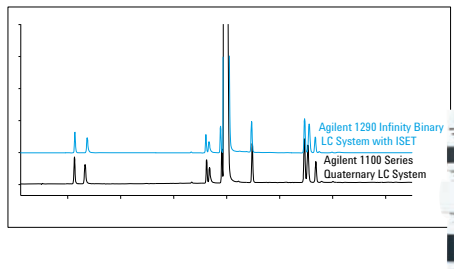
Transferring Methods to the Agilent 1290 Infinity LC Using Intelligent System Emulation Technology (ISET)

Analysis of Metoclopramide Hydrochloride and its Impurities

A.G.Huesgen, Agilent Technologies, Inc, Waldbronn, Germany



Agilent Intelligent System Emulation Technology (ISET) is a function within the Agilent 1290 Infinity LC System that offers seamless transfer of methods from conventional LC systems such as the Agilent 1100 Series Quaternary LC System, which has a higher delay volume and different mixing behaviour, to the 1290 Infinity LC System. When the ISET function is enabled on the 1290 Infinity LC System, almost the same retention times and resolution can be obtained. This Technical Overview describes the transfer of a conventional HPLC method from a 1100 Series Quaternary LC System to the 1290 Infinity LC System. Metoclopramide and its impurities were analysed on both systems. Measurements were done using the 1290 Infinity LC System both with and without ISET enabled. To further improve agreement of retention times and resolution, the fine-tuning option of ISET was deployed. Retention times and resolution of the different experiments were evaluated and compared.

The transfer of a method from a conventional LC to a UHPLC system such as the Agilent 1290 Infinity LC System will always result in significant differences in retention times and resolution of the analysed peaks.

To avoid the need for revalidation of legacy methods, an isocratic hold at the beginning of the run is often deployed or additional delay volume is installed. The disadvantage of these approaches is that only the delay volume is adjusted.

The different mixing behaviour of the two pumps cannot be compensated by these solutions. Using ISET, available in the 1290 Infinity LC, the difference in delay volume and in mixing behaviour is compensated. As a result, retention times and resolution are typically very similar to the original data.

The experiments described in this Technical Overview show how the 1290 Infinity LC with ISET was used to emulate the behaviour of an Agilent 1100 Series Quaternary LC. A method used to analyse metoclopramide and its impurities was transferred to a 1290 Infinity LC System.

Experiments were done with and without using ISET functionality. Further, the fine tuning option of ISET was used to demonstrate how method transfer can be further optimised. Retention times and resolution for all experiments were evaluated and compared.

Experimental

Instrumentation and software

The Agilent 1290 Infinity LC System consisted of the following modules:

- Agilent 1290 Infinity Binary Pump
- Agilent 1290 Infinity Autosampler with Thermostat
- Agilent 1290 Infinity Thermostatted Column Compartment
- Agilent 1260 Infi nity Diode Array Detector

The Agilent 1100 Series LC consisted of the following modules:

- Agilent 1100 Series Quaternary Pump
- Agilent 1100 Series Autosampler
- Agilent 1100 Series Thermostatted Column Compartment
- Agilent 1100 Series Diode Array Detector

*The detector cell of the 1100 Series DAD had a volume of 13µL. Note that ISET (revision 1.0) does not compensate for after-column dispersion volume.

Software: Agilent ChemStation revision C.01.03 and ISET revision 1.0. All LC modules had firmware revisions A.06.32, B.06.32, or B.06.41 or higher, and RC.Net drivers.

Sample

The following mixture of compounds was used for the experiments:

Main: Metoclopramide hydrochloride
X: Bromated metoclopramide
Impurity 1: 4-Amino-5-chloro-2-methoxybenzoic acid (EP C)
Impurity 2: 4-(Acetylamino)-2-hydroxybenzoic acid (EP H)
Impurity 3: 4-Amino-5-chloro-N-2-(diethylaminoethyl)-2-methoxybenzamide N-oxide (EP G)
Impurity 4: 4-Amino-5-chloro-N-2-(diethylaminoethyl)-2-hydroxybenzamide (EP F)
Impurity 5: 4-(Acetylamino)-5-chloro-N-2-(diethylaminoethyl)-2-methoxybenzamide (EP A)
Impurity 6: Methyl 4-(acetylamino)- 2-methoxybenzoate (EP D)
Impurity 7: Methyl 4-(acetylamino)-2-hydroxybenzoate
Impurity 8: Methyl 4-(acetylamino)-5-chloro-2-methoxybenzoate (EP B)
Impurity 9: Methyl 4-amino-2-methoxybenzoate

Chromatographic conditions

Column: Agilent ZORBAX Eclipse Plus C18, 150°— 3.0mm, 3.5µm
Mobile phase: Water + NH4Ac (2.5 g/L), pH 6.99/Acetonitrile
Flow rate: 0.6mL/min
Gradient: 5% ACN at 0 min to 57.5% ACN at 15 min
Stop time: 15 min
Post-time: 5 min
Injection volume: 1µL (with needle wash for 3 s (for the Agilent 1290 Infinity Autosampler only)
Column temp: 37°C
Detection: 275/4nm, Ref. 400/60nm, 5Hz, slit 4nm

Results and discussion

Metoclopramide and its impurities are compounds that react extremely sensitivly to changes in mobile phase composition and temperature.

Therefore, transferring a method for these compounds is a demanding task [2].

Metoclopramide and its impurities were analysed using a conventional method with a 150 x 3.0mm column packed with 3.5µm particles. First, the method was transferred to the 1290 Infinity LC System without using ISET. In the next step, the method was transferred using ISET, see the resulting chromatograms in *Figure 1*, in which the original chromatogram from the 1100 Series LC System was overlaid with the chromatograms measured using the 1290 Infinity LC System with and without ISET.

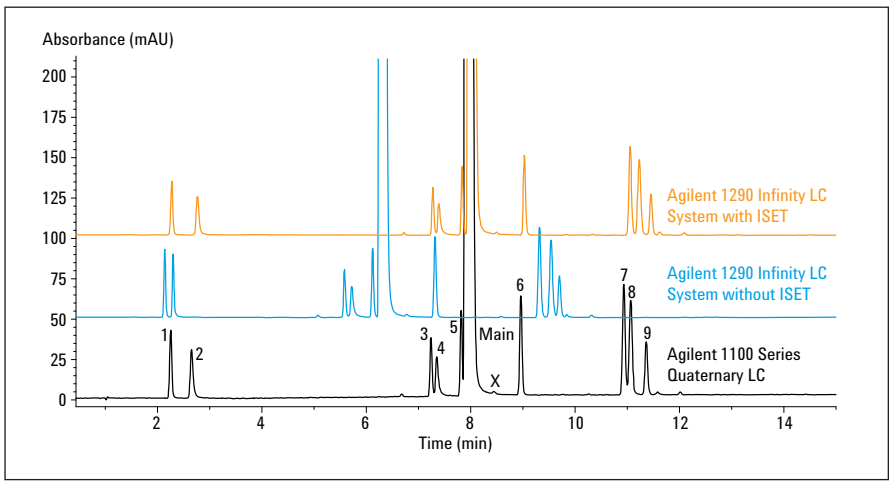


Figure 1. Overlay of original Agilent 1100 Series LC System chromatogram with the Agilent 1290 Infinity LC System chromatograms with and without ISET.

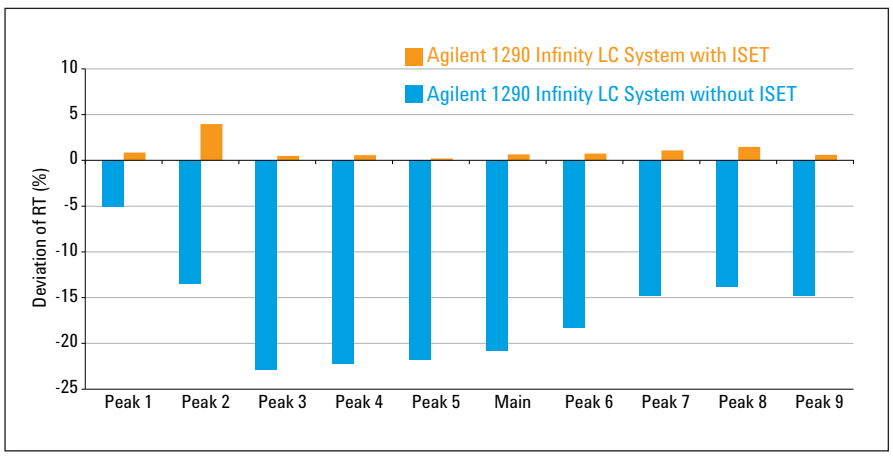
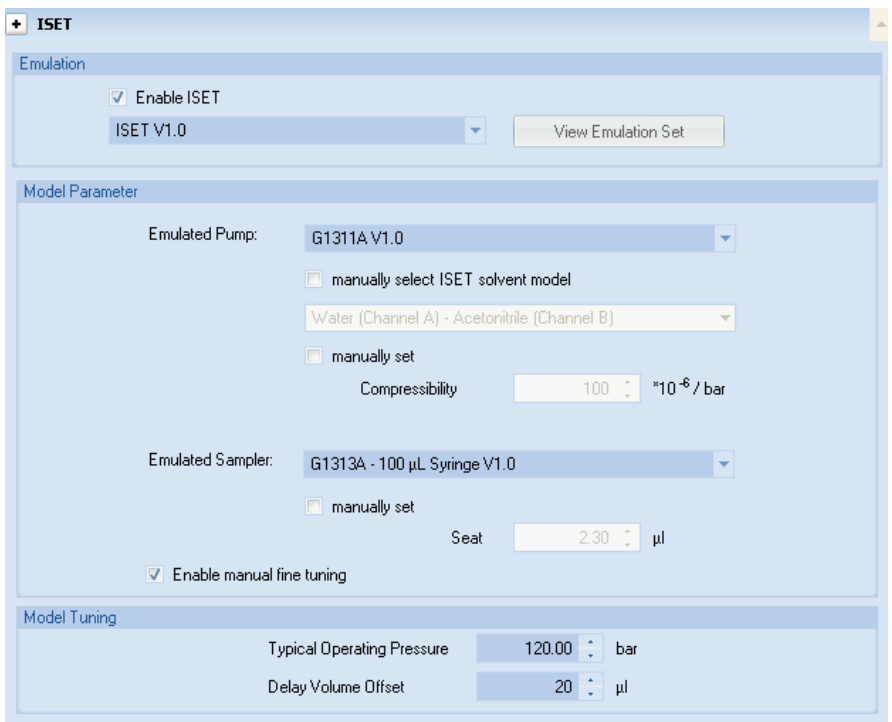


Figure 2. Deviation of retention times from original Agilent 1100 Series LC System data using the Agilent 1290 Infinity LC System with and without ISET.

Without deploying ISET, all peaks obviously shifted to lower retention times. In contrast, when ISET was used, all retention times correlated to the original chromatogram to a great extent. Using the 1290 Infinity LC System with or without ISET gave better resolution in both cases especially for peak 2, the main peak and peak 8. This was mainly due to the lower after-column dispersion volume of the 1290 Infinity LC System. The retention times for all three chromatograms were evaluated and the differences to the original chromatogram were calculated as percentages, *(Figure 2)*.

Without ISET, the retention times shifted between 5 and 23% compared to the original retention times. With ISET, the retention times shifted only by 1.5% to later retention times except for the second peak, which shifted by 4% to a later retention time.

To further improve the agreement between retention times, the Enable Manual Fine Tuning option within ISET was activated, *(Figure 3)*. The retention times shifted to slightly earlier retention times by adding a 20-µL Delay Volume Offset. The maximum backpressure on the Agilent 1100 Series Quaternary LC was about 120 bar for the analysis of metoclopramide. This value was added to the Typical Operating Pressure field within the software. During the formation of gradients the system pressure increases and changes the damper volume of the 1100 Series LCs. The Typical Operating pressure function can be used to compensate for this additional volume.



Applying these new conditions, the agreement between the original 1100 Series data and 1290

Figure 3. ISET software menu for enabling manual fine-tuning.

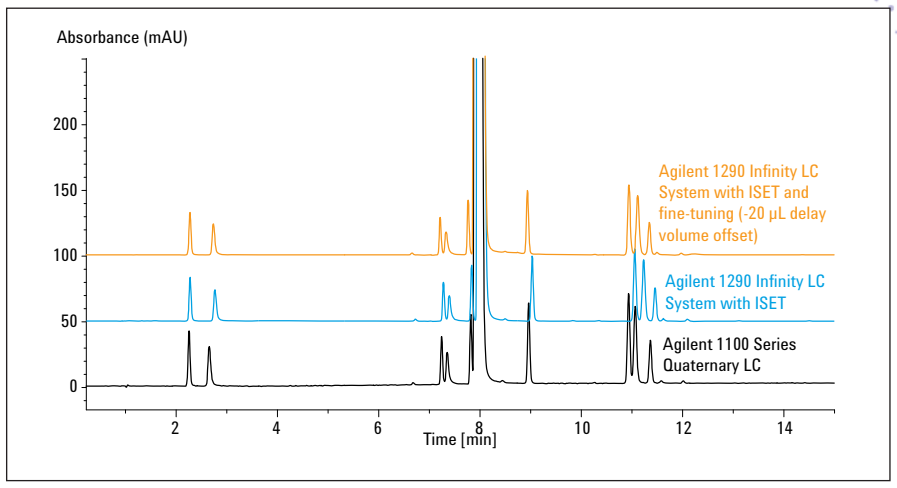


Figure 4. Overlay of the original Agilent 1100 Series LC System chromatogram, the chromatogram of Agilent 1290 Infinity LC System with ISET, and the chromatogram of 1290 Infinity LC System with ISET and with fine-tuning.

Infinity chromatograms with ISET, and with fine-tuning, was further improved, *(Figure 4)*.

The retention times of the original chromatogram and of the chromatogram with the fine-tuning step showed nearly 100% agreement. The results for the three chromatograms with respect to deviation of retention times are summarised in *Figure 5*.

With ISET and additional fine-tuning, the deviation of retention times was less than 3% for the second peak and less than 0.8% for the remaining peaks. The resolution was typically better on the 1290 Infinity LC System. The absolute values for the resolution are combined in *Figure 6*. The agreement for all peaks was very good. The differences are due to the after-column dispersion volumes.

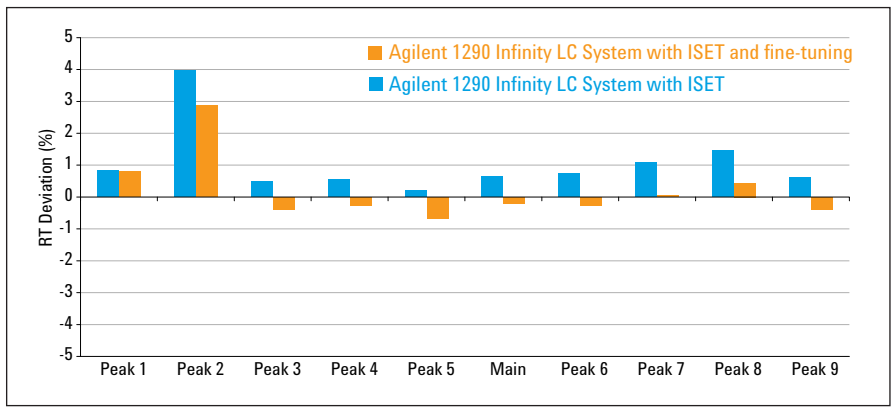


Figure 5. Deviation of retention time from the original Agilent 1100 Series LC System data using Agilent 1290 Infinity LC System with ISET and the Agilent 1290 Infinity LC System with ISET and fine-tuning.

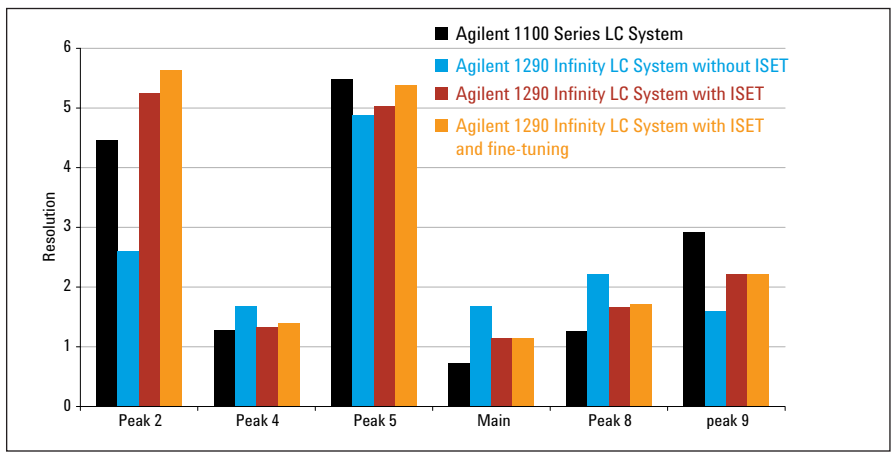


Figure 6. Differences of absolute data for the resolution of Agilent 1100 Series LC System, Agilent 1290 Infinity LC System with and without ISET, and Agilent 1290 Infinity LC System with ISET and additional fine-tuning.

Conclusion

The Agilent Intelligent System Emulation Technology (ISET) facilitates seamless transfer of conventional methods to the 1290 Infinity LC System and thereby achieving excellent agreement of retention times and resolution. A conventional method for the analysis of metoclopramide and its impurities was transferred from the 1100 Series Quaternary LC System to the 1290 Infinity LC System. Enabling the ISET function for the 1290 Infinity LC resulted in deviations for the retention times smaller than 1.5% for all but one peak. Using in addition the fine-tuning option of ISET the deviation of retention times could be reduced to less than 0.8%. The resolution after method transfer to the 1290 Infinity LC System was typically slightly better than for the 1100 Series LC.

References

1. "Agilent 1290 Infinity LC with Intelligent System Emulation Technology", Agilent Brochure, Agilent Technologies publication number 5990-8670EN, 2011.
2. Gerd Vanhoenacker, Frank David, Pat Sandra, Bernd Glatz, Edgar Naegele, "Increasing productivity in the analysis of metoclopramide hydrochloride formulations using the Agilent 1290 Infinity LC system", Agilent Application Note, publication number 5990-3981EN, May 2009.

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