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Degradation Studies of Lacidipine: Identification, Isolation and Structural Characterization of Stress Degradation Products using LCMS, Mass mediated Prep-HPLC, NMR and HRMS

K.V.K. Mohan Pulletikurthi[1,2,*], S.S.K. Chakravarthy Kotha[1], Raju Doddipalla[1], Chidananda Swamy Rumalla[1], Muralidharan Kaliyaperumal[1] and Raghu Babu Korupolu[2]

[1]Department of Medicinal Chemistry, G.V.K. Biosciences Pvt. Ltd., IDA Nacharam, Hyderabad-500076, India
[2]Department of Engineering Chemistry, Andhra University, Visakhapatnam-530003, India

*Corresponding author: E-mail: pulletikurthikrishnamohan@gmail.com; srinumohan@yahoo.co.in

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The stability of lacidipine drug under stress conditions and the identification of the degradation products, according to ICH guidelines Q1A (R2) were investigated in the hydrolytic and oxidative stress conditions. The drug degradation occurred under hydrolytic conditions like (acidic and basic) while it was stable in the oxidative condition. Three degradation products were formed under acidic condition and one degradation product was formed under basic condition, which was separated by using APMS (Auto Purification Mass Spectrometer) and gradient elution with C18 column. The four degradants have not been characterized earlier and in the present study all the structures were established and characterized using NMR spectroscopy (1D and 2D) and HRMS (high resolution mass spectrometer).

Keywords: Lacidipine, Degradation products, Isolation, Identification, NMR, HRMS.

INTRODUCTION

Lacidipine is a calcium channel blocker with antioxidant effect used in the treatment of hypertension and atherosclerosis [1]. The IUPAC name of lacidipine is diethyl (*E*)-4-(2-(3-(*tert*-butoxy)-3-oxoprop-1-en-1-yl)phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate. Among the dihydropyridines, it is one of the most vascular selective because of its high degree of lipophilicity and has a long duration of action. Lacidipine washout rate is slow due to its high membrane partition coefficient and has a long clinical half-life [2]. Lacidipine is an antihypertensive agent and reduces blood pressure and shows greater antioxidant activity than other calcium antagonists [3]. Lacipil or motens are the trade names of lacidipine and allowed for medication in 1991 [4]. Generally, 2-6 mg of lacidipine is administered daily compared with other antihypertensive agents like atenolol (a β-blocker) and enalapril (an ACE inhibitor) [1]. *trans*-Lacidipine is active and used for therapy [5,6].

Solubility of lacidipine is very poor in water as compared to common organic solvents such as methanol, ethanol and acetone. It is susceptible to degradation on exposure to temperature and light [5,6]. Various studies have been carried out to study the stability of lacidipine in formulations and API. This includes spectrophotometric and liquid chromatographic methods [7-10]. Many methods for the determination of lacidipine in biological samples using LC/MS have been reported [11-15]. Non-chromatographic techniques for assay determination are also reported [16-18]. An RP-HPLC/MS method [19] for detection of lacidipine and its related metabolites is also reported. However, this limits only to metabolites and not to other degradation impurities. None of the literature revealed the presently identified degradants and hence establishing them as the novel isolated impurities for lacidipine. Lacidipine is sensitive towards the stress conditions due to this reason it can degrade after sometime [7-9].

EXPERIMENTAL

Lacidipine is a gift sample from a reputed manufacturer in Hyderabad, India. HPLC grade buffers such as formic acid, hydrochloric acid, hydrogen peroxide, ammonium bicarbonate sodium hydroxide and ammonium acetate were purchased from Merck India. Solvents like acetonitrile and methanol were

procured from Thermo-Fisher, India. HPLC grade water from Milli-Q purification system by Millipore.

UPLC-MS: Acquity UPLC-MS (Triple quadrupole MS) from Waters India Pvt Ltd. was used to separate the compounds. Acquity BEH C18 (50 × 2.1) mm, 1.7 µm column using for gradient analysis and the mobile phase consists of 0.1% formic acid in water and acetonitrile as co-solvent. Samples were prepared by using mobile phase in the ratio of 30:70 (0.1% formic acid in water:acetonitrile).

Waters triple quadrupole MS was used in both positive and negative polarity using ESI source with a mass range of 100-1500 Daltons (Da). MS Source parameters such as Capillary voltage (4.0 kV), source temperature (130 ºC) and desolvation temperature (300 ºC), Desolvation gas flow (750 L/h), cone gas flow (75 L/h). and Masslynx software was used.

High resolution mass spectrometry: Samples were analyzed on Thermo Q Exactive orbitrap MS with ESI ion source with alternating positive and negative scan; instrument parameters (source) were: Spray voltage: 3500 V; Aux gas heater temperature: 440 ºC; Capillary temperature: 270 ºC. Sheath gas flow rate: 53; Aux gas flow rate: 14; Sweep gas flow rate: 3. Reserpine was used as an internal standard to check the mass accuracy of the system. Reserpine (monoisotopic mass: 608.2734 Da). Dionex ultimate 3000 LC was controlled by Chromeleon software, Mass data was acquired by using Xcalibur software.

Mass mediated preparative HPLC: Waters Autopurification system with PDA and mass based fraction collector was used to purify the compounds. Masslynx data handling system was used to operate the instrument and Kromosil C18 column (150 × 25) mm, 7 µm was used to purify the mixture of compounds. Mass capillary voltage was maintained at 1.5 kV, Source temperature 150 ºC and desolvation temperature 375 ºC, desolvation gas flow 500 L/h. The cone gas flow was set at 40 L/h. Aqueous ammonium acetate (1 mM) and acetonitrile (30: 70, v/v) was used as a makeup solvent with flow rate of 0.3 mL/min having a split ratio of 1:1000 to the mass detector.

NMR analysis: NMR analysis of lacidipine (API), acid, base degradation products were taken on Bruker 400 MHz. NMR instrument equipped with 5 mm Double Resonance Broad Band Probes (BBO) NMR probe with Z-gradient shim system and autosampler having 60 sample holder capacity. NMR analysis have taken at 298 K probe temperature with fine tuning for the respective nuclei. TMS is used as reference and set at zero ppm, DMSO-d_6 septet at 39.5 ppm in carbon NMR.

One dimensional (1D) analysis: ^{1}H NMR and ^{13}C NMR

Two-dimensional (2D) analysis: Homonuclear 1H-1H gDQCOSY, 1H-13C gHSQC and gHMBC.

Sample preparation

Forced degradation studies: As per ICH stability guidelines [20-27] different kinds of stress parameters *i.e.* acidic, basic, oxidation, thermal and photolytic conditions were employed.

Acid degradation: Lacidipine (500 mg) was added in 5 mL of 0.1 N HCl containing in a round bottom flask. The mixture was refluxed at 60 ºC for 10 h.

Base degradation: Lacidipine (500 mg) was taken in a round bottom flask and 5 mL of 0.1N NaOH was added and refluxed at 60 ºC for 10 h.

Oxidative degradation: Lacidipine (500 mg) was mixed with 5 mL of 3% H_2O_2 and the solution was kept at room temperature for 48 h.

Photolytic degradation: Photolytic degradation was carried out under UV light. Lacidipine (200 mg) was placed in a clean glass plate and kept under UV light for 48 h, as no physical change was observed, thus taken for further studies.

Thermal degradation: Lacidipine (200 mg) was kept at 120 ºC for 48 h in hot air oven, as no physical change was observed and thus taken for further analysis.

For NMR analysis: About 8-10 mg of sample was dissolved in deuterated DMSO and taken for analysis.

Preparation of degradation samples for purification: Acid degraded compound was neutralized with saturated aqueous solution of ammonium bicarbonate and the resultant solution was concentrated and further diluted with 4-5 mL of acetonitrile-water (50:50) mixture. For base degradation, degraded sample was neutralized with 5 N HCl solution and the resultant solution was concentrated and further diluted with 4-5 mL of acetonitrile-water (50:50) mxiture for HPLC purification.

Isolation of acid degradation products: In acid degradation, purification was carried out by using mobile phase A: 0.1% formic acid (aq.) and mobile phase B: acetonitrile using Kromosil C18 column with dimensions (150 × 25 mm) 7 µ and gradient (Time/% of B): 0/30, 2/30, 11/60, 13/70, 14/80, 14.1/100, 17/100, 17.1/30, 20/30. Crude sample was purified in Prep HPLC and the fractions of masses 328.20 (M+H), 400.21 (M+H), 400.23 (M+H), collected separately and lyophilized to get free solid.

Isolation of base degradation products: In base degradation, purification was carried out by using mobile phase A: 10 mM ammonium bicarbonate (aq.) and mobile phase B: acetonitrile using X-Bridge OBD C18 column having dimensions (250 × 19 mm) 5 µ and gradient (Time/% of B): 0/50, 1/50, 11/70, 13/80, 16/100, 16.1/100, 18/100, 18.1/50, 20/50. Crude sample was purified in Prep HPLC and the fractions of mass 456.29 (M+H) collected together and concentrated to get free solid.

Degradation behaviour of lacidipine: No degradation products were found in oxidative, thermal and photolytic conditions for the lacidipine API. The drug was found to be labile to acid hydrolysis as a total of 14.39% degradation was found (0.1N HCl reflux at 60 °C, up to 10 h) with DP-1 4.11%, DP-2 5.28% and DP-3 5.00%, respectively. The drug degradation was also found in base degradation (0.1 N NaOH reflux at 60 ºC up to 10 h) with DP-4 of 9.82%. Degradation details are shown in Table-1. Acid, base, oxidative, thermal and photolytic degradation chromatograms are shown in Fig. 1.

RESULTS AND DISCUSSION

The degradants were observed after specific time interval after each stress study. The resultant degradation samples of all the stress studies were also analyzed individually in UPLC-MS. Four major degradation products were identified, isolated, characterized by HRMS, APMS and NMR (1D and 2D) techniques.

Structural characterization of acid degradation products of DP-1: Isolated DP-1 compound was analyzed by HRMS

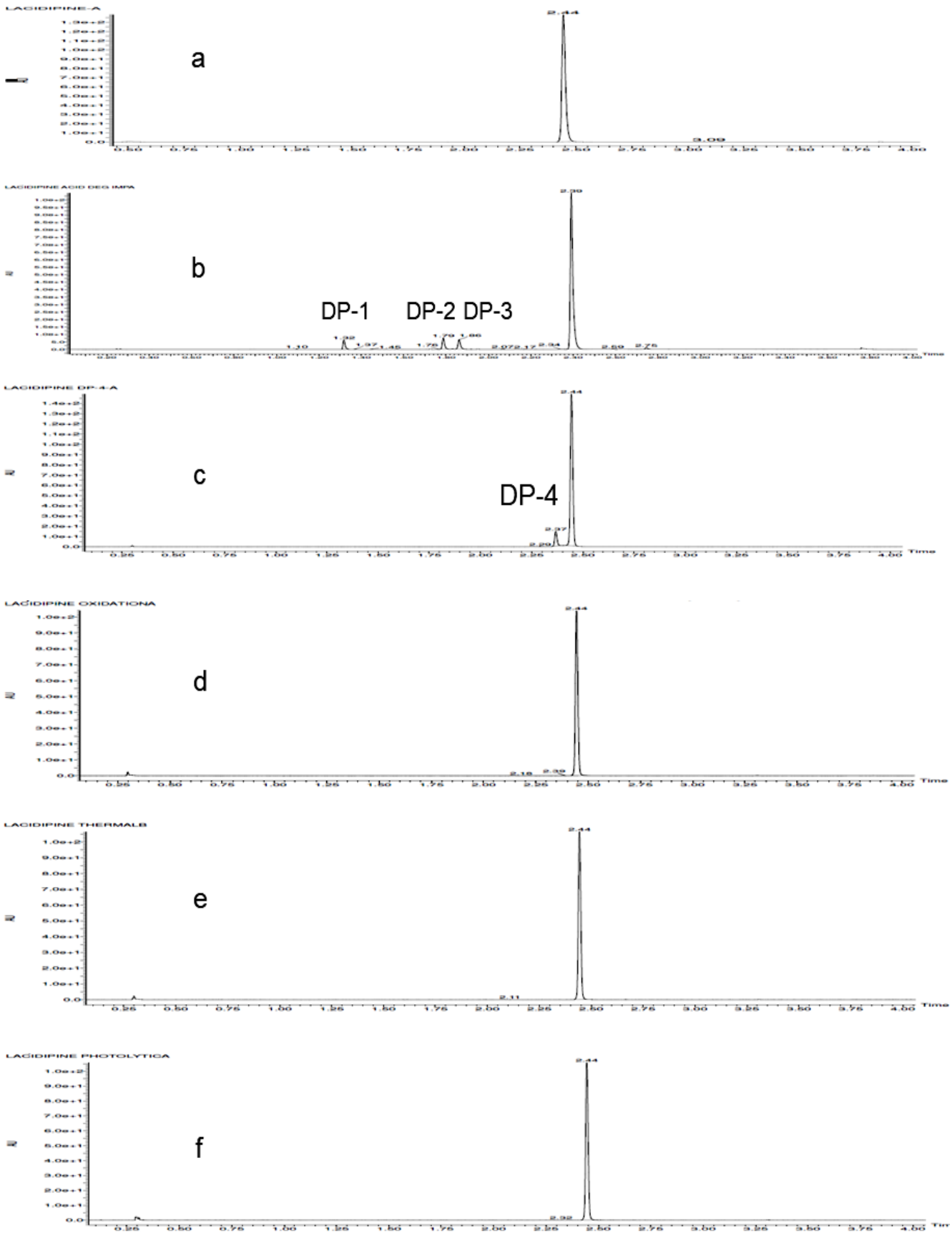

Fig. 1. LCMS chromatograms of lacidipine and lacidipine degradant impurities. (a) Lacidipine (API), (b) acid degradation, (c) alkaline degradation, (d) oxidative degradation, (e) thermal degradation and (f) photolytic degradation

TABLE-1
LACIDIPINE FORCE DEGRADATION STUDIES

Conditions	DP-1 (%)	DP-2 (%)	DP-3 (%)	DP-4 (%)	API (%)
Lacidipine API	–	–	–	–	99.95
Acid (0.1 N HCl)	4.11	5.28	5.00	–	83.88
Base (0.1 N NaOH)	–	–	–	9.82	90.18
Oxidation (3% H_2O_2)	–	–	–	–	99.68
Thermal (120 °C for 48 h)	–	–	–	–	99.61
Photolytic (48 h)	–	–	–	–	99.86

for mass and NMR for structure elucidation. NMR spectra of DP-1 was compared with the NMR of API to get an insight on the changes of groups in the degradation product. Absence of *tert*-butyl group, -NH proton and presence of only one triplet at 1.23 ppm and multiplet at 4.19 ppm confirmed the cleavage of *tert*-butyl group and one of the ethyl ester (Table-2). Further, proton at 13.0 ppm indicated presence of acid group. Two methyl groups were observed at 1.53 and 2.46 ppm indicating symmetry loss of pyridine ring of API. Gradient 1H-13C HSQC experiment confirmed presence of three aliphatic methine groups and absence of Olefinic –CHs in DP-1. gHSQC (Fig. 2b) along with gHMBC (Fig. 2c) helped establish methylene at 3.34 ppm. Taking this data into reference mass was recorded on HRMS. Based on the data from HRMS and NMR, the calculated molecular formula for DP-1 is $C_{19}H_{22}NO_4$ with protonated mass of 328.1534 and error of 2.71 ppm. The tentative structure was derived as below (Fig. 2a).

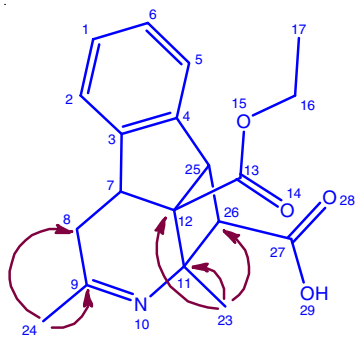

Fig. 2a. Structure of DP-1

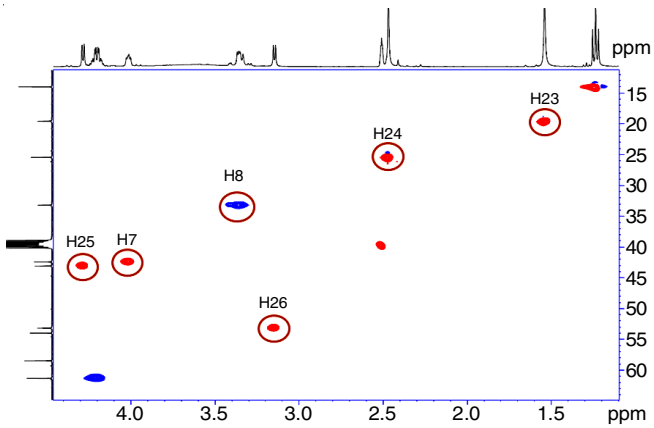

Fig. 2b. gHSQC spectrum of DP-1 with key correlation

TABLE-2
ASSIGNMENTS OF ^{1}H AND ^{13}C NMR SIGNALS OF LACIDIPINE API AND DP-1

API				DP-1			
Atom No.	Type of atom	^{1}H Chemical shift (ppm); Coupling constant (*J*)	^{13}C Chemical shift (ppm)	Atom No.	Type of atom	^{1}H Chemical shift (ppm); Coupling constant (*J*)	^{13}C Chemical shift (ppm)
1	CH	7.30 (t, 8.0 Hz, 1H)	130.51	1	CH	7.32 (m, 1H)	124.18
2	CH	7.33 (d, 7.6 Hz, 1H)	129.72	2	CH	7.28 (m, 1H)	124.31
3	C	–	149.05	3	C	–	143.26
4	C	–	130.51	4	C	–	143.63
5	CH	7.60 (d, 7.6 Hz, 1H)	125.09	5	CH	742 (d, 6 Hz, 1H)	124.18
6	7.12	7.12 (t, 8.0 Hz, 1H)	126.36	6	CH	7.29 (m, 1H)	128.51
7	CH	5.21 (s, 1H)	34.38	7	CH	4.01 (dd, 6.4 Hz, 4Hz, 1H)	42.39
8, 12	C	–	102.62	8	CH2	3.35 (m, 2H)	33.18
9, 11	C	–	145.6	9	C	–	187.8
10	NH	8.84 (s, 1H)	–	10	N	–	–
13, 18	C	–	166.63	11	C	–	58.45
14, 20	O	–	–	12	C	–	53.96
15, 19	O	–	–	13	C	–	170.5
16, 21	CH2	3.77, 3.97 (m, 4H)	58.87	14	O	–	–
17, 22	CH3	1.05 (t, 7.2 Hz, 6H)	13.99	15	O	–	–
23, 24	CH3	2.26 (s, 6H)	18.15	16	CH2	4.19 (m, 2H)	61.31
25	CH	8.37 (d, 16 Hz, 1H)	119.54	17	CH3	1.23 (t, 7.2 Hz, 3H)	13.96
26	CH	6.31 (d, 16 Hz, 1H)	119.54	18			
27	C	–	165.94	19			
28	O	–	–	20			
29	O	–	–	21			
30	C	–	79.55	22			
31, 32, 33	CH3	1.49 (S, 9H)	27.82	23	CH3	1.53 (s, 3H)	19.54
				24	CH3	2.16 (s, 3H)	25.41
				25	CH	4.29 (d, 5.2, 1H)	43.07
				26	CH	3.15 (d, 5.2 Hz, 1H)	53.15
				27	C	–	170.09
				28	O	–	–
				30	OH	13.00 (broad hump)	–

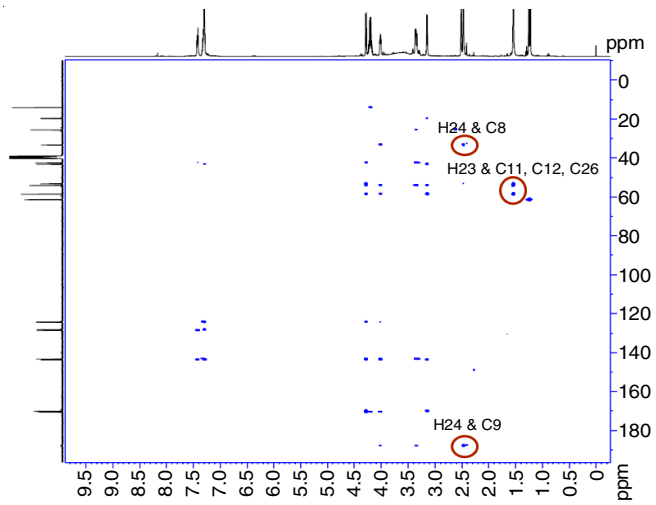

Fig. 2c. ^{1}H-^{13}C gHMBC spectrum of DP-1 with key correlation

Further the correlations were confirmed by COSY (Fig. 2d) and gHMBC. In COSY experiment, H25 correlates with H26 proton. gHMBC helped understand 2J and 3J connectivity's. H24 showed 2J connectivity to C9 carbon and 3J connectivity to C8 carbon. H23 proton showed 2J connectivity to C11 carbon and 3J connectivity to C12 and C26 carbons. α-β unsaturated double bond involved in cyclization with one of the double bond of dihydropyridine ring and lead to form tricyclic compound. The IUPAC name for DP-1 was concluded as 1a′-(ethoxycarbonyl)-1a,3-dimethyl-1,1a,1a′,4,4a,8b-hexahydro-2-azacyclobuta[*j*,*k*]fluorene-1-carboxylic acid. Fig. 2e shows proton NMR spectrum of lacidipine (API), DP-1, DP-2, DP-3 and DP-4.

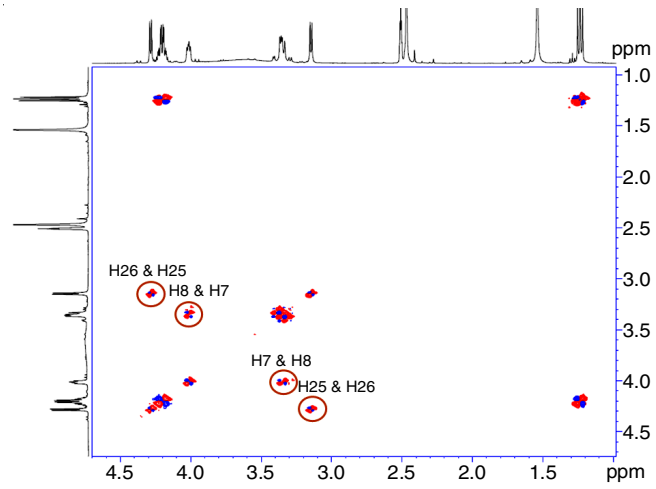

Fig. 2d. gCOSY spectrum of DP-1 with key correlation

Structural characterization of acid degradation products of DP-2: 2D-NMR and mass using HRMS was recorded for DP-2 and compared with that of API. NMR showed absence of *tert*-butyl group and presence of additional exchangeable proton at 12.30 ppm, which was confirmed as carboxylic acid peak by ^{13}C NMR showed a peak at 168.09 ppm. The mass obtained was 400.1747 with tentative molecular formula of $C_{22}H_{26}NO_6$, which gave an error of 2.02 ppm. This was further confirmed by HMBC, which gave correlation of 2J connectivity of H26 and 3J connectivity of H25 with C27 (Fig. 3b). Based on the above key points, the structure of DP-2 was elucidated as shown in Fig. 3a. IUPAC name of DP-2 is (*E*)-3-(2-(3,5-*bis*-(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridin-4-yl)-phenyl)acrylic acid. NMR studies and its full characterization details have been summarized in Table-3.

TABLE-3
ASSIGNMENTS OF ^{1}H AND ^{13}C NMR SIGNALS OF LACIDIPINE DP-2

Atom No.	Type of atom	^{1}H Chemical shift (ppm); Coupling constant (*J*)	^{13}C Chemical shift (ppm)
1	CH	7.30 (t, 8 Hz, 1H)	130.49
2	CH	7.35 (d, 7.6 Hz, 1H)	129.7
3	C	–	149.09
4	C	–	130.67
5	CH	7.60 (d, 7.6 Hz, 1H)	125.07
6	CH	7.12 (t, 7.6 Hz, 1H)	126.33
7	CH	5.21 (s.1H)	34.39
8, 12	C	–	102.67
9, 11	C	–	145.56
10	NH	8.84 (s, 1H)	–
13, 18	C	–	166.7
14, 20	O	–	–
15, 19	O	–	–
16, 21	CH2	3.90 (m, 4H)	58.93
17, 22	CH3	1.04 (t, 6.8 Hz, 6H)	13.99
23, 24	CH3	2.26 (s, 6H)	18.2
25	CH	8.46 (d, 16 Hz, 1H)	143.69
26	CH	6.38 (d, 16 Hz, 1H)	118.99
27	C	–	168.09
28	O	–	–
29	OH	12.3 (broad hump)	–

Structural characterization of acid degradation products of DP-3: On comparison of DP-3 proton NMR data with API, it was understood that α-β unsaturated olefinic CH protons are mislaid of *tertiary*-butyl group and presence of one exchangeable proton at 12.5 ppm, emphasized that *tert*-butyl group got cleaved and formed free acid. The difference between the two methyl group protons at 1.31 and 2.15 ppm showed the loss of dihydropyridine ring symmetry. HSQC (Fig. 4b) revealed the presence of three aliphatic CH protonated carbons in the compound. gCOSY (Fig. 4c) showed the correlation of H25 with H26. ^{1}H-^{13}C gHMBC (Fig. 4d) experiment showed 2J connectivity of H24 to C9, 3J connectivity to C8 carbon and 2J connectivity of H23 to C11, 3J connectivity's to C12 and C26 carbons. The absence of α-β unsaturation and asymmetric nature of dihydropyridine ring indicates that both the bonds were involved in cyclization and lead to form tricyclic compound shown in Fig. 4a. Mass spectrometry analysis using HRMS measured the protonated exact mass of DP-3 as 400.1747 that have 2.02 ppm error for the calculated molecular formula of $C_{22}H_{26}NO_6$. The HRMS spectrum of all the degradants along with API is collectively shown in Fig. 5. The NMR studies and its full characterization details have been tabulated in Table-4. The IUPAC name of DP-3 is 1a′,4-*bis*(ethoxycarbonyl)-1a,3-dimethyl-1,1a,1a′,2,4a,8b-hexahydro-2-azacyclobuta[*j*,*k*]-fluorene-1-carboxylic acid.

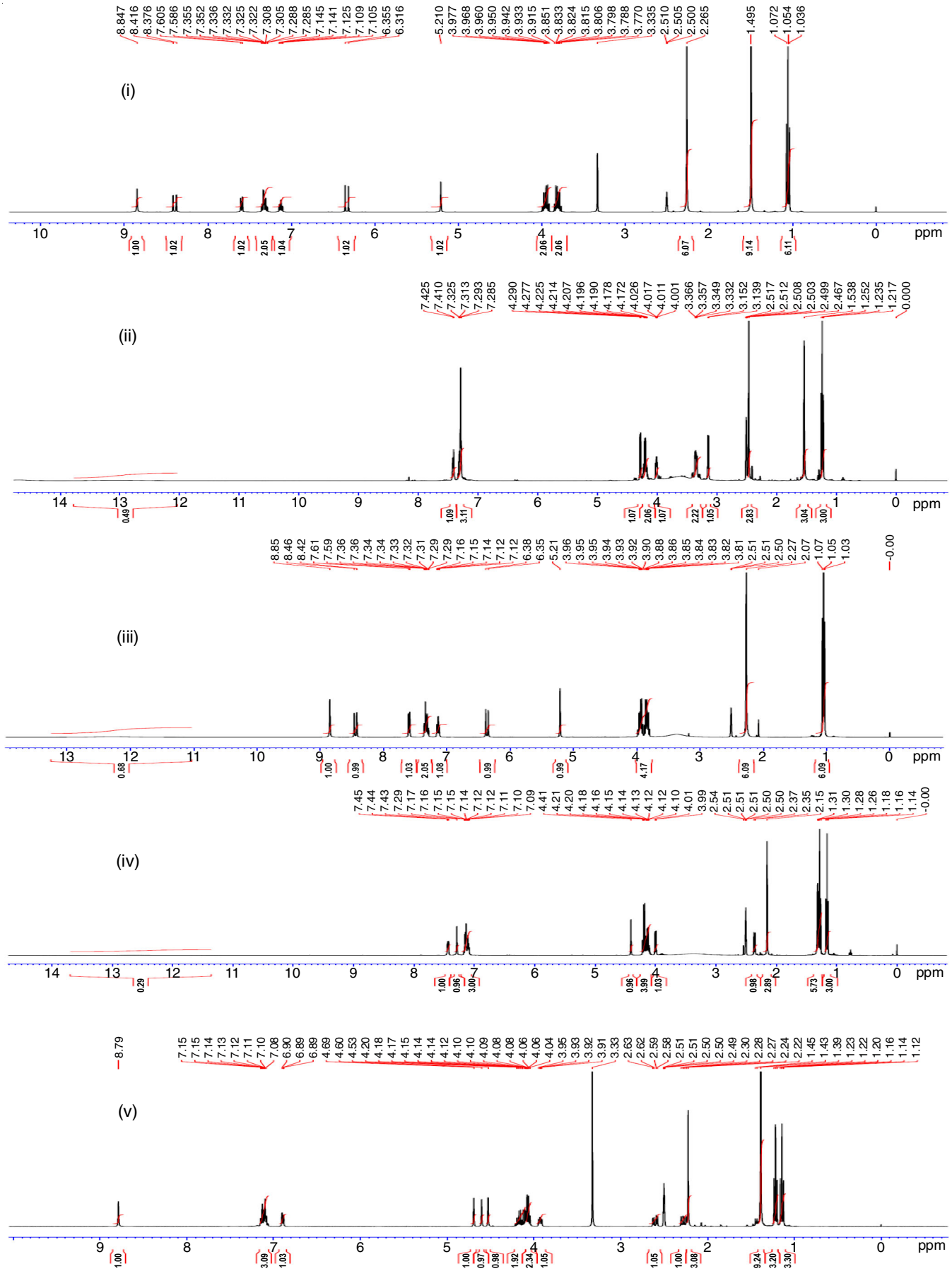

Fig. 2e. Proton NMR spectrum of (i) lacidipine (API), (ii) DP-1, (iii) DP-2, (iv) DP-3 (v) DP-4

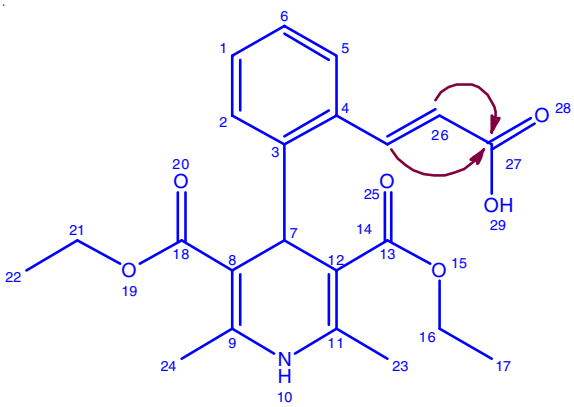

Fig. 3a. Structure of DP-2

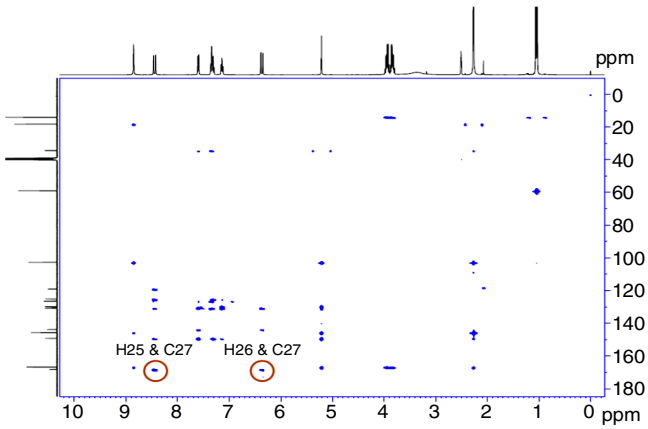

Fig. 3b. ^{1}H-^{13}C gHMBC spectrum of DP-2 with key correlation

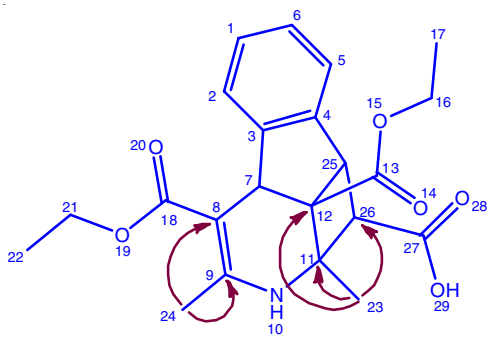

Fig. 4a. Structure of DP-3

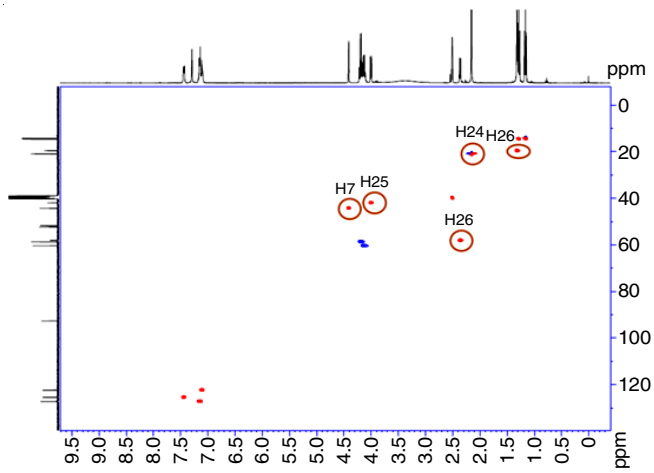

Fig. 4b. gHSQC spectrum of DP-3 with key correlation

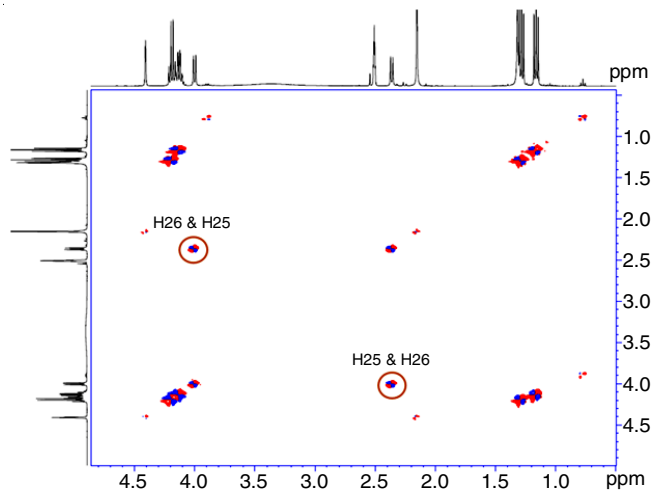

Fig. 4c. gCOSY spectrum of DP-3 with key correlations

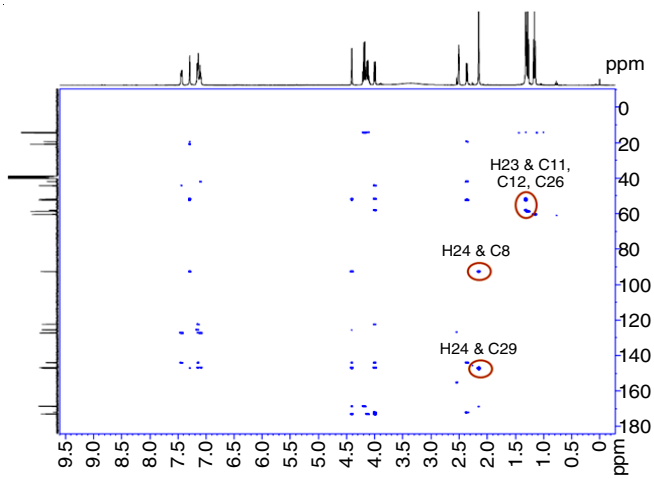

Fig. 4d. ^{1}H-^{13}C gHMBC spectrum of DP-3 with key correlation

Structural characterization of base degradation product (DP-4): The NMR data (Table-5) showed absence of one aliphatic methyl group and α-β unsaturated olefinic protons. Instead new olefinic –CH_2 was observed at 4.52 & 4.60 ppm with its carbon at 93.24 ppm confirming the conversion of methyl group from dihydro pyridine ring to olefinic methylene. This was further supported by gHSQC (Fig. 6b) where two methane protons (H7 & H25) and one methylene group (H26) was observed. The gCOSY (Fig. 6c) data showed correlation of H25 with H26 and *vice-versa*. ^{1}H-^{13}C gHMBC (Fig. 6d) was also recorded to get all 2J and 3J correlations. H7, H23 and H25 showed connectivity to C11 and H24 showed connectivity to C8 & C9. Whereas H5, H26 showed connectivity to C25 and H7, H23, H25 and H26 showed connectivity to C12. The HRMS analysis resulted in protonated mass of 456.2371 with 2.06 ppm error as per the molecular formula ($C_{26}H_{34}NO_{6,}$) derived from the elucidated structure. All the NMR correlation directed to the involvement of α-β unsaturated double bond in cyclization with dihydro pyridine ring and formation of bicyclic compound along with *exo*-olefin shown in Fig. 6a. The IUPAC name of DP-4 is diethyl 9-(2-(*tert*-butoxy)-2-oxoethyl)-3-methyl-1-methylene-1,2,4a,9-tetrahydro-9aH-indeno[2,1-*c*]pyridine-4,9a-dicarboxylate.

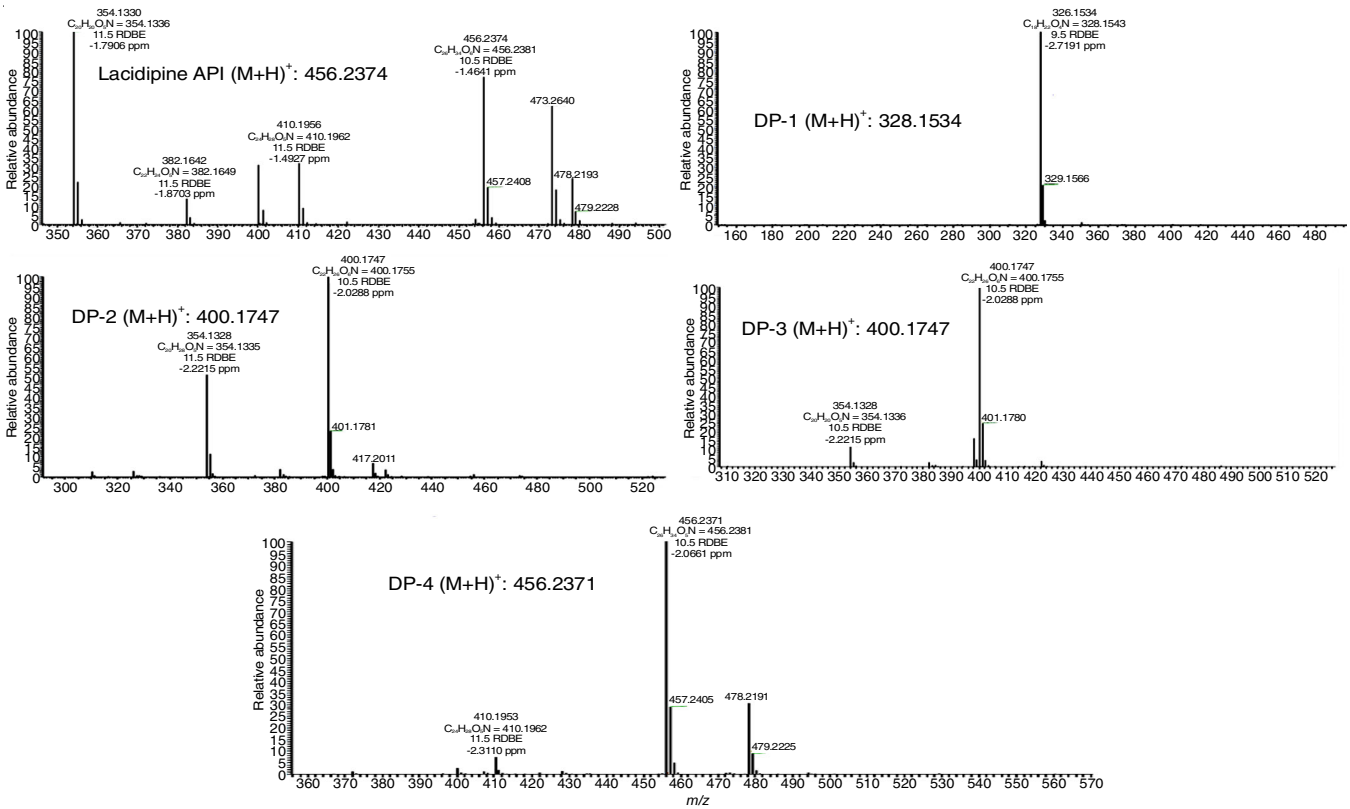

Fig. 5. HRMS spectrum of lacidipine (API), DP-1, DP-2, DP-3 and DP-4

TABLE-4
ASSIGNMENTS OF ^{1}H AND ^{13}C NMR SIGNALS OF LACIDIPINE DP-3

Atom No.	Type of atom	^{1}H Chemical shift (ppm); Coupling constant (*J*)	^{13}C Chemical shift (ppm)
1	CH	7.17 (m, 1H)	127.2
2	CH	7.43 (m, 1H)	125.43
3	C	–	146.75
4	C	–	143.95
5	CH	7.09 (m, 1H)	122.33
6	CH	7.13 (m, 1H)	127.15
7	CH	4.40 (s, 1H)	44.19
8	C	–	92.59
9	C	–	147.11
10	NH	7.29 (s, 1H)	–
11	C	–	52.31
12	C	–	51.71
13	C	–	172.92
14	O	–	–
15	O	–	–
16	CH2	4.14 (m2H)	60.37
17	CH3	1.16 (t, 6.8 Hz, 3H)	14.21
18	C	–	168.58
19	O	–	–
20	O	–	–
21	CH2	4.19 (q, 6.8 Hz, 2H)	58.65
22	CH3	1.28 (t, 7.2 Hz, 3H)	14.49
23	CH3	1.31 (s, 3H)	19.47
24	CH3	2.15 (s, 3H)	20.87
25	CH	4.00 (d, 7.6 Hz, 1H)	41.92
26	CH	2.35 (d, 7.6 Hz, 1H)	58.03
27	C	–	171.92
28	O	–	–
29	OH	12.5 (broad hump)	–

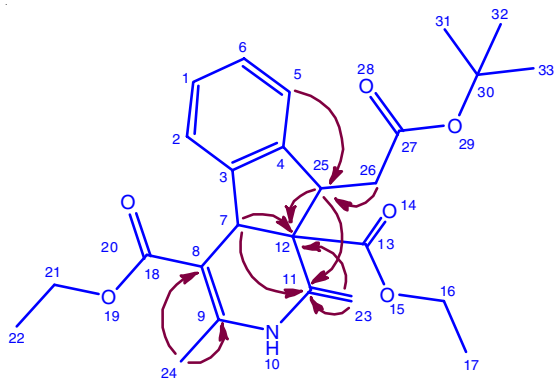

Fig. 6a. Structure of DP-4

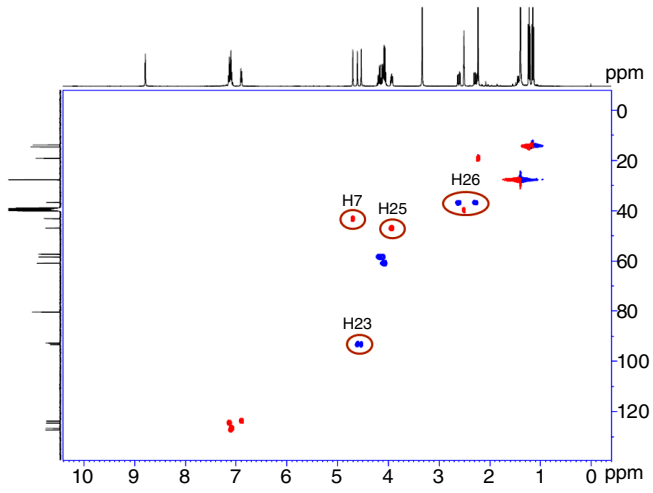

Fig. 6b. gHSQC spectrum of DP-4 with key correlation

TABLE-5
ASSIGNMENTS OF ^{1}H AND ^{13}C NMR SIGNALS OF LACIDIPINE DP-4

Atom No.	Type of atom	^{1}H Chemical shift (ppm); Coupling constant (*J*)	^{13}C Chemical shift (ppm)
1	CH	7.08 (m, 1H)	126.59
2	CH	6.88 (m, 1H)	123.74
3	C	-	145.06
4	C	-	142.49
5	CH	7.14 (m, 1H)	124.5
6	CH	7.11 (m, 1H)	127.28
7	CH	4.96 (s, 1H)	43.17
8	C	-	92.68
9	C	-	148.15
10	NH	8.78	-
11	C	-	140.76
12	C	-	57.26
13	C	-	170.46
14	O	-	-
15	O	-	-
16	CH2	4.06 (q, 7.2 Hz, 2H)	60.85
17	CH3	1.14 (t, 7.2 Hz, 3H)	13.83
18	C	-	167.31
19	O	-	-
20	O	-	-
21	CH2	4.15 (m, 2H)	58.39
22	CH3	1.21 (t, 6.8 Hz, 3H)	14.60
23	CH2	4.52, 4.60 (s, 2H)	93.24
24	CH3	2.22 (s, 3H)	19.12
25	CH	3.93 (dd, 8.8, 6.0 Hz, 1H)	46.91
26	CH2	2.28 (dd, 15.6, 8.8 Hz, 1H), 2.61 (dd, 15.6, 6.0 Hz, 1H)	36.75
27	C	-	170.32
28	O	-	-
29	O	-	-
30	C	-	80.32
31, 32, 33	CH3	1.38 (s, 9H)	27.70

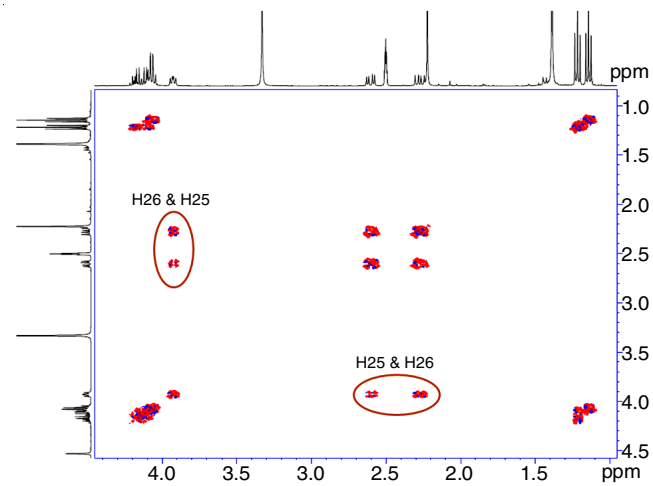

Fig. 6c. gCOSY spectrum of DP-4 with key correlations

Conclusion

In summary, the forced degradation behaviour of lacidipine was studied. Lacidipine was found to be stable under stressed oxidative, thermal and photolytic conditions and labile to acidic and basic degradation. All the four degradants were identified, isolated and characterized structurally.

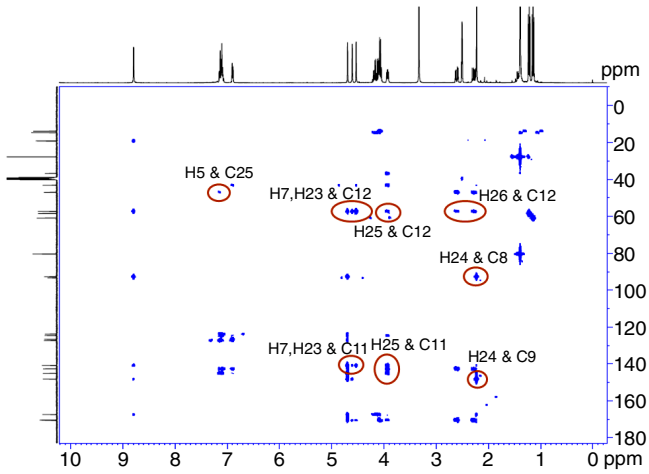

Fig. 6d. ^{1}H-^{13}C gHMBC spectrum of DP-4 with key correlation

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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