Method Transfer from an Agilent 1200 Series LC to an Agilent 1260 Infinity II LC

Proof of Equivalency for the Analysis of Tricyclic Antidepressant Drugs

Application Note

Small Molecule Pharmaceuticals

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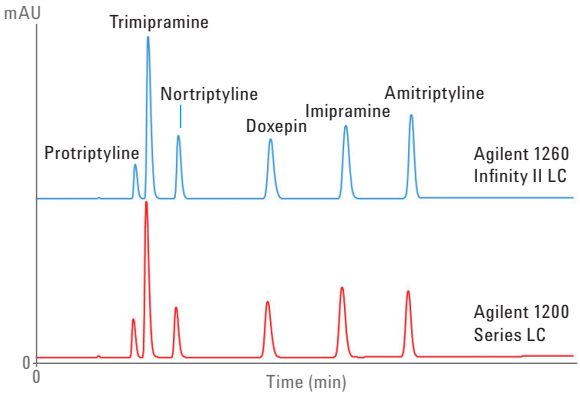
Abstract

Instrument-to-instrument method transfer is an important topic for laboratories in the pharmaceutical and other industries. This Application Note shows the seamless transfer of a conventional LC method for the analysis of tricyclic antidepressant drugs from an Agilent 1200 Series LC to an Agilent 1260 Infinity II LC.



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Introduction

Tricyclic antidepressants (TCAs) are pharmacologically active compounds, named after their chemical structure containing three rings, that are primarily used as antidepressants[1,2]. Originally, TCAs were synthesized as antihistamines (H1 receptor antagonists) in the 1950s[1,2]. TCAs are rapidly absorbed after oral administration[2] and, as most antidepressants, they act as inhibitors of the re-uptake of serotonin or norepinephrine into nerve endings[1,2]. This leads to an increase in the synaptic concentrations of these neurotransmitters[1].

Method transfer is important in QA/QC laboratories, clinical research, and other industries, for example, when replacing older LC instruments with new LC instruments such as the Agilent 1260 Infinity II LC, a reliable, robust, and efficient LC for everyday analysis. This Application Note shows the seamless transfer of a conventional LC method for the analysis of tricyclic antidepressant drugs from an Agilent 1200 Series Quaternary LC to an Agilent 1260 Infinity II LC.

Experimental

Instrumentation

The Agilent 1260 Infinity II LC comprised the following modules:

- Agilent 1260 Infinity II Quaternary Pump (G7111B)
- Agilent 1260 Infinity II Vialsampler (G7129A) with integrated column compartment, 3.0 µL heater (Option #063) and sample cooler (Option #100)
- Agilent 1260 Infinity II Variable Wavelength Detector (G7114A) with standard flow cell (G1314-60186)

The Agilent 1200 Series Quaternary LC comprised the following modules:

- Agilent 1200 Series Quaternary Pump (G1311A)
- Agilent 1260 Infinity High Performance Degasser (G4225A)
- Agilent 1200 Series Autosampler (G1329A) with Agilent 1200 Series Thermostat (G1330B)
- Agilent 1200 Series Thermostatted Column Compartment (G1316A)
- Agilent 1200 Series Variable Wavelength Detector SL (G1314C) with standard flow cell (G1314-60186)

Software

Agilent OpenLAB CDS Version 2.1 (availability planned for September 2016)

Column

Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm (p/n 959993-902)

Chemicals

All solvents were LC grade. Methanol was purchased from Merck (Darmstadt, Germany). Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak, EMD Millipore, Billerica, MA, USA). Ammonium bicarbonate, amitriptyline hydrochloride, doxepin hydrochloride, imipramine hydrochloride, nortriptyline hydrochloride, protriptyline hydrochloride, and trimipramine hydrochloride were purchased from Sigma-Aldrich (Steinheim, Germany).

Sample

A mixture of the tricyclic antidepressant drugs amitriptyline, doxepin, imipramine, nortriptyline, protriptyline, and trimipramine was prepared in water/methanol (50/50; v/v) at a concentration of 50 µg/mL.

Results and Discussion

Figure 1 and Table 2 show the conventional LC analysis of the TCA drugs amitriptyline, doxepin, imipramine, nortriptyline, protriptyline, and trimipramine on the 1200 Series Quaternary LC with the corresponding retention time (RT) and area precision, as well as resolution.

Methods

Table 1. Chromatographic conditions for conventional LC analysis.

Parameter	Description
Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm
Solvent	A) 10 mM Ammonium bicarbonate in water, pH 8 B) Methanol
Gradient	60 %B at 0 minutes, 68 %B at 10 minutes, 90 %B at 20 minutes
Stop time	20 minutes
Post time	10 minutes
Flow rate	1.5 mL/min
Temperature	40 °C
Injection volume	10 µL
Detection	254 nm, peak width > 0.05 minutes

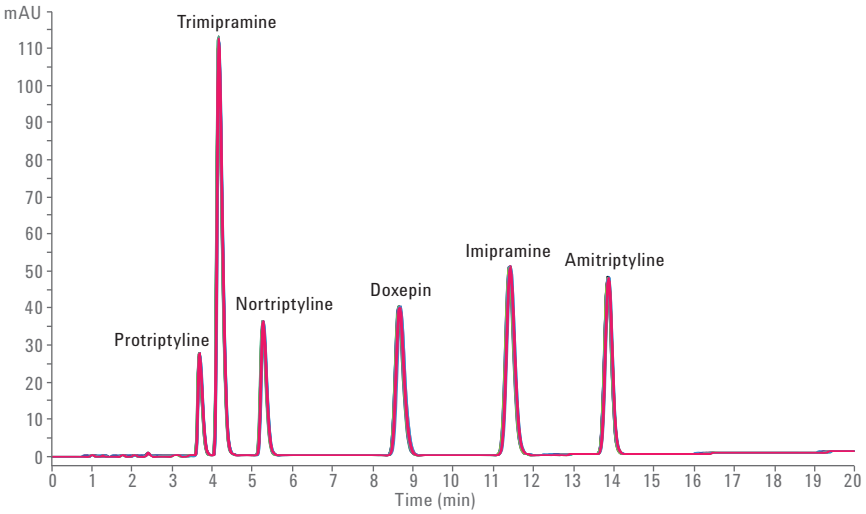

Figure 1. Conventional LC analysis of tricyclic antidepressant drugs on an Agilent 1200 Series Quaternary LC; overlay of 10 consecutive runs.

Table 2. Conventional LC analysis of tricyclic antidepressant drugs on an Agilent 1200 Series Quaternary LC; retention time and area precision were determined from 10 consecutive runs.

Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
Protriptyline	3.69	0.20	211.9	0.17	–
Trimipramine	4.16	0.19	1,054.9	0.11	2.1
Nortriptyline	5.27	0.18	347.5	0.16	4.4
Doxepin	8.66	0.19	553.4	0.18	11.0
Imipramine	11.41	0.11	728.4	0.13	7.4
Amitriptyline	13.86	0.09	580.9	0.10	6.9

The retention of the TCAs is highly sensitive towards small changes in the organic content in the solvent. This is shown in Figure 2 and Table 3, where the RTs of the TCAs obtained with the original gradient (Table 1) and gradients containing 2 % more and 2 % less methanol are compared.

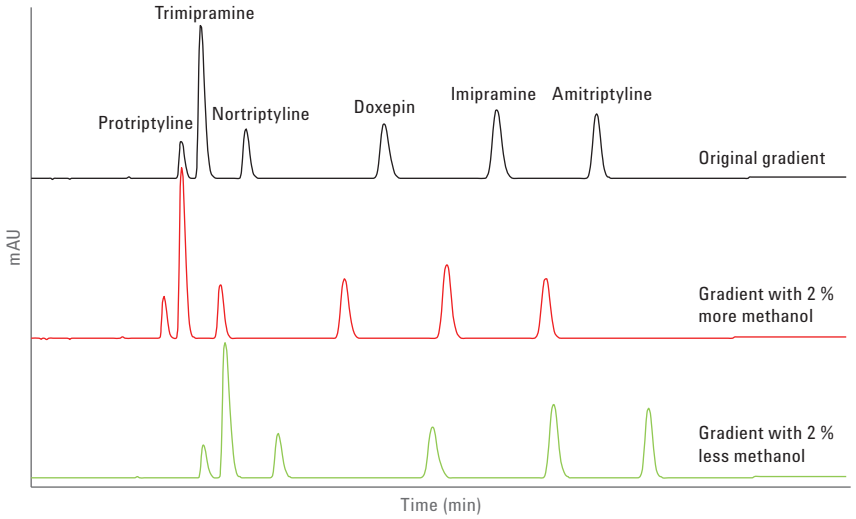

Figure 2. Conventional LC analysis of tricyclic antidepressant drugs on an Agilent 1200 Series Quaternary LC using the original gradient and gradients containing 2 % more and 2 % less methanol.

Table 3. Comparison of retention times for conventional LC analysis of tricyclic antidepressant drugs on an Agilent 1200 Series Quaternary LC using the original gradient and gradients containing 2 % more and 2 % less methanol.

Compound	Original RT (min)	RT deviation with 2 % more methanol (%)	RT deviation with 2 % less methanol (%)
Protriptyline	3.69	-11.2	14.8
Trimipramine	4.16	-11.0	14.4
Nortriptyline	5.27	-11.5	15.1
Doxepin	8.66	-11.0	13.7
Imipramine	11.41	-10.5	12.3
Amitriptyline	13.86	-8.9	9.3

The method for conventional LC analysis of the TCAs was transferred to the 1260 Infinity II LC without any changes. The resulting separation, corresponding RT and area precision, as well as resolution are presented in Figure 3 and Table 4. Excellent retention time and area precision was achieved with the 1260 Infinity II LC.

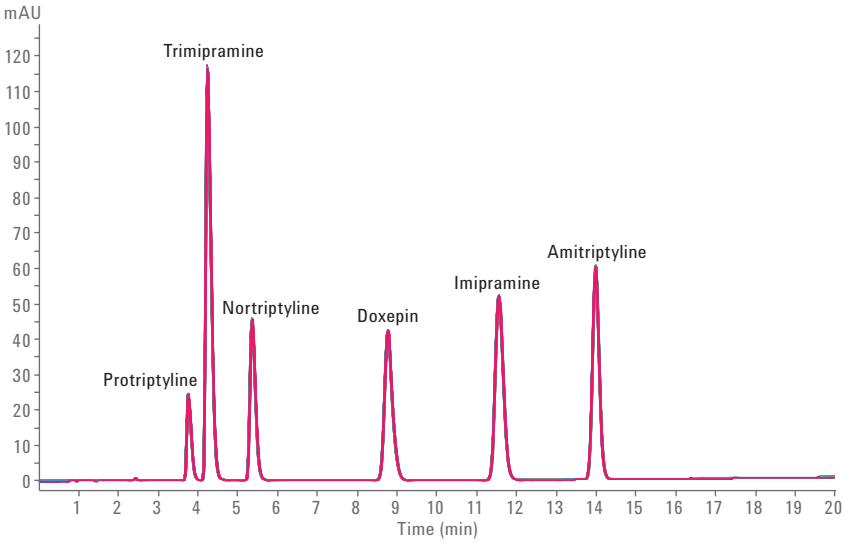

Figure 3. Conventional LC analysis of tricyclic antidepressant drugs on an Agilent 1260 Infinity II LC; overlay of 10 consecutive runs.

Table 4. Conventional LC analysis of tricyclic antidepressant drugs on an Agilent 1260 Infinity II LC; retention time and area precision were determined from 10 consecutive runs.

Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
Protriptyline	3.76	0.06	186.4	0.17	–
Trimipramine	4.24	0.06	1,084.4	0.11	2.1
Nortriptyline	5.36	0.06	434.3	0.09	4.4
Doxepin	8.77	0.03	587.6	0.09	11.1
Imipramine	11.56	0.03	740.7	0.07	7.5
Amitriptyline	13.99	0.02	713.7	0.05	7.0

Figure 4 and Table 5 compare the retention times of the tricyclic antidepressant drugs analyzed on the 1200 Series Quaternary LC and the 1260 Infinity II LC. As shown above, the retention of the TCAs reacts highly sensitively towards small changes in the organic content in the solvent. Even for these compounds and conditions, with a maximum deviation of 1.9 %, an excellent agreement of RTs between the 1200 Series Quaternary LC and the 1260 Infinity II LC was observed. This shows the possibility of seamless transfer of a conventional LC method for the analysis of TCAs from the 1200 Series LC to the 1260 Infinity II LC.

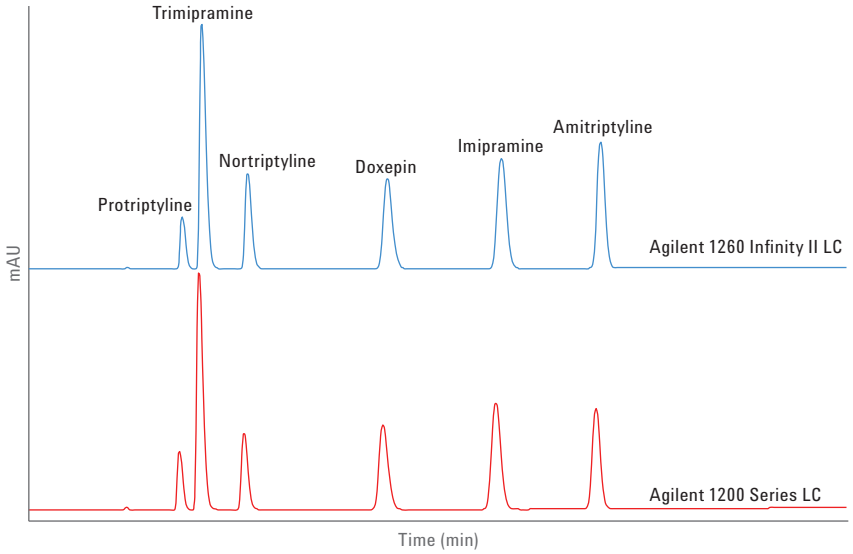

Figure 4. Conventional LC analysis of tricyclic antidepressant drugs on an Agilent 1200 Series Quaternary LC and an Agilent 1260 Infinity II LC.

Table 5. Conventional LC analysis of tricyclic antidepressant drugs on an Agilent 1200 Series Quaternary LC and an Agilent 1260 Infinity II LC; comparison of retention times.

Compound	RT Deviation (min)	RT Deviation (%)
Protriptyline	0.07	1.9
Trimipramine	0.07	1.8
Nortriptyline	0.09	1.7
Doxepin	0.11	1.3
Imipramine	0.15	1.3
Amitriptyline	0.13	0.9

In this Application Note, instrument control of the 1200 Series Quaternary LC and the 1260 Infinity II LC, as well as data analysis, was performed using Agilent OpenLAB CDS software, version 2.1. This version of OpenLAB CDS offers a single software for liquid chromatography, gas chromatography, and mass spectrometry. It provides a flat user interface, and customized and interactive reporting with drag-and-drop template creation. Figure 5 shows an example of the data analysis in OpenLAB CDS. The Peak Explorer available in the data analysis, for example, enables at-a-glance inspection of large data sets.

Agilent InfinityLab columns and supplies work together perfectly with the 1260 Infinity II LC for maximum performance and efficiency of LC workflows. Agilent InfinityLab Poroshell columns, in combination with the pressure range of up to 600 bar of the 1260 Infinity II LC, enable UHPLC analyses, offering time and solvent savings while maintaining or increasing peak resolution. When ordering a 1260 Infinity II LC, the customer has a choice between different InfinityLab Poroshell columns that can be delivered with the system. The InfinityLab Quick Connect fittings (p/n 5067-6166, Quick Connect fitting with a 0.17 × 105 mm capillary) and InfinityLab Quick Turn fittings (p/n 5067-5966) enable tool-free, fast, and easy column installation, ensuring a perfect column connection independent of the user. The setup of the 1260 Infinity II LC on the InfinityLab Flex Bench rack (p/n 5043-1252) enables efficient use of lab space and an ergonomic approach with easy access to the instrument.

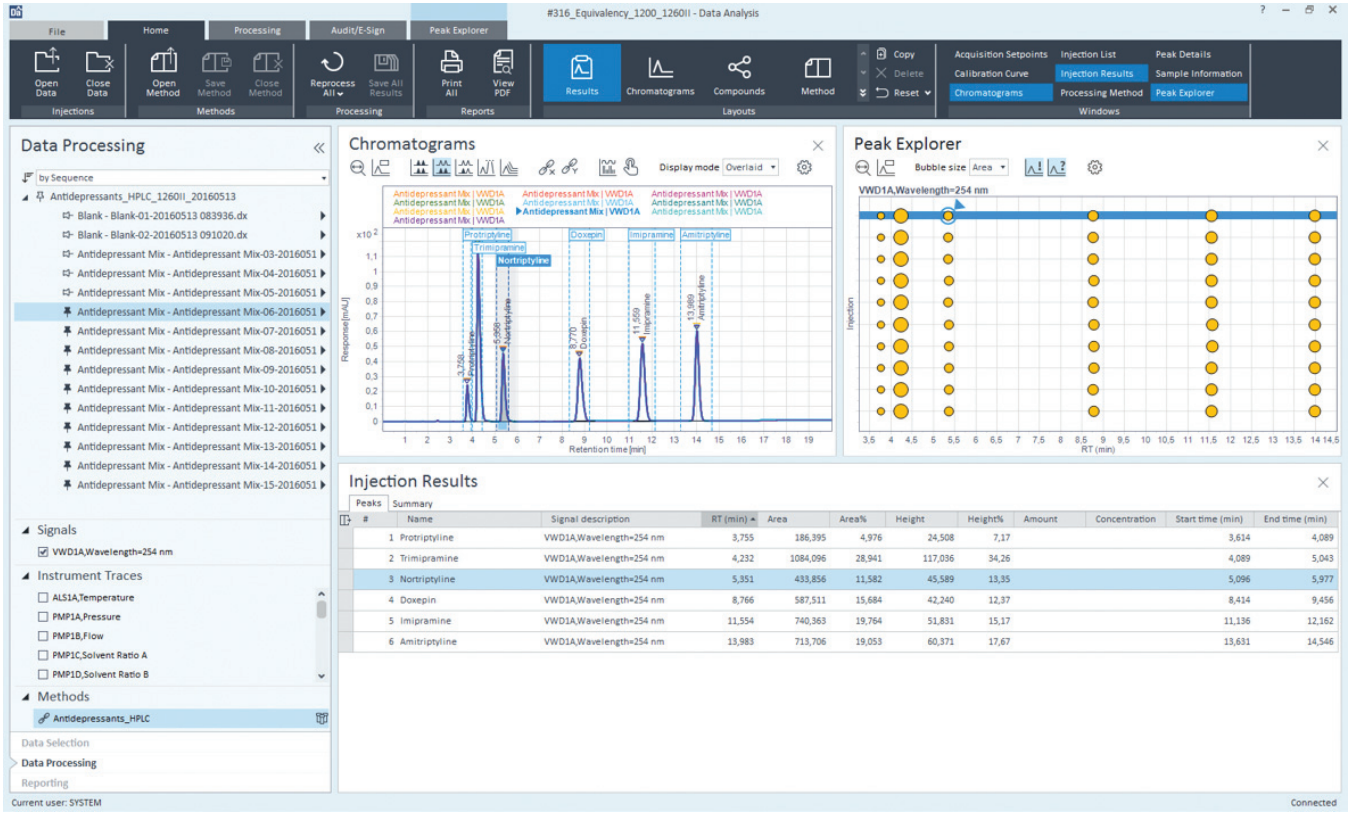

Figure 5. Example of the data analysis in Agilent OpenLAB CDS software, version 2.1.

Conclusions

The transfer of a conventional LC method for the analysis of tricyclic antidepressant drugs from an Agilent 1200 Series Quaternary LC to an Agilent 1260 Infinity II LC showed a maximum retention time deviation of 1.9 %. This proves the equivalency of the Agilent 1260 Infinity II LC compared to the Agilent 1200 Series Quaternary LC for the analysis of tricyclic antidepressant drugs, and shows the possibility of a seamless transfer of a conventional LC method.

References

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2. Gillman, P. K. Tricyclic antidepressant pharmacology and therapeutic drug interactions updated, *British Journal of Pharmacology* **2007**, *151*, 737–748.

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