LC-MS/MS Analysis of β-blockers in Human Plasma using Supported Liquid Extraction Sample Preparation

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Introduction

Simple, efficient sample preparation techniques are essential to the success of high throughput bioanalytical assays. Techniques such as protein precipitation and solid phase extraction are widely used. Liquid-liquid extraction is also popular, but is difficult to automate successfully, and can present problems such as low analyte recovery and emulsion formation. Supported liquid extraction (SLE) is an alternative to traditional liquid-liquid extraction which is easily automated, and hence suited to high throughput assays. SLE provides high analyte recoveries, while eliminating emulsion formation and other liquid handling issues.

This poster describes the development of a 96-well plate based sample preparation method using ISOLUTE® SLE+ Supported Liquid Extraction Plates combined with LC-MS/MS for the analysis of three β-blockers (Metoprolol, Oxprenolol and Propranolol) from human plasma.

The β-blockers were eluted with a range of solvents in order to optimize recoveries, extract cleanliness and precision. Extracts were evaporated to dryness and reconstituted in mobile phase for subsequent LC-MS/MS analysis. Samples were analyzed using a Waters Ultima Pt triple quadrupole mass spectrometer equipped with electrospray ionization.

Experimental Procedure

Reagents

Metoprolol, Oxprenolol and Propranolol (see **Figure 1**) were purchased from Sigma Chemical Co. (Poole, UK). Blank human plasma was obtained through the Welsh Blood Service (Pontyclun, UK). All solvents were HPLC grade from Fisher Scientific (Loughborough, UK).

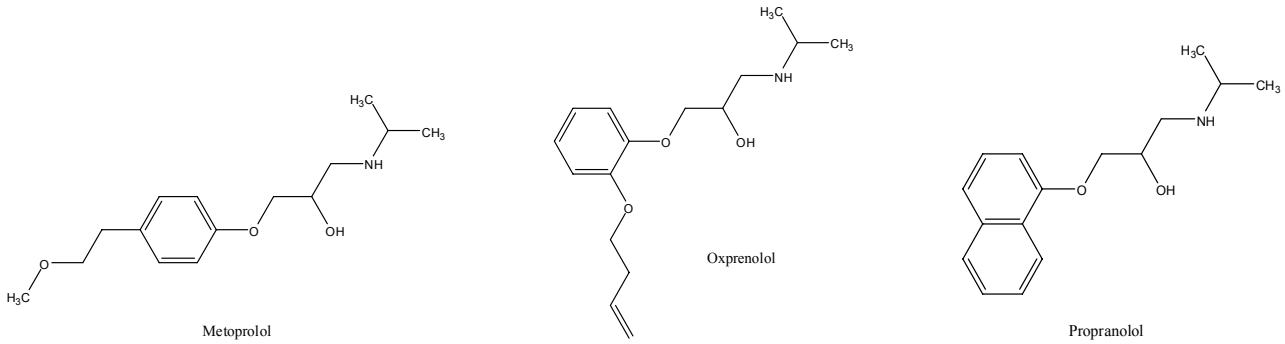

Figure 1. Structures of Metoprolol, Oxprenolol and Propranolol respectively

Supported Liquid Extraction Procedure

Blank human plasma (100 µL) was spiked with various concentrations of the three β-blockers. The plasma was then diluted 1:1 (v/v) with 0.5M NH_4OH prior to loading onto the ISOLUTE SLE+ Supported Liquid Extraction Plate. A pulse of vacuum was applied to initiate flow onto the plate and the samples left to absorb for 5 minutes.

A range of elution solvents were investigated in order to optimize the method. In all cases elution was brought about by the addition of 1 mL of the appropriate solvent. The eluate was evaporated to dryness and the analytes reconstituted in 200 µL of 20:80 (v/v) MeOH/H2O prior to analysis.

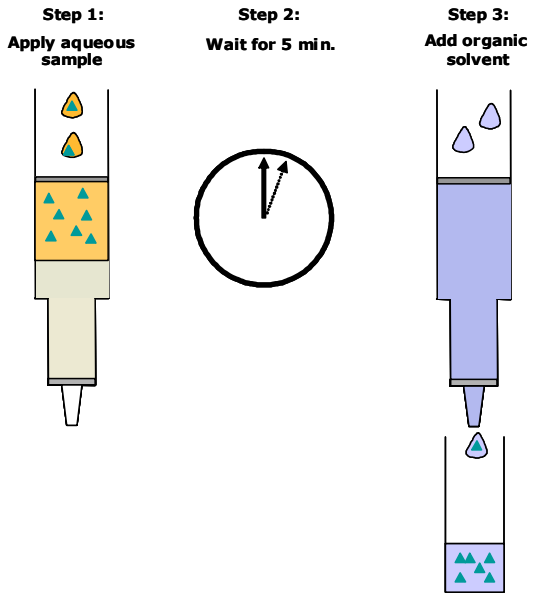

Figure 2. Schematic of the ISOLUTE SLE+ supported liquid extraction procedure. A single well of the 96-well plate is illustrated

Liquid Chromatography

Liquid Chromatography was performed using a Waters 2795 Liquid Handling System (Waters Assoc., Milford, MA, USA). Chromatographic separation was achieved on a Zorbax Eclipse XDB C18 3.5 µm analytical column (100 x 2.1 mm id) equipped with a C8 guard column (both Agilent Technologies, Berkshire, UK). An isocratic mobile phase of 75:25 (v/v) 0.1% aqueous formic acid and MeCN was employed at a flow rate of 0.25 mL/min. Injection volumes were set to 25 µL and all separations were performed at ambient temperature.

Mass Spectrometry

The entire column effluent was directed into a Quattro Ultima Pt triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. Positive ions were acquired in the multiple reaction monitoring mode (MRM) using a desolvation temperature of 350 °C, an ion source temperature of 100 °C and collision gas pressure of 2.6 x 10^{-3} mbar. The base peak in each compound spectrum was attributed to the protonated molecular ion $[M^+H]^+$ and were subsequently used as the precursor ions in the resulting MRM transitions. See **Table 1** for full MRM transitions and ionization conditions.

Table 1. Quattro Ultima Pt mass spectrometer parameters.

Analyte	MRM Transition	Dwell (s)	Cone Voltage (V)	Collision Energy (eV)
Metoprolol	268.1 > 116.1	0.1	55	18
Oxprenolol	266.1 > 72.1	0.1	55	18
Propranolol	260.1 > 116.1	0.1	55	17

Results

Elution Solvent Optimization

In order to determine optimum elution conditions for the three β-blockers, five different solvents / solvent combinations were evaluated: 98:2 (v/v) hexane/3-methyl-1-butanol, 90:10 (v/v) DCM/IPA, DCM, ethyl acetate and MTBE. Recoveries for the five elution solvents are shown in **Figure 2**. With recoveries consistently greater than 80%, the final solvent choice also took into account precision and extract cleanliness. At concentrations of 0.05 and 0.2 ng/mL, a greater level of interference was observed with certain solvents, leading to RSD's of 10–20%. Taking these factors into account, MTBE was selected as the optimum elution solvent.

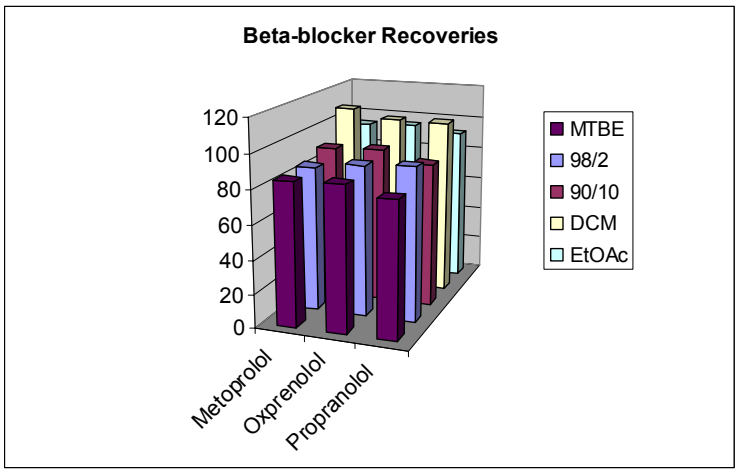

Figure 2. Analyte recoveries for three β-blockers using five elution solvents

Standard Curves

Although no internal standards were used in this work, standard linearity and method conformity was investigated. Standard curves were run over the concentration range 25 pg/mL – 10 ng/mL (**Figure 3**). Good linearity was obtained over the concentration ranges showing coefficients of determination >0.99.

Samples

Extractions were performed at six different concentration levels (n=5), with recoveries > 80%. The results show good inter-day recoveries and RSD's of < 10% (see **Table 2, page 4**) . The lowest extraction level is approaching the LOQ for the three analytes tested. To attain true precision and accuracy the use of an isotopically labelled internal standard would be required. However, from the results attained in this work it is clear supported liquid extraction using ISOLUTE SLE+ is a good sample preparation technique for this application.

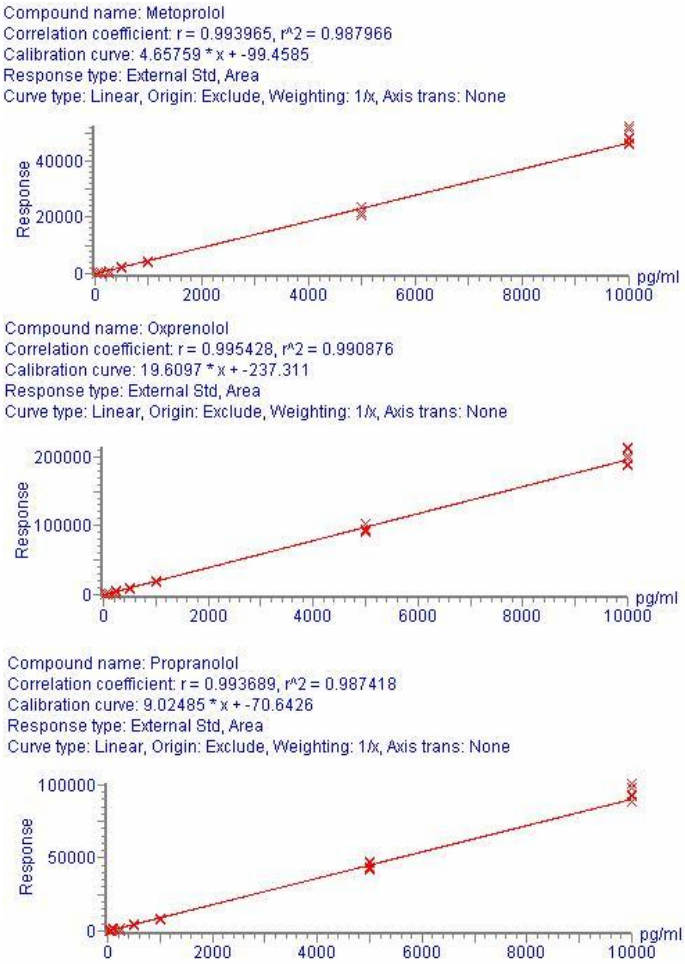

Figure 3. Standard curves for the 3 respective β-blockers over the concentration range 25 pg/mL-10 ng/mL

Biotage

Table 2. Intra- and inter-day recovery and precision for three β-blockers from plasma samples with RSD's in parentheses.

Analyte	Conc (ng/mL)	Day 1 Precision	Day 1 Recovery % (RSD)	Day 2 Precision	Day 2 Recovery % (RSD)
Metoprolol	0.05	0.0501	100 (5.4%)	0.0402	80 (7.7%)
	0.2	0.194	97 (7%)	0.181	91 (8.6%)
	0.5	0.483	97 (4.4%)	0.453	91 (7.7%)
	2.5	2.34	94 (1.8%)	1.89	76 (5.8%)
	5	4.50	90 (2.5%)	4.72	94 (7.2%)
	10	9.30	93 (3.7%)	10.63	106 (6.2%)
Oxprenolol	0.05	0.0496	99 (13.9%)	0.0437	87 (3.7%)
	0.2	0.187	94 (6.5%)	0.182	91 (5.2%)
	0.5	0.468	94 (2.1%)	0.477	95 (3.7%)
	2.5	2.38	95 (2.2%)	2.41	96 (5.9%)
	5	4.62	92 (2%)	4.59	92 (6.5%)
	10	10.03	100 (2.3%)	9.57	96 (5.1%)
Propranolol	0.05	0.0511	102 (11.1%)	0.0504	101 (9.9%)
	0.2	0.207	103 (8.5%)	0.196	98 (8.5%)
	0.5	0.540	108 (8.4%)	0.514	103 (8.6%)
	2.5	2.80	112 (5.2%)	2.50	100 (5.9%)
	5	5.10	102 (8.3%)	4.90	98 (7.6%)
	10	10.72	107 (5.6%)	10.66	107 (9.9%)

Conclusions

1. A supported liquid extraction procedure has been developed for the isolation of Metoprolol, Oxprenolol and Propranolol from plasma
2. Sample processing using the ISOLUTE SLE+ plate was simple, with 96 samples processed in approximately 15 minutes using an 8-port manual pipetter for all liquid dispensing steps.
3. MTBE was found to be the most suitable elution solvent from a range of solvent / solvent combinations evaluated.
4. Minimal ion suppression was observed, and excellent analyte recovery and linearity were obtained for the three β-blockers at all concentration levels, indicating no loss of compound at low concentrations through interaction with the supported liquid extraction media.
5. The LOQ's achieved were approximately 150 pg/mL, 50 pg/mL and 25 pg/mL for Propranolol, Metoprolol and Oxprenolol, respectively.
6. Good inter- and intra-day precision and accuracy were obtained, showing that supported liquid extraction using the ISOLUTE SLE+ can be used for high sensitivity bioanalytical assays.

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