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Identification and structural characterization of major degradation products of tapentadol by using liquid chromatography–tandem mass spectrometry

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ABSTRACT

Tapentadol, a centrally acting analgesic was subjected to hydrolysis (acidic, alkaline, and neutral), oxidation, photolysis, humidity, and thermal stress conditions as per International Conference on Harmonization prescribed guidelines. Tapentadol was found susceptible to oxidative stress that produced two major degradation products DP-I and DP-II. However, it was stable to hydrolysis, photolysis, and thermal stress conditions. A simple, sensitive, and accurate high-performance liquid chromatography stability-indicating assay method (liquid chromatography–mass spectrometer compatible) was developed and validated for identification and characterization of stressed degradation products of Tapentadol. The chromatographic separation of the drug and its degradation products were achieved on Inertsil ODS, C18 (250 × 4.6 mm, i.d., 5 µm) column using a 12.5 mM aqueous ammonium acetate buffer (with 0.2% triethyl amine and final pH of buffer was adjusted to 3.60 with glacial acetic acid): acetonitrile (75:25, v/v) as a mobile phase. The degradation products were characterized by liquid chromatography mass spectrometry and subsequently its fragmentation pathway as well as plausible mechanism for generation of degradation products was also proposed. The stability indicating high-performance liquid chromatographic method was validated with respect to linearity, precision, and accuracy.

GRAPHICAL ABSTRACT
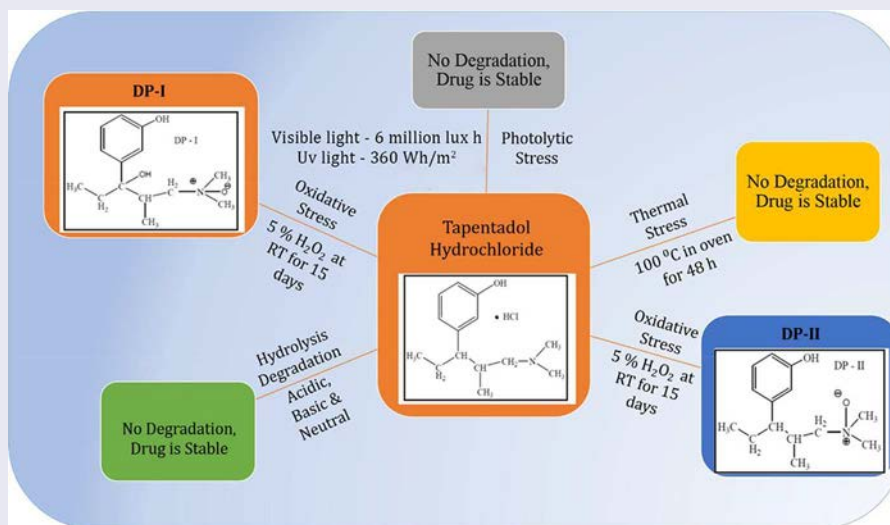
Introduction

Poor drug quality and nonsupportive stability data for expiry date fixation of the finished pharmaceutical product has been a major reason for drug recalls in the past decade. Therefore, stability studies are indispensable part of pharmaceutical product development. Stability studies are a prerequisite for the acceptance and approval of any pharmaceutical product as it ensures quality, safety, and efficacy of drug product throughout its shelf life. These studies are required to be conducted in a planned way by following the guidelines issued by various regulatory authorities such as ICH, WHO, USFDA across the globe.[1–3]

Tapentadol hydrochloride (TAP) is a novel centrally acting oral analgesic. TAP affects the brain and body primarily by activating opioid receptors in the brain, spinal cord, and gastrointestinal tract. In addition, it inhibits the reuptake of
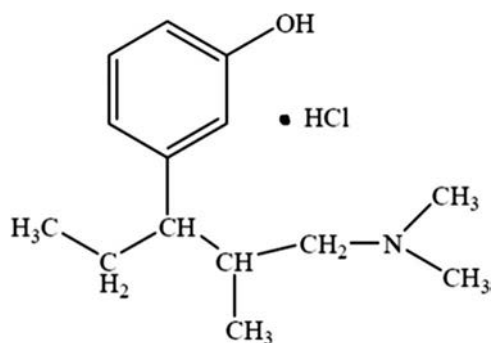
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Figure 1. Structure of TAP (tapentadol hydrochloride).

the brain chemical norepinephrine which possibly has an analgesic effect.[4,5]

Tapentadol hydrochloride was approved by US Food and Drug Administration in November 2008 for the treatment of moderate to severe acute pain including cancer and other related pain as well. It is available as immediate-release tablets in 50, 75, and 100 mg strengths. TAP is chemically 3-[(1R,2R)-3-(dimethyl amino)-1-ethyl-2methylpropyl] phenol monohydrochloride and available in hydrochloride form (Figure 1).

It is not official in any pharmacopoeia. Various analytical techniques such as UV-spectroscopic, spectrophotometric, spectrofluorometric, and RP-HPLC methods have been reported in the literature for estimation of TAP in bulk as well as from its pharmaceutical dosage form.[6–11]

Kathirvel et al., have reported method for impurity profiling of TAP in bulk and its dosage form. No degradation product was found in any stressor as milder conditions were used for degradation.[12,13] However, no information exists in the literature on the degradation behavior and stability indicating LC–MS method of TAP. Kale and Gupta have stated stability indicating method for tapentadol and paracetamol in bulk and combined dosage form that showed degradation by decrease in peak area but no distinct degradation product had been reported. Similarly, Marathe et al. have reported stability indicating RP-HPLC method for the determination of tapentadol in bulk and in pharmaceutical dosage form but not discussed degradation studies by oxidative stress.[14,15] However, no information exists in the literature on the degradation behavior, degradation products, and stability indicating LC–MS method of TAP.

Therefore, the present study focuses on the identification and structural characterization of major degradation products of TAP by using LC–MS–MS. Possible fragmentation pattern for parent drug as well as major degradation products has been proposed. In addition, plausible mechanism for the generation of degradation products and probable structures of major degradation products is also postulated.

Materials and methods

Chemicals and reagents

Pure TAP was kindly gifted by Precise Chemipharma Pvt Ltd, Mumbai, India, certified to contain 99.55% of TAP and used without further purification. Methanol, acetonitrile, glacial acetic acid (GAA), ammonia, triethyl amine (TEA), and ammonium acetate used for mobile phase preparation as well as for the preparation of diluent were HPLC grade and procured from Merck, Mumbai, India.

Hydrochloric acid, sodium hydroxide, and hydrogen peroxide (30% w/v) used for degradation studies and sample preparation were analytical reagent (AR) grade and purchased from SD Fine Chemicals Mumbai, India. Deionized water was prepared using Milli-Q plus purification system Millipore (Bradford, USA) and used throughout the study. Same water was used for the preparation of stressor solutions as well as for diluent preparation.

Apparatus and equipment

Liquid chromatography system consisted of Binary Pumps-Jasco PU-2080 and Solvent Mixing Module-Jasco MX-2080. Rheodyne loop injector with 20-μL fixed loop, equipped with photo diode array (PDA) Detector MD-2015 Plus (Jasco Japan), with *BORWIN-PDA* Software for the data acquisition and data collection. High-precision water bath, EIE 406, EIE instrument Pvt Ltd, Ahmedabad, India was used during degradation studies. Hot air oven, EIE 108, EIE Instruments Pvt Ltd, Ahmedabad, India was used for thermal degradation studies. Temperature and Humidity chamber, model no 0603134, Nova Instruments Pvt Ltd, Ahmedabad, India was used to study combined effect of heat and humidity. Photostability study was performed in photostability chamber, TH 90 S, Thermolab India Pvt Ltd, Mumbai, India.

Liquid chromatography tandem mass spectrometry studies were performed on a system in which LC part consisted of Varian Prostar HPLC, comprising of an online degasser, binary pumps (Prostar 210), with auto-injector and PDA detector (Prostar 335). The MS system consisted of Varian 500 with ion trap mass analyzer (Varian, USA). The data were collected and processed using MS Workstation by Varian Inc., USA *as System Control* software.

Liquid chromatography–mass spectrometry Parameters: Analysis was performed in ESI positive ionization mode. Optimized ESI conditions were as follows. Spray voltage 5 KV, tube lens 80 V, capillary voltage 250 V, capillary temperature 300°C, nitrogen gas flow rate 40 L/min, auxiliary gas helium 30 L/min, split ratio 10:1. In full scan mode, the mass range was set at *m/z* 100–300.

Methods

Chromatographic conditions

The separation of TAP and its degradation products was achieved on a Inertsil ODS, C18 (250 × 4.6 mm; 5 μm) column using a mobile phase consist of acetonitrile and 12.5 mM aqueous ammonium acetate buffer (with 0.2% TEA and final pH of buffer was adjusted to 3.60 with GAA) in the ratio of 25:75 v/v. The flow rate of mobile phase was 1.0 mL/min, the detection wavelength was 285 nm, and the injection volume was 20 μL.

The stability indicating assay method was validated with respect to linearity, precision, accuracy, and specificity as summarized in ICH guidelines Q2R1.[16]

Preparation of solutions

Acid and alkaline hydrolytic stressed samples were appropriately neutralized with equimolar concentrations of NaOH and HCl, respectively, before injecting in HPLC. The methanolic stock solutions of thermal and photo stress samples were prepared to get the concentration of 1 mg/mL and were appropriately diluted with diluent to get concentration 100 μg/mL each of TAP. All the final solutions were filtered through 0.45-μm syringe filter before HPLC and LC/MS analyses.

Table 1. Optimized degradation conditions for TAP.

Degradation type	Degradation condition
Acid hydrolysis	5 N HCl, reflux at 80°C for 5 days
Base hydrolysis	5 N NaOH, reflux at 80° C for 5 days
Neutral hydrolysis	Reflux at 80°C in water for 5 days
Oxidative stress	5% H_2O_2 at room temperature for 15 days
Thermal/humidity degradation	100°C in hot air oven for 48 h Kept at 40°C and 75% RH in a stability chamber for 1 month
Photolytic degradation	Exposed to sunlight in bright sunny day for 12 h Kept in photostability chamber Visible light: 6×10^6 lux h UV light: 360 Wh/m^2

LC–MS–MS study on degradation sample of TAP

For characterization and elucidation of degradation pathway for major degradation product of TAP, LC–MS–MS study of stressed samples was performed. The developed RP-HPLC stability indicating assay method is compatible method. Therefore, the same method was transferred on LC–MS system using same chromatographic conditions using 12.5 mM aqueous ammonium acetate buffer.

Experimental

Forced degradation studies

Forced degradation studies were performed in accordance with ICH guidelines Q1A (R2).[17] TAP was exposed to different stress conditions such as dry heat, hydrolysis, oxidation, photolysis, and combination of heat and humidity. Since TAP is freely soluble in water; all degradation samples were prepared directly in respective stressors.

Stress samples were prepared by taking a concentration of 1 mg/mL of TAP. Acidic and neutral hydrolysis was performed by refluxing TAP with 5 N HCl and water, respectively, at 80°C for 5 days. Base hydrolysis was performed by refluxing TAP with 5 N NaOH for 3 h daily for 5 days. The oxidative stress degradation study was
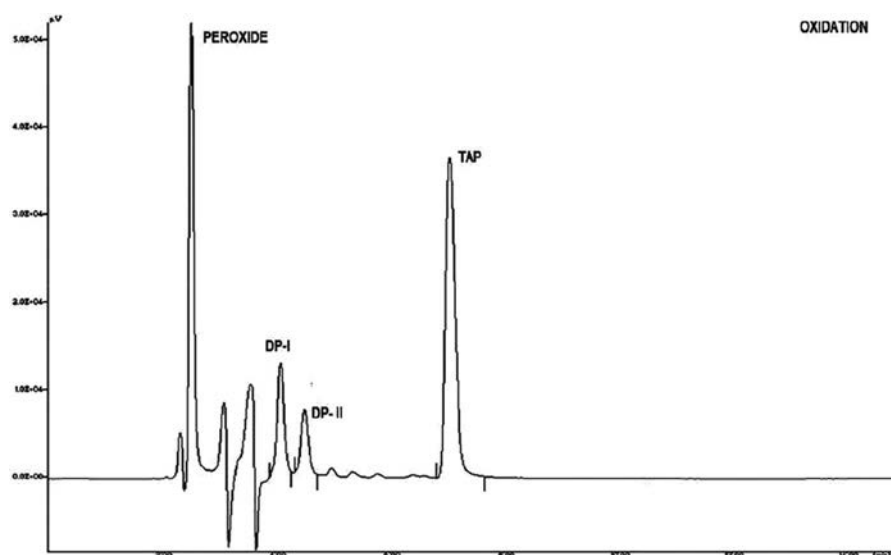

Figure 2. RP-HPLC chromatogram of oxidative degraded TAP (100 μg/mL) in optimized chromatographic conditions.

Table 2. Summary of degradation behavior of TAP.

Degradation condition	Wt. of TAP taken (mg)	Initial peak area (zero time)	Peak area after stress	Approximate % degradation observed	RT of major DP (min) along with peak purity	Approximate % mass balance achieved
Acid hydrolysis	50.2	695480	680980	NSD	–	98.0
Base hydrolysis	50.3	703585	687991	NSD	–	97.8
Neutral hydrolysis	49.8	688798	678001	NSD	–	98.3
Oxidative hydrolysis	49.5	685168	492515	28%	4.1, 995 4.5, 994	97.5
Photolytic stress (sunlight exposure)	50.3	701267	695990	NSD	–	99.2
Photolytic stress (photostability chamber)	50.6	710467	702262	NSD	–	99.0
Thermal (dry heat)	50.1	699450	688956	NSD	–	98.8
Stability chamber (heat and moisture)	50.4	700874	693956	NSD	–	99.1

NSD, no significant degradation.

Table 3. Summary of results obtained from the validation parameters of developed stability-indicating assay method for TAP.

Sr. no.	Validation parameter			Observation
1	System suitability	Retention time (RSD)[a]		0.50
		Peak area (RSD)[a]		0.36
		Theoretical plates (RSD)[a]		0.74
		Asymmetry (RSD)[a]		0.85
2	Linearity and range	Range (µg/mL)		1–150
		Regression equation		$6838.6x + 5663.9$
		correlation coefficient (r)		0.9998
3	Specificity			Specific
4	Limit of detection (µg/mL)			0.047
	Limit of quantitation (µg/mL)			0.14
5	Precision repeatability (mean assay and RSD)[b]			99.60; 0.21
	Intermediate precision (mean assay and RSD)[c]	Interday precision		99.46; 0.63
6	Accuracy study (mean recovery and RSD)[d]			99.9; 0.43
7	Robustness (% assay and % difference in assay value from normal conditions)	Change in organic phase of mobile phase by ±5.0%	−5%	99.10; 0.50
			+5%	99.27; 0.33
		Change in wavelength by ±2 nm	283	99.2; 0.40
			287	99.4; 0.20
		Change in the flow rate of the mobile phase by ±10%	0.90	99.3; 0.30
			1.10	99.5; 0.10
		Change in pH of buffer of mobile phase by ±0.02	−0.02	99.0; 0.60
			+0.02	99.7; 0.10
8	Solution stability	% assay and difference in value of initial sample (Benchtop)	24	99.1; −0.51
			48	98.9; −0.71
		% assay and difference in value of initial sample (Refrigeration)	24	99.2; −0.41
			48	99.0; −0.61

[a]is $n = 5$, [b]is $n = 6$, [c]is the mean value of day-I, -II, and -III observations, [d]is $n = 3$ for each level.

performed with 5% H_2O_2 for 15 days at room temperature. TAP was spread over a petri dish and kept at 100°C in hot air oven for 48 h for thermal degradation study. Solid-state photolytic study was performed by exposing TAP to 6.0×10^6 lux hours of fluorescent light and 360 Wh/m^{-2} UV light in a photostability chamber. Additionally, TAP was also subjected to combination of thermal and humidity stress, at 40°C and 75% RH in a stability chamber for 1
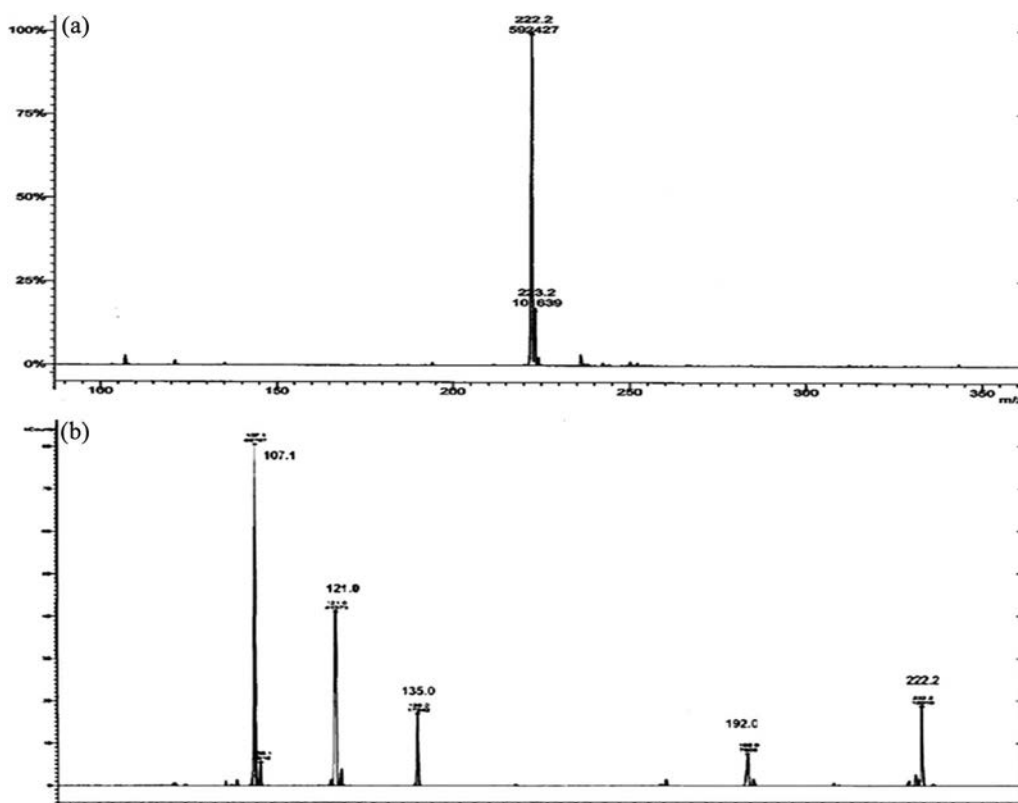

Figure 3. (a) Full scan MS spectra of TAP showing M+1 peak at *m/z* 222.2; (b) MS–MS spectra of TAP by taking 222.2 as precursor ion. *Note*: TAP, tapentadol hydrochloride; MS, mass spectrometry.

Table 4. Summary of LC–MS–MS analysis.

Compound	Molecular ion peak (M+H peak)	MS–MS fragments (m/z)
TAP (parent drug)	222.2	222.2, 192.0 (loss of C_2H_5 from m/z 222), 135.0 (loss of $C_5H_{12}N$ from m/z 222), 121.0 (loss of $-CH_2$ from m/z 135), 107.1 (Base peak: loss of $-CH_2$ from m/z 121)
DP-I (oxidative stress)	254.1	253.2, 252.2, 237.2, 236.3 (loss of H_2O from m/z 254), 193.3 (loss of $-C_2H_6NO$ from m/z 254), 177.4, 176.3 (Base Peak – loss of $-OH$ from m/z 193), 147.3 (loss of $-C_2H_5$ from m/z 176), 146.3, 136.3 (loss of $-C_3H_5$ from m/z 176).
DP-II (oxidative stress)	238.1	234.1, 223.0 (loss of $-CH_3$ from m/z 238), 218.0 (Base Peak: loss of $-OH$ from m/z 236 [formed by loss of $-2H$ from m/z 238 because it is stable]), 205.9 (loss of $-C_2H_5$ from m/z 236), 192.1 (loss of $-C_2H_5$ from m/z 223), 150.3,(loss of $-NOCH_3$ from m/z 192) 149.0, 134.8 (loss of C_5H_9N from m/z 218).

month to assess the probable degradation of TAP in accelerated stress conditions.

All the stressed samples were withdrawn at suitable time intervals and diluted with diluents (ACN/Water, 1:1) to get the concentration of 100 μg/mL. All the optimized degradation conditions applied as per summarized in Table 1.

A minimum of four samples were generated for each condition, i.e., initial (zero time) sample containing the drug with stressor, the drug solution generated after stress exposure, blank solutions stored under normal conditions, and the blank solutions subjected to identical conditions. No peaks were observed in blank solution as well as degraded blank, so there is no need of subtraction of blank.

Results

Degradation behavior of TAP and method validation

Tapentadol hydrochloride was forcefully degraded under thermal, hydrolytic, oxidative, and photolytic stress conditions to assess its intrinsic stability. Degradation sample of TAP showed only single peak of TAP and no additional peak was observed in HPLC chromatogram of acidic, basic, neutral, photolytic, thermal as well as sample stressed to thermal and humidity. Peak area of TAP was almost the same as initial sample along with good mass balance which indicates stability of TAP in these conditions. Initially, in 5% peroxide, some degradation was observed and hence to achieve degradation up to 20%, it was kept for 15 days at room temperature in the dark condition.

Thus results indicate that TAP is susceptible to oxidative stress and get degraded in to two major degradation products (DP-I and DP-II) in 5% H_2O_2 at room temperature when kept for 15 days. Figure 2 shows the degradation products formed under oxidative stress conditions, while it was stable under other degradation conditions. Total two degradation products (DP-I and DP-II) were formed. Table 2 summarizes the degradation behavior of TAP under various stress conditions. Quantification performed by UV–PDA detector, MS used only for the identification of mass.

Developed HPLC–PDA method was successfully validated by evaluating various validation parameters as recommended
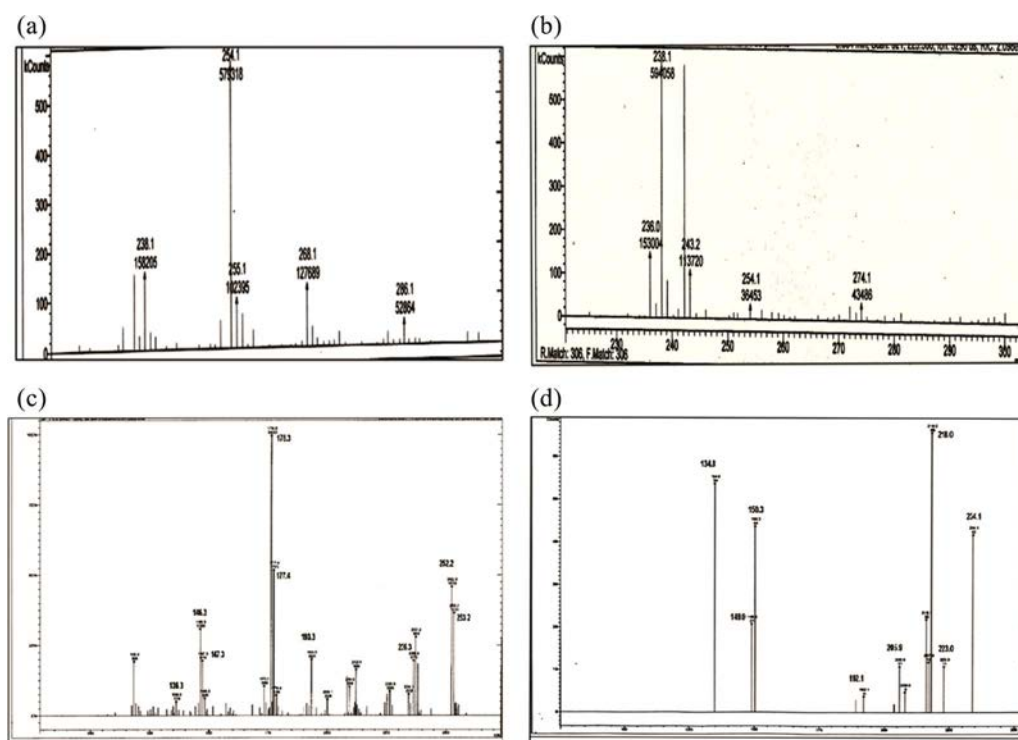

Figure 4. (a) Full scan MS spectra of DP-I showing M+1 peak at m/z 254.1, (b) full scan MS spectra of DP-II showing M+1 peak at m/z 238.1, (c) MS–MS spectra of DP-I by taking 254.1 as precursor ion, (d) MS–MS spectra of DP-II by taking 238.1 as precursor ion.

by ICH guidelines. It was found that results of all validation parameters were within the acceptable criteria. Summary of validation parameters are furnished in Table 3.

LC–MS–MS study of oxidative degradation products of TAP

Degradation products obtained under oxidative stress were subjected to LC–MS–MS study for its characterization and structural elucidation. The developed stability indicating assay method is also LC–MS compatible. Therefore, the same method was transferred on LC–MS system. Parent drug i.e., TAP and degradation mixture as such were subjected to LC–MS analysis and further respective molecular ion was subjected for MS–MS analysis. Figure 3a shows LC–MS spectra for TAP with [M+H]$^+$ peak at 222 m/z which confirms molecular weight of 221 for TAP. Further extensive fragmentation of molecular ion peak was performed by MS–MS and mass spectra of the same is depicted in Figure 3b. The positive ion ESI–MS spectrum of TAP shows an abundant [M+H]$^+$

ion at m/z 222 (Figure 3a) and its MS–MS spectrum (Figure 3b) shows product ion (Fragment ions) as per summarized in Table 4.

Furthermore, oxidative degradation product of TAP, DP-I, and DP-II were subjected to LC–MS analysis and respective molecular ion peak was subjected to MS–MS analysis. Figure 4a and b shows LC–MS spectra of DP-I and DP-II, respectively. MS–MS spectra of molecular ion peak of DP-I and DP-II are depicted in Figure 4c and d, respectively. The degradation product DP-I formed under oxidative stress condition was eluted at 4.1 min. The LC–MS spectrum shows its [M+H]$^+$ ion at m/z 254.1 (Figure 4a) and MS–MS spectrum (Figure 4c) shows product ion as given in Table 4. From the structure of TAP, the observed fractional pattern of protonated DP-II is formed to be highly compatible with the proposed structure.

The degradation product DP-II at m/z 238 [M+H]$^+$ (Figure 4c) was also formed under oxidation stress condition and obtained at RT of 4.5 min. The LC–MS data suggest that it is formed by inclusion of oxygen atom in the parent drug.
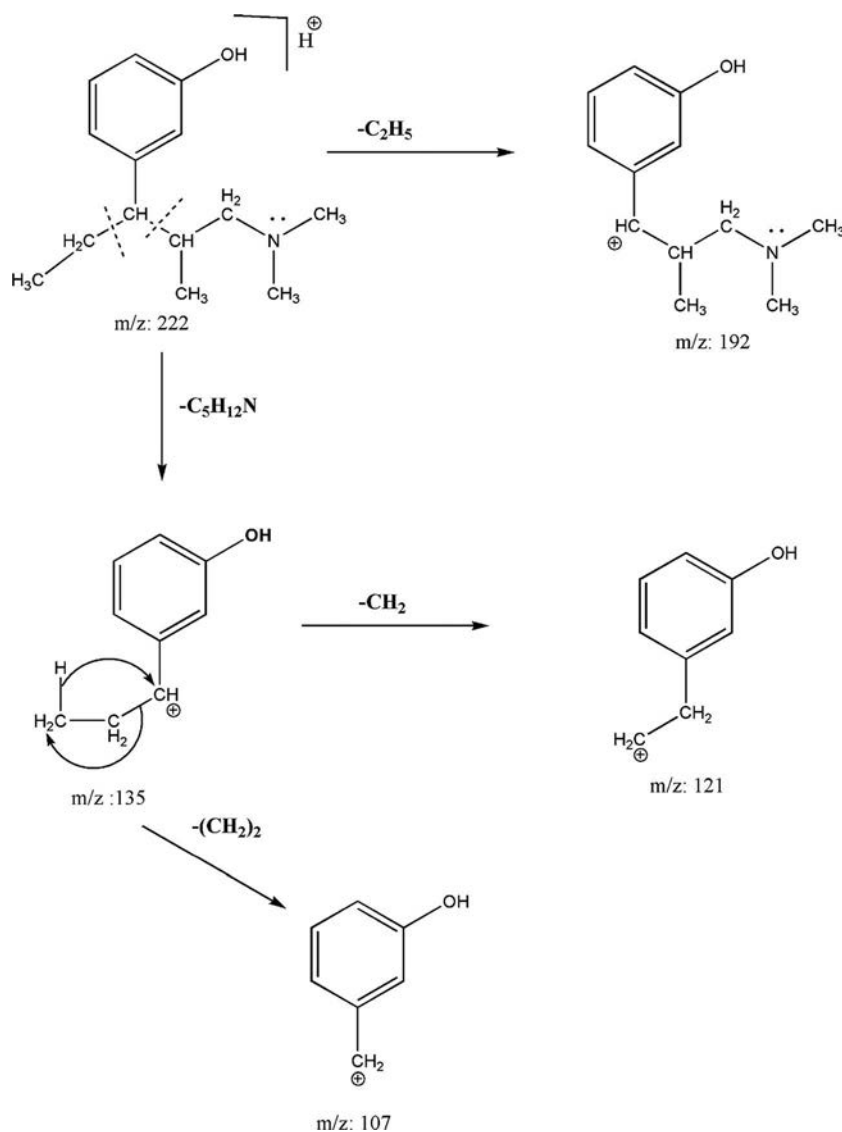

Figure 5. Fragmentation pattern for TAP (parent drug).

Overall summary of LC–MS–MS for parent drug and oxidative degradation product is depicted in Table 4.

Discussion

Method development and optimization of chromatographic conditions

Study of intrinsic stability of molecule gives an idea about its susceptibility for degradation under various conditions.

To perform a detailed stability study of TAP, it was necessary to develop stability-indicating RP-HPLC assay method which can separate TAP as well as its degradation products. When this drug was selected for study, not a single method was reported on degradation behavior of TAP. TAP has pKa value of 9.34 and 10.45.[18] The pKa value suggests that it is a basic drug. As it contains nitrogen in its structure, possibility of peak tailing was also there. Initial method development was started using acetonitrile and water combination. Different

combinations of organic solvent and aqueous solvent were tried before selecting final mobile phase. At 285 nm, degradation products showed good response, when it was checked in PDA detector and also no additional peaks were observed at shorter wavelength or no other interfering/merging of peak was observed as peaks of degradation products as well as tapentadol was pure with good mass balance.

Degradation products and their probable structures

In the literature, various stability indicating HPLC methods have been reported for tapentadol, but no one has observed distinct degradation products. Also, this method is LC–MS compatible, so further can be extended for bioanalytical analysis and bioanalytical estimation. Detail degradation studies have been performed using maximum harsh conditions.

In oxidative stress sample, along with TAP peak, two additional peaks were observed (Figure 2) at RT of 4.1 and
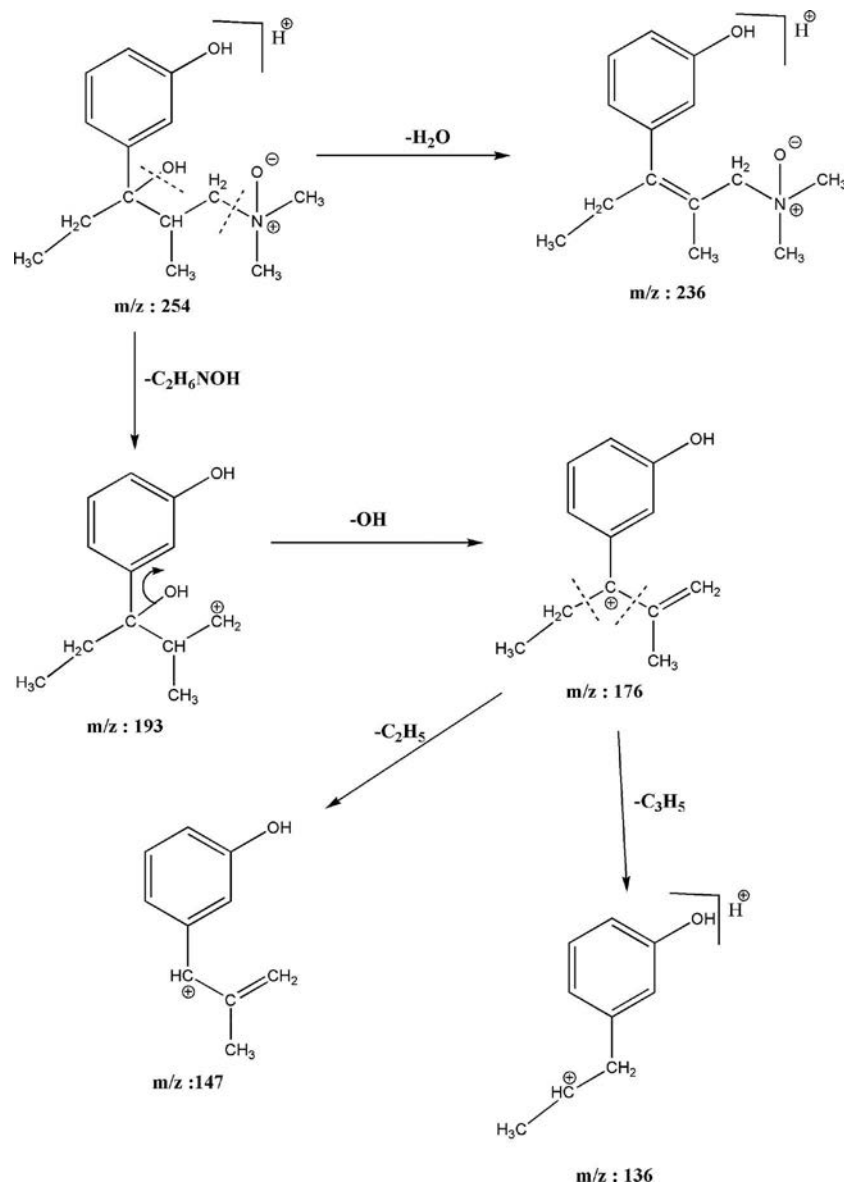

Figure 6. Fragmentation pattern for oxidative degradation product DP-I.

4.5 min. These peaks might be due to degradation of TAP in oxidative stress conditions. The peak area of TAP was also decreased as compared to initial peak area and mass balance was also achieved to approximately 97.5%. No late eluting peak was observed, confirmed by the long run time approximately thrice of the RT of main peak (TAP). Additionally, achieved mass balance indicates that there is no late eluting peak other than that observed in degradation products.

From the LC–MS–MS data, probable fragmentation pattern of parent drug has been proposed and shown in Figure 5, similarly, fragmentation pattern of DP-I is shown in Figure 6 and DP-II in Figure 7. From the LC–MS–MS results and fragmentation pattern probable mechanism for the formation of degradation product, DP-I and DP-II have been proposed and it is presented in Figure 8.

Liquid chromatography tandem mass spectrometry study of DP-I gave molecular ion peak at 254 m/z ratio which was 32 Da more than that of TAP. LC–MS–MS study of DP-II gave molecular ion peak at 238 m/z which was 16 Da more than that of TAP. Both degradation products were observed in oxidative degradation conditions, hence there are chances of inclusion of 2 and 1 oxygen atoms in TAP structure, respectively. TAP possesses tertiary nitrogen in its structure; generally, tertiary amines are susceptible to autoxidation and along with autoxidation product, it also forms stable N-oxide.[19,20]

Therefore, it was predicted that DP-I is autoxidation products of TAP having two oxygen atoms and DP-II is N-oxide of TAP. From the LC–MS–MS fragmentation and plausible mechanism, probable structure of degradation
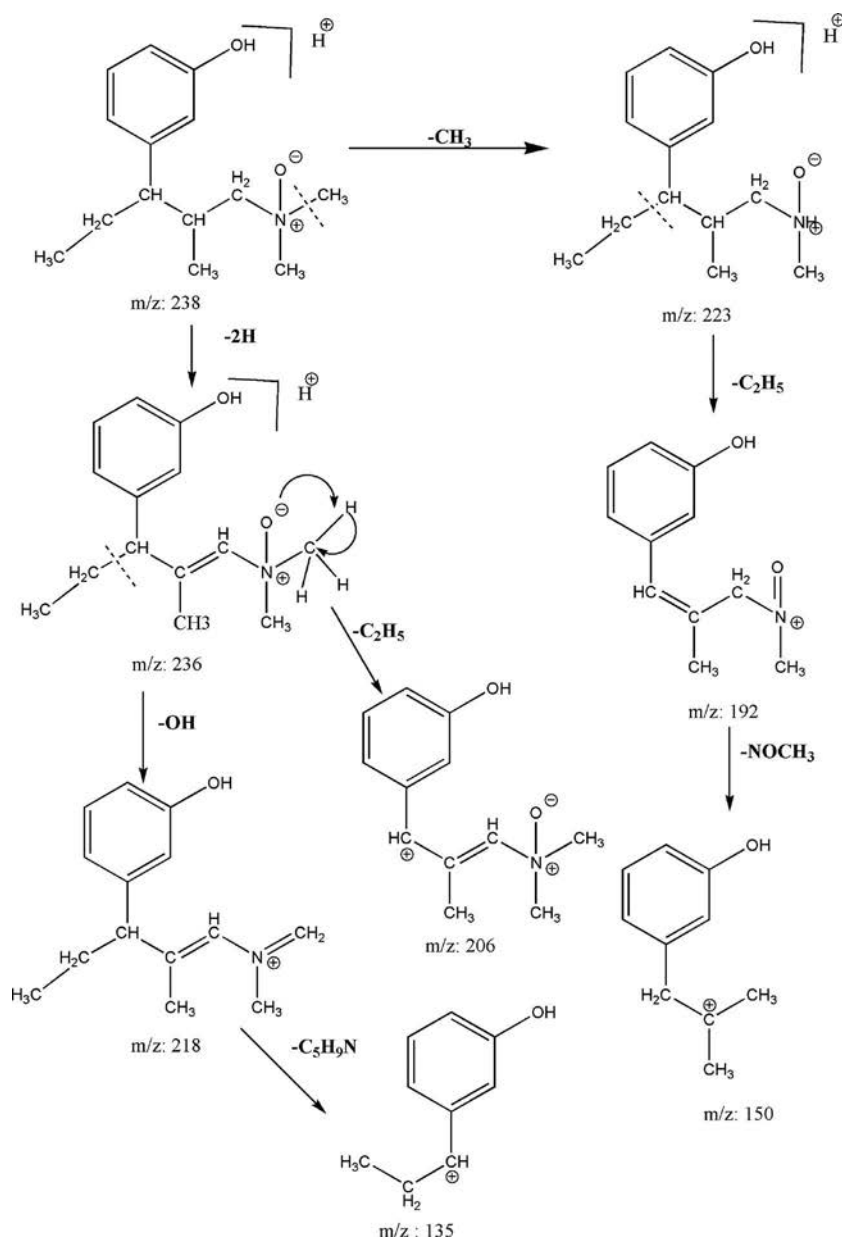

Figure 7. Fragmentation pattern for oxidative degradation product DP-II.
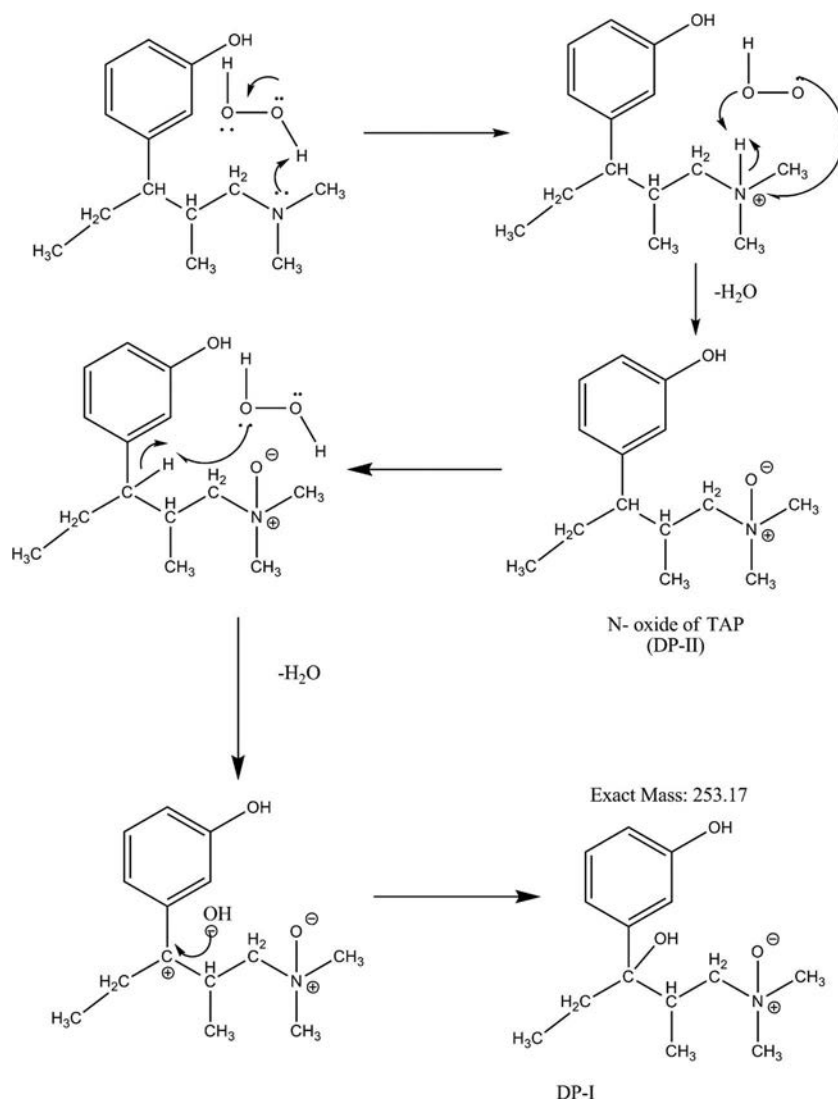
Figure 8. Plausible mechanism for the formation of oxidative degradation products DP-I and DP-II.
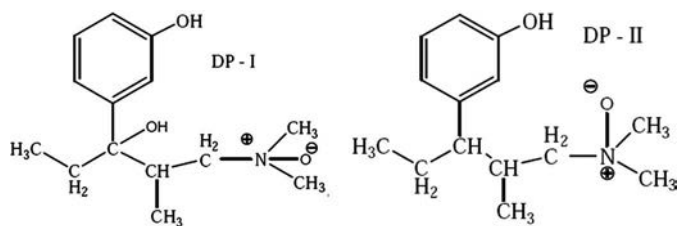

Figure 9. Probable structures for oxidative degradation products of TAP.

products were proposed for DP-I and DP-II as shown in Figure 9.

Conclusions

A simple, sensitive, and accurate stability-indicating reversed-phase high-performance liquid chromatographic method which is also compatible with liquid chromatography mass spectrometer was developed. Degradation behavior of tapentadol was studied in detail under hydrolytic, oxidative, thermal, and photolytic stress conditions. The developed method was able to separate the degradation product and drug from each other with very good resolution.

From LS–MS–MS, it was confirmed that the oxidative degradation product of tapentadol, one degradation product (DP-I) is phenol N-oxide and the second degradation product (DP-II) is N-oxide of tapentadol. The developed method can be further used to separate, quantify, and study the degradation products of tapentadol.

Conflict of interest

The authors state that there are no conflicts of interest pertaining to this manuscript.

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