

# Adding Mass Selective Detection to Improve Analytical Sensitivity and Maximize Confidence in Results for Impurity Profiling by UHPLC

## Application Note

Pharma



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### Abstract

Analytical methods to check the purity of active pharmaceuticals have been described in pharmacopeias. HPLC-UV detection is the most common analytical liquid chromatography technique for impurity profiling in QC laboratories because of its ease-of-use, robustness, and reliability. However, there are many occasions where ultraviolet (UV) detection has limitations, including cases where analytes or impurities have poor UV absorption, peaks coelute, and unknowns need analyzing, especially when it is necessary to investigate out-of-specification (OOS) results. In addition, degradation products may be significantly less UV active than the Active Pharmaceutical Ingredient (API) due to the loss of the chromophore. In these situations, using a mass detector in series with the UV detector adds significant confidence to the analytical results. This Application Note demonstrates the benefits of mass detection for unambiguous peak identification, determining compound coelution, detection of compounds with weak UV absorbance, and compound library searching for added confirmation with the InfinityLab LC/MSD XT system.



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## Introduction

Enalapril maleate (Figure 1) is used to treat hypertension, symptomatic heart failure, and asymptomatic left ventricular dysfunction (Figure 1). The United States and European Pharmacopeia-recommended analytical instrumentation for routine impurity profiling of Enalapril maleate is liquid chromatography (LC) with ultraviolet (UV) detection. There are nine structurally related known impurities for Enalapril maleate. Impurities are typically reported relative to the percentage of the main Active Pharmaceutical Ingredient (API). Usually, a low wavelength of 215 nm is used for UV detection as there are no significant UV-active chromophores for Enalapril maleate and its structurally similar impurities. Percentage area calculations of known or unknown impurities from UV traces are challenging, as the targets do not have a good UV response. Therefore, determining the purity based only on UV peak area can be ambiguous and questionable.

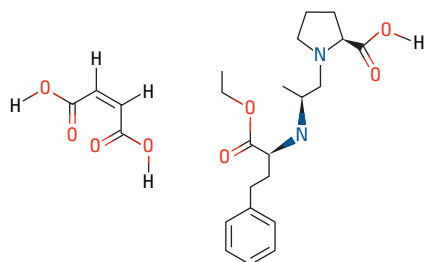


Figure 1. Chemical structure of Enalapril maleate.

Adding single quadrupole (SQ) mass detection helps to address these analytical needs in a typical pharmaceutical lab. The Agilent LC/MSD XT (Mass Selective Detector), with a footprint similar to a typical HPLC system, was specifically designed for easy integration into the chromatographic stack. Mass spectrometry data provided confident detection and identification of the less-UV-active compounds and coeluting impurities of Enalapril maleate. This Application Note discusses the advantages of using mass selective detection for routine QC impurity profiling.

## Experimental Details

### Standards and chemicals

Methanol, formic acid, and Enalapril maleate standards were purchased from Sigma-Aldrich (Santa Clara, CA). Milli-Q water was used in all experiments (Merck, Darmstadt, Germany). All other chemicals used for the study were purchased from Sigma-Aldrich.

## Instrumentation

The LC/MSD system consists of the following modules:

- Agilent 1260 Infinity II Binary Pump (G7112B)
- Agilent 1260 Infinity II Sampler (G7129A)
- Agilent 1260 Infinity II Diode Array Detector (G7117C)
- Agilent InfinityLab LC/MSD XT system with ESI-AJS option (G6135C)

Agilent OpenLAB CDS Software was used for data acquisition, processing, and reporting. OpenLAB CDS provides compliance features that support data integrity with US FDA 21 CFR Part 11, EU Annex 11, and other similar regulations. Agilent 1260 Infinity II LC and Agilent LC/MSD XT are designed to ensure reliability and actionable LC/MS for routine applications.

## Method parameters

The Pharmacopeia-recommended impurity analysis chromatography method uses non-MS-compatible mobile phases. An MS-compatible method using a methanol and water system was developed for this experiment. Tables 1 and 2 list the LC and MS instrument parameters.

## Procedure

One microliter of an Enalapril maleate sample at a concentration of 1,000 µg/mL in methanol was injected into the LC/MS system. To maintain instrument performance with a high concentration of API, the column eluent after UV detection was diverted to waste during the elution of the main API peak using the diverter valve. The LC/MSD XT detected impurities were searched against an in-house library to confirm the identity.

Table 1. Agilent 1260 Infinity II LC Method parameters.

Parameter	Set value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm at 55 °C
Mobile phase A	0.1 % Formic acid in water
Mobile phase B	0.1 % Formic acid in methanol
Gradient	Time    B% 0        20 1        20 12       90
Post run	3 minutes
Flow rate	0.8 mL/min
Injection volume	1 µL, needle wash with methanol, flush port enabled for 7 seconds
Detection UV	a) 215 nm
Acquisition rate	40 Hz
Detection MS	
Mass range	65–600 <i>m/z</i>
Delta EMV	250 V
Scan time	200 ms
Fragmentor voltage	125 V

Table 2. Agilent LC/MSD XT AJS parameters.

Parameter	Set value
Ion source	AJS ESI
Polarity	Positive
Peak filter	0.02 minutes
Dwell time	30 ms
Drying gas temperature	250 °C
Drying gas flow	7 L/min
Sheath gas temperature	300 °C
Sheath gas flow	10 L/min
Nebulizer pressure	30 psig
Capillary voltage positive	1,500 V
Nozzle voltage	0 V
Diverter valve to waste	From 3.39 to 3.99 minutes

## Results and Discussion

### UV Detection is inadequate for impurities with weak chromophores

Figure 2 shows the elution profile of the Enalapril maleate sample, based on the UV data. The data from the UV detector revealed the presence of seven impurity peaks in the sample, and the area percentage purity of the API was 98.7 %. The UV response of the API and its impurities was low, thus limiting the ability to confidently identify the impurities at low concentrations relative to the API.

### MSD Elution profile

The poor UV response of the Enalapril maleate sample can be overcome using mass spectrometry based detection. We used an Agilent LC/MSD XT compact mass selective detector to efficiently track the sample components based on their mass following chromatographic elution. Figure 3 shows the observed elution profile using the LC/MSD XT. The MSD was able to detect two more additional peaks that were not observed by UV detection alone.

### The integrated diverter valve ensures system robustness

MS spectral information facilitates the detection and confirmation of impurities with poor UV activity. However, repeated sample injections of high API concentrations may slowly cause contamination of the MS ionization source. This challenge can be minimized using the built-in diverter valve included in Agilent mass spectrometers. The valve may be set to divert the column eluent flow to waste during the elution of the highly concentrated main peak (1,000 µg/mL). During LC method development, we ensured that the main API peaks were well separated from rest of the impurities. The diverter valve switching is very smooth, and maintains retention time reproducibility, as shown in Figure 4.

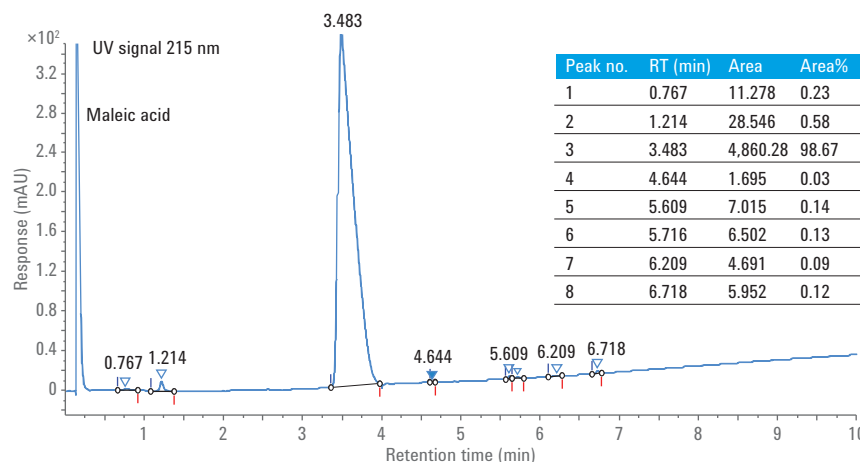


Figure 2. LC/UV elution profile of Enalapril maleate at 215 nm. API at 1,000 µg/mL concentration, showing only ~350 mAU, and confirming the poor UV absorbance.

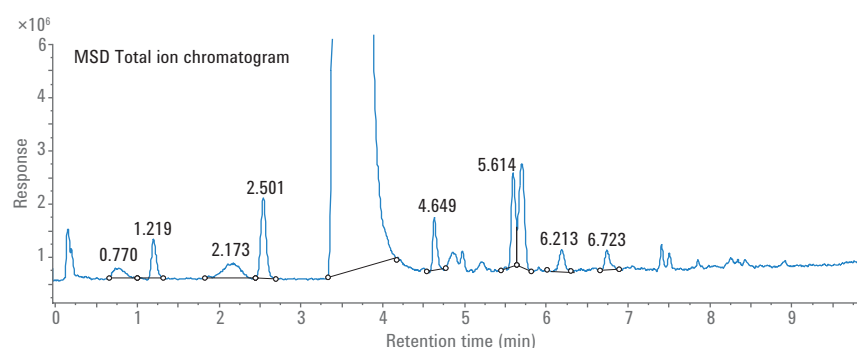


Figure 3. The total ion chromatogram (TIC) from the MS analysis of Enalapril maleate. MS detection revealed additional impurity peaks. For example, the impurity eluting at 2.501 minutes was detected by the MSD, while it was not observed in the UV trace.

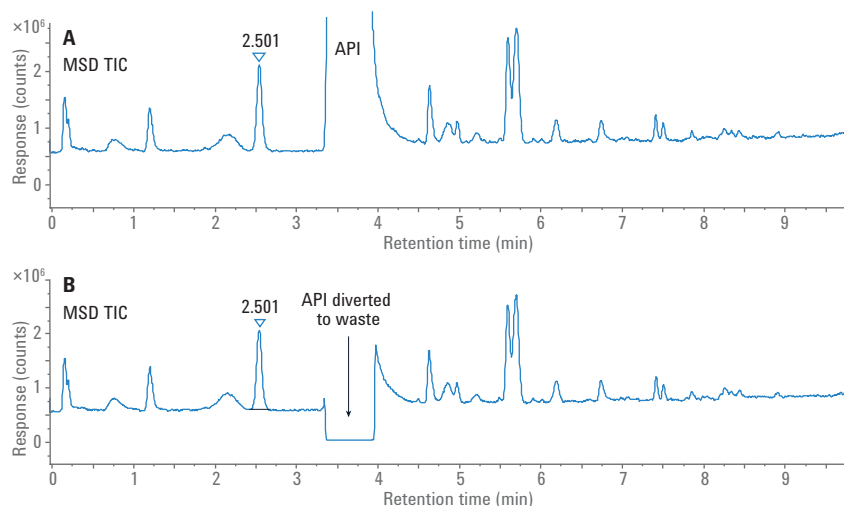


Figure 4. A) Enalapril maleate LC/MSD XT TIC trace. B) Enalapril maleate LC/MSD XT TIC trace with diverter valve enabled to direct high concentration API peak to waste. The superior reproducibility of retention time confirms smooth functioning of the diverter valve.

### LC/MSD XT Detects unexpected non-UV-active impurities

Enalapril maleate and its related impurities were well ionized and detected by the Agilent LC/MSD XT. Two new significant impurities were detected by MS that could not be detected by UV absorbance. For example, one new impurity was found to elute at 2.5 minutes. The  $m/z$  value for this impurity was 280 Da, and Figure 5 shows the extracted ion chromatogram. The mass spectral information of this impurity was searched against a previously created custom library of API-related impurities, and was identified as Impurity B (Figure 6).

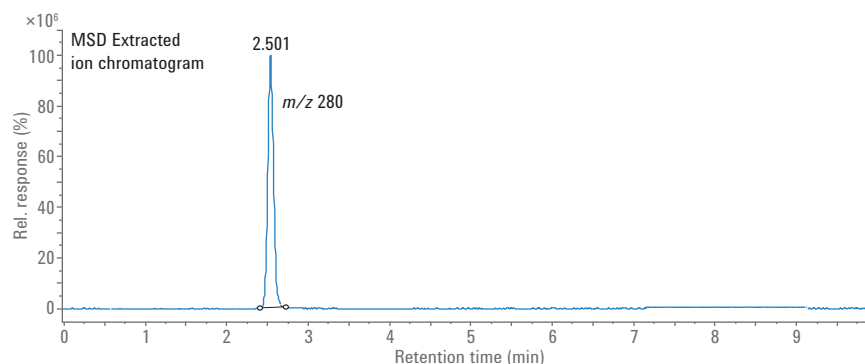


Figure 5. Extracted ion chromatogram (EIC) of an impurity with  $m/z$  280, eluting at 2.5 minutes. This impurity was not found by UV detection.

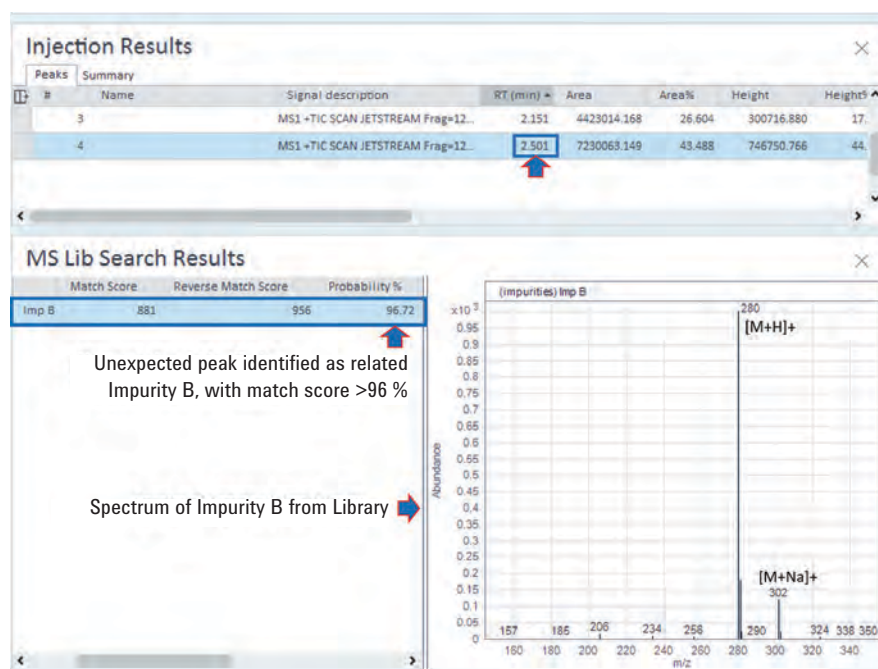


Figure 6. Library search result for impurity with  $m/z$  280. The library search of  $m/z$  280 resulted as Impurity B, with a probability score of >96 %.

## Mass spectrometry detection enables identification of coeluting compounds

Coelution of peaks is a problem when performing impurity profiling, and can lead to an incorrect assessment of API purity. Often, only chromatography peak shapes are taken into consideration, and the UV peak purity function is used to compare the spectra during the LC-UV analysis. This approach may fail if the UV spectra of coeluting peaks are similar. This problem can be eliminated using the Agilent LC/MSD XT in conjunction with UV detection.

For Enalapril maleate API, the UV detection trace showed two relatively well separated peaks at 5.6 and 5.7 minutes (Figure 7A). However, spectral analysis of the LC/MSD XT total ion chromatogram (TIC) trace detected coeluting impurities within the 5.7-minute peak (Figure 7B). The extracted ion chromatogram (EIC) of individual impurities clearly showed coelution of expected impurities (Figure 7C). Figure 8 shows observed MSD spectra of coeluting impurities within the 5.7-minute peak from different time points.

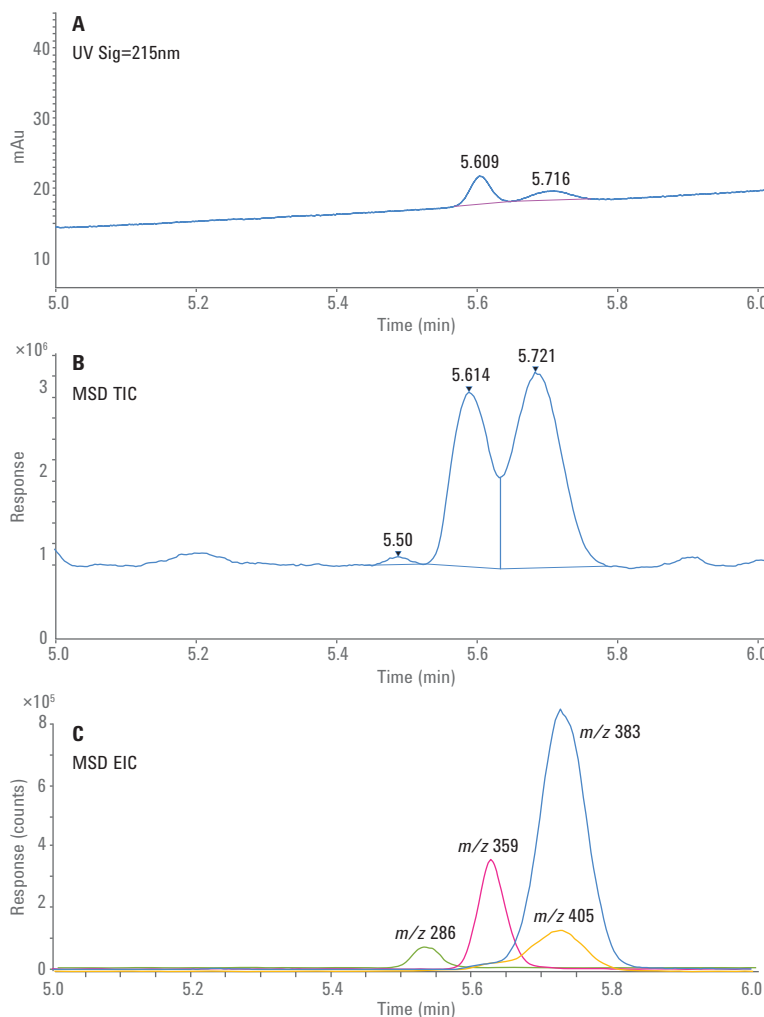


Figure 7. Mass detection facilitates identification of coeluting peaks not observed with UV detection. A) The UV detection trace showing two peaks at 5.6 and 5.7 minutes. B) The LC/MSD XT TIC trace detected an additional low-level impurity at 5.5 minutes. Spectral evaluation of the 5.72-minute peak confirmed the coelution of two peaks with  $m/z$  383 and  $m/z$  405. C) Overlay EIC of all impurities eluting between 5.5 and 5.7 minutes.

## Conclusions

The Agilent LC/MSD XT together with the UV detector helps avoid ambiguity, and gives higher confidence for impurity profiling results. When there are challenging detection issues for impurities, such as peak coelution or poor UV absorbance, mass information from the Agilent LC/MSD XT helps to increase the confidence of impurity profiling results. Compound library creation and search options included in Agilent OpenLAB CDS software assist in identifying compounds in drug discovery and QC environments. The advanced technical controls included in the OpenLAB CDS software provide indispensable features that support data integrity in a typical pharma QA/QC lab.

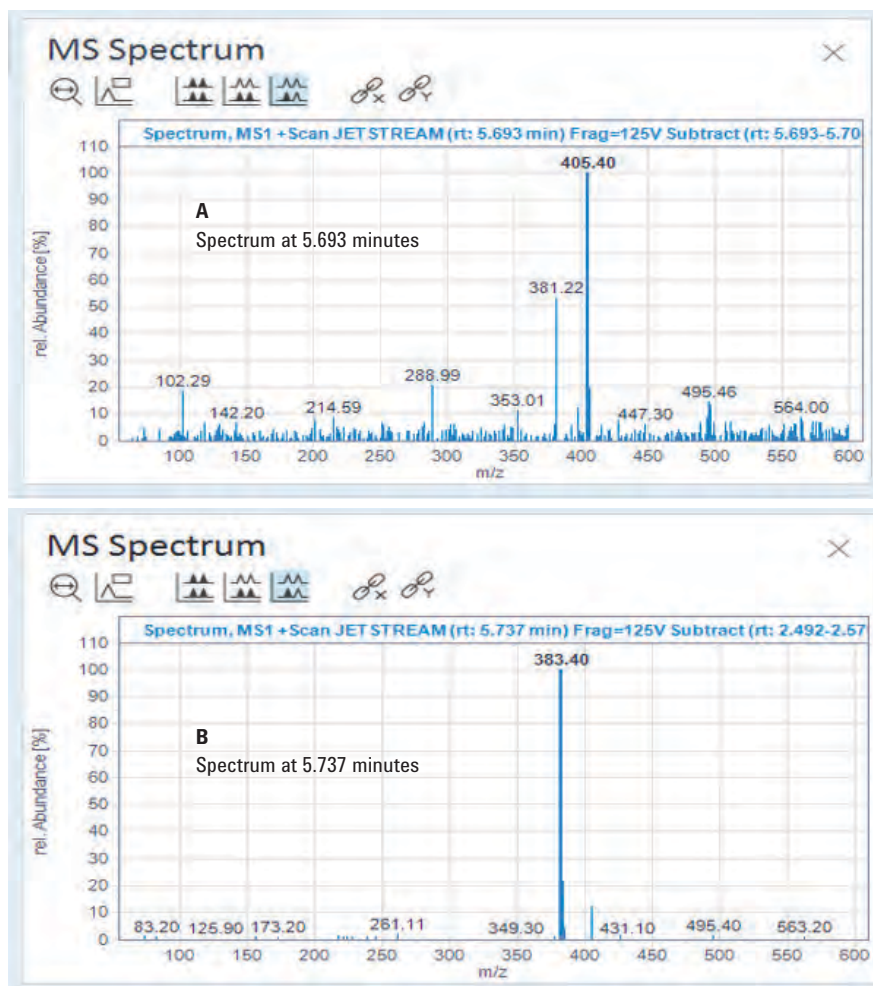


Figure 8. Observed MS spectra of the 5.7-minute peak from the TIC trace. Different spectra from two different time points suggest the possibility of coelution of impurities. The spectrum at 5.693 minutes proposes the presence of an impurity with  $m/z$  405.4 (A), and the MS spectrum at 5.737 minutes proposes the presence of an impurity with  $m/z$  383.4 (B).



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