Increasing productivity in the analysis of impurities in metoclopramide hydrochloride formulations using the Agilent 1290 Infinity LC system

Application Note

Pharmaceuticals

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Abstract

This Application Note evaluates the performance of the Agilent 1290 Infinity LC system for the determination of impurities and related substances of metoclopramide hydrochloride in a pharmaceutical formulation. The translation of a conventional liquid chromatography (LC) method on a 1200 Series HPLC system to an ultra high pressure method on an Agilent 1290 Infinity LC system is discussed. Method translation is relatively easy and temperature fine-tuning is the most important parameter to obtain the same selectivity for the different impurities. Pressures as high as 1070 bar were applied during method development.

The final high productivity method is carried out at 880 bar in an analysis time of 3.5 min which is approximately 4 times faster than the original HPLC method but with the same accuracy. A validation study was carried out to demonstrate the performance of the Agilent 1290 Infinity LC system. Limits of detection for the impurities were as low as 0.001 % w/w relative to the main compound using the new diode array detector (DAD). This is more than one order of magnitude lower than required.

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Introduction

In pharmaceutical analysis present key words are high throughput, high productivity and high resolution. In high productivity, the goal is to develop analytical methods that are approximately 4–5 times faster than those presently used with the prerequisite that accuracy, precision, and repeatability of developed and validated methods are kept intact.

In liquid chromatography (LC), ways to speed up analysis include the use of particles less than 2 µm and/or operation at elevated temperature. Columns packed with particles less than 2 µm can be operated at much higher velocities compared to conventional columns but dedicated LC instrumentation is required.

The Agilent 1290 Infinity LC system was applied for the determination of impurities in a metoclopramide hydrochloride formulation. The system was equipped with a high pressure pump capable of delivering up to 1200 bar and a new fast and sensitive diode array detector (DAD). Method development consisted of the translation of conditions from a conventional instrument (Agilent 1200 Series LC system) equipped with a column packed with 3.5-µm particles to an UHPLC system (Agilent 1290 Infinity LC system) equipped with columns packed with 1.7-µm particles. The data from the two systems were compared and evaluated in terms of accuracy, precision, and repeatability.

Experimental

Instrumentation

A standard Agilent 1200 Series HPLC system and an Agilent 1290 Infinity LC system with the following configurations were used:

1200 Series HPLC		Agilent 1290 Infinity LC system	
G1322A	Vacuum degasser	G4220A	1290 Infinity Binary Pump with integrated vacuum degasser
G1311A	Quaternary pump		
G1313A	Automated liquid sampler	G4226A	1290 Infinity Autosampler
G1316A	Thermostatted column compartment	G1316C	1290 Infinity Thermostatted Column Compartment
G1315B	Diode Array Detector	G4212A	1290 Infinity Diode Array Detector

Solutions

Stock solutions of the impurities and related substances were prepared in methanol. The structures of the compounds together with their EP-code are listed in Table 1. The peak numbering is used throughout the text. The stock solutions were mixed and diluted at the appropriate concentrations with water. The formulation was a solution in water for injection of metoclopramide hydrochloride (5 mg/mL) together with some other substances (confidential composition).

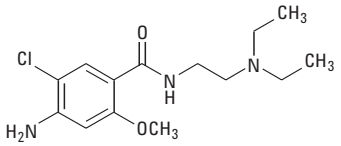
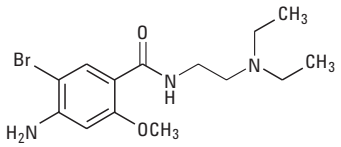
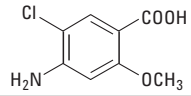
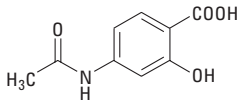
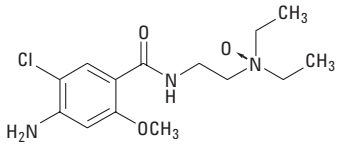
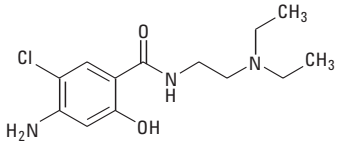
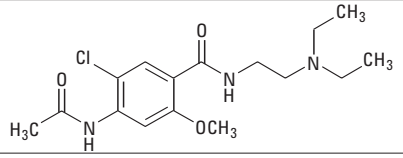
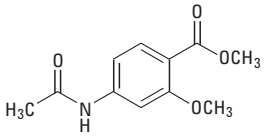
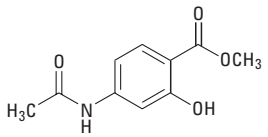
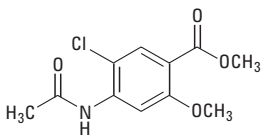
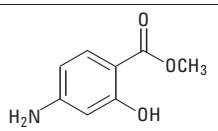
Peak	Name (European Pharmacopoeia code, EP)	Structure
Main	Metoclopramide	
X	Bromated metoclopramide	
1	4-Amino-5-chloro-2-methoxybenzoic acid (EP C)	
2	4-(Acetylamino)-2-hydroxybenzoic acid (EP H)	
3	4-Amino-5-chloro-N-2-(diethylaminoethyl)-2-methoxybenzamide N-oxide (EP G)	
4	4-Amino-5-chloro-N-2-(diethylaminoethyl)-2-hydroxybenzamide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

Table 1
Compounds under investigation.

Results and Discussion

1. Method translation from a 1200 Series LC to a 1290 LC Infinity System

The original method was developed on a 1200 Series HPLC with a linear gradient using a quaternary pump. A chromatogram for a spiked formulation at 0.5% w/w level is shown in Figure 1A. The column used was an XBridge C-18 column packed with 3.5-µm particles. The initial pressure was 140 bar. The method was easily transferred to the 1290 Infinity LC as long as some instrumental differences were taken into account. When the method parameters were copied to the 1290 Infinity LC system retention times were significantly shorter. Selectivity changes were noted and attributed to the difference in delay volume for the two systems. A quaternary pump has a delay volume of 950 µL, while the 1290 Infinity Binary Pump has a reduced volume of 10 µL. An initial isocratic hold time was introduced into the 1290 Infinity LC method to compensate for this difference. After this straightforward correction, the retention times and selectivity were very similar for both systems, while the efficiency for the 1290 Infinity LC was higher than that of the 1200 Series (Chromatogram not shown).

The method was then translated to a 2.1 mm id BEH C18 column packed with 1.7-µm particles. The flow rate was reduced to 0.22 mL/min to maintain the same linear velocity in both columns while the injection volume was decreased from 2 µL to 0.8 µL. The pressure was 380 bar. An initial 0.5 min hold time on the gradient was introduced to compensate for differences in delay volume. Under these conditions the result was very similar (Figure 1B) but slightly faster compared to the result obtained by the column with 3.5-µm particles (Figure 1A).

Efficiency and resolution were improved significantly with the 1290 Infinity setup. The theoretical efficiency can roughly be calculated as the ratio between column length and two times the particle diameter. Therefore, the efficiency of the 1.7-µm particle column should be about double the efficiency of the 3.5-µm column. Consequently, resolution should increase by a factor of 1.4 on the same system since resolution is related to the square root of the efficiency. In this particular case, the resolution enhancement is much higher than theoretically predicted (for example, from 2.6 to 5.9 for peaks 1 and 2). This is due to the lower dead volume and the superior pump drives of the Agilent 1290 Infinity LC system.

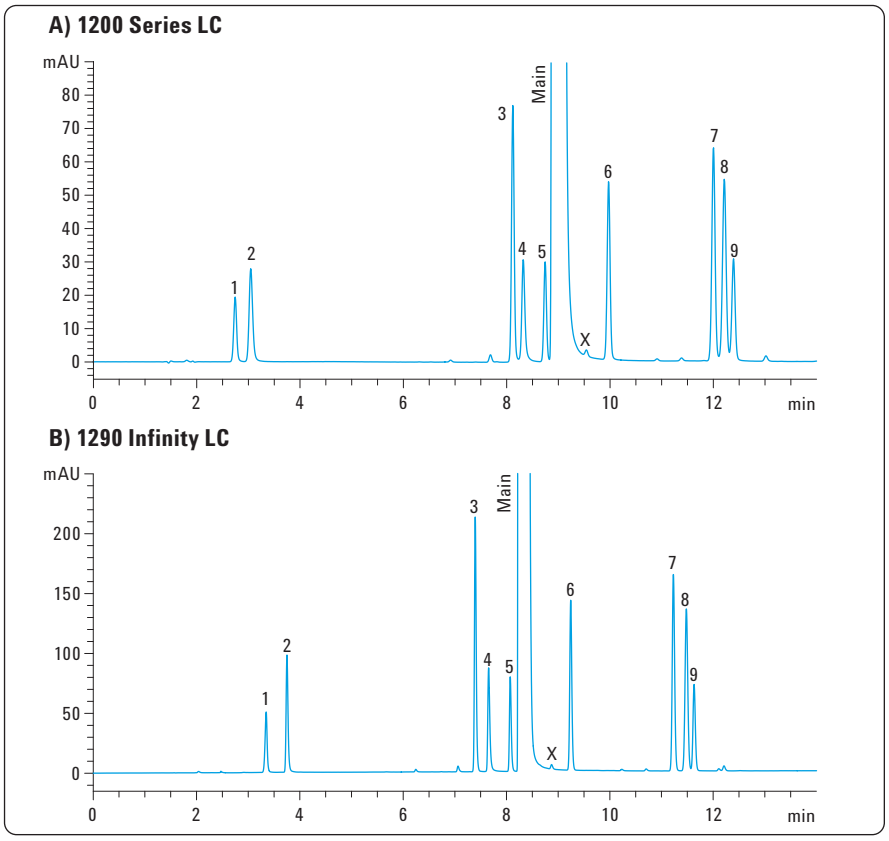


Figure 1.
Method transfer from an Agilent 1200 Series LC system to an Agilent 1290 Infinity system. Sample: formulation spiked at 0.5% w/w level with impurities 1 to 9.

Conditions Figure 1	A	B
Column	XBridge C-18, 150 mm × 3.0 mm, 3.5 µm	BEH C18, 150 mm × 2.1 mm, 1.7 µm
Mobile phase	A = 0.25% w/w ammonium acetate in water B = acetonitrile	
Flow rate	0.45 mL/min	0.22 mL/min
Gradient	0-15 min: 5–57.5% B	0-0.5 min: 5% B isocratic 0.5–15.5 min: 5–57.5% B
Temperature	37 °C	37 °C
Injection volume	2 µL	0.8 µL
Detection	DAD, Signal 275/4 nm, Reference 400/60 nm	
Maximum pressure	**140 bar**	**380 bar**

2. Increasing speed with the Agilent 1290 Infinity LC system

The analysis on the 1.7-µm particle column was performed at 380 bar which is far below the 1200 bar upper pressure limit of the 1290 Infinity LC system pump. Therefore, the flow rate could be increased to shorten the analysis time. When doing this, the gradient time should be reduced in proportion to the flow rate increase in order to maintain the same elution profile.

The operating pressure increased from 380 bar to 1020 bar when the flow rate was changed from 0.22 to 0.66 mL/min. As expected, at very high pressure, or high mobile phase velocity in the column, frictional heat is generated [1,2]. Since retention of some of the analytes is temperature dependent, the selectivity differences at high flow rates were noted. It can be seen that the resolution between compound 5 and the main compound, and of compounds 7 and 8 were especially affected by this temperature change. (Figure 2A).

The heat effect could be counteracted by reducing the temperature of the column from 37 to 32 °C (Figure 2B). Under these conditions good separation was achieved in 4.5–5 min which is about 3 times lower than the original LC method. However, the pressure increased to 1070 bar while the columns are only rated at 1000 bar. Using the column at higher pressures than the maximum rated pressure for a longer time will definitely reduce the column's lifetime and the robustness of the method.

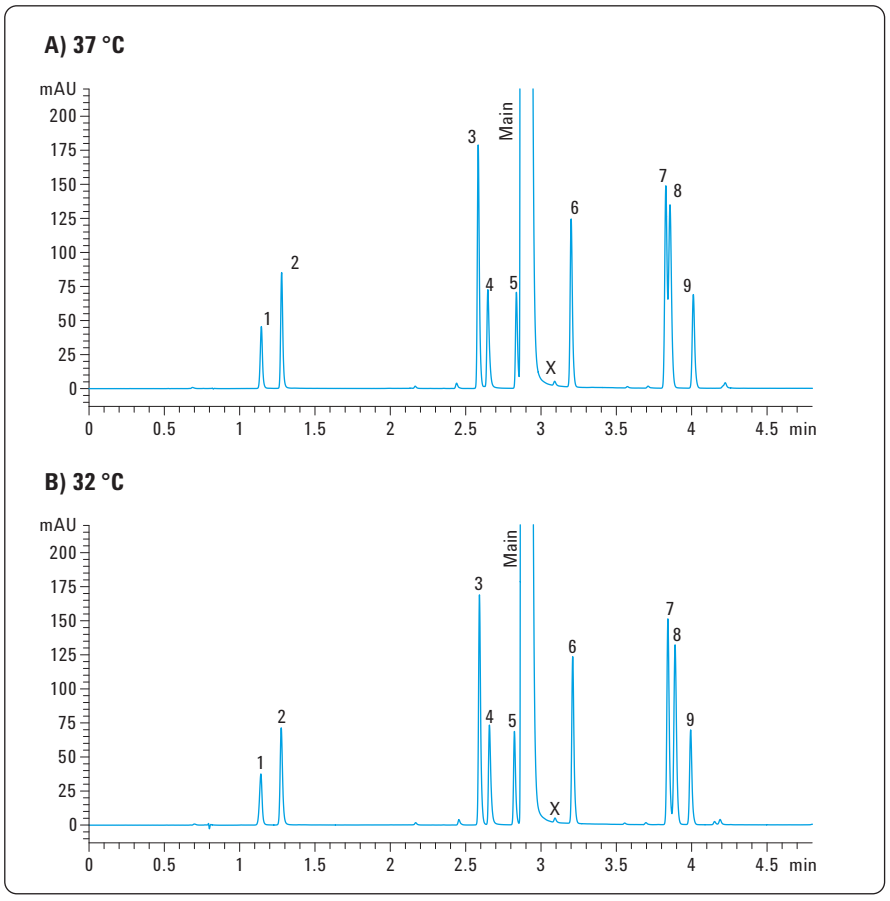


Figure 2.
Influence of frictional heat generation on selectivity and effect of lowering column temperature.
Sample: formulation spiked at 0.5% w/w level with impurities 1 to 9.

Conditions Figure 2	**A**	**B**
Column	BEH C18, 150 mm × 2.1 mm, 1.7 µm	
Mobile phase	A = 0.25% w/w ammonium acetate in water B = acetonitrile	
Flow rate	0.66 mL/min	
Gradient	0-16 min: 5% B isocratic 0.16-5.16 min: 5–57.5% B	
Temperature	37 °C	32 °C
Injection volume	0.8 µL	
Detection	DAD, Signal 275/4 nm, Reference 400/60 nm	
Maximum pressure	**1020 bar**	**1070 bar**

Therefore, for routine application, the column length was decreased from 150 mm to 100 mm to provide a maximum backpressure of 880 bar, at 0.66 ml/min. The gradient hold time and gradient time were reduced further to preserve the selectivity of the original method. The total analysis time was 3.5 min compared to 15.5 min with the original 3.5-µm particle column (Figure 3). The resolution for the initial peaks is 3.8 which still is higher than the value obtained with the 1200 Series LC.

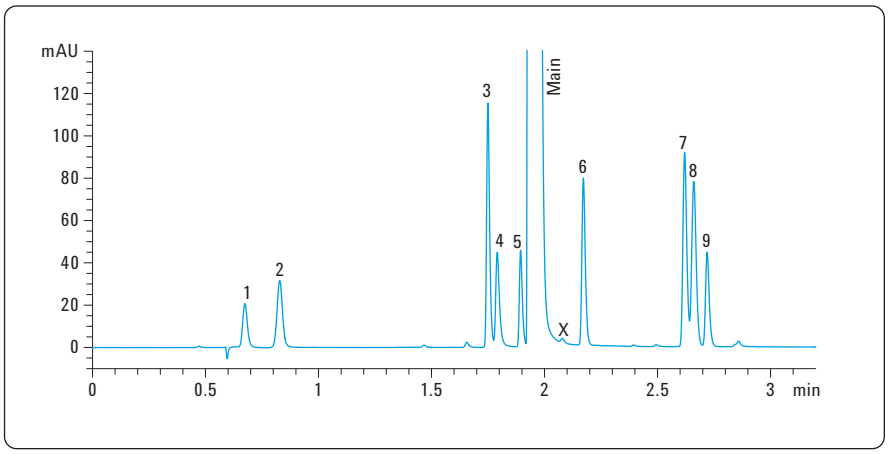


Figure 3.
Result with the final Agilent 1290 Infinity LC system method. Sample: formulation spiked at 0.5% w/w level with impurities 1 to 9.

Conditions Figure 3	Final Agilent 1290 Infinity LC system method
Column	BEH C18, 100 mm × 2.1 mm, 1.7 µm
Mobile phase	A = 0.25% w/w ammonium acetate in water B = acetonitrile
Flow rate	0.66 mL/min
Gradient	0–0.1 min: 5% B isocratic 0.1–3.45 min: 5–57.5% B
Temperature	32 °C
Injection volume	1 µL
Detection	DAD, Signal 275/4 nm, Reference 400/60 nm
Maximum pressure	**880 bar**

3. Method validation

Using the conditions of Figure 3, an analysis was performed on the parameters of linearity, repeatability of injection, and detection limit (Table 2). It was determined that good linearity (>0.999 for all compounds) and injection precision were obtained. RSDs at the 0.005% level, which is close to the limit of detection (LOD) for some compounds was below 8% for all and below 3.5% for most compounds. This is more than acceptable at this level. The chromatograms for analysis at the LOD are shown in Figure 4. The LOD varies between 0.001 and 0.005% w/w relative to the main compound (50–250 pg on-column). This means that the impurities are detected at levels which are 10 to 50 times lower than the reporting threshold.

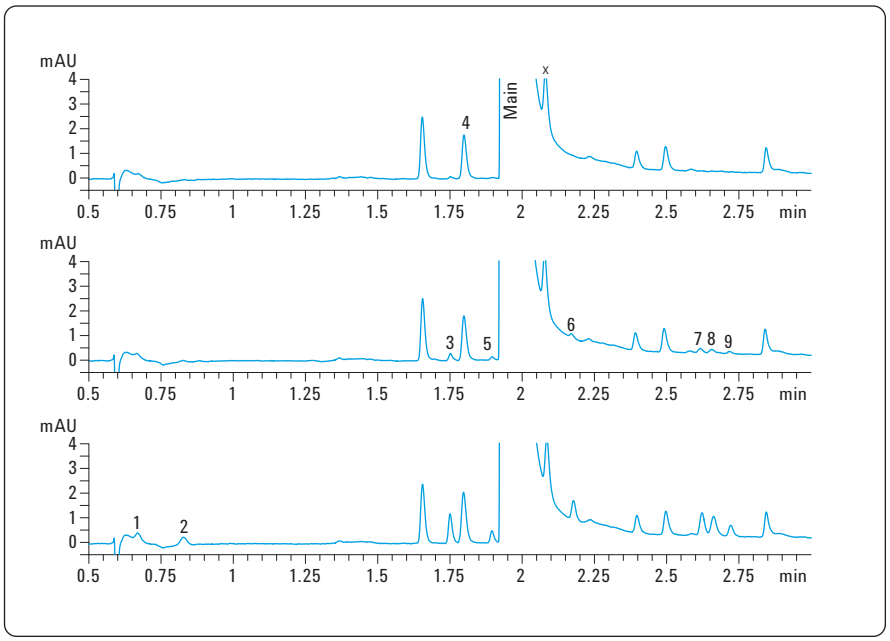


Figure 4.
Analysis of formulation (top chromatogram) and spiked formulations with impurities 1 to 9 at LOD level (middle chromatogram: 0.001% w/w, bottom chromatogram: 0.005% w/w). Conditions: see Figure 3.

Compound	Linearity, R^2[1]	Repeatability of injection, % RSD[2]			LOD	
		Area, 0.005%	Area, 0.05%	% w/w	On-column (pg)	S/N[3]
1	1.0000	4.50	2.65	0.005	250	7.3
2	0.9999	7.54	1.72	0.005	250	8.6
3	1.0000	1.35	0.95	0.001	50	7.9
4	0.9994	1.77	1.74	NA[4]	NA[4]	NA[4]
5	1.0000	2.35	2.24	0.001	50	3.6
6	0.9997	3.32	0.93	0.001	50	4.3
7	1.0000	2.42	0.49	0.001	50	5.3
8	1.0000	1.92	0.81	0.001	50	4.5
9	1.0000	3.61	1.42	0.001	50	3.0

[1] 0.01, 0.02, 0.05, 0.1, 0.2, 0.5%, 1 injection/level
[2] 6 consecutive injections/level
[3] Signal-to-noise ratio, noise was taken from approximately 1–1.25 minutes
[4] No data available, impurity already present in formulation

Conclusion

This application note demonstrates the feasibility of translating existing HPLC methods to fast Agilent 1290 Infinity LC system methods.

Initially, the HPLC analysis developed on a 1200 Series LC was simply transferred to a 1290 Infinity LC System instrument. The method transfer was relatively straightforward if some instrumental characteristics were taken into account. The original column (150 mm × 3.0 mm, 3.5-µm particle size) was then changed to a narrow bore 2.1-mm column with smaller 1.7-µm particles. This significantly increased the resolution between the compounds.

The use of particles less than 2 µm allowed an increase of velocity in the mobile phase to reduce the analysis time without hampering resolution. Frictional heat generation at high pressure and mobile phase velocity changed the selectivity of the method. The column temperature setting was lowered in order to maintain the original selectivity.

The final Agilent 1290 Infinity LC system analysis was carried out on a 100-mm column at 880 bar and was four times faster than the original HPLC method. This Agilent 1290 Infinity LC system method was successfully validated. Limit of detection varied between 0.001 and 0.005% w/w relative to the main compound corresponding to 50–250 pg on-column, which is 10 to 50 times lower than the required reporting level.

References

1. de Villiers A., Lauer H., Szucs R., Goodall S., Sandra P., *J. Chromatogr. A*, 1113 84–91. **2006**
2. Gritti F., Guiochon G., *J. Chromatogr. A*, 1187 165–179. **2008**

www.agilent.com/chem/1290

May 15, 2009
Publication Number 5990-3981EN

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