Determination of degradation products of Metoprolol tablets with the Agilent 1120 Compact LC after method development with the Agilent 1200 Series Rapid Resolution LC system

Application Note

Pharmaceuticals

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Abstract

Conventional LC methods used in routine analyses to characterize or monitor chemicals are often standardized for quality control of pharmaceutical products. This Application Note describes the analysis of several degradation products of metoprolol with the Agilent 1120 Compact LC. The preceding method development was done with the Agilent 1200 Series Rapid Resolution LC (RRLC) system.

This Application Note starts with the final result of method development, to prove that no further development is necessary.[1] It shows that the Agilent 1120 Compact LC works as a reliable and highly robust instrument for standard LC methodology. The transfer from rapid resolution separation material to conventional columns by the Method Translator Software is shown. The results of system suitability and performance tests prove the reliability and quality of the Agilent 1120 Compact LC system for everyday quality control testing.

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Introduction

The Agilent 1120 Compact LC was designed to meet the highest requirements for analytical instrumentation used in routine analysis. In the field of quality control testing, products are often characterized with standard LC methods. Therefore, instruments used for these purposes need optimal cost-of-ownership, high reliability, high flexibility, and ease of use.

In the last few years the method development of new tests with a high-end Agilent 1200 Series RRLC system became familiar. In the Application Note "Agilent 1200 Series LC Method Development Solution for the analysis of degradation products of metoprolol tablets, Agilent Technologies publication 5989-9339EN" the results of the Agilent method development solution are shown.

This Application Note starts with the final results of method development, to prove that no further enhancement is necessary. It then shows that the Agilent 1120 Compact LC works as a reliable and highly robust instrument for standard LC methodology. It also shows that the Agilent 1120 Compact LC can be used for determinations after back transfer from rapid resolution separation material to conventional columns by the Agilent Method Translator Software. The results of system suitability and performance tests in this Application Note prove the reliability and quality of Agilent 1120 Compact LC for everyday quality control testing.

Instrumentation

For method development an Agilent 1200 Series RRLC system and an Agilent 1120 Compact LC with the following configurations were used:
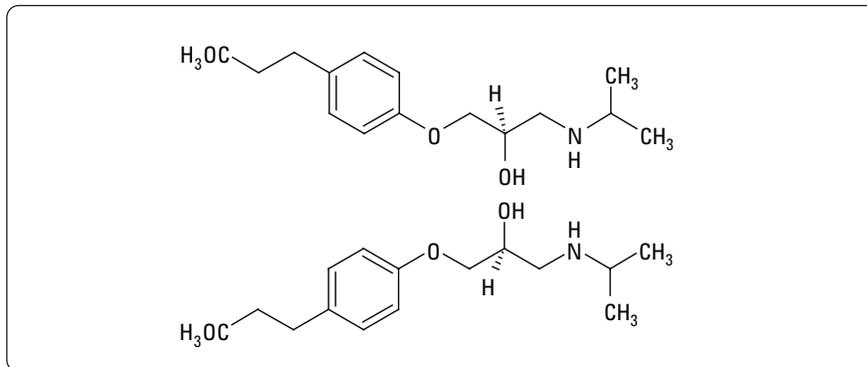
Configuration of the Agilent 1200 Series Rapid Resolution LC System	Configuration of the Agilent 1120 Compact LC
Binary pump (low delay configuration) and vacuum degasser	Gradient pump and vacuum degasser
Wellplate autosampler	Auto sampler
2 Column compartments	Column oven
Diode array detector	Variable wavelength detector
Software: ChemStation B.04.01	Software: EZ-Chrom Elite Compact 3.3

Preparation of samples

Reference samples

Samples were prepared in the same manner as those described in another Application Note.[1] Two 50-mg metoprolol tablets were powdered. One of the samples was dissolved in water. The other tablet was heated to 80 °C for 3 hours, and the residue also dissolved in water. Both solutions were two step filtered by syringe filters: first by an Agilent p/n 5064-8222, 2-µm filter followed by an Agilent p/n 5064-8221, 0.45-µm filter. Five microliters of the resulting liquids were injected.

Structure of Metoprolol (both isomers)

Chromatographic conditions	
Column	
For method development:	Agilent ZORBAX StableBond C18, 50 mm × 2.1 mm, 1.8 µm
For routine testing:	Agilent ZORBAX StableBond C18, 150 mm × 4.6 mm, 5 µm
Mobile Phase A:	Water + 0.2% TFA
Mobile Phase B:	Acetonitrile + 0.16% TFA
Gradient (linear):	Time (min) % B
	0 5
	45 50

Pump settings	
Stop time:	45 min
Post time:	5 min
Flow rate:	2.0 mL/min

Autosampler	
Injection volume:	15 µL

Thermostatted column compartment	
Temperature:	30 °C

Detector	14 µL cell, Peak width: >0.05 min, 1 s response time (10 Hz), Signal: 210 nm

System suitability and performance test

The following limits were used for system suitability testing the reference samples (see Reference Sample Preparation):

Similar peak pattern according to separation with rapid resolution

Precision of areas must be < 2 % RSD.

Precision of retention times must be < 0.5 % RSD.

The following samples were prepared and analyzed, using the limits and settings recorded in Table 1.

Sample	Purpose	Number of injections
Blanc solution	Verify baseline stability and identify artifacts	2
Suitability sample	Verify precision of areas and retention times for reference solution	10

Table 1
Setup for testing.

Results and discussion

Conditions for the method transfer were chosen from the Agilent 1200 Series RRLC method development system (see Preparation of samples).

The first step was to select the same column selectivity and column efficiency. The efficiency for the 50 mm × 2.1 mm, 1.8 µm rapid resolution high throughput (RRHT) column was estimated to nearly 12000 plates whereas for the 150 mm × 4.6 mm, 5 µm conventional column, 10700 plates were calculated. Agilent provides materials with the same selectivity independent of the particle size. As a final result of method development, the Agilent ZORBAX StableBond C18 material was found to separate all compounds.

According to these data, the RRLC parameters were transferred into the Agilent Method Translator to transform them to parameters suitable for conventional HPLC systems by selecting the simple conversion option (see Figure 1).

The Agilent Method Translator software calculates the new LC parameters, which should be used as starting conditions. Some parameters such as the different delay volumes of the pumps and different types of gradient mixing (high pressure versus low pressure) could not be converted satisfactorily. Sometimes these parameters must be adapted by experimentally optimizing the gradient steps.

Figure 1
Conversion of LC parameters found by rapid resolution to parameters suitable for conventional HPLC with Method Translator software.

After transfering the parameters from the 50 mm × 2.1mm, 1.8 µm RRHT column to the 150 mm × 4.6 mm, 5 µm column, the analysis of pure metoprolol tablets with the Agilent 1120 Compact LC system and EZ-Chrom Elite Compact Software obtained chromatograms similar to those in Figure 2. As a result of the Method Translator, the flow was set to 2 mL/min, which prolonged the analysis time without any influence on the resolution.

The chromatogram in Figure 3 shows the appropriate separation of the degradation products from the Metoprolol peak. With the variable wavelength detector, several peaks at 210 nm are detected. The pattern looks very similar to the pattern obtained after separation with the Agilent 1200 Series RRLC system (see "Preparation of samples").
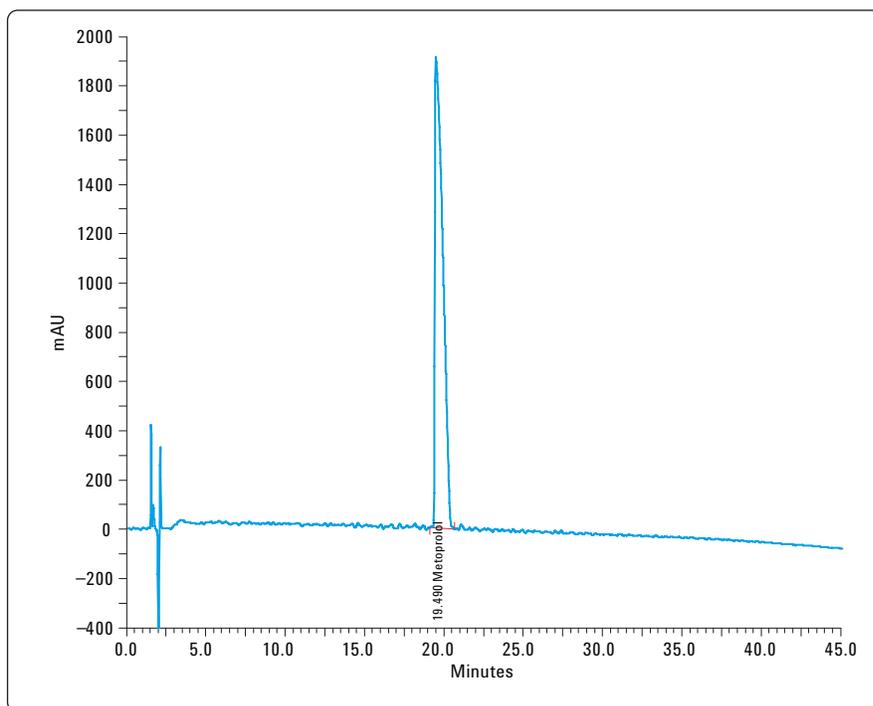

Figure 2
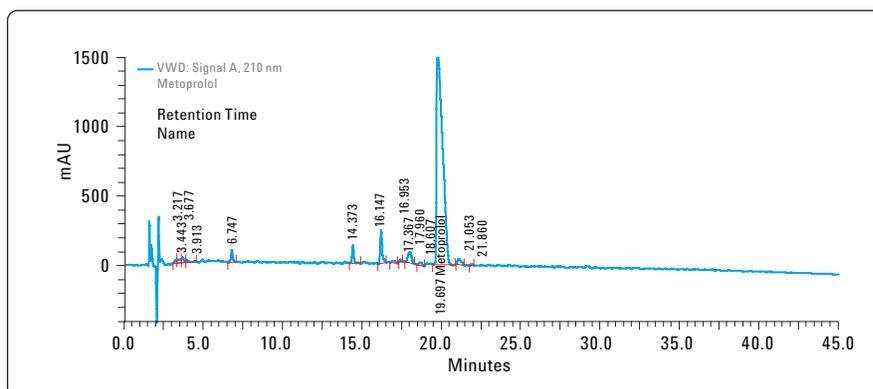**Analysis of metoprolol tablet after powdering, dissolving in water and filtering.**

Figure 3
Analysis of a "stressed" metoprolol tablet after powdering, dissolving in water and filtering.

Compared to the results obtained with the Agilent 1200 Series RRLC system (see Table 2) the chromatogram in Figure 3 shows that the elution order has not changed. It shows that the selectivity does not change with the particle size. Detailed data for some selected peaks (see Figure 4) are listed in Table 3. As a main result it can be emphasized that it is possible to transfer LC parameters from rapid resolution by the Method Translator to conventional LC systems and columns with 5-µm particles.

The adaptation of method development results with rapid resolution to the Agilent 1120 Compact LC was successful. The appropriate separation with the Agilent ZORBAX StableBond C18 material is proven by the data shown in Table 3. High resolution for every peak > 2 shows that this column material is highly suitable for the determination of the degradation peaks of Metoprolol.

To demonstrate the reliability and precision of the Agilent 1120 Compact LC the suitability sample was analyzed 10 times. The data presented in Table 4 show the areas and retention time precision results of all compounds.

To use the system for QC methods the criteria (see System suitability and performance test) must be fulfilled for all compounds due to strong limits . The data for precision of retention times and areas show that all data are within the limits.

The high precision of retention times for all components is also a result of performance and reliability of the pumping system. The high reproducibility of the autosampler is best shown by the data for area precision. These data allocate the Agilent 1120 Compact LC to be used for QC testing.

Parameter	Agilent 1200 SL System	Agilent 1120 Compact LC System
Column-dimensions	50 mm × 2.1 mm	150 mm × 4.6 mm
Particle-size	1.8 µm	5 µm
Flow	0.50 mL/min	2.0 mL/min
Temperature	30 °C	30 °C
Gradient time	0-15 min	0–45 min
Composition	5% to 50%	5% to 50%
Delay volume	Low delay (120 µL)	Normal delay (approx. 1 mL)

Table 2
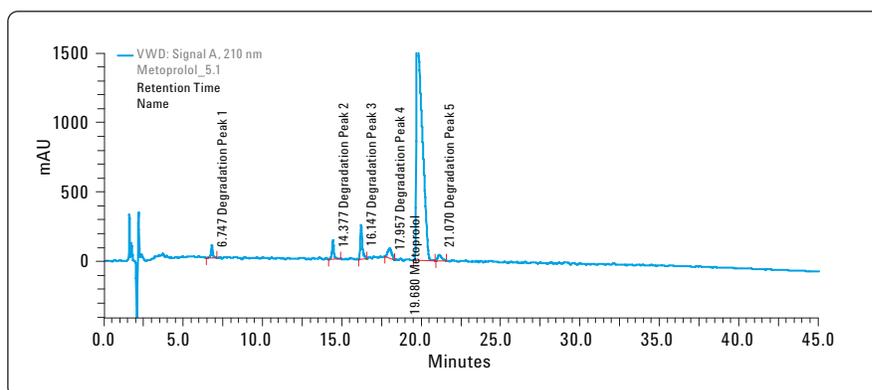**Detailed parameters for instrument setup.**

Figure 4
Selection of some representative peaks of a "stressed" metoprolol tablet for evaluating the stability of the separation.

Compound	Retention-time (min)	Resolution
Degradation peak 1	6.747	–
Degradation peak 2	14.377	37.84
Degradation peak 3	16.147	8.02
Degradation peak 4	17.957	5.23
Metoprolol	19.680	2.78
Degradation peak 5	21.070	2.25

Table 3
Results for retention times and resolution.

Compound	Retention-time (min)	RSD RT n=10	RSD Area n=10
Degradation peak 1	6.747	0	0.633
Degradation peak 2	14.377	0.023	0.747
Degradation peak 3	16.147	0.016	0.702
Degradation peak 4	17.957	0.048	0.601
Metoprolol	19.680	0.063	0.664
Degradation peak 5	21.070	0.048	0.895

Table 4
Suitability sample: Precision of retention times and areas .

Conclusion

The Agilent 1120 Compact LC was designed for conventional chromatography. Users from medium to small size companies, who have high requirements of reliability, ease-of-use and lowest cost-of-ownership for standard LC methodology in a QA/QC environment should be supported with this compact nonmodular LC system.

This Application Note shows the easy transfer of parameters determined by method development with Rapid Resolution (see Table 2). This approach is reliable and provides precision of areas and retention times, and high chromatographic resolution. The results show a system optimized for everyday productivity, which meets the highest requirements for routine analysis.

With the results shown in Table 3 the resolution of all main peaks was found to be greater than 2. The results in Table 4 show that all criteria for the precision of determination, such as area and retention times are fulfilled. The results allow the use of the Agilent 1120 Compact LC in QA/QC laboratories to determine degradation products of Metoprolol tablets.

All results show the applicability of the Agilent 1120 Compact LC for quality control testing with reduced costs per system and improved simplicity of use. In addition to the instrument capabili-ties, the new version of the EZChrom Elite compact software allows the full control of the Agilent 1120 Compact LC with a wide range of features for data analysis and reporting of results.

The high resolution results show that selectivity and performance of the Agilent ZORBAX StableBond material is independent of the particle size. The data also show that the design of the LC systems eliminates band broadening and peak distortion, and enhances the method transfer. In summary, an Agilent 1200 Series RRLC system provides fast method development and back transfer of results to conventional HPLC. The Agilent 1120 Compact LC is qualified for such an approach and meets the highest requirements for ordinary productivity.

References

1.
Agilent 1200 Series LC Method Development Solution for the analysis of degradation products of Metoprolol tablets, Agilent Technologies publication 5989-9339EN, **2009**

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