System suitability testing for Tramadol quality control with the Agilent 1120 Compact LC and ZORBAX C-18 columns

Application Note

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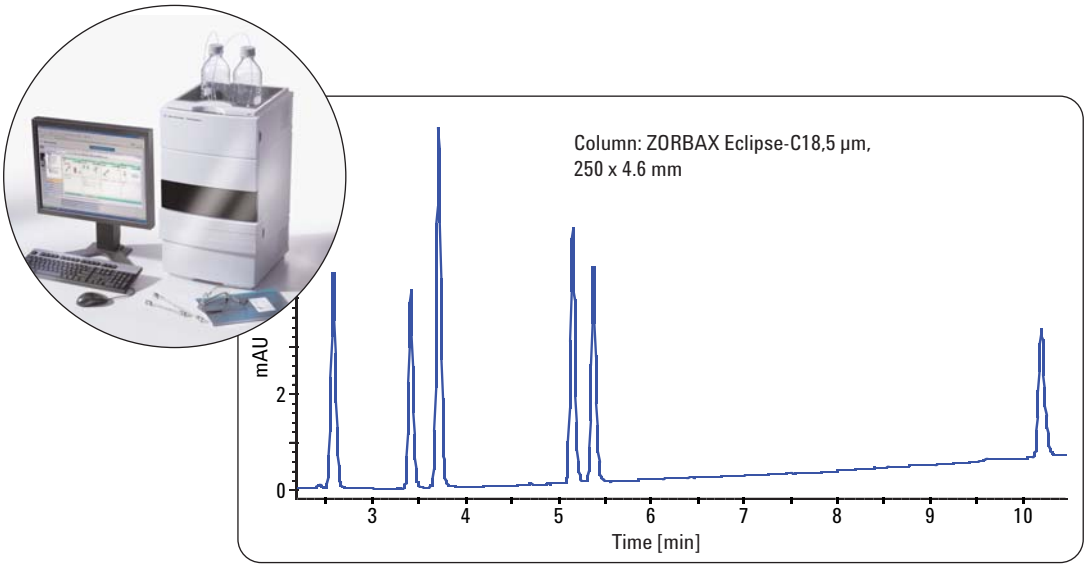

Abstract

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 the highly precise retention times and peak areas. 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.

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Introduction

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. Currently SST measurements have become a part of the analytical procedures, and are also recommended by the pharmacopeias and accepted by the United States Food and Drug Administration (FDA).

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 the analgesic drug Tramadol and potential impurities from its production.

Figure 1
Agilent 1120 Compact LC.

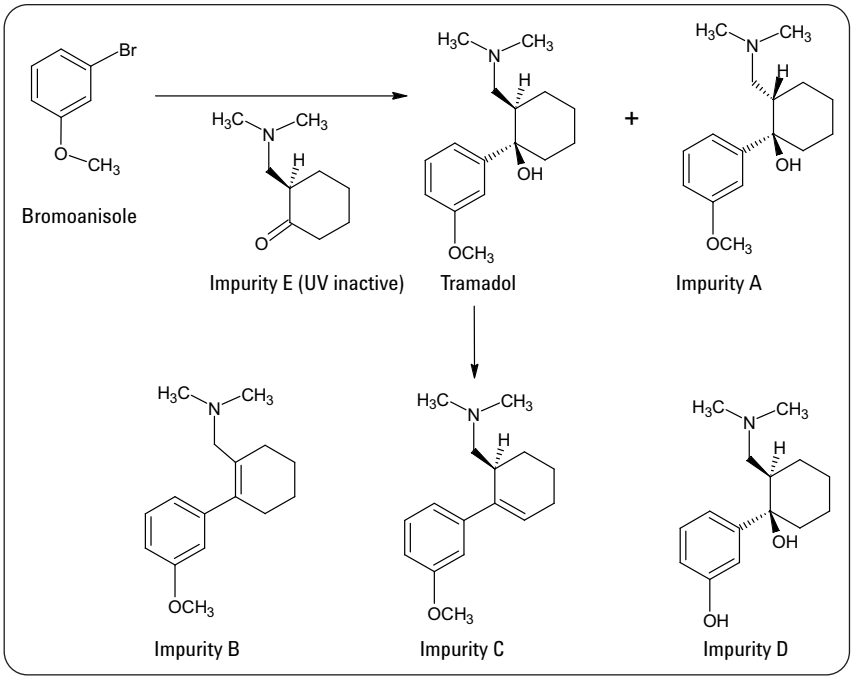

Figure 2
Structures of the compounds from the synthesis of Tramadol.

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)

A ZORBAX Eclipse XDB C18, 5 µm, 250 x 4.6 mm, was used.

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

Structures of compounds used

Figure 2 shows the synthesis of Tramadol, highlighting both starting materials and potential byproducts. This study focused on analysis of all of these compounds

except for impurity E, which is an ultraviolet (UV)-inactive starting material.

Sample preparation

Stock preparation: 2 mg/mL of Tramadol and 5 mg/mL each of starting material (3-bromoanisole) and impurities A, B, C, and D were prepared as the six stock solutions.

System suitability sample: A test mix for system suitability was prepared with Tramadol at 10 µg/mL and all other starting materials/impurities at 5 µg/mL each. This test mix was injected six times for the calculation of system suitability.

Chromatographic parameters

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

- Sample: Tramadol; impurities A, B, C, and D; and 3-bromoanisole
- Column: ZORBAX Eclipse XDB C18, 5 µm, 250 x 4.6 mm,
- Mobile phases:
  A = water + 0.2 % TFA,
  B = acetonitrile + 0.16 % TFA
- Flow rate: 1.2 mL/min
- Gradient: at 0 min 30 %B, at 9 min 85 %B, then hold the ratio for three more minutes
- Injection volume: 10 µL
- Autosampler programmed with a wash vial (using acetonitrile) for cleaning the needle exterior
- Run time: 12 min
- Post time: 5 min
- Column oven: 30 °C
- VWD: 270 nm, peak width (PW) > 0.05 min
- Diluent / blank: 30:70 acetonitrile:water

Sequence table

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

Line	Location	Sample name	# Injections	Injection volume (µL)
1	Vial 1	Blank	3	10
2	Vial 2	System suitability	6	10
3	Vial 3	Blank	1	10

Table 1
Sequence table.

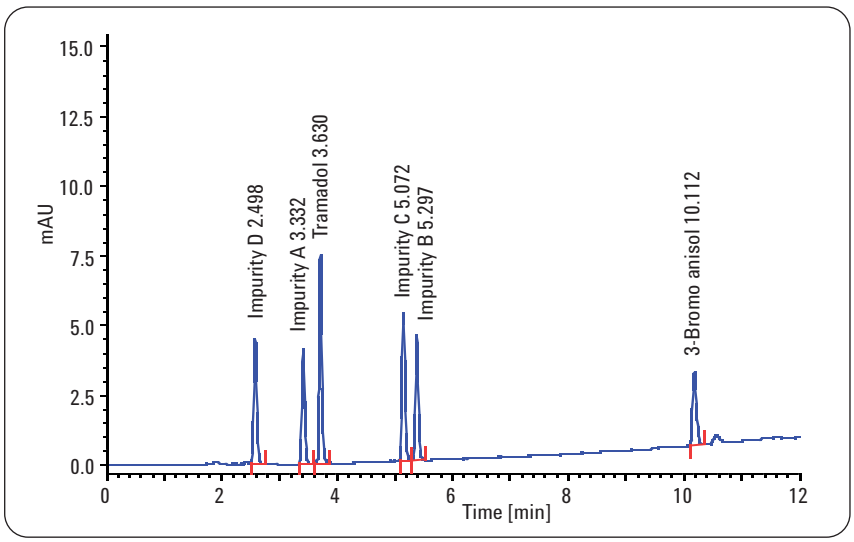

Figure 3
Chromatogram of Tramadol with impurities and starting material.

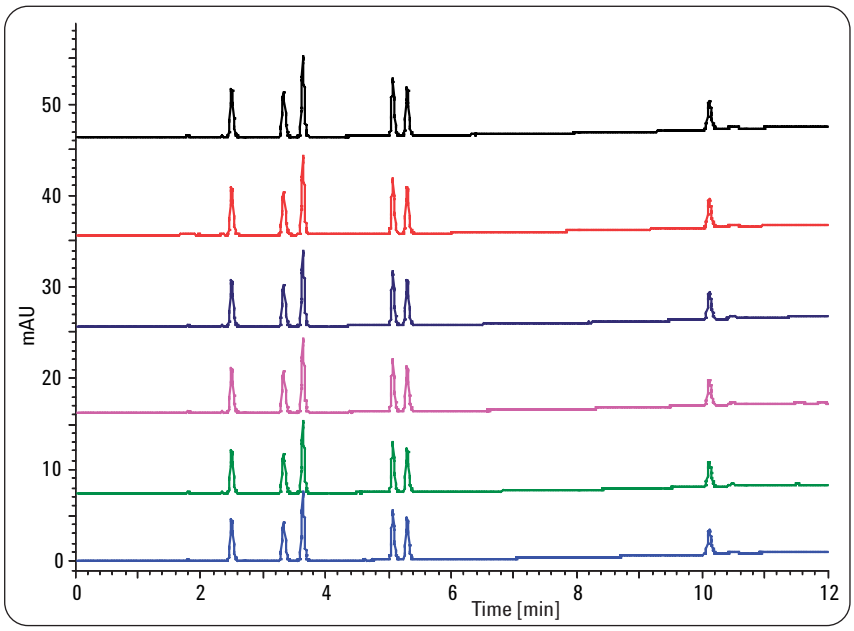

Figure 4
Overlay of 6 repetitive chromatograms..

Results and discussion

In figure 3, an example chromatogram for system suitability testing shows excellent resolution. The separation time was 12 minutes and the total run time (including time for re-equilibration) could be limited to 17 minutes. The mobile phase contained trifluoroacetic acid as modifier, which improved peak shape.

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

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

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.

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 Tramadol demonstrate that the Agilent 1120 Compact LC meets the stringent performance requirements for pharmaceutical QA/QC analysis.

Compound	Results on 250 x 4.6 mm Agilent TC-C18 5 µm column								Passed
	Amount (µg/mL)	RSD of RT (%)	RSD of area (%)	Reso-lution	PW (min)	Asymmetry	Theoretical plates	RSD of height (%)	(yes/no)
Tramadol	10.1	0.037	0.150	3.23	0.05	1.16130	>25000	0.207	Yes
Impurity A	5.2	0.068	0.234	8.91	0.05	1.14739	>20000	0.347	Yes
Impurity B	5.3	0.013	0.191	2.47	0.05	1.14858	>50000	0.439	Yes
Impurity C	5.2	0.017	0.165	15.83	0.05	1.17513	>50000	0.199	Yes
Impurity D	5.1	0.066	0.237	NA	0.05	1.16080	>11500	0.413	Yes
3-Bromoanisole	5.2	0.008	0.212	45.17	0.07	1.28923	>60000	0.281	Yes

*N/A = not applicable

Table 3
System suitability test results.

Conclusion

The Agilent 1120 Compact LC is ideally suited for QA/QC of pharmaceuticals because the system gives excellent precision for retention times and areas. In this study, the precision for retention times was < 0.07 % RSD and for areas of baseline-separated peaks was < 0.25 % RSD.

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