Application Note 20686

A Comparison of the Extraction of Beta Blockers from Plasma Using Solid-Supported Liquid-Liquid Extraction and Traditional Liquid-Liquid Extraction

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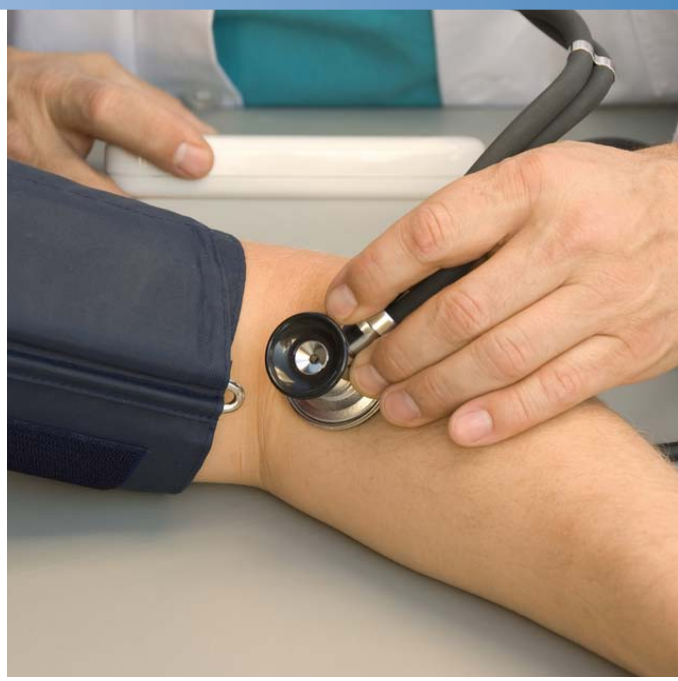
Key Words
SLE, HyperSep SLE, beta blockers, liquid-liquid extraction, LLE, Accucore

Abstract
By using Thermo Scientific™ HyperSep™ SLE instead of traditional liquid-liquid extraction (LLE) methods, recovery of the analytes can be improved and the extraction time significantly reduced. This was achieved with the use of smaller sample volumes and buffer volumes, providing cost savings.



Introduction

Beta blockers (or beta antagonists) are a category of drugs used to treat a number of medical complaints, such as hypertension, angina, heart failure, and heart attacks. Beta blockers are designed to stop the functioning of a naturally occurring compound, noradrenaline. Noradrenaline is a chemical released in the body that can cause the arteries to narrow and the heartbeat to increase.

Beta blockers can be extracted using solid-supported liquid-liquid extraction (SLE). SLE has time and cost benefits over conventional liquid-liquid extraction (LLE) while maintaining the ability to provide clean extracts free of matrix interferences. HyperSep SLE lends itself to the extraction of components of moderate to low polarity (logP >2) and has several advantages over LLE, including:

- Uses less solvent
- Does not produce emulsions
- Easily automated
- Takes less time
- Applicable to small sample volumes

HyperSep SLE uses a solid support of packed diatomaceous earth to support an aqueous sample. The aqueous sample is first loaded onto the support and allowed to adsorb. Small volumes of extraction solvent are then passed through the packing to allow the analytes to partition between immiscible layers. This can be carried out several times and the individual extraction volumes combined and dried down before being reconstituted and analyzed.

This application uses Thermo Scientific™ Accucore™ HPLC columns, which use Core Enhanced Technology™ to facilitate fast, highly efficient separations. The particles are not totally porous, but instead have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. The carbon loading of Accucore C18 provides high retention of non-polar analytes via a predominantly hydrophobic interaction mechanism. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

The extraction of three beta blockers from plasma using HyperSep SLE is demonstrated in this application.

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Experimental Details

Sample Handling	Part Number
Fisher Scientific™ HPLC grade water	W/0106/17
Fisher Scientific HPLC grade methanol	M/4056/17
Fisher Scientific Analytical grade formic acid	F/1900/PB08
Fisher Scientific HPLC grade ammonia solution	A/3295/PB05
Fisher Scientific HPLC grade ethyl acetate	E/0906/17
Thermo Scientific premium vial	60180-600

Sample Handling Equipment	Part Number
Thermo Scientific™ UltraVap™ high speed sample concentrator	CLS-229070
Thermo Scientific HyperSep glass block manifold	60104-232
Thermo Scientific™ FinnPipette™ (100-1000 µL)	642090
Thermo Scientific FinnPipette (10-100 µL)	4642070
Thermo Scientific FinnPipette (1-10 µL)	4642040

SLE Sample Preparation		Part Number
Compounds:	Metoprolol, propranolol, pindolol	
Matrix:	Human plasma	
Cartridge type:	HyperSep SLE 200 mg / 3 mL	60109-200-3-7
Sample pretreatment:	Aliquot 200 µL of spiked plasma and add 200 µL of 0.5 M ammonium hydroxide. Mix well.	
Application stage:	Load 400 µL of the pretreated plasma onto the HyperSep SLE cartridge.	
Adsorption stage:	Apply a pulse of vacuum. Allow to load under gravity and wait 5 minutes.	
Elution stage:	Elute with 2 x 1 mL of ethyl acetate under gravity. Apply gentle vacuum to collect excess elution solvent.	
Additional stage:	Dry down samples and reconstitute in 200 µL of water / methanol (95:5 v/v) (ensure that the temperature is less than 40 °C when drying down samples) and sonicate for 5 minutes.	

Liquid-Liquid Sample Preparation	
Compounds:	Metoprolol, propranolol, pindolol
Matrix:	Human plasma
Sample pretreatment:	Aliquot 500 µL of spiked plasma and add 500 µL of 0.5M ammonium hydroxide into a 2 mL tube. Mix well.
Elution stage:	Add 1 mL of ethyl acetate, shake/invert for 15 minutes, centrifuge for 10 minutes at 3000 rpm, and transfer supernatant to a vial. Repeat.
Additional stage:	Dry down samples and reconstitute in 500 µL of water / methanol (95:5 v/v) (ensure that the temperature is less than 40 °C when drying down samples) and sonicate for 5 minutes.

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific™ Accela™ 1250 HPLC	
Column:	Accucore C18 2.6 µm, 50 x 2.1 mm	17126-052130
Mobile phase A:	Water + 0.1% formic acid	
Mobile phase B:	Methanol + 0.1% formic acid	
Gradient:	10%–40% B in 2.5 minutes	
Flow rate:	0.7 mL/min	
Column temperature:	45 °C	
Wavelength:	220 nm	
Injection details:	2 µL	
Injection wash:	Water	

Solutions

Primary standards of each of the compounds were prepared at 3 mg/mL in methanol.
Spiking standard contained 0.5 mg/mL of each compound, which was spiked into plasma to achieve a concentration of 0.05 mg/mL.

Results

Extraction from human plasma was carried out on a HyperSep SLE 200 mg/3 mL cartridge and by traditional liquid-liquid extraction. Replicate extractions of the beta blocker mix showed that the HyperSep SLE gave much higher recoveries and comparable reproducibility results to LLE (Table 1). By giving greater recoveries, HyperSep SLE enables the use of smaller sample and modifier volumes, has comparable solvent usage, and significantly reduces the extraction time (Tables 2 and 3).

The analysis was carried out on an Accucore C18 2.6 µm, 50 x 2.1 mm column. As shown in Figure 1, the three beta blockers eluted in under three minutes.

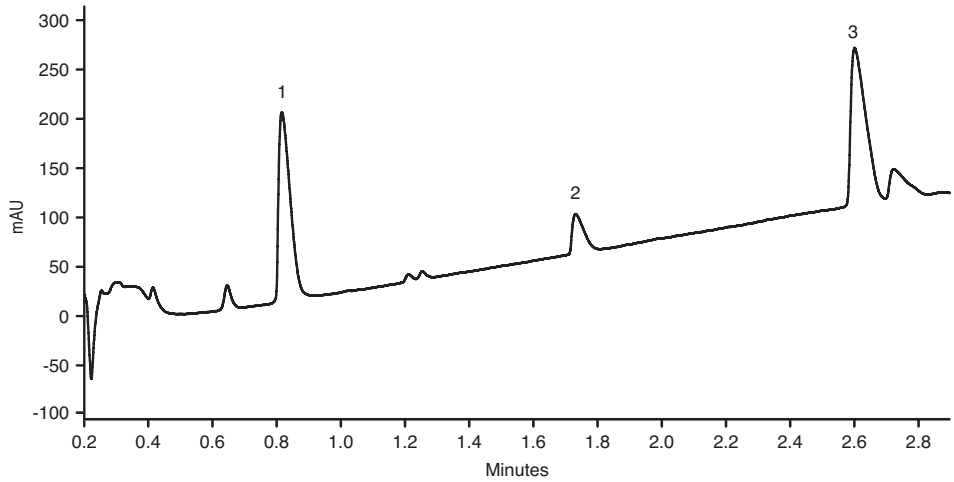

Figure 1: Chromatogram for pindolol (1), metoprolol (2), propranolol (3) separated on an Accucore C18 2.6 µm, 50 x 2.1 mm column.

	Pindolol	Metoprolol	Propranolol
SLE average area	475450	95125	500669
SLE overspike average area	543217	119682	568619
SLE % recovery	88	79	88
LLE average area	188494	36912	354773
LLE overspike average area	636083	143958	593757
LLE % recovery	30	26	60
SLE %RSD	9	10	13
LLE %RSD	9	10	8

Table 1: Method recovery for the beta blocker mix extracted by HyperSep SLE and traditional liquid-liquid extraction (LLE). (Data calculated from six replicate extractions.)

	SLE	LLE	SLE Savings
Sample volume (µL)	200	500	300
Sample modifier volume (µL)	200	500	300
Elution solvent volume (µL)	2000	2000	0
Extraction time for 96 samples (min)	30	140	110

Table 2: Sample savings by using SLE instead of traditional liquid-liquid extraction (LLE)

Extraction of 96 Samples	Time SLE (min)	Time LLE (min)	SLE Time Savings (min)
Pipette out sample	5	5	0
Pipette out modifier	5	5	0
Transfer to SLE	5	X	-5
Wait for SLE to absorb	5	X	-5
Add elution 1	5	5	0
Shake	X	15	15
Centrifuge	X	4 x 10	40
Transfer	X	5	5
Add elution 2	5	5	0
Shake	X	15	15
Centrifuge	X	4 x 10	40
Transfer	X	5	5
Total	30	140	110

Table 3: Solvent and time savings by using SLE instead of traditional liquid-liquid extraction (LLE)

Conclusion

HyperSep SLE yields results with excellent recoveries for the extraction of beta blockers from human plasma. The recoveries were three times higher compared to traditional liquid-liquid extraction and produced in one-fifth of the time. HyperSep SLE requires lower sample and buffer volumes and significantly less extraction time. Therefore, HyperSep SLE can reduce sample time costs and increase sample throughput.

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