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Short communication

A multidisciplinary approach to identify a degradation product in a pharmaceutical dosage form

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ABSTRACT

An unknown degradation product found in non-MS compatible HPLC analysis was studied using a multidisciplinary approach. The unknown was separated and isolated from other components in the drug product by HPLC followed by ion trap MS to obtain MS^n fragmentation patterns. Its chemical formula was determined using a high resolution time-of-flight mass spectrometer (TOF MS). Nuclear Magnetic Resonance (NMR) was used to elucidate the molecular structure. The impurity was identified as 5-hydroxymethyl furfural, which was a degradation product of lactose, one of the excipients used in the formulation of this dosage form.

1. Introduction

LC/MS has been widely used in the pharmaceutical industries due to its high sensitivity and selectivity. The technique has excellent sensitivity for the detection of trace level of impurities and degradation products observed in pharmaceutical drug development. Ion trap MS provides structure-rich information while TOF MS determines the chemical formula of the compound of interest. The use of these two complimentary techniques increases the identification confidence [1,2]. In certain complex situations, however, LC/MS alone cannot determine the final structure. The combination of MS and NMR techniques is therefore necessary for structure confirmation. A multidisciplinary approach including LC/MS and NMR has proven to be a powerful tool for the structure elucidation of unknowns [3–5].

During the stability testing of a pharmaceutical dosage form, an unknown peak was observed to increase over time by HPLC. At the 24-month time point, the impurity was above the permissible limit of 0.2% for this product triggering an impurity identification study. As the established analytical LC method is not MS compatible, a modified HPLC protocol was developed to separate and collect the impurity fraction. Multiple analytical techniques were utilized to identify the structure of the impurity including ion trap MS for the ion fragmentation pattern, TOF MS for the potential chemical formula, and NMR for the identification and confirmation of the chemical structure. Further forced degradation studies of the formulated placebo revealed the source of this degradation product.

2. Experimental

2.1. Liquid chromatography

As the original ion-pairing LC method is not MS compatible, a new method was developed for LC/MS analysis and isolation. The separation was achieved using a Waters XTerra MS C18 column (4.6 mm × 150 mm, 3 μm). Mobile phase A contained 0.03% formic acid in water and mobile phase B contained 100% acetonitrile. A Waters 2690 Alliance HPLC system and a Waters ACQUITY UPLC System were used for ion trap MS and TOF MS, respectively.

2.2. Mass spectrometry

The MS/MS analysis was conducted using an ion trap MS (LCQ^{deca}, ThermoElectron, San Jose, CA, USA) equipped with an electrospray ionization (ESI) source. Mass range acquired was from m/z 100 to 1000. All data were acquired and processed using Xcalibur V2.0 software. The accurate mass measurements were conducted on a high resolution TOF MS (LCT Premier, Waters, Manchester, UK). The mass spectrometer was operated at a W-

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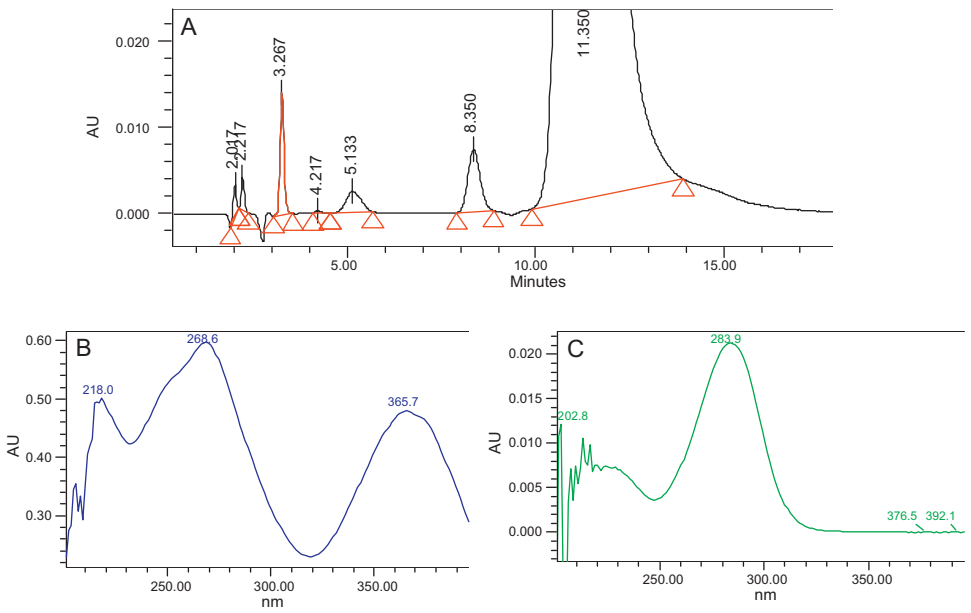

Fig. 1. (A) LC chromatogram of a dosage form after stability storage at 25 °C/65% RH for 24 months using the original ion-pairing LC method; (B) UV spectrum of the drug substance at 11.3 min; (C) UV spectrum of the unknown at 3.3 min.

mode with a resolution of 11,500. All analytical data were acquired and processed using Masslynx V4.1 software.

2.3. NMR

The fractions of the impurity were collected manually from the HPLC runs. The fractions were concentrated with a nitrogen flow before reconstituted with DMSO-d_6 (Cambridge Isotope Laboratory, Andover, MA, USA) for NMR analysis. NMR experiments were carried out on a Bruker AVANCE 600 MHz spectrometer equipped with a 3 mm $^1H/^{13}C$ DUI CryoProbe with Z gradient. Structure elucidation was performed using standard 1D and 2D experiments including ^1H, 2D correlation spectroscopy (COSY), heteronuclear multiple-bond correlation (HMBC) experiments, and selective NOE experiments.

3. Results and discussion

3.1. LC/MS studies

Fig. 1 shows the chromatogram of a drug product stability sample. An unknown peak was found at 3.3 min. As the peak increased over time under stability storage condition (25 °C/65%RH), the unknown is a degradation product. The UV spectral comparison in Fig. 1 B and C shows that the unknown has only one maximum absorbance at 285 nm, while the drug substance has three absorbance maxima at 218, 269, and 366 nm. This significant difference implies that the unknown may not be structurally related to the drug substance.

Fig. 2 shows the LC/MS analysis results from the isolated fraction. The peak of interest at around 5 min has the same UV spectrum as the unknown (Fig. 1C). Fig. 2B shows the mass spectrum of the unknown. The ion at m/z 127 was tentatively assigned as a molecular ion while m/z 167.7 may be an acetonitrile adduct $[M+ACN+H]^+$ ion. The ion at m/z 214 was due to incomplete background subtraction. Fig. 2C–E shows MS^n results. The ion at m/z 168 has a neutral loss of 41 Da due to the loss of acetonitrile. The ion m/z 127 can have neutral losses of 18 (H_2O) and 28 (most likely CO). Further fragmentation of m/z 81 failed to form any ions, indicating that this fragment is very stable and may be highly conjugated. A neutral loss of 18 Da and then 28 Da is a typical fragmentation pattern for a carboxylic acid. However, a negative ion MS scan did not generate a deprotonated $[M-H]^-$ ion peak at m/z 125, indicating the unknown is not a carboxylic acid. Therefore, the hydroxyl (–OH) group and the carbonyl (CO) group are not structurally connected to each other. A TOF MS was then used to define its potential chemical formula. Two major ions of m/z 127.0364 and m/z 168.0650 were detected. The mass difference of 41.0286 Da corresponds to the molecular weight of acetonitrile. Accurate mass of m/z 127.0384 suggests a chemical formula of $C_6H_7O_3$ with a deviation of −1.1 mDa from its theoretical mass. Therefore, the chemical formula of this degradation compound is $C_6H_6O_3$.

3.2. NMR studies

^1H NMR spectrum of the impurity is shown in Fig. 3A. A total of 6 protons are observed for the unknown: one aldehyde proton (δ 9.54 ppm, s), two aromatic protons (δ 6.60 ppm, d, δ 7.50 ppm, d), a methylene group (2H, δ 4.50 ppm, d) and an exchangeable proton (δ 5.57 ppm, t, confirmed by deuterium exchange experiment). COSY, NOE and HMBC correlation data confirmed these resonances all belong to the unknown of interest. COSY data suggests the unknown has a $-CH_2OH$ group. The two aromatic protons are coupled to each other with a $^3J_{HH}$ of 3.5 Hz, suggesting they are on a 5-member ring. Based on the NMR results and the LC/MS results the structure of the unknown was determined to be 5-hydroxymethyl furfural. The proposed structure was confirmed by comparing the NMR spectrum of the unknown with that of the standard 5-hydroxymethyl furfural, Fig. 3B. As shown in the figure, the unknown has the same chemical shifts, coupling patterns and integrals as those of the standard.

3.3. Formation of 5-hydroxymethyl furfural

The formulated placebo and then lactose excipient alone were stressed with 1 N HCl solution heated at 80 °C for 1 h. Both samples showed the same unknown peak, indicating that the unknown came from lactose, one of the excipients used in the formula-

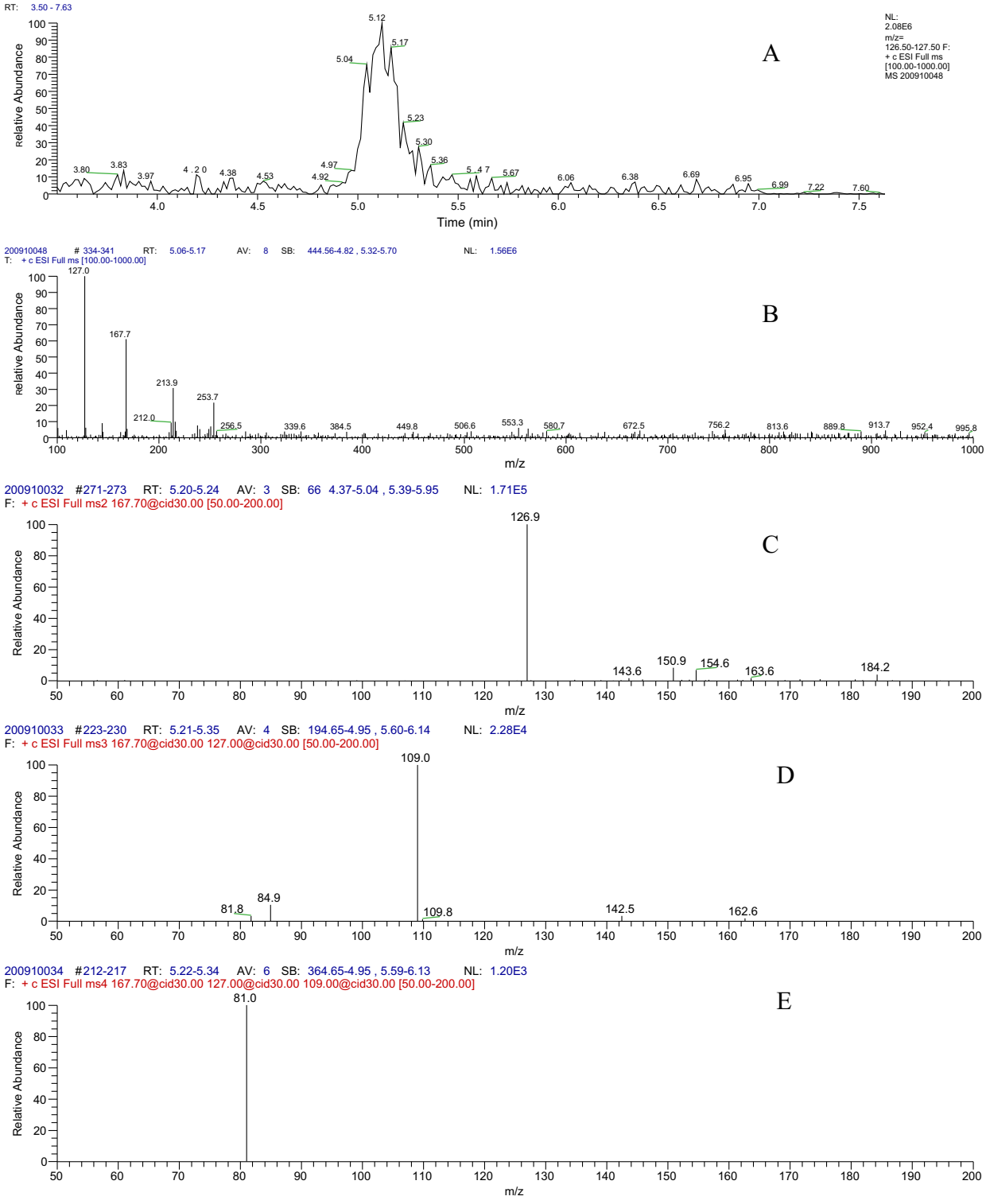

Fig. 2. (A) Extracted ion (*m*/*z* 127.0) chromatogram of the isolated fraction; (B) mass spectrum of the unknown at 5.1 min; (C) MS/MS spectrum of *m*/*z* 168; (D) MS/MS/MS spectrum of *m*/*z* 168 > 127; (E) MS/MS/MS/MS spectrum of *m*/*z* 168 > 127 > 109.

tion. Formation of 5-hydroxymethyl furfural from lactose has been reported in the literature. It was found in the heating of milk [6], in cookies during the baking process [7], and during roasting of coffee [8]. It was further demonstrated that either the glucose or galactose portion of the lactose may serve as the origin of hydroxymethyl furfural [9]. The mechanism may include acidic hydrolysis of lactose to form glucose, which subsequently undergoes several dehydration steps to form 5-hydroxymethyl furfural, as illustrated in Fig. 4. The presence of 5-hydroxymethyl furfural in a pharmaceutical dosage form could have an impact on drug stability. It may induce drug degradation due to its chemically active hydroxyl group and aldehyde group. When lactose is used in the formulation of a dosage form, 5-hydroxymethyl furfural should be considered as one of the reaction precursors to cause drug degradation if the drug substance contains functional group(s), such as amine or carboxylic acid.

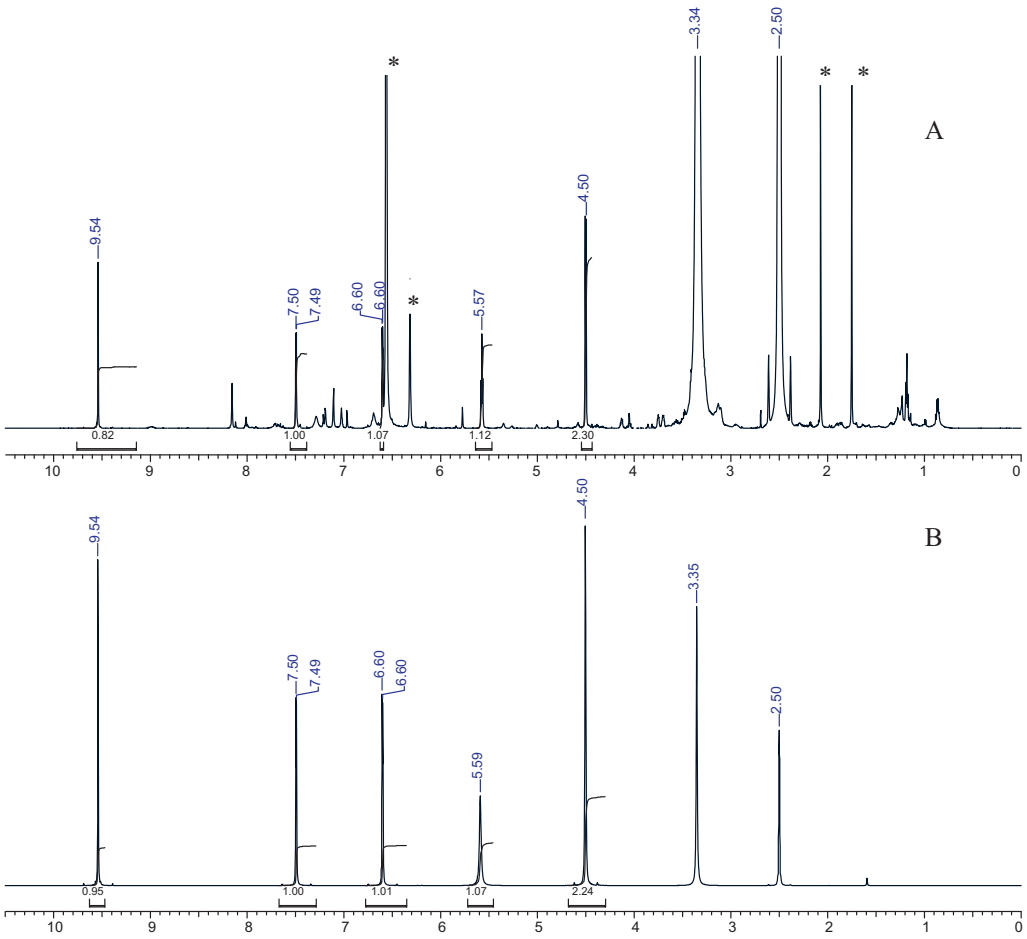

Fig. 3. ^{1}H NMR spectra of the unknown in DMSO-d_6 (A) and 5-hydroxymethyl furfural standard (B). Resonances at 2.50 and 3.34 ppm are DMSO-d_6 and HOD, respectively. Signals marked with * were observed in excipient fractions, and are not related to the impurity.

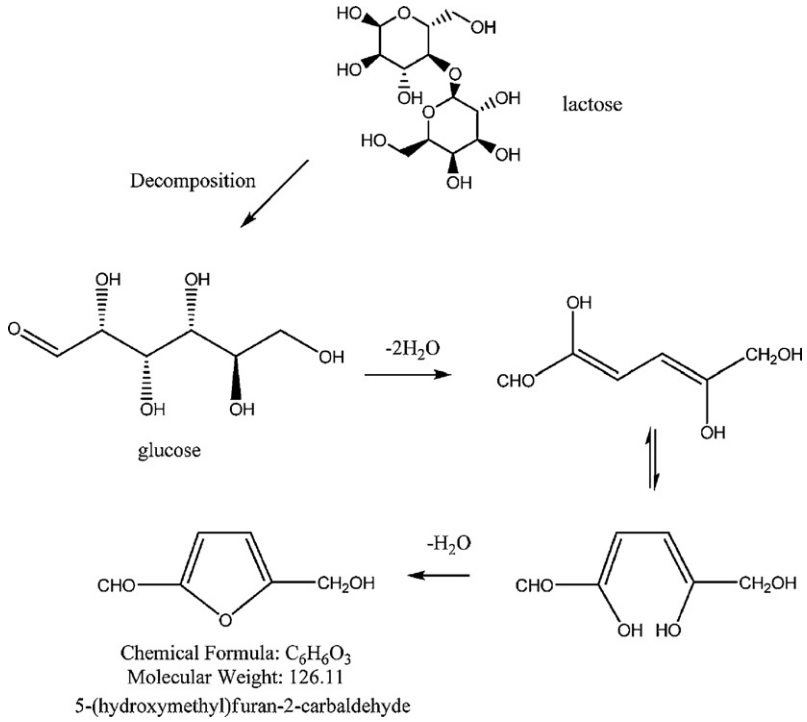

Fig. 4. Formation of 5-methylhydroxyl furfural from lactose degradation.

4. Conclusion

The unknown degradation product found in a pharmaceutical dosage form was identified with a multidisciplinary approach. A MS-compatible HPLC method was developed for peak isolation and LC/MS analysis. The isolated fraction was studied using a tandem mass spectrometer for ion fragmentation patterns and a high resolution mass spectrometer for chemical formula/elemental composition. Using NMR in combination with the MS results, the unknown has been identified and confirmed as 5-hydroxymethyl furfural. The compound is a degradation product of lactose, one of the excipients used in the formulation. The reaction involves acidic hydrolysis of lactose to form glucose, followed by several dehydration steps under heat.

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