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Short communication

Identification of a drug degradation product found in a stressed dosage form using LC/MS^n, LC/TOF MS and on-line H/D exchange MS

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ABSTRACT

An unknown degradation product was found when a dosage form was stressed under base and heat. Comprehensive LC–MS studies have been conducted to identify this degradation product. The approach included the use of an ion-trap MS for MS^n ion fragmentation patterns, a time-of-flight (TOF) MS to measure the accurate mass for its potential chemical formula, and on-line hydrogen/deuterium (H/D) exchange LC–MS to determine the number of exchangeable hydrogen atoms in the gradation product. Based upon the above LC–MS results, the unknown was identified to result from the conversion of a trifluoromethyl moiety in the drug substance to a carboxylic acid under the combination of thermal and base stress. Different ion fragmentation pathways between the drug substance and its degradation product were discussed. The reaction mechanism was proposed to be nucleophilic substitution through S_N2 mechanism.

1. Introduction

Understanding drug degradation in formulated product is critical in pharmaceutical development as drug stability and degradation products could have significant impacts on formulation development, analytical method development, package development, storage conditions and shelf-life determination, safety and toxicology concerns. The structures of degradation products over the identification threshold must be identified, and their levels must be carefully monitored and controlled during drug formulation development, according to the International Conference on Harmonization Guidelines (ICH A3B) [1]. In the formulation development, pharmaceutical stress testing has been widely used to predict drug stability problems, develop stability-indicating analytical methods, and identify degradation products and their reaction pathways [2,3]. Understanding degradation mechanism is very important to design a formulation with suitable compositions, to define product manufacturing conditions, and to choose suitable packaging and storage conditions. In this study, we carried out stress testing of a formulated dosage form during formulation development. One of the degradation products was found under base and thermal stress. Various MS-related techniques were applied in this study. Ion-trap MS was used to acquire MS/MS data while TOF MS was used to determine potential chemical formula. Furthermore, on-line H/D exchange LC/MS was conducted to determine labile hydrogen atoms, from which specific functional groups can be derived. The combination of LC/MS^n, LC/TOF MS and on-line H/D exchange LC/MS provides detailed structural information of this degradation product. The benefit of this combination approach has been demonstrated in some studies [4,5]. Furthermore, fragmentation pathway and related chemical reaction mechanism were also discussed based upon the experimental results. The proposed structure of this base-degradation product was further understood through mechanistic explanation.

2. Experimental

2.1. Liquid chromatography

The separation was achieved using a Waters XBridge C18 column, 3.0 mm x 150 mm, with particle size of 3.5 μm. Mobile phase A contains 0.1% trifluoroacetic acid in water and mobile phase B contains 0.1% trifluoroacetic acid in acetonitrile. The HPLC gradient program was: 0.8 ml/min flow rate; 0–5.0 min from 25% B to 25% B, 5.0–20.0 min from 25% B to 50% B, 20.0–20.1 min from 50% B to 80% B, and held for 5 min, then come back to the initial condition 25% B and held for 5 min; column temperature was at 40 °C, sample temperature was held at room temperature. All HPLC analyses were carried out by an Agilent 1100 HPLC system. An Agilent PDA detector was used to monitor UV–vis signals at 310 nm.

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2.2. Mass spectrometry

Two mass spectrometers were applied in this study. The first MS instrument was a linear ion-trap MS (LTQ XL, ThermoScientific Corporation, San Jose, CA, USA) equipped with an ESI source in a positive ionization mode. All LC/MS^n studies were carried out using this instrument. Mass range acquired was from m/z 100 to m/z 1000. ESI conditions: capillary temperature 300 °C, capillary voltage 27 V, sheath gas 35 (arbitrary unit), auxiliary gas 0 (arbitrary unit), and collision energy 30%. All LC and MS data were acquired and processed using ThermoScientific Xcalibur V2.1 software. The second MS instrument was a high resolution TOF MS (LCT Premier, Waters, Manchester, UK) for accurate mass measurement to determine the chemical formula. The TOF MS was operated in a W-mode with a resolution of 11,000. The capillary voltage and sampling cone voltage were set at 3000 V and 10 V, respectively. The desolvation gas was set at a temperature of 300 °C with a flow rate of 700 L/h and the cone gas flow was set at 0 L/h. The ion source temperature was set at 120 °C. All analyses were acquired using Leucin-enkephalin as a "Lock Spray" to ensure accurate mass assignment. The mass range was set from 200 to 800 m/z. Mass spectral data were collected in a centroid mode. A Waters ACQUITY UPLC System (Milford, MA, USA) was interfaced to this TOF MS. All analytical data were acquired and processed under Waters Masslynx V4.1 software.

2.3. Forced decomposition of a dosage form

Transfer 2 dosage forms in a 200 mL volumetric flask; add 100 mL of water and acetonitrile in a ratio of 1:1, sonicate for 30 min and shake 30 min. Add 10 mL of 1 N NaOH solution. Heat the solution at 80 °C for 1 h. Cool to room temperature. Then add 10 mL of 1 N HCl to neutralize the solution pH. Dilute to 200 mL with sample solvent. Pipet 4.0 mL of the above solution into a 10 mL of volumetric flask and dilute to 10 mL with sample solvent.

3. Results and discussion

3.1. LC/MS/MS studies

Fig. 1A shows the chromatogram of a dosage form after being stressed with NaOH at 80 °C for 1 h. The drug substance eluted at 15 min while an unknown peak with increasing intensity appeared at 3.4 min in the chromatogram. The earlier retention time of this degradation product than its parent drug substance in a reverse phase LC chromatogram indicates that the unknown is more polar than its parent compound. Fig. 1B and C show the mass spectral data of the drug substance. It has an [M+H] ion at m/z 456 and fragment ions at m/z 438 and m/z 316. The drug substance has two major functional groups: a carboxylic acid group on one end and a trifluoromethyl group on the other end. Two competitive ion fragmentation processes occur in the MS/MS process as illustrated in Fig. 2. When protonation was on a carboxylic acid, a neutral loss of 18 Da (H_2O) was observed to form a weak ion at m/z 438. On the other hand, the observed strongest ion (m/z 316) cannot come from the fragmentation of either carboxylic acid or trifluoromethyl group. Protonation on an aromatic Ring 2 (R_2) causes one of the aromatic π bonds to be dissociated. The resulting positive charge can then be transferred to anon-aromatic Ring 3 (R_3) through a series of hydrogen re-arrangements. When the charge is located on one of the carbon atom on the Ring 3, ion cleavage could occur on the carbon–carbon bond between R_2 and R_3. This ion cleavage leads to the formation of a stable leaving group of R_3–COOH while the remaining positive charge (m/z 316) on the R_2 ring can be delocalized by a highly conjugated ring system (R_2–NH–R_1).

In comparison, the unknown has an [M+H] ion at m/z 432, Fig. 1D. Its molecular weight is 24 Da lower than that of the drug substance. The odd number molecular weight indicates that it has an odd number of nitrogen atoms. Since the drug substance has three nitrogen atoms, two of them are on the aromatic rings and one amine acts as a bridge between two rings, it is chemically unlikely to lose or gain two nitrogen atoms during base/thermal stress. Therefore, the degradation product should still contain three nitrogen atoms. Comparison of Fig. 1C and E indicates that the degradation product has a completely different ion fragmentation pattern from its parent drug substance. Its protonated molecular ion (m/z 432) can be fragmented to form a very stable ion m/z 388. The resulting neutral loss of 44 Dais most likely due to decarboxylation (–CO_2), a characteristic pattern for a carboxylic acid. As the drug substance does not have a neutral loss of 44 Da even though it contains one carboxylic acid, the observed decarboxylation must come from a newly formed carboxylic acid. In addition, a neutral loss of 18 Da was also observed in the MS/MS spectrum, indicating this degradation product should have a hydroxyl group. The low intensity of m/z 414 ion implies that this substructure is not conjugated.

3.2. Accurate mass measurement

A high resolution TOF MS was used to determine the elemental composition of this compound. The mass accuracy was first checked by measuring the drug substance to ensure the mass accuracy. The measured mass matches well with the known chemical formula with a deviation of −0.5 mDa from its theoretical value. The unknown has an accurate mass of m/z 432.1918. Twenty six formulae match this value within amass tolerance of 3 mDa. As this unknown must have 3 nitrogen atoms, only 4 out of 26 chemical formulae meet this criterion. In addition, this unknown should have the same 25 carbon atoms as its parent drug substance as it is unlikely to lose or add carbon atoms based on the ring structure and the nature of base-induced chemical reactions. Furthermore, the observed neutral loss of 44 Da indicates the presence of an additional carboxylic acid. Therefore, the final selection criteria for all potential chemical formulae should be 3 nitrogen atoms, 25 carbon atoms and not less than 2 oxygen atoms. Based on this selection rule, the only chemical formula matching all criteria is $C_{25}H_{26}N_3O_4$. The measured mass has a deviation of −0.5 mDa from the theoretical value of this formula. In comparison of the drug substance, this degradation product contains no fluorine atoms but has two additional oxygen atoms. With accurate mass measurement, the chemical formula of this unknown has been determined with a high confidence.

Based upon the ion fragmentation patterns obtained from tandem MS and chemical formula obtained from TOF MS, a tentative structure for this degradation product was proposed. Under base and heat conditions, the –CF_3 moiety of the drug substance was replaced by –COONa. The resulting di-sodium salt was converted to its corresponding di-carboxylic acid during HPLC analysis with TFA acidic mobile phases.

3.3. H/D exchange LC–MS

The drug substance has 2 exchangeable hydrogen atoms and therefore its measured $[M_D+D]^+$ was m/z 459, 3 Da higher than its protonated $[M_H+H]^+$. In comparison, the $[M_D+D]^+$ ion of this unknown is 4 Da higher than its $[M_H+H]^+$ ion (m/z 436 vs. m/z 432), indicating that the degradation product contains 3 exchangeable hydrogen atoms. The result is fully consistent with the proposed chemical structure. Furthermore, the major fragment ion of the $[M_D+D]^+$ ion at m/z 392 is also 4 Da higher than the major fragment

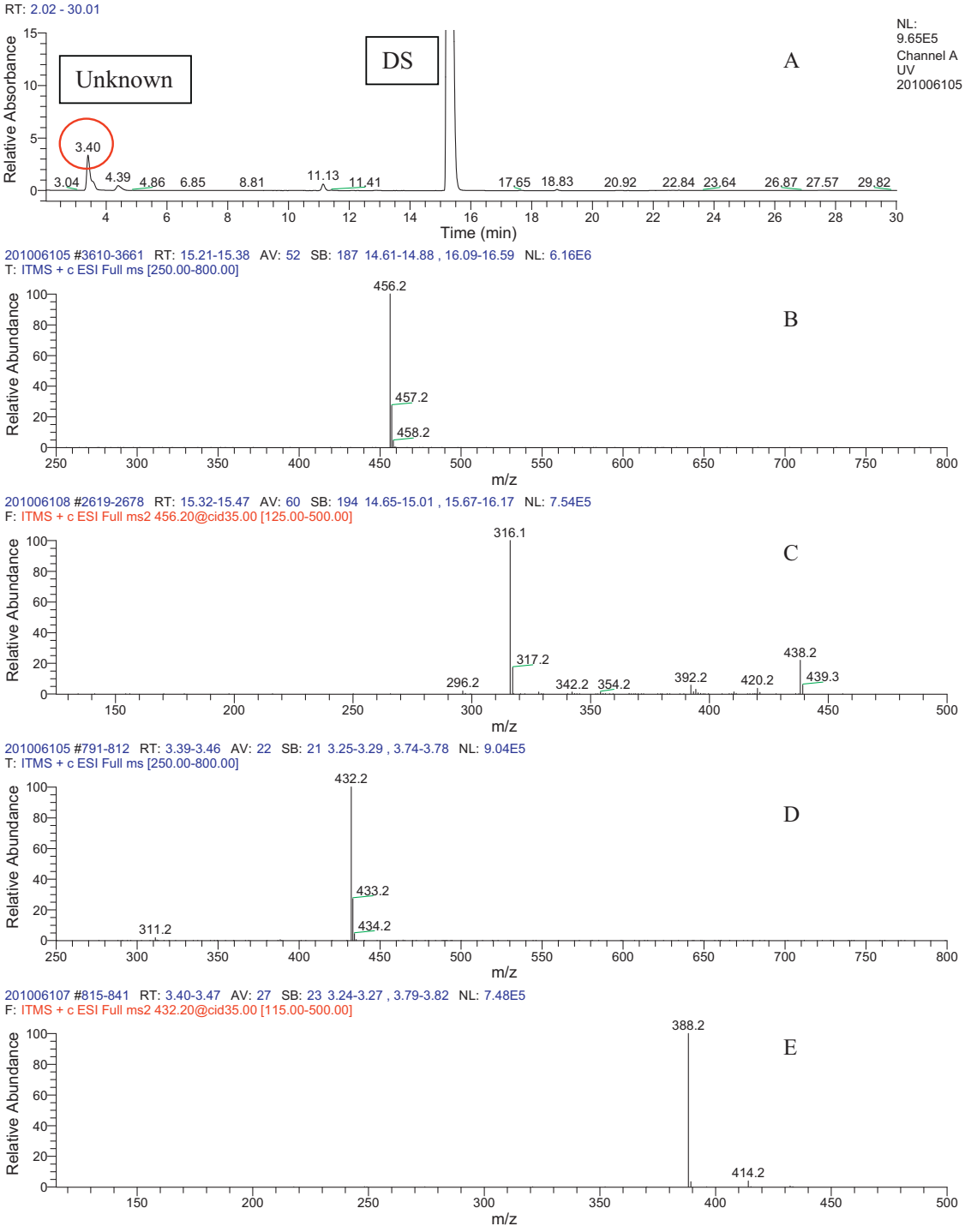

Fig. 1. (A) Chromatogram of abase-stressed dosage form; (B) mass spectrum of the drug substance eluted at 15 min; (C) MS/MS spectrum of *m*/*z* 456; (D) mass spectrum of the degradation product eluted at 3.4 min; (E) MS/MS spectrum of *m*/*z* 432.

ion *m*/*z* 388 detected with protonated solvents. All H/D exchange experimental results are shown in bold in parentheses in Fig. 3.

3.4. Proposed fragmentation pathways

In most LC–MS/MS studies, the core substructure of a drug substance can usually be found to be related to its degradation product. It seems to be an exception in this case. Comparison of Fig. 1C and E shows that this base-induced degradation product has a completely different ion fragmentation pattern from its parent drug substance. This is because the formation of fragmentation ions in the MS/MS process depends on two main factors: the accessibility of a leaving group and the stability of a remaining charge species. Fragmentation ions will be strong if the leaving group is easy to form and the remaining charge is stabilized by de-localization. Fig. 3 shows that there are two competitive ion fragmentation pathways for this degradation product. Protonation on the hydroxyl group of a carboxylic acid yields a neutral loss of 18 Da (H_2O). The resulting low intensity ion *m*/*z* 414 does not have a stable structure as the charge is only localized on the carbonyl group. The other

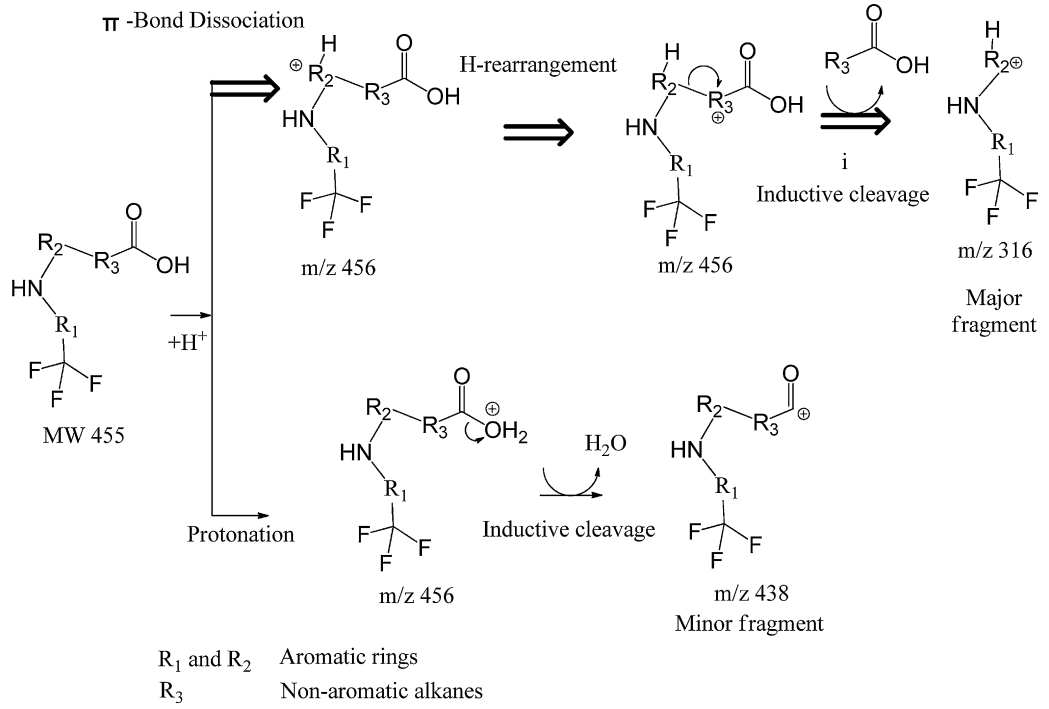

Fig. 2. Proposed ion fragmentation pathways of the drug substance.

fragmentation pathway is to have protonation on the aromatic ring (R_1) to dissociate one of the carbon–carbon double bond (π-Bond dissociation). The resulting positive charge (*m*/*z* 388) can be stabilized by a highly conjugated ring system (R_1–NH–R_2) after carbon dioxide (CO_2) is released as a stable leaving group. This fragmentation pathway is more favorable as it involves not only an easy-leaving group but also a stable fragment ion structure. This fragmentation pathway, however, could not readily occur if a

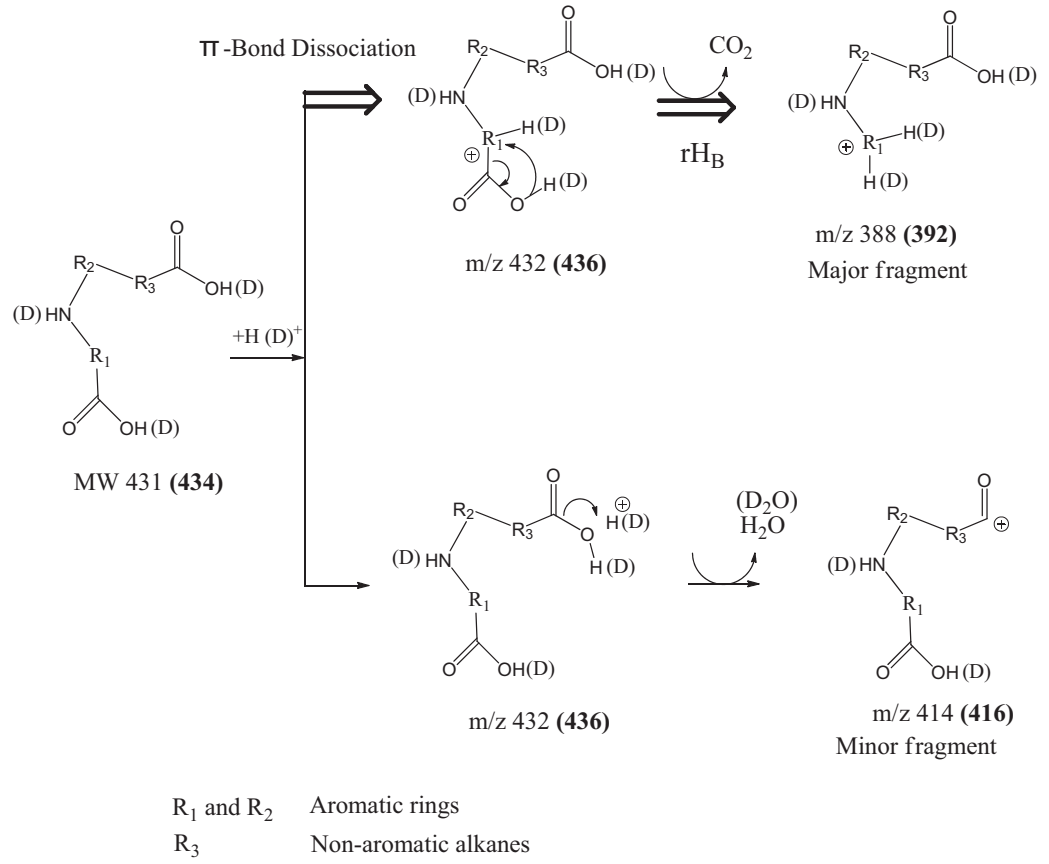

Fig. 3. Proposed ion fragmentation pathways of the drug degradation product.

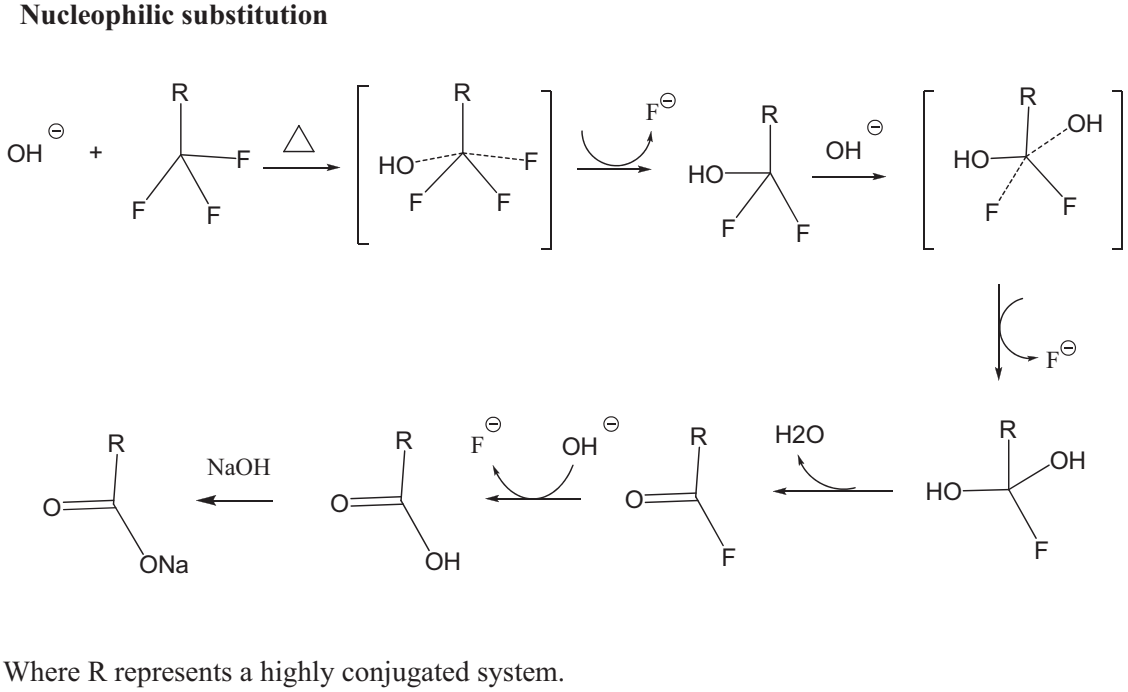

Fig. 4. Proposed drug substance degradation mechanism under base and heat.

carboxylic acid is not conjugated to an aromatic ring. That is why the drug substance cannot have a neutral loss of 44 Da (decarboxylation) in its MS/MS spectrum even though it contains a carboxylic acid.

In summary, the proposed structure is highly consistent with all LC–MS data including MS^n, accurate mass and H/D exchange experiments. The compound is structurally more polar than the drug substance as the result of replacing $–CF_3$ moiety with –COOH. Its MS/MS fragmentation ions have well-defined substructures and its fragment pathways are fully understood. During the course of this investigation, an authentic compound of this degradation product has been synthesized by organic chemists at a different site. The above tentative structure from this LC–MS work has been positively confirmed.

3.5. Proposed reaction mechanism

The degradation reaction mechanism under heat and base is illustrated in Fig. 4.The drug substance contains a trifluoromethyl moiety (R_1–C–F_3), in which three fluorine atoms can be displaced by stronger bases. The base posses an unshared pair of electrons and is seeking a relatively positive site to share their electrons. These basic and electron-rich reagents are also called necleophilic reagents. Hydroxide ion ($:OH^-$) is a stronger necleophilic reagent than fluorine ion ($:F^-$). During base stress, a large number of hydroxide ions could attack the positive site of the trifluoromethyl moiety, in which the carbon atom is connecting to the aromatic ring (R_1). This nucleophilic attack on a tetrahedral carbon, through S_N2 mechanism, involves a transition state containing pentavalent carbon. The existing C–F bond must be partially broken to permit the attachment of the hydroxide ion nucleophilic reagent. The attacking hydroxide ion becomes partially bonded to the reacting carbon atom before the incipient fluorine ion has become wholly detached form it. The reaction involves several steps of nucleophilic substitution followed by dehydration to form a new carboxylic acid. The final product in the stressed solution is a carboxylic anion/salt. The prerequisite for this reaction to occur are the following: (1) trifluoromethyl moiety is connected to an aromatic ring and (2) thermal process is needed to activate the reaction.

4. Conclusion

An unknown degradation product found in a base-stressed dosage form has been comprehensively studied using various LC–MS approaches. With tandem MS, the fragmentation pattern of this unknown was found to be quite different from that of the drug substance. A TOF MS was also used to determine its chemical formula. Based on these results, a tentative structure was proposed. On-line H/D exchange LC–MS was then applied to confirm the proposed structure. The difference in fragmentation pathways between the degradation product and its parent drug substance was due to the changes in their molecular structures. The reaction mechanism of converting a trifluoromethyl moiety to a carboxylic acid under base and heat was also discussed. The formation of this degradation product was proposed to be nucleophilic substitution through S_N2 mechanism involving a pentavalent carbon transition state.

References

[1] U.S. Food and Drug Administration, Guidance for Industry, Q3B Impurities in New Drug Products, 2006.
[2] S. Baertschi, P. Jansen, Stress testing: a predictive tool, in: S. Baertschi (Ed.), Pharmaceutical Stress Testing, Taylor & Francis Group, Boca Raton, FL, 2005, pp. 13–50.
[3] P. Jansen, K. Smith, S. Baertschi, Stress testing: analytical considerations, in: S. Baertschi (Ed.), Pharmaceutical Stress Testing, Taylor & Francis Group, Boca Raton, FL, 2005, pp. 141–172.
[4] G. Chen, B.M. Warrack, A.K. Goodenough, Analysis of impurities and degradants in pharmaceuticals by high resolution tandem mass spectrometry and on-line H/D exchange LC/MS, Am. Pharm. Rev. 13 (2010) 20–27.
[5] R.P. Shah, S. Singh, Identification and characterization of a photolytic degradation product of telmisartan using LC–MS/TOF, LC–MSn, LC–NMR and on-line H/D exchange mass studies, J. Pharm. Biomed. Anal. 53 (2010) 755–761.