Developing a fast, generic method for rapid resolution LC with quadrupole MS detection

Combining the Agilent 6140 quadrupole MS and multimode source with the Agilent 1200 Series Rapid Resolution LC system

Application Note

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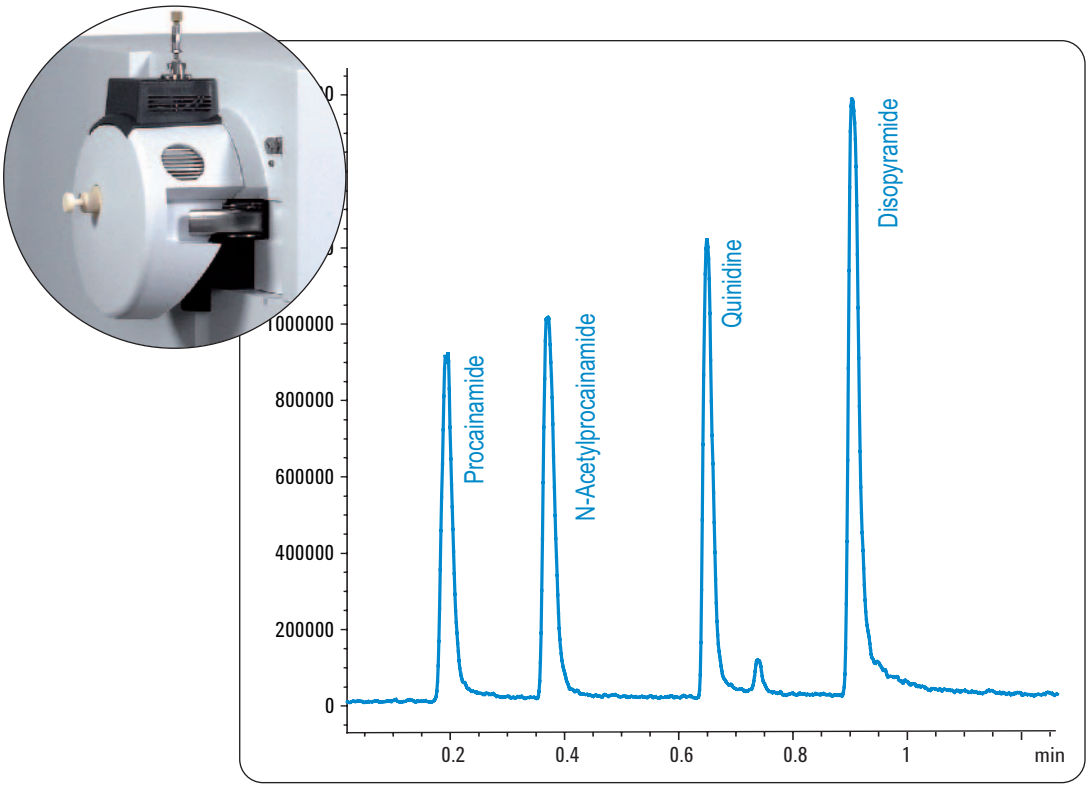

Abstract

In this Application Note the Agilent 6140 quadrupole MS, equipped with the multimode source, is combined with the Agilent 1200 Series Rapid Resolution LC system, to develop a fast, generic LC/MS method for:

- analysis of 35 pharmaceutical compounds from 17 compound classes
- reduction of the method run time from 2.1 to 0.8 minutes

Agilent Equipment

- 1200 Series Rapid Resolution LC system
- 6140 quadrupole MS detector
- Multimode Source

Application Area

- High throughput MS analysis in drug discovery

Agilent Technologies

Introduction

With the introduction of the Agilent 1200 Series Rapid Resolution LC system[1], it is now possible to run sub-2-micron particle columns at high flow rates and achieve run times of less than 1 – 2 minutes. With increased temperatures, 4.6 mm i.d. columns can be operated at flow rates of 5 mL/min but this flow rate is too high for electrospray (ESI) or atmospheric pressure chemical ionization (APCI). Therefore, usually 2.1 mm i.d. columns are used in a system with MS detection but these columns are also operated at flow rates above 1 mL/min. With a conventional source the maximum flow rate is around 1 mL/min (depending on the mobile phase composition). With the Agilent multimode source[2] with integrated, additional drying using IR emitters, the maximum flow rate can be increased up to 2 mL/min. Further, the multimode source offers the possibility of simultaneous ESI and APCI ionization, which makes it the ideal source for fast, generic MS detection. In combination with the fast scanning Agilent 6140 quadrupole MS[3] it is the ideal solution for fast and ultra-fast LC analysis with single quadrupole MS detection.

Experimental

Equipment

The Agilent 1200 Series Rapid Resolution LC/MS system contained the following modules:

- Agilent 1200 Series binary pump SL
- Agilent 1200 Series high-performance autosampler SL
- Agilent 1200 Series thermostatted column compartment SL
- Agilent 1200 Series diode array detector SL (micro flow cell: 2 µL, 3 mm path length)
- Agilent 6140 quadrupole LC/MS with multimode source

The system was controlled using the Agilent ChemStation (rev. B.03.01. SR1).). The new capillary that provides fast POS/NEG switching is standard on Agilent 6100 Series instruments shipped after August 2007. Earlier instruments would require the following in addition to the B.03.01.SR1 or later software:

- One resistive capillary (Agilent part number G1960-80060)
- One narrow-bore capillary (Agilent part number G1960-20310).

Results and discussion

Moving towards a generic method

Generic methods for liquid chromatography with UV detection often use trifluoro acetic acid (TFA) as ion-pairing modifier to achieve small peak widths and good peak shapes (figure 1A). For applications with MS detection TFA is usually avoided because it can lead to substantial ion suppression for certain compounds and therefore modifiers such as formic or acetic acid are preferred. On the other hand TFA cannot be replaced with formic or acetic acid for all application to maintain chromatographic quality (figure 1B).

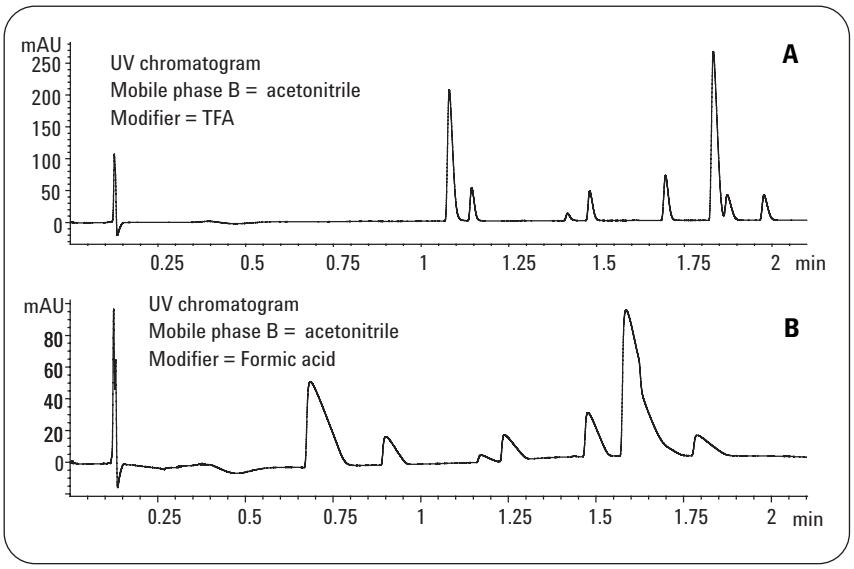

Figure 1
Influence of different modifiers on the chromatographic performance.

Whereas electrospray ionization (ESI) requires a modifier, atmospheric pressure chemical ionization (APCI) is controlled by vapor chemistry. The use of acetonitrile in APCI can prevent ion formation in both positive and negative ion polarity and, therefore, methanol is the preferred organic mobile phase for maximum analyte coverage. One thing that has to be considered when using methanol as organic mobile phase in chromatography is that the viscosity is higher than for acetonitrile. Therefore, the maximum flow rate until the maximum system back pressure is reached is lower than for acetonitrile. Further, the maximum viscosity for a water/methanol mixture is reached at about 50/50 v/v. This means the maximum system back pressure is not reached at the beginning but somewhere in the middle of the run. A flow rate must be selected that ensures that the system pressure is not exceeded even later in the run. Keeping the above mentioned facts in mind, the general rules for a generic method are:

- To ensure ionization of the compound, the multimode source should be operated in mixed mode
- Use acetonitrile as organic phase and formic or acetic acid as modifier if possible
- TFA should only be used if chromatography requires it
- If ionization is done in APCI or mixed mode (Multimode source) methanol should be used as mobile phase.

Sample name	Sample	Compound	Totals formulae	Monoisotopic mass
UH_TM_AH01	Antihypertensive drugs	Captopril	$C_9H_{15}NO_3S$	217.08
		Enalapril	$C_20H_{28}N_2O_5$	376.20
UH_TM_AA01	Antiasthmatic drugs	Enprofylline	$C_8H_{10}N_4O_2$	194.08
		Theobromine	$C_7H_8N_4O_2$	180.06
		Theophylline	$C_7H_8N_4O_2$	180.06
UH_TM_AB01	Antibacterial drugs	Trimethoprim	$C_{14}H_{18}N_4O_3$	290.14
		Furazolidone	$C_8H_7N_3O_5$	225.04
		Nalidixic acid	$C_{12}H_{12}N_2O_3$	232.08
		Penicillin-G	$C_{16}H_{18}N_2O_4S$	334.10
UH_TM_AH01	Antihistaminic drugs	Chlorpheniramine	$C_{16}H_{19}N_2Cl$	274.12
		Promethazine	$C_{17}H_{20}N_2S$	284.13
		Tripelennamine	$C_{16}H_{21}N_3$	255.17
UH_TM_AA02	Antiarrythmic drugs	Disopyramide	$C_{21}H_{29}N_3O$	339.23
		N-Acetylprocainamide	$C_{15}H_{23}N_3O_2$	277.18
		Procainamide	$C_{13}H_{21}N_3O$	235.17
		Quinidine	$C_{20}H_{24}N_2O_2$	324.18
UH_TM_TAD01	Tricyclic antidepressant drugs	Imipramine	$C_{19}H_{24}N_2$	280.19
		Protriptyline	$C_{19}H_{21}N$	263.17
		Trimipramine	$C_{20}H_{26}N_2$	294.21
UH_TM_S01	Sulfa drugs	Sulfadiazine	$C_{10}H_{10}N_4O_2S$	250.05
		Sulfamerazine	$C_{11}H_{12}N_4O_2S$	264.07
		Sulfamethazine	$C_{12}H_{14}N_4O_2S$	278.08
UH_TM_CCB01	Calcium channel blockers	Nifedipin	$C_{17}H_{18}N_2O_6$	346.12
		Nimodipin	$C_{21}H_{26}N_2O_7$	418.17
		Nisoldipin	$C_{20}H_{24}N_2O_6$	388.16
		Nitrendipin	$C_{18}H_{20}N_2O_6$	360.13
UH_TS_05	Antidepressant drugs	Bupropion	$C_{13}H_{18}NOCl$	239.11
UH_TS_07	Antiepileptic drugs	Phenytoin	$C_{15}H_{12}N_2O_2$	252.09
UH_TS_08	Antitussive drugs	Dextromethorphan	$C_{18}H_{25}NO$	271.19
UH_TS_12	Analgesic drugs	4-Hydroxyantipyrine	$C_{11}H_{12}N_2O_2$	204.09
UH_TS_15	Antianginal drugs	Verapamil	$C_{27}H_{38}N_2O_4$	454.28
UH_TS_18	Antiinflammatory drugs	Naproxen	$C_{14}H_{14}O_3$	230.09
UH_TS_20	Androgen drugs	Testosteron	$C_{19}H_{28}O_2$	288.21
UH_TS_23	Glucocorticoid drugs	Prednisolon	$C_{21}H_{28}O_5$	360.19
UH_TS_24	Muscle-relaxing drugs	Papaverin	$C_{20}H_{21}NO_4$	339.15

Table 1
Analyzed pharmacological compounds.

Application example

To test a generic method 35 pharmacological compounds were selected and analyzed using a fast, generic method with a run time of less than 2 minutes. There are other possibilities to reduce the cycle time, for example, alternating column regeneration, however, this was not the focus of this study but is described in other publications. Since the compounds belong to completely different compound classes from steroids to antibacterial drugs, TFA was used as modifier. The multimode source was operated in mixed mode, and methanol was used as organic mobile phase. The list of analyzed compounds is shown in table 1.

The following method was used to analyze the compounds:

- Column: Agilent ZORBAX SB-C18 2.1 x 50 mm, 1.8 µm
- Solvents: A: water + 0.05 % TFA B: methanol + 0.045 % TFA
- Gradient: 0 min, 10 %B; 1.7 min, 92.5 %B
- Flow rate: 1.6 mL/min
- Stop time: 1.7 min
- Post time: 0.2 min
- Injection volume: 1 µL
- Column temperature: 80 °C
- Diode array detector: 230 nm/40 (ref. 550 nm/50)
  Peak width: > 0.0025 min (80 Hz)
  Flow cell: Micro flow cell (2 µL, 3 mm path length)

Multimode source, mixed mode, positive scan, ultra-fast scan:

- Scan: 150–800 m/z
- Step size: 0.2 (fixed)
- Peak width: 0.01 min
- Drying gas flow: 7 L/min
- Neb. pressure: 60 psig
- Drying gas temp.: 350 °C
- Vaporizer temp.: 250 °C
- Cap. voltage: 2000 V
- Corona current: 2 µA
- Charging voltage: 2000 V

Figure 2 shows four example MS chromatograms for different compound classes. Under the selected, generic conditions only the two compounds phenytoin and naproxen did not ionize very well but still gave good MS spectra. Only two compounds – imipramine and protryptiline – did not separate well.

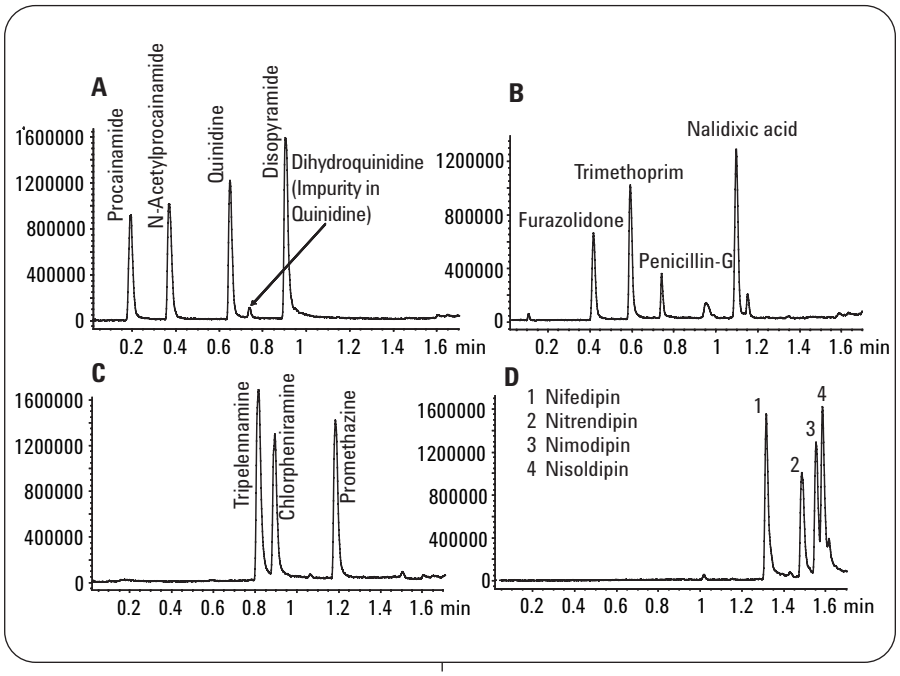

Figure 2
A) Antiarrythmic drugs B) Antibacterial drugs
C) Antihistaminic drugs D) Calcium channel blockers

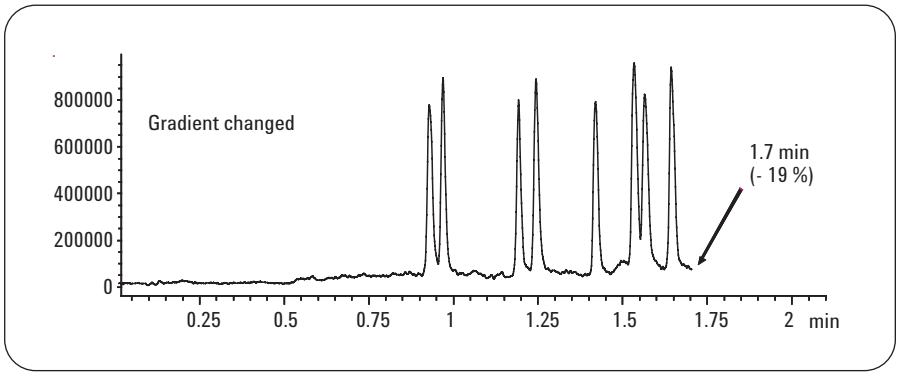

Figure 3
Optimized gradient, MS chromatogram (TIC).

Moving towards an ultra-fast method

The multimode source does not only offer the possibility of simultaneous ESI and APCI ionization but also higher flow rates can be used due to drying using infrared emitters. Therefore, different steps were done to reduce the run time of the sample and method shown in figure 1A, from 2.1 to below 1 minute. As a starting point for optimization, the gradient slope was changed until no significant reduction in resolution was discovered. With the optimized gradient the runtime could already be reduced by 19 % (figure 3).

In the next step the automatic delay volume reduction feature of the Agilent ChemStation was turned on. This feature removes the autosampler volume from the flow path after the sample is transferred to the column. To ensure leaving the autosampler capillaries and flow path in a defined condition after removing it from the flow path, it is important to add an isocratic part to the method prior to the gradient. With the removal of the autosampler delay volume, the run time could be reduced by 28 % compared to the starting conditions (figure 4).

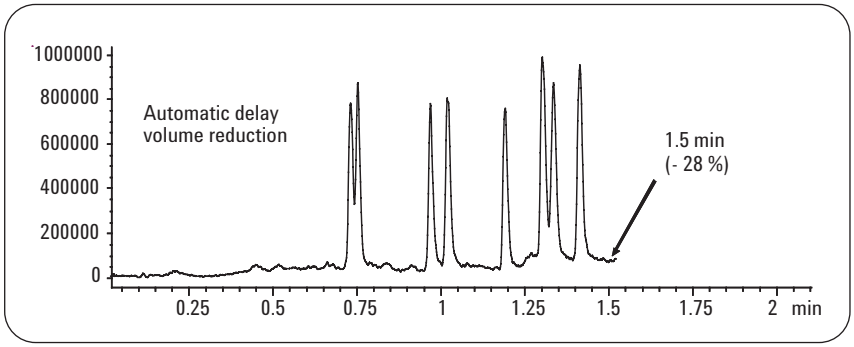

Figure 4
Automatic delay volume reduction, MS chromatogram (TIC).

In the next step the flow was increased from 1.2 to 1.6 mL/min, which shortened the run time to 1.2 minutes without compromising too much resolution. This can be done because of the almost flat van- Deemter curve for sub-2-micron particles for higher flow rates (figure 5).

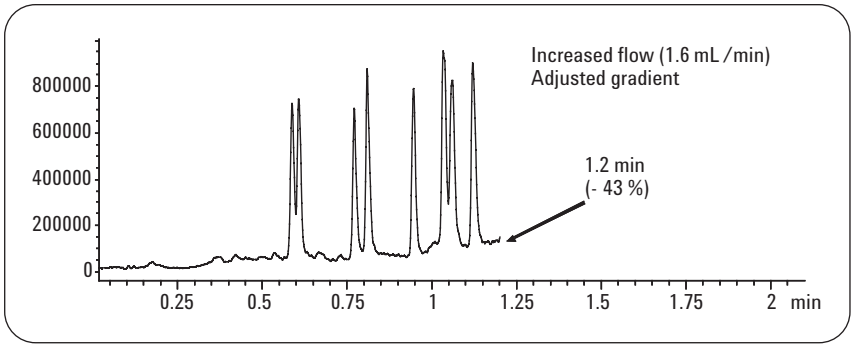

Figure 5
Increase of flow rate, MS chromatogram (TIC).

With increasing flow the back pressure also increases and at a certain point the maximum back pressure of the system will be reached. However, if the temperature, at which the column is used is also increased, the viscosity of the mobile phase is decreased and the system back pressure is lowered. Since the separation was done on an Agilent ZORBAX Eclipse Plus C18 2.1 x 50 mm, 1.8 µm column, the maximum column temperature was 60 °C. Changing to an Agilent ZORBAX StableBond C18 column with the same dimensions allowed a further increase of the temperature to 90 °C (figure 6).

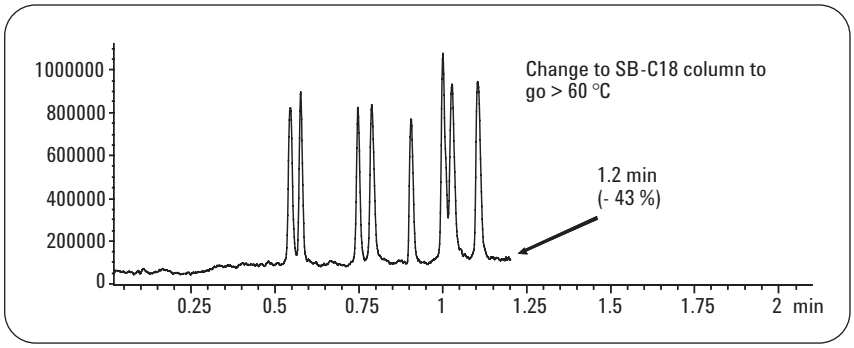

Figure 6
Change to ZORBAX Stable Bond column, MS chromatogram (TIC).

With the increased temperature the flow could be further increased up to the maximum flow rate of 2 mL/min that can be used with the multimode source. With the final method the run time could be reduced from 2.1 minutes (figure 7a) down to 0.8 minutes (figure 7b) with almost the same performance.

In the example shown here the run time of the method could be reduced from 2.1 minutes down to 0.8 minutes (reduction by 62 %) with very similar performance. This was achieved by using steeper gradients, increased temperature and higher flow rates, which was only possible because the multimode source can be operated at these high flow rates.

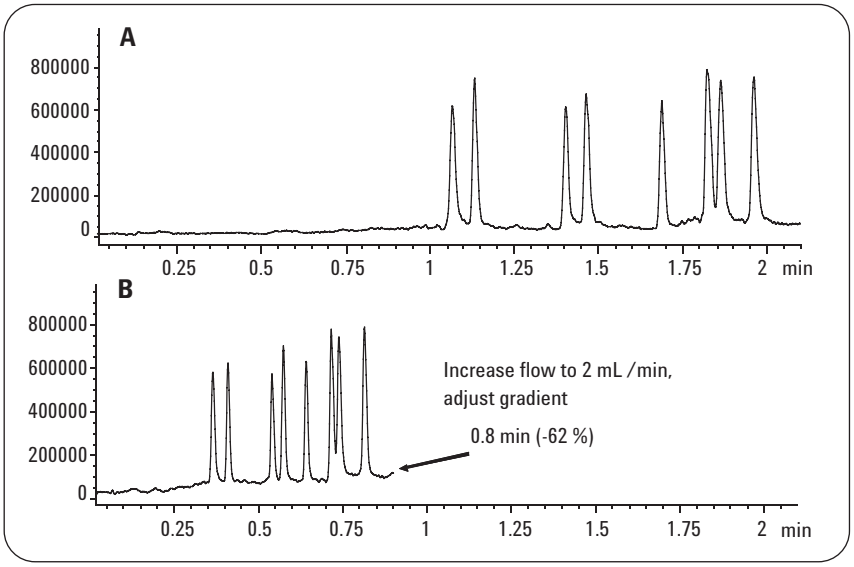

Figure 7
A) and B) Separation with initial and final method.

Conclusion

In this Application Note a generic method was developed on the Agilent 1200 Series Rapid Resolution LC system in combination with the Agilent 6140 quadrupole MS equipped with the multimode source. This method was used to analyze 35 pharmaceutical compounds from 17 different compound classes. Finally, the generic method was further optimized to reduce the run time from 2.1 down to 0.8 min without compromising chromatographic quality.

References

1.
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2.
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