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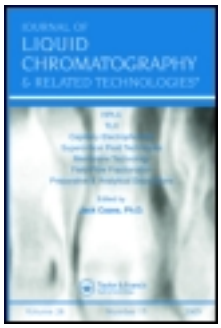

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FORCED DEGRADATION STUDY OF TORASEMIDE: CHARACTERIZATION OF ITS DEGRADATION PRODUCTS

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Forced Degradation Study of Torasemide: Characterization of its degradation products

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Abstract

Torasemide was subjected to forced degradation studies. Stress conditions were varied concerning hydrolysis (acid, base and neutral), oxidation, photolysis and thermal degradation in order to identify the potential degradation products and consequently establish the possible degradation pathways and intrinsic stability of the drug. The study was performed according to ICH guidelines and drug was found to be relatively stable in the solid form. It showed that torasemide degraded significantly under acidic, neutral and alkaline conditions and resulted in formation of degradation product R2. When temperature was increased the degradation was accelerated. Also, the drug showed slight instability under extreme oxidative stress conditions which resulted in formation of two degradation products in total. The drug and degradation products have been separated employing gradient elution method on Zorbax SB C analytical column. To characterize the degradation products LC–MS was applied. The mass fragmentation pattern was established using single quadrupole and ion trap mass analyzers. Finally, the most possible degradation mechanism of torasemide in different experimental conditions was proposed.

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KEYWORDS: torasemide, forced degradation study, degradation pathway, HPLC-DAD, HPLC-MS/MS

INTRODUCTION

Torasemide is a loop diuretic used for edema associated with heart failure, including pulmonary edema, and with renal and hepatic disorders. It is also used in the treatment of hypertension, either alone or with other antihypertensives. Chemically, torasemide is 1-isopropyl-3-(4-m-toluidinopyridine-3-sulphonyl) urea.

The literature search revealed several reported analytical approaches for the determination of torasemide and its metabolites in human plasma and urine. These include high–performance liquid chromatography (HPLC) with UV or electrochemical detection,gas chromatographic–mass spectrometric method (GC–MS)and capillary zone electrophoretic method with an experimental design approach.Also, LC–MS and capillary electrophoretic methods for the determination of torasemide in the mixture of diuretics in urine were found. Torasemide drug substance is official in the U.S. Pharmacopoeia (USP), British Pharmacopoeia (BP) and European Pharmacopoeia (EP) where HPLC methods for determination of torasemide and/or its impurities have been described . Recently, Patel et al. developed and validated UPLC method for determination of impurities related to torasemide tablets.

As the degradation mechanism and pathways of torasemide has been lacking, it was decided to perform the forced degradation of torasemide active pharmaceutical ingredient (API) and torasemide tablets drug product (DP), in order to identify the potential

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degradation products and consequently establish the possible degradation pathways and intrinsic stability of the drug. The forced degradation study was carried out in accordance with ICH guidelines. The substance has been exposed to acid, base, oxidising agent, higher temperature and photolytic extreme conditions. For impurity characterization LC–DAD and LC–MS techniques were selected. Mass detection was selected due to its ability to provide an unequivocal identification of the components of a mixture even if they are not completely separated from each other and therefore can reveal the presence of potential unknown impurities in the sample. The final objective of this study was to prove that the method for simultaneous determination of torasemide and its impurities, we have previously published, is stability-indicating.

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EXPERIMENTAL

Chemicals And Reagents

The standard substances of torasemide, impurity R2 (4-(3-methylphenylamino)-3-pyridinesulfonamide) and Diuver® tablets (containing 10 mg of torasemide) were obtained from Pliva (Zagreb, Croatia). Acetonitrile (Merck, Darmstadt, Germany), ammonium formate (Sigma Aldrich, Steinheim, Germany), formic acid (Merck, Darmstadt, Germany), hydrochloric acid (Merck, Darmstadt, Germany), sodium hydroxide (Merck, Darmstadt, Germany) and hydrogen peroxide (Lach Ner, Neratovice, Czech Republic) were also used. All reagents were of analytical grade. Water for chromatography was deionized using an "Easy pure RF" (Barnstead, Dubuque, USA) purification system.

Equipment

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Analysis of the samples was carried out on the LC–MSD system Agilent 1100 series (Agilent Technologies, Germany) with binary pump, degasser, thermostated autosampler, thermostated column compartment, DAD detector and a single quadrupole mass analyzer (G1946D). Data collection and processing were performed using Agilent Chemstation software (Agilent Technologies, Germany). For additional identification of peaks the LCQ Advantage (Thermo Fisher Scientific, USA) quadrupole ion trap mass spectrometer coupled with Surveyor HPLC system (Thermo Fisher Scientific, USA) was used.

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drying gas (N) flow rate, 12.0 L min; nebulizer gas pressure, 60 psig; temperature, 350 °C; capillary voltage, 3000 V; gain, 2. The mass spectrometer operated in full scan mode (100-500 m/z).

••••••••••••••ion trap

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acetonitrile–water (50:50, v/v) mixture and diluted with mobile phases A and B (50:50, v/v) to produce the expected torasemide concentration of 0.1 mg mL for LC–DAD analysis and 0.01 mg mL for LC–MS analysis.

Forced Degradation

Stress studies of drug substance and drug formulation were carried out under the conditions of dry heat, hydrolysis, oxidation and photolysis, as described in ICH guidelines. For the stress studies of drug substance, torasemide stock solution in concentration of 1 mg mL in the acetonitrile–water (50:50, v/v) was used. Acid hydrolysis was performed by mixing 1mL of torasemide stock solution in the 10-mL volumetric flasks with 1mL of 0.1 mol L, 0.5 mol L and 1.0 mol L HCl solutions, respectively, and the mixtures were kept at room temperature. Alkaline hydrolysis and neutral hydrolysis were carried out in a similar manner with 0.1 mol L, 0.5 mol L and 1.0 mol L NaOH and water, respectively. For oxidative stress studies, 1mL of torasemide stock solution was mixed in the 10-mL volumetric flasks with 1mL of 3% and 10% HO. All experiments were repeated at higher temperature of 70 °C, except for oxidative stress studies which only were performed at room temperature. Photolytic studies with torasemide in solution were carried out by dissolving torasemide in the acetonitrile–water (50:50, v/v) in concentration of 1 mg mL and exposing the solutions to an overall illumination of 1.2 million lux hours and an integrated near ultraviolet energy of 200 watt hours/square meter according to the ICH guidelines.Thermal (70 °C) and photolytic degradations were also conducted on solid drug substance and drug product.

The specified stress conditions and time points (up to 7 and 21 days for drug substance

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and drug product, respectively) were selected in order to result in 5–20% degradation of torasemide. Prior to analysis, samples were withdrawn at appropriate time, neutralized (in case of acid and alkaline hydrolysis) and diluted with mobile phases A and B (50:50, v/v) to concentration of 0.1 mg mL for LC–DAD and 0.01 mg mL for LC–MS.

Several control samples were prepared for comparison with the stressed samples: the blank solution stored under normal conditions, the blank subjected to stress in the same manner as the drug and zero time sample (freshly prepared sample solution). For photolytic degradation dark control (sample solution stored in the refrigerator and protected from light) was analyzed along with stressed samples. The blank sample for the drug product studies was the placebo solution.

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RESULTS AND DISCUSSION

Degradation Of Torasemide

Degradation of torasemide under various stress conditions was investigated by LC–DAD and confirmed the formation of degradation products when API solution was subjected to thermal degradation, hydrolysis and oxidation. The summary of results with relative retention times (RRt) of the drug and the degradation products are presented in Table 1, and typical chromatograms in Figure 1. The peak purity angles of the peaks were much less than the thresholds demonstrating that all peaks were pure and free of co-eluting components. The mass balance (% assay + % sum of all degradants) results were calculated for all stressed samples and found to be more than 99%.

Identification of degradation product R2 was evaluated by LC–DAD and LC–MS using standard substance of R2. Mass and DAD spectra were compared for in both sample and

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standard solution, and it showed to be identical. For other peaks identification was performed using LC–MS analysis. Mass spectra of torasemide and degradation products are presented in Figure 2.

Thermal Degradation And Hydrolysis

The API showed stability in acidic (0.1 mol L, 0.5 mol L, 1 mol L HCl), alkaline (0.1 mol L, 0.5 mol L, 1 mol L NaOH) and neutral solutions at room temperature, and there was no significant degradation after 7 days. In neutral conditions at 70 °C, degradation of 6.5% was observed after 24h. Only, introduction of 1.0 mol L NaOH at 70 °C decreased the hydrolysis to 1.1% after 24h and degradation of 7.2% was achieved after 7 days. The drug showed higher sensitivity to acidic conditions and degradation of 18.7% after 24h was obtained with 1.0 mol L HCl at 70 °C (no degradation was observed with lower concentrations of acid). In both, hydrolysis as well as thermal degradation, one degradation product at RRt 0.66 was observed.

Peak at RRt 0.66 showed identical DAD spectrum and retention time as degradation product R2. The full identification was achieved by LC–MS analysis. Peaks, in both the standard solution and the sample showed a molecular ion at *m/z* 264 [M+H] and fragment ions *m/z* 183 and 168 (Figure 2b).

Photolysis

There was no significant degradation of the API in solution form when exposed to light according to ICH guideline, showing that it was stable against photolytic stress conditions.

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Oxidation

Preliminary experiments for oxidative degradation were performed with hydrogen-peroxide solutions in concentrations of 3% suggested in the literature but no degradation was achieved, proving that API is rather stable against oxidation. When the concentration of HO was increased to 10% API solution showed degradation in total of 9.4% after 7 days. Two peaks at RRt 0.53 and 1.18 were detected with LC–DAD.

Compound at RRt 0.53 (impurity 1) was ionizated with large energy using both single quadrupole and ion trap mass analyzers. Two ions were detectable at mass spectrum in positive mode at *m/z* 302 and 286 (Figure 2d). On the larger ionization and fragmentation energies increases the possibility of adduct ions formation which could explain the ion *m/z* 302 [M+Na]. Namely, it could be degradation product R2 N-oxide, as the chemical structure of degradation product R2 could be easily oxidized. Therefore, fragment ion at *m/z* 286 could be the result of oxygen cleavage in N-oxide moiety. Molecular ion of this degradation product [M+H] would be *m/z* 280. The actual structure of this compound might be determined using NMR or LC–NMR methods, but this was not necessary because this degradation product is not key degradant.The identification of the degradation product structure might be needed also if it was demonstrated that this degradation product appeared in long term or accelerated stability testing.

DAD spectrum of impurity 2 at RRt 1.18 was very similar to the torasemide DAD spectrum, indicating intact basic torasemide structure. The peak was formed under oxidative conditions and it was assumed that the oxygen bonded to the torasemide molecule. The assumption was confirmed when ESI–MS spectra were recorded. The

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peak showed a molecular ion at *m/z* 365 [M+H] and fragment ions at *m/z* 306 and 280 with the difference of 16 more than corresponding ions of torasemide (molecular ion at *m/z* 349 [M+H] and fragments at *m/z* 290 and 264), proving the similar fragmentation pattern and oxygen bonding.

Additional proof of similar fragmentation pattern was obtained by LC–MS analysis and results are presented in Table 2.

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Solid-State Studies

Solid drug substance and drug product were very stable under extreme temperature (70 °C) and extreme photolytic conditions during 21 days and no degradation was observed.

Fragmentation Pattern Of Torasemide And Key Degradation Product R2

The presence of similar fragment ions in the mass spectra (Table 1 and 2) indicated a probable fragmentation pattern and structures of torasemide degradation products. The torasemide fragmentation starts with a breaking of the lateral NH-CO group in the urea structure (*m/z* 290) and continues with elimination of lateral carbonyl moiety (*m/z* 264). After that there is two possible ways for formation of additional fragments: cyclization between position 5 of pyridine and position 6 of phenyl ring on one hand (*m/z* 183), and between lateral sulfonamide moiety and position 2 of phenyl ring on the other hand (*m/z* 247). Finally, the fragment *m/z* 168 is created by elimination of remaining methyl group in the fragment *m/z* 183. The similar pattern was supposed for degradation product R2 as shown at Figure 3.

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Proposed Degradation Pathways Of Torasemide

Torasemide has one very vulnerable bond in the structure where the hydrolysis is expectable. The sulfonylurea moiety undergoes hydrolysis under acid-catalyzed conditions. Water addition leads to loss of an amine and formation of a carbamic acid derivative. Acid-catalyzed loss of carbon dioxide from the carbamic acid derivative yields the corresponding sulfonamide. The hydrolysis is possible also in basic and neutral solutions but only in increased temperature conditions. The product of hydrolysis is degradation product R2.

Pyridine has the ability to oxidize in the presence of hydrogen peroxide, forming N-oxide. The mechanism involves nucleophillic attack of the nitrogen free electrons to the peroxy acid. Oxidation is also possible at secondary amine moiety resulting in formation of hydroxylamine, but this is not a common degradation pathway. Hydroxylamines may not always be observed or may be difficult to isolate.

The proposed degradation pathways are presented at Figure 4.

Development And Validation Of Stability-Indicating Method

Stress studies were carried out in order to achieve a suitable stability-indicating LC–DAD and LC–MS methods. Complete evaluation of chromatographic behavior and establishment of optimal experimental conditions for analysis of torasemide and its impurities were determined with the assistance of experimental design. It was decided to employ 3 full factorial design as suitable kind of optimization design. After the optimization procedure, the validation was performed according to the validation

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protocols that comply with the international guidelines on method validation. The selectivity, linearity, accuracy, precision (repeatability), limits of detection and quantification were determined both, for LC–DAD and LC–MS methods. Both methods met all validation criteria. Development and validation are thoroughly described in our previous work.

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CONCLUSIONS

Torasemide was found to be stable in the solid form, but unstable in solution when submitted to thermal degradation and hydrolysis. Degradation occurred in neutral, acidic and alkaline medium with increased temperature conditions. The major degradation product which was formed corresponds to degradation product R2. Under the extreme oxidative stress conditions two degradation products were formed and are proposed to be torasemide N-oxide and degradation product R2 N-oxide. Under photolytic stress conditions torasemide degradation was not observed. The proposed LC–DAD and LC–MS methods developed in our previous paper showed stability-indicating power and suitability for the assay of torasemide and its potential degradation products.

ACKNOWLEDGMENTS

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TABLE 1 Summary results of API solution stress studies

Stress conditions	Neutral hydrolysis/Thermal	Acidic hydrolysis/Thermal	Alkaline hydrolysis/Thermal	Oxidation		Photolysis
	70°C 24h	1M HCl, 70°C 24h	1M NaOH, 70°C 7 days	10% HO7 days		ICH conditions
Drug assay (%)	93.5	81.3	92.8	89.8		100
Degradation products	R2	R2	R2	Impurity 1	Impurity 2	/
% of present impurities	6.5	18.7	7.2	3.4	6.0	/
RRt	0.66	0.66	0.66	0.53	1.18	/
Molecular ion	264 [M+H]	264 [M+H]	264 [M+H]	302 [M+Na]	365 [M+H]	/
Major fragments	183/168	183/168	183/168	286	306/280	/

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TABLE 2 MSfragmentation of impurity 2 and torasemide

Compound	Molecular ion	Collision energy	MS(ion intensity)	Collision energy	MS(ion intensity)
Impurity 2	365	30	280 (100)	35	263 (100)
					246 (25)
			306 (40)	40	289 (100)
					246 (30)
Torasemide	349	30	264 (100)	40	247 (85)
					230 (55)
					219 (100)
					183 (30)
					201 (25)
			290 (40)	37	247 (85)
					230 (60)
					219 (100)
					183 (30)
					201 (30)

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Figure 1 Representative LC–DAD chromatograms of torasemide reference solution (a) and stressed samples under hydrolysis (b) and oxidation (c).

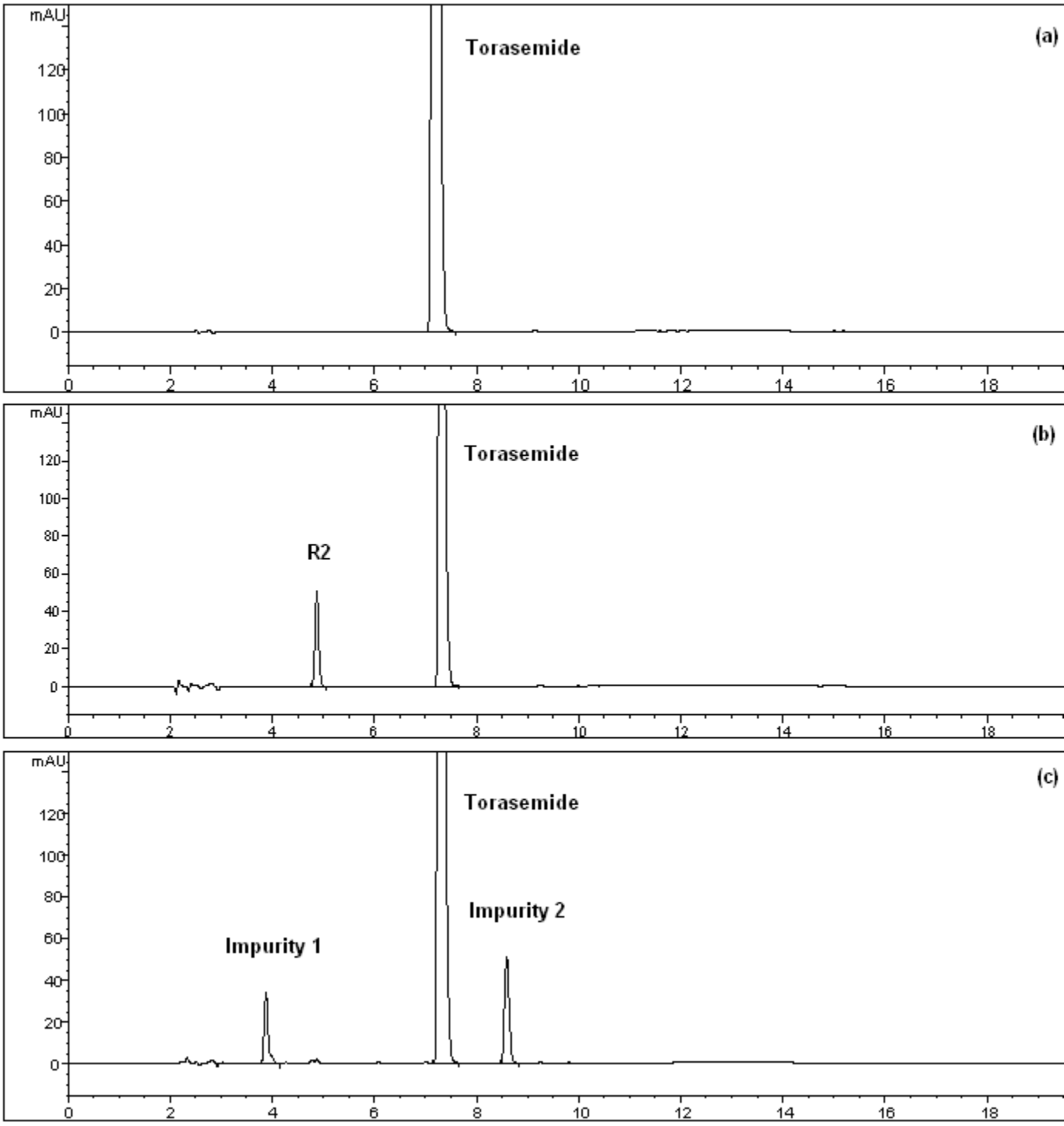

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Figure 2 Mass spectra in positive ionisation mode of torasemide (a) and degradation products: R2 (b), impurity 2 (c) and impurity 1(d).

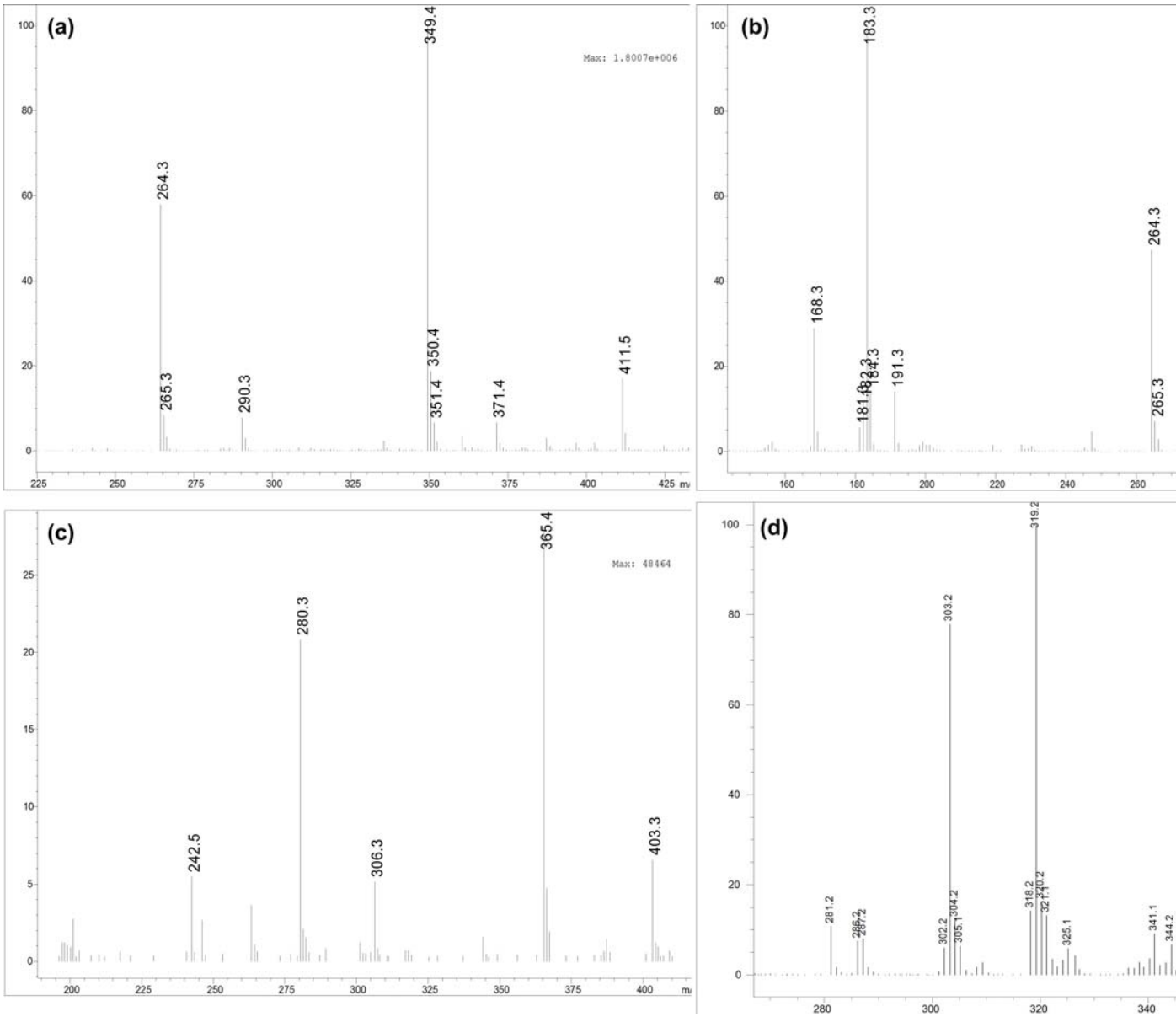

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Figure 3 Fragmentation pattern of torasemide and degradation product R2.

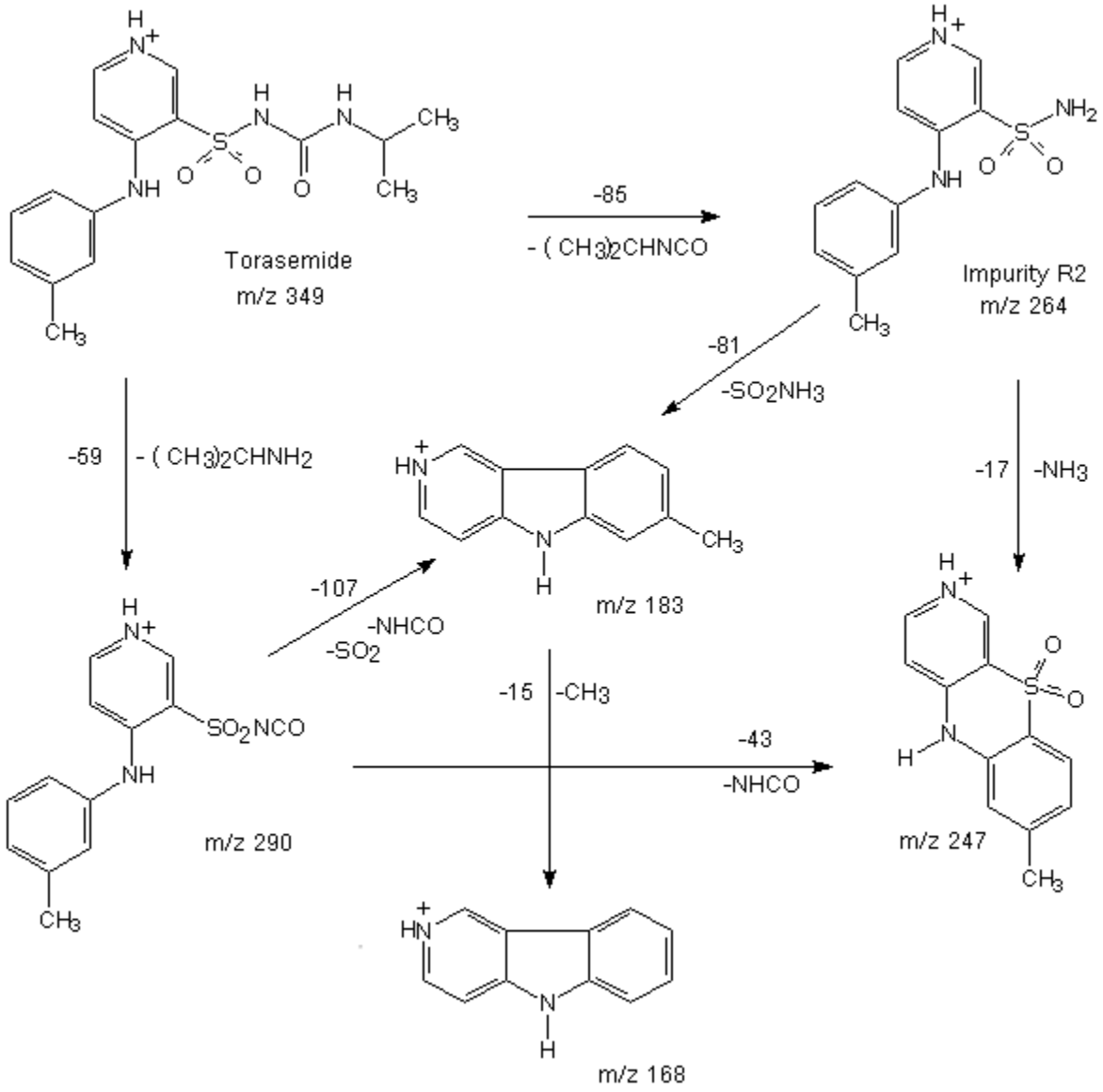

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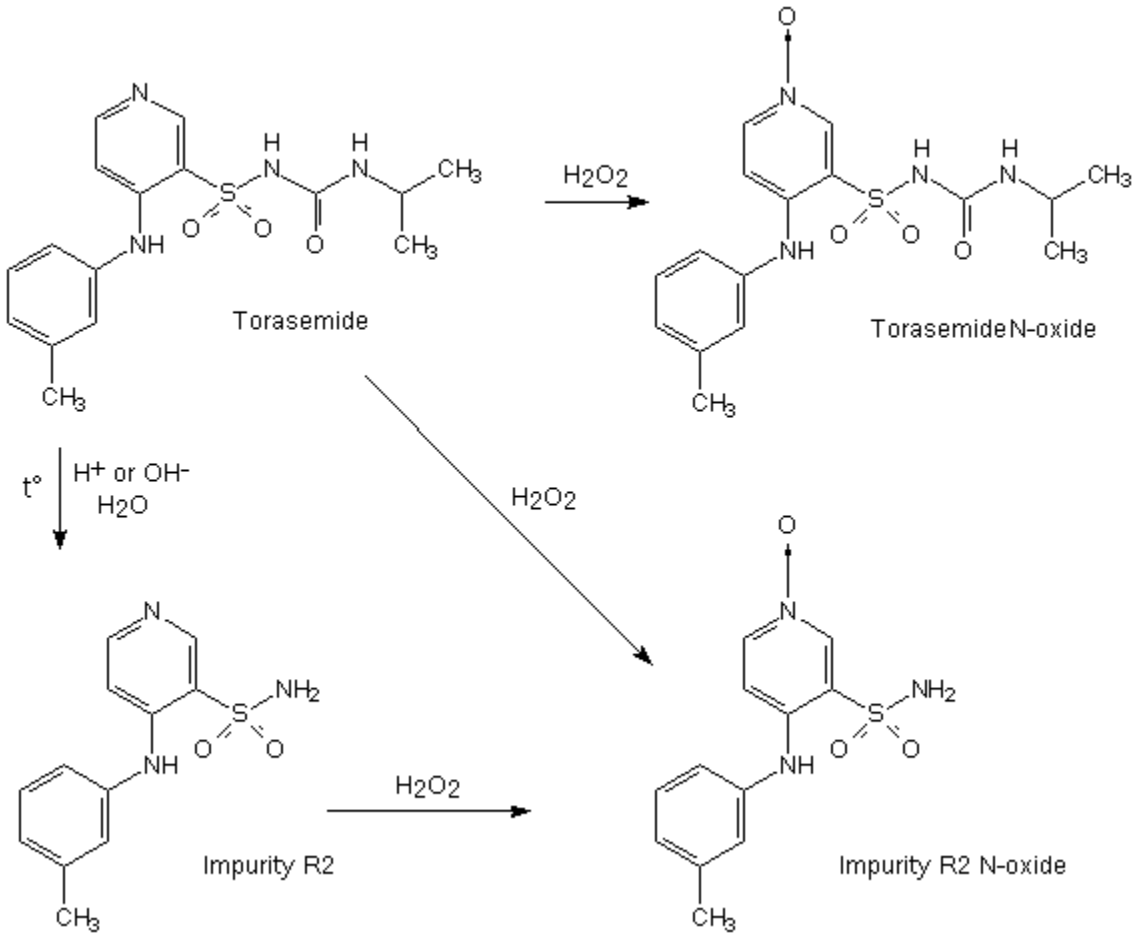
Figure 4 Proposed degradation pathways of torasemide.



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