Journal of Pharmaceutical and Biomedical Analysis 55 (2011) 435–445

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

LC, LC–MS/TOF and MS^n studies for the identification and characterization of degradation products of nelfinavir mesylate

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ARTICLE INFO

Article history:
Received 27 October 2010
Received in revised form 14 February 2011
Accepted 17 February 2011
Available online 24 February 2011

Keywords:
Nelfinavir mesylate
Stress studies
LC
LC–MS/TOF
MS^n
Degradation pathway

ABSTRACT

The objective of the present investigation was to separate, identify and characterize the major degradation products (DPs) of nelfinavir mesylate generated under hydrolytic, oxidative, photolytic and thermal stress conditions as advised in International Conference on Harmonization (ICH) guideline Q1A(R2). The drug was found to degrade under acidic, basic, oxidative and photolytic stress, while it was stable in neutral and thermal stress conditions. A total of three degradation products were formed, which were separated on a C-18 column employing a gradient HPLC method. A complete mass fragmentation pathway of the drug was first established with the help of multi-stage (MS^n) and MS/TOF accurate mass studies. Then stressed samples were subjected to LC–MS/TOF studies, which provided their fragmentation pattern and accurate masses. The mass spectral data were employed to characterize the DPs and assign structures to them. The total information was also used to establish the degradation pathway of the drug. The degradation products were identified as 3-hydroxy-*N*-((2*R*,3*R*)-3-hydroxy-1-(phenylthio)butan-2-yl)-2-methylbenzamide and (3*S*,4a*S*,8a*S*)-*N*-*tert*-butyl-2-((2*R*,3*R*)-2-hydroxy-3-(3-hydroxy-2-methylbenzamido)-4-(phenylsulfinyl)butyl)decahydroisoquinoline-3-carboxamide.

1. Introduction

Nelfinavir mesylate (Fig. 1) is (3*S*,4a*S*,8a*S*)-*N*-*tert*-butyl-2-((2*R*.3*R*)-2-hydroxy-3-(3-hydroxy-2-methylbenzamido)-4-(phenylthio)butyl)decahydroisoquinoline-3-carboxamide methanesulfonate. It is a potent and selective human immunodeficiency virus 1 (HIV-1) protease inhibitor [1,2]. Various high-performance liquid chromatographic (HPLC) methods are reported for the investigation of the drug in biological fluids [3–5]. There exist a few reports on stability-indicating methods of the drug by using HPLC and high-performance thin layer chromatography (HPTLC) [6–8]. The drug was also reported for its simultaneous determination with other HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors by using HPLC [9–15], ion-pair HPLC [16], HPLC–MS [17], LC–MS–MS [18].

No study so far has been reported on the systematic characterization of degradation products of nelfinavir mesylate under stress conditions prescribed by ICH Q1A(R2) [19] and World Health Organization [20]. The advantage of stress testing under variety of conditions and the characterization of degradation products is that these studies provides a comprehensive information on the degradation pathway of the drug. Hence the ultimate objective of the present investigation was to: (i) degrade nelfinavir mesylate under conditions of hydrolysis, oxidation, photolysis and thermal stress, (ii) resolve the formed degradation products on an HPLC column and validate the developed HPLC method, (iii) characterize them by LC–MS/TOF and MS^n studies, and (iv) postulate the degradation pathway of the drug based on the stress degradation behaviour.

2. Experimental

2.1. Drug and reagents

Pure nelfinavir mesylate was obtained as gratis sample from Macleods Pharmaceutical Laboratories (Mumbai, India) and it was used without further purification. Analytical reagent (AR) grade sodium hydroxide (NaOH) was purchased from S.D. Fine-Chem Ltd. (Mumbai, India), hydrochloric acid (HCl) and HPLC grade methanol (MeOH) from Merck Specialities Pvt. Ltd. (Mumbai, India) and hydrogen peroxide (H_2O_2) from Qualigens Fine Chemicals Pvt. Ltd. (Mumbai, India). Buffer salts and all other chemicals were bought from local suppliers. Ultra pure water obtained from Millipore water purification system (Molsheim, France) was used throughout the studies.

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doi:10.1016/j.jpba.2011.02.020

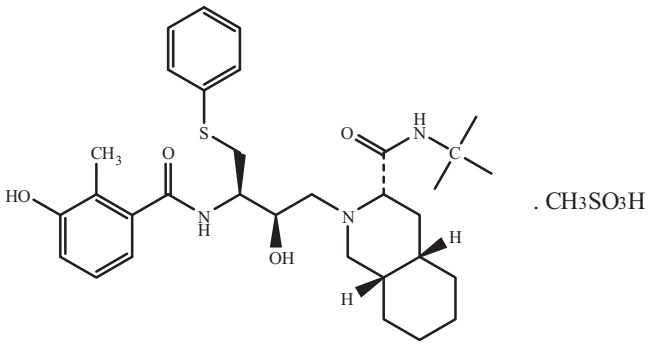


Fig. 1. Structure of nelfinavir mesylate.

Table 1
Stress conditions for optimum degradation.

Stress condition	Concentration of stressor	Exposure condition	Duration
Hydrolysis			
Acid	1 N Hcl		8 h
Neutral	H_2O	80 °C	48 h
Base	1 N Hcl		24 h
Oxidation	0.3% H_2O_2	RT	8 h
Photolysis	8500 lx. h fluorescent and 0.05 W/m^2 UV		
Acid	0.01 N HCl		14 d
Neutral	H_2O	40 °C/	14 d
Base	0.01 N NaOH	75% RH	14 d
Solid	–		14 d
Thermal	–	50 °C	21 d

2.2. Equipments

A high-performance liquid chromatography (HPLC) system from PerkinElmer (Shelton, CT, USA) was used for LC studies, which consists of an on-line degasser, sample injector (Rheodyne sample loop 20 μl), UV–visible detector (Series 200), pump (Reciprocating, series 200) and a computer system loaded with Total Chrome Navigator (version 6.3.1) software. The LC/MS system consists of a 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) and a MicrOTOF-Q mass spectrometer (Bruker Daltonics, Bremen, Germany). The LC part comprised of an on-line degasser (G1379A), binary pump (G131A), auto-injector (G1313A), column oven (G1316A) and diode-array detector (G1315B). The system was controlled by combination of Hyphenation Star (version 3.1) and MicrOTOF Control (version 2.0) software. Multi-stage mass spectrometric (MS^n) studies were carried out on Finnigan Mat LCQ ion-trap equipment (San Jose, USA). Its LC part consists of a P4000 pump, an AS3000 autosampler, a UV6000LP PDA detector, and a SCM1000 degasser (all parts of the equipment were from Spectrasystem, USA). The same was controlled by Xcalibur (version 2.0) software. Other equipments used were a pH meter (Labindia, Mumbai, India), weighing balance (Shimadzu, AUX220, Kyoto, Japan) and a micro-pipette (Erba Biohit, Mannheim, Germany). In all studies, separations were achieved on a C-18 column 250 mm × 4.6 mm i.d., particle size 5 μm; Kromasil (Eka Chemicals AB, Bohus, Sweden). Precision water baths equipped with MV controller (Thermostatic Classic Scientific India Ltd., Mumbai, India) were used for stress studies. Degradation experiments in acid, base and neutral conditions were performed using a dry-bath (Labline Sun Scientifics Ltd., New Delhi, India). The solid state thermal stress studies were carried out in dry-air oven (NSW Limited, New Delhi, India). Photolytic studies were carried out in a photostability chamber (Thermolab, Th-400G Mumbai, India), set at 40 ± 1 °C/75% ± 3% RH and equipped with an illumination bank on inside top, consisting of a combination of two black light UV lamps (OSRAM L 18W/73) and four white fluorescent lamps (PHILIPS Col 86) in accordance with option two of the ICH guideline Q1B [21]. The samples were placed at a distance of 9 in. from the light bank. Both fluorescent and UV lamps were switched on simultaneously.

Table 2
Parameters for MS/TOF studies in positive ESI mode.

Mode	Parameters for $M+H^+$	Parameters for fragment study
Source		
End plate offset (V)	−500	−500
Capillary (V)	−4500	−4500
Nebuliser (Bar)	1.0	1.0
Dry gas (L/min)	5.0	5.0
Dry temperature (°C)	180	180
Transfer		
Funnel 1 RF (Vpp)	100	100
Funnel 2 RF (Vpp)	200	200
ISCID energy (eV)	0.0	4.0
Hexapole RF (Vpp)	200	160
Quadrupole		
Ion energy (eV)	5.0	5.0
Low mass (*m/z*)	300	220
Collision cell		
Collision energy (eV/z)	5.0	50
Transfer time (μs)	100	75
Collision RF (Vpp)	320	260
Pre-pulse storage (μs)	4.0	4.0
Detector		
Source (V)	−1200	−1200

2.3. Stress studies

The stress studies were carried out under the conditions of hydrolysis, photolysis, oxidation and dry heat as mentioned in ICH guideline Q1A(R2) [19]. The approach suggested by Singh and Bakshi [22] was adopted for these studies. The drug solution was

Table 3
Interpretation of MS/TOF and MS^n data of fragments of the drug.

Peak no.	Experimental mass	Best possible molecular formulae	Theoretical mass	Error in mmu	RDB	Possible parent fragment	Difference from parent ion	Possible losses corresponding to difference
0	568.3120	$C_{32}H_{46}N_3O_4S$	568.3204	−8.354	11.5			
1	467.2334	$C_{27}H_{35}N_2O_3S$	467.2363	−2.890	11.5	0	101.0786	$C_5H_{11}NO$
2	330.1144	$C_{18}H_{20}NO_3S$	330.1158	1.440	9.5	1	137.119	$C_9H_{15}N$
3	312.1054	$C_{18}H_{18}NO_2S$	312.1053	0.124	10.5	2	18.0090	H_2O
4	202.0864	$C_{12}H_{12}NO_2$	202.0863	0.145	7.5	3	11.0190	C_6H_5S
5	190.1588	$C_{13}H_{20}N$	190.1590	−0.226	4.5	1	277.0746	$C_{14}H_{14}NO_2S$
6	179.0532	$C_{10}H_{11}OS$	179.0525	0.688	5.5	3	151.0633	$C_8H_9NO_2$
7	161.0424	$C_{10}H_9S$	161.0419	0.453	6.5	6	18.0108	H_2O
8	135.0428	$C_8H_7O_2$	135.0441	0.045	5.5	4	67.0436	C_4H_5N
9	123.0248	C_7H_7S	123.0263	−1.497	4.5	7	38.0176	C_3H_2
10	107.0469	C_7H_7O	107.0491	−2.241	4.5	8	27.9959	

Table 4
MS^n fragmentation of the drug.

MS^n	Precursor ion	Product ions
MS^2	568	467, 330, 190
MS^3	467	330, 190
	330	312
MS^4	312	202, 179
MS^5	202	135, 107
	179	161, 123
MS^6	135	107
	161	123

prepared in methanol at a concentration of 2 mg mL^{-1}. The 50:50 v/v ratio of the drug solution and stressor (e.g., HCl, NaOH, water) was used in all the stressed conditions in solution state. Acidic, alkaline and neutral hydrolysis were carried out by heating in 1 N HCl, 1 N NaOH and water at 80 °C for 8 h, 24 h and 48 h, respectively. The oxidative study was carried out in 0.3% H_2O_2 at room temperature for 8 h. For thermal stress testing, the drug was sealed in glass vials and placed in a dry-air oven at 50 °C for 21 d. Photolytic studies of the drug in the solution state were carried out in 0.01 N HCl, 0.01 N NaOH and water by exposing it for 14 d to a combination of UV and fluorescent light in a photostability chamber set at accelerated conditions of temperature and humidity (40 ± 1 °C/75% RH). Solid state photolytic studies were performed by exposing a thin layer of the drug to light under similar conditions as that of the solution state. A parallel blank set was kept in the dark for 14 d. The optimized stressed conditions are enlisted in Table 1.

2.4. Preparation of samples for HPLC analyses

The samples were withdrawn at suitable time intervals and diluted four times with H_2O/MeOH (50:50 v/v) before injection into HPLC. Also, all the stressed samples were mixed in equal volume and used for HPLC method development.

2.5. HPLC method development, optimization and validation

A literature search indicated that most of the reported HPLC methods for nelfinavir mesylate were developed by using acetonitrile as an organic modifier. Since the detection wavelength for nelfinavir mesylate was 252 nm, hence it was thought worthwhile to develop a simple and cost effective LC method by using methanol instead of acetonitrile. Various trials were conducted to achieve an adequate separation of the drug and its DPs. The separation was tried using different proportions of MeOH (A) and potassium dihydrogen phosphate buffer (10 mM) (B) and also by varying the pH of buffer with phosphoric acid. HPLC studies were performed initially on all reaction solutions individually, and then on a mixture of degraded drug solutions. The flow rate, injection volume and detection wavelength were 1 mL min^{-1}, 20 μL and 252 nm, respectively. The developed method was validated with respect to various parameters outlined in the ICH guidelines Q2 (R1) [23]. A stock solution containing 1 mg mL^{-1} drug was prepared in methanol and the linearity was established by using the concentration in the range of 20–120 μg mL^{-1}. The solutions were prepared in triplicate and analyzed by injecting 20 μL into HPLC. The intra-day and inter-day precision were established by analyzing 60 μg mL^{-1}, 80 μg mL^{-1} and 100 μg mL^{-1} drug solutions three times on the same day and the next day, respectively. Accuracy was determined by spiking a mixture of stressed samples with three known concentrations of the drug, viz., 60 μg mL^{-1}, 80 μg mL^{-1} and 100 μg mL^{-1} in triplicate and then determining the percent recovery of the added drug. Specificity of the method was established by determining peak purity using a PDA detector. In fact, both peak purity as well as the resolution was determined for all the DP peaks, in addition to the drug peak, to prove that the developed method was selective in nature.

Table 5
LC–MS/TOF data of DPs (I–III) along with their possible molecular formulae and major fragments.

DPs	Experimental mass	Best possible molecular formula	Theoretical mass	Error in mmu	RDB	Major fragments (error in mmu, chemical formula)
I	330.1123	$C_{18}H_{20}NO_3S^+$	330.1158	−3.540	9.5	311.2921 (22.79, $C_{18}H_{35}N_2O_2^+$) 196.1345 (19.05, $C_{11}H_{18}NS^+$)
II	584.3094	$C_{32}H_{46}N_3O_5S^+$	584.3152	−5.868	11.5	458.2973 (-4.03, $C_{26}H_{40}N_3O_4^+$) 359.2300 (-2.91, $C_{21}H_{31}N_2O_3^+$) 339.2045 (-2.20, $C_{21}H_{27}N_2O_2^+$) 311.2919 (22.59, $C_{18}H_{35}N_2O_2^+$) 251.2109 (-0.88, $C_{15}H_{27}N_2O^+$) 220.0978 (0.98, $C_{12}H_{14}NO_3^+$) 195.1508 (1.61, $C_{11}H_{19}N_2O^+$) 166.1260 (3.36, $C_{10}H_{16}NO^+$)
III	584.3095	$C_{32}H_{46}N_3O_5S^+$	584.3152	−5.868	11.5	458.2972 (-4.03, $C_{26}H_{40}N_3O_4^+$), 359.2301 (-2.91, $C_{21}H_{31}N_2O_3^+$) 339.2051 (-2.20, $C_{21}H_{27}N_2O_2^+$) 251.2108 (-0.88, $C_{15}H_{27}N_2O^+$) 220.0977 (0.98, $C_{12}H_{14}NO_3^+$) 195.1513 (1.61, $C_{11}H_{19}N_2O^+$) 166.1258 (3.36, $C_{10}H_{16}NO^+$)

Table 6
Linearity data for nelfinavir mesylate.

Conc. (μg mL^{-1})	Average area	±SD	RSD (%)	Slope	Correlation coefficient (R^2)
20	439536.49	4913.64	1.12		
40	965386.80	12,442.43	1.29		
60	1476953.61	19,472.15	1.32	514878	0.9993
80	1984321.03	10,967.99	0.55		
100	2453732.00	17,603.61	0.72		
120	3049204.71	22,571.78	0.74		

Table 7
Intra-day and inter-day precision studies.

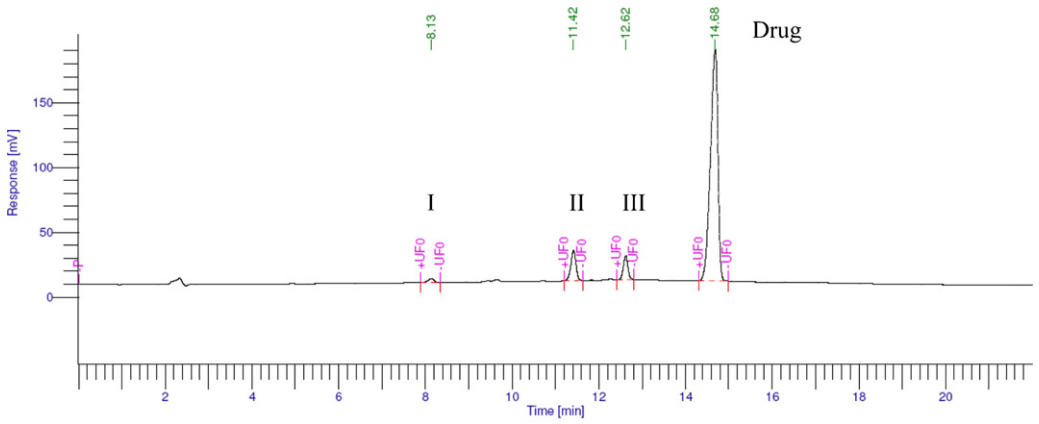
Conc. ($\mu g\,mL^{-1}$)	Intra-day precision	Inter-day precision
	Measured conc., ±SD ($\mu g\,mL^{-1}$), RSD (%)	Measured conc., ±SD ($\mu g\,mL^{-1}$), RSD (%)
60	58.29 ± 0.183, 0.31	58.97 ± 0.868, 1.45
80	79.59 ± 0.271, 0.33	79.99 ± 1.027, 1.29
100	99.38 ± 0.476, 0.41	101.24 ± 0.739, 0.74

Table 8
Recovery studies of nelfinavir mesylate.

Spiked conc. ($\mu g\,mL^{-1}$)	Calculated spiked conc. ($\mu g\,mL^{-1}$), ±SD ($\mu g\,mL^{-1}$), RSD (%)	Recovery (%)
60	58.74, ± 0.159, 1.42	97.91
80	79.35, ± 0.621, 1.08	99.19
100	99.52, ± 0.506, 0.44	99.52

Table 9
Retention time, relative retention time and peak purity.

Drug/degradation products	Retention time	Relative retention time	Peak purity index
DP-I	8.13	0.55	0.998580
DP-II	11.42	0.77	0.999964
DP-III	12.62	0.85	0.999972
Drug	14.68	1.00	0.999952



Peaks	I	II	III
Stress conditions	A	O	O
	B	PA	PA

Fig. 2. Chromatogram showing separation of degradation products (I–III) and drug in the mixture of stress samples. Keys: I–III: degradation products; A: acid; B: base; O: oxidative; PA: photoacid.

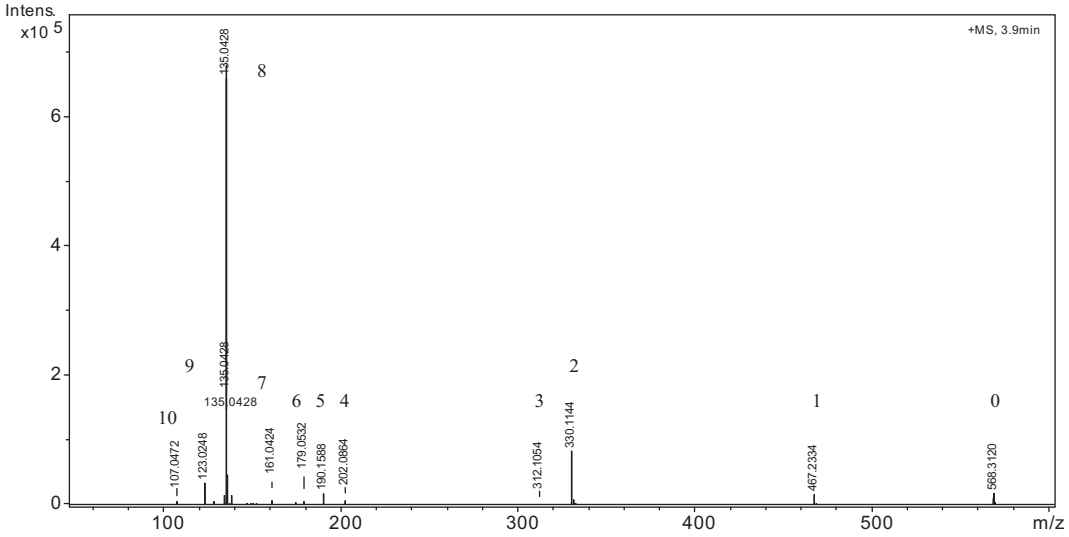


Fig. 3. Line spectra of drug obtained in MS/TOF study.

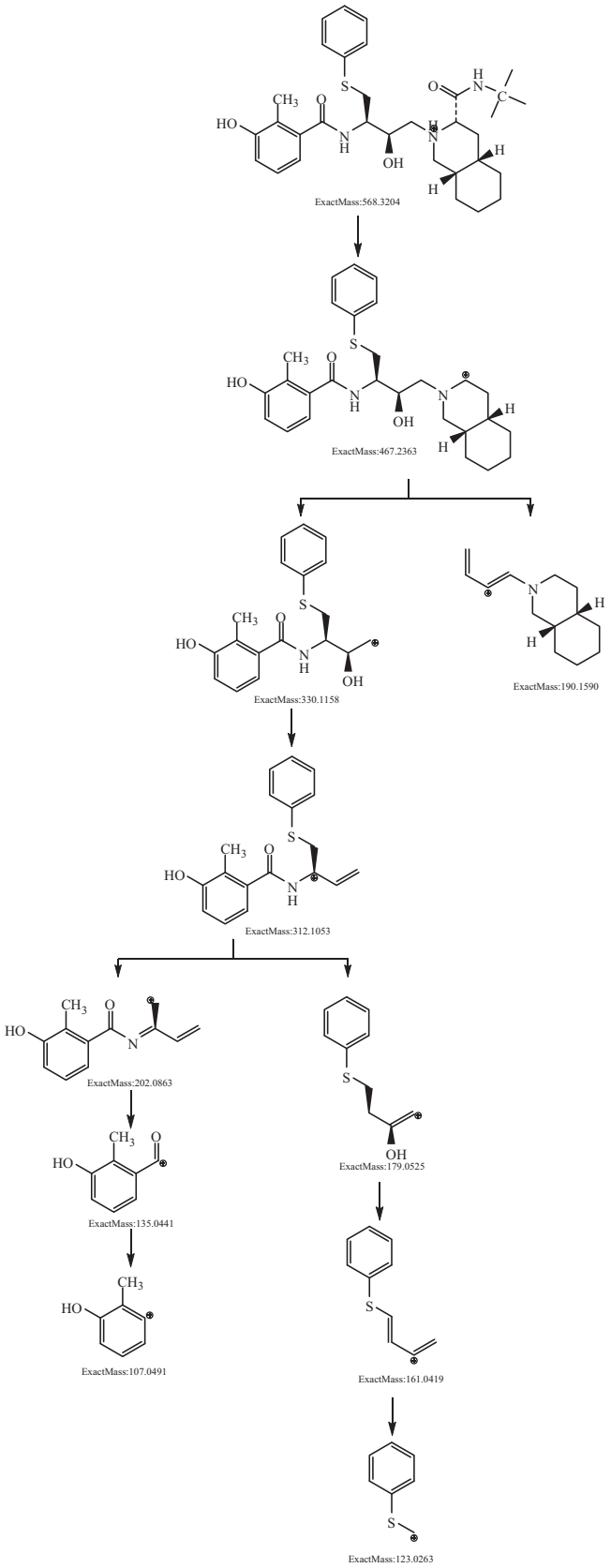


Fig. 4. Fragmentation pathway of drug along with the exact masses of the fragments.

2.6. MS/TOF and MS^n studies on drug

The fragmentation pattern of the drug was established by carrying out MS/TOF and MS^n studies in positive electrospray ionization (ESI) mode in the mass range of 50–1500 daltons (Da). The drug was directly infused using a syringe pump into MS/TOF at a concentration of 5 $\mu g\,mL^{-1}$ in methanol:water (50:50 v/v). The mass parameters were suitably optimized to get clear information about the molecular ion peak of the drug. These were further modified to get complete fragmentation of the drug. The optimized mass parameters are listed in Table 2. In MS/TOF studies, the masses of the peaks were recorded up to fourth decimal precision. High purity nitrogen was used as the nebulizer as well as the auxiliary gas. In the subsequent step, the information on the origin of each individual fragment was obtained by subjecting the drug to multi-stage mass (MS^n) studies in positive ESI mode. Fragmentation of various precursor ions formed in MS^n studies was achieved at different collision energies (Table 3). The fragments obtained in MS^n studies are listed in Table 4.

2.7. LC–MS/TOF studies on degradation products

The stressed samples were subjected to LC–MS/TOF analysis using the same parameters as optimized for the drug (Table 2). A previously developed gradient LC method was used for the analysis however, phosphate buffer was replaced by ammonium acetate of the same concentration and pH. The identity of each degradation product was established with the help of LC–MS fragmentation analyses and accurate *m/z* values.

3. Results and discussion

3.1. Method development and validation

An acceptable separation was achieved by using buffer of pH 4.0 along with the organic modifier in a gradient mode (T_{min}/A:B; T_0/60:40; T_9/88:12; T_{13}/80:20; T_{17}/60:40; T_{22}/60:40). The developed method was validated with respect to linearity, precision, accuracy, specificity and selectivity (Table 5). The linearity data are shown in Table 6. The response for the drug was found to be linear in the investigated concentration range. The values of slope and correlation coefficient (R^2) were 514878 and 0.9993, respectively. Inter-day and intra-day precision data are given in Table 7. The RSD values ranging from 0.31% to 0.41% for intra-day precision and from 0.74% to 1.45% for inter-day precision studies respectively, confirmed that the method was sufficiently precise. The percent recovery ranged between 97.91% and 99.52%, when accuracy was tested on spiking a mixture of stressed samples with the three known concentrations of the drug, viz., 60 $\mu g\,mL^{-1}$, 80 $\mu g\,mL^{-1}$ and 100 $\mu g\,mL^{-1}$ (Table 8). The method proved to be specific to each peak, which was indicated through peak purity data obtained using a PDA detector. The retention time, relative retention time and peak purity data of drug and DPs are shown in Table 9.

3.2. Degradation behaviour

As evident in Fig. 2, the drug degraded primarily into three DPs (denoted as DPs I–III in accordance with the sequence in which the peak appeared from left to right in the chromatogram). DP-I was formed in both acid and alkaline stress solutions, while DP-II and DP-III were formed in oxidative and photoacid stress conditions. The later two were formed as major products. The drug was found to be stable under all other stress conditions, including heating in

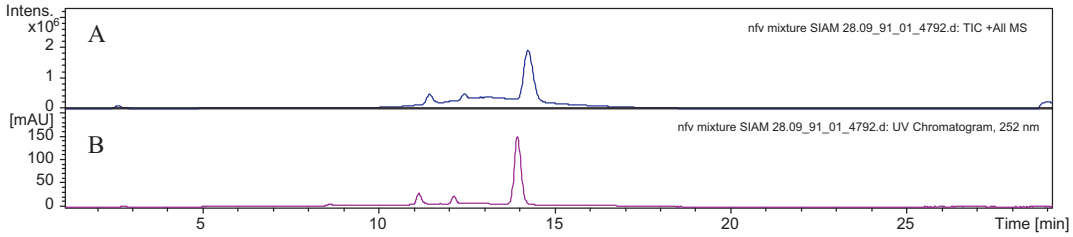


Fig. 5. Total ion current (TIC) chromatogram (A) and UV chromatogram (B) of mixture of degradation products.

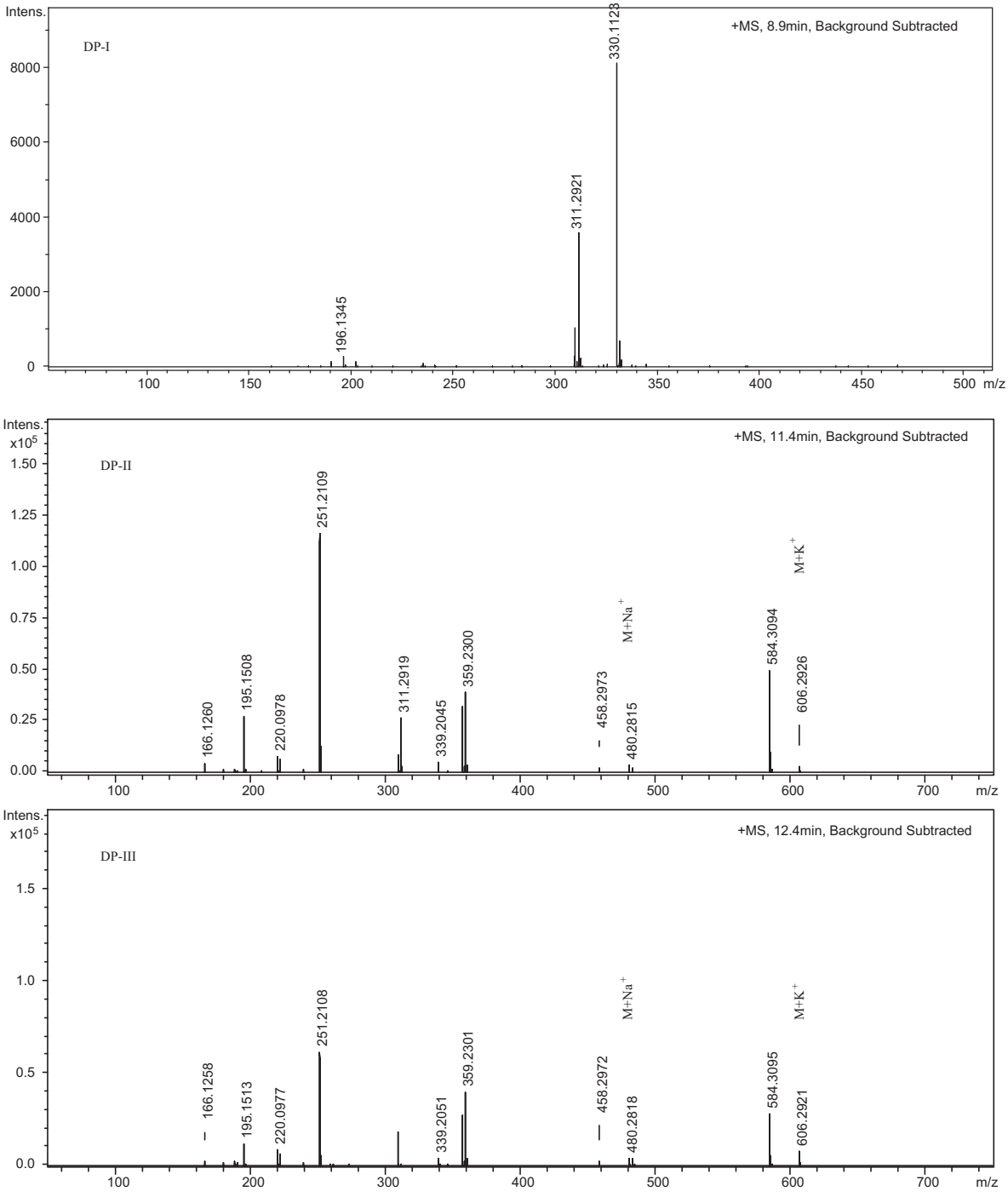


Fig. 6. Line spectra of degradation products (DPs-I–III) obtained in LC–MS/TOF studies.

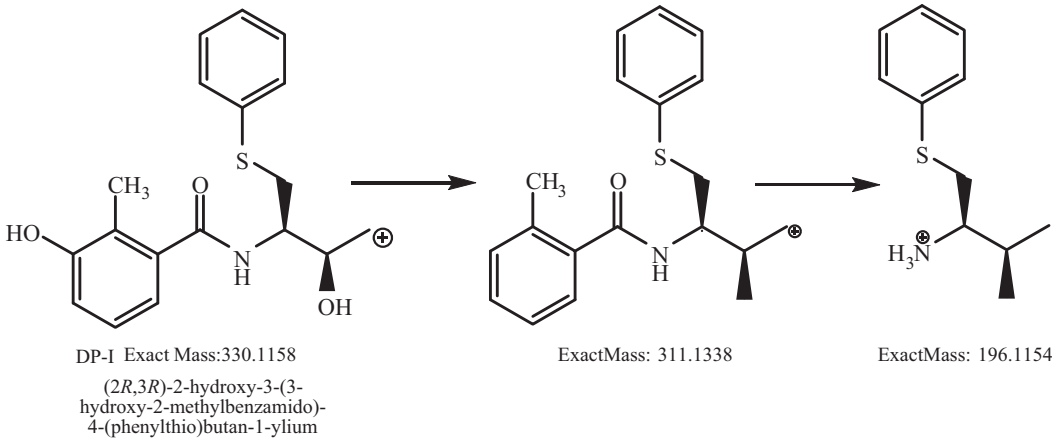


Fig. 7. Fragmentation pathway of DP-I.

water, thermal stress, photoneutral, photobase and photolytic solid stress conditions (Fig. 3).

3.3. Fragmentation pathway of the drug

Fig. 4 shows line spectrum of the drug obtained in MS/TOF studies. A total of 10 fragments were formed from the drug. The fragments are labelled 1–10, while the drug is numbered 0. The most probable molecular formula for each fragment was calculated from experimental accurate mass values with the help of Elemental Composition Calculator. The data are shown in Table 3. The data obtained from MS^n studies are shown in Table 4. These were helpful to establish origin of each fragment and also in understanding the fragmentation pathway of the drug. The major fragments of the drug had *m/z* values of 467.2334, 330.1144 and 135.0428. For the elucidation of structures of fragments, multi-stage mass studies (MS^n) were conducted by taking the fragments in a sequential order. In MS^2 studies, the parent of *m/z* 568 fragmented into two major ions of *m/z* 467 and 330. The latter was subjected to MS^3 studies where ion of *m/z* 467 fragmented into daughters of *m/z* 330 and 190, while ion of *m/z* 330 reduced into fragments of *m/z* 312. In MS^4 study, the daughter of *m/z* 312 resulted in the formation of two new fragments of *m/z* 202 and 179. In a similar manner, MS^n studies were extended up to MS^6.

The results showed that the major fragment of *m/z* 467 was formed on the loss of *N*-*tert*-butylformamide moiety ($C_5H_{10}NO$) from the drug, while the fragment of *m/z* 330 was formed on further loss of the decahydroisoquinoline moiety ($C_9H_{17}N$). The fragment of *m/z* 330 lost a water molecule and 3-hydroxy-2-methylbenzamide moiety to result in fragments of *m/z* 312 and 179, respectively. Both these fragments followed a different pathway subsequently. The fragment of *m/z* 312 on the loss of phenylthio moiety resulted in the formation of an ion of *m/z* 202. The complete fragmentation pathway of the drug is shown in Fig. 4.

3.4. LC–MS/TOF studies on stressed samples

The UV and total ion chromatogram (TIC) obtained in LC-MS/TOF study on the mixture of stressed samples are shown in Fig. 5. The mass spectra of DPs I–III are shown in Fig. 6. The experimental masses, possible molecular formulae and major fragments of DPs are enlisted in Table 5.

3.5. Postulated structures of degradation products

The structure elucidation of DP-I, DP-II and DP-III was achieved with the help of their major fragments observed in MS/TOF studies (Fig. 6) and comparison with fragmentation pattern of the drug (Fig. 5).

3.5.1. DP-I (*m/z* 330)

As shown in Fig. 6, apart from parent ion of *m/z* 330.1123, DP-I fragmented into two ions of *m/z* 311.2921 and 196.1345. The parent ion had almost the same mass (330.1144) as generated during MS/TOF study on the drug (Fig. 3). It indicated that, DP-I was formed on the loss of a moiety of 238 Da from the drug. Taking the help of Mass Frontier Software and Elemental Composition Calculator, a best possible molecular formula was generated for DP-I, showing that *N*-*tert*-butyldecahydroisoquinoline-3-carboxamide moiety of the drug underwent hydrolysis involving cleavage of loosely held carbon-nitrogen bond. The structure and the fragmentation pathway of DP-I are outlined in Fig. 7.

3.5.2. DP-II and DP-III (*m/z* 584)

As shown in Fig. 6, the fragments generated in LC–MS/TOF studies for DP-II and DP-III were almost similar to each other. The direct attack of oxygen on sulphur atom of the phenylthio moiety resulted in the formation of oxidative degradation products. Based on the fragmentation similarity between DP-II and DP-III, it was assumed that the two could be isomeric to each other. Both DP-II (584.3094) and DP-III (584.3095) had *m/z* of ~16.00 mass units more than the drug. The best possible structures and the fragmentation pathways are outlined in Figs. 8 and 9, respectively.

3.6. Postulated degradation pathway of the drug

The proposed degradation pathway of the drug under variety of stress condition is shown in Fig. 10. Under acid and alkaline stress conditions, the *N*-*tert*-butyldecahydroisoquinoline-3-carboxamide moiety of the drug underwent hydrolysis and resulted in the formation of DP-I. Under oxidative and photoacid stress conditions, two common isomeric degradation products, DP-II and DP-III, were generated on oxidation of sulphur atom of the phenylthio moiety of the drug. The mechanism of the formation of all the three degradation products are shown in Fig. 11.

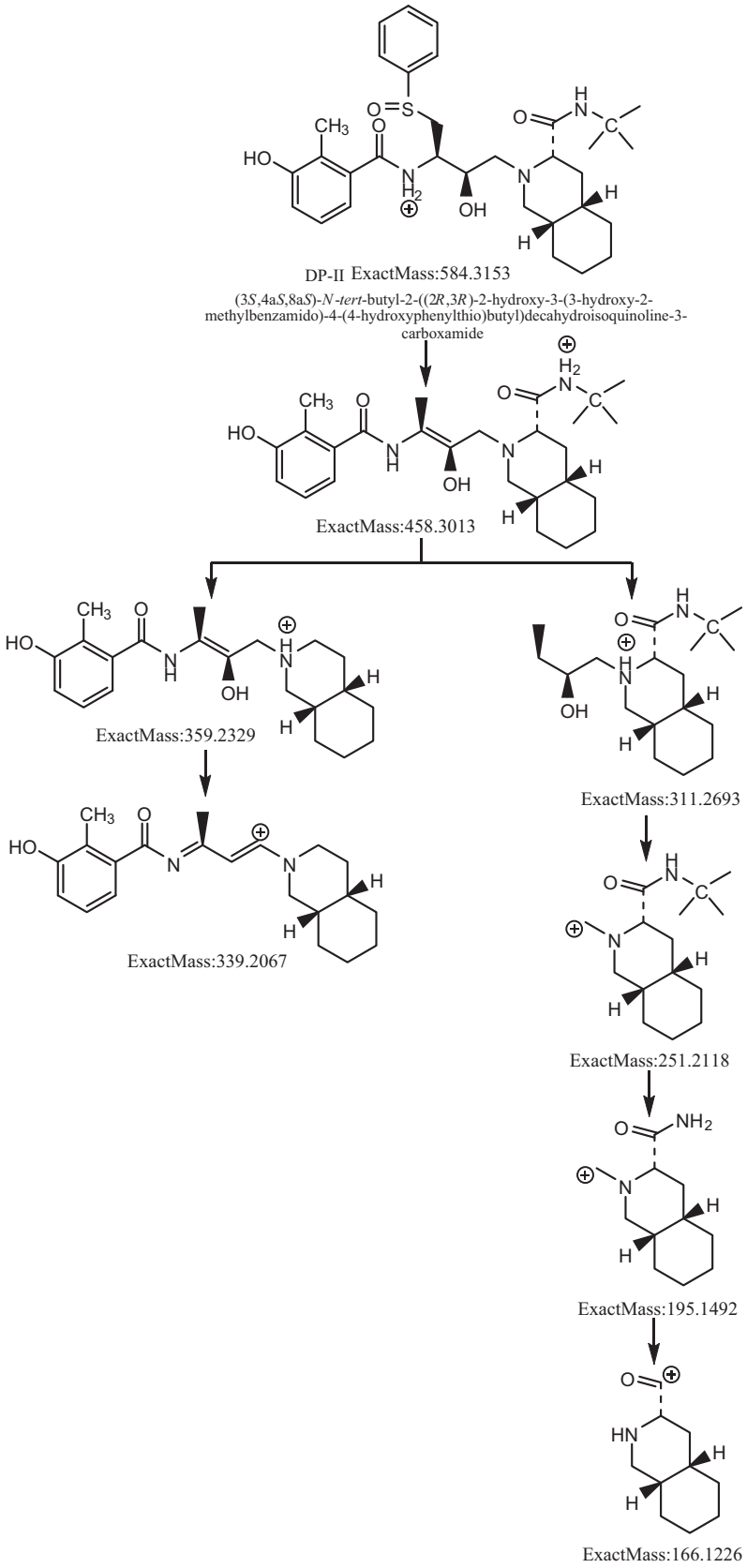


Fig. 8. Fragmentation pathway of DP-II.

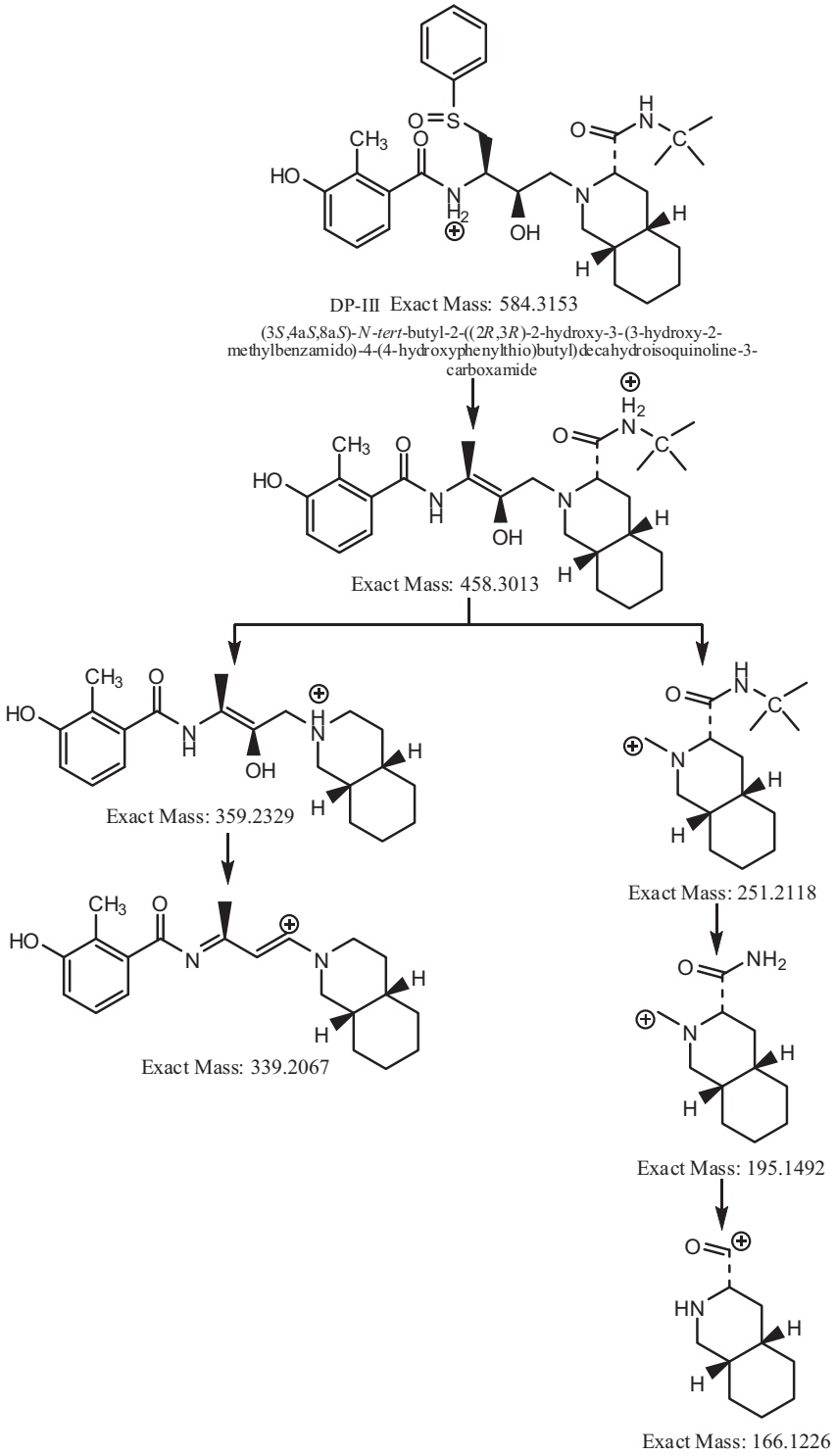


Fig. 9. Fragmentation pathway of DP-III.

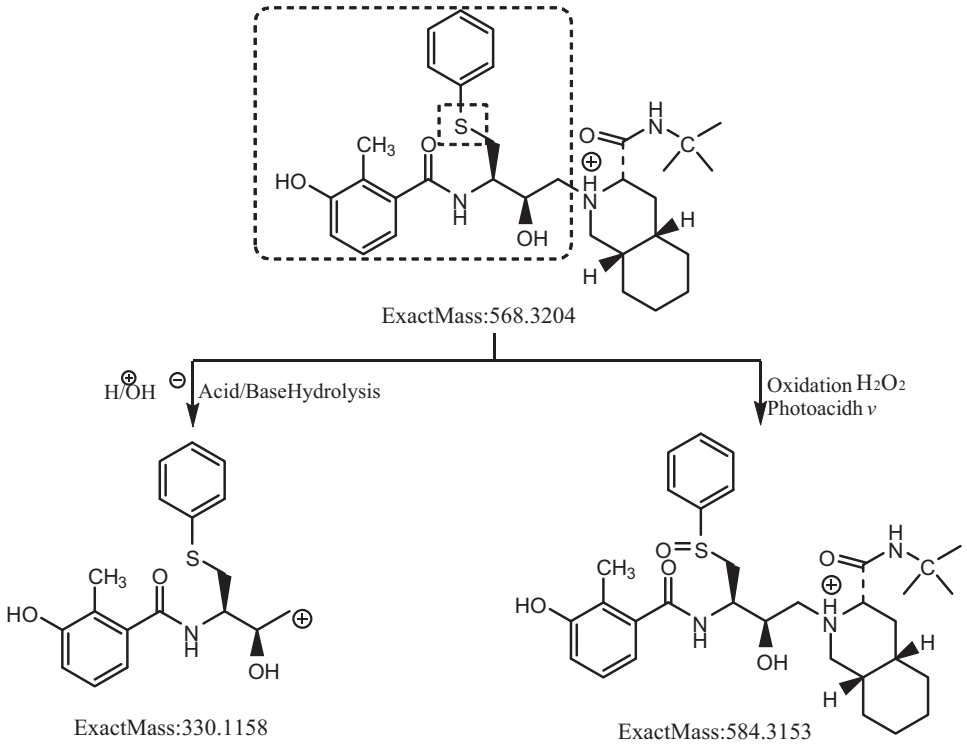


Fig. 10. Degradation pathway of the drug.

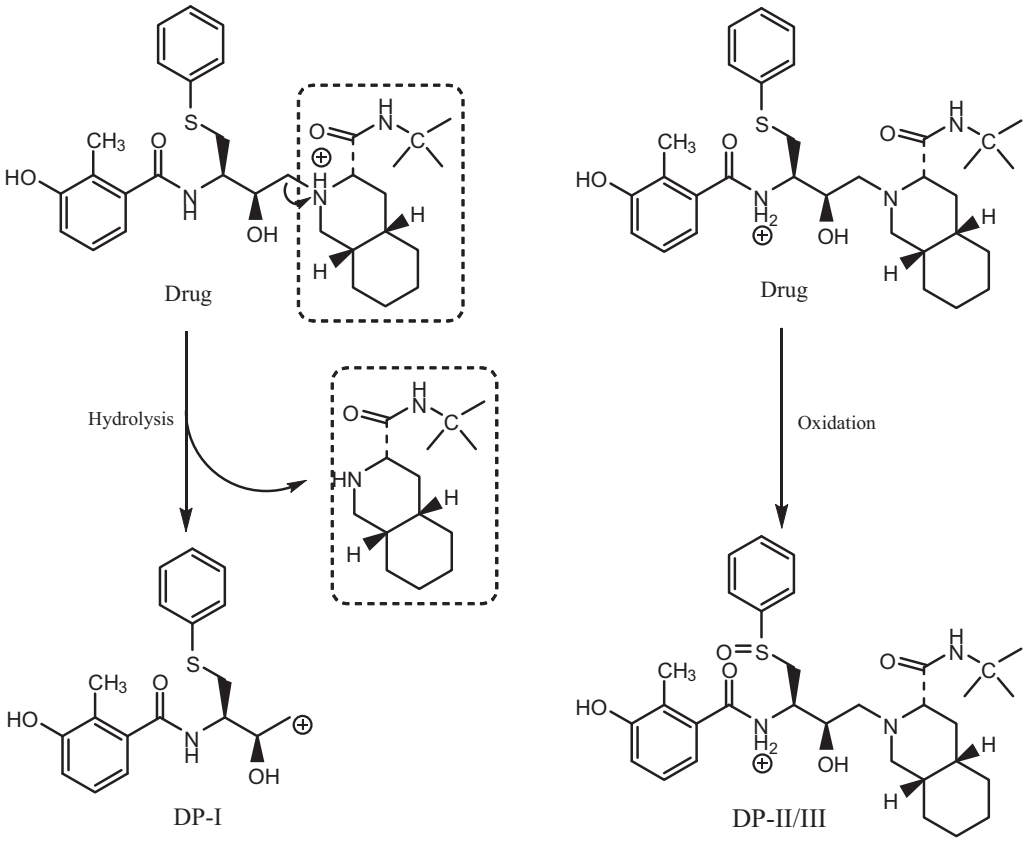


Fig. 11. Mechanism of formation of DPs.

4. Conclusions

Degradation behaviour of nelfinavir mesylate was explored by exposing it to ICH defined stress conditions. The drug degraded under acidic, basic, oxidative and photoacid stress, while it was found to be stable under all other stress conditions. HPLC analysis revealed the formation of three degradation products (DPs I–III), all of which were found to be previously unknown. While DP-I was formed as a common hydrolytic degradation product under both acid and alkaline stress conditions. Two major isomeric DPs (DP-II and DP-III) were generated under oxidative and photoacid stress. To resolve their structures, mass fragmentation pattern of the drug was established with the help of MS/TOF and MS^n studies. The degradation products were then characterized with the help of LC–MS/TOF data and comparison of the same to that for the drug. The complete degradation pathway of the drug and mechanism of the formation of degradation products were also established. This information is being reported for the first time.

Acknowledgements

The authors wish to extend their thanks to Prof. Saranjit Singh, Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research, S.A.S. Nagar Mohali, Punjab, India, for extending LC–MS/TOF and LC–MS^n facilities and helpful discussion.

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