High-Throughput LC/MS/MS for cytostatic drugs with an Agilent 1290 Infinity LC System coupled to a tandem mass spectrometer

Application Note

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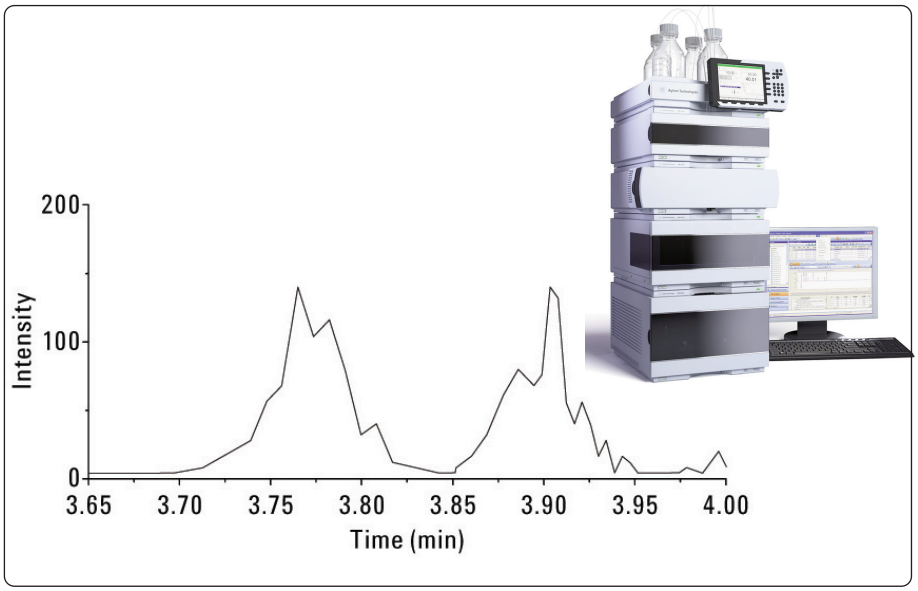

Abstract

This Application Note demonstrates the capabilities of the Agilent 1290 Infinity system to increase the speed and resolution of conventional HPLC separations with additional benefit for mass spectrometric analysis when the Agilent 1290 Infinity system is used as an MS front end.

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Introduction

Cytostatic drugs are used to treat cancer. Because of health risks associated with hazardous drugs such as antineoplastics, several guidelines exist for the safe handling of these substances. Routine occupational exposure measurements are taken at pharmaceutical manufacturing sites during production, at specialized pharmacies during preparation, and at hospitals and health care centers during application. Analysis of these occupational exposure measurements requires a fast and sensitive detection method. The LC/MS/MS technique meets all requirements and is the method of choice.

The introduction of the Agilent 1290 Infinity LC system has improved LC/MS/MS methods in several ways. The pressure stability of the Agilent 1290 Infinity LC system is up to 1200 bar, which is a significant improvement in comparison to Agilent 1100 Series and 1200 Series LC systems. In addition, the small dwell volume (the volume from the point of mixing solvent A and B up to the column inlet) is a considerable technical innovation over conventional HPLC systems. The Agilent 1290 Infinity LC system has a dwell volume of 45 µL (up to 135 µL when using the Jet Weaver) which shortens analysis time, improves detection sensitivity, and reduces organic solvent consumption.

This Application Note presents a comparison of an Agilent 1100 Series pump and the new Agilent 1290 Infinity LC system coupled to a tandem mass spectrometer. This comparison clearly indicates the improvements of an Agilent 1290 Infinity LC system over an Agilent 1100 Series LC system.

To perform the comparison, it was necessary to develop a method to separate cytostatic drugs with the Agilent 1290 Infinity LC system. Comparisons could then be made with the current internal method using an Agilent 1100 Series pump with an Agilent 1290 Infinity LC system. Commercially available method development software was used to find the best method parameters to develop an optimal method. Four basic chromatographic runs were performed to determine the best temperature and solvent gradient.

After that, a method was developed and experimentally confirmed with high agreement between simulation and experiment.

Experimental

The chromatographic calculations were done with Analyst Software version 1.5.

Agilent 1100 Series LC system

For the determination of cytostatic drugs by the current internal LC/MS/MS method, an Agilent 1100 Series LC system was used including:

- Agilent 1100 Series Binary Pump (G1312A)
- Agilent 1100 Series Degasser (G1379A)
- CTC-PAL Autosampler
- MayLab MistraSwitch column oven

Agilent 1290 Infinity LC system

For method development, an Agilent 1290 Infinity LC system was used. The system consisted of:

- Agilent 1290 Infinity LC System Binary Pump with integrated degasser (G4220A)
- Agilent 1290 Infinity LC System High Performance Autosampler (G4226A)
- Agilent 1290 Infinity LC System Thermostatted Column Compartment SL (G1316B)

Tandem mass spectrometer

An API 3000 triple quadrupole mass spectrometer equipped with a TurboIonSpray interface operating at 450 °C with ion spray probe voltages of 5000 V was used to detect the cytostatic drugs. The parameter settings for nebulizer, curtain, and collision gases (all nitrogen) were 15, 12, and 6 arbitrary units, respectively. The antineoplastic drugs were detected by multiple reaction monitoring (MRM). The pause time was 5 ms and the dwell time was 100 ms. Table 1 shows the MRM parameters for the analyzed compounds.

Analyte		Q/V	Q_1 [amu]	Q_3 [amu]	DP [V]	FP [V]	CE [eV]	CPX [V]
Gemcitabine (1)	$[M+H]^+$	Q	264	112	56	280	27	8
		V	264	95	56	280	59	6
Ifosfamide (2)	$[M+H]^+$	Q	261	92	36	200	37	10
		V	261	154	36	200	33	10
Cyclophosohamide (3)	$[M+H]^+$	Q	261	140	61	320	31	8
		V	261	233	61	320	37	16
Etoposide (4)	$[M+H]^+$	Q	589	229	16	130	21	16
		V	589	185	16	130	47	12
Paclitaxel (5)	$[M-Na]^+$	Q	830	549	101	330	35	38
		V	830	248	101	330	45	18
Docetaxel (6)	$[M-Na]^+$	Q	876	308	100	310	43	18
		V	876	591	100	310	35	36

Q: Quantification MRM; V: Verification MRM; DP: Declustering potential; FP: Focusing potential; CE: collision energy; CXP: Cell exit potential.

Table 1
MRM parameters for the analyzed compounds.

Analytes

The mixture of cytostatic drugs is an internal standard diluted in deionized water with a pH of 3 adjusted with 0.1 mol/L hydrochloric acid.

	Figure 1a	Figure 1b	Figure 1c
Agilent LC system	1100 Series	1290 Infinity LC	1290 Infinity LC
Stationary phase (Shimadzu Shim-pack XR-ODS)	50 × 3.0 mm, 2.2 µm	50 × 3.0 mm, 2.2 µm	50 × 2.0 mm, 2.2 µm
Solvents	A: H2O + 0.1 % HCOOH B: ACN + 0.1 % HCOOH		

	Figure 1a		Figure 1b		Figure 1c	
Solvent gradient	Time [min]	% B	Time [min]	% B	Time [min]	% B
	0.00	5	0.00	7	0.00	7
	0.55	5	0.59	24	0.39	24
	12.00	80	1.51	31	1.01	31
	13.00	5	3.14	31	2.09	31
	20.00	5	3.24	56	2.16	56
			6.50	56	4.00	56
			7.00	7	4.50	7
			10.70	7	6.15	7

	Figure 1a	Figure 1b	Figure 1c
Injection volume	50 µL	10 µL	20 µL
Concentration injected standard	250 ng/mL	500 ng/mL	0.1 ng/mL
Analytes on column	12.5 ng	5 ng	0.002 ng
Temperature	35.0 °C	31.3 °C	31.3 °C
Pressure drop	150 bar	95 bar	175 bar

Table 2
Description of the chromatographic conditions of Figure 1.

Results and discussion

Figure 1a shows the separation of six cytostatic drugs using the current internal Agilent 1100 Series LC/MS/MS method. The analytes were separated within approximately 13 minutes. However, the runtime of this method was approximately 20 min due to re-equilibration of three column volumes. Each analysis used 1.70 mL of acetonitrile. This method achieved a limit of detection of 0.1 ng/mL except for paclitaxel (0.5 ng/mL) based on a signal-to-noise ratio of 3:1.

Figure 1b shows the separation of the same cytostatic drugs using the newly developed method on an Agilent 1290 Infinity LC/MS/MS system. The same HPLC column with a diameter of 3.0 mm was used. The analytes were separated in approximately 6.5 minutes. Considering the re-equilibration time of three column volumes, the method had a run time of 10.7 minutes. Each analysis consumed 1.01 mL of acetonitrile.

A comparison of Figures 1a and 1b shows that the Agilent 1290 Infinity LC/MS/MS system separates the analytes two times faster than the Agilent 1100 Series LC/MS/MS system. This can be attributed to the small 45-µL dwell volume of the 1290 Infinity pump.

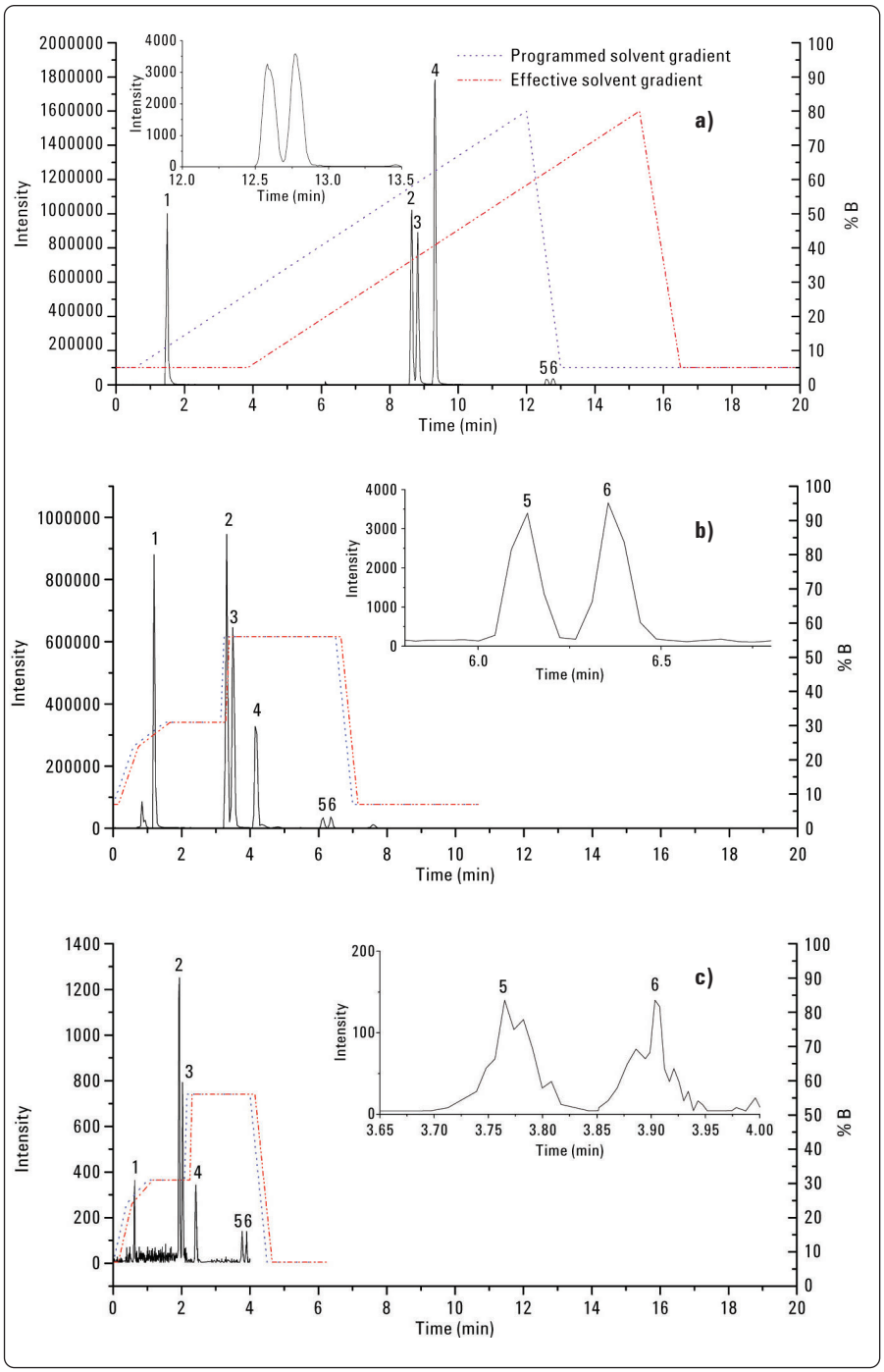

Figure 1
Separation of six cytotoxic drugs using different Agilent LC systems. For details see experimental section.

In contrast, the Agilent 1100 Series LC system exhibits a dwell volume of 1000 µL. At a flow rate of 300 µL/min, the programmed solvent gradient reaches the column inlet with a delay of 3 minutes. Furthermore, the early eluting peaks elute under isocratic conditions and cannot be affected by the solvent gradient.

Because of the small dwell volume of 45 µL for the Agilent 1290 Infinity LC system, the programmed solvent gradient reaches the column inlet with a delay of 9 seconds.

Furthermore, the 1290 Infinity LC system is well-designed. Individual modules such as the pump, autosampler, and column oven are arranged to minimize extra column volume. Due to the short gradient time in Figure 1b, a 40% reduction of organic solvent consumption can be obtained.

The separation shown in Figure 1b can be improved either by increasing the flow rate or by using narrower column diameters. To reduce organic solvent consumption, the 3 mm id column was replaced by a 2 mm id column, and a separation of the cytostatic drug mixture was performed at a flow rate of 300 µL/min. Figure 1c shows the separation of six antineoplastic drugs using the Agilent 1290 Infinity LC system with a 2 mm id column. The analytes were separated in 4 min. The method run time was 6.2 min including a re-equilibration time of three column volumes. Therefore, each analysis needed 0.64 mL of acetonitrile.

Figure 1c also shows significant separation improvement using a 2 mm id column while the resolution of the critical peak pair is not affected. In addition, narrower peaks are observed due to increased band compression from the shortened gradient time. Comparing the current internal method (Figure 1a) with the newly developed method using the Agilent 1290 Infinity LC system (Figure 1c) it is shown that analysis time is reduced by a factor of 3. In addition, consumption of organic solvent is reduced by 62 %, while detection sensitivity is improved by 3 to 7-times (Table 3).

A further decrease in analysis time is possible by increasing the flow rate up to 500 µL/min, however, in this case the cycle time of the API 3000 mass spectrometer was the limiting factor because of the minimum dwell time of 50 ms.

Analyte	LOD_A [ng/mL]	LOD_C [ng/mL]	Improvement
Gemcitabine (1)	0.10	0.02	5
Ifosfamide (2)	0.10	0.02	5
Cyclophosohamide (3)	0.10	0.02	5
Etoposide (4)	0.10	0.02	5
Paclitaxel (5)	0.50	0.07	7
Docetaxel (6)	0.10	0.03	3

Table 3
Comparison of Limits of Detection of the current in-house method (LOD_A) using an Agilent 1100 Series LC/MS/MS system and of the newly developed method (LOD_C) using an Agilent 1290 Infinity LC/MS/MS system.

Conclusion

It could be shown that the hyphenation of the Agilent 1290 Infinity LC system with a tandem mass spectrometer is very suitable to develop fast LC/MS/MS methods. The separation of six cytostatic drugs succeeded in four minutes by using acetonitrile as an organic modifier in the mobile phase. In addition, the presented methods highlight that fast HPLC separations are only possible using HPLC systems with very small dwell volumes. The Agilent 1100 Series LC system shows a dwell volume of approximately 1000 µL. If a flow rate of 300 µL/min is used, the programmed solvent gradient reaches the column inlet with a delay of about three minutes, so that the elution of the early eluting analytes occurs under isocratic conditions. Using the Agilent 1290 Infinity LC system with a dwell volume of 45 µL at a flow rate of 300 µL/min, the programmed solvent gradient reaches the column inlet after 9 seconds and enables fast separations within a few minutes. We could also show that the consumption of the organic modifier can be reduced by 62 %. Finally, a 3 to 7-times improvement in detection sensitivity could be achieved.

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January 1, 2010
Publication Number 5990-5193EN

Agilent Technologies