Application Note

Clinical Research

Chiral separation of methamphetamine and amphetamine on an Agilent InfinityLab Poroshell 120 Chiral-V column with detection by LC/MS

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Abstract

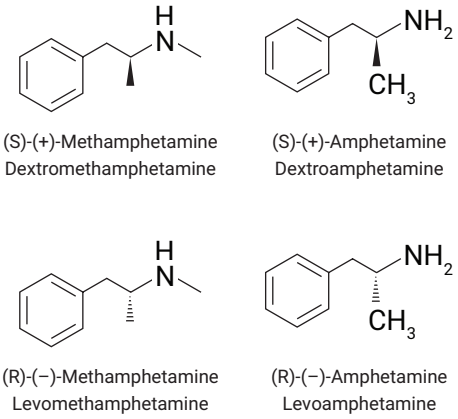
An Agilent InfinityLab Poroshell 120 Chiral-V 2.1 × 150 mm, 2.7 µm column (p/n 683775-604) was used to analyze methamphetamine and amphetamine by LC/MS, using a mobile phase of methanol with 0.1 % acetic acid and 0.02 % ammonium hydroxide. The analysis was accomplished in 5 minutes, with a resolution Rs of 1.9 or better for racemic amphetamine and methamphetamine.

Introduction

Superficially porous particle columns are a popular tool in liquid chromatography. Superficially porous particle columns generate high efficiency at lower pressure relative to their totally porous particle column counterparts[1]. This is primarily due to a shorter mass transfer distance and substantially narrower particle size distribution of the particles in the column[2]. The current trend with superficially porous particles is reducing particle size for further efficiency improvements. The higher efficiency can be used to speed up analyses, or improve results by increasing resolution and sensitivity.

Using LC/MS in the analysis of therapeutics and metabolites has become increasingly popular due to its selectivity, sensitivity, and speed. For the analysis of amphetamines, LC/MS eliminates the need to derivatize, and facilitates direct, 100 % detection. However, mass spectrometry alone is unable to distinguish between stereoisomers, since it characterizes compounds solely in terms of mass. Consequently, separations are required before the mass spectrometer.

Methamphetamine and amphetamine are chiral molecules (Figure 1). For both compounds, the D-enantiomer has greater biological activity than the L-enantiomer. Both the individual enantiomers and the racemate of methamphetamine are controlled substances (Class A in Europe and Schedule II in the US). Levomethamphetamine is the chemical precursor of the anti-Parkinson's drug Selegiline[3]. Selegiline is also metabolized into levomethamphetamine and levoamphetamine[4,5]. This has caused users to test positive for amphetamines[6,7]. The traditionally used method for analysis, immunoassay, is unable to distinguish between the enantiomers, and can give incomplete, inconclusive results.



(S)-(+)-Methamphetamine
Dextromethamphetamine

(S)-(+)-Amphetamine
Dextroamphetamine

(R)-(−)-Methamphetamine
Levomethamphetamine

(R)-(−)-Amphetamine
Levoamphetamine

Figure 1. Chemical structures of amphetamine and methamphetamine enantiomers.

This study demonstrates the UHPLC performance of a 2.7 µm Agilent InfinityLab Poroshell 120 Chiral-V column using LC/MS, including the baseline resolution of two racemic pairs.

Experimental

An Agilent 1290 Infinity LC system with an Agilent 6460 triple quadrupole LC/MS was used in this experiment. The system was modified from its standard configuration to have low system volume and dispersion. Table 1 shows the instrument configuration details. Table 1 lists the Agilent InfinityLab Poroshell 120 Chiral-V 2.1 × 150 mm, 2.7 µm column used in this work. Table 2 shows the LC and MS parameters.

The methamphetamine and amphetamine samples were bought as a racemic mixture from Cerriliant, Round Rock, Texas, USA, at 1 mg/mL in methanol. The samples were diluted in mobile phase prior to injection. Acetic acid was bought from Sigma-Aldrich. Ammonium hydroxide was bought from GFS Chemicals as Veritas Grade double-distilled, Columbus, Ohio USA. Methanol was bought from Honeywell (Burdick and Jackson). Water was 0.2 µm filtered 18 MW from a Milli-Q system (Millipore).

Table 1. Instrument configuration details.

Parameter	Value
LC	
Column	Agilent InfinityLab Poroshell 120 Chiral-V 2.1 × 150 mm, 2.7 µm (p/n 683775-604)
Mobile phase	Methanol:acetic acid:ammonium hydroxide 1,000:1:0.2 (isocratic)
Flow rate	0.25 mL/min
Column temperature	20 °C
Injection volume	0.2 µL
MS	
Ionization mode	ESI positive (Agilent Jet Stream)
Gas temperature	300 °C
Gas flow	5.0 L/min
Sheath gas temperature	250 °C
Capillary voltage	3,500 V
Nozzle voltage	500 V

Table 2. Standards and MRM values.

Analyte	Precursor ion (*m/z*)	Product ion (*m/z*)	Fragmentor voltage	Collision energy	Cell accelerator voltage	Dwell time
Methamphetamine	150.1	91.1	75	20	4	200
Methamphetamine	150.1	65.1	75	44	4	200
Amphetamine	136.1	119	90	4	3	200
Amphetamine	136.1	91	90	16	3	200

Results and discussion

Figure 2 shows the separation of (R)-(−)- and (S)-(+)-methamphetamine and (R)-(−)- and (S)-(+)-amphetamine on an InfinityLab Poroshell 120 Chiral-V 2.1 × 150 mm, 2.7 µm column. With LC/MS detection, baseline chromatographic resolution is not necessary for all compounds, as the detector resolves the analytes by their specific mass fragments. However, when enantiomeric compounds are present, baseline chromatographic resolution is necessary. In this case, chromatographic resolution for methamphetamine and amphetamine enantiomers were found to be two or better at 20 °C, providing good integration and quantitation of these two isomers. Figure 3 shows resolution and retention time at three temperatures, and Table 3 presents a summarization of them. Increasing temperature has the effect of decreasing retention time and increasing selectivity, while lowering column pressure decreases mobile phase viscosity.

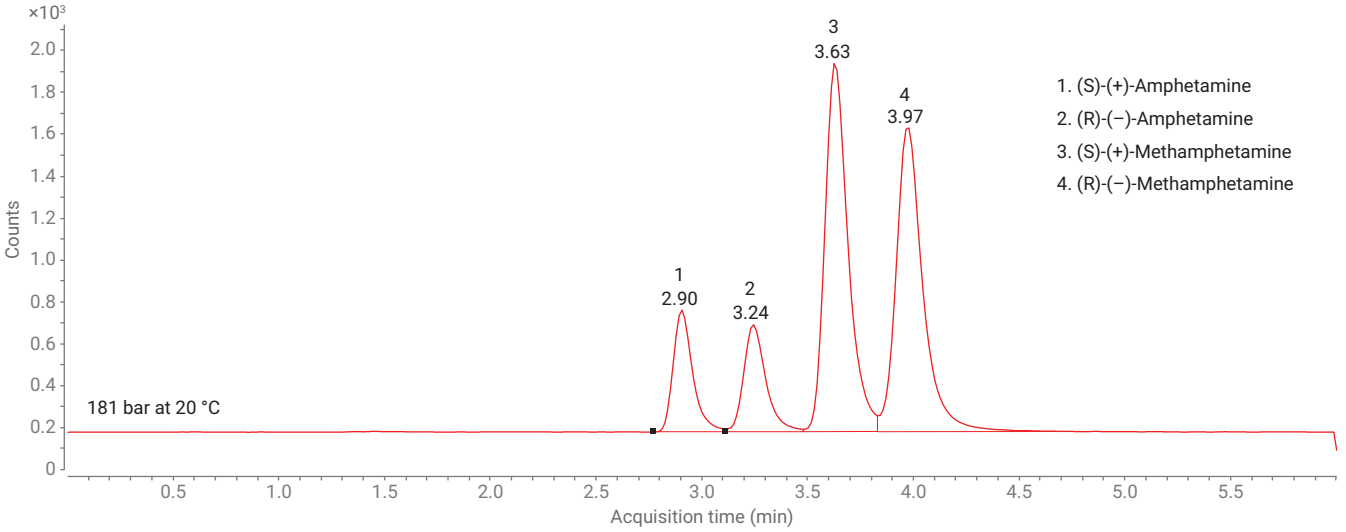


Figure 2. Analysis of methamphetamine and amphetamine using an Agilent InfinityLab Poroshell 120 Chiral-V 2.1 × 150 mm, 2.7 µm column. Injection volume 0.2 µL with 5 µg/mL each of methamphetamine and amphetamine. The column was connected to the MS using 320 mm, 0.075 mm id tubing to reduce extra-column broadening.

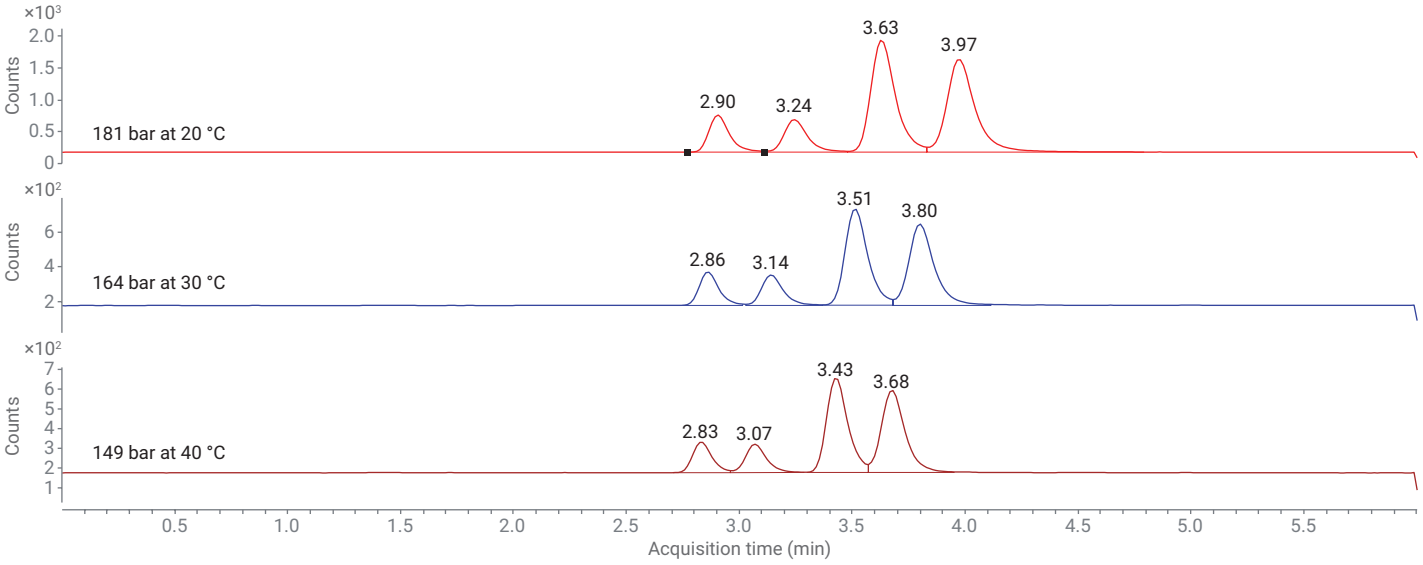


Figure 3. Analysis of methamphetamine and amphetamine using an Agilent InfinityLab Poroshell 120 Chiral-V 2.1 × 150 mm, 2.7 µm column at different temperatures. Flow rate 0.25 mL/min, 1 L MeOH, 1 mL acetic acid, 200 µL NH_4OH.

Conclusions

The Agilent InfinityLab Poroshell 120 Chiral-V 2.7 µm column was used to accomplish a separation of chiral methamphetamine and amphetamine by LC/MS. The high efficiency of this small superficially porous particle column provided sufficient resolution to resolve the racemic pairs at baseline.

Table 3. Retention and resolution at varied temperatures.

Temperature (°C)	Retention time (min)				Resolution		
	Peak 1	Peak 2	Peak 3	Peak 4	Peaks 1–2	Peaks 2–3	Peaks 3–4
20	2.39	3.24	3.63	3.94	2.1	2.1	1.8
30	2.86	3.14	3.51	3.80	1.8	2.2	1.6
40	2.83	3.07	3.43	3.68	1.5	2.2	1.4

Peak 1: (S)-(+)-Amphetamine
Peak 2: (R)-(−)-Amphetamine
Peak 3: (S)-(+)-Methamphetamine
Peak 4: (R)-(−)-Methamphetamine
Sample: 5 µg/mL each of (±)methamphetamine and (±)amphetamine diluted in mobile phase.
Flow rate: 0.25 mL/min, 1 L MeOH, 1 mL acetic acid, 200 µL NH_4OH.

References

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