Agilent LC/MSD XT Single Quadrupole Open Access System for Synthetic Chemists

Automated, Confident, Fast LC/MS Analysis is Only a Few Clicks Away

Application Note

Pharma and Biopharma

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Agilent LC/MSD XT with the Agilent 1260 Infinity II LC System.

Abstract

Compound identification, purity analysis, and impurity detection are examples of important analytical measurements that occur during the pharmaceutical drug development processes. Cost-efficient technology advancements, which provide faster and more accurate measurements are desirable to enhance productivity within the pharmaceutical industry. Using the Agilent Liquid Chromatograph/Mass Selective Detector XT (LC/MSD XT) in open access mode provides tremendous user flexibility to automate and optimize tasks such as compound confirmation and purity/impurity analysis. The Agilent 1260 Infinity II Liquid Chromatograph (LC) and the LC/MSD XT, based on single quadrupole technology, are both highly robust and suitable for the open access laboratory. Agilent MassHunter Walkup software has been designed to simplify the sample submission process for various types of complex LC/MS analyses in a typical multiuser environment. Using the Agilent LC/MS open access system with MassHunter Walkup software, chemists can analyze their own samples with minimal training. The software automatically simplifies and executes data analysis and reporting.

Agilent Technologies

Introduction

LC/UV analysis is a widely accepted analytical technique for purity analysis in pharmaceutical manufacturing and contract research organizations. The coupling of LC with MSD adds higher confidence in compound identification and purity analysis for quantitative and qualitative characterization. Best-in-class features of the LC/MSD hardware and software are designed to support walk-up LC/MS methods for analyzing small molecules as well as biomolecules in a multiuser environment. The user does not need to be familiar with Agilent ChemStation or Agilent MassHunter software to operate the system, instead only a few simple sample submission steps need to be followed.

An administrator can set up the LC/MSD system as ready-to-use for various advanced tasks such as compound confirmation, purity checking, and impurity identification even by an untrained user. A simplified intuitive software interface, combined with robust hardware, allows high quality and reliable LC/MS analysis for the synthetic chemistry lab.

Open Access Instrumentation

Agilent Walkup hardware

The Agilent LC/MSD Walkup system consists of the following modules:

- Agilent 1260 Infinity II binary pump (G7112B)
- Agilent 1260 Infinity II sampler (G7129A) with external tray (p/n G1313-60004) and waste tube (p/n G1313-27302)
- Agilent 1260 Infinity II diode array detector HS (G7117C) with an Agilent Max-Light cartridge cell
- Agilent LC/MSD XT with multimode source enabled using fast 30 ms polarity switching for positive and negative mode acquisitions

The multimode source combines the functionality of both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) into a single source. This source can be operated as dedicated ESI, dedicated APCI, or the preferred mixed ESI/APCI mode. The combination of the multimode source with fast polarity switching along with LC/MS walkup software provides universal detection across a wide range of molecular characteristics with a simplified user experience.

Agilent walkup software

Agilent MassHunter Walkup can be integrated with Agilent OpenLAB, and an administrator can manage many systems remotely. The software packages used for the MSD walkup setup were:

- Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, Rev. C.01.07
- Agilent MassHunter Walkup Software for LC/MS and LC Systems, V: C.02.01.
- Analytical Studio Reviewer (ASR), V: G3772AA, B.02.01.

Agilent MassHunter Walkup administrative software helps to manage users, methods, instrument configurations, and report creation. A detailed description of the MassHunter Walkup administrative software is further described in Agilent publication 5991-6534EN.

MassHunter Walkup software is simple and effortless. The sequence creation, method selection, and sample loading steps are intuitive, and users do not have to be experts in LC/MS hardware or software. Details of user sample loading steps are described in Application Note 5991-6535EN.

Walkup software suggests the vial positions in the external tray (up to 17 vials), and the user has the flexibility to prioritize samples. Automatic discard of injected vials (with optional delay) can also be configured using the walkup administrative interface.

Agilent walkup software can be configured for automated data processing and emailing of the reports. Reports generated by Agilent OpenLAB CDS ChemStation Edition or Agilent Analytical Studio Reviewer (ASR) can create individual or batch reports, and are highly customizable to show the desired information.

Experimental Details

Chemicals and reagents

Methanol was LC/MS grade, and LC/MS eluent additive grade ammonium formate and ammonium fluoride were used (Sigma-Aldrich, USA). Milli-Q water was used in all experiments (Merck, Darmstadt, Germany). All other chemicals and standards were bought from Sigma-Aldrich (USA). A library of 20 compounds was bought from Sigma-Aldrich (USA), and was used for the demonstration of walkup experimentation.

Method parameters

The 18-compound library used for this work included analytes with a wide polarity range. Three walkup methods were developed for different polarity ranges. The user selects the method from the list according to the polarity of the expected analyte. Table 1 describes three gradient programs: polar, nonpolar, and highly nonpolar. The Agilent LC/MSD XT can acquire mass-to-charge data up to 3,000 Da. The mass range of 100–1,000 Da was selected for the analysis of small molecule library samples. Tables 1 and 2 list the LC and MS instrument parameters.

Results and Discussion

Agilent LC/MSD XT Open Access system for rapid compound confirmation

The Agilent LC/MSD XT open access system is equipped with a multimode source. The multimode source is a highly versatile ion source that allows both ESI and APCI. The benefit of the multimode source for rapid compound confirmation in open access environments is demonstrated by screening a library of 18 small molecule samples with a wide range of physiochemical properties. Use of the multimode source with positive and negative polarity switching expands the probability of ionization for a large variety of different compound classes.

Table 1. Agilent 1260 Infinity II LC method parameters.

Parameter	Value			
Column	Agilent InfinityLab Poroshell 120, EC-C18, 2.1 × 50 mm, 2.7 µm at 45 °C			
Mobile phase A	5 mM Ammonium formate and 1 mM ammonium fluoride in water			
Mobile phase B	5 mM Ammonium formate and 1 mM ammonium fluoride in methanol			
Gradient	Time	%B		
		Gradient 1[a]	Gradient 2[b]	Gradient 3[c]
	0	15	50	70
	0.7	60	85	95
	2	60	85	95
Post run	2 minutes			
Flow rate	0.6 mL/min			
Injection volume	1 µL, needle wash with acetonitrile, flush port enabled for 7 seconds			
Detection UV	254 nm using Agilent 10 mm Max-Light cartridge flow cell			
Acquisition rate	40 Hz			

[a] For polar compounds
[b] For nonpolar compounds
[c] For highly nonpolar compounds

Table 2. Agilent 6135B MSD multimode parameters.

Parameter	Value
Ion source	Multimode
Polarity	Positive and negative
Drying gas temperature	250 °C
Drying gas flow	6 L/min
Nebulizer pressure	30 psig
Vaporizer temperature	250 °C
Capillary voltage positive	2,000 V
Nozzle voltage positive/negative	2,000/2,000 V
Corona current positive/negative	1/1 µA
Charging voltage positive/negative	2,000/2,000 µA
Peak width	0.02 minutes
Ionization switch delay	50 ms
Polarity switch delay	30 ms
MS detection signal	1. ESI positive 2. APCI positive 3. APCI negative 4. ESI negative
Scan range (*m/z*)	100–1,000 m
Fragmentor voltage	Positive mode: 160 V Negative mode: 130 V
Gain	1
Threshold	50

A short 2-minute gradient method was used for compound confirmation in an open access environment. Each sample was injected onto the LC/UV/MSD system. An LC gradient suitable to the polarity of each analyte was chosen. UV detection was based on the general detection of compounds by absorbance. Adding MSD as an extra detector allows the user to confirm the target compound based on molecular mass. Table 3 gives the list of compounds used for the study.

The tabulated results show the benefit of adding MSD using a multimode source with positive and negative polarity. For example, sodium hexane sulfonate did not absorb UV, but was detected by the MSD in negative mode. For many other analytes, APCI positive mode was found to be better than ESI positive mode. As another example, pravastatin showed a higher response in negative mode than in positive mode.

MSD detection adds confidence to compound confirmation

Molecular mass information from MSD adds confidence to purity results from UV detection. Sulfamethizole, (molecular weight 270) was separated using gradient 1 conditions for polar compounds, and UV and MSD data were collected. The elution time was 0.4 minutes, and compound purity information from UV detection was found to be >99 %. The observed *m/z* value using the multimode source in positive and negative mode was 271 *m/z* and 269 *m/z* respectively. Molecular weight information from the MSD signal helped confirm the target compound.

Table 3. List of compounds used.

Compound	Name	Molecular weight
1	Pemirolast	228
2	Sulfamethizole	270
3	Sulfamethazine	278
4	Sodium hexanesulfonate	188
5	Amitriptyline	277
6	BMS-193885	590
7	BMS-493	255
8	Buspirone	385
9	Butorphan	311
10	Pravastatin	446
11	Sulfadimethoxine	310
12	Fosinopril	585
13	Nefazodone	470
14	Paclitaxel	876
15	Clopidogrel	321
16	Trazodone	371
17	Zofenopril	429
18	Ketoconazole	530

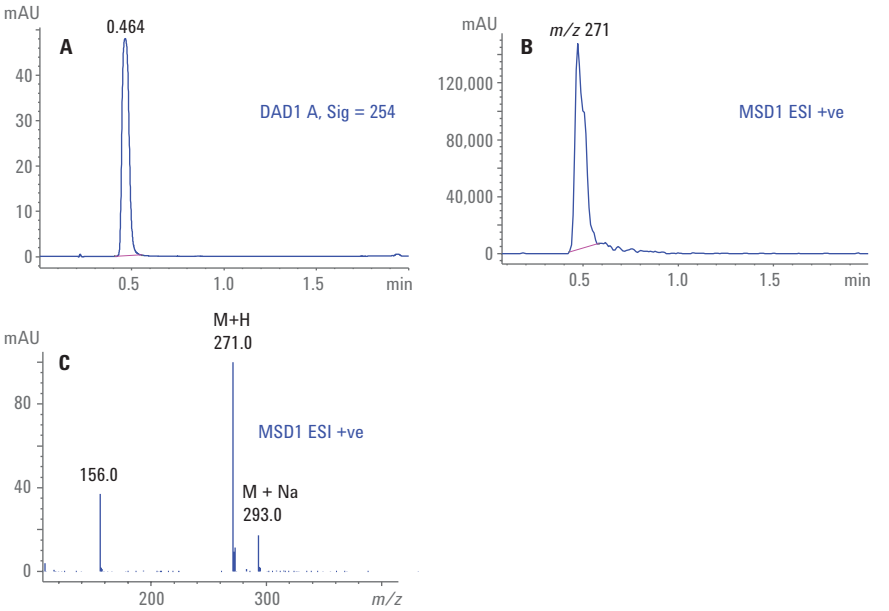

Figure 1. LC/MS results of sulfamethizole (molecular weight 270). From the UV signal (A), the compound is >99 % pure, and mass information from the MSD signal confirmed the identity of the target. B) ESI positive trace. C) MS spectrum of sulfamethizole from positive ionization.

Open Access with MSD offers confident purity assessment

When multiple compounds are present, compound identification and purity assessment based on only UV signals are challenging. Molecular mass information from the MSD helps to locate the target peak and assess the compound purity. Figure 2 explains this scenario using fosinopril analysis as an example. The fosinopril (molecular weight 585), was analyzed using gradient 3 method parameters, for highly nonpolar compounds, and the UV trace showed four significant peaks (Figure 2A). Confident assessment of percentage purity of the sample is challenging without identifying the correct target peak. From the MSD data, it was confirmed that the peak of interest was the one eluting at 1.4 minutes. The accuracy of *m/z* values observed from ESI positive/negative and APIC positive/negative traces offered higher confidence to the UV purity results.

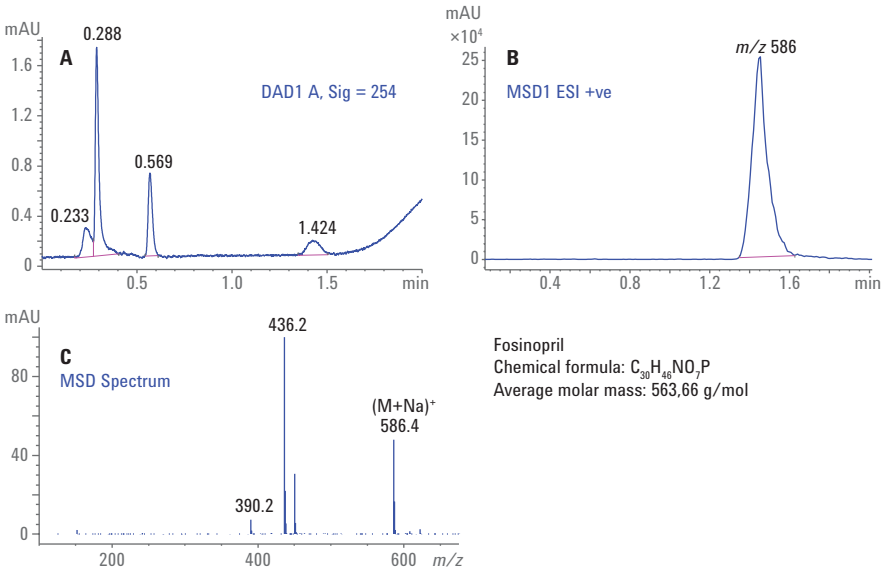

Figure 2. LC/MS results from the purity analysis of fosinopril with molecular weight 585. A) UV detection profile of fosinopril with four significant chromatographic peaks. Mass information from MSD signals identified peak at 1.4 minutes as the target (purity ~15 %). B) ESI positive trace. C) MS spectrum of fosinopril (*m/z* 586) from positive ionization.

MSD with multimode source with polarity switching enhances the ionization of a wide range of different compound classes

Polarity switching allows MS data to be acquired in both positive and negative ionization polarity simultaneously during a single chromatographic run. Agilent LC/MSD can perform fast polarity switching, and collect more scans from a particular chromatographic peak without compromising data quality. This feature is a tremendous timesaver because it helps acquire MS data in both polarities in a single chromatographic run, and helps identify unknown analytes regardless of their preferred polarity. Two examples are discussed to elaborate the benefit of polarity switching:

- Positive polarity is the favored mode to identify nefazodone (Figure 3)
- Negative polarity is the favored ionization mode for sodium hexane sulfonate (Figure 4)

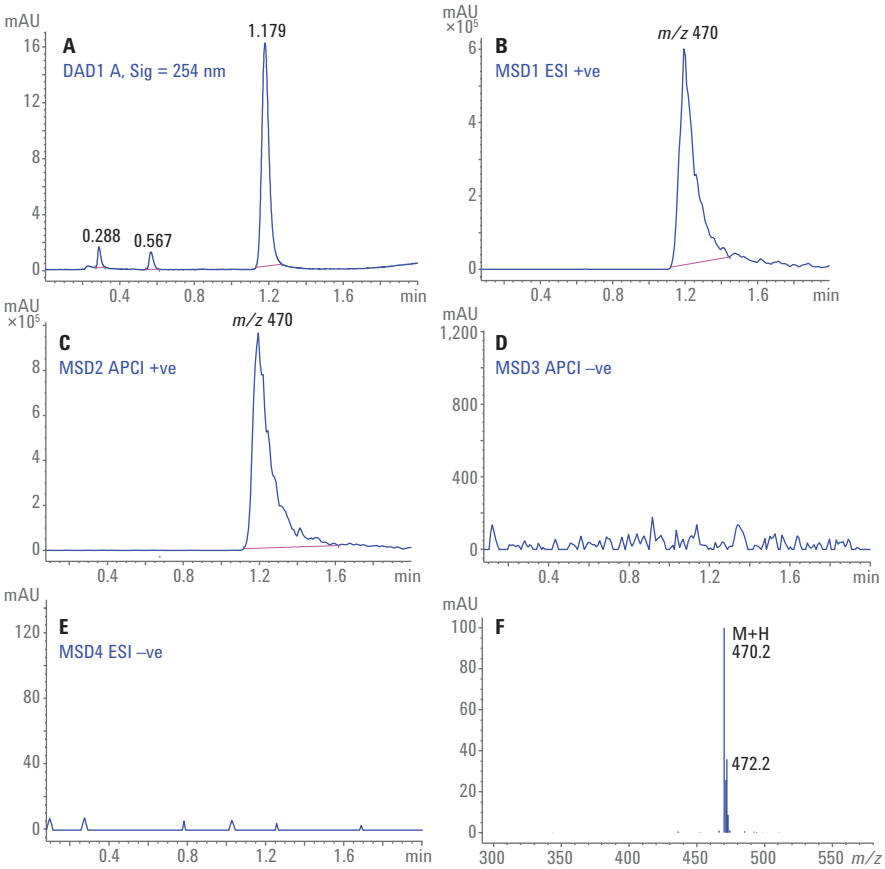

Figure 3. Nefazodone, *m/z* 470, showed good ionization in positive polarity and no ionization observed in negative polarity. A) UV signal of nefazodone. B) ESI positive trace. C) APCI positive trace. D) APCI negative trace and E) ESI negative trace. F) MSD spectrum of nefazodone.

Automated data processing using ASR

The intuitive ASR software allows automated data analysis and quick review. ASR reports are highly customizable to show only the desired information. ASR reports can be generated for a single sample or for a batch of multiple samples. ASR results can be e-mailed to the user as specified in the preconfigured options set by the system administrator. Figure 5 shows an e-mail report that contains the result of fosinopril in a PDF format. Using ASR, data processing and visualization are greatly facilitated for quick review.

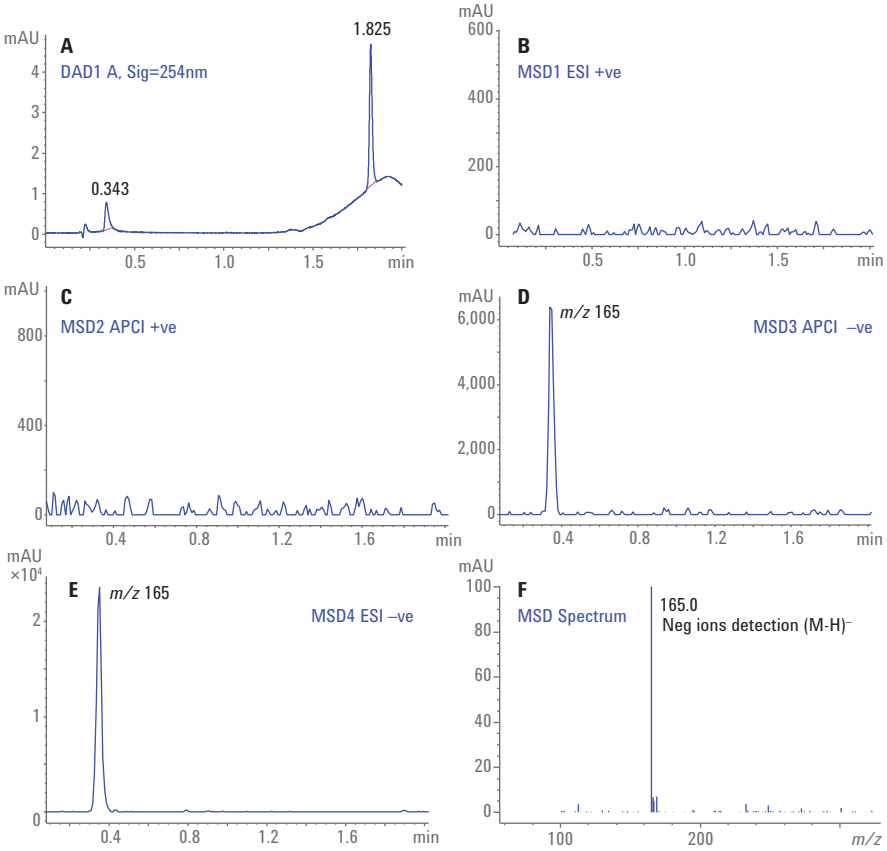

Figure 4. Sodium hexane sulfonate, *m/z* 165, was ionized in negative polarity and no ionization observed in positive polarity. A) UV signal of sodium hexane sulfonate. MSD data confirmed the smaller peak at 0.343 minutes as the target compound. Sodium hexane sulfonate was not detected by ESI positive and APCI positive (B and C). However, negative ESI and APCI mode (E) favored to ionization of sodium hexane sulfonate (D and E). F) The MSD spectrum of sodium hexane sulfonate.

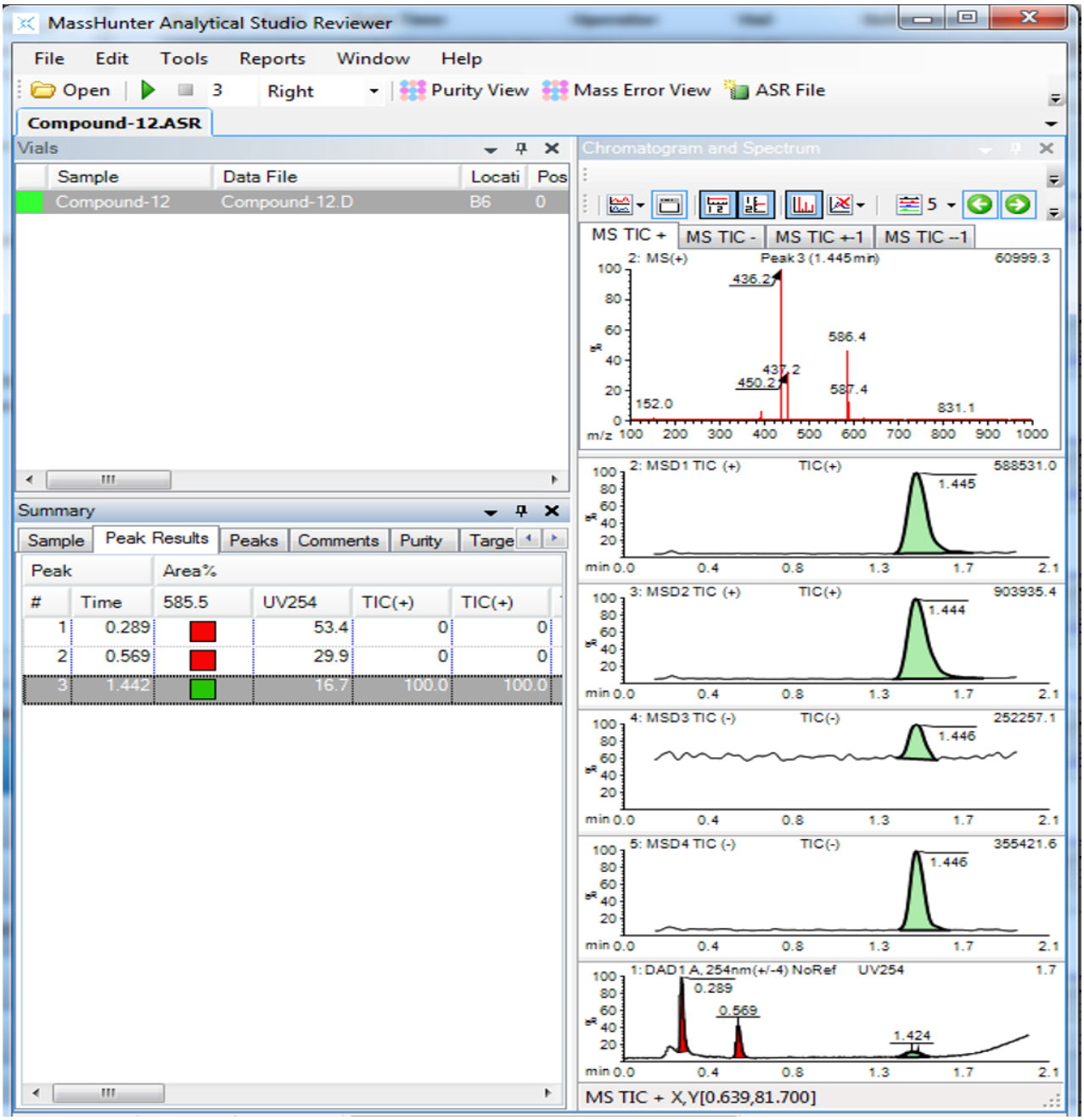

Figure 5. Snapshot of ASR report for fosinopril. The compound purity based on UV signal peak area, retention time, MS spectra, and peak color coding for easy visualization are included in the ASR report for quick review at a glance.

Walkup results summary

Table 4 summarizes the compound confirmation results and response comparison from UV and MSD. Target response from UV detection, ESI (positive/negative), and APCI (positive/negative) detections are summarized using various shades of green. The most intense ionization is marked with a star symbol. The table summarizes the benefit of using MSD in addition to UV detection for routine purity or compound identification analysis.

Conclusion

The combination of the robust Agilent 1260 Infinity II LC coupled with the Agilent LC/MSD XT and Agilent MassHunter Walkup software is an easy-to-operate and efficient system for routine multiuser laboratory LC/UV/MS analysis. Use of mass spectrometry and UV detection in an open access mode allows nonexpert MS users to easily perform compound identification and purity analysis. Intuitive software enables easy sample submission, analysis, and data reporting without previous LC/MS expertise. Also, a broader range of compound classes can be analyzed in a single run using the multimode source and fast polarity switching. Automated data analysis using Agilent MassHunter Analytical Studio Reviewer software helps ensure simplified data review and reporting for the synthetic or medicinal chemist.

Table 4. Compound confirmation results summary.

	Compound	Gradient	m/z	RT	UV	ESI+	APCI+	APCI–	ESI–
1	Pemirolast	1	229	0.83			★		
2	Sulfamethizole	1	271	0.48			★		★
3	Sulfamethazine	1	279	1.1			★		
4	Sodium hexanesulfonate	1	165	0.34					★
5	Amitriptyline	2	278	1.4			★		
6	BMS-193885	2	591	1.1			★		
7	BMS-493	2	256	0.5			★		
8	Buspirone	2	386	1.3			★		
9	Butorphan	2	312	0.45			★		
10	Pravastatin	2	447	0.6					★
11	Sulfadimethoxine	2	311	0.32			★		
12	Fosinopril	3	586	1.4		★			
13	Nefazodone	3	470	1.1			★		
14	Paclitaxel	3	877	0.4			★		
15	Clopidogrel	3	322	1.1			★		
16	Trazodone	3	372	0.5			★		
17	Zofenopril	3	430	0.4			★		
18	Ketoconazole	3	531	0.7			★		

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