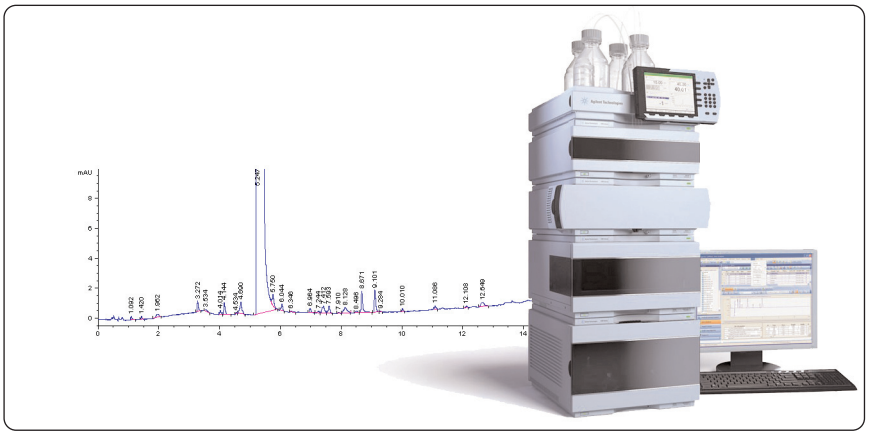
Analytical instrument qualification and system validation according to USP Chapter <1058> for the Agilent 1290 Infinity LC system

Application Note

Pharmaceutical QA/QC



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Abstract

According to USP Chapter <1058> analytical instruments must be qualified before use. This Application Note will show a setup for testing the Agilent 1290 Infinity LC System. Metoclopramide was chosen as example substance to acquire data for testing accuracy, precision, and linearity and the results are presented.

The results of independent gradient testing with the Jet Weaver show steep gradient shapes and high plateau accuracy. The setup for different columns to achieve comparable separations starting with 5 µm material (250 mm × 4.6 mm), 3.5 µm material and columns with sub-2 µm materials of different vendors are shown. This versatility makes the system applicable for standard LC methodology as well as where highest separation power is needed.

The results for the determination of the precision of areas (<2% required), and retention times (<0.05% required) show that all criteria for qualified instruments are fulfilled. The coefficients for linearity for all components are better than 0.999. No carryover was detected.

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Introduction

USP Chapter <1058>[1] describes the relevant guidelines for analytical instrument qualification. These guidelines are not mandatory and allow different approaches. If analytical instruments are used in FDA regulated environments, related procedures are recommended. USP guidelines are mandatory only if any USP monographs require qualified instruments for a specific analysis, or if any regulated testing is applied.

The USP Chapter <1058> divides laboratory tools and instruments into three categories (A, B, C). Group A includes tools such as magnetic stirrers, Group B lists balances and pH-meters and Group C contains complex instruments like HPLCs or mass spectrometers. Depending on the complexity of the instrument and its application, the effort for qualification increases. The 4Q-model (design qualification, installation qualification, operational qualification, performance qualification) supports the guidelines of the instrument qualification for Group C instruments. Since it has been in use for 10 years, many users are familiar with the model.[2]

This model is applicable to all types of instruments because it is flexible and allows all laboratories to define test procedures and acceptance criteria .

The Agilent 1290 Infinity LC System belongs to category C, where testing according USP Chapter <1058> is necessary. This Application Note will show a setup for testing the instrument. Metoclopramide was chosen as an example substance to acquire data for testing of accuracy, precision and linearity. For setup of the method, the results of previous method developments were used.[3]

Experimental

Instrumentation

Table 1 shows the configuration of the Agilent 1290 Infinity LC system that was used. Several columns were used to show the performance.

Part No	Module
G4220A	1290 Infinity Binary pump with integrated vacuum degasser and different solvent mixers
G4226A	1290 Infinity Autosampler
G1316C	1290 Infinity Column Compartment
G4212A	1290 Infinity Diode Array Detector
Software:	Chemstation B.04.02

Table 1
Configuration of the Agilent 1290 Infinity LC system

Preparation of samples

Reference samples

The stock solution was prepared by mixing two different liquid formulations of metoclopramide hydrochloride (each 5 mg/mL, each 1 mL). One millilitre of the mixture was diluted with 4 mL of methanol to yield a concentration of 1 mg/mL for the main component. The resulting solution was diluted to the 0.01% concentration of the impurities.[3] Figure 1 shows the structure of metoclopramide.

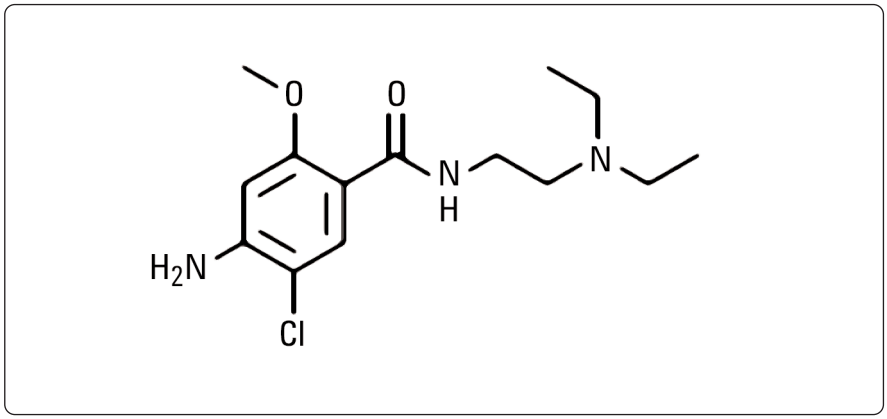

Figure 1
Structure of metoclopramide.

Chromatographic conditions

Columns:

- Agilent TC C18(2), 250 mm × 4.6 mm, 5 µm
- ZORBAX Eclipse Plus C18, 150 mm × 2.1 mm, 3.5 µm
- ZORBAX RRHD Eclipse Plus C18, 150 mm × 2.1 mm, 1.8 µm
- Waters BEH C18, 150 mm × 2.1 mm, 1.7 µm

Mobile phases: Gradient testing

- Mobile phase A: water
- Mobile phase B: water + 0.5% acetone (v/v)

Time	% A	% B
0.00	100.00	0.00
3.00	100.00	0.00
3.01	90.00	10.00
6.00	90.00	10.00
6.01	52.00	48.00
9.00	52.00	48.00
9.01	48.00	52.00
12.00	48.00	52.00
12.01	10.00	90.00
15.00	10.00	90.00
15.01	0.00	100.00
18.00	0.00	100.00
18.01	48.00	52.00
21.00	48.00	52.00
21.01	52.00	48.00
24.00	52.00	48.00
24.01	100.00	0.00
27.00	100.00	0.00

Table 2
Gradient.

Gradient for separation of metoclopramide and impurities

- Mobile phase A : 0.25% w/w ammonium acetate in water
- Mobile phase B : acetonitrile

The setup for all columns was found by using the parameters determined in Agilent publication number 5990-3981EN for the BEH column for starting conditions, converting them with the Method Translator Software[4]. (Instrument conditions are shown in Table 3.)

Setup for testing

USP Chapter <1058> defines tests and limits for the evaluation of HPLC systems. Typically, tests are used to evaluate pump performance, autosampler performance, the stability of temperatures, or the accuracy of optical detectors.

The chromatographic performance of the pump is shown by plotting and evaluating gradient mixing capabilities with a tracer and retention time precision. The autosampler can be validated by calculating the area precision of equal injection volumes, the correlation of calibration curves or the determination of the carryover. These results are only valid, if the detection system is stable enough to deliver reproducible data with sufficient sensitivity and high signal-to-noise values.

The following setup is the selection from a typical assortment of tests for system suitability with a reference sample :

- Determination of pump performance depending on dwell volumes by gradient tests
- Establishment of a chromatographic separation to achieve data for long time evaluations
- Similar peak pattern and resolution according to selected column with respect to particle size and column dimension and adapted to gradient shape and flow
- Precision of areas must be < 2 % RSD.
- Precision of retention times must be < 0.5 % RSD.
- Linearity should be $R^2 > 0.999$

	Agilent TC C18(2), 250×4.6mm, 5µm,	Eclipse Plus C18, 150×2.1mm, 3.5µm	RRHD Eclipse Plus C18, 150×2,1mm, 1.8µm	Waters BEH C18, 150×2.1mm, 1.7µm
Flow rate	1.058 mL/min	1.058 mL/min	0.221 mL/min	0.221 mL/min
Gradient	0-25 min 5-57% B	0-15 min 5-57% B	0-15 min 5-57% B	0-15 min 5-57% B
Temperature	37 °C	37 °C	37 °C	37 °C
Injection volume	8 µl	2 µl	1 µl	1 µl
Detection	DAD, Signal	275/4, Reference	400/60	standard cell (10 mm path length)
Data rate	2 Hz	2 Hz	40 Hz	40 Hz
Maximum pressure	138 bar	145 bar	310 bar	435 bar

Table 3
Instrument conditions.

There are more tests available to evaluate the accuracy of the optical unit of the detector. These tests can be established in addition to those mentioned, but are often part of a special setup available during diagnosis, and are not evaluated with a chromatographic test.

The samples in Table 4 were prepared and analyzed with these limits and settings for testing.

Results and discussion

USP Chapter <1058> defines tests and limits for the evaluation of HPLC modules and HPLC systems. The results shown here illustrate this process.

The accuracy of gradient mixing, as well as flow accuracy, are the main tests for evaluating a gradient pump. Gradient mixing becomes more and more influenced by the dwell volume. The testing setup must be variable to eliminate the effects of the mixer used.

The Agilent Jet Weavers for high efficiency mixing allow the use of different mixing volumes. The delay and dwell volume of the pumps have an influence on the separation for narrow bore applications combined with fast gradients. Enlarging the dwell volumes, thereby increasing retention times, could affect the resolution, and the gradient shape because of dispersion effects and different flush-out behavior. Chromatograms can appear different due to different mixers and mixing volumes. Figure 2 shows the different but steep gradient shapes, resulting in very short response times depending on the mixer. It also shows high accuracy of the gradient steps independent of the mixer used.

Sample	Purpose	Number of injections
Blanc solution	Verify baseline stability and identify artifacts	3
Suitability sample	Verify precision of areas and retention times for reference solution	10
Calibration	Verify linearity	3 for each level
Highest concentration and Blanc solution	Verify carryover	3 of each sample

Table 4
Setup for testing.

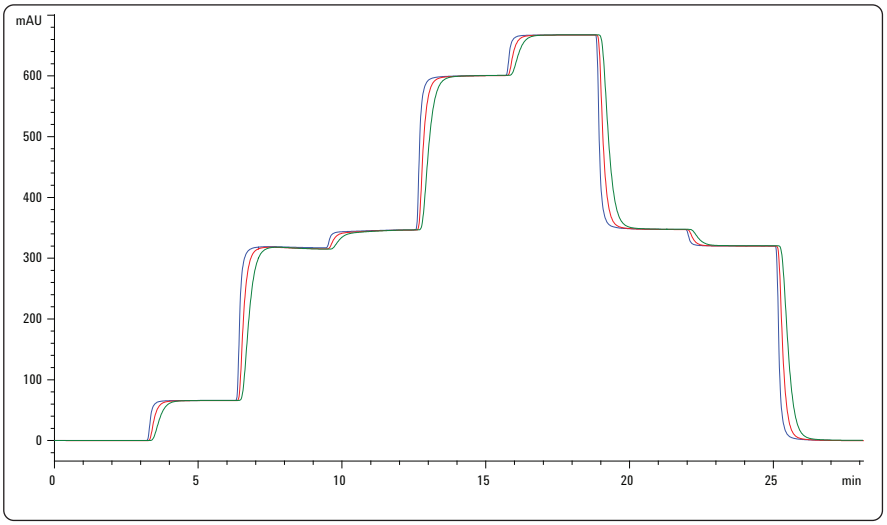

Figure 2
Gradient test depending on the volume of the mixers (blue-35 µL, red-135 µL, green-300 µL).

The next test evaulates the functionality of the system with different columns. For that purpose, four different columns with different particle sizes were tested (Figure 3).

In addition, the flow precision was evaluated using the retention time stability of some selected components in the mixture. With the same setup, the precision of the autosampler can be calculated, if the test sample is injected a minimum of 10 times and the relative standard deviation (RSD) of the areas is calculated. The data in Table 5 show high stability of retention times and high precision of areas even at low levels of impurities.

The accuracy of retention times is not only influenced by the flow precision but also by the temperature. The remarkable high stability is also a demonstration of excellent temperature stability in the column compartment.

The test for linearity shows correlation coefficients for all components greater than 0.999 which prove the high performance of the autosampler.

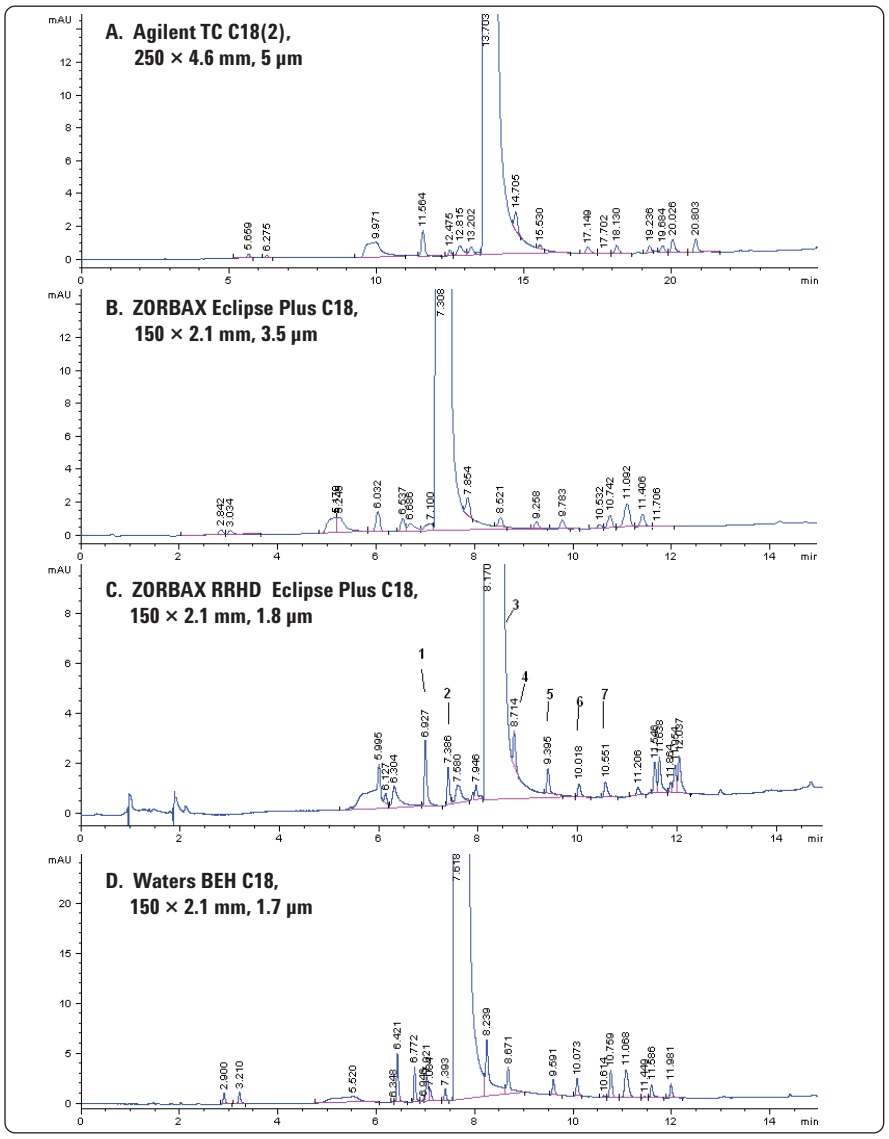

Figure 3
Separation of metoclopramide and its impurities.

	Retention times		Areas		Linearity
	Mean	RSD	Mean	RSD	R^2
Impurity 1	6.927	0.030	3.765	1.36	0.9994
Impurity 2	7.386	0.025	2.170	1.49	0.9997
Metoclopramide 3	8.170	0.039	10190.182	0.58	0.9998
Impurity 4	8.714	0.035	1.707	1.48	0.9993
Impurity 5	9.395	0.013	1.496	1.58	0.9993
Impurity 6	10.018	0.039	1.023	1.55	0.9990
Impurity 7	10.551	0.039	1.971	1.46	0.9993

Table 5
Determination of the precision of areas and retention times in Figure 3C.

A further test to evaluate sampler performance is the determination of carryover. Figure 4A shows the chromatogram after an injection of the highest concentration of metoclopramide. No carryover can be seen (Figure 4B).

The chromatogram in Figure 5 shows further method optimization, where the analysis time was shortened to improve the capacity of the system. It can be seen that all peaks are eluted within 10 minutes.

Conclusion

The Agilent 1290 Infinity LC system is designed to provide highest speed, resolution and sensitivity. A new power range allows operation with any particle type, any column dimension, or any mobile and stationary phase. The 1290 Infinity LC is the first system that allows method transfer from any Agilent HPLC System.

To use this system for quality control testing and development, as well as in an FDA regulated environment, it is necessary to meet the criteria of the new USP Chapter <1058>. These new regulations enforce procedures for testing and evaluating the applicability and versatility of the equipment before use.

This Application Note shows a selection of tests that can be established to evaluate an LC system.

Figure 2 shows the results of gradient testing. Independent of the Jet Weavers used and mixing volumes, the gradient shapes are steep and show high accuracy of each plateau.

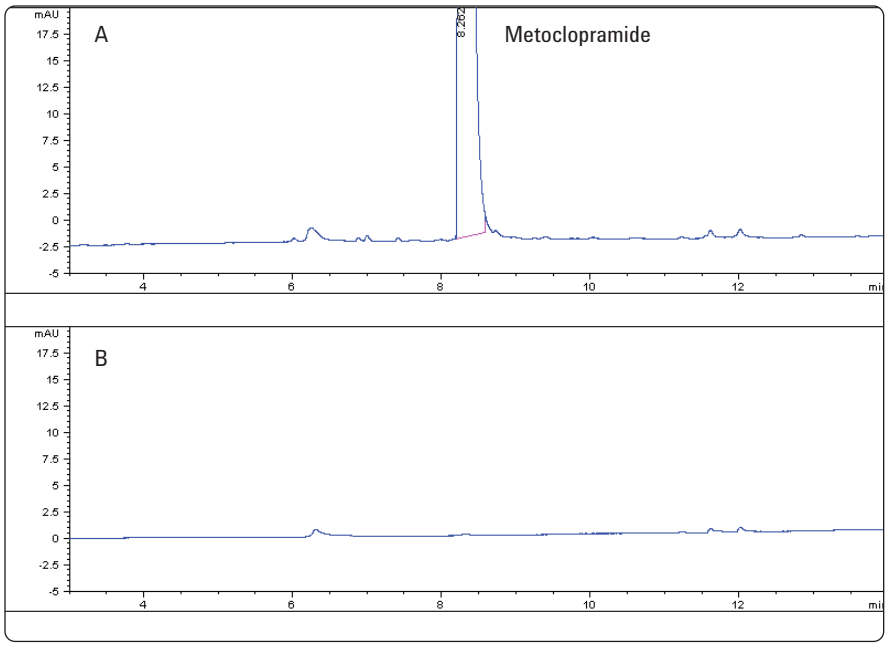

Figure 4
Determination of carryover. A. Injection of sample with highest concentration of metoclopramide. B. Injection of a blank solvent sample.

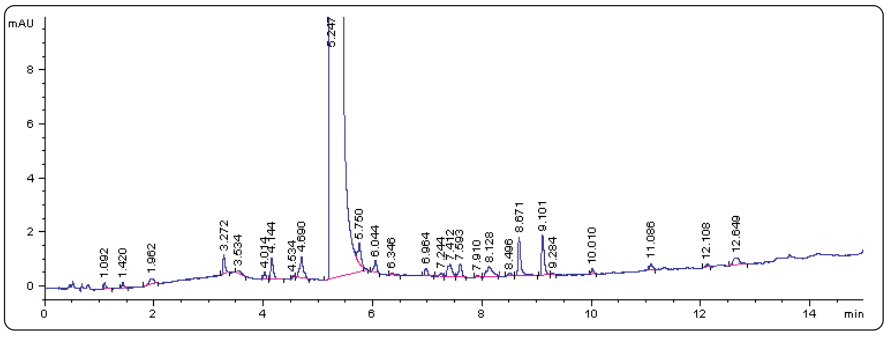

Figure 5
Separation of metoclopramide and its impurities on a ZORBAX RRHD Eclipse Plus C18, 150 × 2.1 mm, 1.8 µm, speed optimized (flow: 0.44 ml/min).

One of the great benefits of the system is the use of any column dimension or particle size. This was proven by the comparison of the separation power of a developed method with different column sizes. Figure 3 shows very similar chromatograms achieved from a column with 5 µm material (250 mm × 4.6 mm), a column with 3.5 µm material, and columns with sub-2 µm materials from different vendors. This versatility allows the use of the system for standard LC methodology as well as methods in which the highest separation power is needed.

The results shown in Table 5 show that all criteria for the precision of the determination (areas, retention times) are fulfilled. The coefficients of linearity for all components are better than 0.999. This is not only proven for the main component but also for the impurities at the 0.01%-level. No carryover was detected (Figure 4).

All results explicitly show the applicability of the 1290 Infinity LC system for quality control testing and development as well as in an FDA regulated environment.

In addition, the speed optimization test confirms that the system provides excellent separation possibilities (Figure 5).

The results of method transfer show that the selectivity and performance of the Agilent ZORBAX Eclipse Plus C18 material is independent of the particle size. The data also show the excellent flow design of the Agilent 1290 Infinity LC system, assuring the user that no band broadening or peak distortion will occur, hindering the separation power.

In summary, the data presented in this Application Note has illustrated the versatility and reliability of the Agilent 1290 Infinity LC system. This system allows fast method transfer to and from any column or particle size, allowing its use for almost any applications.

The Agilent 1290 Infinity LC system is qualified for the criteria of USP Chapter <1058> and will meet the highest requirements for every LC application.

References

1. United States Pharmacopiea, Chapter <1058>, Analytical Instrument Qualification, USA, 2008.

2. Analytical Instrument Qualification and System Validation, January 1, 2009, Agilent Technologies publication number 5990-3288EN.

3. Increasing productivity in the analysis of impurities in metoclopramide hydrochloride formulations using the Agilent 1290 Infinity LC system, Agilent Technologies publication number 5990-3981EN.

4. http://www.agilent.com/chem/hplc2uhplc

www.agilent.com/chem/1290

December 7, 2009
Publication Number 5990-4781EN

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