Heart-cut 2D-LC/MS approach for pharmaceutical impurity identification using an Agilent 6540 Q-TOF LC/MS System

Application Note

Pharmaceuticals

Author

Siji Joseph
Agilent Technologies, Inc.
Bangalore, India

Abstract

This Application Note describes a heart-cut two-dimensional LC/MS method for the identification of a close eluting impurity observed in Duloxetine drug substance using a non-MS compatible reverse phase LC method. The analysis was performed using an Agilent 1260 Infinity LC system coupled with anAgilent 6540 UHD Q-TOF LC/MS system. Agilent ZORBAX Eclipse Plus columns were used for the first and second dimensional separation. In the first LC dimension, a non-MS compatible liquid chromatography (LC) method was performed. Using a column switching valve, the specific unknown impurity was diverted to the second LC column, and was separated using an MS compatible mobile phase from Pump 2. Accurate mass MS and MS/MS data of the impurity were acquired using a 6540 UHD Q-TOF. Data processing using Agilent MassHunter and Agilent Molecular Structure Correlator (MSC) software enabled quick identification of the unknown impurity. Efficient and unambiguous impurity profiling is possible with minimum user intervention when using this method.

Agilent Technologies

Introduction

Detection and identification of impurities in the active pharmaceutical ingredients (API's) are critical in the pharmaceutical industry. Increasing stringency in the regulatory environment demands sensitive and convincing analytical approaches for the determination of impurities in API's. ICH guidelines require that impurities at or above 0.1% in the drug substance are identified[1]. Better understanding of the impurity gives insights to control the chemical reaction process and achieve higher API purity. At times, the impurities may be structurally similar to the API, therefore, sophisticated and selective analytical methods are critical. Conventional analytical approaches for impurity profiling often involve multiple instrument platforms and can be time-consuming and laborious.

Techniques such as LC/MS/MS are widely used for impurity identification in pharmaceutical industries due to its high sensitivity, selectivity, and speed of analysis. Coupling high pressure liquid chromatography (HPLC) system with quadrupole time-of-flight (Q-TOF) enables accurate mass measurement of both precursor and fragment ions. Data processing using advanced algorithms such as molecular feature extraction (MFE) and molecular formula generation (MFG) of the Agilent Mass Hunter Qualitative Analysis software along with MassHunter Molecular Structural Correlator software (MSC) allows impurity identification and structure elucidation. Identification of eight European pharmacopeia specified impurities in atenolol API using this approach is well described in Agilent publication number 5991-1375EN[2].

There are limits to LC/MS with respect to the use of nonvolatile buffers as mobile phases. If an original LC method is developed with nonvolatile buffers, then the chromatographer has to invest additional time to develop an equivalent LC method with an MS compatible mobile phase. Furthermore, it may add additional challenges and uncertainty if an impurity elution order changes with the newly developed MS-compatible LC method. This limitation can be eliminated with a heart-cut approach employing two reversed phase LC conditions. The approach described in this Application Note uses a two dimensional LC technique to transfer an impurity of interest from the first column eluted with non-MS compatible mobile phase to the second column which is under MS compatible LC condition. This approach can be extended to analyses of many pharmaceutical ingredients and related impurities.

Experimental

Instruments

LC/MS consisted of an Agilent 6540 UHD Q-TOF with Jet Stream source, and an Agilent 1260 Infinity Binary LC system. The individual modules and columns used are as follows:

- Agilent 1260 Infinity Series Degasser (G1379B) for the first and second dimensions
- Two Agilent 1260 Infinity Binary Pumps (G1312B) for the first and second dimension
- Agilent 1260 Infinity High-performance Autosampler (G1367D)
- Agilent 1260 Infinity Thermostatted Column Compartment with 2-Position/6-Port column switching valve (G1316B)
- Agilent 1290 Infinity Diode Array detector (G4212A) with Max-Light flow cell (4.0 µL volume, 60-mm path length) (G4212 A)
- Column 1: Agilent ZORBAX Eclipse Plus C-18, 4.6 × 250 mm, 5.0 µm (p/n 959990-902)
- Column 2: Agilent ZORBAX Eclipse Plus C-18, 4.6 × 75 mm, 3.5 µm (p/n 959933-902)

Software

Agilent ChemStation software B.04.03 was used to acquire LC-UV data and to quantify area percentage of impurities, and MassHunter Workstation (version B.04.00) was used for LC mass spectrometry data acquisition. MassHunter Qualitative Analysis software (B.04.00) was used for data processing. MassHunter MSC software (version B.05.00) was used to facilitate structure elucidation of impurities.

Reagents and Materials

LC/MS grade acetonitrile, methanol and formic acid were purchased from Fluka (Germany). Highly purified water from a Milli Q system (Millipore Elix 10 model, USA) was used for mobile phase preparation. Potassium dihydrogen phosphate was purchased from Fluka (Germany). Standards of duloxetine and its impurity were purchased from Varda biotech (India).

Workflow

Figure 1 shows the instrument block diagram used for the experiment. The impurity analysis of Duloxetine API was performed as per the HPLC method described in Reference 3. The overall chromatographic elution profile using Agilent ZORBAX Eclipse Plus C-18, 4.6 × 250 mm, 5.0 µm column was evaluated.

The impurity was eluted at 8.7 minutes using the HPLC method 1 (Table 1). Later, in a separate injection, the unknown impurity was transferred to Column 2 by changing the valve position between 8.2 minutes to 9.0 minutes using a 2-Position/6-Port column switching valve. During this heart-cut time, Column 2 is also connected to Pump 2 which was running at 95% aqueous of MS compatible mobile phase with a T-connector. The total flow through Column 2 during this time was the sum of the flows from Pumps 1 and 2. The flow from Pump 2 contains high percentage of aqueous component that reduces the overall organic content in the mobile phase, and, thus, impurity can be retained in Column 2. After 9 minutes, the column switch valve was changed back to the original position and eluent from Column 1 using Pump 1 was redirected to DAD and continued the UV analysis.

From 9 minutes on, the flow of Column 2 depended entirely on Pump 2. Pump 2 remained at 95% aqueous for 12 minutes. This was found to be sufficient to wash out all buffers from Column 2. Up to this time, Column 2 flow was directed to the waste using Q-TOF inlet rheodyne. From 12 minutes on, the gradient was operated using Pump 2 to elute the trapped impurity from Column 2. The eluent was directed to the Q-TOF mass spectrometer and data were acquired in an auto MS/MS mode.

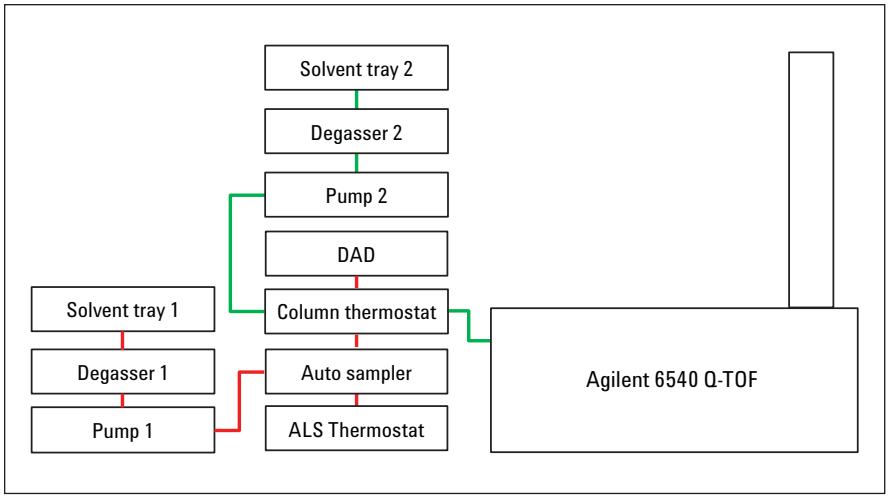


Figure 1
Instrument block diagram used for the experiment. The column compartment includes two columns and connected with a six port switch over valve.

The illustrative representation of the valve positions during the heart-cut method is shown in Figure 2 and the entire workflow is summarized in Figure 3.

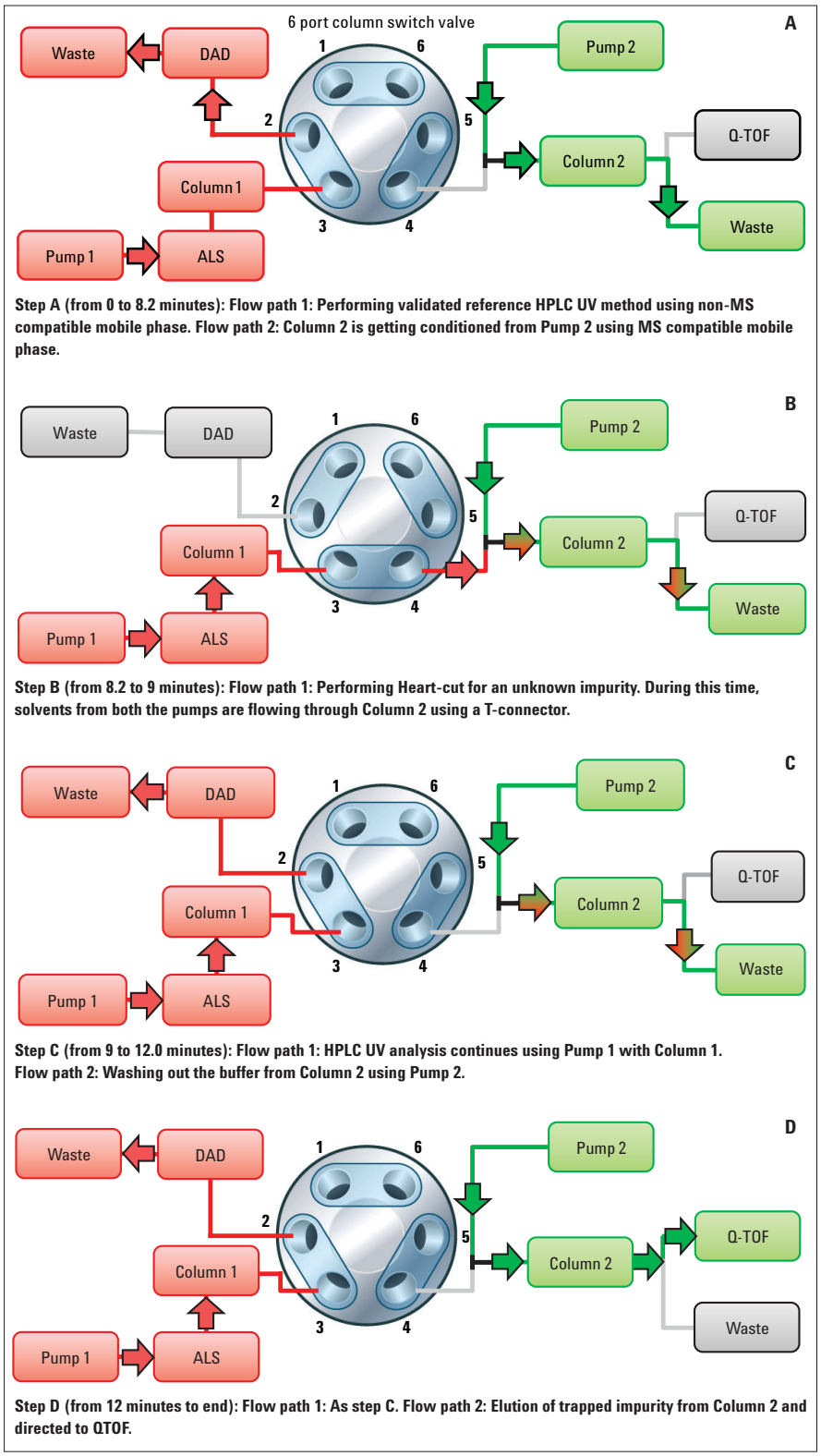


Figure 2
Valve positions before, during, and after the heart-cut process. Red color indicates the flow path (1) with non-MS compatible mobile phase and green color indicates the flow path (2) with MS compatible mobile phase.

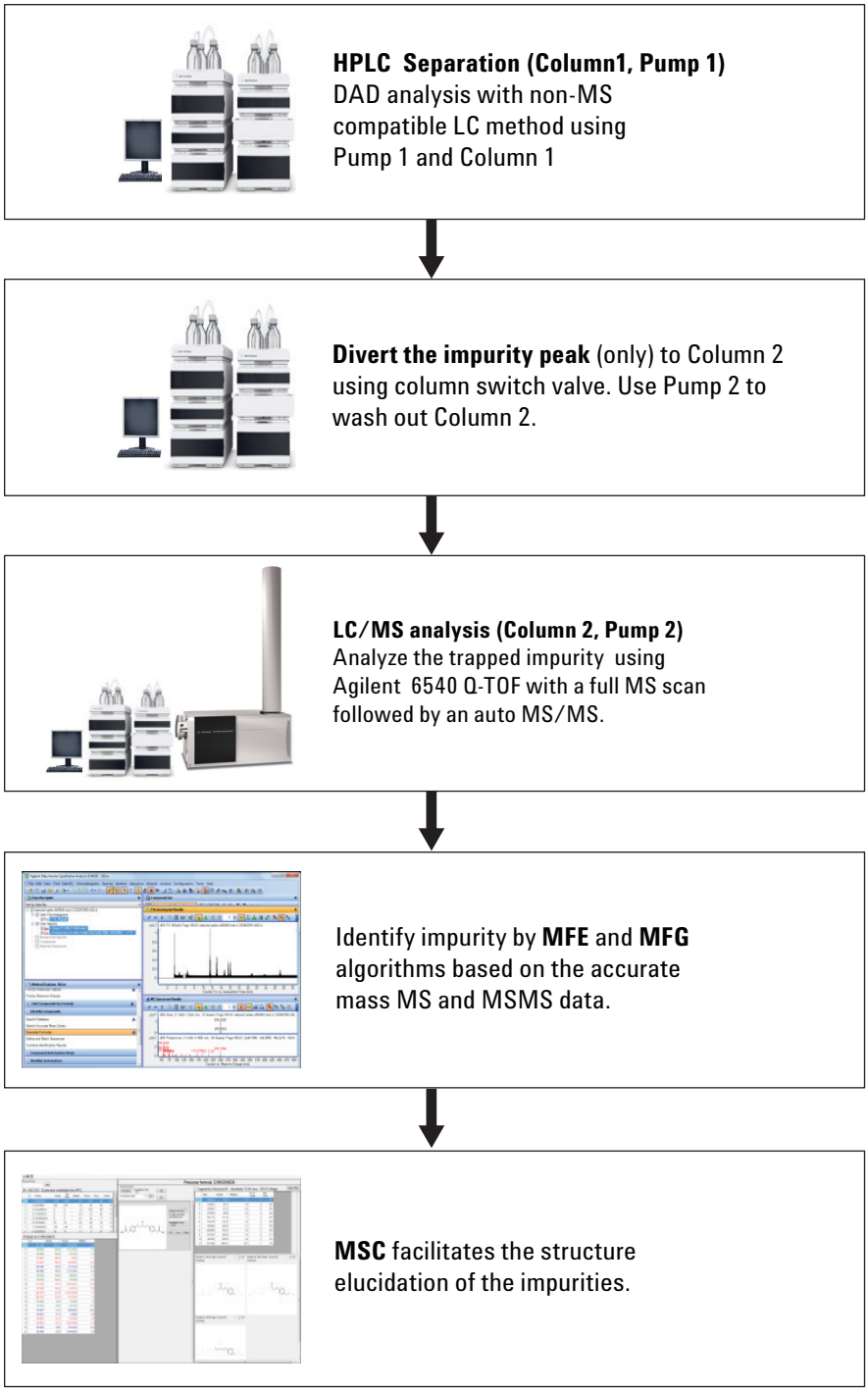


Figure 3
Workflow for impurity identification using a heart-cut approach.

Instrument parameters

Tables 1 and 2 summarize the instrument parameters.

Parameter	HPLC:1 (non-MS compatible) conditions	HPLC: 2 (MS compatible) conditions		
Column	Agilent ZORBAX Eclipse Plus C-18, 4.6 × 250 mm, 5.0 µm	Agilent ZORBAX Eclipse Plus C-18, 4.6 × 75 mm, 3.5 µm		
Flow rate	1.0 mL/min	As gradient		
Mobile phase A	Isocratic: 20 mM Phosphate buffer: acetonitrile: methanol; 55:37:8	0.1% formic acid in water		
Mobile phase B	Not applicable	0.1% formic acid in acetonitrile		
Detection	DAD:229 nm	Q-TOF		
Injection vol	5 µL	Not applicable		
Needle wash	Activated for 8 seconds using methanol	Not applicable		
Pump mode	Pump:1, Isocratic for 30 minutes	Pump:2, Gradient		
		Time	%B	Flow
		0	5	0.5
		9	5	0.5
		10	5	0.7
		17	60	0.7
		17.1	5	0.5
		25	5	0.5

Table 1
Agilent 1200 Series LC instrument parameters.

Q-TOF MS and auto MS/MS conditions	
MS instrument	Agilent 6540 Q-TOF
Ion source	AJS ESI
Acquisition mode	2 GHz, Ext dynamic range
Ion polarity	Positive mode
Drying gas temperature	325 °C
Drying gas	10 L/min
Nebulizer	45 psig
Sheath gas temperature	375 °C
Sheath gas flow	12 L/min
VCap	4,000 V
Nozzle voltage	500 V
Fragmentor	90 V
Acquisition	MS followed by auto MS/MS

Table 2
Agilent 6540 Q-TOF parameters.

Results and Discussion

Figure 4 shows the elution profile of duloxetine API with LC-UV analysis. The area percentage of the unknown impurity (retention time: 8.7 minutes) was approximately 0.1%. Figure 5 shows the total ion chromatogram (TIC) obtained from the Q-TOF during the heart-cut analysis.

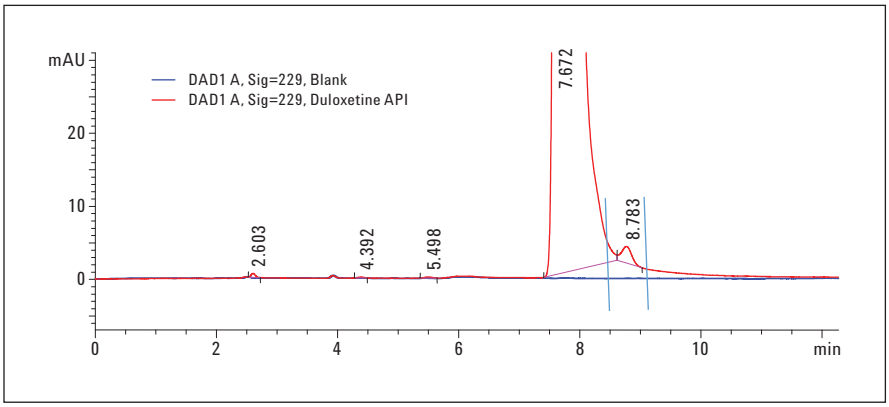


Figure 4
Elution profile of duloxetine API using LC-UV analysis. (The heart cut region of the baseline is marked).

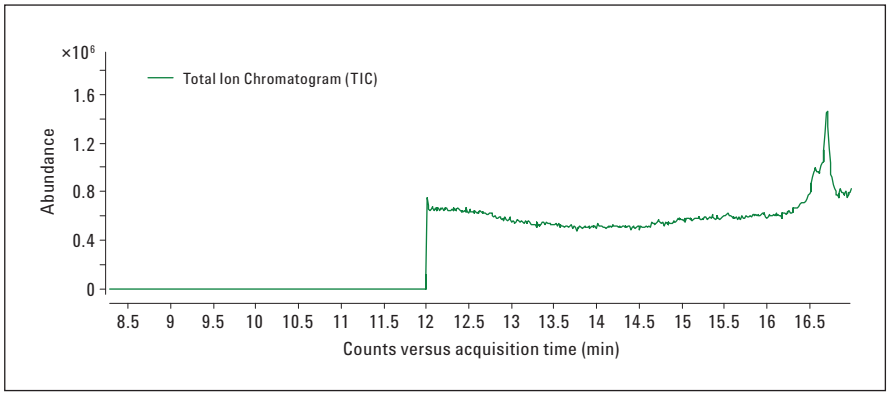


Figure 5
The total ion chromatogram (TIC) obtained from QTOF analysis.

Data analysis was performed using the MFE and MFG algorithms of the MassHunter Qualitative Analysis software. Using an MFE algorithm, high resolution accurate mass spectral information of all the sample components was extracted. From the acquired data, the MFE algorithm listed two entities, the *m/z* values of the entities were 298.1257 and 312.1416 (Figure 6). The *m/z* 298.1257 corresponds to the duloxetine API and the second entity corresponded to the unknown impurity. Using the MFG algorithm, a relevant list of candidate molecular formulas for each entity was tabulated and it ranked them according to the relative probabilities (Figure 7).

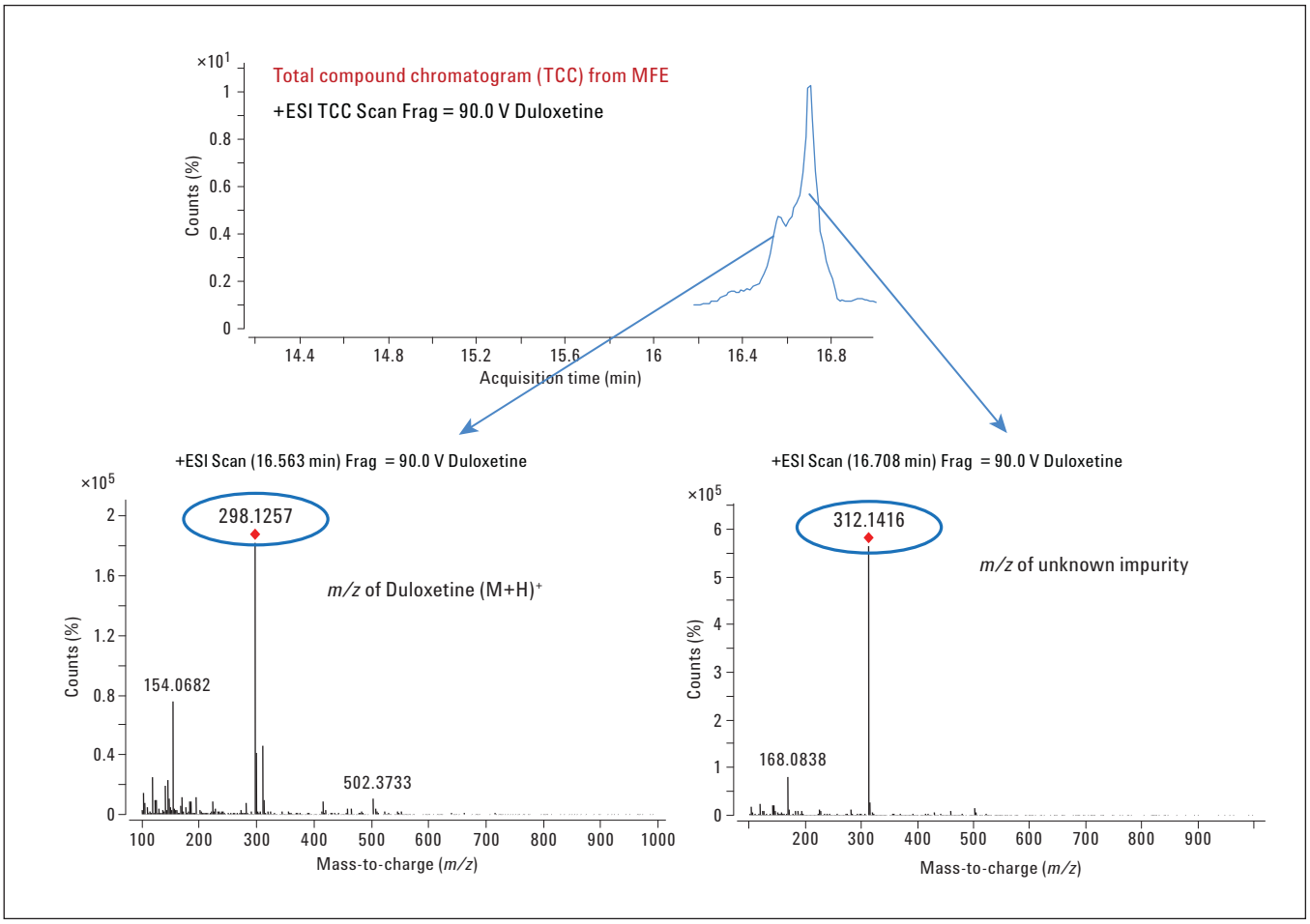


Figure 6
Mass spectra of the API (298.1257) and impurity (312.1416).

MFE

Show/Hide	Cpd	Label	Formula	Score	Mass	Avg Mass	Mass (MFG)	Diff (MFG, ppm)	Diff (MFG, mDa)	Base Peak	RT
TRUE	1	Cpd 1: 16.555			297.1184	297.2948				298.1257	16.557
TRUE	2	Cpd 2: 16.701			311.1343	311.3752				312.1416	16.701

MFG

Show/Hide	Cpd	Label	Formula	Score	Mass	Avg Mass	Mass (MFG)	Diff (MFG, ppm)	Diff (MFG, mDa)	Base Peak	RT
TRUE	1	Cpd 1: C18 H19 NOS	C18 H19 NOS	98.18	297.1184	297.3848	297.1187	1.07	0.32	298.1257	16.557
TRUE	2	Cpd 2: C19 H21 NOS	C19 H21 NOS	98.54	311.1343	311.3995	311.1344	0.29	0.09	312.1416	16.701

Best	Formula	Score	Mass	Mass (MFG)	Score (MFG)	Diff (abs. ppm)	Diff (mDa)	ID Source	Diff (ppm)	RT
TRUE	C19 H21 N O S	98.54	311.1343	311.1344	98.54	0.29	0.09	MFG	0.29	16.701

Species	Ion Formula	m/z	Height	Score (MFG)	Score (MFG, MS)	Score (MFG, mass)	Score (MFG, abund)	Score (MFG, iso. spacing)
(M+H)+	C19 H22 NOS	312.1417	562137.9	98.54	98.54	99.94	95.18	99.79

Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
545200.1	76.8	100	312.1417	0.1	562137.9	100	79.2	312.1416	0.29
119921.6	16.9	22	313.1448	0.1	115726.6	20.6	16.3	313.1447	0.3
38075.8	5.4	7	314.1412	–0.5	27089.3	4.8	3.8	314.1417	–1.62
6252.5	0.9	1.1	315.1424	0.6	4925.6	0.9	0.7	315.1419	1.82
693.8	0.1	0.1	316.1441	0.9	264.4	0	0	316.1432	2.85

Figure 7
MFE/MFG results for Duloxetine and impurity.

The accurate mass information of the precursor ion and fragment ions of API and unknown impurity from the MFE/MFG algorithm were uploaded to the MSC software and searched against the ChemSpider database to retrieve all possible structures. The details of the MSC software and multiple approaches to identify impurities can be found in Agilent publication 5991-1375EN[2]. Multiple candidate structures were retrieved for both entities with their calculated correlation scores. One of the listed entities was confirmed to be Duloxetine API. Figure 8 shows a screen shot of results from MSC software for unknown impurity. The overall MFG score for the selected precursor ion, the rank of the MSC proposed structure, and the structure correlation score for impurity are highlighted in red circles. The structure with the highest correlation score (93.26%) corresponds to methyl derivative of Duloxetine API.

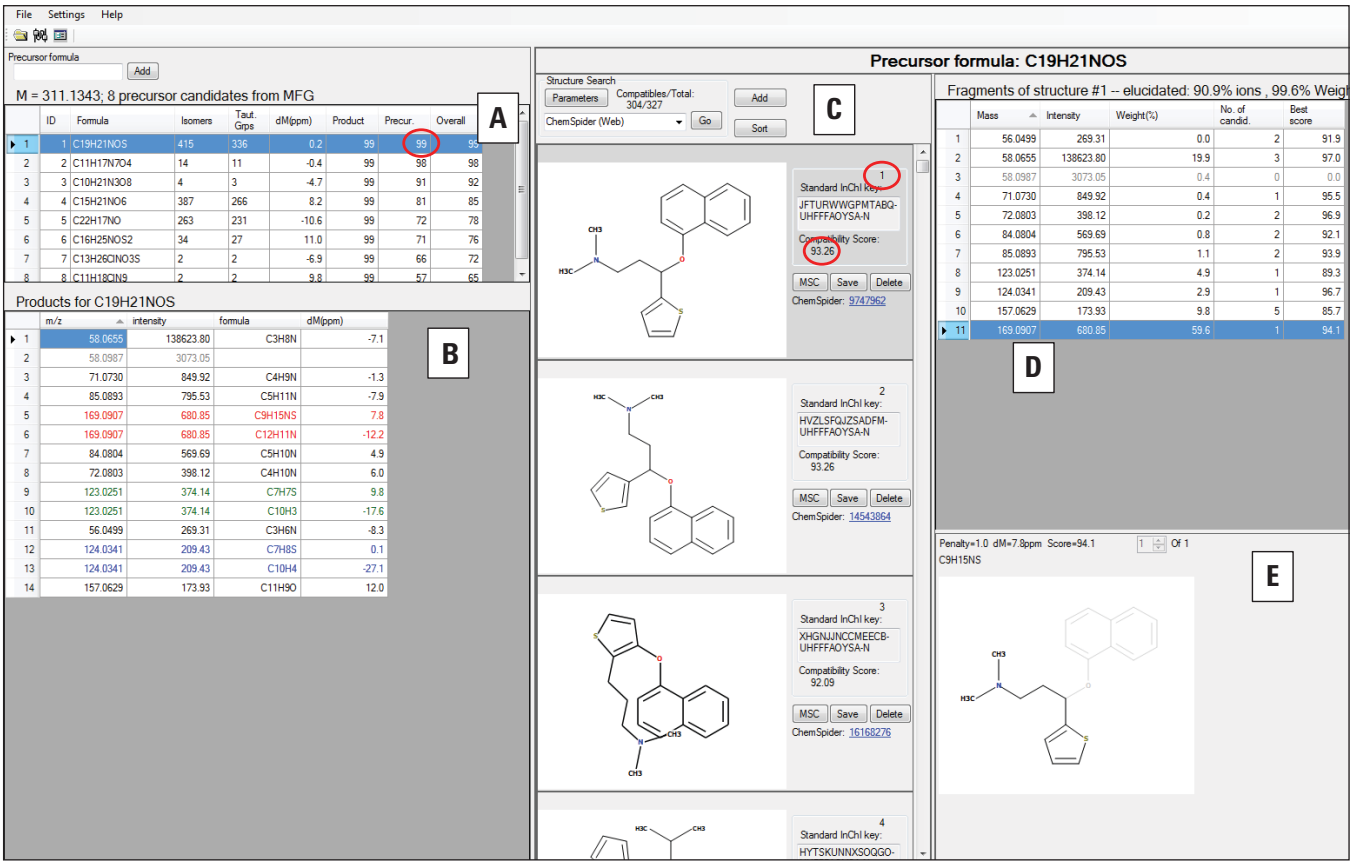
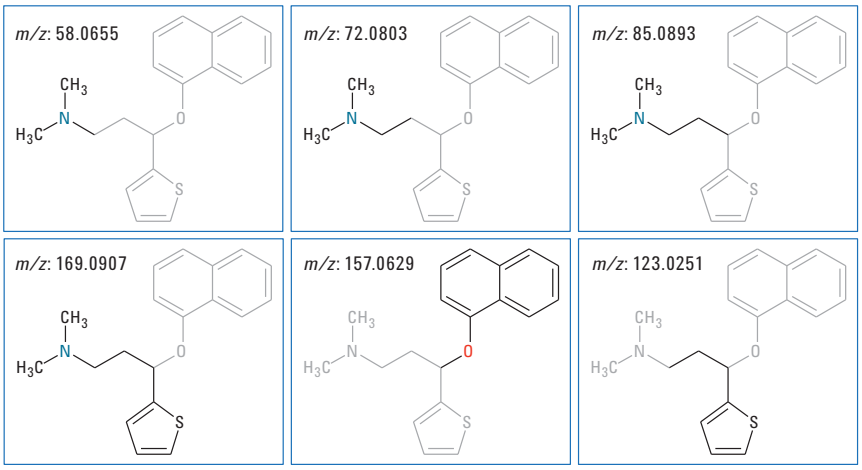


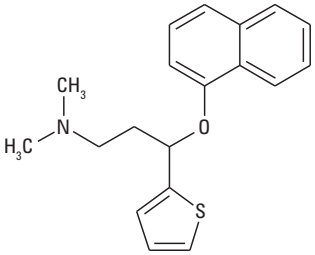
Figure 8
Screen shot of MSC results for Identification of unknown impurity.
A: List of possible molecular formulas for the precursor ion of impurity
B: MFG results for the product ions of the impurity
C: Candidate structures for the unknown impurity
D: Fragment ions for the candidate structure selected in panel C
E: Substructure assignments for a selected fragment ion in panel D

Figure 9 summarizes the structure elucidation of the unknown impurity from the MSC. This impurity was further confirmed by spiking the impurity standard.

Main fragments	Mass	dM (ppm)	Best score
1	58.0655	–7.1	97.0
2	72.0803	6.0	96.9
3	85.0893	–7.9	93.9
4	123.0251	9.8	96.7
5	157.0629	12.0	85.7
6	169.0907	7.8	94.1



Final structure of the impurity



Molecular formula	$C_{19}H_{21}NOS$
Calculated mass	311.1344
Measured mass	311.1343
dM (ppm)	0.3

Figure 9
Structure elucidation of duloxetine impurity (m/z: 311.1344) demonstrating wide usability of MSC software to assign structures for each fragment ions.

Figure 10 shows the increased area percentage from the API sample spiked with the impurity standard.

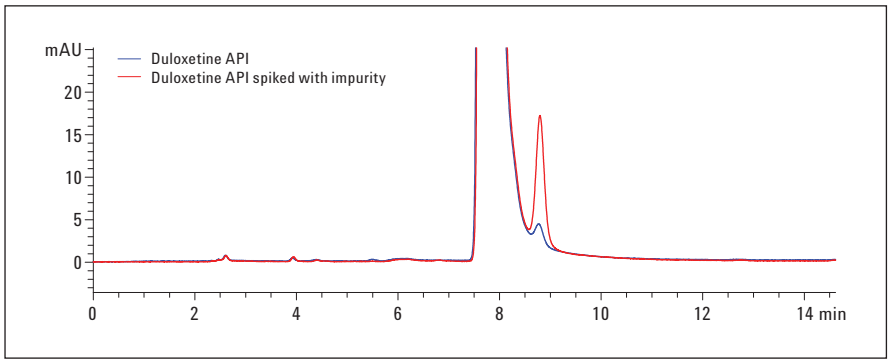


Figure 10
Area percentage increment of impurity peak using DAD detection when methyl derivative of Duloxetine was spiked into the API.

Conclusion

This Application Note demonstrates a workflow for the selective identification of an impurity. This workflow used a heart cut two-dimensional LC/MS method to identify an impurity in Duloxetine API. This method eliminated the need to develop an MS compatible LC method, for a non-MS compatible pharmacopeia method to perform accurate mass analysis of the impurity of the interest. It enabled specific selection of a close eluting, low abundant unknown impurity in the API for MS analysis. An Agilent 6540 Q-TOF along with advanced software tools (MSC, MFE, and MFG) software enabled quick and reliable identification of unknown compounds.

References

1.
International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Steering Committee, March 30, **1995**.

2.
Agilent publication 5991-1375EN "Pharmaceutical Impurity Identification and Profiling Using Agilent Q-TOF LC/MS Combined with Advanced MassHunter Data Processing Software" dated November 21, **2012**.

3.
Validated reverse phase method for duloxetine from *International Journal of Comprehensive Pharmacy* (IJCP), Vol 01, and Issue 03. Viriyala R. K. *et al*. Pharmacie Globale (IJCP), 3 (03) **2010**.

www.agilent.com/chem

© Agilent Technologies, Inc., 2013

Published in the USA, March 1, 2013
5991-1873EN

Agilent Technologies