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Understanding the degradation pathway of a poorly water-soluble drug formulated in PEG-400

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Abstract

VX-497 is a poorly water-soluble compound. It is formulated in PEG-400 and encapsulated in softgel capsules. Although the drug product is stable at refrigerated conditions, many degradation peaks have been observed at accelerated storage conditions. An investigation utilizing high performance liquid chromatography-mass spectrometry (HPLC–MS) was conducted to understand the degradation mechanism of the active pharmaceutical ingredient (VX-497) in PEG-400 formulation. Results revealed that the degradation was mainly caused by the reaction between VX-497 with moisture (hydrolysis) and PEG-400 (PEGylation). The numerous degradation peaks observed in the samples stored at accelerated conditions were PEG adducts covalently attached to portions of the VX-497 molecule, which were confirmed by comparison with synthetic markers. Investigation also found that an impurity, which was present in the VX-497 drug substance, reacted with PEG-400 following the same reaction mechanism, and generated additional impurities in the VX-497 drug product. By changing the process for drug substance synthesis, pure batches of VX-497 were obtained. Furthermore, it was found that the reaction between VX-497 and PEG-400 was temperature and time dependent. When the drug product was manufactured at 45 °C and the processing time was controlled, the PEG degradants and by-products were reduced to non-detectable levels, resulting in greatly improved drug product quality. This paper presents an integrated effort among analytical, process, and formulation scientists on how to develop a better drug product by understanding the fundamental issues of the drug product, namely the degradation mechanism.

Keywords: Degradation mechanism; PEG-400 formulation; HPLC; HPLC–MS

1. Introduction

VX-497 is a new vertex compound that is an inhibitor of inosine 5′-monophosphate dehydrogenase (IMPDH), an enzyme that catalyzes a key step in the *de novo* guanosine nucleotide biosynthetic pathway. VX-497 is administered orally and is being developed as a potential treatment for hepatitis C. The structure of VX-497 is presented in Fig. 1 in Section 3.

VX-497 is a poorly water-soluble compound and thus, the compound is formulated in PEG-400 and supplied as a liquid-filled softgel capsule drug product for oral administration. Since PEG-400 solubilizes many poorly water-soluble compounds and it is a water-miscible solvent, it is routinely employed as a solubilizing agent in liquid-filled softgel capsules [1]. PEG-400 is considered as an inert excipient, although its concentration-dependent effect on drug GI transit has been reported [2,3]. Besides its popular usage in drug formulation, PEGylation technology that conjugates PEG to proteins, peptides, or small molecules has been considered a successful drug delivery approach since the 1970s [4–6], and the PEGylated drug products have provided clinically and chemically better product than the non-conjugated form [7–11].

While there are many advantages of using PEG in drug development and previous efforts have generated numerous publications and patents, research on PEGylation of low molecular-weight drugs has not yet provided commercially available PEG products [12]. The drawback of using PEG as a bulk excipient in drug formulation is associated with how PEGy-

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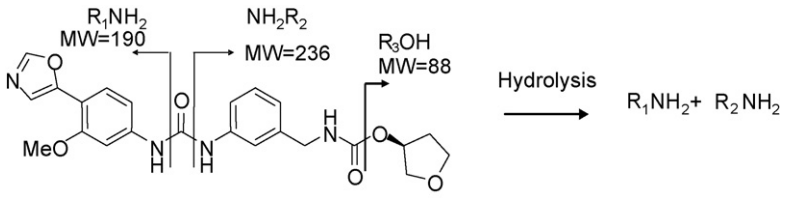

Fig. 1. Degradation pathway of VX-497 by hydrolysis.

lated drugs are synthesized, i.e., the linkage between PEG and the parent molecules can be easily formed through ester, carbonate, and amide bonds [11]. Thus, the chemical stability of an active pharmaceutical ingredient in the PEG-400 formulation is very much dependent on the molecular structure. Although the safety of the PEGylated degradants are not a major concern [9,10], the non-PEGylated by-product degradants of the parent molecule can be a concern especially when there are genotoxic moieties in the parent molecule [13]. Thus, it is extremely important to understand the degradation pathway of the formulated drug product, so that precautionary steps can be taken to control the degradation rate and the level of degradation products during the product manufacturing process and storage.

It is commonly acknowledged in the pharmaceutical industry that PEG-400 is unstable under excessive heat, generating peroxide and other degradants [14,15]. However, very few reports can be found regarding an active pharmaceutical ingredient (API) reacting with PEG-400 at high temperature to result in degradation of the API, suggested by scarce publications. This paper presents a study of the degradation pathway and the stability of an active pharmaceutical ingredient (API) VX-497 in a PEG-400 based formulation and the impact of the study results on drug product quality improvement. Characterization of the major degradation products by high performance liquid chromatography and mass spectrometry is also discussed.

2. Materials and methods

2.1. Reagents

The chemical reagents were directly purchased from different manufacturers: PEG-400 (NF) and ammonium acetate (HPLC grade) were from Fisher Chemicals, acetonitrile and methanol (HPLC grade) were from J.T. Baker, tetrahydrofuran (HPLC grade, inhibitor free) was from Sigma–Aldrich, and acetic acid (Glacial, ACS reagent) was from Spectrum Chemical Manufacturing Corp. Water used in the experiments was Milli-Q grade (18.2 mΩ). VX-497 reference standard, VX-497 drug substance and drug product were Vertex properties.

2.1.1. PEG-CO-NHR$_1$ preparation

At ambient temperature, 2.10 g PEG-400, 10 mL ethyl acetate (EtOAc), and 850 mg (5.26 mmol) triphosgene were added into a 50-ml round bottom flask. The reaction was cooled to 0 °C and then 680 mg (5.26 mmol) diisopropylethylamine was added. The reaction mixture was stirred for 1 h at 0 °C, and then 1.00 g (5.26 mmol) R_1NH_2 was added (see Fig. 1 for its detailed structure). The reaction was allowed to warm slowly to ambient temperature, concentrated to dryness and the final product was red oil.

2.1.2. PEG-CO-NHR$_2$ preparation

One gram (2.81 mmol) of the known process Impurity 1 (see Fig. 1 for structure) was added into a 50-ml round bottom flask, and then 10 mL CH_2Cl_2 and 2.10 g PEG-400 were added. Seven hundred and twenty-five milligrams (5.62 mmol) diisopropylethylamine was added while stirring the reaction solution at ambient temperature. The solution was then heated to reflux and held at reflux for 20 h. The reaction was allowed to warm slowly to ambient temperature, concentrated to dryness and the final product was brown oil.

2.1.3. PEG-400 and VX-497 reaction

Twenty-five milligrams VX-497 and 600 mg PEG-400 (clear liquid) were added into a clear glass container, and then the container was placed in an 80 °C oven for 12–14 h. The reaction yielded a slightly yellow product. Sample containing only PEG-400 were also held in the 80 °C oven for the same length of time. The control samples of the heated reaction mixture and the heated PEG-400 were left at room temperature.

2.1.4. Thermal degradation of softgel capsule fill solution

Fill solution from softgel capsules was added into a glass container and placed into an 80 °C oven for 24 h.

2.1.5. Sample preparation for standard VX-497

VX-497 standard was transferred into a volumetric and diluted with methanol to make a 0.5 mg/mL solution. The standard was sonicated for 5 min and then mixed well.

2.1.6. Sample preparation for PEG-400 and VX-497 (or placebo) reaction product or fill solution

An appropriate amount of liquid reaction product was weighed and added into a volumetric flask to make a 0.5 mg/mL solution. It was diluted to volume with methanol, sonicated for 5 min, and then mixed well. All reaction samples (the samples which were kept at 80 °C and room temperature) were prepared in the same manner.

2.1.7. Sample preparation for softgel capsules (thermally or non-thermally degraded)

In a small beaker, one softgel capsule (25 mg VX-497) was broken, sonicated for 5 min in a small amount of methanol, and then the liquid was transferred into a 50-mL volumetric flask. The beaker and capsule were rinsed three times with methanol and then the entire contents were transferred into the volumetric

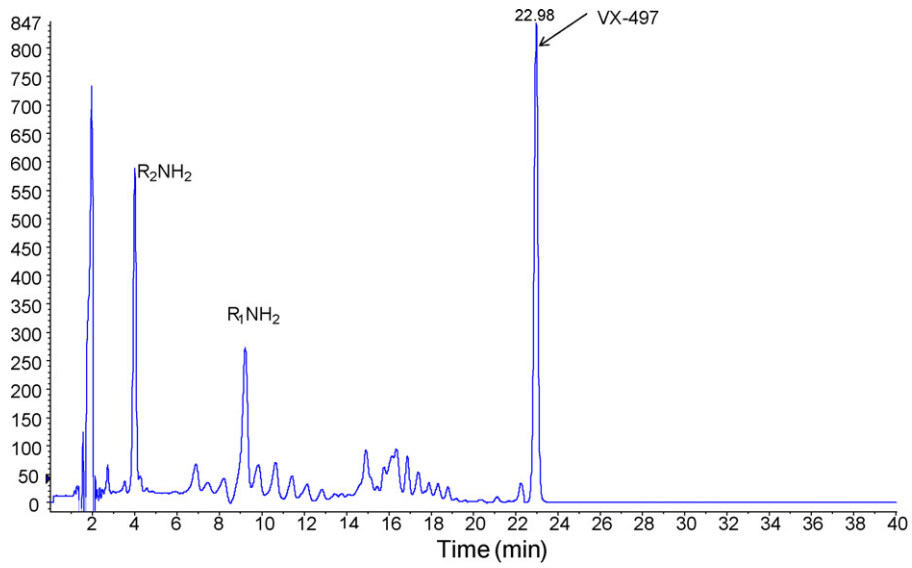

Fig. 2. Chromatogram of VX-497 25 mg softgel capsules that have been stored at room temperature in bulk for 3 years, Lot E. This chromatogram was generated using HPLC method 1.

flask. The flask was diluted to volume with methanol and mixed well.

2.2. HPLC method 1

The high-pressure liquid chromatographic (HPLC) method used an Agilent Zorbax SB-CN, 150 mm (length) × 4.6 mm (i.d.), 5 μm column with mobile phase A composed of 5 mM Acetate Buffer, pH 5.7:Acetonitrile, 80:20 (v/v), and mobile phase B composed of 5 mM Acetate Buffer, pH 5.7:Acetonitrile, 10:90 (v/v). The HPLC conditions for method 1 can be described as follows: holding at 0% B for 5 min, ramping up the gradient from 0% B to 40% B in 20 min, holding at 40% B for another 5 min, and ramping down the gradient to 0% B in 5 min followed by equilibration for 15 min at 0% B. Other parameters were flow rate: 1.0 mL/min, the detection wavelength: 240 nm with reference off, column temperature: 35 °C, and injection volume: 10 μL.

2.3. HPLC method 2

The method used a YMC ODS-AQ column, 100 mm × 4.6 mm (i.d.), 3 μm (particle size), 120 Å (pore size). The method used 95:4:1 (v/v/v) 10 mM ammonium acetate, pH 5.0: acetoni-

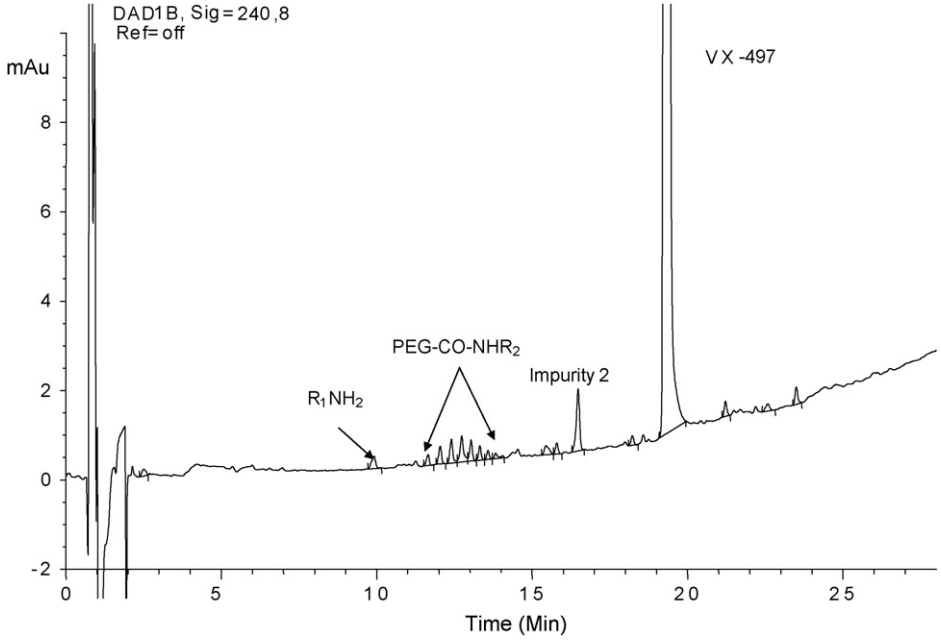

Fig. 3. Chromatogram of VX-497 drug product at release (Lot JM-9). The peak labelled as Impurity 2 is a known process impurity. The chromatogram was generated using HPLC method 1.

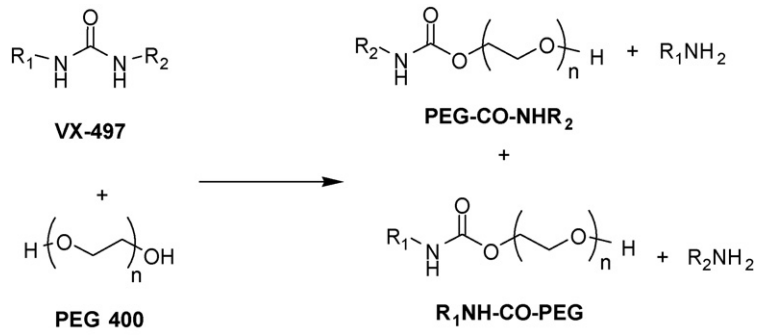

Fig. 4. Reactions between VX-497 and PEG-400.

trile: THF as mobile phase A and 10:72:18 (v/v/v) 10 mM ammonium acetate, pH 5.0:acetonitrile: THF as mobile phase B. The HPLC conditions for method 2 can be described as follows: holding at 6% B for 2 min, ramping up the gradient from 6% B to 52% B in 25 min, and ramping down the gradient to 6% B in 1 min followed by equilibration for 5 min. Other parameters were flow rate:1.5 mL/min, detection wavelength: 240 nm with reference off, column temperature: 35 °C, and injection volume: 20 μL.

2.4. HPLC–MS conditions

An Applied Biosystem API 3000 triple quadrupole was employed for the HPLC–MS(/MS) analysis. A splitter was placed after the HPLC–UV detector to allow 0.2–0.4 mL/min eluent split from the 1 mL/min HPLC flow to the mass spectrometer. The interface temperature was set at 350 °C, and the electrospray voltage was set at 5 kV.

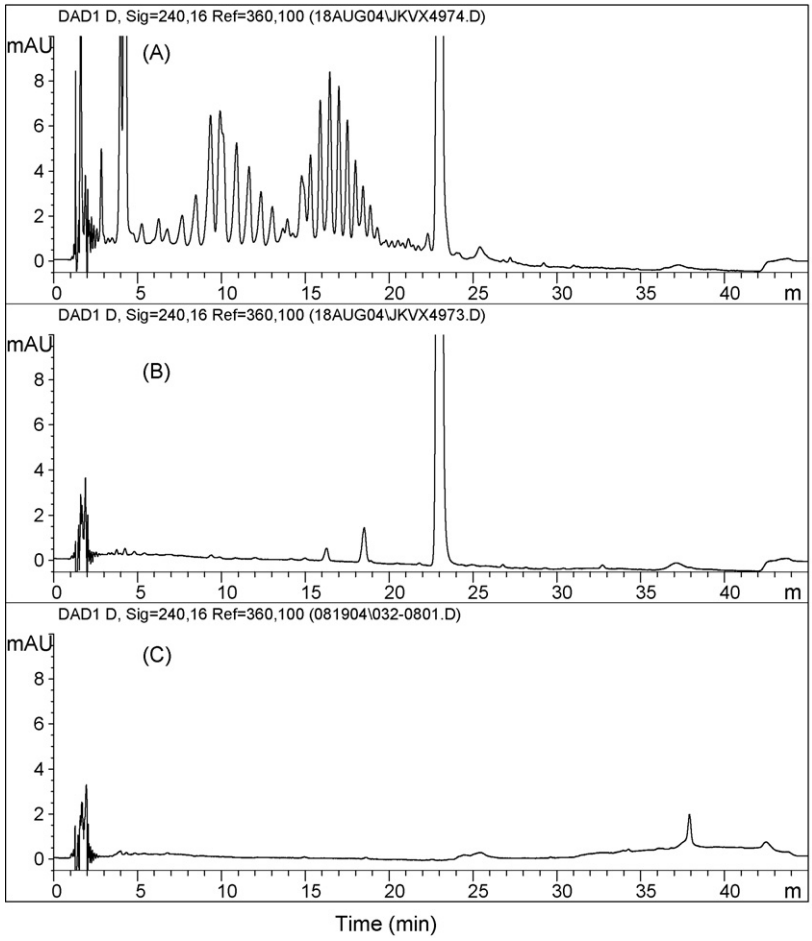

Fig. 5. Chromatograms resulted from the reaction between PEG-400 and VX-497, (A) PEG-400 and VX-497 reacted at 80 °C for 12–14 h, (B) PEG-400 and VX-497 reacted at room temperature for 12–14 h, and (C) PEG-400 was heated at 80 °C for 12–14 h. Chromatograms were generated using HPLC method 1.

3. Results and discussions

3.1. Understanding and identification of the degradation pathway

Hydrolysis of VX-497 was investigated and it was found that VX-497 generates two degradation products by hydrolysis of the urea bond (Fig. 1). These products have been consistently observed in the chromatograms of stability samples that were placed in stability chambers at 5 °C, 25 °C/60% RH, 30 °C/65% RH, and 40 °C/75% RH. Since the VX-497 softgels contain about 7% moisture, hydrolysis of VX-497 is inevitable.

Representative chromatograms from the analysis of a drug product lot that was stored at room temperature for 3 years in bulk packaging (capsules were stored in a low density polyethylene bag which was kept in a cardboard box) and from the initial stability time point are presented in Figs. 2 and 3, respectively. In addition to the two expected hydrolysis degradants, R_1NH_2 and R_2NH_2, there are also a number of other peaks that display a polymeric distribution pattern.

Since the drug product formulation contains mainly PEG-400 which is a nucleophile that can react with VX-497 at the urea carbonyl, it is reasonable to believe that these polymer peaks are chemical reaction products between VX-497 and PEG-400, as hypothesized in Fig. 4. As indicated in Fig. 4, the reaction should yield the following degradants: R_1NH_2 paired with PEG-CO-NHR_2 adducts and R_2NH_2 paired with PEG-CO-NHR_1 adducts. This proposed reaction mechanism is consistent with those reported in the literature on how the PEGylated drugs are synthesized [11].

If this hypothesis is true, a group of polymeric peaks containing the subunits of PEG-400 should be observed as the degradation products, and they should also be evidently observed in the chromatograms of samples that were stored at accelerated storage conditions, since higher temperature will increase the reaction rate. In fact, these polymeric peaks were confirmed in

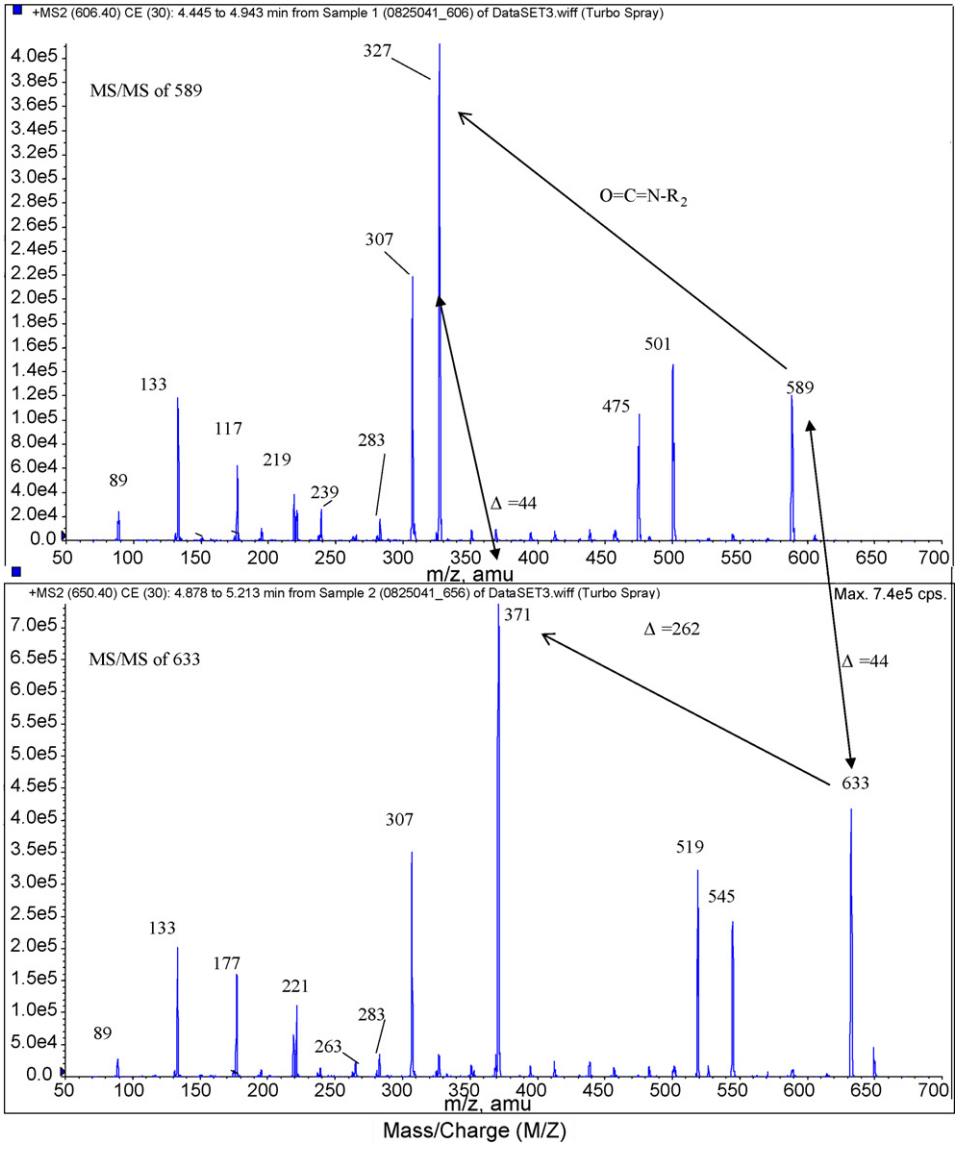

Fig. 6. Representative MS/MS spectra of two adjacent PEG-CO-NHR_2 adducts.

the chromatograms of the stability samples stored at the accelerated conditions, such as 40 °C/75% RH (data not shown) and 30 °C/65% RH (Fig. 2). Interestingly, these polymer peaks were also found in a chromatogram obtained immediately after the drug product was manufactured (Fig. 3), although at the level of below the quantitation limit (<0.05% wt/wt) which suggested that PEGylation may have occurred during the manufacturing process.

3.2. Identification and characterization of PEGylated degradation products

In order to prove the hypothesis and confirm the identity of the polymeric peaks, experiments were performed by mixing PEG-400 with VX-497 and heating the mixture at 80 °C for 12–14 h along with a pure PEG-400 sample. Two control samples of the heated samples were stored at room temperature. The purpose of the control experiment was to determine if VX-497 would react with PEG-400 at room temperature and the degradation of PEG-400 at 80 °C. Analyses of all the samples revealed that VX-497 reacted with PEG-400 at 80 °C and generated two groups of polymer peaks that chromatographically match in retention time to those peaks observed in stability studies (Fig. 5A). However, VX-497 did not react with PEG-400 at room temperature (Fig. 5B). In addition, degraded PEG-400 alone did not show any polymer peaks in the chromatogram, since PEG-400 itself does not contain any chromophores (Fig. 5C). All the experimental samples were then analyzed by LC–MS. The masses listed in the columns under Group 1 and Group 2 refer to the peaks shown in Fig. 5A. Within each group, the two adjacent peaks have a mass interval of 44 Da, further supporting the suggestion that they are polyethylene glycol subunits.

Several peaks from each group were subjected to collision-induced dissociation to generate fragmentation (MS/MS) spectra. As shown in Fig. 6 (MS/MS spectra for Group 1 adducts), neutral loss of a mass of 262 corresponds to the pro-

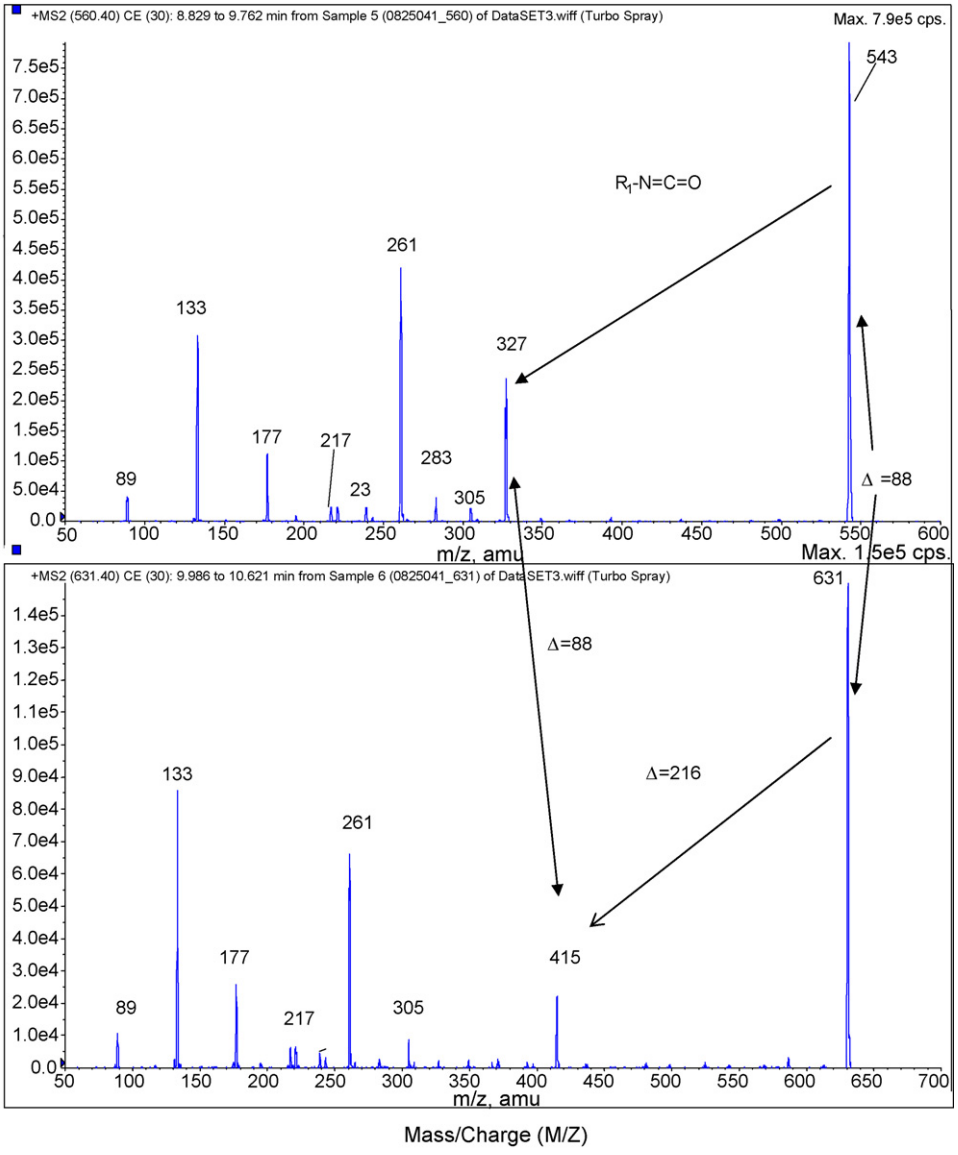

Fig. 7. Representative MS/MS spectra of two non-adjacent PEG-CO-NHR$_1$ adducts.

Table 1
Calculated masses of PEG-400 and PEG-adducts found in MS analysis

Cal. PEG-400 masses	Group 1	Group 2
238	500	454
282	544	498
326	588	542
370	632	586
414	676	630
458	720	674
502	764	718
546	808	762

posed structure related to R_2NH_2, while neutral loss of a mass of 216 corresponds to the proposed structure related to R_1NH_2 (Fig. 7). The neutral loss of 262 and 216 can also been seen in Table 1 by subtracting the corresponding PEG-400 masses from the Group 1 and Group 2 masses in each row. Moreover, because R_2NH_2 is relatively fragile compared to R_1NH_2, other neutral losses from the carbamate moiety were observed, including R_3OH (loss of 88, see Fig. 1 for the detailed structure) and 114. To confirm that the 44 Da mass interval is from PEG-400, peaks that were not adjacent were selected for MS/MS study. They still followed the $n \times 44$ Da mass rule where the difference is 2×44 as shown in Fig. 7. These data support the proposal that the two groups of degradation products consist of PEG-400 adducts of VX-497 related substances.

The absolute confirmation of the PEG-adducts was accomplished by chromatographically matching (in retention time) those peaks to independently synthesized markers. The PEG-CO-NHR_2 marker was synthesized by reacting PEG-400 with the known process impurity (Impurity 1 which contains R_2NH_2), while the PEG-CO-NHR_1 marker was synthesized by react-

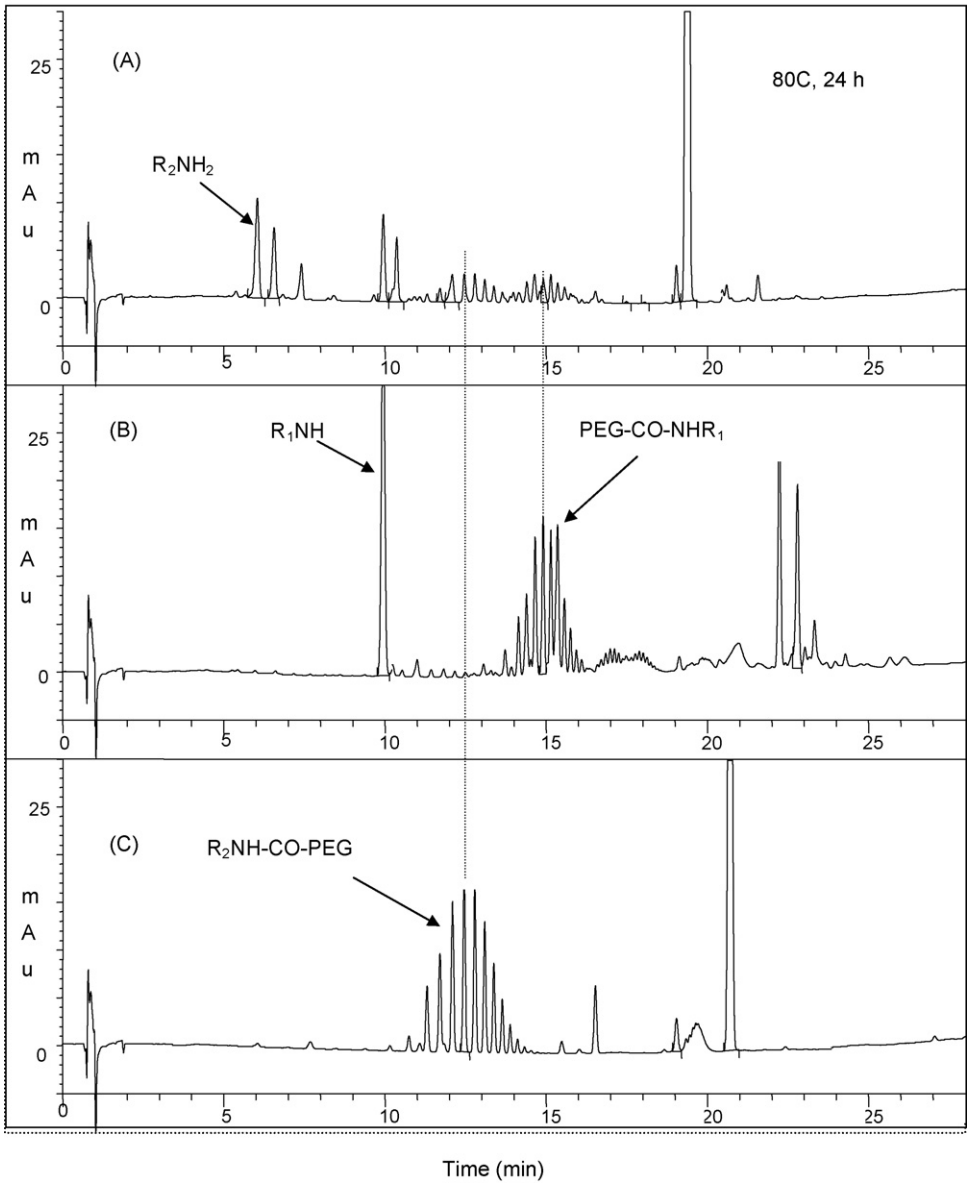

Fig. 8. Chromatograms of VX-497 25 mg softgel capsule and markers, (A) Lot-JM-8 incubated at 80 °C for 24 h, (B) Marker of PEG-CO-NHR_1 adducts, and (C) Marker of PEG-CO-NHR_2 adducts. Chromatograms were generated using HPLC method 2.

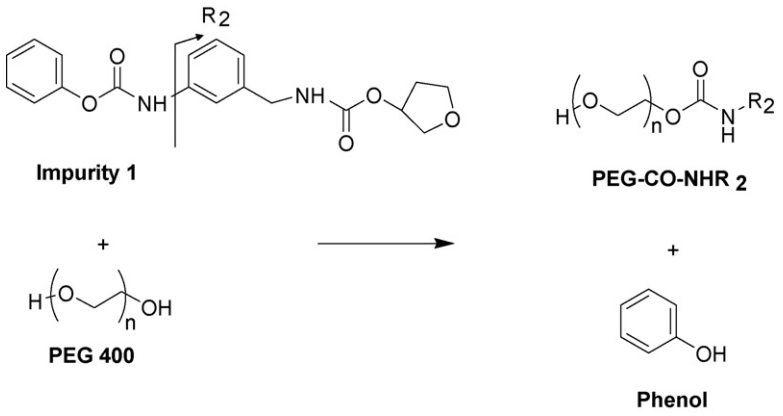

Fig. 9. Reaction mechanism between PEG-400 and the known process Impurity 1.

ing PEG-400 with phosgene and then R_1NH_2. The matching chromatograms are shown in Fig. 8.

3.3. Understanding the formation of PEG-adducts and using this information to improve the drug product manufacturing process

Having determined the mechanism for the formation of the PEG adducts, it was understandable that a minor amount of PEG adducts were observed in the chromatograms at product release (Fig. 3). The manufacturing process involved mixing PEG-400, VX-497, and other excipients at 75–80 °C for 60 min. The high temperature may have contributed to the generation of initial levels of PEG adducts since PEG-400 reacts with VX-497 at 80 °C.

As the manufacturing temperature for mixing VX-497 with the excipient mixture was lowered, it was found that the level of PEG adducts decreased correspondingly, thus confirming the relationship between temperature and the formation of the PEG-adducts. When the mixing temperature was lowered to 45 °C, the R_1NH-CO-PEG adducts were not observed (data not shown), however, the PEG-CO-NHR_2 adducts were still present. After further investigation, it was found that the known process Impurity 1 (see Fig. 1 for structure) was present in the drug substance. Impurity 1 reacted with PEG-400 (Fig. 9) as how the PEG-CO-NHR_2 marker was synthesized, and contributed to the formation of PEG-CO-NHR_2 adducts. Mass balance for this reaction was obtained by comparing the amount of Impurity 1 originally present in the drug substance to the total amount of PEG-CO-NHR_2 formed in the corresponding drug product (see Fig. 10A

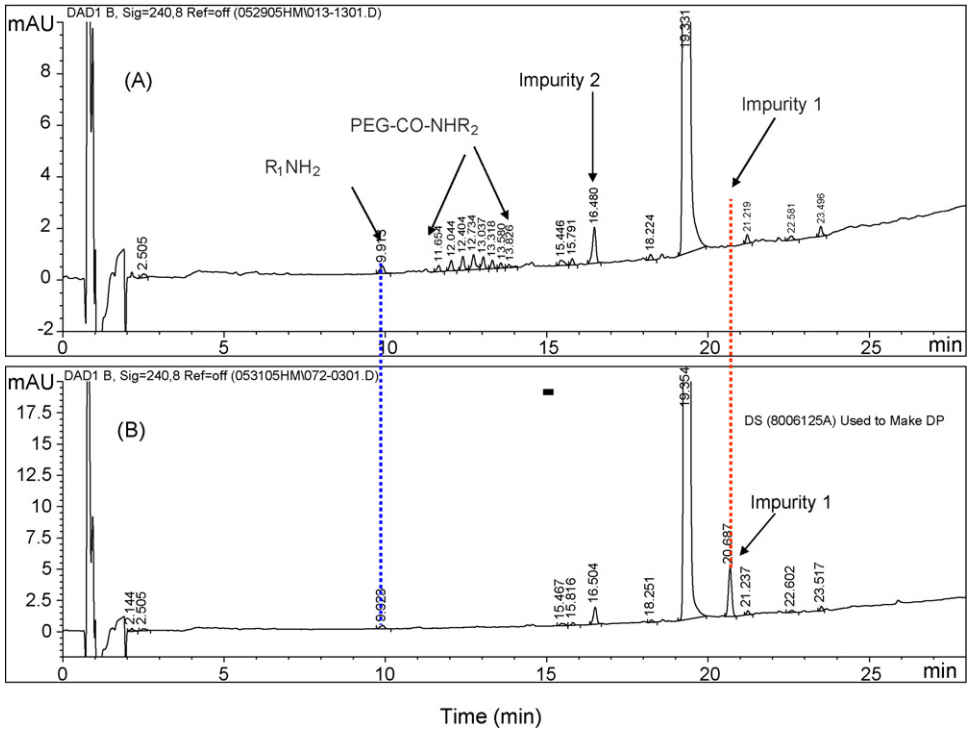

Fig. 10. (A)Chromatogram of drug product Lot JM-9 at release (made from a batch of drug substance, Lot 8, that contains Impurity 1), total amount of PEG-CO-NHR_2 adduct is 0.28%. (B) Chromatogram of drug substance Lot 8, amount of Impurity 1 is 0.20% (w/w). Chromatograms were generated using HPLC method 2.

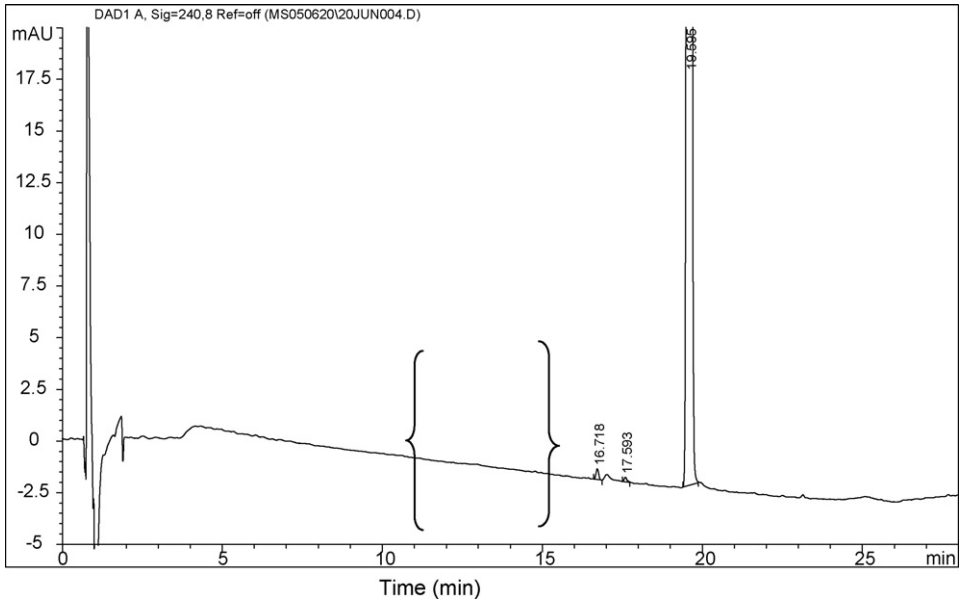

Fig. 11. Analysis of VX-497 fill solution prepared at 45 °C using a batch of drug substance lot-L1 that has purity of 99.9%. This chromatogram was generated using HPLC method 2.

and B). In Fig. 10A, the R_1NH_2 degradant was observed since the manufacturing process (mixing temperature remained at 75 °C) did not change at the time for the production of this Lot. A peak labelled as Impurity 2 (also mentioned in Fig. 3) at retention time of 16.48 min was another known process impurity that did not react with PEG-400, thus it is not relevant to the scope of this study.

With the analytical information about the reaction between Impurity 1 and PEG-400, the synthesis process for the drug substance was improved. The new process for the drug substance was able to remove Impurity 1 which yielded new batches of material with high purity (99.9%). When the high purity drug substance was used to manufacture the drug product at 45 °C, neither hydrolytic degradation products nor PEG-CO-NHR_2 were observed as shown in Fig. 11. Moreover, the drug product manufacturing process was also modified such to ensure that all excipients to be dissolved while keeping VX-497 intact during the manufacturing of the fill solution for VX-497 softgel capsules.

4. Conclusions

The VX-497 drug product degradation pathway was identified and the related degradation products were characterized and confirmed. The degradation products of VX-497 drug product were resulted primarily from the reaction between VX-497 with moisture and PEG-400. The major degradants were R_1NH_2, R_2NH_2, PEG-carbamate adducts, and PEG-amino-oxazole adducts.

This study revealed that one can place critical control steps in place to improve the quality of the drug product by understanding the degradation mechanism. Two significant changes were made (1) removal of the know process Impurity 1 in the drug substance so that the chance for PEG adduct formation during the drug product manufacturing phase was eliminated and (2) shortening of the mixing time and lowering the mixing temperature for VX-497 and the excipient during the drug product manufacturing process so that the reaction between VX-497 and PEG-400 as well as the thermal degradation of VX-497 was prevented. With the process improvements for both the drug substance and the drug product, the degradation products in the final VX-497 drug product were controlled to a non-detectable level.

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