Solid Supported Liquid Extraction (SLE) for LC/MS/MS Bioanalysis

Application Note

Pharmaceuticals

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Abstract

Solid supported liquid extraction (SLE) is a commonly practiced sample preparation method due to its simplicity. Without conditioning and washing steps, sample preparation time can be reduced substantially. When the use of SLE as a sample preparation technique is combined with UHPLC, laboratory productivity can be greatly increased without compromising the accuracy and precision of the data.

Introduction

Biological samples with basic drug compounds are often prepared by cation-exchange solid phase extraction (SPE). Simpler sample preparation methods are always sought for increased throughput in bioanalysis. The Agilent VersaPlate SLE plate is an ideal solution for large numbers of samples because it eliminates many of the steps conventionally required in SPE methods, such as conditioning, equilibration, and washing.

This application note chose human plasma as the biological sample matrix. The performance and applicability of VersaPlate SLE as a practical laboratory protocol are presented with precision and accuracy data.

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Materials and Methods

The methanol was LC/MS grade. 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.

The sample preparation procedure for the isolation of β-blockers using Agilent VersaPlate SLE was as follows.

1. Dilute spiked plasma with 2 % ammonia at 1:1.
2. Load 0.3 mL of spiked and diluted plasma onto the VersaPlate.
3. Apply a slight vacuum to initiate flow until the whole sample volume is soaked by the sorbent, then stop the vacuum.
4. Wait 5 minutes for sample adsorption.
5. Elute twice with 0.9 mL of EtOAc with a slight vacuum.
6. Evaporate and reconstitute in 0.15 mL of initial mobile phase.

Conditions

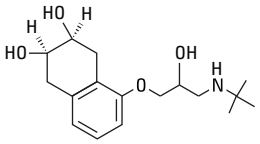
Column:	Agilent ZORBAX Eclipse Plus Phenyl-Hexyl, 2.1 × 100 mm, 3.5 µm (p/n 959793-912)
Sample prep:	Agilent VersaPlate preassembled 96-well plate for Hydromatrix and ChemElut, 260 mg (p/n 75430260)
Samples:	See Table 1
Eluent:	A, 0.1 % Formic acid B, MeOH + 0.1 % Formic acid
Injection volume:	3 µL
Flow 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 an 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

Table 1. β-Blockers under investigation.

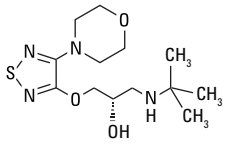
	Nadolol	Pindolol	Metoprolol	Timolol	Acebutolol (internal standard)
log P	0.81	1.75	1.6	1.83	1.71
pKa	9.67	9.25	9.68	9.21	9.20
MRM	310.2 → 254.1	249.2 → 116.2	268.2 → 56.1	317.2 → 74.2	337.2 → 116.2
Collision energy	12	12	28	20	16

Structures

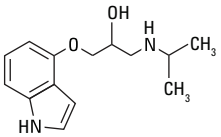
Nadolol



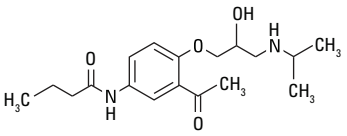
Timolol



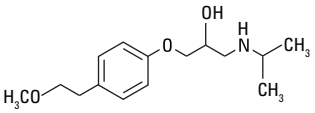
Pindolol



Acebutolol



Metoprolol



Results and Discussion

All compounds were well separated chromatographically on an Eclipse Plus Phenyl-Hexyl column (Figure 1). Excellent calibration curve linearity was achieved ($R^2 \geq 0.995$) over the entire calibration range of 0.1 to 100 ng/mL (Figure 2).

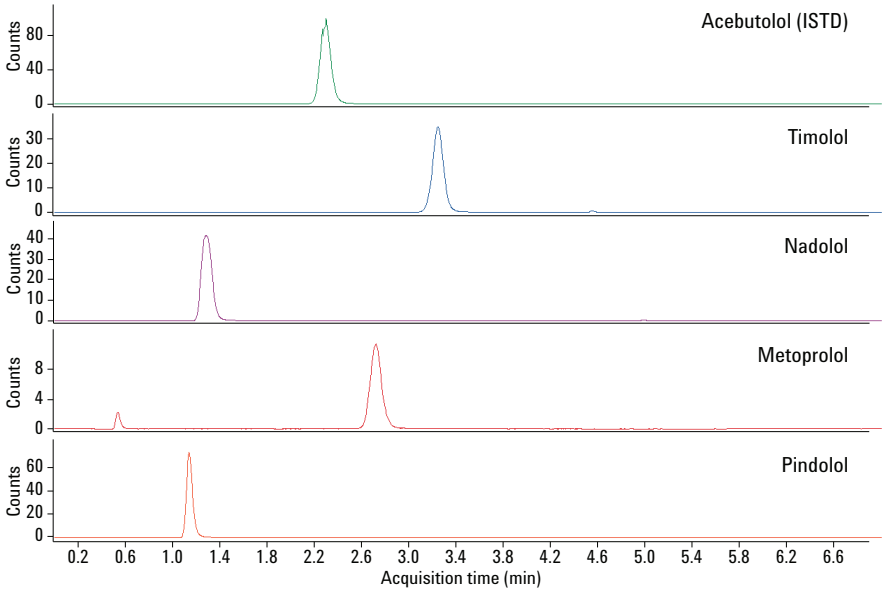


Figure 1. MS chromatograms of human plasma sample spiked with β-blockers.

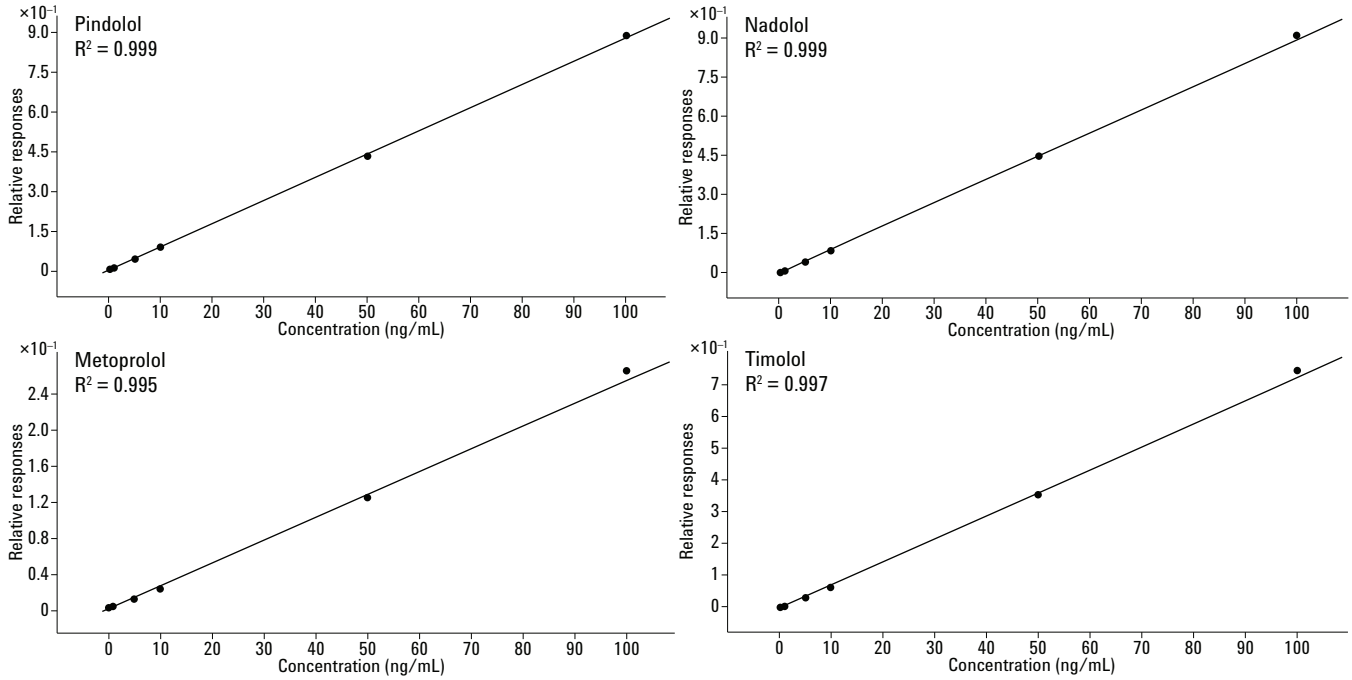


Figure 2. Calibration curves for five β-blockers in human plasma from 0.1 to 100 ng/mL (seven data points).

Recoveries were 81.6 to 105.8 %, 9 for low, mid, and high concentrations (n=8 at each level). Precision and reproducibility were reflected in the low %RSDs achieved for the study (≤ 4.5 %), further demonstrating the suitability of using the SLE plate (Table 2).

Conclusions

For small sample volumes combined with a large number of human plasma samples, the Agilent VersaPlate format SLE worked flawlessly. Unlike conventional SPE sample preparation, SLE did not require conditioning, equilibration, or washing steps. By adopting the liquid-liquid extraction concept, but without requiring special glassware or duplicated extraction steps, the solid-supported liquid-liquid extraction process, using simple filtration in the VersaPlate format SLE, was an excellent way of achieving reproducible data.

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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	0.5	0.9988	81.6	3.28	93.7	2.40	97.0	3.64
Pindolol	0.5	0.9994	95.4	4.45	101.4	4.23	104.4	2.59
Metoprolol	0.5	0.9950	86.2	3.31	98.0	1.47	105.3	1.32
Timolol	0.5	0.9971	87.1	2.24	99.7	1.93	105.8	1.36

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