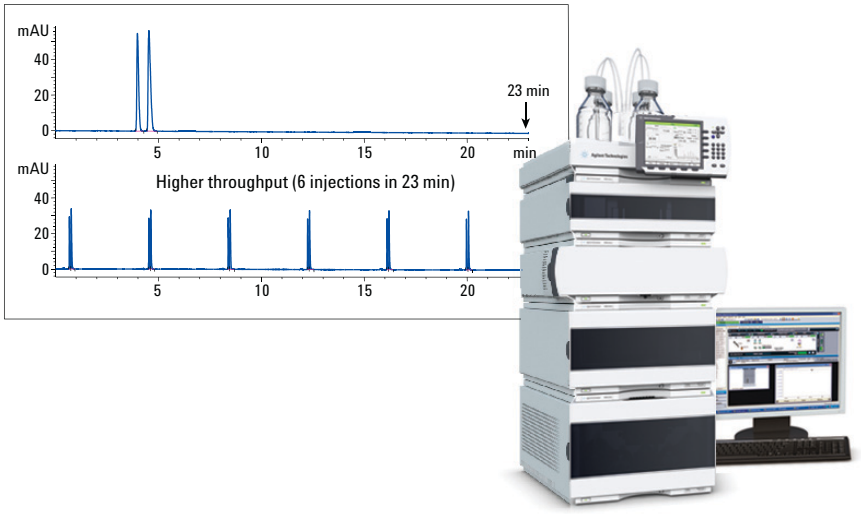
Reducing analysis time and solvent consumption for isocratic USP assay methods with current and proposed USP guidelines using the Agilent 1290 Infinity LC System

An efficient way to reduce cost of analysis for tramadol assay

Application Note

Pharmaceutical QA/QC



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Abstract

In this Application Note a high pressure liquid chromatography (HPLC) isocratic method for a tramadol assay was optimized for varying column dimensions. The column dimensions were varied according to the currently existing or recently proposed Interim Revision Announcements (IRAs) of United States Pharmacopeia (USP) <621> chromatography general chapters. The intention was to achieve lower cost for assay analysis by saving analysis time and solvent. The Agilent 1290 Infinity LC System was used with Agilent Poroshell and sub-2 µm columns were used to show the cost savings. The newly developed methods met USP system suitability requirements and ensured excellent performance for routine analysis without method revalidation[1]. An 83% cost saving was achieved along with higher throughput and lesser solvent consumption compared to the existing USP method.

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Introduction

Providing accurate and efficient chromatographic results in shorter time while consuming less solvent is one way to improve productivity of lab operations. The efficiency of a chromatographic separation can be evaluated by the length/particle size (L/dp) ratio. From this equation, it is clear that the efficiency is proportional to the column length and inversely proportional to the particle size. Therefore, the chromatographic efficiency can be improved by simple modifications in column dimensions.

The USP method for tramadol (a best-selling analgesic drug) HPLC assay is an isocratic run on a 250-mm long column with 5-µm L1 particles[2]. An Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5-µm column (p/n 959990-902) column was used to perform tramadol system suitability under USP conditions (Experiment 1). Official guidelines are available from USP on allowed deviations for column dimensions (Table 1)[1].

Within the current guidelines, the particle size can be reduced up to 50%, to 2.5 µm. The Agilent Poroshell 120 packing has a solid core of 1.7 µm with a porous 0.5-µm thick outer layer and a total particle size of 2.7 µm. This column can be treated like a sub-2 µm column regarding performance. The use of an Agilent Poroshell column allows a reduction of the column length to achieve the same performance by maintaining a comparable L/dp ratio. To verify the acceptability of this modification for tramadol assay, a system suitability testing was performed using an Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7-µm column (p/n 695975-902) (Experiment 2).

USP is under discussion to relax the current restrictions on particle size for isocratic separations and incorporate a new limit based on L/dp ratio. The new proposed column dimension deviation limits for isocratic analysis is shown in Table 2[3]. After the approval of the new proposed plan, the Agilent 1.8-µm particle columns can also be used for tramadol assay analysis as replacement for 5 µm. For a 250-mm column with 5-µm particle size, the L/dp ratio is 250,000 µm /5 µm, and gives a value of 50,000. With a particle size of 1.8 µm instead 5 µm, a column length of 100 mm gives a higher L/dp value and promises better performance. When the column length is reduced by a factor of 2.5, analysis time will also reduced by this factor. The column flow can be further minimized by reducing the id.

The enhanced time and solvent saving as per the latest USP Interim Revision Announcement (IRA) proposal was illustrated in Experiment 3 by performing system suitability testing of tramadol using a ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8-µm column (p/n 959758-902). The new flow rate for Experiment 3 was calculated using Equation 1 mentioned in the USP IRA proposal on general chromatography chapters <621> which is currently open for discussion and will be published in USP36-NF31 1S[3].

$$F_2 = F_1 \times \left(\frac{dc_2^{\,2} \times dp_1}{dc_1^{\,2} \times dp_2} \right) \qquad \text{Equation 1}$$

Where F_1 and F_2 are the flow rates for the original and modified conditions, respectively; dc_1 and dc_2 are the respective column diameters and dp_1 and dp_2 are the particle sizes.

All these combinations such as reduction in column length and internal diameter along with the use of smaller particle size, can contribute to significant savings in analysis time and solvent. This results in higher throughput, less cost of analysis, and improved productivity of the lab without compromising on chromatographic performance.

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

Table 1
Currently allowed column deviations as per USP <621> recommendations.

Column parameter	USP limit for deviation
Length and particle size	No limit, but keep constant L/dp ratio or should be between -25% to +50% of the original L/dp ratio
Internal diameter	No limit, but keep constant linear velocity
Flow rate	±50%

Table 2
Proposed column deviations for isocratic run as per USP Interim Revision Announcements (IRAs).

Experimental

Instruments

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

The systems was controlled using the Agilent ChemStation C.01.03 software.

Chromatographic parameters

The mobile phase for the isocratic run was prepared as per the USP method. A premixed 30:70 solution of acetonitrile and water with 0.05% trifluoroacetic acid was used as the mobile phase. The detection was done at 270 nm. The detailed chromatographic method parameters for each experiment are tabulated in Table 3.

Reagents and materials

Commercially available USP reference standards for tramadol and corresponding related compound A were purchased from USP-India Private Ltd, Hyderabad, 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. Trifluoroacetic acid was used as additive and purchased from Aldrich (India).

Procedure

The system suitability solution was prepared as per USP method for tramadol described in USP 34–NF 29.

System suitability solution:
0.05 mg/mL each of USP tramadol hydrochloride RS and USP tramadol hydrochloride related compound A (RCA) in mobile phase. Using all three experimental conditions, system suitability testing for tramadol assay method was performed.

Parameter	USP method (Experiment 1)	Experiment 2	Experiment 3
Injection volume (µL)	20	8	1.7
Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm (p/n 959990-902)	Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-902)	Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)
Flow rate (mL/min)	1.0	1.0	0.58
Acquisition rate (Hz)	10	10	20

Table 3
Detailed chromatographic parameters for all the three experiments.

Results and discussion

System suitability results

The peaks were well separated under all three experimental conditions, as shown in the chromatograms in Figure 1. The data in Figure 1 illustrates the reduction in analysis time for tramadol assay while reducing the column length from 250 mm to 100 mm. The observed back pressure with the 1.8-µm column was above 600 bars. Here, the 1290 Infinity LC System can operate at higher pressures (up to 1,200 bar) and is best suitable for developing high throughput UHPLC methods with higher column back pressure. Six replicate injections were made under each set of experimental conditions to assess injector precision and were evaluated by calculating the relative standard deviation (RSD) for area and retention time. The system suitability results obtained under all three experimental conditions were tabulated in Table 4 and the results were found to be within the acceptance criteria.

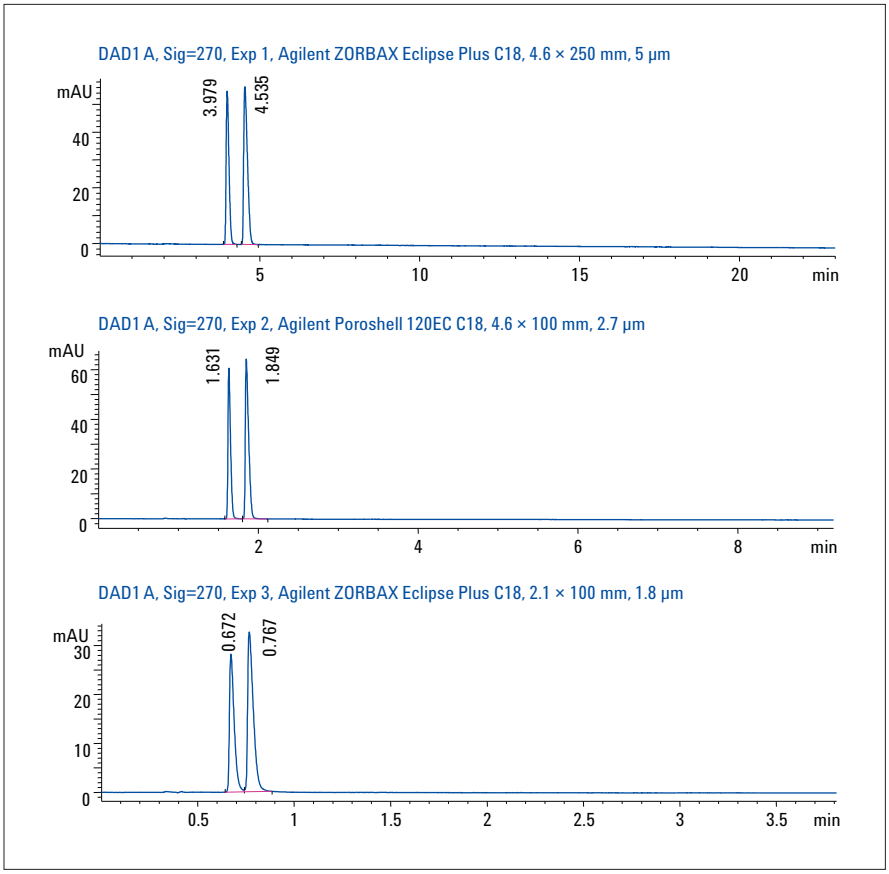

Figure 1
Separation of tramadol system suitability mix in USP method and newly developed cost saving methods. For uniformity, five times of the main peak retention time was considered as the run time.

No.	Test	Results			
		Limit	Exp. 1 (USP)	Exp. 2	Exp. 3
1	Resolution between tramadol related compound A and tramadol	NLT 2.0 *	2.9	3.2	2.0
2	RT RSD (%)	NMT 2.0 #	0.09	0.08	0.07
3	Area RSD (%)	NMT 2.0 #	0.14	0.13	0.34
4	RRT of related compound A	0.9	0.88	0.88	0.88

*NLT: Not less than
#NMT: Not more than.

Table 4
System suitability results obtained under all three experimental conditions.

Cost of analysis

For the experiments, the cost of analysis was calculated with the cost of acetonitrile as US $ 60/L, the running cost of an instrument as US $ 80/hour, and the cost associated with waste solvent disposal as US $ 1.5/L. The approximate time required to complete one injection for tramadol USP assay method is approximately 22.7 minutes and the total analysis cost is approximately US $ 30.6/injection. However, by adopting the conditions of Experiment 2, using a Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7-µm column, the total cost of analysis can be reduced to US $ 12.5 and productivity can be increased by 2.5 times. Likewise, the total expense using a ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8-µm column under the conditions of Experiment 3 was calculated as only US $ 5.1 and the time required to complete assay analysis was just 3.84 minutes. The graphical representation of analysis time, solvent consumption, and cost of analysis for all three experiments is given in Figure 2. Agilent sub-2 µm columns can be efficiently used to improve the speed of analysis. While achieving higher throughput, narrow bore sub-2 µm columns consume less solvent than conventional HPLC methods. The example shown here illustrates that by reducing the column length from 250 mm to 100 mm and the particle size from 5 µm to 2.7 µm, 59% of solvents can be saved. Reducing the particle to 1.8 µm with a narrow bore column, results in a 90% saving of solvents.

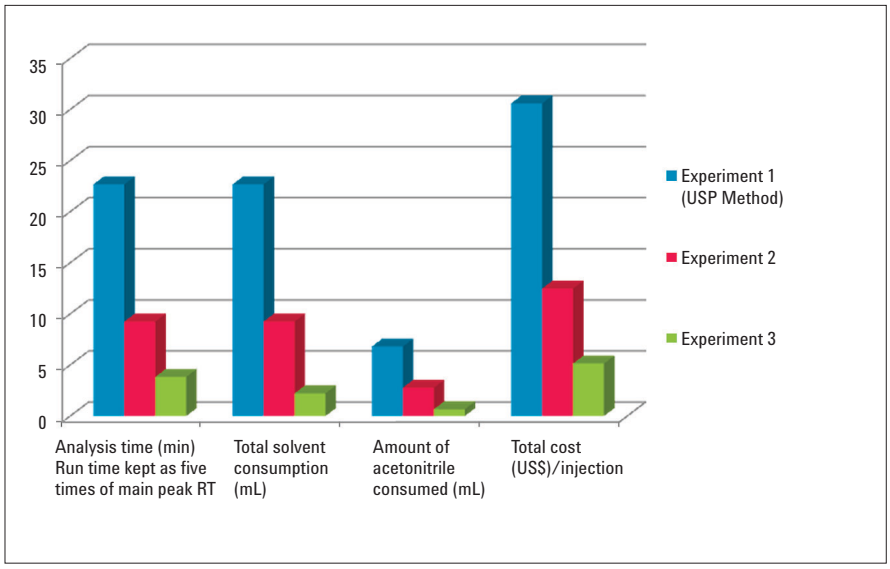

Figure 2
Solvent, time and total cost calculation for USP and newly developed cost saving methods.

Conclusion

Two new methods were developed for tramadol assay by modifying existing USP recommended column dimensions. The usability of these new methods for daily assay purpose was confirmed by performing system suitability testing with each new method. In the first method, column length and particle size were varied within the USP<621> limits that are currently in place resulting in a total cost saving of US$ 18/injection which is 59% of the USP method. In the second method, an Agilent sub-2 µm column was used as per the latest USP Interim Revision Announcements. This reduced the run time from 22.7 minutes to 3.8 minutes without compromising on system suitability criteria. With the time required to complete one injection under USP conditions, one can complete six injections using this method. That means, by using an Agilent 1290 Infinity LC System with Agilent sub-2 µm columns for UHPLC method development a 6-fold increment in lab productivity can be achieved. When moving from a HPLC method using a 25-cm, 5-µm column to a UHPLC method with a 10-cm, 1.8-µm column, 90% of solvents and 83% of analysis time could be saved.

References

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

2.
USP method for Tramadol assay, USP34–NF29 (**2010**).

3.
USP Pharmacopeial Forum (PF) 38(2) In-Process Revision: <621> chromatography (USP36-NF31 1S), Page 17 (**2012**).

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Published in the USA, August 1, 2012
5991-0733EN

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