Analysis of β-Blockers Using the Agilent 1290 Infinity II LC Controlled Through Waters Empower Software

Application Note

Small Molecule Pharmaceuticals

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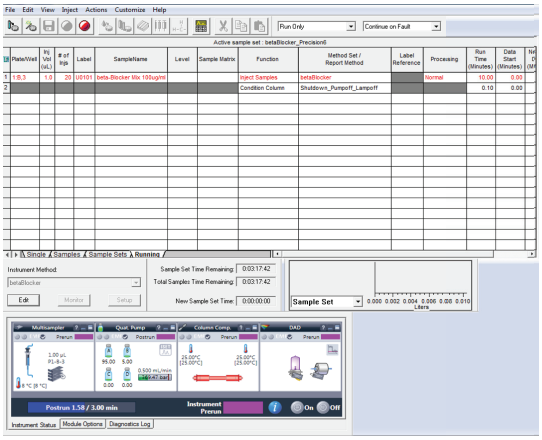
Abstract

Agilent Instrument Control Framework (ICF) allows third-party data acquisition and processing software to control Agilent LCs. In this Application Note, an Agilent 1290 Infinity II LC was controlled through Waters Empower 3 software for the separation of six β-blockers with subsequent data processing and data analysis. The precision of retention time and area for each compound was calculated to evaluate the performance of the LC system.



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Introduction

Agilent Instrument Control Framework (ICF) gives software providers an easy and fast implementation of Agilent LCs in their data acquisition and processing software. ICF contains an instrument driver standard (RC.NET) that provides a simple programming interface for third-party software connectivity, and eliminates the time-consuming development of their own native driver for Agilent LCs[1]. With ICF, control of Agilent LCs by Waters Empower is possible without a compromise in functionality, for certain LC modules.

In this Application Note, a mixture of six β-blockers was analyzed using an Agilent 1290 Infinity II LC system equipped with an Agilent 1290 Infinity II Flexible Pump. Beta blockers, also known as β-adrenoceptors antagonists, belong to a class of drugs blocking B1 or B2 adrenergic receptors. It has been discovered that several β-blockers, such as propranolol, have an anticancer effect as a first-line therapy for infantile hemangiomas[2].

Retention time (RT) and area precision for the six β-blockers were determined to evaluate the performance of the LC system.

Experimental

Instrumentation

The Agilent 1290 Infinity II LC System used for the experiments consisted of the modules listed below:

- Agilent 1290 Infinity II Flexible Pump (G7104A)
- Agilent 1290 Infinity II Multisampler (G7167B), equipped with integrated sample cooler (Option #100
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B), equipped with a 10 mm Max-Light cartridge cell

Solvents and samples

All solvents used were LC grade, and purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak).

The following β-blockers were used for the experiments:

- Atenolol
- Pindolol
- Labetalol
- Propranolol
- Timolol
- Acebutolol

All compounds were purchased from Sigma-Aldrich St. Louis, Missouri, US. The β-blockers were prepared at 10 mg/mL in 50 % DMSO in water, and were further diluted in 10 mM ammonium formate (pH 3) to a final concentration of 0.1 mg/mL, with the exception of acebutol, which was diluted to a final concentration of 0.2 mg/mL.

Column

Agilent ZORBAX Bonus-RP, 2.1 × 50 mm, 1.8 µm (p/n 857768-901)

Software

Waters Empower 3 (build 3471) with Waters ICS version 2.1 and Agilent ICF version A.02.03 were used to control the Agilent 1290 Infinity II LC, and to perform the data analysis.

Method

Table 1. Chromatographic conditions for the analysis of β-blockers.

Parameter	Value
Mobile phase	A) 10 mM ammonium formate, pH 3 B) methanol
Gradient	0 minutes – 5 %B 5 minutes – 38 %B 6 minutes – 80 %B
Stop time	7 minutes
Post time	10 minutes
Flow rate	0.5 mL/min
Injection volume	1 µL with standard needle wash, sample cooled at 8 °C
Column temperature	25 °C
Detection	280/4 nm, reference 360/80 nm Data rate 10 Hz Spectra 190 to 400 nm

Results and Discussion

The Agilent 1290 Infinity II LC was configured through the Configuration Manager in the Empower software. After configuration, the 1290 Infinity II LC appeared in the Empower data acquisition screen (Figure 1).

The 1290 Infinity II LC system was prepared for running the samples by purging the pump. The method-set, including the instrument method, was created, followed by data acquisition. For data evaluation, the processing and reporting function (Process Data) of Empower was used.

Retention time and area precision

For the study described in this Application Note, a mix of six β-blockers was separated on a reversed-phase LC column, 2.1 × 50 mm, packed with 1.8 µm particles. Figure 2 displays an overlay of 10 subsequent runs.

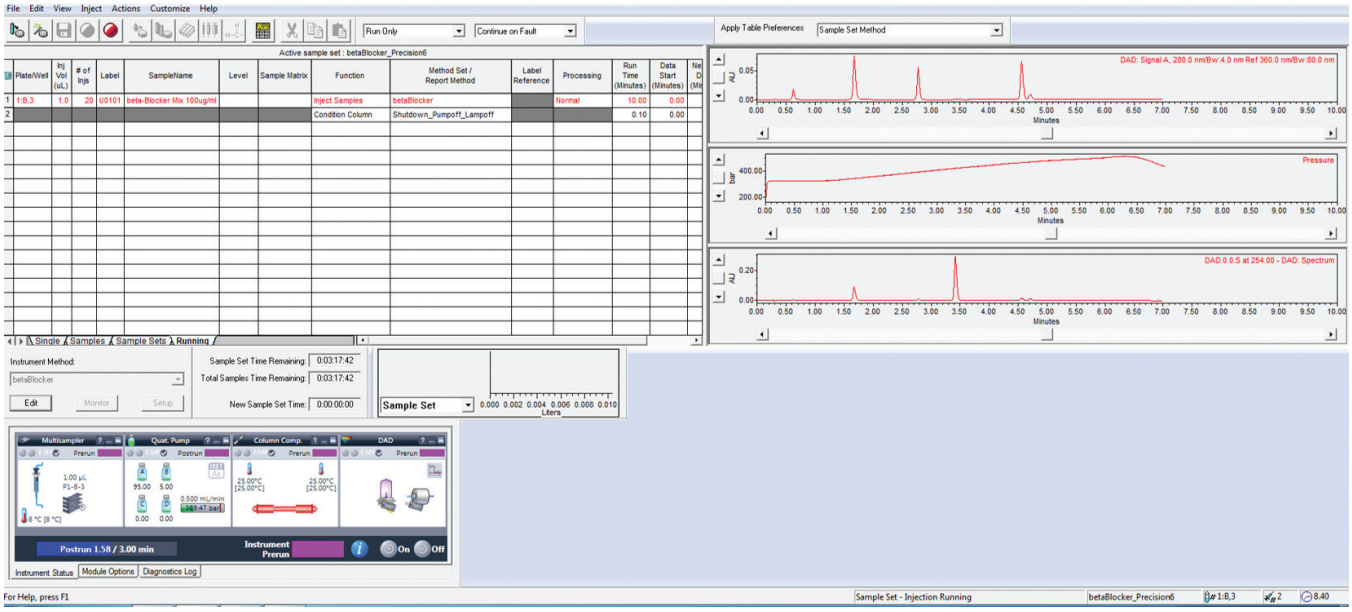

Figure 1. Agilent instrument status screen using Waters Empower and Agilent ICF.

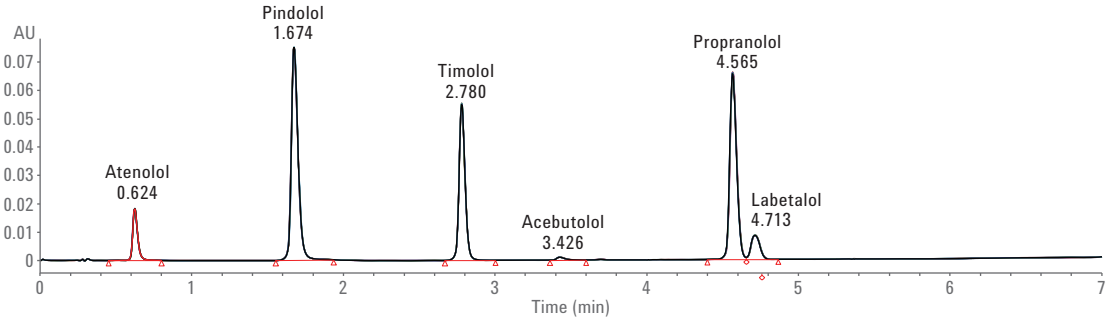

Figure 2. Analysis of six β-blockers, showing an overlay of 10 consecutive runs with each chromatogram acquired at 280 nm.

The RT precision and area precision of these runs were calculated using Empower. Excellent precision was found for RT and area precision, with relative standard deviations (RSDs) below 0.1 and 1.31 %, respectively (Table 2).

Table 2. RT precision, area precision, and resolution for the analysis of the β-blocker mix. For calculation of RSD, 10 consecutive runs were used, and for RT and resolution, the mean of the 10 runs were used.

Compound	RT (min)	RT RSD (%)	Area RSD (%)	Resolution
Atenolol	0.62	0.091	0.876	–
Pindolol	1.67	0.044	0.340	15.37
Timolol	2.78	0.010	0.263	14.78
Acebutolol	3.42	0.032	1.309	8.48
Propranolol	4.57	0.018	0.260	13.84
Labetalol	4.71	0.018	0.327	1.51

Conclusion

Agilent Instrument Control Framework enables control of Agilent LCs through third-party software such as Waters Empower. For the study described in this Application Note, an Agilent 1290 Infinity II LC was configured under Empower, together with the entire data acquisition and data analysis. For the analysis of six β-blockers, excellent RT and area precision values were obtained. Agilent ICF, in combination with Empower, enables access to almost all Agilent instrument features and the excellent performance characteristics of Agilent LCs such as the 1290 Infinity II LC. In addition, ICF facilitates the data analysis, with complete functionality within the Waters Empower environment.

References

1. Agilent Instrument Control Framework, *Agilent Technologies Technical Overview*, Publication Number 5990-6504EN, **2001**.

2. Fernandez-Pineda, I., Williams, R., Ortega-Laureano, L., Jones, R., Cardiovascular drugs in the treatment of infantile hemangioma, *World J. Cardiol.*, **2016**, *8(1)*, 74–80.

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