Automated LC/MS/MS Bioanalysis of β-blockers in Human Plasma

Application Note

Pharmaceuticals

Author

Mike Chang

Agilent Technologies, Inc.

Abstract

A mixture of compounds with varying log P values, such as β-blockers in plasma, can present difficulties in sample preparation for LC/MS/MS analysis. Thus, an analyte-independent sample preparation method, regardless of log P values, is an ideal step prior to LC/MS/MS bioanalysis. Agilent Captiva ND Lipids is a quick and easy-to-use sample preparation solution through filtration.

Introduction

Sensitive and fast instruments, such as UHPLC systems with triple-quadrupole detection, are growing in popularity. It has become critical to prepare biological samples as quickly and cleanly as possible to fully appreciate the benefits of UHPLC without extended maintenance.

Biological samples have abundant interferences originating from the matrix, which need to be overcome in LC/MS/MS bioanalysis. Without an appropriate sample preparation method, those interferences can damage sensitive and expensive instruments. Preparing clean samples by removing interferences as much as possible is critical. With the emergence of UHPLC technology, faster analysis is becoming the main focus of high throughput laboratories so that the system is used to its full extent. Being able to fully automate the sample preparation process is one of the keys to success in laboratory operation.

This application note demonstrates how quickly human plasma samples can be prepared for LC/MS/MS bioanalysis using automation without compromising accuracy and precision.

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Materials and Methods

The methanol was LC/MS grade. The MeOH containing 0.1 % formic acid was prepared by adding 1 mL of formic acid to 1 L of MeOH, and 0.1 % formic acid was prepared by adding 1 mL of formic acid to 1 L of water.

To begin sample preparation, a 96-well collection plate (bottom), CaptiVac collar (middle), and Captiva ND Lipids 96-well plate (top) were stacked as shown in Figure 1.

The sample preparation procedure for the isolation of β-blockers using Agilent Captiva ND Lipids was as follows.

1. Add 0.6 mL of MeOH to Captiva ND Lipids 96-well plate.
2. Add 0.2 mL of spiked human plasma to the plate.
3. Mix loaded samples five times by aspirating and releasing the loaded sample.
4. Apply vacuum to filter samples; leave the vacuum on until filtration is complete.
6. Place the 96-well collection plate in an automatic sampler for LC/MS/MS bioanalysis.

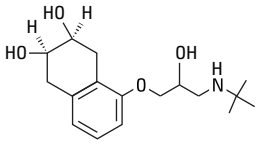
Figure 1. Stacked Agilent Captiva ND Lipids with a CaptiVac collar and 96-well collection plate (above). The bottom image shows the CaptiVac collar alone.

Conditions

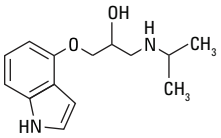
Column:	Agilent ZORBAX Eclipse Plus Phenyl-Hexyl, 2.1 × 100 mm, 3.5 µm (p/n 959793-912)
Sample prep:	Agilent Captiva ND Lipids (p/n A59640002I)
Samples:	See Table 1
Eluent:	A, 0.1 % formic acid B, MeOH + 0.1 % formic acid
Injection volume:	3 µL
Flow rate:	0.6 mL/min
Gradient:	Time (min) % B 0 30 2 30 3.5 80 4.5 80 4.6 30 7 30
LC/MS/MS:	Agilent 1290 Infinity LC with Agilent 6460 Triple Quadrupole LC/MS
Drying gas:	300 °C, 7 L/min
Sheath gas:	325 °C, 8 L/min
Nebulizer:	45 psi
Capillary:	3,500 V (positive)
Nozzle voltage:	0 V

Structures

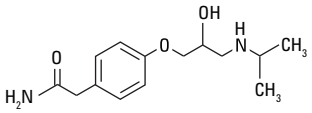
Nadolol



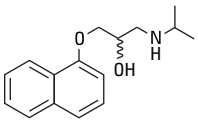
Pindolol



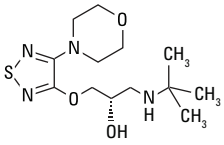
Atenolol



Propranolol



Timolol



Acebutolol

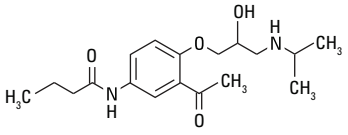


Table 1. β-Blockers under investigation.

	Nadolol	Pindolol	Atenolol	Propranolol	Timolol	Acebutolol (internal standard)
log P	0.81	1.75	0.16	3.48	1.83	1.71
pKa	9.67	9.25	9.60	9.42	9.21	9.20
MRM	310.2 → 254.1	249.2 → 116.2	267.2 → 56.2	260.2 → 56.1	317.2 → 74.2	337.2 → 116.2
Collision energy	12	12	28	28	20	16

Results and Discussion

Superior chromatographic separation was achieved by the Eclipse Plus Phenyl-Hexyl column, as shown on the MS chromatogram in Figure 2.

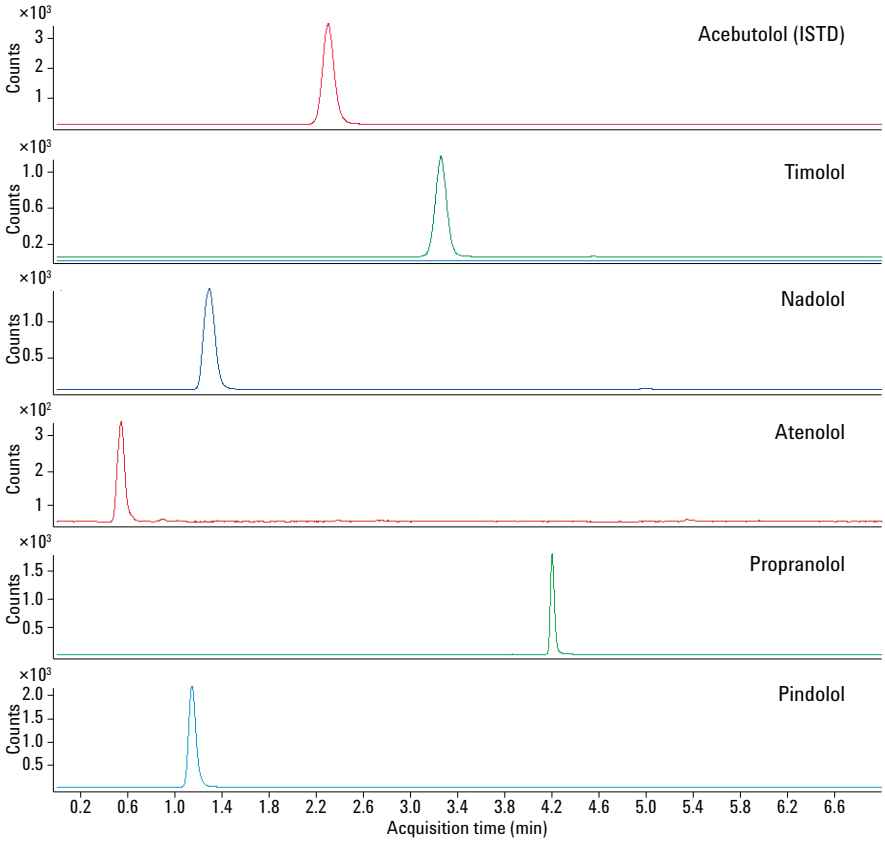


Figure 2. MS chromatograms of human plasma sample spiked with β-blockers.

Calibration curves for all compounds showed excellent linearity with R^2 values of 0.996 or greater (Figure 3).

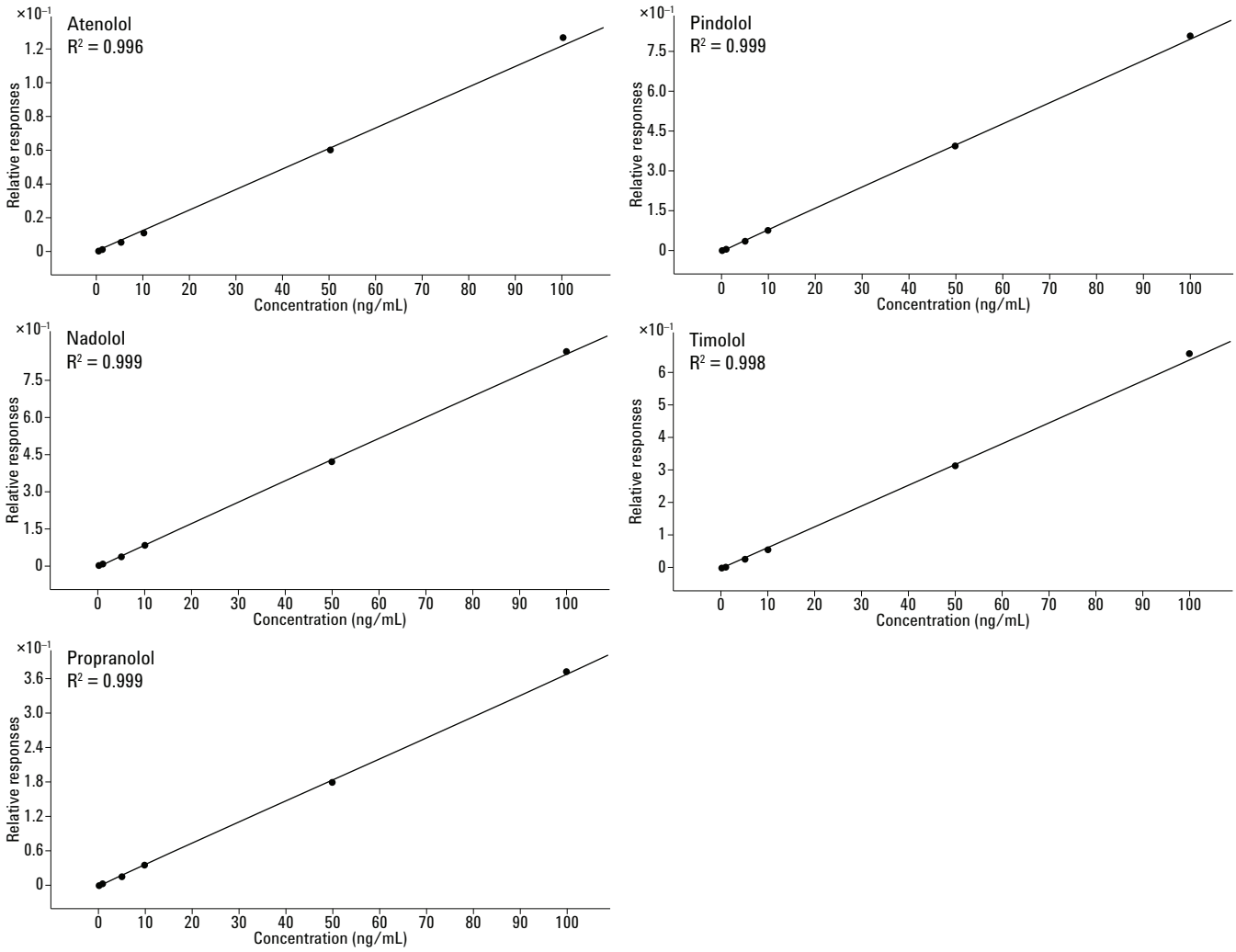


Figure 3. Calibration curves for five β-blockers in human plasma from 0.1 to 100 ng/mL (seven data points).

Recovery data for all five β-blockers ranged from 85 to 111.5 % throughout low, mid, and high concentrations. Most %RSDs were within a range of 3 %. Limits of quantitation (LOQs) for all compounds were 0.1 ng/mL in human plasma, except for propranolol, which had an LOQ of 0.5 ng/mL.

Captiva ND Lipids did not require any washing, evaporation, or reconstitution steps during the entire sample preparation process. It offered not only increased productivity but also brought great reproducibility as indicated by the excellent detection limits, precision, and accuracy data summarized in Table 2.

Streamlined human plasma sample preparation using a Captiva ND Lipids plate was ideally suited for a high throughput environment. Multiple samples were generated in a short period of time using this simple filtration process under vacuum in just a few minutes.

Conclusions

By using Captiva ND Lipids plates in human plasma sample preparation, processing a large number of biological samples in a timely manner was easily achieved. All sample preparation steps could be automated by making use of a robotic liquid handler for even higher throughput. No conditioning, equilibration, or washing steps were involved in any part of the sample preparation, and great linearity and recovery data were obtained.

Table 2. Summary of data with limits of quantitation, calibration curve linearity (R^2), recovery, and %RSD (n=8 for each concentration).

			5 ng /mL (low)		50 ng/mL (mid)		100 ng/mL (high)	
	LOQ (ng/mL)	Linearity, R^2	% Recovery	%RSD	% Recovery	%RSD	% Recovery	%RSD
Nadolol	0.1	0.9990	100.9	10.09	95.3	1.97	96.6	0.77
Pindolol	0.1	0.9990	96.8	4.45	103.4	2.66	105.4	1.06
Atenolol	0.1	0.9964	85.3	8.43	93.1	1.19	95.3	1.70
Propranolol	0.5	0.9988	111.5	8.1	100.9	2.39	107.6	1.57
Timolol	0.1	0.9974	90.31	1.8	100.1	1.23	104.5	1.46

For More Information

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