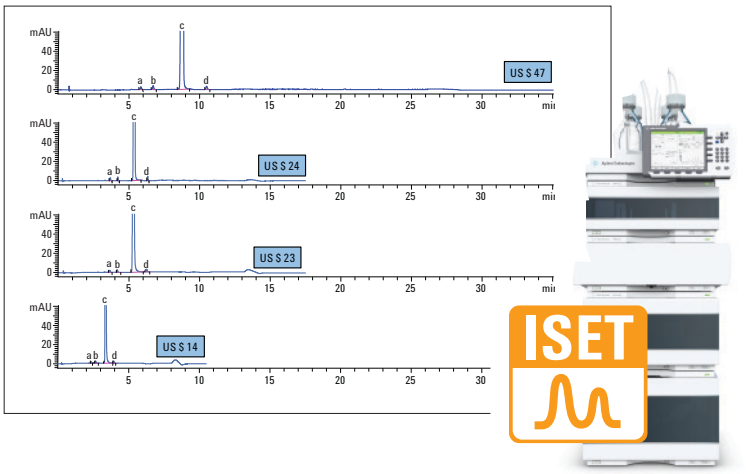
# Reducing total analysis cost for generic drugs within USP <621> allowed limits

## Saving solvent, time, and cost for a risperidone assay method using Agilent sub-2 µm columns and an Agilent 1290 Infinity LC System with ISET

## Application Note

Pharmaceutical QA/QC





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## Abstract

This Application Note describes an approach to significantly reduce cost of analysis and increase throughput of liquid chromatography (LC) under pharmacopeia guidelines using the example of a risperidone assay method. Cost reduction was achieved by saving time and solvent by varying column dimensions used for chromatographic separation. The variation of column dimensions were made as per the United States Pharmacopeia (USP) guidelines on allowed deviations for column dimensions thereby eliminating the need for method revalidation. Three new shorter columns were chosen and new gradient parameters were derived using the automated Agilent Method Translator. The Agilent 1290 Infinity LC System with Intelligent System Emulation Technology (ISET) was used for the instrumentation. The cost of analysis per injection can be reduced from US $ 47 to US $ 14 by varying column dimensions within USP guidelines. The reliability of the new cost effective methods for assay purpose was confirmed by analyzing risperidone tablets.


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## Introduction

Risperidone is one of the important block buster drugs used as a typical antipsychotic[1]. The USP assay method for risperidone runs for approximately 35 minutes and uses a 100 × 4.6 mm column with a 3-µm L1 packing. Assuming that the cost of methanol is US $ 25/L and the cost for running an instrument is US $ 80/hour, the total cost for risperidone analysis amounts to US $ 47 per injection. This includes the cost of US $ 1.5/L for solvent waste disposal. A significant amount of solvent and analysis time can be saved by reducing column dimensions. USP has clear guidelines on permitted column dimension deviations[2]; this is summarized in Table 1.

In this Application Note, the use of Agilent Polaris C18-Ether 4.0 × 100 mm, 3 µm column to carry out the standard USP analysis (Experiment 1) is described. Since the column internal diameter was different than the USP recommendation, the flow rate was adjusted to maintain linear velocity constant. To reduce the analysis cost, three additional smaller columns were selected. For the adoption of any column dimension modification, a system suitability test as per pharmacopeia should be performed and criteria should be met[3]. The risperidone USP System Suitability Mixture contains the active pharmaceutical ingredient (API) and approximately 0.2% each of the following impurities: a) Z-Oxime, b) 9-Hydroxyrisperidone, and c) 6-Methylrisperidone.

| 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**

The system suitability test for risperidone includes:

a) Identification of the peaks in the system suitability mix using relative retention time (RRT)

b) Measurement of the resolution between Z-oxime and 9-hydroxyrisperidone (limit: not less than 2.8)

c) Calculation of the tailing factor for risperidone (limit: not more than 1.5) and

d) Calculation of the relative standard deviation (RSD) (limit: not more than 2.0% for the risperidone peak).

An Agilent 1290 Infinity LC System with ISET was used and emulated to various different instrument modes according to the column dimensions used. The ISET algorithm delivered identical gradient mixing conditions as selected other instruments and eliminated the variation due to difference in delay volumes[4]. A risperidone tablet assay using the new shorter methods was performed to demonstrate the usability for quantifying the API from real samples.

## Experimental

### Instruments

The 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

| Column parameter | USP recommendation | Experiment 1 | | Experiment 2 | | Experiment 3 | | Experiment 4 | |
|---|---|---|---|---|---|---|---|---|---|
| | | Actual | % Deviation | Actual | % Deviation | Actual | % Deviation | Actual | % Deviation |
| length | 100 mm | 100 mm | 0 | 50 mm | –50 | 50 mm | –50 | 30 mm | –70 |
| Diameter | 4.6 mm | 4.0 mm | –13.0 | 4.6 mm | 0 | 2.1 mm | –47.5 | 2.1 mm | –47.5 |
| Particle size | 3 µm | 3 µm | 0 | 1.8 µm | –40 | 1.8 µm | –40 | 1.8 µm | –40 |

**Table 2**
**Dimensions of columns used and their deviations from the original USP method.**

In addition to the USP method (Experiment 1), three additional methods (Experiments 2, 3, and 4) were performed with modified column dimensions within the allowed deviation limit. Savings in time and solvent were evaluated and compared to the original pharmacopeia method. Table 2 shows the column parameter details of columns used in all four experiments.

## Reagents and materials

The USP reference standards for risperidone and corresponding impurities were purchased from USP-India Private Limited (Hyperbad, India).

Methanol 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. Other chemicals like ammonium acetate, and acetic acid to make the mobile phases were purchased from Aldrich (India). Risperidone 2-mg tablets were purchased locally.

| | 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 | 8 µL (USP injection volume is 10 µL) | 5 µL | 1.0 µL | 0.6 µL |
| Column | Agilent Polaris C18-Ether 4.0 × 100 mm, 3 µm (p/n A2021100X040) | Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 5 µm (p/n 959993-902) | Agilent ZORBAX Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (p/n 959741-902) | Agilent ZORBAX Eclipse Plus C18, 2.1 × 30 mm, 1.8 µm (p/n 959731-902) |
| Flow rate | 1.13 mL/min | 1.5 mL/min | 0.31 mL/min | 0.31 mL/min |
| Gradient | At 0 min 30% B<br>At 1 min 30% B<br>At 20 min 95% B<br>At 25 min 95% B<br>At 27 min 30% B<br>At 35 min 30% B | At 0 min 30% B<br>At 0.5 min 30% B<br>At 10 min 95% B<br>At 12.5 min 95% B<br>At 13.5 min 30% B<br>At 17.5 min 30% B | At 0 min 30% B<br>At 0.5 min 30% B<br>At 10 min 95% B<br>At 12.5 min 95% B<br>At 13.5 min 30% B<br>At 17.5 min 30% B | At 0 min 30% B<br>At 0.3 min 30% B<br>At 6 min 95% B<br>At 7.5 min 95% B<br>At 8.1 min 30% B<br>At 10.5 min 30% B |
| Acquisition rate | 10 Hz | 10 Hz | 10 Hz | 20 Hz |

**Table 3**
**Chromatographic parameters used for all four experiments.**

## Chromatographic parameters

The buffers and mobile phases were prepared as per the USP method. The buffer solution was prepared by dissolving 15.4 g ammonium acetate in 1 L of water and adjusting the pH to 6.5 using 10% acetic acid. Mobile phase A was prepared by mixing 100 mL of buffer solution with 150 mL of methanol in a 1 L volumetric flask and diluted with water to volume. Mobile phase B was prepared by mixing 100 mL of buffer solution with 850 mL of methanol in a 1 L volumetric flask, and diluting with water to volume. The column temperature was maintained at 35 °C and the detection was set at 275 nm. The detailed chromatographic method parameters used for each experiment are shown in Table 3.

## Procedure

The system suitability solutions are prepared as per USP assay method for risperidone described in USP 34–NF 29 (1 mg/mL solution of USP Risperidone System Suitability Mixture). System suitability testing and API assay using extracted sample from 2.0 mg risperidone tablet were performed using all four columns. Relative retention time (RRT) of all the peaks, resolution 9-hydroxyrisperidone with respect to Z-oxime, tailing factor and RSD values for the risperidone peak were computed for all the four experimental conditions. Selecting the appropriate instrument models suitable for each column dimensions was done by emulating the 1290 Infinity LC using ISET. Shorter gradient time parameters were calculated using the Agilent Method Translator in simple conversion mode. Savings in total time and solvent were calculated for each experiment.

## Results and discussion

### Separation and detection

Experiments 1–4 were performed using the following instrument/column configurations:

- Experiment 1:(USP method) Polaris C18-Ether 4.0 × 100 mm, 3 µm column, Agilent 1290 Infinity Binary LC System with ISET emulating to Agilent 1100 Series Binary LC System
- Experiment 2: ZORBAX Eclipse Plus C18 4.6 × 50 mm, 1.8 µm (length approximately 1/2 of original length, original id and smaller particle size), Agilent 1290 Infinity LC System with ISET emulating Agilent 1260 Infinity Binary LC System
- Experiment 3: ZORBAX Eclipse Plus C18 2.1 × 50 mm, 1.8 µm (same length as Experiment 2, but 2.1 mm id, smaller particle size).
- Experiment 4: ZORBAX Eclipse Plus C18 2.1 × 30 mm, 1.8 µm (minimum length according to USP limits, and 2.1 mm id, smaller particle size) Experiments 3 and 4 were performed using the Agilent 1290 Infinity LC System without ISET.

All the peaks in the system suitability mix were well separated in all four experiment conditions and the chromatograms are shown in Figure 1.

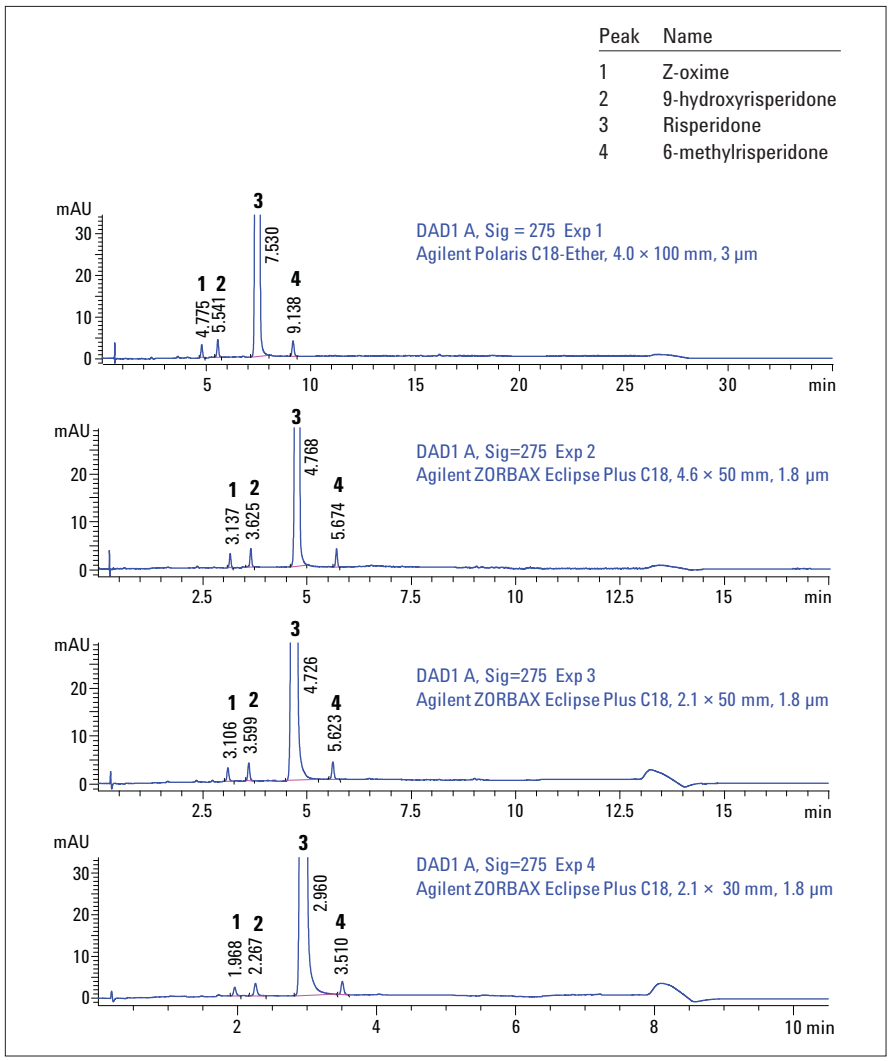


**Figure 1**
**Separation of risperidone system suitability mix in four different experiment conditions.**

System suitability results from all the experiments are tabulated in Table 4.

The system suitability test results were found to be within the acceptance criteria. This was true even for a 30-mm ZORBAX Eclipse Plus C18 column (Experiment 4). The use of shorter columns significantly reduced analysis time and total solvent for the gradient cycle, saving a total of 93.8% solvent and 70% time by adjusting the column dimensions within the USP limits (cost reduction of US $ 33 per injection, Figure 2).

## Analysis of the risperidone tablets

The tablet's manufacturer claims that each tablet contains 2.0 mg API/tablet. Using the USP method (Experiment 1) we obtained a calculated amount of 1.7 mg API/tablet. The assay value calculated using the newly developed shorter methods (Experiments 2, 3, and 4) also gave same results as Experiment 1. This confirms the utility of using the shorter methods for cost effective assay analysis.

| SI no. | Test | USP limit | Results | | | |
|---|---|---|---|---|---|---|
| | | | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 4 |
| 1 | Resolution between Z-oxime and 9-hydroxyrisperidone | NLT 2.8 | 5.97 | 7.48 | 6.01 | 4.58 |
| 2 | USP Tailing factor Risperidone | NMT 1.5 | 0.623 | 0.661 | 0.789 | 0.933 |
| 3 | RSD RT for Risperidone | NMT 2.0% | 0.02% | 0.02% | 0.02% | 0.03% |
| 4 | RSD Area for Risperidone | NMT 2.0% | 0.18% | 0.32% | 0.39% | 0.79% |
| 5 | RRT for Z-Oxime | 0.67 | 0.66 | 0.68 | 0.68 | 0.68 |
| 6 | RRT for 9-Hydroxyrisperodone | 0.76 | 0.76 | 0.78 | 0.78 | 0.79 |
| 7 | RRT for 6-methyl risperidone | 1.2 | 1.19 | 1.17 | 1.17 | 1.17 |

**Table 4**
**System suitability results for all four experiments (NLT = not less than, NMT = not more than).**

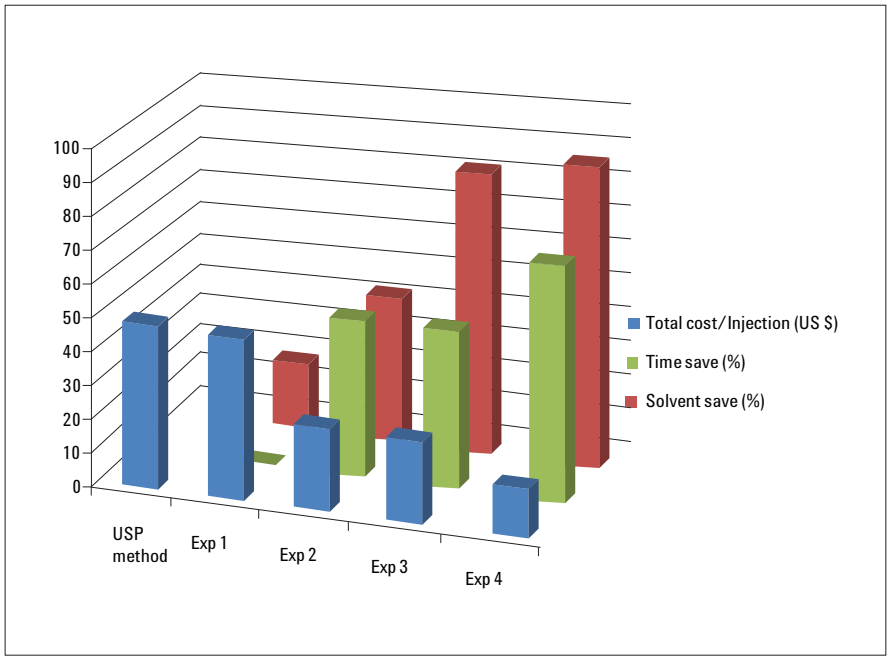


**Figure 2**
**Solvent, time and total cost calculation for all four experiments.**

## Conclusion

- An easy approach to reduce the total cost of analysis for a generic drug, such as risperidone, by using the Agilent 1290 Infinity LC System with ISET is shown.
- The Agilent 1290 Infinity LC System with ISET allowed smooth transferring of method from one to another different instrument model.
- The cost reduction was achieved by varying USP recommended column dimensions within the allowed deviation limits, thereby avoiding the need for method revalidation.
- The usability of the new methods was confirmed by performing system suitability analysis.
- Using this approach, 70% of solvents and more than 90% analysis time can be saved amounting to a total cost advantage of US $ 33/injection.

## References


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**www.agilent.com/chem/ISET**


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