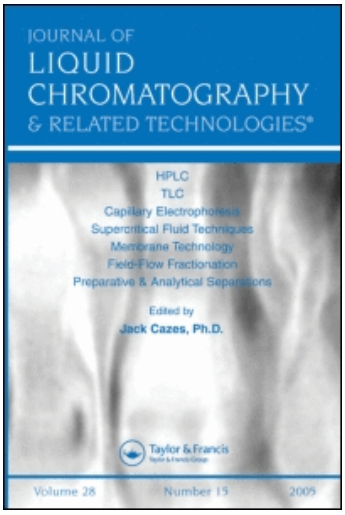

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Identification of Drug Meglumine Interaction Products Using LC/MS and Forced Degradation Studies

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Abstract: In this work we report the identification of unknown degradation products observed during the accelerated stability studies of a Hepatitis C Virus inhibitor drug product by using LC/MS and forced degradation studies. These degradation products are formed through chemical interaction between the Active Pharmaceutical Ingredient (API) and meglumine, an excipient in the drug formulation.

Keywords: Drug-excipient interaction, Forced degradation, LC/MS, Meglumine

INTRODUCTION

Identification of unknown degradation product in a drug formulation is critical for understanding of the degradation chemistry and chemical stability of the drug formulation.[1] During accelerated stability studies of a Hepatitis *C* Virus (HCV) inhibitor drug product, two unknown impurity peaks were observed. These impurity peaks grew with time on stability and exceeded the ICH identification threshold[2] at the 2 month time point (0.24%, area ratio). This paper reports the identification

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of these unknown degradation products using Electrospray LC/MS in conjunction with forced degradation studies.

EXPERIMENTAL

Materials

Meglumine was purchased from USP (Rockville, MD), LOT F0D385. Stability samples, Placebo and API standard were obtained internally.

HPLC-DAD

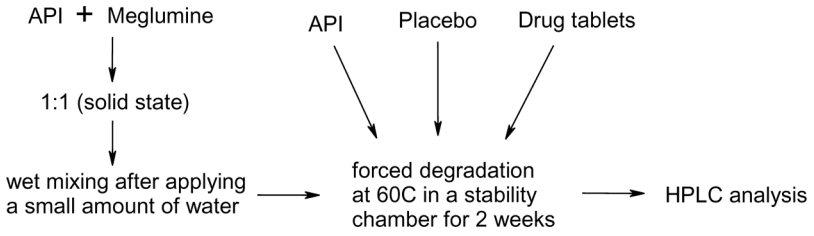
HPLC-DAD analyses were performed on a Waters Alliance HPLC system (Model 2695) equipped with a PDA detector. The HPLC parameters are as below: column: Inertsil ODS-3, 4.6 mm × 150 mm, 3 μm, S/N 5FI82428; flow rate: 1.1 mL/min; mobile phase: A: 0.1% Formic Acid in Water, B: 0.1% Formic Acid in 90/10, Methanol/Acetonitrile; injection volume 10 μL. The HPLC gradient is given in Table 1.

LC/MS

LC/MS experiments were performed on a LTQ linear ion trap mass spectrometer (ThermoFisher, MA, USA) coupled with an Agilent 1100 HPLC system. The HPLC method for LC/MS is adopted from the HPLC method described above. Key mass spectrometer parameters are as follows: ionization mode: positive ion electrospray; mass range: 50–1800 (full scan). MS^n experiments were performed automatically using the data dependent scan function.

Table 1. HPLC gradient

% B	0.00
5.00	45
30.00	55
40.00	70
40.01	100
44.00	100
45.01	45
47.01	45



Scheme 1. Forced degradation protocol for investigation of the API-meglumine interaction product.

Forced Degradation Studies

For investigation of the origin of the degradation products, API was stressed along with meglumine at a 1:1 ratio in slurry for two weeks in a stability chamber (Sanyo, Model XR-5) at 60°C. For comparison, the API, drug product tablets and a placebo were also stressed in parallel. The resulting samples were then analyzed using HPLC-DAD. The protocol of forced degradation studies is shown in Scheme 1.

RESULTS AND DISCUSSIONS

HPLC-DAD and LC/MS Results

The HPLC/UV chromatogram of the drug product sample from accelerated stability studies exhibits two unknown impurity peaks at relative retention times (RRT) of 0.42 and 0.44, respectively as shown in Figure 1. Since the unknown impurities grew on stability, they are actual degradation products in the drug product. The two unknowns exhibit identical UV and mass spectra, indicating that they are likely isomers. The UV spectra of the unknowns have some common features in the long wavelength range with that of the API (not shown), indicating that the unknowns are likely related to the API.

Figure 2 shows the ESI (Electrospray Ionization)[3] mass spectrum of one of the two unknown isomeric degradation products. The molecular weights of the unknowns determined by ESI/MS are 847 (nominal mass). The difference between the measured molecular weights of the unknowns and that of API is 195 amu, which is consistent with the molecular weight of one of the excipients, meglumine,[4] in the formulation. Thus the unknowns are likely the interaction products of API with meglumine.

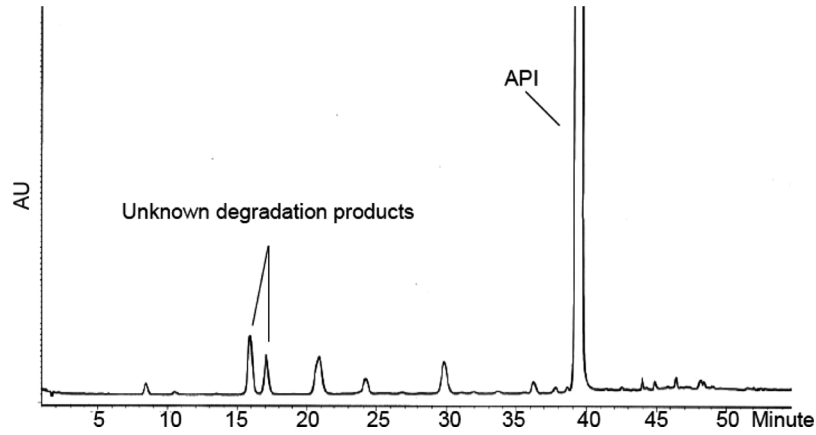


Figure 1. HPLC/UV chromatogram of a representative accelerated stability sample stored at 40°C/75RH for 8 months.

Results of Forced Degradation Studies

Figure 3 shows the chromatograms of the stressed samples. It is very clear that the two unknown impurities are related to the API, because they did not appear in the stressed placebo and sample diluent. Further, the forced degradation studies also confirmed that the two unknowns are API-excipient interaction products because these degradation products were not formed in the stressed API. The formation of the two unknown degradation products in the stressed API-meglumine mixture provided

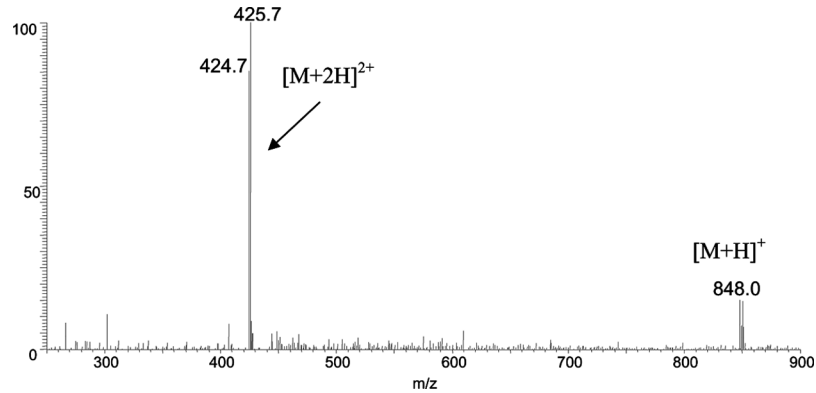


Figure 2. A representative ESI mass spectrum of the two isomeric unknown degradation products.

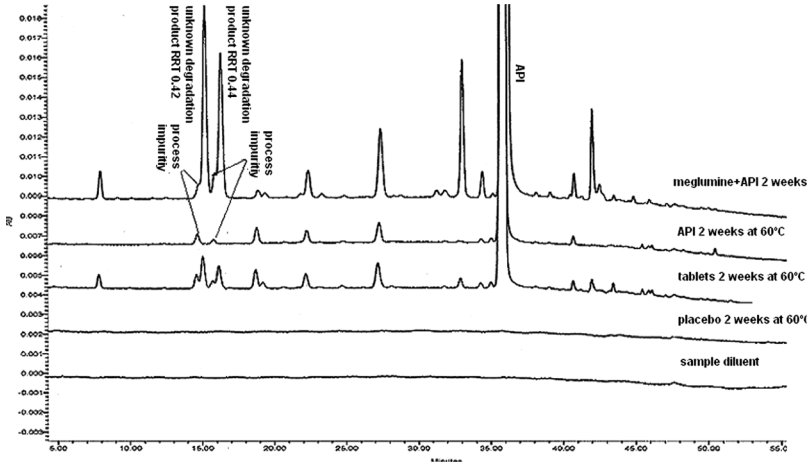


Figure 3. Overlay of HPLC-UV chromatograms of stressed samples.

direct evidence that the unknowns observed during the accelerated stability studies were formed through interaction between the API and meglumine in the formulation.

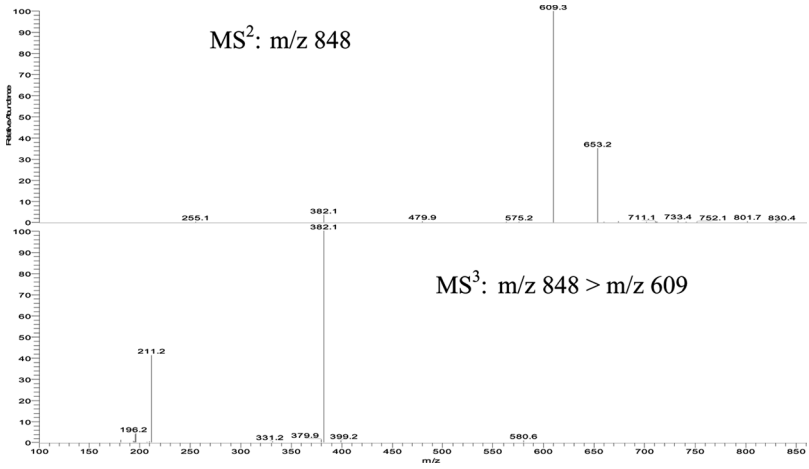
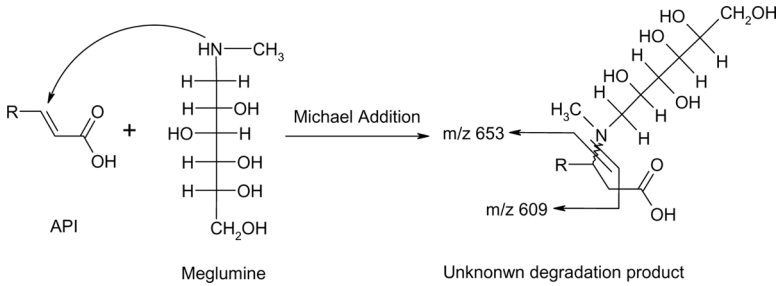


Figure 4. MS^2 and MS^3 spectra of the protonated molecules of the unknown degradation products, m/z 848.



Scheme 2. Mechanism of formation of the API-meglumine adducts.

DISCUSSIONS

The API molecule contains an α, β-unsaturated double bond system. In the solid dosage formulation of the HCV inhibitor, meglumine was used as an basifier and solubilizing agent. Meglumine is a secondary amine. The nitrogen atom in meglumine is a nucleophile and can attack the 2, and 4-positions of the α, β-double bond system to formed nucleophilic addition products. Because the 1, 2-addition products were not observed in LC/MS, the isomeric degradation products observed in the accelerated stability studies were proposed as a pair of stereo isomers formed through 1, 4-nucleophilic addition (Michael Addition),[5] as shown in Scheme 2.

The proposed structures were further confirmed by the MS/MS data of the protonated molecules of the degradation products, as shown in Scheme 2 and Figure 4.

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