Agilent 1290 Infinity LC
The ideal partner for MS – Part 4

Increased sensitivity by enhanced separation between analyte and matrix with the Agilent 1290 Infinity LC

Application Note

Chemical and Pharmaceutical Analysis, Food Safety

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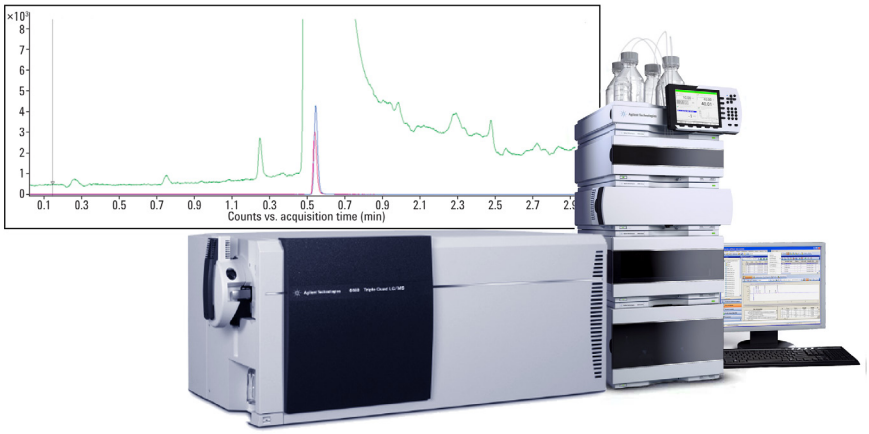

Abstract

This Application Note demonstrates the advantage of using an Agilent 1290 Infinity LC system with 1.8 µm columns as the front-end of an Agilent 6460 Triple Quadrupole MS system. This enables the achievement of better separation of the analyte compound from matrix compounds compared to conventional HPLC separation on a 5 µm column. The presented data shows an increase in peak height and sensitivity by decreased peak width and decreased matrix suppression caused by improved separation on an Agilent 1290 Infinity LC used in combination with 1.8 µm particle size columns.

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Introduction

Today, the analytical chemist is faced with the challenge to detect and quantify compounds in very low trace amounts buried in high amounts of complex matrixes. This is especially important for the determination of pesticides in different food matrixes in modern LC/MS analysis. A typical problem is the prevention of matrix suppression of the analytes. Matrix suppression occurs during the ionization of an analyte in the ion source of a mass spectrometer when other compounds are present in large excess at the same time, resulting from coelution out of the LC column. Modern UHPLC systems, which have the capability to work with 1.8 µm particle columns, can help solve this problem by minimizing matrix suppression effects due to improved separation of the analyte compounds from matrix components and producing sharper peaks.

This Application Note shows the advantage of using an Agilent 1290 Infinity LC system with 1.8 µm columns as the front end of a triple quadrupole MS instrument to achieve better separation of analyte compound from matrix compounds compared to conventional HPLC separation on a 5 µm column. As a matter of principle, the effect of matrix suppression is shown with a two compound mixture and by an example with plasma matrix. Finally, the effect is examined with a complex food matrix in a multi pesticide method. The presented data shows an increase in peak height due to an improved separation, sharper peaks and less ion suppression achieved with the Agilent 1290 Infinity LC running 1.8 µm columns.

Experimental

Equipment:
An Agilent 1290 Infinity LC system consisting of the following modules was used:
Agilent 1290 Infinity Binary Pump
Agilent 1290 Infinity High Performance Autosampler
Agilent 1290 Infinity Thermostatted Column Compartment
Agilent 6460 Triple Quadrupole Mass Spectrometer

Columns:
1) Agilent ZORBAX Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm
2) Agilent ZORBAX Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm
3) Agilent ZORBAX Eclipse Plus C18, 2.1 × 150 mm, 5 µm

Software for data acquisition and data analysis:
MassHunter Data Acquisition software
MassHunter Optimizer software
MassHunter Qualitative Data Analysis software

HPLC Method:

Solvent A:	Water + 0.1% formic acid
Solvent B:	Acetonitrile + 0.1% formic acid
Flow rate:	0.8 mL/min.
Gradient 1 (50 mm column):	0 min 5% B; 2.0 min 95% B; 2.5 min 95% B
Stop time:	2.5 min
Post time:	2 min
Gradient 2 (150 mm column):	0 min 5% B; 15.0 min 95% B; 16.0 min 95% B
Stop time:	16 min
Post time:	3 min
Injection volume:	1 µL Needle wash: 6 sec in MeOH
Column temperature:	35 °C

MS Method:

Source:	Sheath gas temperature: 350 °C, sheath gas flow: 11 L/min
Capillary gas temperature:	300 °C
Capillary gas flow:	5 L/min
Nebulizer pressure:	50 psi
Capillary:	4,000 V
Nozzle voltage:	500 V
Polarity:	positive
MRM settings:	see table 1

Samples:
Stock solutions (100 µg/mL) of: Sulfamethazine (MW 278.0), Sulfamethizole (MW 270.0), Verapamil (MW 454.1). Ehrenstorfer Pesticide Mix 44, content see Table 1, concentration 10 ng/mL each.

Compound name	Ion (m/z)	MRM transition	Fragmentor (V)	Collision energy (V)
Sulfamethizole	271.02	271.03 → 156.0	110	24
Sulfamethazine	279.09	SIM mode only	130	
Verapamil	455.29	455.29 → 165.1	190	24
Content of Ehrenstorfer Pesticide Mix 44				
Atrazinedesethyl	188.07	188.07 → 146.0	115	12
Atrazine	216.10	216.10 → 174.0	130	4
Chlorotoluron	213.08	213.08 → 72.1	105	20
Methabenzthiazuron	222.07	222.07 → 96.0	100	56
Metobromuron	259.01	259.01 → 91.0	100	32
Metolachlor	284.14	284.14 → 176.1	95	24
Cyanazine	241.10	241.10 → 214.1	120	12
Diuron	233.03	233.03 → 72.1	90	20
Hexazinone	253.17	253.17 → 171.1	100	12
Metoxuron	229.08	229.08 → 72.1	115	24
Monolinuron	215.06	215.06 → 99.0	70	35
Sebuthylazine	230.12	230.12 → 174.0	130	12
Isoproturon	207.15	207.15 → 165.1	100	8
Linuron	249.02	249.02 → 182.0	110	12
Metazachlor	278.11	278.11 → 105.0	75	48
Simazine	202.09	202.09 → 124.1	120	12
Terbuthylazine	230.12	230.12 → 104.0	125	32

Table 1
MRM transitions of used compounds. Fragmentor voltages and collision energies determined with MassHunter Optimizer. Dwell time: 10 ms.

Results and Discussion

In an atmospheric pressure ionization (API) source of a mass spectrometer, the effluent from the liquid chromatography is pneumatically sprayed into an electrical field countercurrent to a heated gas stream (electrospray ionization, ESI). Under these conditions, the formed spray droplets evaporate and ions are formed on the surface of the droplets. The droplets shrink to a critical size where the Coulomb forces become too strong and the droplets explode. The free ions are then drawn into the mass spectrometer by the electrical field. If compounds are coeluting from the liquid chromatography column, they compete in the formation of ions. If one compound is in a large excess, this compound can suppress the ionization of the minor compound and decrease its MS signal.

This effect was demonstrated in principle by coeluting sulfamethazine (100 ng/µL) and sulfamethizole (100 pg/mL) from the LC column, where sulfamethazine was present in a 1,000-fold excess (Figure 1). The comparison of the intensity of the MRM signal of sulfamethizole coeluting with a large excess of sulfamethazine shows about 25% less signal intensity compared to the signal in the absence of the large excess of sulfamethazine. This decrease in signal intensity by ion suppression is commonly called matrix effect.

Precipitated blood plasma is a matrix often encountered in clinical and forensic samples. To generate the sample, blood plasma is diluted with about a 3-fold excess of acetonitrile which precipitates the proteins comprised in the plasma. After removal of the precipitant by centrifugation, the supernatant is directly used for LC/MS injection. The advantage is the fast sample preparation, but the disadvantage is that some matrix components remain in the solution, especially glycerophospholipides and lysophospholipides. Common to all phospholipids is a phosphatidylcholine moiety. This part of the molecule can be cleaved off by collision induced dissociation (CID) and detected in a precursor ion scan experiment at *m/z* 184 (Figure 2A).

The molecular weight of glycerophospholipides and lysophospholipides is typically between 400 *m/z* and 800 *m/z*. This means all matrix poshoplipids can be detected and their position in the gradient form LC separation can be determined to avoid any suppression by coelution overlap with the analyte. There are still other components in the matrix which cannot be detected in such an experiment. For their localization, a suppression profile was acquired (Figure 2B). An example drug, verapamil, was taken and the triple quadrupole was run in MRM mode optimized for this compound. First, a blank was acquired with these settings.

In a second experiment, a blank matrix sample was injected and verapamil (10 pg/µL) was infused into the column effluent between column outlet and MS sprayer by a syringe with a T-piece (250 µL/h). The suppression profile was generated by subtraction of the MRM trace of the second experiment from the blank. It is shown, that the highest suppression occurs at the end of the run after 1.9 minutes, exactly where the phospholipids elute

(Figures 2A and 2B). Other early eluting polar compounds cause suppression at the beginning of the run between 0.2 and 0.3 minutes. In the middle of the run, where typical pharmaceutical compounds elute, there is no significant suppression, and separation is not a challenging task. In this example, a plasma sample spiked with verapamil was compared to a standard of verapamil at the same concentration (10 pg/µL) to determine the suppression (Figure 2C). The signal is suppressed by about 30% at the retention time in this gradient (gradient 1, column 1).

The influence of separation becomes more critical when matrix complexity increases which is often the case in food matrixes. In such a case, separation performance becomes important and the advantage of an UHPLC which is able to produce the necessary back pressure to work with 1.8 µm columns compared to an HPLC instrument which can only work with the classical 5 µm columns becomes obvious. A matrix from ginger, which is rich in background compounds, was taken as an example. This matrix was separated on a 2.1 × 150 mm, 5 µm column (column 3) and on a 2.1 × 150 mm, 1.8 µm column (column 2). The difference could be seen immediately by the achieved separation with better resolution in the chromatogram of the 1.8 µm column (Figures 3A and 4A). The back pressure of the 1.8 µm column at the flow rate of 0.8 mL/min is about 940 bar and the back pressure of the 5 µm column is approximately 160 bar at starting conditions. The separation performance with higher resolution and narrower peaks achieved with the 1.8 µm column can separate the compounds of interest much better from the matrix and proceed to a better detection performance.

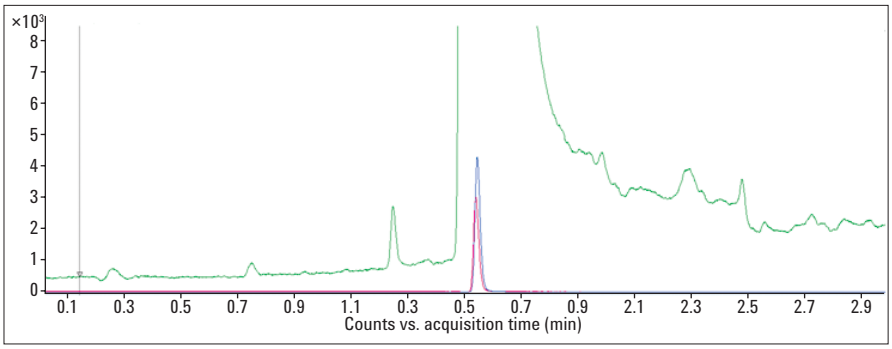

Figure 1
Principal effect of matrix ion suppression. A compound in large excess (sulfamethazine, 100 ng/µL, green, SIM trace) is coeluting from the LC together with a minor compound (sulfamethizole, 100 pg/µL, red, MRM trace) of interest and suppresses their signal by ionization competition compared to the standard solution of the same concentration without presence of matrix (blue, MRM trace).

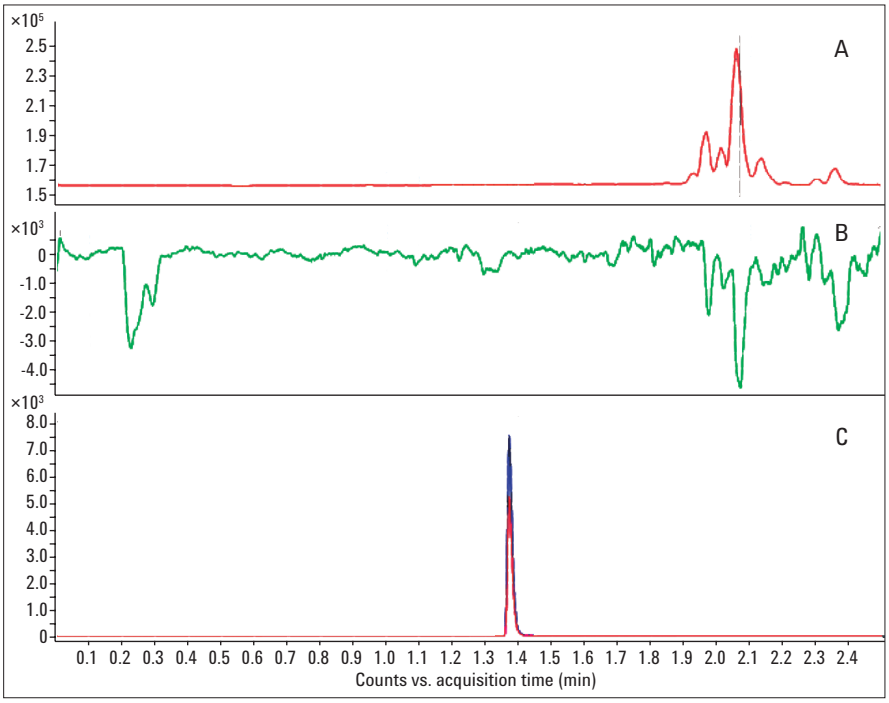

Figure 2
A) Precursor ion scan experiment with a precipitated plasma sample, 400 - 800 *m/z* → 184 *m/z*, for the detection of phospholipids.
B) Determination of the matrix effect for verapamil in plasma in MRM mode optimized for verpamil.
C) Verapamil in plasma (red) compared to a verapamil standard (blue) at the same concentration.

To demonstrate the influence of the matrix with suppression effects, the following experiment was done. A MRM method was developed for a defined mixture of pesticides (Table 1). The pesticide mixture (100 pg/µL, each component) was infused into the column effluent between the end of the column and the MS ESI sprayer by a syringe (250 µL/h). After the acquisition of a blank, the ginger sample was injected. To visualize the suppression effects, the sample separation was subtracted from the blank (Figures 3B and 4B). The resolution of the matrix compounds is much better on the 1.8 µm column than on the 5 µm column, peaks are much sharper and as a direct consequence matrix suppression occurs at more defined retention times (Figures 3B and 4B). With this higher resolution, it is expected that matrix effects are minimized for the measurement of pesticides in such a sample by using 1.8 µm columns on an Agilent 1290 Infinity LC system capable of delivering the necessary back pressure as front end for mass spectrometric analysis.

To compare the performance of both separations for a multi pesticide analysis, the mixture was spiked into ginger matrix to a final concentration of 100 pg/µL for each pesticide. The sample was measured with a 5 µm column and with a 1.8 µm column with the same method and the final result was compared to a measurement of the standard mixture (Figure 5). For the three most intense peaks, an ion suppression between measurement of the standard and measurement in matrix of about 10–15% could be seen with the 1.8 µm column (Figures 5A and 5B). The comparison between measurement of the pesticides in matrix on the 1.8 µm column and on the 5 µm column shows up to 50% less intense peaks for the separation on the 5 µm column (Figures 5B and 5C). The comparison of the measurement of the standard on

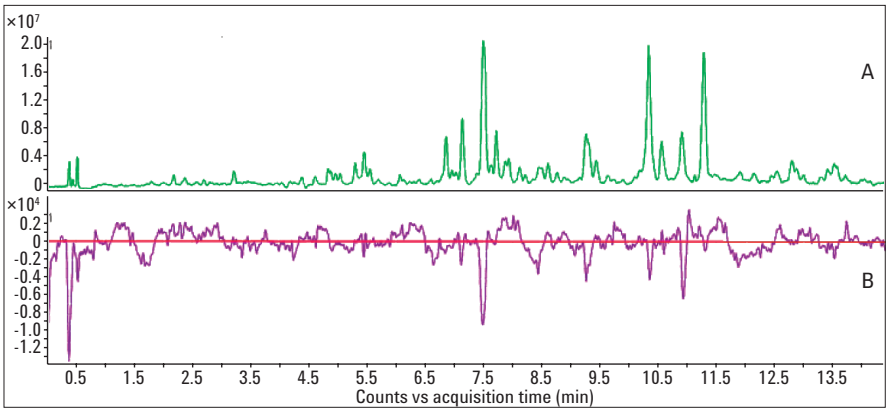

Figure 3
A) Separation of compounds comprised in a matrix of ginger on a 2.1 × 150 mm, 5 µm column (column 3) at 0.8 mL/min, applying gradient 2 at about 160 bar at starting conditions (ESI TIC).
B) Matrix suppression profile TIC MRM for a mixture of 17 pesticides. Pesticide concentration 100 pg/µL each, infused after the column by syringe at 250 µL/hour.

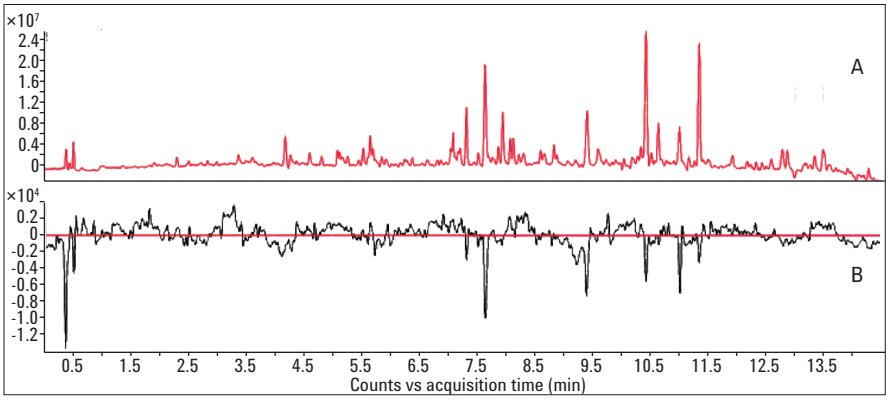

Figure 4
A) Separation of compounds comprised in a matrix of ginger on a 2.1 × 150 mm, 1.8 µm column (column 2) at 0.8 mL/min, applying gradient 2 at about 940 bar at starting conditions (ESI TIC).
B) Matrix suppression profile TIC MRM for a mixture of 17 pesticides. Pesticide concentration 100 pg/µL each, infused after the column by syringe at 250 µL/hour.

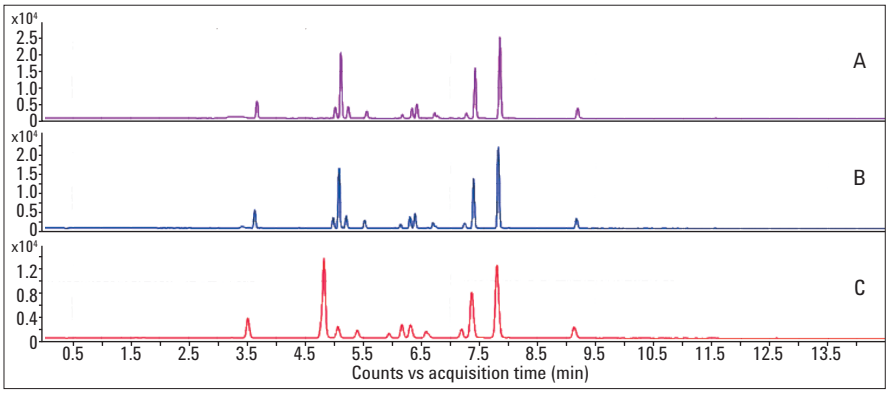

Figure 5
Measurement of ion suppression effects in a multi-pesticide method in ginger matrix (TIC MRM).
A) Standard of the pesticide mixture containing 17 compounds (some are coeluting) at 100 pg/µL each.
B) Measurement of the 17 pesticides in ginger matrix with a 1.8 µm column (940 bar at starting conditions).
C) Measurement of the 17 pesticides in ginger matrix with a 5 µm column (160 bar at starting conditions).

the 1.8 µm column and in matrix with a 5 µm column gives signal intensities even lower than 50% due to unresolved coelution of matrix compounds and peak broadening (Figures 5A and 5C).

This could be seen more accurately if the peaks were extracted individually and compared. As an example the peak of Monolinuron was extracted (Figure 6). Compared to the measurement in standard, an ion suppression of 17.5% could be seen for the measurement with a 1.8 µm column (Figures 6A and 6B) and an additional 25% decrease compared to the 5 µm column (Figures 6B and 6C).

Conclusion

This Application Note demonstrates the influence of ion suppression on peak height starting from principal examples up to examples of highest complexity. For an example of a multi-pesticide analysis in a complex sample, it is shown that the ion suppression critically depends on the quality of the separation. Superior results were achieved by using 1.8 µm columns compared to 5 µm columns. Due to the higher back pressure of the 1.8 µm columns the best separation performance with the minimized ion suppression and therefore most intense peaks even in complex matrixes were achieved with an Agilent 1290 infinity LC system as the front end for pesticide analysis by triple quadrupole mass spectrometry.

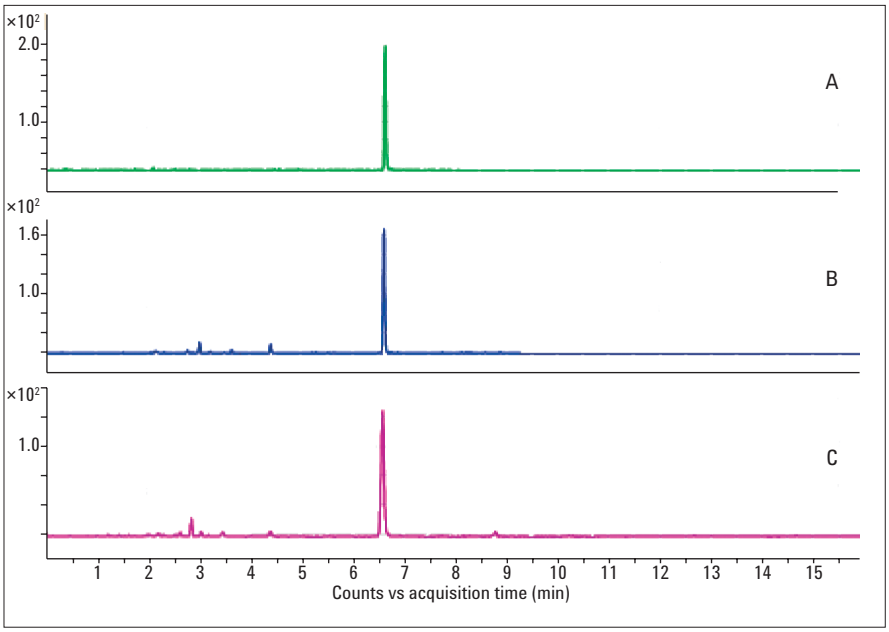

Figure 6
Ion suppression for the pesticide compound monolinuron, eluting at 6.6 minutes, measured by MRM.
A) Monolinuron MRM from measurement in the standard pesticide mixture at 100 pg/µL.
B) Measurement of monolinuron in ginger matrix with a 1.8 µm column.
C) Measurement of monolinuron in ginger matrix with a 5 µm column.

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