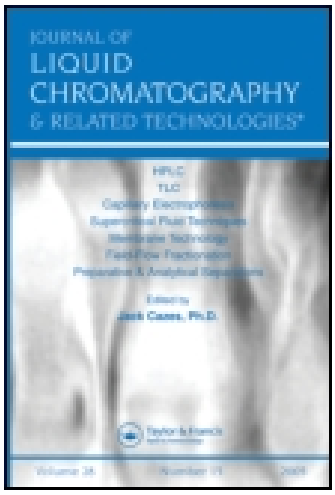

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information: http://www.tandfonline.com/loi/ljlc20

Stability Indicating RP-HPLC Studies for the Estimation of Irbesartan and Amlodipine Besylate in Pharmaceutical Formulations and Identification and Characterization of Degradants Using LC-MS

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Accepted author version posted online: 20 May 2014.Published online: 10 Oct 2014.

To cite this article: Kamala Bodapati , Jayathirtha Rao Vaidya , Sridhar Siddiraju & D. Gowrisankar (2015) Stability Indicating RP-HPLC Studies for the Estimation of Irbesartan and Amlodipine Besylate in Pharmaceutical Formulations and Identification and Characterization of Degradants Using LC-MS, Journal of Liquid Chromatography & Related Technologies, 38:2, 259-270, DOI: 10.1080/10826076.2014.903851

To link to this article: http://dx.doi.org/10.1080/10826076.2014.903851

Journal of Liquid Chromatography & Related Technologies, 38: 259–270, 2015

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826076.2014.903851

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Stability Indicating RP-HPLC Studies for the Estimation of Irbesartan and Amlodipine Besylate in Pharmaceutical Formulations and Identification and Characterization of Degradants Using LC-MS

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A simple, specific, accurate, and stability-indicating reversed-phase high-performance liquid chromatography method was developed for simultaneous estimation of irbesartan and amlodipine besylate in pharmaceutical formulations. The chromatographic separation was achieved on a Zorbax CN column using a mixture of 1 mM potassium dihydrogen phosphate (pH 3.0) and acetonitrile (70:30, v/v) as the mobile phase at a flow rate of 0.9 mL/min. Detection was carried out at 240 nm. The calibration curves were linear ($R^2 \geq 0.99$) over a concentration range of 6–42 μg/mL for irbesartan and 2–14 μg/mL for amlodipine besylate. The retention times of irbesartan and amlodipine besylate were 9.6 and 7.6 min, respectively. For stability studies, irbesartan and amlodipine besylate stock solutions were subjected to acid, alkali hydrolysis, chemical oxidation, and dry heat degradation. The degraded product peaks were well resolved from the pure drug peak with significant difference in their retention times and resolution. To elucidate structures of degradation products, the LC-MS method was used. The proposed method was successfully validated in accordance to the ICH guidelines acceptance criteria.

Keywords: amlodipine besylate, degradation, irbesartan, RP-HPLC method, stability studies, validation

Introduction

Irbesartan (Fig. 1) is chemically described as 2-butyl-3-[*p*-(*o*-1H tetrazol-5-ylphenyl) benzyl]-1, 3-diazaspiro [4.4] non-1-en-4-one. Its empirical formula is $C_{25}H_{28}N_6O$, with molecular weight of 428.5. Irbesartan is an angiotensin II receptor antagonist used for the treatment of hypertension.[1,2]

Amlodipine besylate (Fig. 1) is a potent dihydropyridine calcium antagonist useful in the management of hypertension and angina pectoris.[3–5] It is known as 3-ethyl-5-methyl 4rs)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5 dicarboxylate, benzenesulfonate. It is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and blood pressure.

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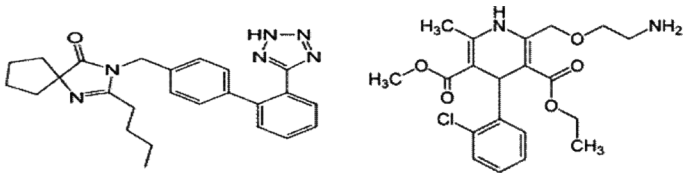
A combination of antihypertensive agents can better control blood pressure and reduce the number and severity of side effects than a monotherapy in patients with multiple cardiovascular risk factors.[6] Both calcium channel blockers and angiotensin II type 1 receptor inhibitors were shown to be efficacious in reducing cardiovascular risk. Irbesartan and amlodipine besylate fixed-dose combinations have been demonstrated in numerous clinical trials to be highly effective in lowering blood pressure and suggest that the combined use might be more effective in treating hypertension than a monotherapy.[7] Aimix®, a new single-pill combination therapy of irbesartan and amlodipine besylate, is approved for the treatment of hypertension.

Literature survey revealed that numerous high-performance liquid chromatography (HPLC) methods have been reported for estimation of irbesartan[8–10] and amlodipine besylate[11–15] individually in pharmaceutical preparations has been reported. To date, no reversed-phase high-performance liquid chromatography (RP-HPLC) analytical method has been reported for the simultaneous determination of irbesartan and amlodipine besylate in pharmaceutical formulations. For quality control and



(a) (b)

Fig. 1. Structures of irbesartan (a) and amlodipine besylate (b).

product development of irbesartan associated with amlodipine besylate, it is recommended to perform the quantitation of irbesartan and amlodipine besylate simultaneously. In addition, evaluation of stability is an important part of drug product development process. The purpose of stability testing is to confirm how quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as light, humidity, and temperature. This enables recommendation of storage conditions, retest periods, and shelf life to be established. In the present study, attempts were made to develop a simple, precise, and accurate HPLC method for the simultaneous estimation of the ingredients of this combination in presence of their degradation products. This article describes the development and validation of a stability-indicating isocratic RP-HPLC method for simultaneous determination of irbesartan and amlodipine besylate in the presence of their degradation products as per the ICH guidelines. The synthesized products were confirmed with LC-MS/MS analysis.

Experimental

Materials and Methods

Reference samples of irbesartan and amlodipine besylate were obtained from Hetero Drugs Limited (Hyderabad, India). HPLC-grade water and acetonitrile were purchased from Rankem (Mumbai, India). Analytical-grade potassium dihydrogen phosphate and sodium hydroxide were also purchased from Rankem (Mumbai, India), whereas analytical-grade orthophosphoric acid, hydrochloric acid, and hydrogen peroxide were purchased from Merck (Mumbai, India).

HPLC Instrumentation and Conditions

An HPLC system (JASCO 2080 (Jasco International Limited, Tokyo, Japan)) consisting of a Zorbax CN column (5 μm particle size, 4.6 × 150 mm) and a binary pump was used for the study. Aliquot of 20 μL of the samples was injected into the column through a Rheodyne 7725 loop. A UV-2075 detector (JASCO) was used, and the output signal was monitored and integrated by JASCO-BORWIN software. An isocratic mobile phase consisting of a mixture of 1 mM potassium dihydrogen phosphate (pH 3.0) and acetonitrile (70:30, v/v) was delivered at a flow rate of 0.9 mL/min. Mobile phase was filtered through a 0.45 μm nylon filter and degassed by ultrasonication prior to use. Wavelength was selected by scanning standard solutions of both drugs over 200–400 nm wavelength using a JASCO V-550 UV-Vis spectrophotometer (Jasco International Limited, Tokyo, Japan).

LC-MS Conditions

LC-MS Instrument and Conditions

Chromatographic Parameters

A Shimadzu Prominence LC-20 AD liquid chromatography system equipped with a Samsung computer using LC Solution software connected to FCM power source, SPD-M20A Prominence DAD detector, SIL-20A HT Prominence auto sampler, isocratic pump system, DGU-20 A5 Prominence degasser, and Zorbax CN column (5 μm particle size, 4.6 × 150 mm) was used for the study. An isocratic mobile phase consisting of a mixture of HPLC-grade water and acetonitrile (35:65, v/v) was used to separate the degradants from the stressed samples and delivered at a flow rate of 1 mL/min into the electrospray ionization chamber of the mass spectrometer.

Mass Spectrometric Parameters

Characterization was achieved with MS detection in a positive ion mode for the degradants using an AB Sciex API–4000 (Applied Biosystems, Foster City, CA, USA) equipped with a Turboionspray™ interface at 200°C. The source parameters, namely, the nebulizer gas, interface voltage, interface current, q-array RF voltage, and detector voltage, were set at 1.5 L/min, −3.5 kv, 0.3 μA, 10.1 v and 0.90 kv, respectively. Total scan speed was set at 1000 μs.

Standard and Sample Preparation

Standard stock solution of irbesartan and amlodipine besylate (1 mg/mL) was prepared in methanol. Working solutions for calibration and controls were prepared by appropriate dilution in mobile phase. Calibration samples were prepared at concentration levels of 6, 12, 18, 24, 30, 36, and 42 μg/mL for irbesartan and 2, 4, 6, 8, 10, 12, and 14 μg/mL for amlodipine besylate.

Preparation of Dosage Form

Twenty tablets each of irbesartan (Avapro, HETERO LABS LTD, Hyderabad, India) were weighed and triturated with proportional quantity of amlodipine besylate API to obtain a homogeneous mixture. They were extracted with methanol to obtain 1 mg/mL. From this, a mixture of solution containing 100 μg of amlodipine besylate and irbesartan was prepared and injected.

Method Validation

The proposed method was validated according to the ICH guidelines with respect to linearity, accuracy, precision, limit of detection and quantification, specificity, robustness, and system suitability. Stress studies would indicate specificity in presence of degradants, as specificity is the ability to assess the analyte in the presence of degradant products, impurities, excipients, and so on. Since tablets for this clinical trial

combination were not available, specificity studies were carried out in compounded formulation with common excipients like lactose, microcrystalline cellulose, magnesium stearate, sodium starch glycolate, and anhydrous calcium hydrogen phosphate.

Linearity

Linearity was determined by injecting solutions with concentrations of 6–42 μg/mL for irbesartan and 2–14 μg/mL for amlodipine besylate.

Precision

It is the measure of the degree of repeatability of an analytical method under normal operation and it is expressed as the percent relative standard deviation for a statistically significant number of samples. Precision studies were performed at three levels: repeatability (intra-day precision), intermediate precision (ruggedness), and reproducibility. Repeatability was determined by injecting samples ($n = 12$) of each drug for three different concentration levels. Ruggedness was investigated by two different analysts on two different days and reproducibility was determined in two different laboratories by injecting the same three samples.

Accuracy

Accuracy study was performed by spiking 50%, 100%, and 150% of amlodipine besylate and irbesartan working standards.

LOD and LOQ

Limit of detection (LOD) is the lowest analyte concentration of a sample that can be detected by the analytical method but do not have quantitatively appropriate value. Limit of quantification (LOQ) is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were determined by using signal-to-noise ratio, that is, 3 for LOD and 10 for LOQ.

Robustness

It is defined as a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness is measured by changing flow rate, pH of the buffer, temperature of the column, and mobile phase composition. These changes help in evaluating the impact on the method.

Forced Degradation Studies

Acid Degradation

Precisely 10 mg each of irbesartan and amlodipine besylate working standard were weighed and transferred into a 50 mL volumetric flask. To the this solution, 10 mL of 0.1 N HCl was added and it was sonicated for 5 min and refluxed under heat at 60°C for 24 hr. After 24 hr, the collected solution was neutralized using 0.1 N NaOH and diluted up to the mark with mobile phase. As much as 1 mL of this solution was pipetted into a 10 mL volumetric flask, diluted up to the mark with mobile phase, sonicated for 5 min, filtered through a 0.45 μm filter, and injected into the HPLC system.

Base Degradation

Precisely 10 mg each of irbesartan and amlodipine Besylate working standard were weighed and transferred into a 50 mL volumetric flask. To this solution, 10 mL of 0.1 N NaOH was added and sonicated for 5 min. This was refluxed under heat at 60°C for 24 hr. After 24 hr, the collected solution was neutralized using 0.1 N HCl and diluted up to the mark with mobile phase. As much as 1 mL of this solution was pipetted into a 10 mL volumetric flask and diluted up to the mark with mobile phase. It was sonicated for 5 min and filtered through a 0.45 μm filter and injected into the HPLC system.

Thermal Degradation

Precisely 10 mg each of irbesartan and amlodipine besylate working standard were weighed and transferred into a 50 mL volumetric flask and placed in controlled temperature oven at 80°C for 48 hr. As much as 1 mL of this solution was pipetted into a 10 mL volumetric flask and diluted up to the mark with mobile phase. It was sonicated for 5 min and filtered through a 0.45 μm filter and injected into the HPLC system.

Oxidative Degradation

Precisely 10 mg each of irbesartan and amlodipine besylate working standard were weighed and transferred into a 50 mL volumetric flask. As much as 10 mL of 3% hydrogen peroxide (H_2O_2) was added to this solution and sonicated for 5 min. It was refluxed under heat at 60°C for 24 hr and 1 mL of the solution was pipetted into a 10 mL volumetric flask and diluted up to the mark with mobile phase. It was sonicated for 5 min and filtered through a 0.45 μm filter and injected into the HPLC system.

Results and Discussion

Method Development and Optimization of Chromatographic Conditions

To develop a rapid, sensitive, and simple assay method that was suitable for stability check, different options were evaluated to optimize detection and chromatography parameters. The method development includes mobile phase selection, flow rate, column type, and injection volume.

Methanol and acetonitrile were tried in different ratios with buffers such as potassium dihydrogen phosphate and sodium hydrogen phosphate as well as acid additives such as phosphoric acid and acetic acid in varying strengths using different columns such as Zorbax, Kromasil, Inertsil, and Nucleosil. It was observed that 1 mM potassium dihydrogen phosphate (pH 3.0) and acetonitrile (70:30, v/v) as the mobile phase was most appropriate to give best sensitivity, efficiency, and peak shape. The use of a chromatography

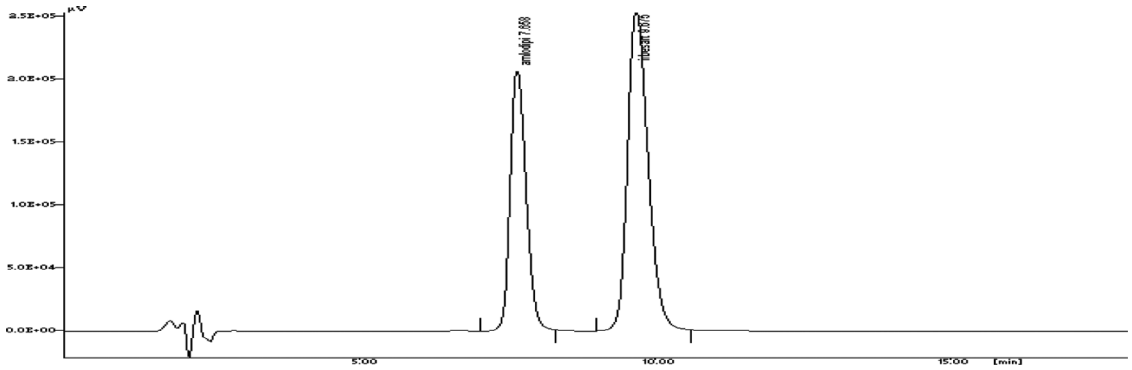


Fig. 2. Simple chromatogram of standard amlodipine besylate and irbesartan (100 μg/mL).

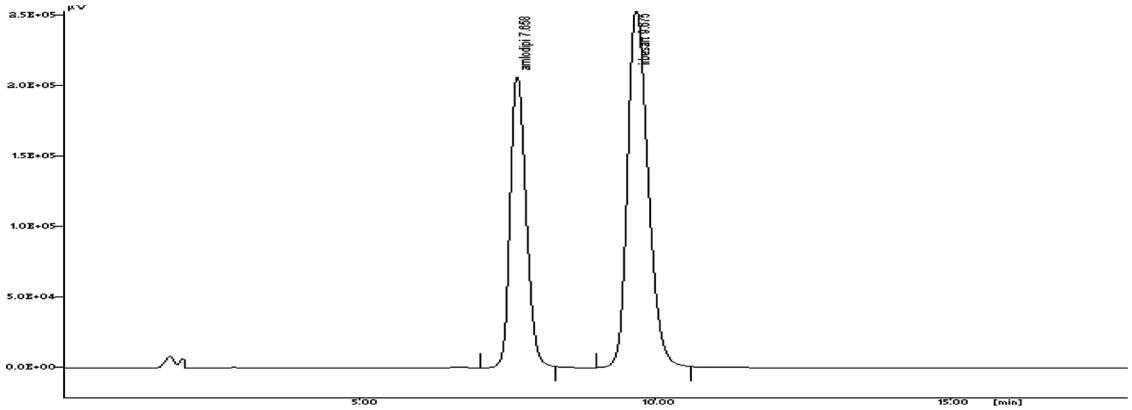


Fig. 3. Simple chromatogram of synthetic mixture of amlodipine besylate and irbesartan (100 μg/mL).

column Zorbax CN column (5 μm particle size, 4.6 × 150 mm) helped in the separation and elution of amlodipine besylate and irbesartan form the degradants in a stipulated run time. The total chromatographic run time was 15 min for each run (Figures 2 & 3).

Method Validation

Accuracy

Accuracy of the method was developed by recovery studies. Individual recovery of irbesartan ranges from 98.6% to 100.8%, with mean recovery of 99.69% and %RSD of 0.4%. Individual recovery of amlodipine besylate ranges from 99.1% to 100.47%, with mean recovery of 99.76% and %RSD 1.1%. The recovery of irbesartan and amlodipine besylate by the proposed method was satisfactory as %RSD is not more than ±2.0% and the mean recovery between 101.4% and 98.3%. The accuracy results of irbesartan and amlodipine besylate are presented in the Tables 1 and 2, respectively.

Precision

The intra- and inter-day precision were determined by analyzing 6, 7 μg/mL of amlodipine besylate and 18, 20 μg/mL of irbesartan on the same day, respectively, and 6, 7, 12 μg/mL and 18, 20, 36 μg/mL on consecutive days, respectively. The intermediate precision was determined by changing column brand, and whole experiment was

Table 1. Recovery of irbesartan

Concentration (%)	Area	Amount added (mg)	Amount found (mg)	Recovery (%)
50%	509867.00	13.50	13.60	100.69
100%	648725.40	18.00	17.80	99.00
150%	796124.60	22.50	22.30	99.37
Mean				99.68
±Standard deviation				0.08
±Relative standard deviation				0.39

Table 2. Recovery of amlodipine besylate

Concentration (%)	Area	Amount added (mg)	Amount found (mg)	Recovery (%)
100%	260794.09	6.00	6.03	100.47
150%	308503.40	7.50	7.48	99.70
Mean				99.70
±Standard deviation				0.05
±Relative standard deviation				0.99

Table 3. Intra-day precision

Intra-day precision ($n = 12$)			
Drug	Concentration (μg/mL)	Mean + SD	CV
amlodipine besylate	6	6.06 + 0.008	0.13
	7	7.06 + 0.012	0.16
Irbesartan	18	18.05 + 0.01	0.05
	20	20.05 + 0.0054	0.02

Table 4. Inter-day precision

Inter-day precision ($n = 18$)			
Drug	Concentration (μg/mL)	Mean + SD	CV
amlodipine besylate	6	6.05 + 0.01	0.17
	7	7.06 + 0.014	0.19
	12	12.06 + 0.09	0.07
irbesartan	18	18.05 + 0.01	0.05
	20	20.05 + 0.008	0.03
	36	36.05 + 0.008	0.02

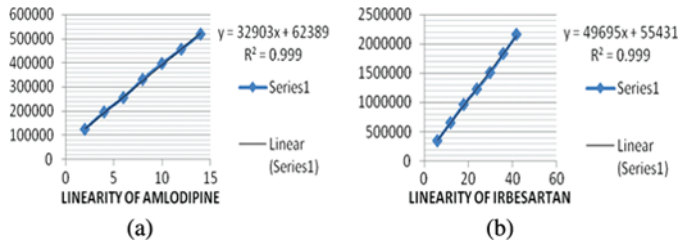


Fig. 4. Linearity of (a) amlodipine besylate and (b) irbesartan.

conducted by different analysts on different instruments. The results of intra-day and inter-day precisions are represented in Tables 3 and 4, respectively.

Linearity

Linearity was obtained by plotting the area of amlodipine besylate and irbesartan against concentration for 7 points ($n = 3$). The linearity was found to be from 2 to 14 μg/mL for amlodipine besylate and 6 to 42 μg/mL for irbesartan, respectively. Typically, regression equations were $Y = 32903x + 62389$ ($R^2 = 0.999$) for amlodipine besylate and $Y = 49695x + 55431$ ($R^2 = 0.999$) for irbesartan, respectively. Linearity is represented in Figure 4.

Limit of Detection and Limit of Quantification

LOD and LOQ for amlodipine besylate and irbesartan were determined at a signal-to-noise ratio of 3:1 and 10:1. The LODs for amlodipine besylate and irbesartan were 0.005 and 0.02 μg/mL, respectively, and the LOQs were 0.02 and 0.06 μg/mL, respectively, for a 20 μL injection volume.

Robustness of the Method

Changing the parameters like pH, mobile phase flow rate, mobile phase composition, the method was found to be robust. The robustness was determined as per USP guidelines. There were no significant variations in system suitability parameters of method; retention time, asymmetry factor, plate count, and resolution. Data are summarized in Table 5.

System Suitability

For system suitability, six replicates of working standard sample were injected and the parameters such as plate

Table 5. Robustness

		Retention time		Asymmetry factor		Plate count		Resolution	
Parameter		AMB	IRB	AMB	IRB	AMB	IRB	AMB	IRB
Flow rate mL/min	0.8	10.86	13.77	1.45	1.41	4827	5099	—	4.17
	1.2	7.28	9.26	1.37	1.32	4454	4602	—	4.04
pH	3.1	7.20	9.17	1.76	1.33	4825	4564	—	3.96
	3.3	7.70	9.70	1.16	1.33	4826	4565	—	3.99
Mobile phase composition	75:25	7.42	9.20	1.17	1.33	4825	4564	—	3.98
	65:35	7.67	9.80	1.26	1.29	4800	4514	—	3.96

AMB, amlodipine besylate; **IRB**, irbesartan.

Table 6. System suitability parameters for the proposed method

Parameter	Result	
	Amlodipine besylate	Irbesartan
Theoretical plates	4799	4413
Asymmetry	1.24	1.28
LOD (μg/mL)	0.005	0.02
LOQ (μg/mL)	0.02	0.06

number (*N*), height equivalent theoretical plates (HETP), and peak asymmetry of samples were calculated. The results are shown in Table 6.

Stress Degradation Studies for Clinical Trial Combination Amlodipine Besylate and Irbesartan

Development and Optimization of Chromatographic Conditions for LC-MS

Degradation studies were carried out by using isocratic LC method on positive ESI mode. Initially, degradation peaks were found to co-elute with main drugs. Different logical

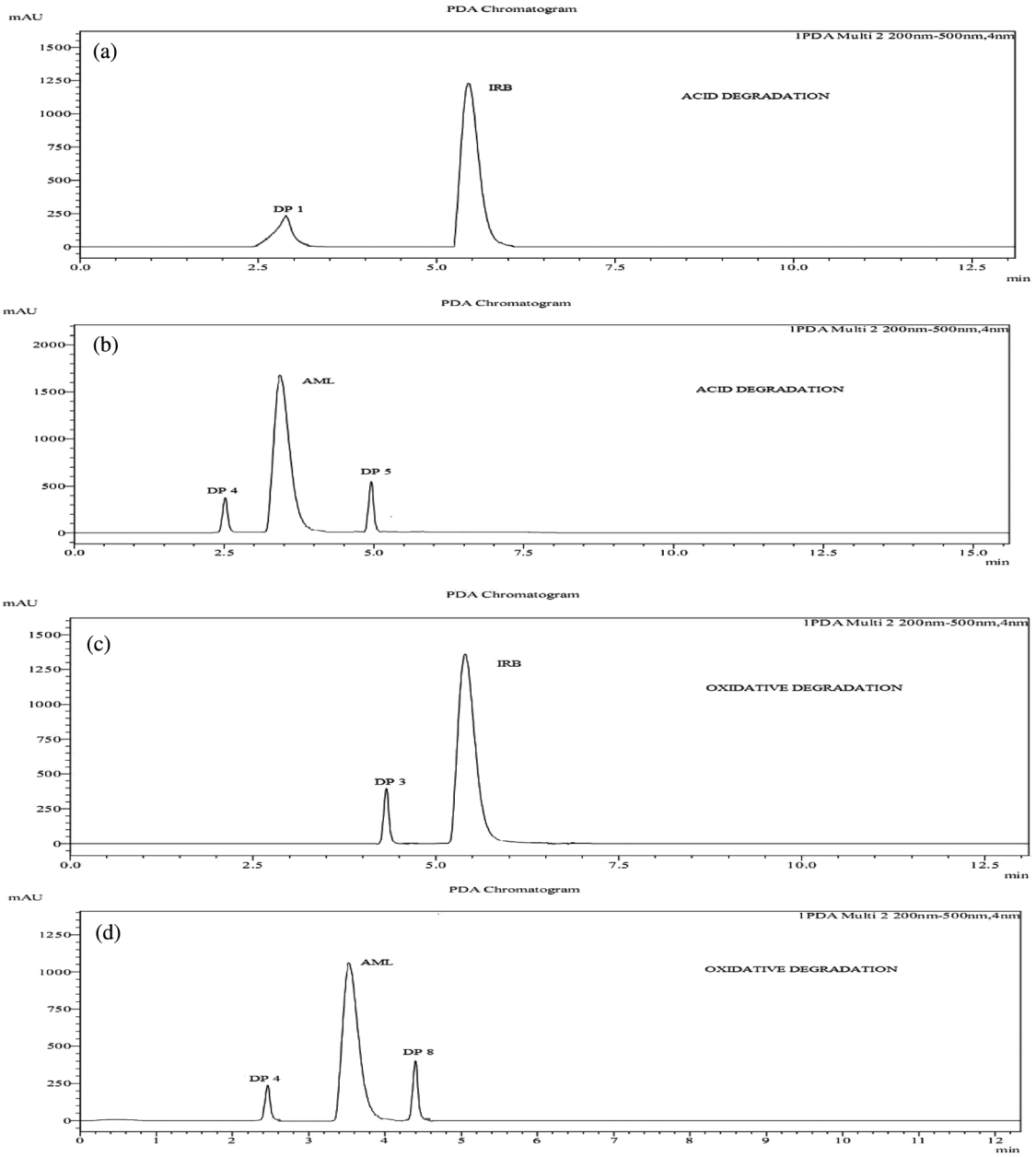


Fig. 5. (a) Acid degradation study of irbesartan; (b) Acid degradation study of amlodipine besylate; (c) Alkaline degradation study of irbesartan; (d) Alkaline degradation study of amlodipine besylate; (e) Oxidative degradation study of irbesartan; (f) Oxidative degradation study of amlodipine besylate.

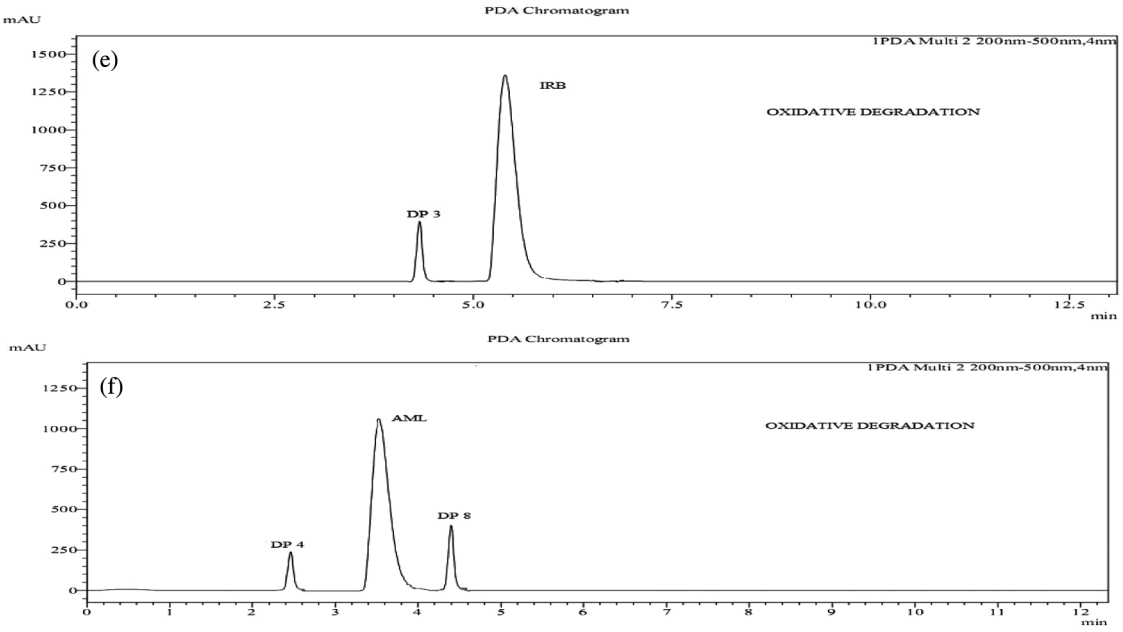
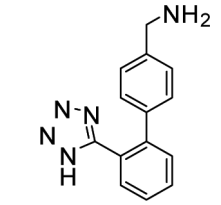
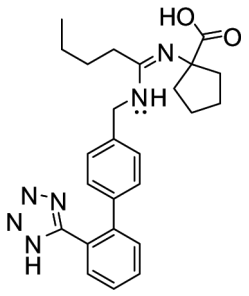
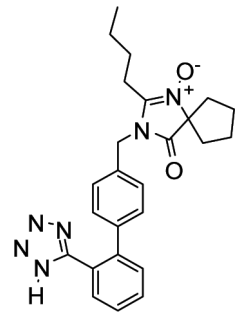


FIG. 5. Continued.

Table 7. Summary of degradant products of irbesartan

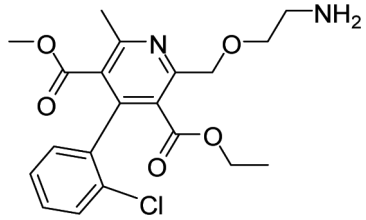
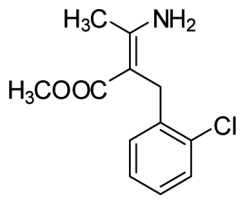
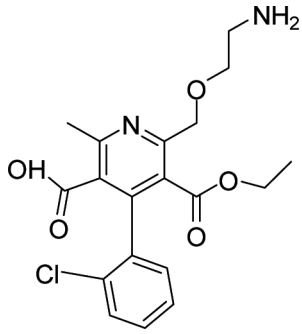
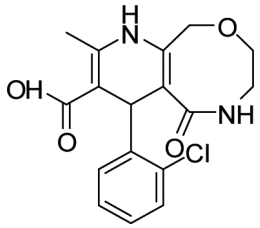
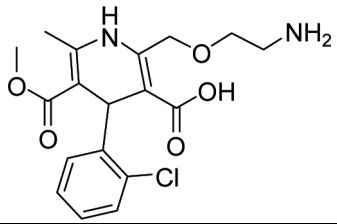
DPs	Structure	m/z	Elemental composition
DP1		**252.00**	$C_{14}H_{14}N_5$ 2-(1H-tetrazol-5-yl)biphenyl-4yl)methanamine.
DP2		**447.25**	$C_{25}H_{31}N_6O_2$, (z)-1-(1-((2′-(1H-tetrazol-5yl)biphenyl-4-yl)-methylamino)pentylideneamino)cyclopentane carboxylic acid
DP3		**445.23**	$C_{25}H_{29}N_6O_2$ structure 3-((2′-(1H-tetrazol-5yl)biphenyl-4-yl)methyl)-2-butyl-40 × 0-1,3diazaspiro[4.4]non-1-ene-1-oxide

modifications like change in flow rate and mobile phase composition were attempted to obtain good separation between drug and degradation products. As phosphate buffer is not used in LC-MS, the mobile phase was changed to acetonitrile and water. Best separation was achieved using mobile-phase acetonitrile and water in the ratio of 35:65, respectively. The degradant products formed were identified with the help of mass spectrometer. The degradation of drug under different conditions and corresponding chromatograms are shown in Figure 5a–5f. A summary of degradant products of amlodipine besylate, and irbesartan is given in Tables 7 and 8, respectively. The proposed degradation pathways for amlodipine besylate and irbesartan are shown in Figures 6 and 7, respectively.

Acid Degradation Studies

For acid degradation, initially the drugs were exposed to 0.1 N HCl for 2 hr, but there were no additional peaks observed, so the time of exposure was increased to 24 hr after this degradation was observed, which was contributed

Table 8. Summary of degradant products of amlodipine besylate

DPs	Structure	m/z	Elemental composition
DP4		**407.12**	$C_{20}H_{24}ClNO_2$ 3-ethyl-5-[-methyl-2-((2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methylpyridine-3,5dicarboxylate
DP5		**239.00**	$C_{12}H_{15}ClNO_2$ (E)-methyl-3-amino-2-(2-chlorobenzyl)but-2-enoate
DP6		**393.11**	$C_{19}H_{22}ClN_2O_5$ 6-((2-aminoethoxy)methyl)-4-(2-chlorophenyl)-5-(ethoxycarbonyl)-2-methylnicotinicacid
DP7		**349.08**	$C_{17}H_{18}ClN_2O_4$ 4-(2-chlorophenyl-2-methyl-5oxo-4,5,6,7,8,10-hexahydro-1H-pyrido[3,2-f][1,4]oxazocine-3-carboxylicacid.
DP8		**381.11**	$C_{18}H_{22}ClN_2O_5$ 2-((2-aminoethoxy)methyl)-4-(2-chlorophenyl)-5-(methoxycarbonyl)-6-methyl-1,4-dihydropyridine-3-carboxylicacid

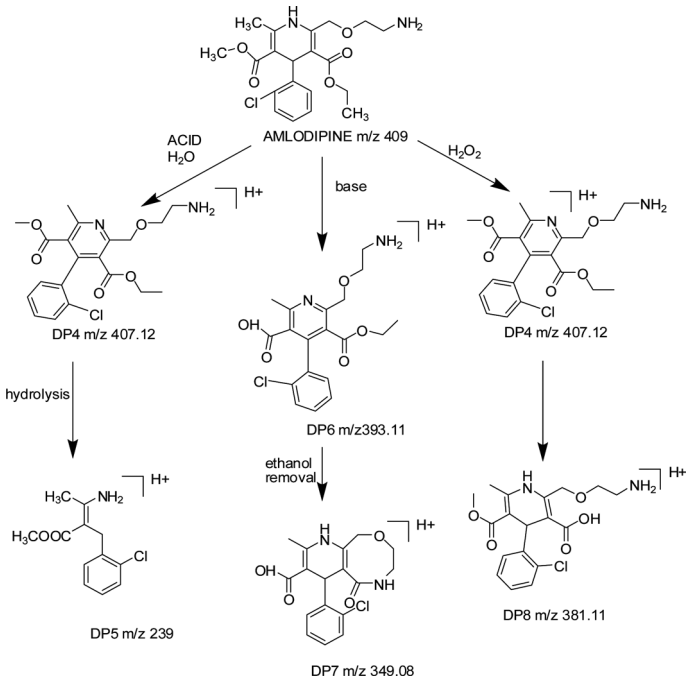


Fig. 6. Proposed degradation pathway for amlodipine besylate.

by both irbesartan and amlodipine besylate. In total, three degradation products (DPs) were formed; DP1 was formed by irbesartan with retention time at 2.88. DP4 and DP5 were formed by amlodipine besylate at 2.4 and 4.99 min. The chromatogram is shown in Figure 5a and 5b.

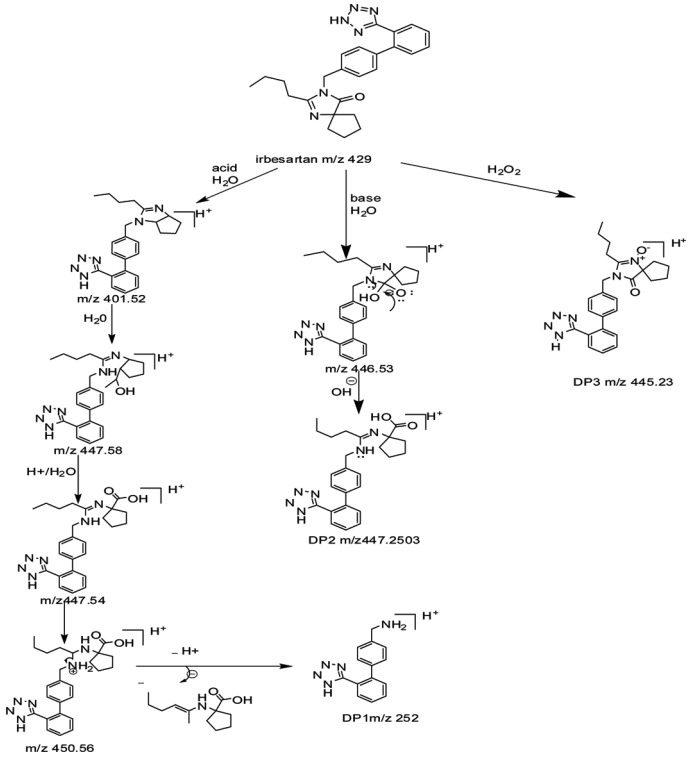


Fig. 7. Proposed degradation pathway for irbesartan.

Alkaline Degradation Studies

Two drugs showed sufficient degradation after 24 hr when refluxed in 0.1 N NaOH, forming three DPs; DP2 was formed by irbesartan with retention time at 2.8 min, and DP6 and DP7 were formed by amlodipine besylate with retention times at 1.9 and 4.8 min, respectively, as shown in Figure 5c and 5d.

Oxidative Degradation

Two drugs showed sufficient degradation in 3% H_2O_2 after keeping the solution for 24 hr in the dark at room temperature, forming one degradant product by irbesartan DP3 with retention time at 4.4 min and two degradant products by amlodipine besylate DP4 and DP8 with retention times at 2.4 and 4.4 min, respectively, as shown in Figure 5e and 5f.

Thermal Degradation

For thermal degradation, both drugs were exposed to 80°C for 24 hr. No degradation was observed; then the time of exposure was increased to 48 hr, yet there were no degradation peaks observed.

Characterization of Amlodipine Besylate and Irbesartan and Identification of their Degradation Products Using LC-MS/MS and MS^n Experiments

MS/MS of Irbesartan

The positive ESI-MS of irbesartan shows an abundant $[M+H]^+$ ION at m/z 429 (Figure 8a)

MS/MS of Amlodipine Besylate

The positive ESI-MS of amlodipine besylate shows an abundant $[M+H]^+$ ION at m/z 409 (Figure 8b).

MS/MS of DP1 (m/z 252)

The degradation product DP1 at m/z 252 $[M+H]^+$; $C_{14}H_{14}N_5$ was eluted at 2.88 min under acidic conditions. From the structure of irbesartan, DP1 can be assigned the structure, 2-(1H-tetrazol-5-yl) biphenyl-4yl) methanamine (Figure 8c).

MS/MS of DP2 (m/s 447)

The degradation product DP2 at m/z 447$[M+H]^+$; $C_{25}H_{31}N_6O_2$ was eluted at 2.8 min under alkaline conditions. From the structure of irbesartan, DP2 can be assigned the structure (z)-1-(1-((2′-(1H-tetrazol-5yl)biphenyl-4-yl)methylamino)pentylideneamino)cyclopentane carboxylic acid (Figure 8d).

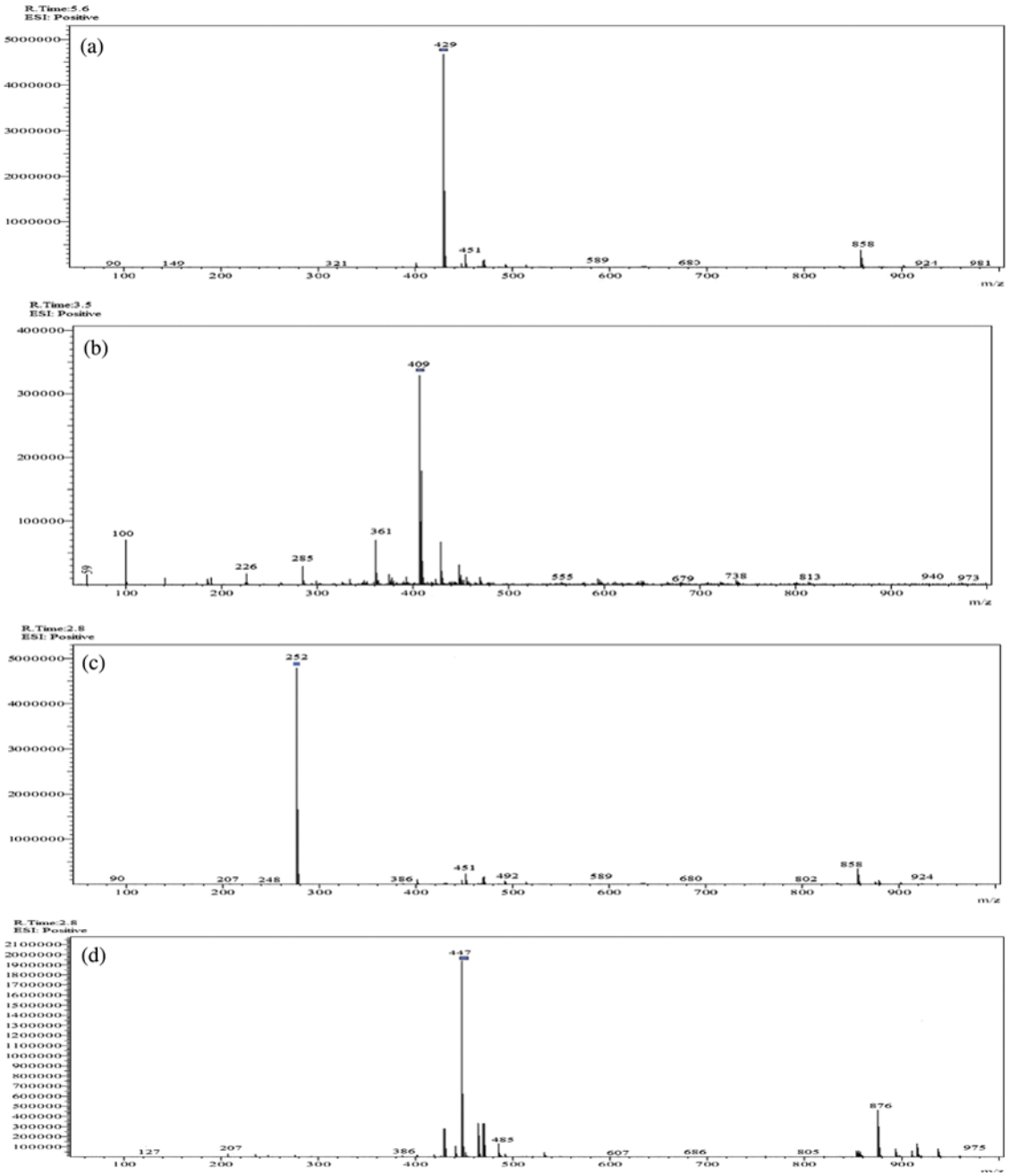


Fig. 8. (a) LC-ESI-MS/MS spectrum of $[M + H]^+$ ion (m/z 429) irbesartan. (b) LC-ESI-MS/MS spectrum of $[M + H]^+$ ion (m/z 409) amlodipine besylate. (c) LC-ESI-MS/MS spectrum of $[M + H]^+$ ion (m/z 252) of DP1. (d) LC-ESI-MS/MS spectrum of $[M + H]^+$ ion (m/z 447) of DP2. (e) LC-ESI-MS/MS spectrum of $[M + H]^+$ ion (m/z 445) of DP3. (f) LC-ESI-MS/MS spectrum of $[M + H]^+$ ion (m/z 407) of DP4. (g) LC-ESI-MS/MS spectrum of $[M + H]^+$ ion (m/z 239) of DP5. (h) LC-ESI-MS/MS spectrum of $[M + H]^+$ ion (m/z 393) of DP6. (i) LC-ESI-MS/MS spectrum of $[M + H]^+$ ion (m/z 349) of DP7. (j) LC-ESI-MS/MS spectrum of $[M + H]^+$ ion (m/z 381) of DP8.

MS\MS of DP3 (m\z 445)

The degradation product DP3 at m/z 445$[M + H]^+$; $C_{25}H_{29}N_6O_2$ was eluted at 4.4 min under oxidative conditions. From the structure of irbesartan, DP3 can be assigned the structure 3-((2′-(1H-tetrazol-5yl)biphenyl-4-yl)-methyl)-2-butyl-40 × 0-1,3diazaspiro[4.4]non-1-ene-1-oxide (Figure 8e).

MS\MS of DP4 (m\z 407) and DP5 (m\z 239)

The degradation product DP4 at m/z 407$[M + H]^+$; $C_{20}H_{24}ClNO_2$ was eluted at 2.4 min and DP5 at m/z 239$[M + H]^+$; $C_{12}H_{15}ClNO_2$ was eluted at 4.9 min under acidic conditions. From the structure of amlodipine besylate, DP4 can be assigned the structure 3-ethyl-5-[-methyl-2-((2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methylpyridine-3,5-dicarboxylate, and DP5

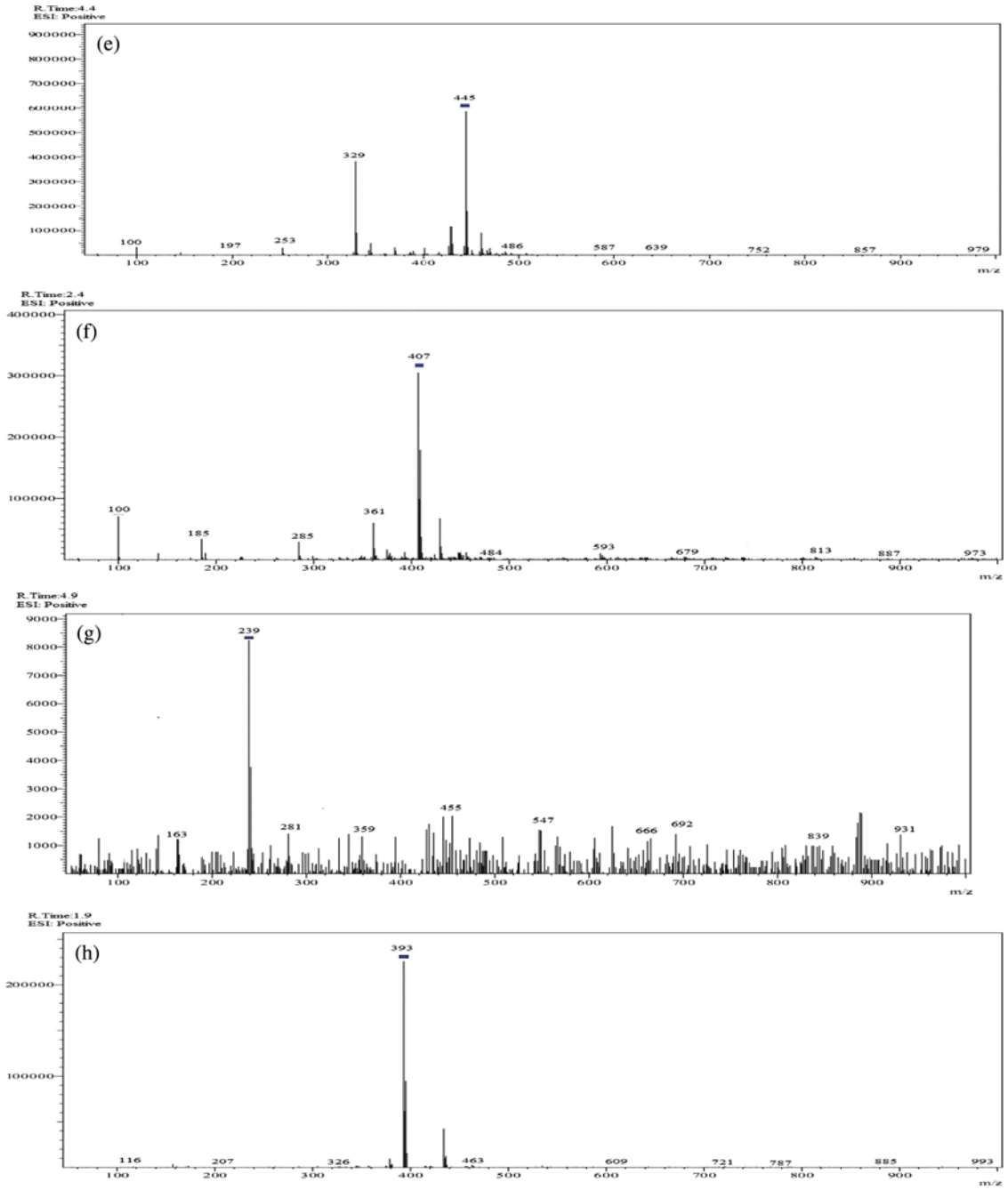


FIG. 8. Continued.

can be assigned the structure (E)-methyl-3-amino-2-(2-chlorobenzyl)but-2-enoate (Figure 8f and 8g), respectively.

MS/MS of DP6 (m/z 393) and DP7 (m/z 349)

The degradation product DP6 at m/z 393$[M + H]^+$; $C_{19}H_{22}ClN_2O_5$ was eluted at 1.9 min and DP7 at m/z 349$[M + H]^+$; $C_{17}H_{18}ClN_2O_4$ was eluted at 4.8 min under alkaline conditions. From the structure of amlodipine besylate, DP6 can be assigned the structure 6-((2-aminoethoxy)methyl)-4-(2-chlorophenyl)-5-(ethoxycarbonyl)-2-methylnicotinicacid and DP7can be assigned the structure 4-(2-chlorophenyl-2-methyl-5oxo-4,5,6,7,8,10-hexahydro-1H-pyrido[3,2-f][1,4]oxazocine-3-carboxylicacid (Figure 8h and 8i), respectively.

MS/MS of DP8 (m/z 381) and DP4 (m/z 407)

The degradation product DP8 at m/z 381$[M + H]^+$; $C_{18}H_{22}ClN_2O_5$ was eluted at 4.4 min and DP 4 at m/z 407$[M + H]^+$; $C_{20}H_{24}ClNO_2$ was eluted at 2.45 min under oxidative conditions. From the structure of amlodipine besylate, DP8 can be assigned the structure 2-((2-aminoethoxy)methyl)-4-(2-chlorophenyl)-5-(methoxycarbonyl)-6-methyl-1,4-dihydropyridine-3-carboxylicacid and DP 4 can be assigned the structure 3-ethyl-5-[-methyl-2-((2-aminoethoxymethyl)-4-(2-

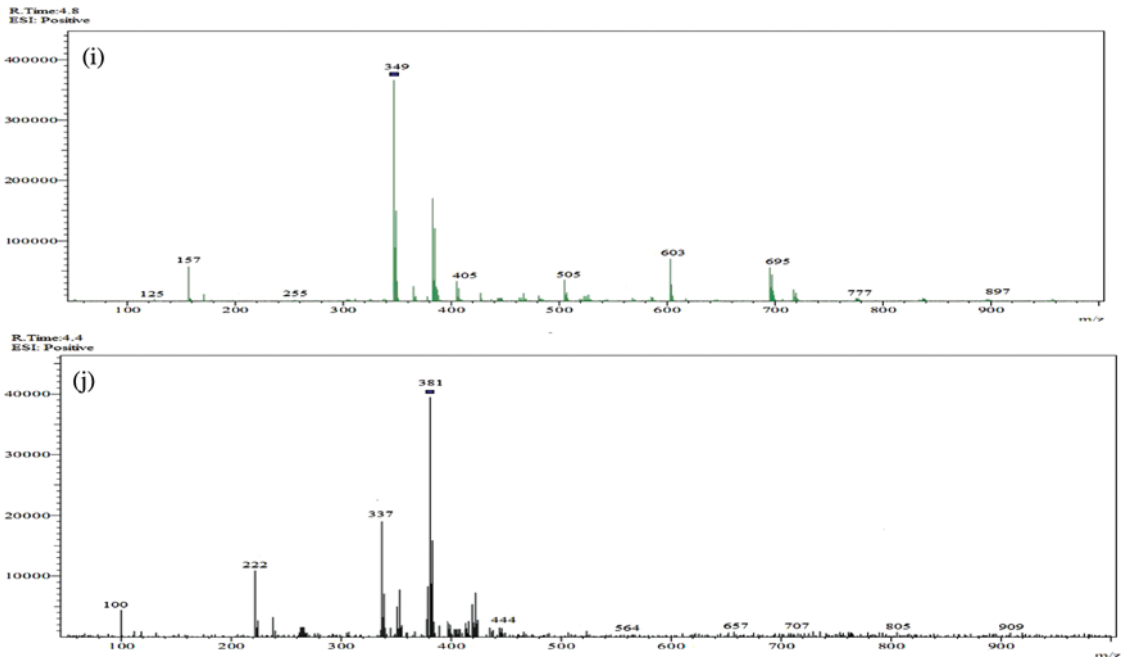


FIG. 8. Continued.

chlorophenyl)-6-methylpyridine-3,5-dicarboxylate (Figure 8j and 8f), respectively.

Conclusion

The reversed-phase HPLC method for analysis of amlodipine besylate and irbesartan mixture in their pharmaceutical preparation was precise, specific, and accurate and with a short run time. The method was validated as per the ICH guidelines and all the data obtained were satisfactory for all the method validation parameters tested. A stability-indicating LC-MS method was developed. The study is an example of stability-indicating assay following the recommendations of the ICH guidelines. The method has the ability to separate these drugs from their degradation products and related substances and can be applied to the analysis of samples obtained during accelerated stability testing. In total, eight degradation products were formed. Irbesartan formed three DPs in acidic, alkaline, oxidative conditions. Four DPs were formed by amlodipine besylate in acidic, alkaline, and oxidative conditions. Both drugs were stable in thermal degradation studies. All these degradation products have been characterized using LC-MS/MS experiments.

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