System suitability testing for Aripiprazole quality control with the Agilent 1120 Compact LC and Agilent TC C18(2) columns

Application Note

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Abstract

The Agilent 1120 Compact LC is the system of choice for conventional, analytical-scale liquid chromatography. It is an integrated LC designed for ease of use, performance, and reliability. It is well-suited for the analysis of drugs due to highly precise retention times and peak areas and low detection limits. This Application Note shows:

- Excellent retention time precision, with relative standard deviation (RSD) < 0.07 %.
- Excellent area precision, with RSD < 0.25 % for baseline-separated peaks.
- Excellent height precision, with RSD < 0.25 % for baseline-separated peaks.

Agilent Equipment

- Agilent 1120 Compact LC
- Agilent TC C18(2) column

Application Area

- Pharmaceutical industry: QA/QC

Agilent Technologies

Introduction

In the Pharmaceutical industry, liquid chromatography is a versatile tool for separation of individual analytes. Innumerable methods have been developed for analysis on HPLC as it is proven to be very reliable and reproducible at all stages from drug discovery to manufacturing. As a result, it is necessary to verify that any HPLC system performs within an acceptable range of precision and accuracy every day.

System Suitability Testing (SST) is a measure of instrument performance on a day-to-day basis. These tests ensure that the method and the HPLC system can generate results of acceptable accuracy and precision. The criteria selected is based on critical chromatographic parameters such as resolution, reproducibility in retention time, peak area and height, column efficiency and their variation (Standard Deviation) within acceptable limits which are defined during the method validation experiments.

In this Application Note, we focus on this final validation step and evaluate the suitability of the Agilent 1120 Compact LC system for the analysis of Aripiprazole and its precursors.

Figure 1
Agilent 1120 Compact LC.

Experimental

Equipment

The Agilent 1120 Compact LC system included:

- A gradient pump with low-pressure mixing
- An autosampler with vial tray
- A column compartment for a column up to 250 mm in length
- A variable wavelength detector (VWD)

The column was an Agilent TC (Typical Carbon load) C18(2) column, 150 x 4.6 mm, 5 µm particle size.

The instrument was controlled by Agilent EZChrom Elite Compact Compliance software.

Compounds

1-(2,3-Dichlorophenyl)piperazine hydrochloride (starting material-1) and 7-(4-bromobutoxy)-3,4-dihydrocarbostyril (starting material-2) are the two starting materials for the synthesis of Aripiprazole. The synthetic scheme is shown in figure 2. For this study, the two starting materials are treated as impurities.

Preparation of samples

Stock preparation: 2 mg/mL of the Aripiprazole and 5 mg/mL of starting material-1 and -2 were prepared as individual stock solutions. Aripiprazole and the two starting materials were initially dissolved in methanol (20 % of the total make-up volume), and diluent was then added up to the marks.

System suitability sample: A test mix for system suitability was prepared with 10 µg/mL Aripiprazole and each of the impurities at 5 µg/mL.

Chromatographic parameters

The chromatographic method was set up such that all compounds were baseline-separated. The conditions were:

- Sample: 1-(2,3-dichlorophenyl)piperazine, 7-(4-bromobutoxy)-3,4-dihydrocarbostyril, and Aripiprazole
- Column: Agilent TC-C18(2), 150 x 4.6 mm, 5 µm
- Mobile phases: A = water + 0.2 % trifluoroacetic acid (TFA), B = acetonitrile + 0.16 % TFA
- Flow rate: 1.0 mL/min
- Gradient: at 0 min 30 %B, at 7 min 70 %B, then hold the ratio

for two more minutes
- Injection volume: 10 µL
- Autosampler programmed with a wash vial (using acetonitrile) for rinsing exterior of the needle
- Run time: 9 min
- Post time: 5 min
- Column oven: 40 °C
- VWD: 254 nm, peak width (PW) > 0.05 min
- Diluent / blank: 60:40 acetonitrile:water

Sequence table

Based on the recommendations by ICH (International Conference on Harmonization) for system suitability tests, the sequence table shown as table 1 was set up in the Agilent EZChrom Elite Compact Compliance software.

Results and discussion

In figure 3, an example chromatogram for system suitability testing shows excellent resolution. The separation time was nine minutes; the total run time (including the re-equilibration) could be limited to 14 minutes. The mobile phase contained trifluoroacetic acid as modifier, which improved retention and peak shape.

When analyzing drugs with UV detection, precision of retention times is of utmost importance. The precision of retention times and areas was determined from 10 replicate injections of system suitability sample. Figure 4 shows an overlay of 10 consecutive runs.

The acceptance criteria for this system suitability study are tabulated in table 2.

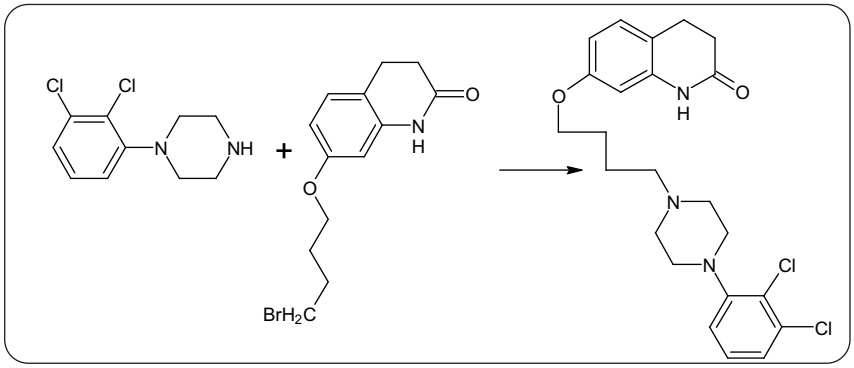

Figure 2
Structures of starting material-1 and -2 and the product Aripiprazole.

Line	Location	Sample name	# Injections	Injection volume (µL)
1	Vial 1	Blank	3	10
2	Vial 2	System suitability	10	10
3	Vial 3	Blank	1	10

Table 1
Sequence table.

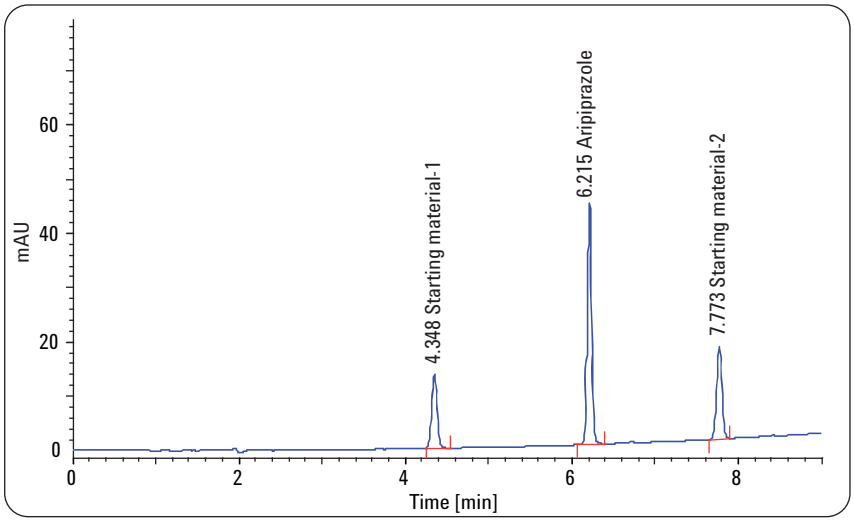

Figure 3
Chromatogram of Aripiprazole and its impurities.

Parameter	Limit
RSD of retention time (RT)	< 0.07 %
RSD of area	< 1.00 %
Resolution	> 2.00
Asymmetry	< 2.00
Theoretical plates	> 2000
Peak width	< 0.08 min
RSD of height	< 0.50 %

Table 2
Acceptance criteria.

Compound	Amount (µg/mL)	RSD of RT (%)	RSD of area (%)	Reso-lution	PW (min)	Asymmetry	Theoretical plates	RSD of height (%)	Passed (yes/no)
Starting material-1	5.15	0.060	0.215	N/A*	0.07	0.99668	> 20500	0.197	Yes
Aripiprazole	10.1	0.042	0.101	16.96	0.06	1.00471	> 60000	0.219	Yes
Starting material-2	5.4	0.019	0.154	14.93	0.07	0.98137	> 60000	0.075	Yes

*N/A = not applicable

Table 3
System suitability test results.

The results of the system suitability testing are shown in figure 4 and are summarized in table 3.

These results of the system suitability test for Aripiprazole demonstrate that the Agilent 1120 Compact LC meets the stringent performance requirements for pharmaceutical QA/QC analysis.

Conclusion

For the analysis of drugs in routine QA/QC, it is very important to have highly precise, accurate, and robust LC systems. This enables reliable analysis of pharmaceutical drugs and their impurities. The Agilent 1120 Compact LC system is well-suited for this application because it delivers the needed data quality and is based on a proven robust design.

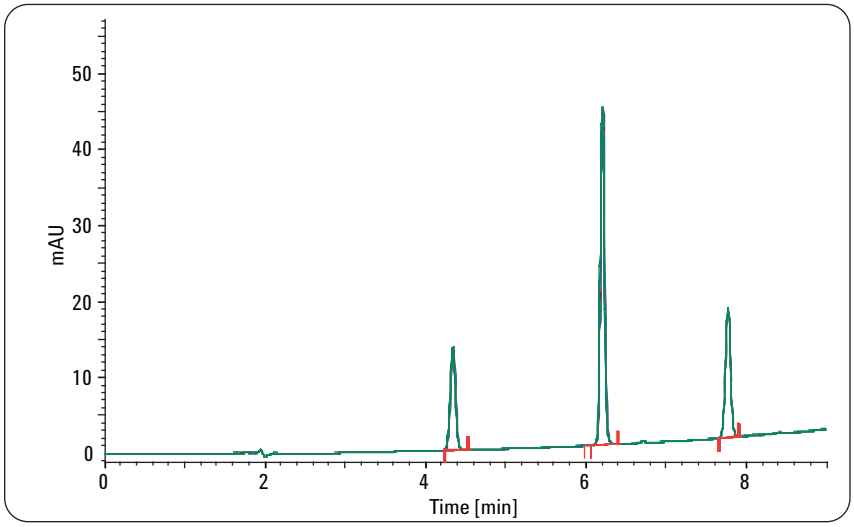

Figure 4
Overlay of 10 repetitive chromatograms.

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