

# Agilent 1290 Infinity II LC with ISET— Emulation of the Agilent 1100 Series Binary LC for Analysis of Tricyclic Antidepressant Drugs

## Application Note

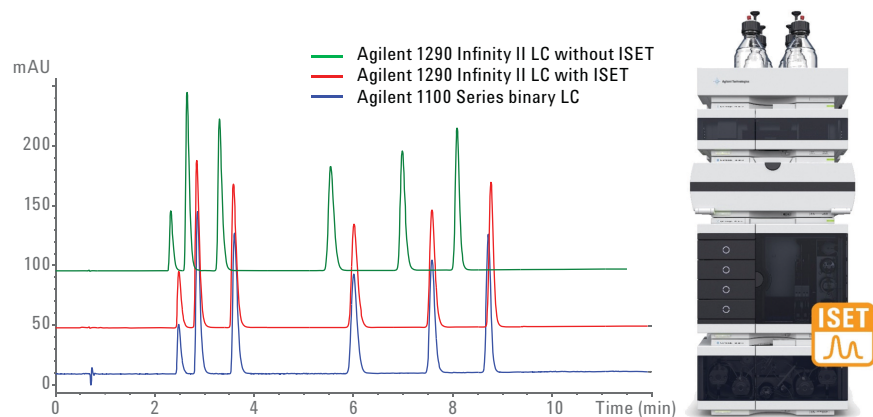
Small Molecule Pharmaceuticals

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### Abstract

Agilent Intelligent System Emulation technology (ISET) in the Agilent 1290 Infinity II LC facilitates the transfer of methods from conventional HPLC systems such as the Agilent 1100 Series binary LC. This Application Note illustrates the advantages of ISET for the analysis of six tricyclic antidepressants. The 1290 Infinity II LC, with ISET enabled, achieved a 99 % agreement of retention times in contrast to the same system with ISET disabled.



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## Introduction

Agilent Intelligent System Emulation technology (ISET) provides for the emulation of different liquid chromatography systems for seamless method transfer from one instrument to another<sup>1,2</sup>. Seamless LC method transfer is possible without changing the original methods, for example, by adding isocratic steps, resulting in the same chromatographic results. This allows the user to run legacy methods without the need for any modifications to the instrument or original methods – making the Agilent 1290 Infinity II LC a truly universal system for executing other HPLC and UHPLC methods. However, the user has the option to benefit from the wide power range and superior performance of the 1290 Infinity II LC.

Tricyclic antidepressants were one of the first-used ataractics against depressive diseases. They are named after their characteristic chemical structure, containing three annulated rings. All components have mood-enhancing effects, which become noticeable usually after two to four weeks. Tricyclic antidepressants inhibit the resumption of the neurotransmitter serotonin, noradrenalin, and dopamine into the neurons of the brain. Typically, the concentrations of those neurotransmitters are too low in the brain of persons with depressive diseases. The increased availability after using tricyclic antidepressants helps to balance the lack of neurotransmitter.

The analysis of tricyclic antidepressants is important in clinical chemistry as well as in forensic toxicology. High-performance liquid chromatography (HPLC) is a robust and reliable tool typically used for the determination and quantification in serum, blood, or urine.

This Application Note describes the separation of a mix of six tricyclic antidepressants. It demonstrates that methods from an 1100 Series binary LC can be transferred easily to a 1290 Infinity II LC without the need to change the original method, and that the same retention times are achieved by simply enabling the ISET function.

## Experimental

### Instrumentation

All experiments were carried out on an 1100 Series binary LC and on a 1290 Infinity II LC, comprising the following modules.

Agilent 1100 Series binary LC:

- Agilent 1100 binary pump (G1312A)
- Agilent 1100 autosampler (G1313A)
- Agilent 1100 thermostatted column compartment (G1316A)
- Agilent 1100 diode array detector (G1315B)

Agilent 1290 Infinity II LC:

- Agilent 1290 Infinity II high-speed pump (G7120A)
- Agilent 1290 Infinity II multisampler (G7167B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)
- Agilent 1290 Infinity II diode array detector (G7117B)

### Chromatographic conditions

Table 1. Chromatographic conditions.

Parameter	Value
Mobile phase	A) Ammonium bicarbonate buffer 10 mM, pH 8 B) Methanol
Flow rate	1 mL/min
Gradient	0 minutes, 60 %B 5 minutes, 68 %B 10 minutes, 90 %B
Stop time	12 minutes
Post time	5 minutes
Needle wash mode	Standard wash
Injection volume	1 µL
Column temperature	50 °C
Detection	254/10 nm, reference 360/100 nm, > 0.025 minutes (0.5 seconds response time), 10 Hz

### Column

Agilent ZORBAX Eclipse XDB-C18, 4.6 × 75 mm, 3.5 µm (p/n 966967-902)

### Software

Agilent OpenLab CDS ChemStation Edition for LC and LC/MS systems, Rev. C.01.07 [22 with ISET 4]

### Sample

Mix of six tricyclic antidepressants (in order of elution): protriptyline, trimipramine, nortriptyline, doxepin, imipramine, and amitriptyline.

### Chemicals

All solvents used were LC grade. Methanol was purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak). The ammonium bicarbonate and the tricyclic antidepressants were purchased from Sigma-Aldrich, St. Louis, Missouri, U.S.

## Results and Discussion

For the analysis of tricyclic antidepressants, a conventional method was chosen as already presented in an Agilent Application Note in 2002<sup>3</sup>. The method included a conventional HPLC column, 4.6 × 75 mm, packed with 3.5 μm particles. The method was used on an 1100 Series binary LC, on a 1290 Infinity II LC without ISET, as well as on a 1290 Infinity II LC with ISET. Generally, method transfer from conventional instrumentation to UHPLC systems can be problematic due to different mixing behavior as well as significantly lower delay and transition volumes of modern UHPLC systems (Figure 1). The retention times of the six tricyclic antidepressants were shorter on the 1290 Infinity II LC compared to the retention times on the 1100 Series binary LC. This effect would be visible on every other modern UHPLC instrument in a similar way.

Two approaches are commonly applied to solve this problem:

1. Addition of an isocratic step at the beginning of the gradient
2. Increasing of delay volume by the addition of extra tubing

Both methods can compensate smaller delay volumes, but mixing performance or transition volume are not affected. Without any compensation, the retention time shifts were between 6 and 9 % for all six analytes (Table 2). Figure 2 shows the method transfer from the 1100 Series binary LC to the 1290 Infinity II LC with and without ISET enabled.

After enabling ISET, the retention times agreed well with the retention times of the original chromatogram. In contrast to the retention times on the 1290 Infinity II without ISET, the deviation was below 1 % for all analyzed compounds (right column in Table 2). A shift of less than 5 % is regarded as tolerable in accordance with Intelligent System Emulation technology<sup>4</sup>.

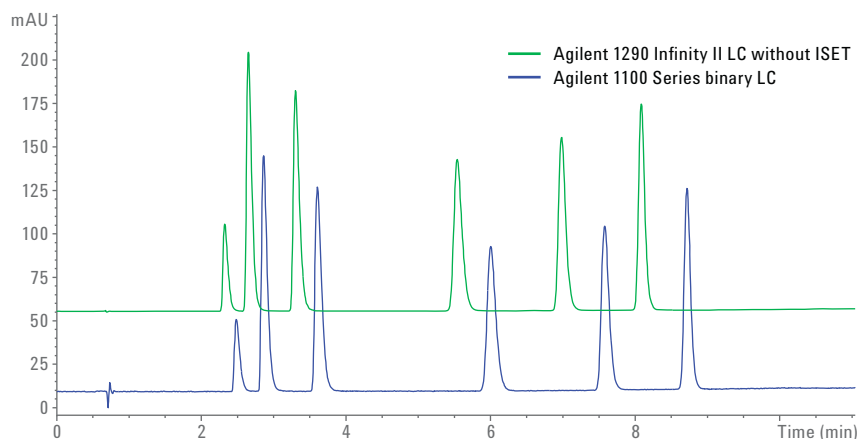


Figure 1. Transfer of a conventional method for separation of six tricyclic antidepressants from the Agilent 1100 Series Binary LC to the Agilent 1290 Infinity II LC System without ISET enabled.

Table 2. Retention times and deviations on an Agilent 1100 Series binary LC and Agilent 1290 Infinity II LC.

	Agilent 1100 Series Binary LC	Agilent 1290 Infinity II LC			
	RT (min)	RT without ISET (min)	RT with ISET (min)	Deviation without ISET (%)	Deviation with ISET (%)
Protriptyline	2.483	2.324	2.481	6.40	0.08
Trimipramine	2.861	2.651	2.839	7.34	0.77
Nortriptyline	3.605	3.303	3.571	8.38	0.94
Doxepin	6.004	5.541	5.995	7.71	0.15
Imipramine	7.578	6.984	7.561	7.84	0.22
Amitriptyline	8.716	8.086	8.758	7.23	-0.48

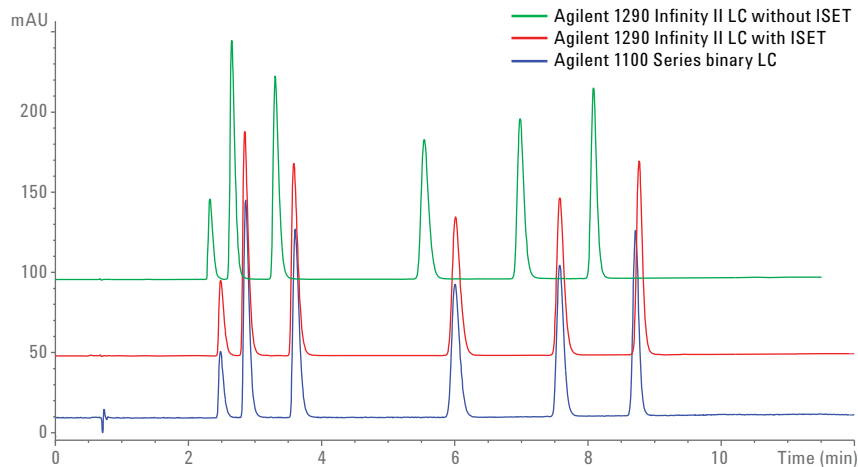


Figure 2. Overlay of chromatograms of a conventional method used on an Agilent 1100 Series binary LC, an Agilent 1290 Infinity II LC with ISET, and an Agilent 1290 Infinity II LC System without ISET.

The precision of retention times and areas obtained on a 1290 Infinity II LC with ISET was significantly better compared to those from a 1100 Series LC, (Table 3).

## Conclusion

The emulation of other HPLC or UHPLC instruments using the Agilent 1290 Infinity II LC with the ISET function allows users to run existing HPLC or UHPLC methods without modifications of instruments or methods, and achieve identical chromatograms (with over 99 % agreement of retention times). This was shown for the analysis of six tricyclic antidepressant drugs with a conventional method run on an Agilent 1100 Series binary LC as well as on an Agilent 1290 Infinity II LC with ISET enabled. In addition, the precision of retention times and areas was significantly improved.

Table 3 Comparison of RT and area precision between an Agilent 1100 Series binary LC and an Agilent 1290 Infinity II LC.

	RSD RT (%)		RSD Area (%)	
	Agilent 1100 Series Binary LC	Agilent 1290 Infinity II LC	Agilent 1100 Series Binary LC	Agilent 1290 Infinity II LC
Protriptyline	0.091	0.068	0.072	0.158
Trimipramine	0.106	0.049	0.238	0.127
Nortriptyline	0.09	0.009	0.043	0.101
Doxepin	0.073	0.007	0.262	0.145
Imipramine	0.057	0.007	0.307	0.137
Amitriptyline	0.032	0.006	0.222	0.132

## References

1. Gratzfeld-Huesgen, A., Agilent 1290 Infinity Binary LC with ISET – Emulation of the Waters Alliance 2695 LC System Analyzing Analgesics, *Agilent Technologies Application Note*, publication number 5991-2792EN, **2013**.
2. Gratzfeld-Huesgen, A., Seamless instrument-to-instrument method transfer from an Agilent 1100/1200 Series LC to an Agilent 1290 Infinity LC using Intelligent System Emulation Technology (ISET), *Agilent Technologies Application Note*, publication number 5990-9113EN, **2011**.
3. Huber, U., Analysis of Tricyclic Antidepressant Drugs by HPLC, *Agilent Technologies Application Note*, publication number 5988-7626EN, **2002**.
4. Agilent 1290 Infinity II with ISET, *Agilent Technologies User Manual*, part number G4220-90314, **2015**.

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