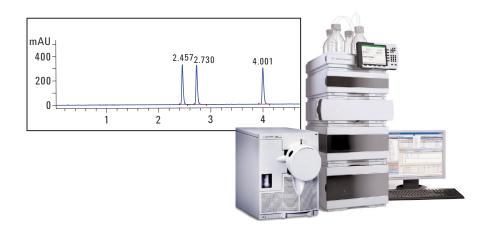


Agilent 1290 Infinity LC The ideal partner for MS — Part 2

Complementary, orthogonal detection by simultaneous UV and MS

Application Note

Pharmaceutical and Chemical



Author

Edgar Naegele Agilent Technologies, Inc. Waldbronn Germany

Abstract

This Application Note demonstrates the use of DAD-UV detection and ESI-MS detection of compounds with different spectroscopic and ionization behavior in screening experiments. The compatibility of UV and MS detection is shown with example compounds of different spectroscopic and ionization properties. In addition, the Agilent 1290 Infinity LC Diode Array Detector is compared to the Agilent 1200 Series Diode Array Detector SL.



Introduction

The screening of samples for their content is a widely used application including UV detection and mass spectrometric detection. This approach is typically used in quality control screening of pharmaceutical libraries, quality control screening of natural products, screening for pollutants in environmental samples, food control samples, and analytical walk-up solutions.

The detection by UV and mass spectrometry interact as complementary techniques in common screening approaches. It is not unusual that compounds which have UV activity do not ionize in electrospray mass spectrometry (ESI-MS) and vice versa. Compounds which undergo ionization in ESI-MS are often only detectable at one polarity, positive or negative. Therefore, it is desirable that a broad range of UV wavelengths is monitored and measured, a typical approach for a diode array detector (DAD). The mass spectrometer must be able to switch between positive and negative electrospray ionization polarity in a way, which is fast enough to follow the chromatographic separation.

This Application Note demonstrates the use of DAD-UV detection and ESI-MS detection of compounds with different spectroscopic and ionization behavior in screening experiments. The compatibility of UV and MS detection is shown with example compounds of different spectroscopic and ionization properties. Additionally, a comparison of the Agilent 1290 Infinity DAD (G4212A) to the Agilent 1200 Series DAD SL (G1315C) is shown.

Results and discussion

In special types of experiments such as screenings and walk-up solutions, LC/MS combines UV detection with the mass spectrometric detection of electrospray ionization giving comparable or complementary results. The separation of compounds in such a sample, and detection by UV and ESI-MS is shown in Figure 1. The detection by UV which was performed with the Agilent 1290

Experimental

Equipment:

Agilent 1290 Infinity LC system containing an Agilent 1290 Infinity Binary Pump, Agilent 1290 Infinity High Performance Autosampler, 1290 Infinity Thermostatted Column Compartment, Agilent 1290 Infinity DAD and Agilent 6140 Single Quadrupole LC/MS system with ESI source.

Column: Software for data acquisition and Agilent ZORBAX SB C18, 50 × 2.1 mm, 1.8 μm

Software for data acquisition and data analysis:

ChemStation B.04.03

HPLC Method:

Solvent A: Water + 0.1% formic acid
Solvent B: Acetonitrile + 0.1% formic acid

Flow rate: 0.5 mL/min

Gradient: 0 min 5% B; 10 min 95% B

Stop time: 10 min
Post time: 3 min
Injection volume: 1 µL
Needle wash: 6 s in MeOH
Column temperature: 35 °C

Diode array detector: 10 mm standard cell, wave length 260/4 nm, Ref. 360/16 nm,

slit 4 nm, data rate 40Hz

MS Method:

Source: Gas temperature: 350 °C, nebulizer pressure: 45 psi,

gas flow: 11 L/min, positive polarity

Signal 1: Positive polarity
Signal 2: Negative polarity
Scan: 100–1000 m/z

Sample: Solution of: 1) Sulfamethazine (MW 278.0), 2) Sulfachloropyridazine

(MW 284.0), 3) Hexanesulfonic acid (MW 166.0), 4) Sulfadimethoxine (MW 310.0), 5) Carbazole (MW 167.0), Crystall violet (MW 407.2), 7) 9-Phenanthrol (MW 194.0), each at a concentration of 100 ng/µL

Infinity DAD, set on the wavelength 260 nm, is shown in Figure 1A. In this UV trace, it is seen that the sample contains five major compounds and one minor compound, which are detectable at 260 nm (other peaks are due to minor impurities in the compounds used to generate the sample). This does not

show the full capabilities of the Agilent 1290 Infinity DAD, which is able to detect compounds at different wavelengths in parallel showing the complete spectral information comprised in the sample. For that purpose, all spectra where acquired and can be shown in a 3-dimensional plot over time, wavelength

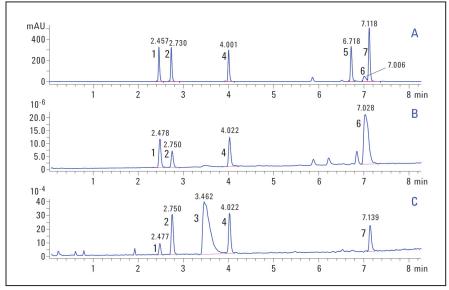


Figure 1
Separation and detection of sample compounds by UV and ESI-MS.

A) Detection by DAD UV at 260 nm. B) Detection by positive scan ESI-MS. C) Detection by negative scan ESI-MS.

and intensity (Figure 2). This plot presents the spectra of the five major compounds and shows that there is no other compound which has UV activity at another wavelength as the used one and the used wavelength is around the maximum absorbance wavelength for all compounds. It would be possible to measure other compounds with a maximum intensity at a different wavelength, because the DAD can acquire eight separate channels at different wavelenghts at the same time.

Comparable and complementary data can be acquired by adding a single quadrupole mass spectrometer such as the Agilent 6140 Single Quadrupole LC/MS system to the LC. This ionizes the compounds by electrospray ionization and allows measurement in positive and negative ion mode (Figures 1B and 1C). The total ion chromatogram from the positive electrospray ionization shows four major compounds and gives the [M+H]+ ion (Compound 1: m/z 279.0, Compound 2: *m/z* 285.0, Compound 4: *m/z* 311.0, Compound 6: *m/z* 372.0 (MW - CI-)). Compounds 1, 2 and 4 can be detected by UV and under positive electrospray ionization conditions. Whereas Compound 6 has little UV activity but good ionization under positive electrospray conditions. The total ion chromatogram from the negative electrospray ionization shows five major compounds and gives the [M-H]- mass information (Compound 1: m/z 277.0, Compound 2: *m/z* 283.0, Compound 3: m/z 165.0, Compound 4: 309.0, Compound 7: *m/z* 193.0). Compound 3 shows only response in negative electrospray ionization, Compound 7 has UV activity and response in negative electrospray ionization.

Detection of Compounds 1, 2 and 4 is easiest because they have UV activity. Also, the compounds respond in positive and negative electrospray ionization (Figure 3). Compounds 6 and 7 show only UV activity and a positive or negative mass spectrum, respectively, but this is still sufficient for proper identification. Whereas, identification

of Compounds 3 and 5 is somewhat more difficult because they show only negative mass spectrum or a UV spectrum, respectively. In Compound 5, the UV spectrum is very characteristic and could be identified by comparison to a spectral library (Figure 4). The result from this experiment clearly shows the value of adding an Agilent 1290 Infinity

LC System with Agilent 1290 Infinity DAD to a mass spectrometer to gain comparative and complementary data. With the Agilent 6140 single quadrupole MS, it is possible to scan 10,000 amu/s in the ultrafast mode and to switch between positive and negative polarity in 20 msec for high speed separations.

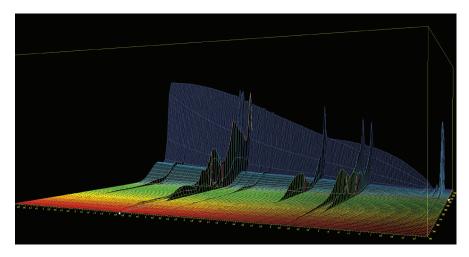


Figure 2
The 3-dimensional plot of wavelength over time and intensity, acquired with the Agilent 1290 Infinity DAD to show the complete spectral information comprised in the sample (X-axis: 0 min on the right side to 10 minutes, Y-axis: intensity in mAU, Z-axis: 190 nm to 400 nm on the front side).

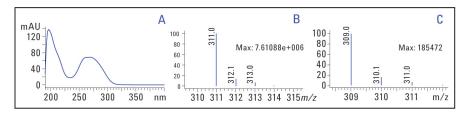


Figure 3 Complete spectral information of compound 4. A) UV spectrum with maximum 255 - 280 nm. B) Positive mass spectrum, m/z 311.0. C) Negative mass spectrum, m/z 309.0.

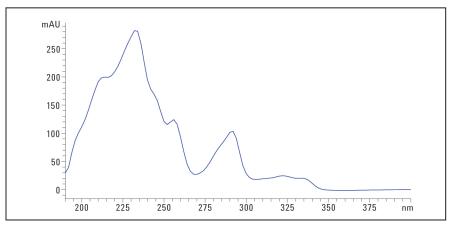


Figure 4
UV spectrum of Compound 5.

In the context of this work, an additional comparing evaluation of the Agilent 1290 Infinity DAD (G4212A) to the Agilent 1200 Series DAD SL (G1315C) was done to compare their performance. For this performance comparison, the peaks of Compounds 1, 2, and 4 where measured with the Agilent 1290 Infinity DAD equipped with the 10 mm Max-Light standard cell (G4212-60008) and with the Agilent 1200 Series DAD SL equipped with the 10 mm cell (G1315- 60022), the 6 mm cell (G1315-60025) and the 3 mm cell (G1315-60024). The obtained data are compared to show the influence on peak width, peak area and peak height (Table 1).

Comparing the Agilent 1290 Infinity DAD with 10 mm Max-Light cell to the Agilent 1200 Series DAD SL with 10 mm cell, it can be seen that the peaks are about 0.006 min (about 0.4 sec.) broader at half height and about 15% less in height, but the area remains unchanged. The loss in peak height and gain in peak broadness due to the larger volume of the flow cell results in less sensitivity for the Agilent 1200 Series DAD SL.

Comparing the results of the Agilent 1200 Series DAD SL with 10 mm cell to the 6 mm cell and the 3 mm cell, a decrease in peak width at half height can be seen, which is due to the shorter cell flow path length and lower flow cell volume. The peak width obtained with the 3 mm cell is comparable to the result obtained for Agilent 1290 Infinity DAD with the 10 mm cell. However, peak area and peak height declined due to the shorter flow path. The peak area and the peak height declined about 40% when exchanging the 10 mm cell with the 6 mm cell in the Agilent 1200 Series DAD SL. With the 3 mm cell the peak area and peak height declined down to

1290 DAD G4212A, 10 mm cell (G4212-60008)				
Peak	RT (min)	Width (min)	Area (mAU*s)	Height (mAU)
1	2.457	0.0294	620.36	323.79
2	2.730	0.0306	646.19	319.40
4	4.001	0.0325	631.61	295.17
1200 DAD G1315C, 10 mm cell (G1315-600022)				
Peak	RT (min)	Width (min)	Area (mAU*s)	Height (mAU)
1	2.533	0.0350	621.95	273.74
2	2.842	0.0368	642.18	269.67
4	4.127	0.0395	631.03	245.46
1200 DAD G1315C, 6 mm cell (G1315-6025)				
Peak	RT (min)	Width (min)	Area (mAU*s)	Height (mAU)
1	2.530	0.0334	370.22	170.04
2	2.844	0.0353	381.69	166.04
4	4.126	0.0384	374.02	148.53
1200 DAD G1315C, 3 mm cell (G1315-60024)				
Peak	RT (min)	Width (min)	Area (mAU*s)	Height (mAU)
1	2.514	0.0295	193.22	100.20
2	2.824	0.0313	199.43	97.83
4	4.107	0.0334	195.17	89.64

Table 1

Data obtained from Agilent 1290 DAD and Agilent 1200 Series DAD SL to compare the influence of different flow cells on peak width, peak area, and peak height.

one third compared to the 10 mm cell. The retention times are about 0.1 min higher in all cases compared to the Agilent 1290 Infinity DAD due to longer connecting cell capillaries. This result demonstrated the higher sensitivity of the Agilent 1290 Infinity DAD compared to the Agilent 1200 Series DAD SL even with the cell of the same path length. This is due to the improved cell design of the Max-Light cell. Furthermore the Max-Light cell leads to much less peak broadening due to its low dispersion volume. The valuable Max-Light cell can be protected from damages from overpressure due to MS sprayer blockage by an additional pressure relief valve between DAD and MS (Agilent in-line pressure relief valve kit, G4212-68001). This valve does not affect the performance of the separation by influence on the peak width.

Conclusion

The results from these experiments clearly show the value of adding an Agilent 1290 Infinity LC system with an Agilent 1290 Infinity DAD to a mass spectrometer to gain comparative and complementary data. The use of DAD-UV detection and ESI-MS detection of compounds with different spectroscopic and ionization behavior is demonstrated and the compatibility of UV and MS detection is shown through example compounds of different spectroscopic and ionization properties. A comparison of the Agilent 1290 Infinity DAD to the Agilent 1200 DAD SL demonstrates the superior detection capabilities regarding UV sensitivity and lower dispersion volume of the Agilent 1290 Infinity DAD.

www.agilent.com/chem/lc

© Agilent Technologies, Inc., 2011 July 1, 2011 Publication Number 5990-7720EN

