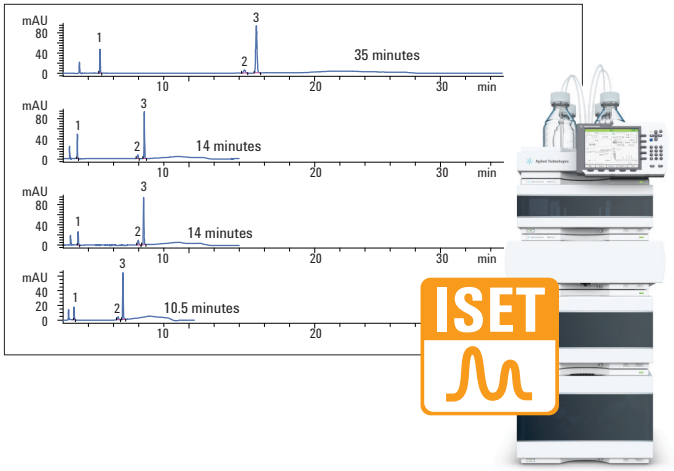
Developing faster methods for generic drugs within USP <621> allowed limits

Higher throughput and cost reduction for purity analysis of Olanzapine tablets using the Agilent 1290 Infinity LC System with ISET

Application Note

Pharmaceutical QA/QC



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Abstract

This Application Note describes an approach to reduce cost per analysis by reducing analysis time and solvent consumption through varying column dimensions. The modifications are made according to the United States Pharmacopeia (USP) guidelines on allowed deviations for column dimensions and thus eliminate the need for method revalidation. The USP method of organic impurities for olanzapine tablets was used to demonstrate the approach. The USP method was efficiently converted in to three shorter gradients using Agilent Poroshell 120 EC C8 columns of various dimensions within the allowed USP limit. The Agilent 1290 Infinity LC System with Intelligent System Emulation Technology (ISET) was used for the experiments to emulate other HPLC system configurations depending on the column dimensions used. The results proved that smart selection of column dimensions can save more than 90% solvent and more than 70% time compared to the original Pharmacopeia method.

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Introduction

For every liquid chromatography method listed in the pharmacopeia, recommendations for column dimensions and packing materials are given in addition to other method parameters. There are general guidelines to chromatographers which clearly state what deviations are permitted for these column parameters. If the deviations are within the allowed limit, no method revalidation is required, but system suitability criteria should be met.[1]

olanzapine is one of the best selling block buster drugs on the global pharma market. The USP method of analysis for organic impurities in olanzapine tablets takes approximately 35 minutes, using a 250 × 4.6 mm column with 5-µm L7 packing[2]. The USP <621> guideline on permitted column dimension deviations for LC methods is given in Table 1.

The system suitability test for the olanzapine tablet includes:

a) Resolution of the active pharmaceutical ingredient (API) peak with one of the impurities

b) Tailing factor and relative standard deviation (RSD) of retention time (RT) of the API peak

c) Signal-to-noise for a diluted sample of the API

Three different smaller column dimensions are selected by varying column length, diameter, and particle size within the allowed deviation on column dimension and system suitability testing. Total cost savings in gradient time and solvent consumption was calculated while meeting the system suitability results. The use of a smaller column length and diameter reduced analysis time and solvent consumption. Smaller particle sizes promised uncompromised resolution of peaks. Different instrument models with different characteristics either from the same or different vendors offer different delay volumes and may result in a compromise on critical separations while performing Pharmacopeia methods. This issue was eliminated by using ISET with the Agilent 1290 Infinity LC System. The ISET emulation algorithm delivers identical gradient mixing conditions as selected other instruments and gives matching retention time and chromatographic resolution. ISET ensures the uncompromised performance of a 1290 Infinity LC System as a universal LC system.

Column parameter	USP limit for deviation
Length	± 70%
Internal diameter	No limit, but keep constant linear velocity
Particle size	– 50%

Table 1
Allowed column deviations as per USP <621> recommendation.

Experimental

Instruments, software and columns

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

- Agilent 1290 Infinity Binary Pump with integrated vacuum degasser (G4220 A) and 35 µL Jet Weaver mixer.
- Agilent 1290 Infinity High Performance Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Diode Array Detector (G4212A) with Max-Light flow cell (1.0 µL dispersion volume, 10-mm path length) (G4212-60008)
- Software: Agilent ChemStation C.01.03

In addition to the USP recommended method, three more methods were performed with modified column dimensions (within the allowed USP deviation limit) to evaluate the time and solvent savings in comparison to the original pharmacopeia method. The details of columns dimensions with observed deviations for all four experiments are tabulated in Table 2.

Column parameter	USP recommendation	Experiment 1 (Original USP method)		Experiment 2		Experiment 3		Experiment 4	
		Actual	% Deviation	Actual	% Deviation	Actual	% Deviation	Actual	% Deviation
Length	250 mm	250 mm	0	100 mm	–60	100 mm	–60	70 mm	–70
Diameter	4.6 mm	4.6 mm	0	4.6 mm	0	2.1 mm	–54	2.1 mm	–54
Particle size	5 µm	5 µm	0	2.7 µm	–46	2.7 µm	–46	2.7 µm	–46

Table 2
Various column dimensions percentage deviations used for the experiments.

Reagents and materials

USP reference standards for olanzapine and corresponding related compounds B and C were purchased from USP-India Private Limited (Hyperbad, India). Acetonitrile was of 'super gradient grade' and was purchased from Lab-Scan (Bangkok, Thailand). Highly purified water from a Milli Q water purification system (Millipore Elix 10 model, USA) was used for the experiment. Chemicals for making buffers, phosphoric acid, sodium hydroxide, sodium dodecyl sulfate, and edetate disodium were purchased from Aldrich (India).

Chromatographic parameters

The buffers and mobile phases were prepared as per the USP method. The details of the buffers, mobile phases and diluent preparation used for this experiment are given in Table 3. The column temperature was maintained at 35 °C and the detection was done at 220 nm. The detailed chromatographic method parameters used for each experiment are tabulated in Table 4.

Procedure

The system suitability, standard, and sensitivity solutions were prepared as per USP method for the olanzapine tablet described in USP 34–NF 29.

System suitability solution: 20 µg/mL of USP olanzapine, and 2 µg/mL each of USP olanzapine related compound B and C in diluent.

Standard solution: 2 µg/mL of USP olanzapine RS in diluent

Sensitivity solution: 0.4 µg/mL of USP olanzapine in diluent, from the standard solution.

Method transfer for all the experiments were carried out using Agilent Method Translator (v:2) in *Simple conversion* mode. System suitability testing was performed using all the four experiment conditions.

Buffer	Details
Buffer 1	3.3 mL/L of phosphoric acid. Adjust with 50% NaOH to a pH of 2.5.
Buffer 2	8.7 g/L of sodium dodecyl sulfate in Buffer 1
Buffer 3	18.6 mg/L of edetate disodium (EDTA) in Buffer 2
Mobile phase A	Acetonitrile and Buffer 2 (12:13)
Mobile phase B	Acetonitrile and Buffer 2 (7:3)
Diluent	Acetonitrile and Buffer 3 (2:3)

Table 3
Buffers, mobile phases and diluent as per USP method.

	Agilent 1290 Infinity Binary LC System with ISET			
Parameter	Experiment 1: Emulated as Agilent 1100 Series LC	Experiment 2: Emulated as Agilent 1260 Infinity LC	Experiment 3: Without ISET	Experiment 4: Without ISET
Injection volume	20 µL	8 µL	1.7 µL	1.3 µL
Column	Agilent ZORBAX Eclipse Plus C8, 4.6 × 250 mm, 5 µm	Agilent Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm	Agilent Poroshell 120 EC-C8, 2.1 × 100 mm, 2.7 µm	Agilent Poroshell 120 EC-C8, 2.1 × 75 mm, 2.7 µm
Flow rate	1.5 mL/min	1.5 mL/min	0.31 mL/min	0.31 mL/min
Gradient	At 0 min: 0% B At 10 min: 0% B At 20 min: 100% B At 25 min: 100% B At 27 min: 0% B At 35 min: 0% B	At 0 min: 0% B At 4 min: 0% B At 8 min: 100% B At 10 min: 100% B At 10.8 min: 0% B At 14 min: 0% B	At 0 min: 0% B At 4 min: 0% B At 8 min: 100% B At 10 min: 100% B At 10.8 min: 0% B At 14 min: 0% B	At 0 min: 0% B At 3 min: 0% B At 6 min: 100% B At 7.5 min: 100% B At 8.1 min: 0% B At 10.5 min: 0% B
Acquisition rate	5 Hz	10 Hz	10 Hz	10 Hz

Table 4
Detailed chromatographic parameters for all the four experiments.

Results and discussion

Separation and detection

The USP recommended column dimension is 4.6 × 250 mm, 5-µm packing L7 column which is a typical column dimension for LC system configurations with a pressure limit of 400 bar. The analysis using this USP recommended column dimension (experiment 1) was carried out using an Agilent 1290 Infinity Binary LC System with ISET emulating to an Agilent 1100 Series Binary Pump. An Agilent ZORBAX Eclipse Plus C8 column was used here. For experiment 2, an Agilent Poroshell 120 EC-C8 100 × 4.6 mm, 2.7-µm column (length about 1/3 of original length) was selected. Analysis using smaller particle sized columns may require LC systems which have higher pressure withstanding capabilities. To address this, experiment 2 was performed using an Agilent 1290 Infinity LC System with ISET emulating an Agilent 1260 Infinity Binary Pump which has a pressure limit of 600 bar. For experiment 3, an Agilent Poroshell 120 EC-C8 100 × 2.1 mm, 2.7-µm column (same length as experiment 2, but with a 2.1 mm id) was selected and for experiment 4, an Agilent Poroshell EC-C8 75 × 2.1 mm, 2.7-µm column (minimum length according to USP limits, and 2.1 mm id) was selected. Using narrow bore columns may demand Ultra High Pressure LC (UHPLC) systems with low delay and dispersion volumes. Experiments 3 and 4 were performed using an Agilent 1290 Infinity LC System without ISET. The peaks are well separated in all four experiments, and Figure 1 shows the observed chromatograms. The system suitability results obtained from all the four experiments are tabulated in Table 5, and the results are found to be within the acceptance criteria.

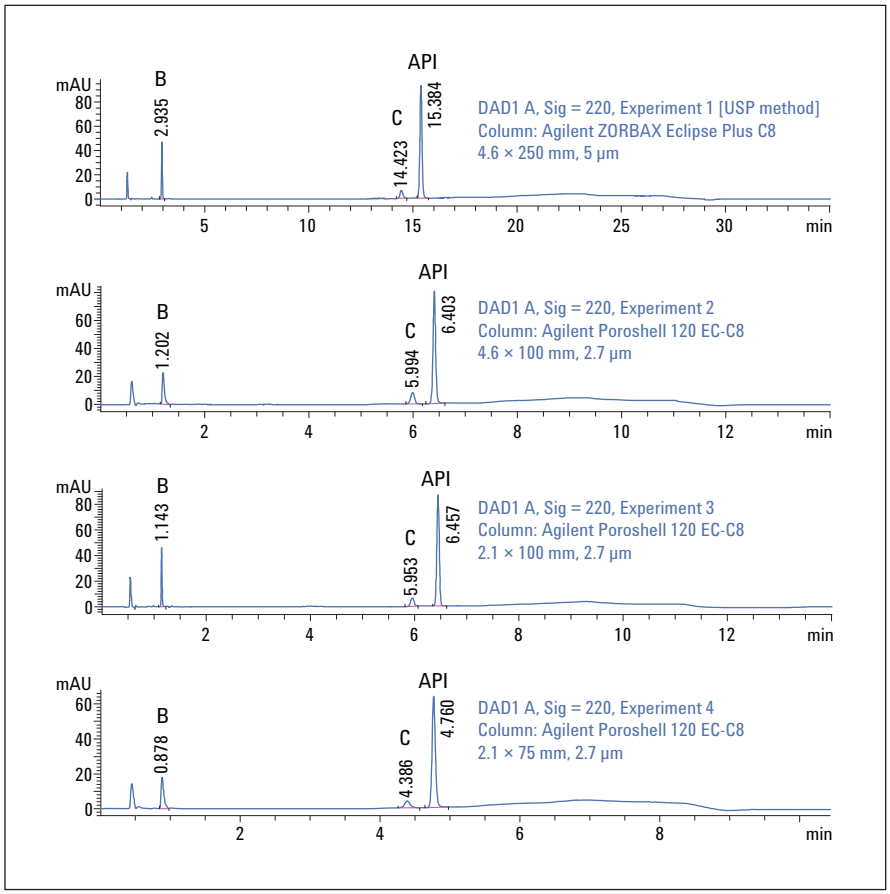

Figure 1
Separation of Olanzapine system suitability mix in USP method and newly developed cost effective methods.

No	Parameter	Limit	Results			
			Exp 1	Exp 2	Exp 3	Exp 4
1	Resolution between Olanzapine and related compound C (system suitability solution)	NLT* 3.0	4.31	4.79	3.63	3.33
2	USP Tailing factor for Olanzapine (system suitability solution)	NMT** 1.5	1.082	0.984	1.034	1.077
3	RSD RT of Olanzapine peak (Standard solution)	NMT** 2.0%	0.07	0.07	0.02	0.10
4	RSD Area of Olanzapine peak (Standard solution)	NMT** 2.0%	0.33	0.33	0.31	0.26
5	Signal-to-noise ratio for Olanzapine peak (Sensitivity solution)	NLT* 10	>13	>13	~50	>38

*NLT: Not less than,
**NMT: Not more than

Table 5
System suitability results for all four experiments.

The system suitability results were found to be within the acceptance criteria even with a 75-mm Poroshell 120 EC C8 column. The resolution between olanzapine and Impurity C was one of the critical parameters to be monitored for the system suitability testing. The system suitability limit for resolution between the olanzapine peak and the Impurity C peak was greater than 3 for a 250-mm column, and the results with modified column dimensions met this requirement. The USP tailing factor for the API peak also passed in all trials. The small values for area and RT RSD confirmed the precision and accuracy of the UHPLC system. Using a shorter column reduced the analysis time and increased the throughput. A reduced flow rate from 1.5 mL/min to 0.31 mL/min with a 2.1-mm id column reduced the total solvent consumption 1/10 fold. Using the experiment 3 and 4 conditions, a 3-fold increment in signal-to-noise was observed and this confirmed the gain in sensitivity with narrow bore columns with smaller particle size. As a result of the savings in time and solvent consumption, a total cost saving factor was calculated for each experiment (Figure 2). In experiments 2, 3, and 4, approximately 60%, 91.7% and 93.8% of acetonitrile could be saved respectively. Experiments 2 and 3 were approximately 60% and experiment 4 was approximately 70% faster than the original Pharmacopeia method. The cost of acetonitrile was calculated with US $60/L, as well as a cost factor for the disposal of solvent waste. The running cost of the HPLC system was calculated as US $80/hour. The method developed in this Application Note (experiment 4) can potentially save a total of US $34 per injection compared to the original USP method.

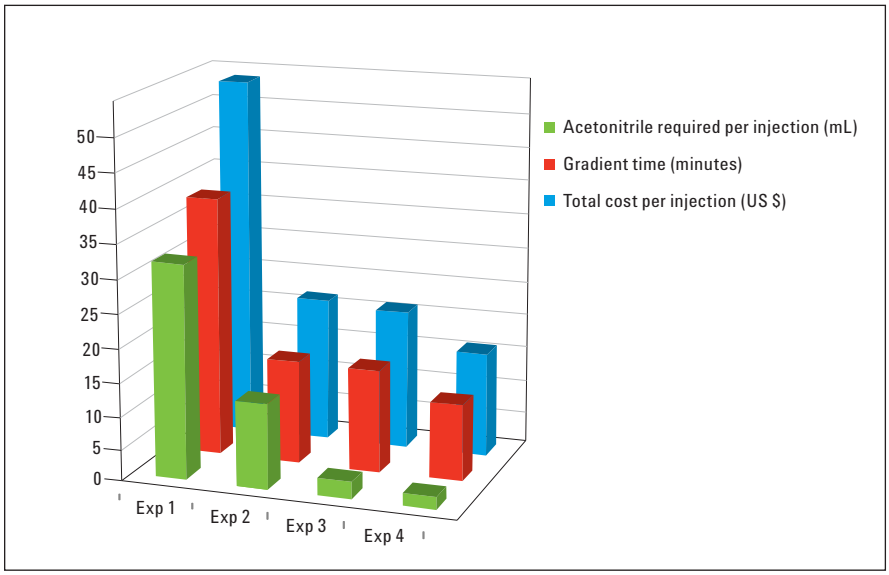

Figure 2
Solvent, time and total cost calculation for all experiments.

Analysis of the olanzapine tablet

The effective usage of the developed, cost effective method with shorter gradients for high throughput was demonstrated by analysis of the olanzapine tablet samples. The API was extracted from olanzapine tablets as per the USP protocol and analyzed using the Pharmacopeia and the three developed methods. The label claim on the tablet as per the manufacturer was 2.5 mg of API and the calculated amount from all the four chromatographic assay methods was about 2.4 mg. The presence of 0.1–0.2% of Impurity C (confirmed by retention time) was observed in the chromatographic separation. The calculated percentage area of API and Impurity C were found to be similar. The chromatograms obtained from the olanzapine tablet analysis using all four methods are shown in Figure 3. Area distribution of API and impurity peaks with calculated API percentage is given in Table 6.

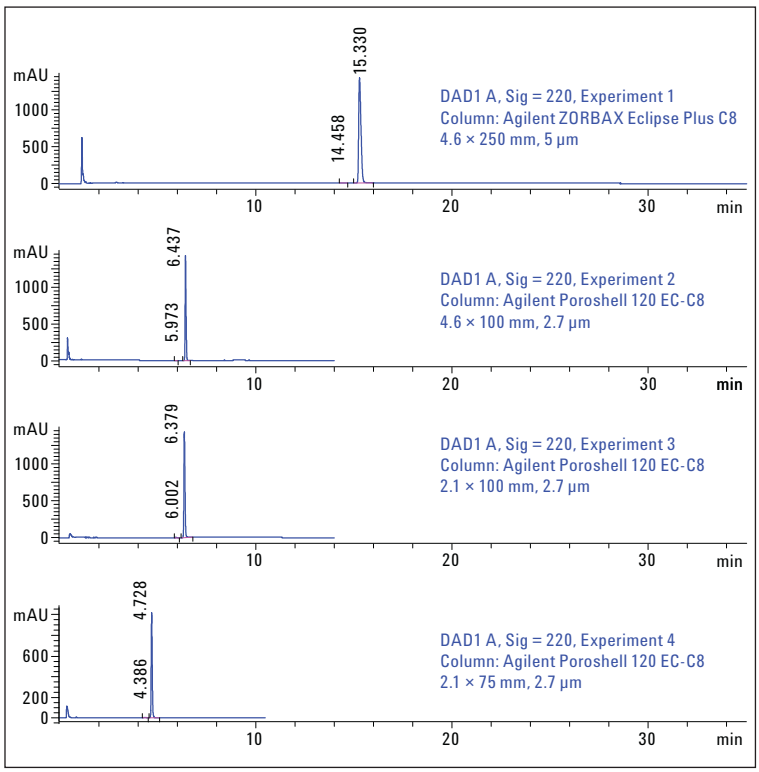

Figure 3
Chromatograms of extracted Olanzapine samples from tables using USP and newly developed methods.

Experiment	Peak	Area	Area%	Calculated API content (mg)
1	Impurity C	13.9	0.119	2.38
	API	11674.9	99.881	
2	Impurity C	5.6	0.118	2.34
	API	4731.8	99.882	
3	Impurity C	8.4	0.174	2.35
	API	4832.2	99.826	
4	Impurity C	6.2	0.168	2.36
	API	3676	99.832	

Table 6
Area percentage of API and impurity C along with calculated amount of API observed from Olanzapine tablet analysis.

Conclusion

- The original column dimension for the USP organic impurity analysis for the olanzapine tablet was adapted to shorter dimensions as allowed by the USP guidelines for column deviations.
- Revalidation is not required as the modifications incorporated are within the USP limits.
- Deriving new gradient parameters for each modified column dimension was performed with the Agilent Method Translator.
- An Agilent 1290 Infinity LC System with Intelligent System Emulation Technology (ISET) was efficiently used to emulate to other different instrument configurations.
- System suitability testing was performed with modified column dimensions using the new methods and the results were within the acceptance criteria.
- The usage of smaller column dimensions reduced the analysis time and solvent consumption and thus reduced the cost per analysis.
- Smart selection of column dimensions within the Pharmacopeia allowed limit reduces the total cost per analysis by up to 70% and increased the high throughput.

References

1.
"Validation of analytical methods" Agilent Publication Number 5990-5140EN, **2011**

2.
USP method for olanzapine tablet, USP34–NF29

www.agilent.com/chem/ISET

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