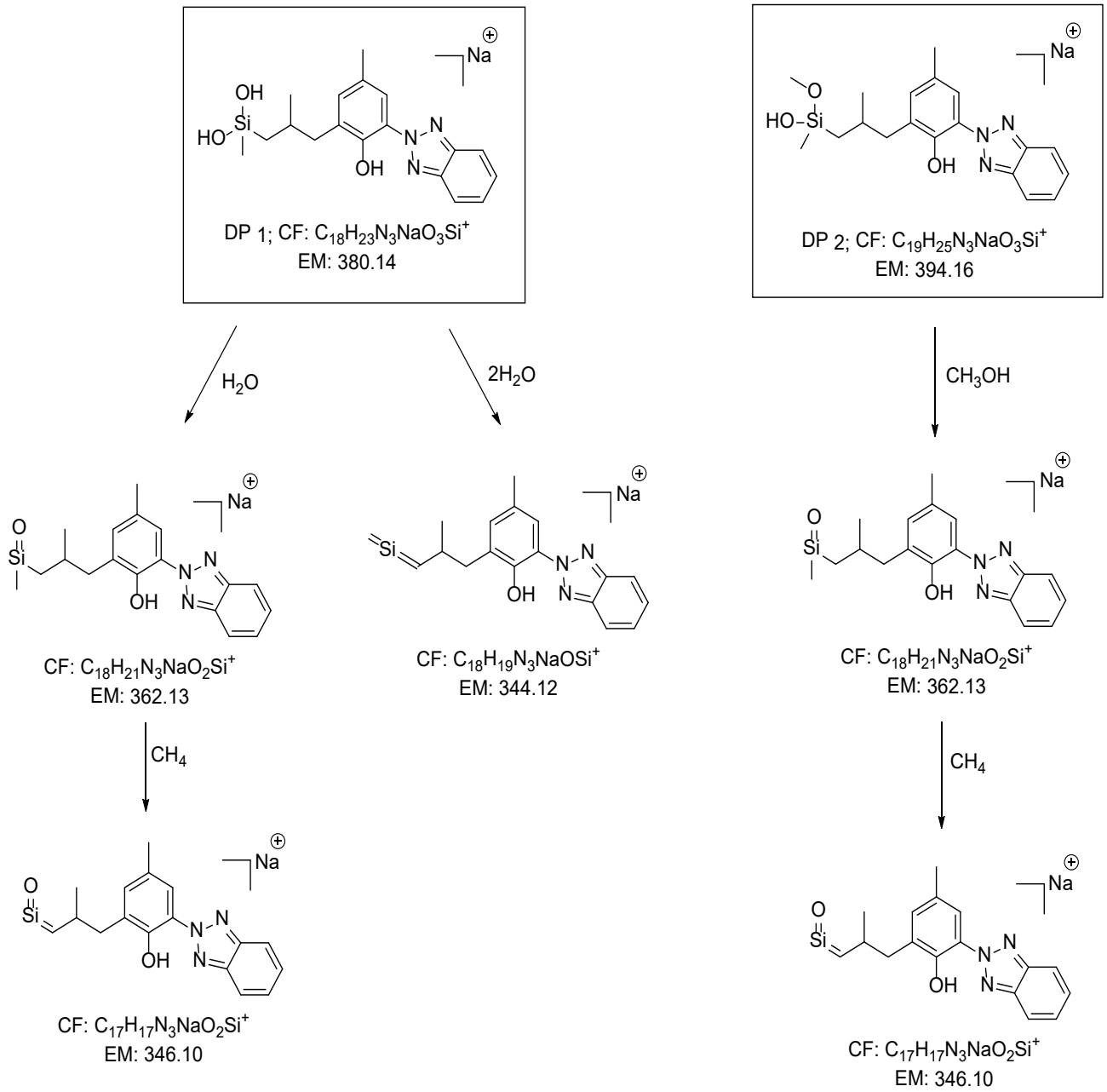

Fig. 4: Mass fragmentation pathway of (a) DP1 and (b) DP2.

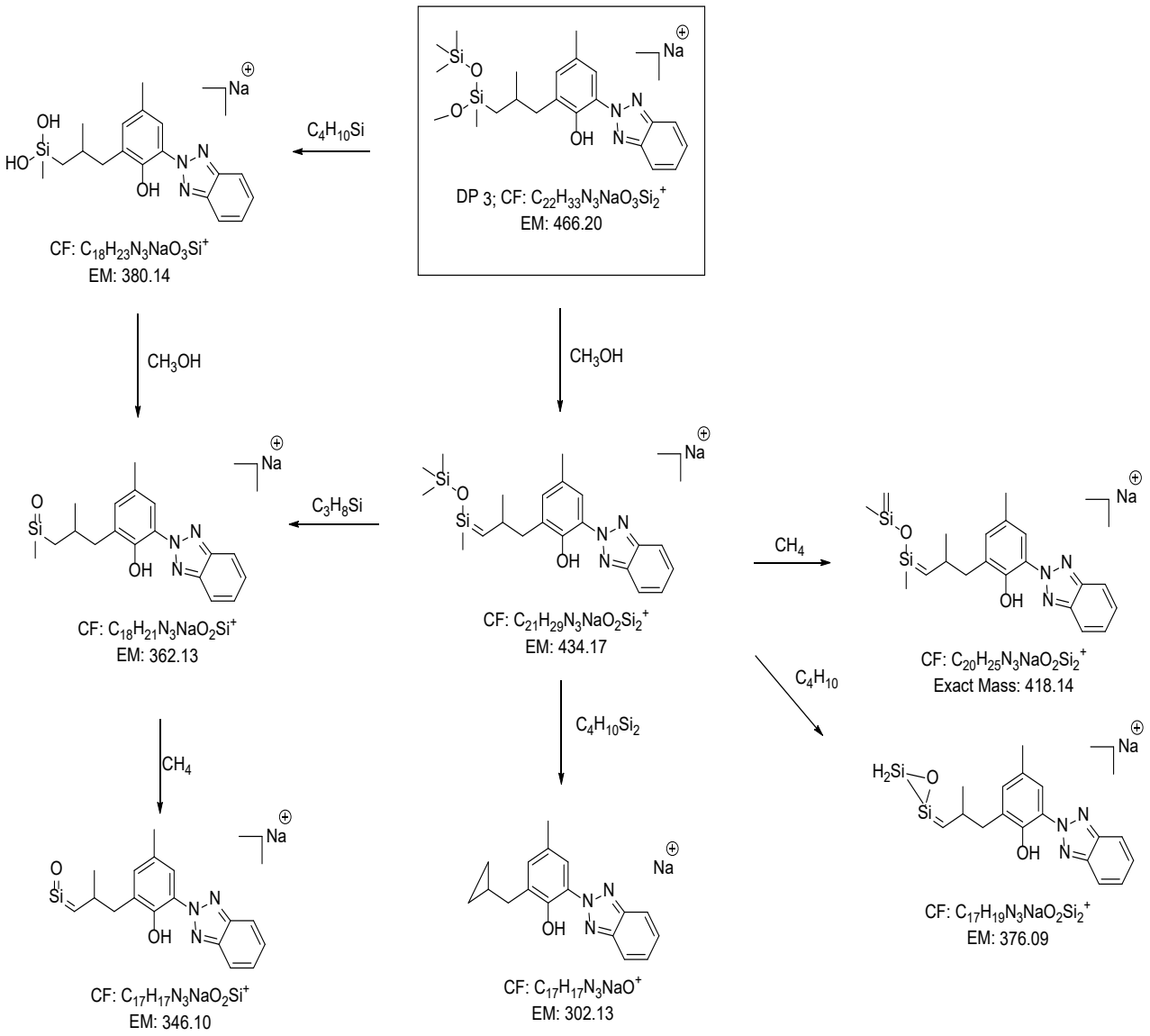

Fig. 5: Mass fragmentation pathway of DP3.

CONCLUSION

LC-MS compatible stability indicating UPLC method was developed and validated to describe the stress degradation behavior of DRT under various conditions like hydrolysis (acid, base and neutral), oxidation, photolysis, and thermal. This developed method can be used by the industry for the estimation of DRT in the API, formulations and stability samples. In the present study, we considered LC/MS/MS and ESI/QTOF/MS/MS ionization techniques for structural characterization and mass accuracy for the DRT and its three degradation products DP1, DP2 and DP3, respectively formed under acidic and basic conditions. The possibility to synthesize and develop reference standards and monitor their presence in the stability samples from the identified degradation products of this study. This developed method to determine and characterization study may be useful for the same class drug analysis.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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