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Original Article

Development and validation of HPTLC method for estimation of dofetilide in pharmaceutical dosage form and determination of its degradation profile by MS-MS method

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ABSTRACT

Background: Dofetilide is the class III antiarrhythmic drug used as a potassium channel blocker approved by US FDA in 1999 for the maintenance of sinus rhythm in individuals prone to atrial flutter and atrial fabrication. Currently there is no HPTLC-MS method reported for systematic characterization of degradation products dofetilide.
Methods: As per the ICH guidelines, the HPTLC method for the determination of dofetilide both in bulk and pharmaceutical formulation has been developed and validated. The degradation products were identified and characterized by using MS/MS. The Rf value was found to be 0.52±0.3. The degradation study was performed as per ICH guidelines (Q2R1). Isolation of the degradation product by HPTLC method and categorized by MS/MS method.
Results: The linearity of the method was found suitable over the range 100-600 ng/band with r^2 of 0.998. Dofetilide was subjected to stability studies, the drug was found to degrade under various stress conditions. The recovery was found in the range of 98-101%. HPTLC-MS/MS method showed a possible degradation mechanism of 7 degrading products. The degradation of the drug under various stress conditions indicates the storage conditions for the drug and drug product during its shelf life.
Conclusion: The HPTLC method developed for its linearity, range, precision studies, LOD and LOQ can be used for the routine quality control of the drug dofetilide in bulk drugs. The degradation pathway of a drug can help in the future to identify the impurities and for the impurity profiling of dofetilide.

Keywords: Stability indicating, HPTLC, HPTLC-MS/MS degradation studies, dofetilide

INTRODUCTION

Dofetilide (Figure 1) is the class III antiarrhythmic drug used as a potassium channel blocker approved by US FDA in 1999 for the maintenance of sinus rhythm in individuals prone to atrial flutter and atrial fabrication with a very potent dosage form in 0.25, 0.250 and 0.500 mg capsules of Dofetilide. (Wells, Khairy, Harris, Anderson, & Balaji, 2009; Krafte, & Volberg, 1994; Woolf, Miler, & Gosting, 1962). This dosage form is manufactured by Pfizer. (Wells et al., 2009; Aktas, Shah, & Akiyama, 2007; Al-Dashti, & Sami, 2001). The method refined by HPTLC was vali-

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dated for linearity range, interday and intraday precision, Limit of Quantitation (LOQ) and Limit of Detection (LOD) according to ICH Q2R1 guidelines. (2005: ICH Guidelines)

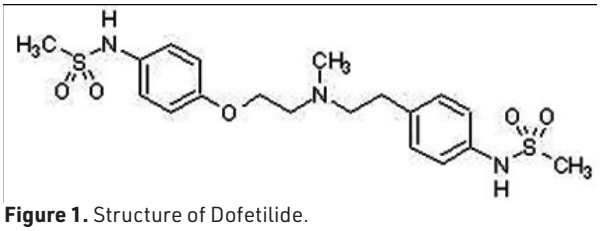

Figure 1. Structure of Dofetilide.

Dofetilide is a drug that can target potassium channels present in the cardiac region with good potency. (Bhole, Naksarkhre, & Bonde, 2019; Udin, Sung, Hunge, & Hu, 2019; Qile, Henriett, & David, 2019; Chi, Liu, & Wang, 2017; Kaddar, Pilote, Wong, Caillier, & Patoine, 2013; Mounsey, & DiMarco, 2007; Clusin, 2003), this can be an alternative therapy or considered as an aid for the currently available methods (catheter-based ablation and alternative pharmacological approach for the treatment of atrial arrhythmias.) According to ICH guidelines - Q1A (R2) the degradation of the drug under various stress conditions may help for the determination of stability of the drug. The forced degradation studies of drug substance or product should be evaluated for the development of stability-indicating methods. As per the prescribed guidelines, 20% degradation is within the acceptable range. As per the Literature review there are some methods reported for quantification of Dofetilide in pure form or in its pharmaceutical dosage form. (Udin et al., 2019; Chi et al., 2017; Bhole, Shinde, Chitlange, & Wankhede, 2015). On the other hand, high performance thin-layer chromatography (HPTLC) is a promising sustainable alternative to HPLC in some analysis. As HPTLC, separations has several advantages, it takes a short time for analysis. Moreover, it requires few nanoliter injection volumes. Furthermore, minimal use of solvent and no prior extraction steps compared to HPLC. However, There are very few HPTLC determination methods are available for the drug dofetilide as per literature. Some of these methods also report the degradation behavior under some stress conditions. As the solvent used in these methods are not very cost effective. It was also noted that there is an emerging need to perform a systematic characterization of degradation products by using MS-MS technique (Bhole, Biradar, & Bonde, 2018). So the current study was performed to develop selective and highly sensitive, specific, cost-effective and precise HPTLC method for determination of Dofetilide in presence of their degradation products and also in its dosage form. Moreover, the isolated stressed sample of Dofetilide was characterized by MS/MS method and also its possible degradation pathway was proposed.

MATERIALS AND METHODS

Chemicals and Reagents

Toluene, methanol, triethylamine, dofetilide

Instrumentations

Precoated aluminium plates with silica gel 60 F254 plates (Merck, Germany; supplied by Merck India, Mumbai, India). Camag Linomat V (Muttenz, Switzerland). The pressure requirement for the sample application is 4 µL. Dimension 360 mm x 510 mm x 410 mm (Width x Length x Height)]. Camag 100 µL syringe (Hamilton, Bonaduz, Switzerland). Camag twin trough glass chamber (10 x 10 cm and 10 x 20 cm). It has the dual-wavelength function (254 and 366 nm) [Dimension: 477 mm x 343 mm x 285 mm (Length x Width x Height)].

Chromatographic Conditions

4 µL of sample and standard solutions are applied on the TLC plate by using Camag Linomat V automatic sample applicator in the form of band (band with: 6 mm, and the distance is 5.6 mm between two bands) using micro-syringe. In twin trough glass chambers, the plates were saturated (10 minutes) with the mobile phase of composition toluene: methanol: triethylamine (7:2.5:0.5, v/v/v). Ascending development was performed up to a distance of 8 cm by placing the plates in the mobile phase. Later the development, the plates were dried in air and a densitometric scanning (slit dimensions: 5 × 0.45) was performed at 231 nm using Camag TLC scanner III operated in reflectance–absorbance mode (Bhole et al., 2018; Bonthagarala, 2003).

Analysis Formulation

Preparation of Standard Stock Solution

Accurately weighed 12.5 mg Dofetilide, transferred to 100.0 mL volumetric flask, and dissolved in AR grade 25 methanol by ultra-sonicating for 10 min and volume was made up to the mark using the methanol to give a stock solution of concentration 0.125 mg/mL or 125 µg/mL. Further dilution 8 ml stock solution to 10 mL (concentration100 µg/mL) to above solution of dofetilide was applied on the TLC plates in the range 0.1 to 0.6 µL i.e. 100 – 600 ng/band of dofetilide with the help of Hamilton syringe using LINOMAT-V automatic sample applicator. The plate was then developed in an optimized mobile phase. Different mobile phase combinations were tested and finally this ratio selected for the method development on HPTLC. The mobile phase ratio is methanol: toluene: triethylamine (7:2.5:0.5) was selected as it gives good resolution and peak symmetry for dofetilide. The Rf value for dofetilide was found to be 0.52 respectively (Fegade, Bhole, Patil, & Chaudhari, 2009; Rakibe, Tiwari, Mahajan, Rane, & Wakte, 2018).

Preparation of Sample Solution

(Each capsule contains 0.500 mg of Dofetilide) 25 capsules shells were opened and the powder was weighed equivalent to 12.5 mg (13.75 mg with excipients) of the Dofetilide sample into a 100 ml clean volumetric flask added about 40 ml of diluents and sonicated up to 10 min to completely dissolve and diluted up to the mark with diluents. The final concentration of the stock solution was 125 µg/ml. then further dilution. Further dilution 8 ml to 10 ml (concentration 100 µg/ml). (Jadhav, Nimbalkara, Mathad, & Mali, 2013).

Selection of Working Wavelength (λmax)

The UV spectrum of 4 µg/mL of Dofetilide in methanol, the spectrum was recorded by scanning in the range (200 nm to 400 nm). From the UV spectrum wavelength selected as 231 nm. The spectrum was shown in Figure 2 (Shivashankar, & Gandhimathi, 2005).

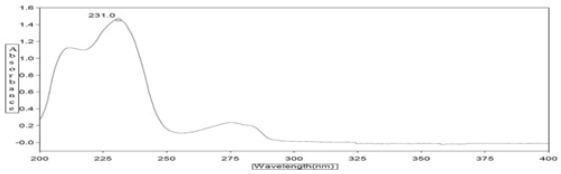

Figure 2. UV-VIS spectrum was found to be 231 nm for Dofetilide.

Method Validation

The method was validated in compliance with ICH guidelines.

Linearity

The linearity of the method was evaluated at the five equal-spaced concentrations (Ceresole, Moyano, Pizzorno, & Segall, 2007) by diluting the standard stock solution to give solution over the range of 100-600 ng/band of dofetilide. A calibration curve was constructed at six linear concentrations of dofetilide (0.1 to 0.6 µg/mL). solutions were injected into the chromatographic system, after getting the results plotted a graph concentration versus an area to evaluate correlation coefficient.

Acceptance criteria: Correlation coefficient Not less than 0.99.

Accuracy & Recovery

Accuracy solutions prepared into three levels (80%, 100%, and 120%). For each level the preparations were prepared individually. 80%, 100% & 120% Solutions were prepared with different drug weights with a constant weight of placebo in the manner of sample preparation.

Acceptance Criteria: % Recovery should be 98.0 to 102.0 Acceptance Criteria: % RSD for nine preparations recover values should be ≤ 2.0

Precision

Method precision, validation parameter investigated using the six individual sample preparations as reported above. Six samples were injected individually into the chromatographic system and calculated the % assay of individual samples. Repeatability and Intermediate precision (Intraday and Interday precision).

Acceptance Criteria: % Assay should be 95.0 to 105 & %RSD for six preparations assay should be ≤ 2.0.

Robustness

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. By introducing small changes in the mobile phase composition, mobile phase volume and duration of chamber saturation with the mobile phase, the effects on the Rf value of drugs were examined. The composition of the mobile phase was changed slightly (± 0.1 mL for the component). Robustness of the method was performed as per the standard guidelines.

Acceptance Criteria: System suitability should be within the acceptance criteria: System suitability should be within the acceptance criteria.

Limit of LOD and LOQ

The LOD and LOQ of the developed method were calculated by using the standard method

Acceptance Criteria: ≤ 2

Forced Degradation Studies

The stability of the drug was studied at various stress conditions as indicated by the ICH guidelines Q1A (R2) for acid hydrolysis with 0.1 M HCl, base hydrolysis with 0.1M NaOH, 3% hydrogen peroxide for oxidation, thermal degradation at 40°C, 60°C, 80°C, and photolysis. Accurately weighed the quantity of capsule powder equivalent to about 12.5 mg of Dofetilide was transferred separately to six different 10.0 ml volumetric flask, (flask no. 1, 2, 3, 4, 5 and 6). To flask no.1, 2 and 3, followed by the addition of 3.0 mL of 0.1 M HCl, 3.0 mL of 0.1 M NaOH, 3 mL water for neutral hydrolysis and 3% H_2O_2 respectively. The content of flask no. 1, 2, 3 and 4 were heated in a water bath at 80°C for 1 hrs 30 min, 30 min, 1 hr and 2 hrs 30 min respectively. The flask no. 5 containing powder kept in a hot air oven at 600°C for 10 min to study the effect of heat on the sample (heat degradation). Flask no. 6 containing powder was exposed to UV-radiations for 72 hrs to study the effect of light on the sample (photodegradation). The Whatman filter paper no 42 is used for filtration. From the filtrate, the 1.0 mL solution was diluted to 10 mL with the mobile phase. The diluted solution was analyzed similarly as described under the analysis of marketed formulation. The typical dendrogram was shown in Figure 3.

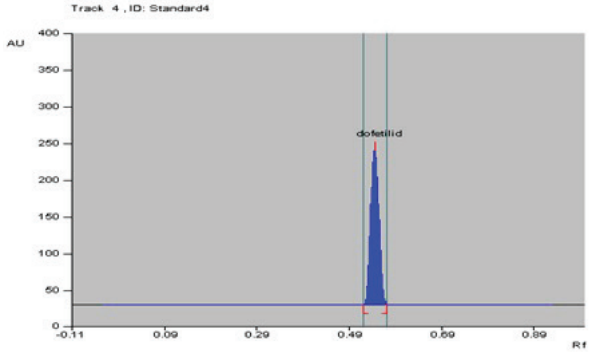

Figure 3. Typical densitiogram of Dofetilide retention factor: 0.52 (±0.03).

RESULT AND DISCUSSION

These studies were performed to develop a sensitive method, and an economically and less time-consuming HPTLC technique, which may be implemented for the determination of Dofetilide in pharmaceutical formulation. The HPTLC method was developed as per the stated method. Such as a large number of samples handle easier to scan the band and the detector response is higher. The chromatographic saturation is 10 minutes. Many trial and error methods were tried by using various solvents with altering polarity and in the diverse extent to obtain superior resolution and sharp peaks with acceptable Rf values (0.1–0.6 µg/band). Amongst the various mobile phase blend tested, mobile phase consisting of toluene: methanol: triethylamine (7:2.5:0.5 v/v/v) shows enhanced resolution and sharp

peaks with Rf values of 0.5205±0.05 of dofetilide. The linearity graph and linearity table are shown in Figure 4 and Table 1.

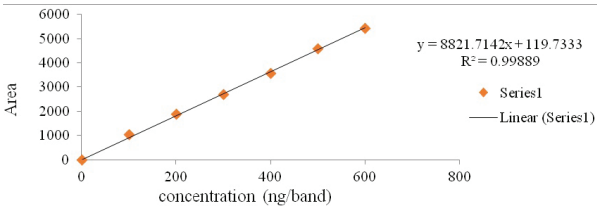

Figure 4. Linearity graph.

Table 1. Calibration parameter.

Parameters	Dofetilide
Linearity Range	100ng/band
Linearity Equation	Y=8221.742x+119.7333
Correlation Coefficient	0.998

Accuracy (Recovery)

To determine the accuracy of the said methods, accuracy studies were carried out as per ICH guidelines. The determination was performed in triplicate at each level was shown in Table 2.

Table 2. Recovery study.

Level of recovery	% Recovery*	S.D.	R.S.D.
80%	98.93	0.29	0.29
100%	99.37	0.45	0.46
120%	99.65	0.12	0.12

***Mean of three determinations**

Precision

The precision study was performed as per the standard method. The % RSD for intraday and interday precision is less than 2, indicating the precision of the method as shown in Table 3.

Table 3. Precision of developed method.

Precision parameter	% label claim	S.D.	%R.S.D.
Repeatability	98.03	1.12	1.14
Intraday	99.70	0.10	0.10
Interday	99.20	0.20	0.20

*Mean of three determinations

Robustness

The mobile phase composition changed within a range of ±1 ml. Moreover, the chamber saturation time was varied in the range of and ±2.5 min, respectively. The effect of these changes on both the Rf values and peak area was shown in Table 4.

Table 4. Result of robustness study

Robustness study			
Factor	**Level**	**Area**	**Rf**
Mobile Phase Composition (±0.1 ml)			
6.9:2.6:0.5	-0.1	3670	0.53
7:2.5:0.5	0	3660	0.52
7.1:2.4:0.5	+0.1	3675	0.55
%RSD		0.21	
Duration for Chamber saturation (±5 min)			
5 min	-5 min	3670	0.49
10 min	0 min	3675	0.52
15 min	+5 min	3690	0.57
Volume of mobile phase (±1 ml)			
9.0 ml	-1 ml	3699	0.50
10.0 ml	0 ml	3674	0.52
11.0 ml	+1 ml	3620	0.52

*Mean of three determinations

LOD and LOQ

Limit of detection and Limit of quantification were done separately and found to be 0.17 ng/band for dofetilide. LOQ was found to be 0.51 ng/band for Dofetilide.

Analysis of Formulation

Twenty-five capsules (Tikosyn) were weighed and crushed to obtain a fine powder. The average weight of the capsule was calculated. Accurately weighed the quantity of capsule powder equivalent to about 500 mcg of Dofetilide. The content of the drug was close to 100%. The result was summarized in Table 5 and 6.

Table 5. Marketed formulation

	Tikosyn		Label claim: 500 mcg	
Sr. No.	**Wt. of capsule powder (mcg)**	**Peak area***	**Amount found (mcg)**	**% label claim**
1.	550	3669	492	98.40
2.	550	3637	480	96.00
3.	550	3680	490	98.00
4.	550	3669	490	98.00
5.	550	3680	497	99.40
6.	550	3643	492	98.40

*Mean of three determinations

Table 6. Statistical validation for analysis of marketed formulation

Drug	Amount of drug found (mcg)	% Label claim*	S.D. (±)	R.S.D.
Dofetilide	490.16	98.03	1.12	1.14

* Mean of six determination

Forced Degradation Studies

Dofetilide was found to degrade in various stress conditions (acid, alkaline, and oxidation). Utmost degradation was observed in the said conditions, (alkaline, acid, neutral, heat, and photodegradation). Percentage assay of active substance along with Rf values of degradation products is summarized in Table 7 and Figures 5-10. (alkaline, acid, neutral, heat, and photodegradation stress conditions). Acid degradation shows two peaks.

Table 7. Result of degradation studies

Sr. No.	Stress Condition	Temperature and Time	% assay of active substance	Rf of degraded product
1	Acid (0.1 M HCl)	80°C for 1hr 30 min	89.96	0.03, 0.60
2	Alkali (0.1 M NaOH)	80°C for 30 min	86.96	0.07, 0.20 0.58
3	Neutral (H_2O)	80°C for 1 hr	91.89	0.07, 0.12, 0.16, 0.23
4	Oxide (3 % H_2O_2)	80°C for 2hr 30 min	91.64	0.08
5	Thermal	60°C for 10 min	91.25	0.08, 0.16, 0.23
6	Photo Degradation	24 hr	90.71	0.60, 0.64

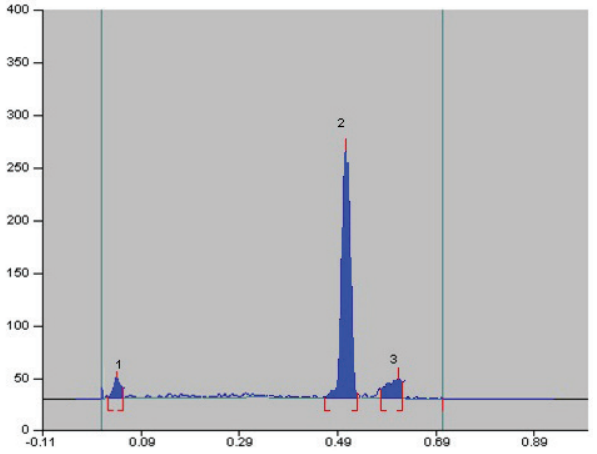

Figure 5. Densitogram of acid (0.1 M HCl) treated sample.

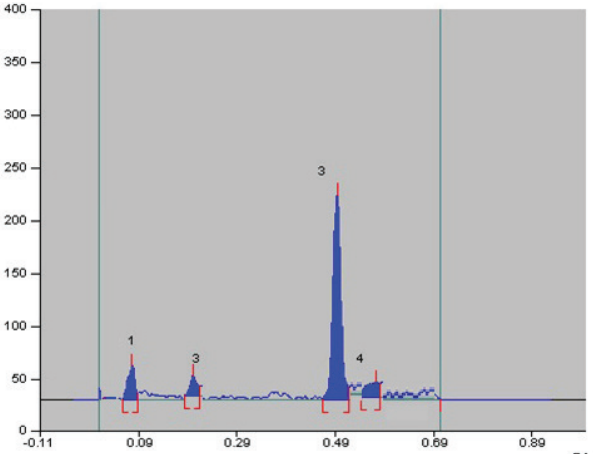

Figure 6. Densitogram of alkali (0.1M NaOH) treated sample.

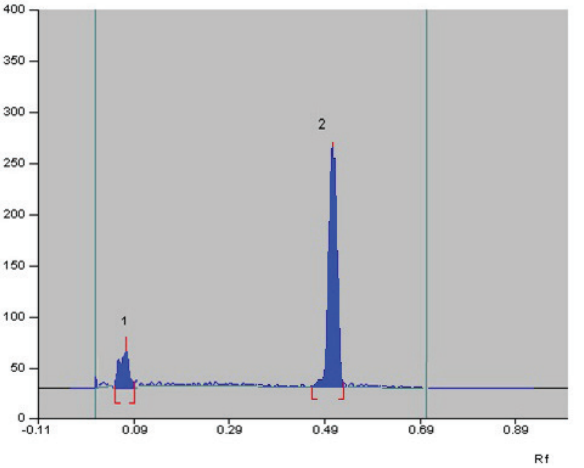

Figure 7. Densitogram of oxide (3% H_2O_2) treated sample.

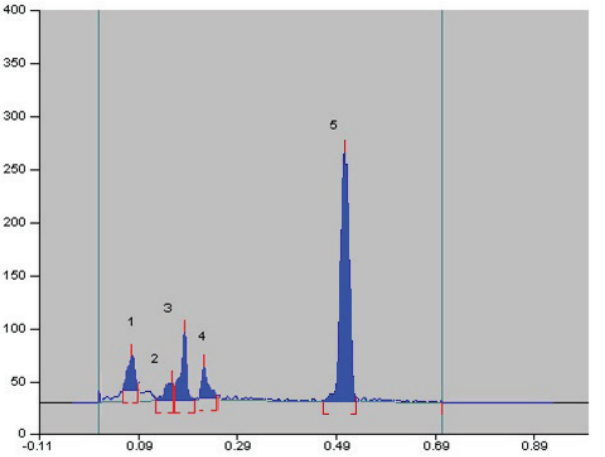

Figure 8. Densitogram of the sample exposed to neutral hydrolysis.

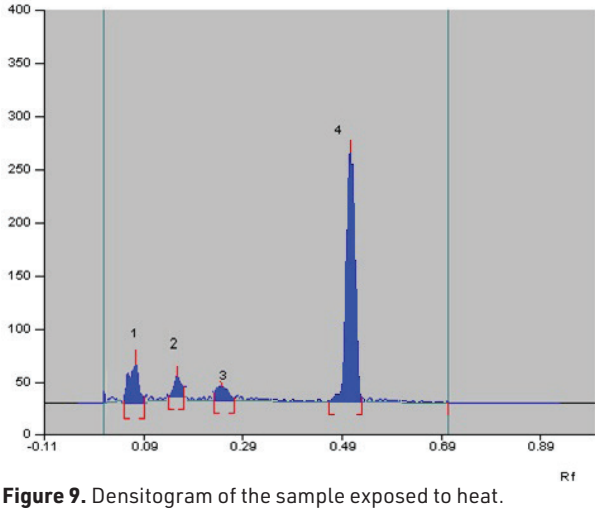

Figure 9. Densitogram of the sample exposed to heat.

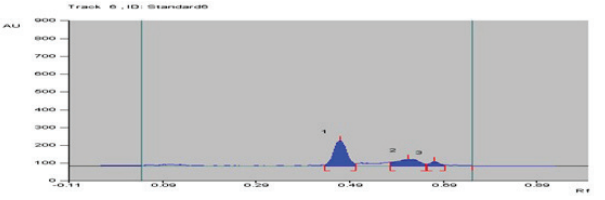

Figure 10. Densitogram of the sample exposed to photodegradation.

Separation, Characterization of Degradation Product by HPTLC and by MS/MS (Tandem Mass Spectroscopy) Method

Isolation of degradation product by using HPTLC method

An accurately weighed quantity of 10.0 mg of Dofetilide was transferred to10.0 mL of volumetric flask than add 3.0 ml of distilled water flask no.1 3.0 mL of 0.1 M acid in flask no 2, 0.1 M NaOH in flask no 3, 3% of H_2O_2 in flask no 4. The forced degradation study was carried out by exposing samples to the stress condition as 0.1 M HCl, 0.1 M NaOH, neutral, oxide for degradation, contents of the flask were reflux in a water bath at 80°C for 1 hr 30 min, 30 min, 1 hr and 2 hr 30 min respectively. After the respective time intervals, all the flasks were removed and allowed to cool. Then the samples were applied on the TLC plate with the sample volume of about 10 µL/ band, 4 band of degraded sample and 1 band of std. were applied. Then the TLC plate was allowed to develop under optimized chromatographic conditions for Dofetilide. After development of the plate these plates were kept under the UV chamber on the basis of Rf value of the std. and degradation product they are marked and that portion of TLC plate was cut and allowed it to extract into methanol. Then the IR spectra and MS-MS spectra was recorded for interpretation of the probable structure of the degradation product. The diagrammatic procedure was given below degradation of sample in acid, alkali, oxide and neutral stressed condition.

The said degradation products were then subjected to MS/MS studies by using positive electrospray ionization (ESI) mode (mass range of 50–1500 daltons). The drug (concentration of 6 µg/mL) was directly infused using a syringe pump into MS/MS in methanol: water (50:50 v/v) as a solvent system. This is for the optimization of mass parameters which inform about the molecular ion peak of the drug. These were further modified to get complete fragmentation of the drug. High purity nitrogen was used as the nebulizer as well as the auxiliary gas (Bhole, Naksarkhre, & Bonde, 2019; Bhole et al., 2018). Fragmentation of various precursor ions formed in MS/MS studies was achieved at different collision energies The seven degrading products were observed in the four neutral stress conditions, two in acidic stress condition, two in alkaline stress condition and one in oxidative stress condition. (Molecular formula C19H27N3O5S2; Molecular weight: 441.56) i.e. DP-1, DP-2, DP-3, DP-4, DP-5, DP-6, DP-7. The correlation of the degradation product in Dofetilide and its stress conditions were shown in Figures 11-16 (mass spectrum) and fragmentation pattern shown in Figures 17-20.

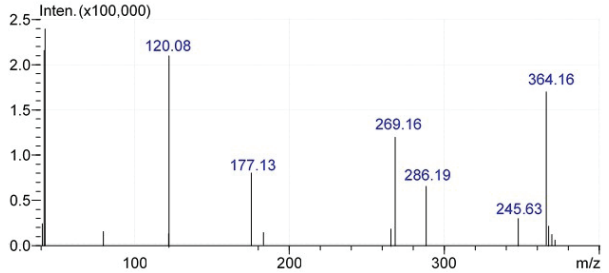

Figure 11. MS/MS spectrum of the DP-1(m/z 364.17).

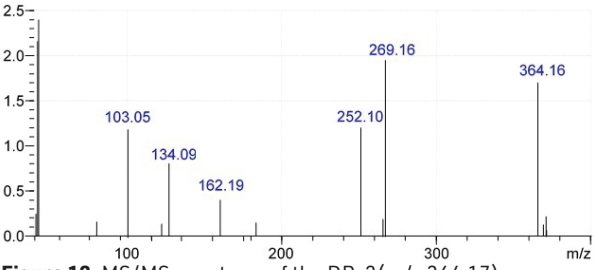

Figure 12. MS/MS spectrum of the DP-2(m/z 364.17).

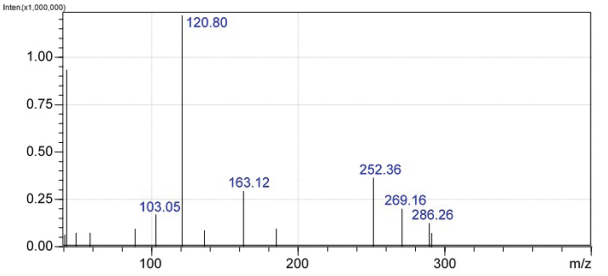

Figure 13. MS/MS spectrum of the DP-3 and DP-4 (m/z 285.18).

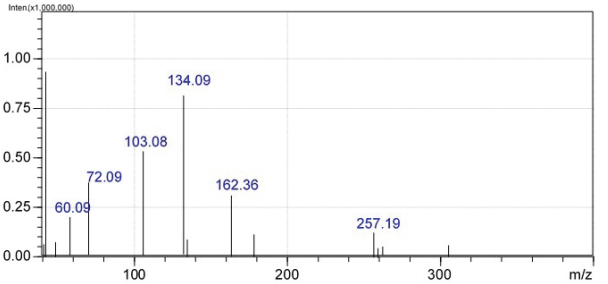

Figure 14. MS/MS spectrum of DP-5 (m/z 257.13).

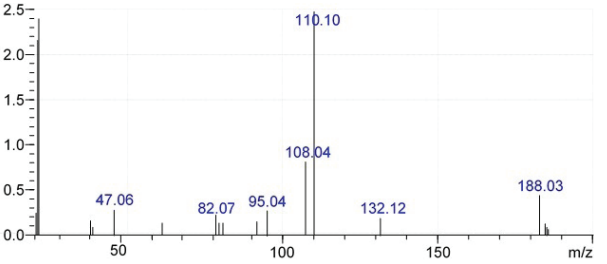

Figure 15. MS/MS spectrum of DP-6 (m/z 188.04).

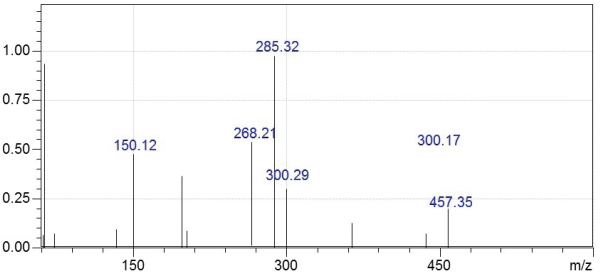

Figure 16. MS/MS spectrum of DP-7 (m/z 363.14).

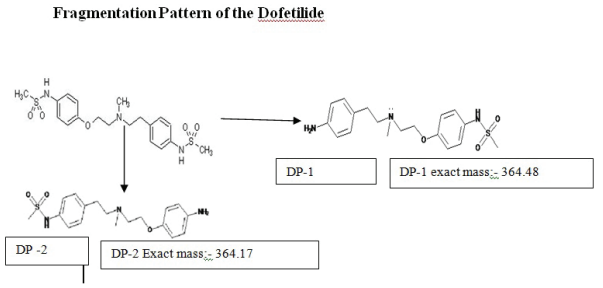

Figure 17. Possible degradation pathway in neutral condition.

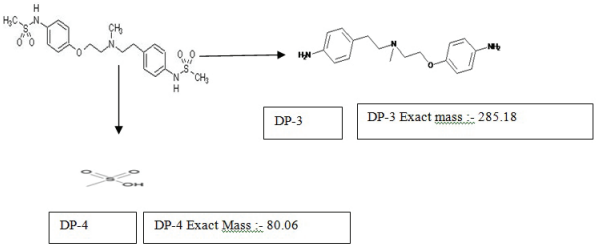

Figure 18. Possible degradation pathway in alkali stress condition.

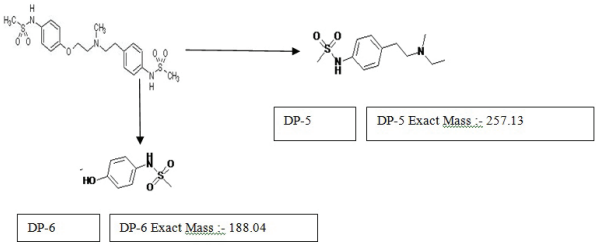

Figure 19. Possible degradation pathway in acid stress condition.

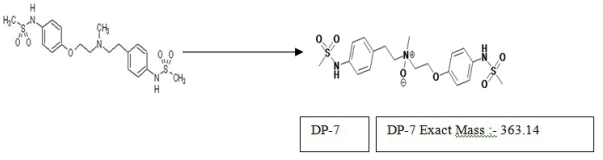

Figure 20. Possible Degradation pathway in oxidative stress condition.

CONCLUSION

The HPTLC method developed for its linearity, range, precision studies, LOD and LOQ can be used for the routine quality control of the drug dofetilide in bulk drugs. The degradation of the drug under various stress conditions indicates the storage conditions for the drug and drug product during its shelf life. Moreover, the degradation pathway of a drug can help in the future to identify the impurities and for the impurity profiling of dofetilide.

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