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Qualitative analysis of 4 impurities in enalapril using liquid chromatography-ion trap time of flight hybrid mass spectrometry

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Introduction

Enalapril Maleate is the maleate salt of enalapril, the ethyl ester of a long acting angiotensin converting enzyme inhibitor. Enalapril is a pro-drug; it is bioactivated by hydrolysis of the ethyl ester to enalaprilat, which is the active angiotensin conveting enzyme inhibitor. Enalapril has side effects: fainting; urinating more; chest pain, etc.

However, by-products, unknown impurities and their structures are a big concern. Currently, high-performance liquid chromatography (HPLC) has been the essential method for analysis of enalapril. Atmospheric pressure ionization sources, such as electrospray ionization (ESI) are routinely used in drug impurities analysis for sensitivity.

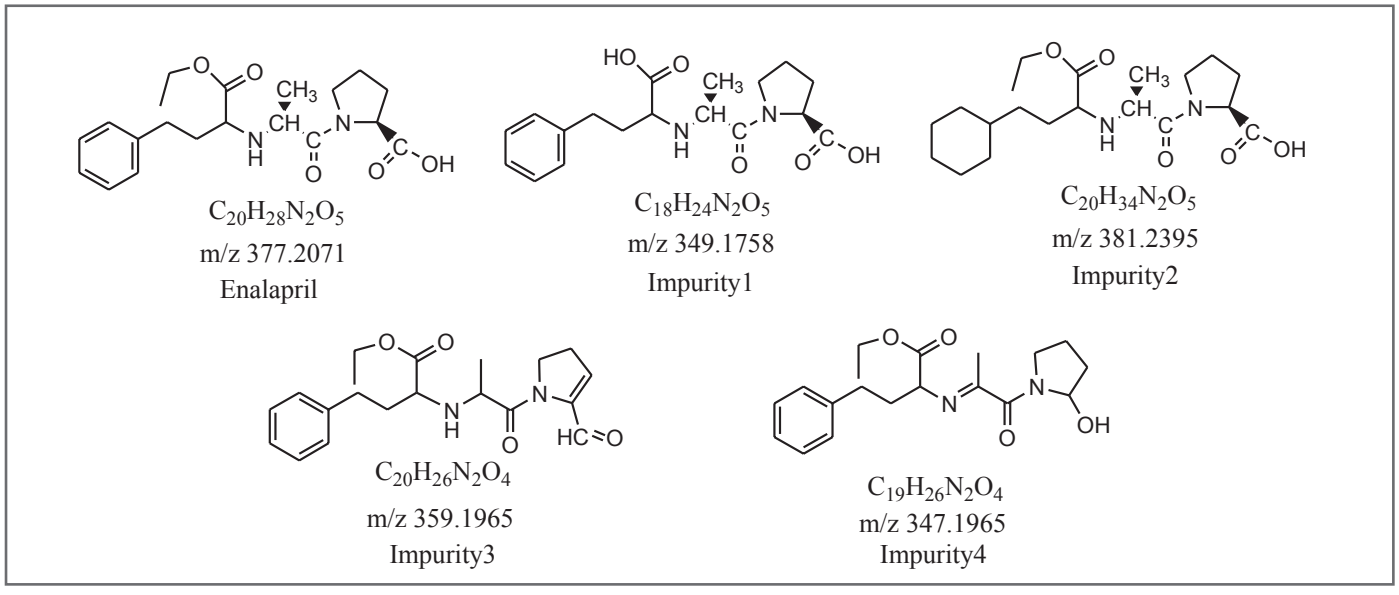

Fig. 1 Molecular structure of Enalapril and 4 impurities.

A liquid chromatography/electrospray ion trap time-of-flight mass spectrometry (LCMS-IT-TOF) was used in this study. It has the ability to provide multistage tandem spectra (MS^n) with accurate masses (error<5 ppm) in both MS and MS^n modes. Tof analyzer allows fast acquisition of full spectra with high sensitivity and elevated mass resolution (12000 FWHM). With this technique, we can acquire reliable structural information about the ionized molecule and the product ions, based on the exact mass and the multistage tandem mass spectrometric analyses (MS^n). In addition, as the mass/charge ratio gets smaller, the number of candidates gets smaller, too. Since mass/charge ratios of the product ions in the highest order spectra are usually less than 200 Da and the neutral losses are usually less than 100 Da, their formulae could be confirmed with significantly higher confidence.

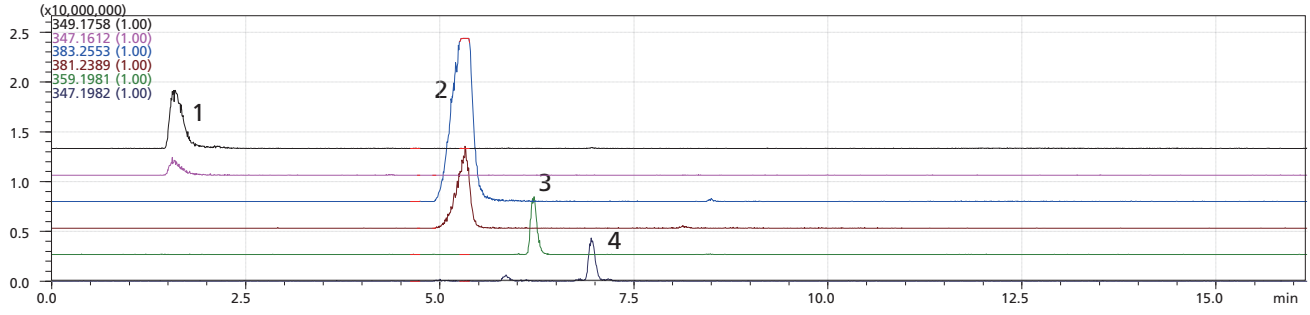

Fig. 2 chromatogram of 4 impurities.

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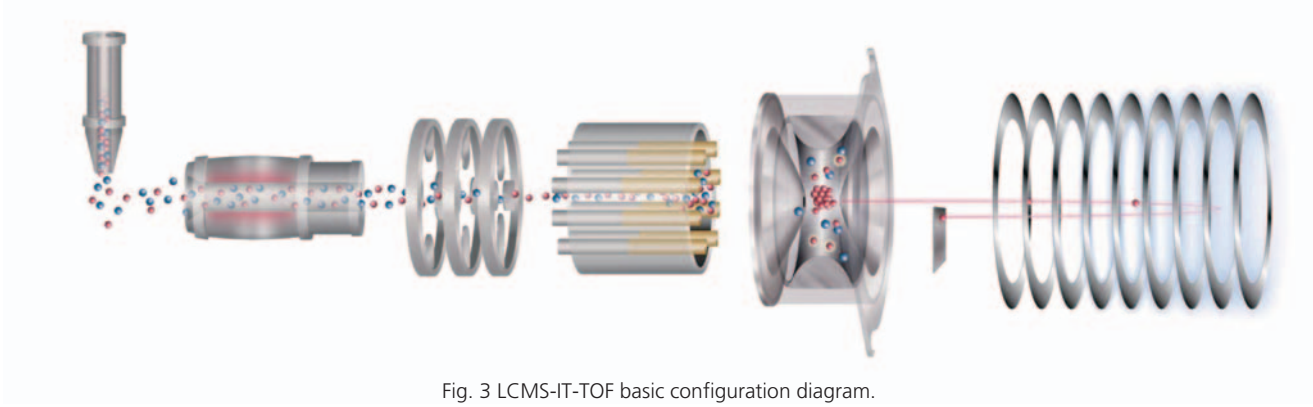

Fig. 3 LCMS-IT-TOF basic configuration diagram.

Experimental

Enalapril samples were separated on a Shimadzu Shim-pack XR-ODS column (2.0 mm I.D.×75 mm L., 2.2 µm) with isocratic elution using 10 mM ammonium acetate and acetonitrile as mobile phase. High concentration of enalapril (300 mg/L) was injected to concentrate impurities. Flow path switching valve was used in the instrument, cutting the enalapril main constituent to waste line in order not to contaminate MS detector. Chromatogram is shown in Fig. 2 Four impurities were separated and detected by LCMS-IT-TOF. MS^n spectra (n≥4) of each impurity were obtained in both positive and negative ion mode. Formulae, chemical structures of impurities in an Enalapril sample were suggested with supporting results on the probable fragmentation pathways are shown in Fig. 4-5.

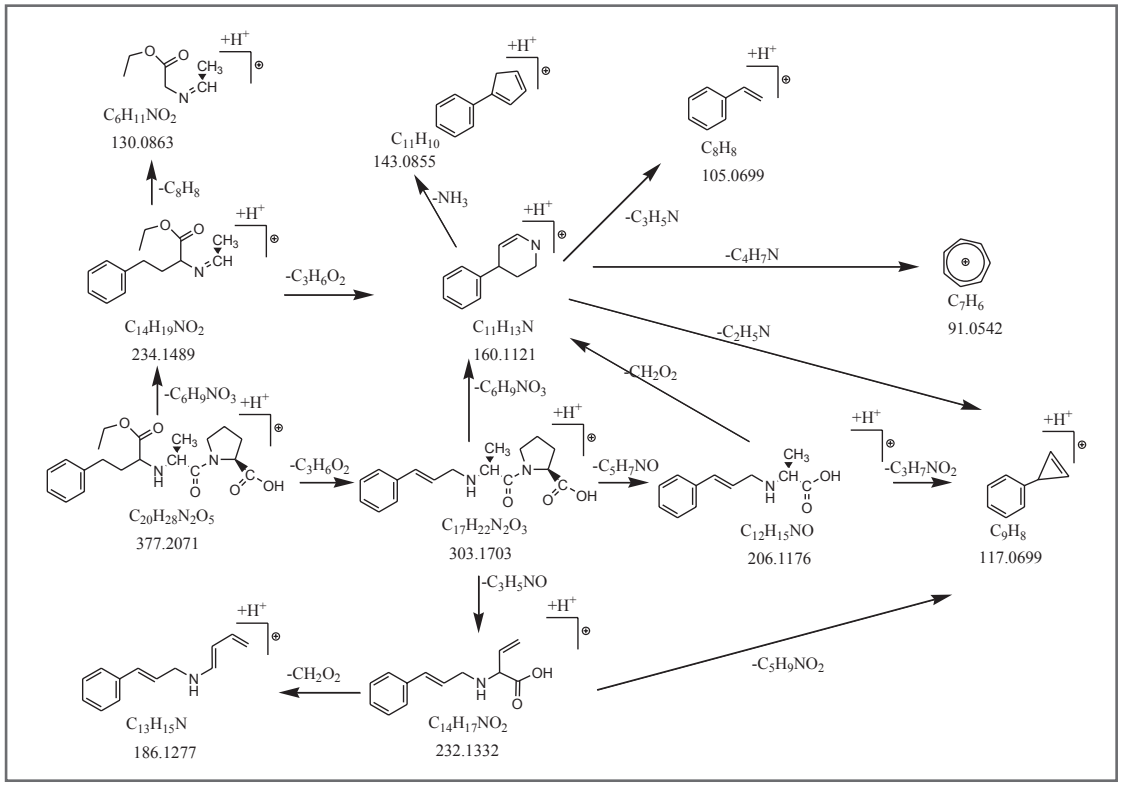

Fig. 4 Probable fragmentation pathways of Enalapril.

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Results and discussion

Protonated molecules of impurity 1 (*m/z* 349.1758) gave product ions at *m/z* 303.1703 with a neutral loss of *m/z* 46.0055, which was CH_2O_2. Using formula predict software impurity 1 was $C_{18}H_{24}N_2O_5$.

Similar MSn measurement was observed between impurity 1 and enalapril. This could be explained by loss of two methylene group (perhaps carboxylic acid ethyl ester) from enalapril structure. Formula predict of impurity 2 (*m/z* 381.2395) shows a formula as $C_{20}H_{34}N_2O_5$, contains 6 more H atom compared to enalapril. When *m/z* 381 was selected as the precursor ion to perform the MS^2 product ion scan experiment, a product ion at *m/z* 335.1976、307.2027, 291.2078, 273.1972, 263.2129 and 192.1394, all have corresponding response loss of 6 H in enalapril. Product ion at *m/z* 183.0775, 170.0823 were both observed at impurity 2 and enalapril. *m/z* 183.0775, 170.0823 is formed by the loss of benzene ring form enalapril. So the structure of impurities 2 may be explained by deoxidation of benzene ring from enalapril. Also we may get the formulae for impurity 3 and 4 are $C_{20}H_{26}N_2O_4$ and $C_{19}H_{26}N_2O_4$.

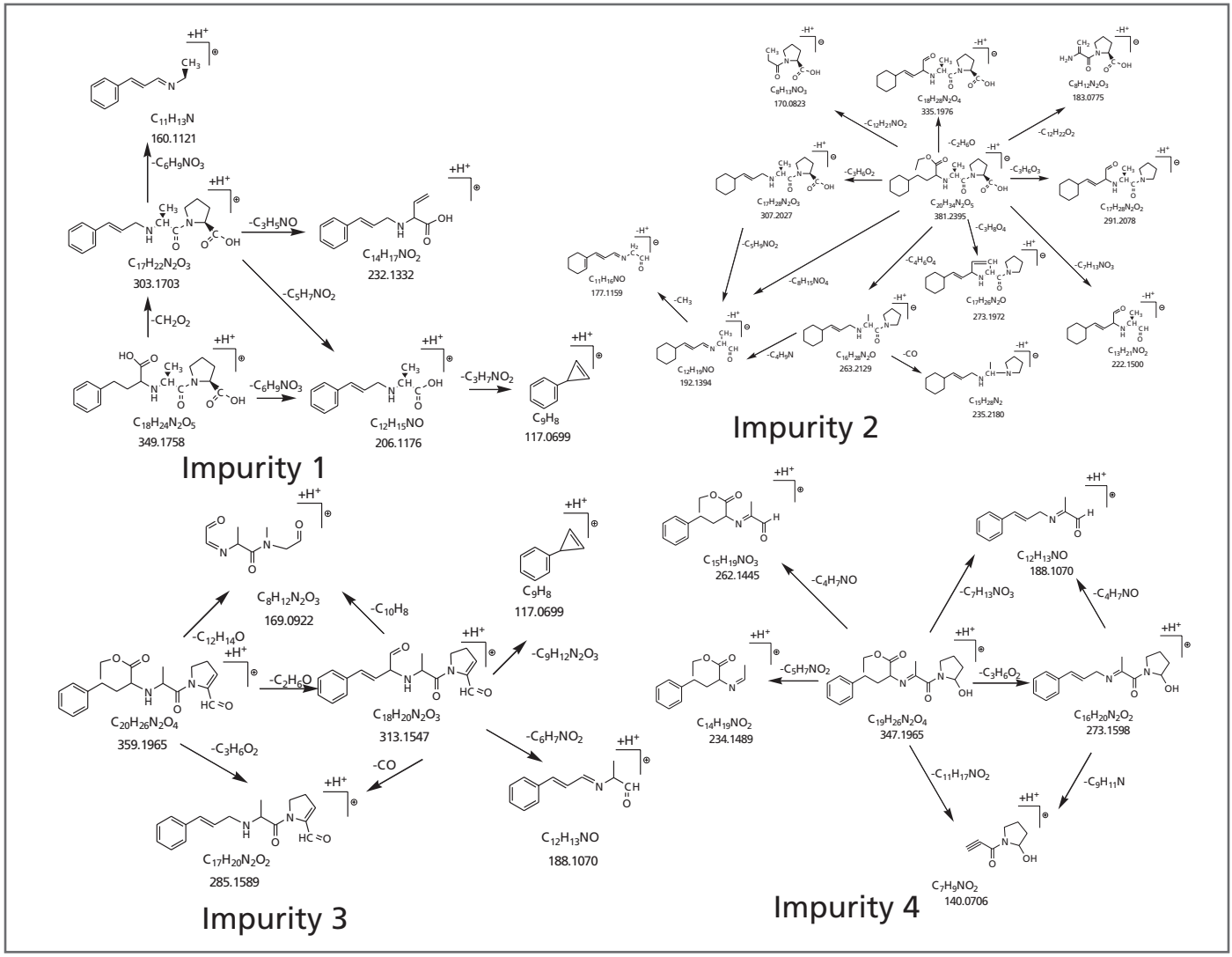

Fig. 5 Probable fragmentation pathways of 4 impurities.

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Conclusions

To our knowledge, this is the first complete characterization of the fragmentation of enalapril impurities, mainly are its by-products, using LCMS-IT-TOF tandem mass spectrometry with accurate mass measurements.

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