Purity Assessment of Drug Discovery Compound Libraries Using an Agilent Single Quadrupole LC/MS System Coupled to Diode Array and Evaporative Light Scattering Detectors

Application Note

Drug Discovery

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Abstract

Medicinal and analytical scientists supporting pharmaceutical medicinal chemistry efforts usually assess the purity of synthesized compounds prior to selecting them for biological activity. This application note demonstrates a fast and automated purity assessment of a 30 compound library using one generic method and a multidetector system. The use of the Agilent 6130 Series Single Quadrupole LC/MS System coupled to both an ultraviolet (UV) and an evaporative light scattering detector (ELSD), enables the detection of impurity peaks that may be missed by using only one mode of detection. Agilent Analytical Studio Reviewer software provides a simplified user data processing interface for compound confirmation and purity assessment.

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Introduction

With an emphasis on generating high-quality drug candidates efficiently, and reducing risk of failure, many analytical groups in drug discovery and development are emphasizing stringent purity criteria for compound libraries. This puts greater importance on monitoring purity using multiple detection methods to ensure that impurities do not enter the compound lots being tested for biological safety and efficacy. This application note presents Agilent solutions for purity assessment of compound libraries using a multidetection system that simplifies the workflow for medicinal and process chemists.

A 30-compound library, synthesized using combinatorial chemistry methods, was used in this experiment. Since impurities may lack a UV chromaphore and therefore not show absorbance or poorly ionize, an Agilent 1260 Infinity Diode Array Detector (DAD), a combination of a 6130 Series Single Quadrupole LC/MS System, and an Agilent 1260 Infinity Evaporative Light Scattering Detector (ELSD) were used. This enabled the detection of UV- and non UV-active impurities. Mass spectrometry and ELSD complement each other. Non-ionized compounds missed by MS can be detected by ELSD.[1] The LC method used a 7 minute generic LC gradient on a sub-2 µm column. The use of sub-2 µm particles ensures higher theoretical plates and, thus, greater resolution compared to larger particle size columns of the same dimension. This complete setup alleviates the need to develop individual purity methods for each compound. An Agilent Analytical Studio Reviewer (ASR) provides access to LC/MS data through an intuitive user interface which allows the user to simultaneously review chromatograms from the multidetectors, purity area percentages, and sample locations in one window. ASR also provides a rapid assessment of compounds that fail purity tests.[2]

Previously, a solution for purifying drug compounds was presented using Agilent mass-triggered preparative LC and a fraction collection system.[3] This application note presents a solution for purity assessment using Analytical Studio Reviewer that includes UV, MS, and ELSD detectors.

Experimental

Synthesis of library compounds

A library of 30 different compounds was synthesized from the scaffold structure shown in Figure 1. The compounds were synthesized by combinatorial methods using metal-mediated catalysts. Metal catalysts contribute to formations of additional impurities which can make purity assessment challenging.

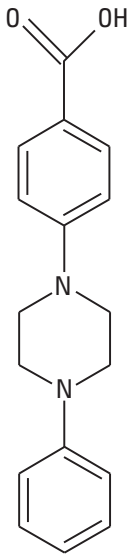

Figure 1. Scaffold used to make the library compounds.

A generic method containing water and acetonitrile with 0.1 % formic acid was used to evaluate the purity of a 30 compound library. The UV detector was set to three wavelengths, 210 nm, 254 nm, and 280 nm for detection of impurities.

ELSD optimization was readily achieved by testing a set of 10 predefined ELSD methods. These 10 methods had different values for evaporative temperature, nebulizer temperature, and gas flow. The results showed that one of the methods (Table 1) gave the best ELSD signal and this was selected for all experiments.

Table 1. LC/MS conditions.

LC/MS conditions		
Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 1.8 µm (p/n 959964-902)	
TCC	40 °C	
Sample thermostat	6 °C	
Sample diluent	100 % methanol	
Mobile phase	A: Water with 0.1 % formic acid, B: Acetonitrile with 0.1 % formic acid	
Gradient	Time (min) 0 1.0 4.0 5.0 5.5 7.0 7.01 Post time: 1 minute	% B 10 10 75 75 95 95 10
DAD	Peak width > 0.1minutes 210 nm, 254 nm, and 280 nm	
UV flow cell	2 µL (capacity), 3 mm (flow path), 120 bar (maximum pressure)	
Flow rate	1.0 mL/min	
Injection volume	10 µL, 10 second needle wash with 100 % mobile phase B	
T connector	Split ratio ELSD:MS 82:18. Split ratio obtained by connecting 0.12 mm PEEK tubing of varying length after the T connected[1]	
ELSD (G4260B) parameters	Nebulizer temperature	80 °C
	Evaporator temperature	80 °C
	Evaporator gas flow	0.90 Standard Liter per Minute (SLM)
	Smoothing	3.0 seconds
	LED intensity	100 %
	PMT gain	4
MS acquisition	Scan range	50–950 amu
	Peak width	0.05 minutes
	Step size	0.1
	Polarity	positive and negative
	Cycle time	0.84 sec/cycle
	Fragmentor	90 V
	Gas temperature	300 °C
	Flow	10 L/min
	Nebulizer	15 psig
	Sheath gas temperature	250 °C
	Sheath gas flow	7 L/min
	Capillary	3,000 V
	Negative	2,500 V
	Nozzle voltage	1,500 V for both pos and neg
Purity assessment	Analytical Studio Reviewer B.02.01	
	Sample purity option	
	Target mass detection	
	% of BPI of target found	8
	% of BPI of target confirm	20

Results and Discussion

Confirmation of library compound synthesis

MS positive mode is used for molecular weight (MW) confirmation of the compounds. ASR calculates the ratio of the abundance (area) of the target ion (preassigned by the user) to the abundance of the base ion and reports it as a percentage of base peak intensity (% BPI) (Figure 2). The user defines the minimum value of % BPI to confirm the compound as found and color codes the sample green. The red color on one compound shows that it was below the % BPI threshold value. The % BPI value of 20 % was chosen in this experiment. Figure 2 shows that the masses of almost all the compounds matched with the masses assigned in the sequence table, confirming the synthesis of the desired compounds.

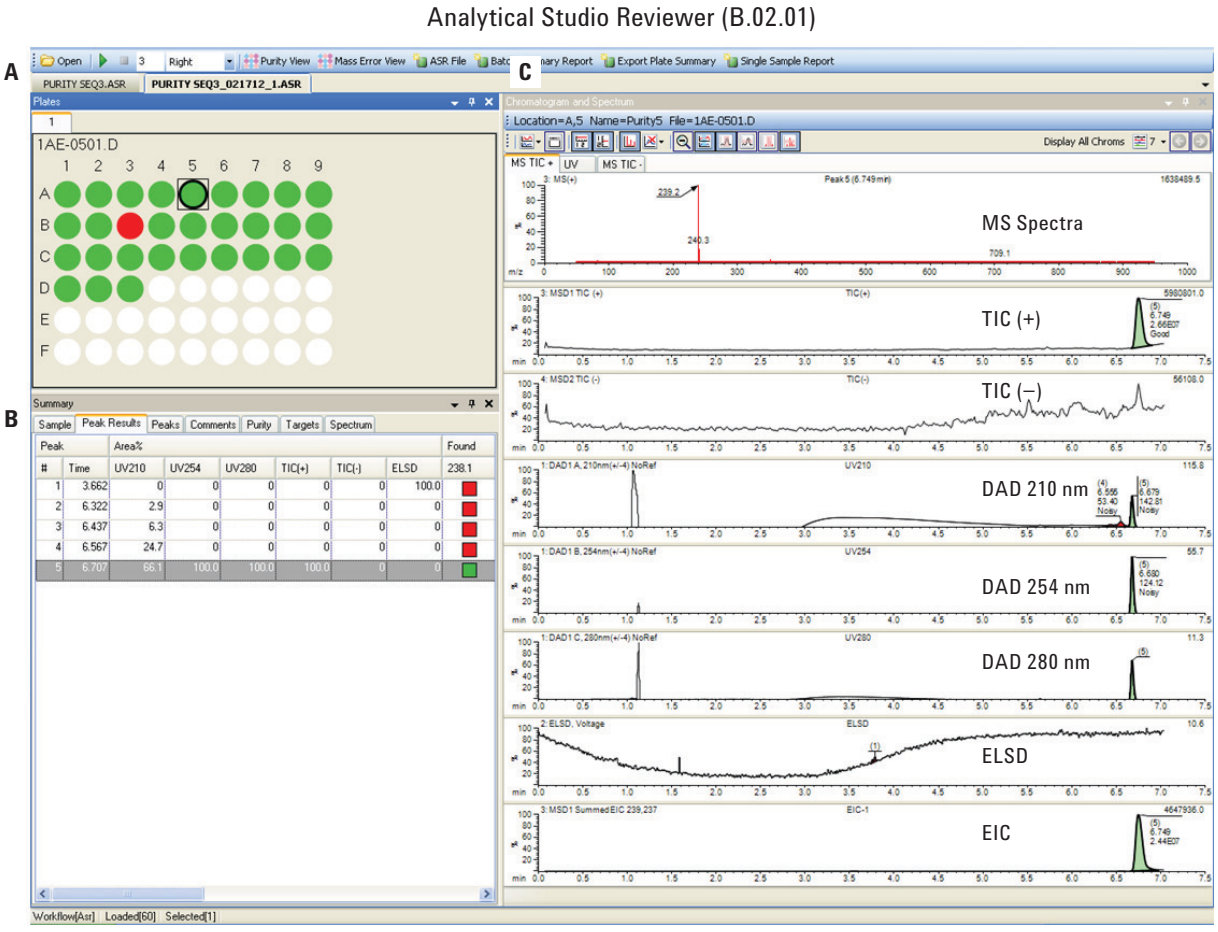

Figure 2. The green symbols represent those compounds whose masses were detected (A). The area percentage of each peak from individual detectors is displayed in table format (B). The multiple chromatogram view option shows the stacking or overlay of a large number of chromatograms for simultaneous review from orthogonal detectors (C).

Determination of compound purity

ASR determines the purity of compounds based on the peak area percentage from any one of the detectors. The color code (Figure 3A) indicates the degree of purity. The pink color is defined as purity > 80 % area percentage, while the blue color is in the range of 20–80 % and the orange is below 20 % area percentage. The results using MS as purity assessment detector, show that one third of the library compounds have purity greater than 80 % (Figure 3). Due to differences in the ionization efficiencies of impurities or adducts, it is possible to arrive at different conclusions about the purity. ASR allows users to view results from different detectors such as ELSD to confirm the purity assessment. When the purity was assessed using ELSD as the primary detector, 67 % compounds show purity > 80 %. This suggests that MSD can detect more impurities, and that ELSD or UV can be used to further confirm the purity results from MSD.

Purity View in Analytical Studio Reviewer

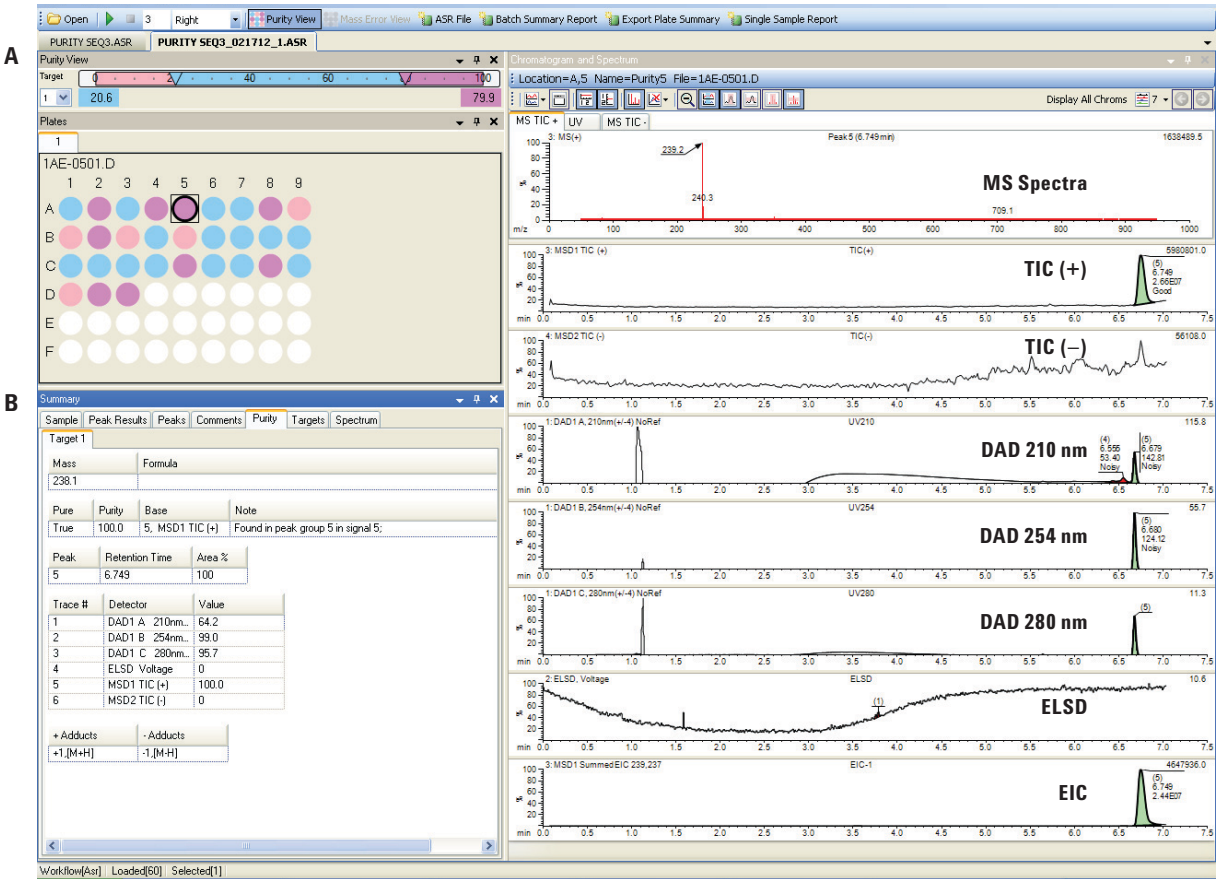

Figure 3. The purity tab of the ASR displays those compounds that are pure (A). The area % within each chromatogram is used to determine the purity of the peak (B).

Increased sensitivity with the Agilent Jet Stream source

The compound library was analyzed using a 6130B Series Single Quadrupole LC/MS System with an ESI source or an Agilent Jet Stream source (AJS). The results from the AJS source showed enhanced signal intensity compared to that from the ESI source, leading to the detection and integration of additional peaks (Figure 4A). The peak area of the major signal was 2.27 × 10^7 with the AJS source while peak area of the same peak was 1.07 × 10^7 with the ESI source, suggesting a 2-fold increase in the signal intensity. The purity view of ASR (Figure 4A and 4B) shows nine compounds having purity > 80 % with the AJS source compared to 14 compounds when using the ESI source. In Figure 4B, one compound (number 16, marked as B7) is marked as 100 % pure by the ESI source while the same compound was shown as 70 % pure by the AJS source (Figure 4A). The AJS source may provide more accurate purity assessment for library compounds.

Figure 4. The comparison of purity view of the same compound library analyzed using the AJS source (A) and the ESI source (B).

Conclusions

The combination of the Agilent 6130 Series Single Quadrupole LC/MS System with an AJS source, an Agilent 1260 Infinity Diode Array Detector, an Agilent 1260-ELSD Infinity Evaporative Light Scattering Detector, and the Agilent Analytical Studio Reviewer provides a versatile tool for rapidly screening library compounds.
A short generic LC method, using a sub-2 µm particle size column, eliminates the need to develop multiple purity methods for each compound. Moreover, multiple detectors offer complementary detection of impurity peaks that might be missed by a single detector.

The AJS source provided enhanced MS signal intensity for more accurate assessment of compound purity. In addition, Analytical Studio Reviewer software offers a quick and simple solution for data review and navigation of compounds, significantly reducing data analysis time.

References

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3. Configuring a Mass-based Fraction Collection System for Highest Purity Mass Triggered Prep LC System. Application note. Agilent publication 5990-4845EN.

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