Comparison of Biotage® Extrahera™ vs Manual Sample Processing Using a Vacuum Manifold

Extraction of Beta Blockers from Plasma Using ISOLUTE® SLE+

Automated sample preparation using the Biotage® Extrahera™ was compared to an equivalent manual method utilizing a vacuum manifold. A selection of beta blocker drugs were extracted from pooled stripped plasma using a supported liquid extraction procedure. ISOLUTE® SLE+ 200 µL sample volume plates, part number 820-0200-P01 were used for extraction.

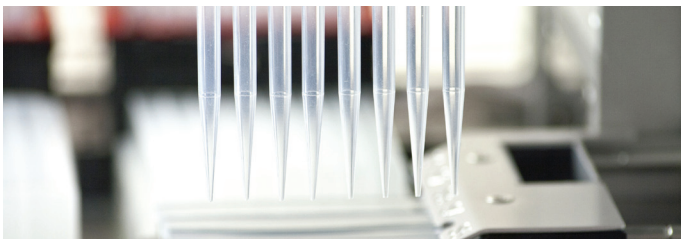
Resulting extracts from both preparation methods were subsequently analyzed by LC-MS/MS.

Procedure

A pooled plasma sample was prepared in a sufficient quantity to run a full 96-well plate for each processing method. This pooled plasma sample was fortified with Metoprolol, Propanolol and Alprenolol at a concentration of 200 ng/mL respectively. Pindolol was added as an internal standard at a concentration of 200 ng/mL

From this pooled plasma sample 150 µL was transferred to all wells of two 96-well plates.

All subsequent aspects of sample preparation were performed in duplicate on two separate plates utilizing either Extrahera or manual preparation using an air-displacement pipette.



The pooled plasma sample was pre-treated 1:1 (v/v) with 0.5M Ammonium Hydroxide (150 µL).

After pre-wetting the pipette tips via aspirate/dispense cycling and to mix the samples, 200 µL of the pre-treated sample was loaded to each well of the ISOLUTE® SLE+ plates. Flows were initiated using a pulse of positive pressure (Extrahera) or vacuum (manual method).

After leaving for 5 minutes to allow the sample to completely absorb into the plates, elution was performed by the application of 1 mL of 95:5 (v/v) dichloromethane:propan-2-ol to the ISOLUTE® SLE+ plates.

The extracts were collected in 2 mL 96-well collection plates under gravity elution, and as a final step to recover all available solvent from the media, by applying a pulse of positive pressure (Extrahera) or vacuum (manual method).

The extracts were evaporated to dryness in a TurboVap® 96 at 37 °C and reconstituted in 250 µL of 85:15 (v/v) water/methanol solution.

The plates were mixed on an orbital shaker for 10 minutes.

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HPLC Conditions

Instrument: Waters Alliance 2795

Column: ACE Excel 2 C18-AR 50 x 2.1mm id column

Mobile Phase: 50:50 (v/v) 0.1 % (v/v) formic acid/acetonitrile at 0.25 mL/min

Injection Volume: 25 µL

Experimental Precautions

- Both plates were evaporated side by side on the same evaporation instrument (TurboVap® 96).
- During analysis on the LC-MS system samples were injected alternately from the two plates to reduce the effect of any sample stability issues.
- The same batch/bottles of samples, reagents and solvents were used for both methods.

Mass Spectrometry

Instrument: Waters Quattro Ultima Pt

MRM Conditions

Analyte	Transition	RT (min)	Dwell (sec)	Cone (V)	Col Energy (V)
Metoprolol	268.1 to 116.1	2.7	0.4	55	18
Propanolol	260.1 to 116.1	4.8	0.08	55	16
Alprenolol	250.1 to 116.1	4.7	0.08	55	17
Pindolol	249.1 to 116.1	1.6	0.4	55	16

Results

Average peak area data was calculated for all three compounds to compare any improvements in analyte recovery between Extrahera and manually processed samples.

Peak area ratio data was also generated for all samples by referencing the analyte vs. Pindolol. This provides standardized data to allow a comparison of the % RSD of the Extrahera vs. manual data sets.

	Extrahera Metroprolol Peak Area Ratio Summary	Manual Metroprolol Peak Area Ratio Summary
Average Metropolol Peak Area	186759	189717
Improvement (%) vs. Manual Method	-1.6	-
Average IS Peak Area	548257	564335
Improvement (%) vs. Manual Method	-2.8	-
Average Peak Area Ratio	0.340	0.335
% RSD of Extrahera Extraction	2.5	3.2
Improvement (%) vs. Manual Method	19.3	-

	Extrahera Propranolol Peak Area Ratio Summary	Manual Propranolol Peak Area Ratio Summary
Average Metropolol Peak Area	117460	120435
Improvement (%) vs. Manual Method	-2.5	-
Average IS Peak Area	548257	564335
Improvement (%) vs. Manual Method	-2.8	-
Average Peak Area Ratio	0.216	0.214
% RSD of Extrahera Extraction	5.1	5.6
Improvement (%) vs. Manual Method	8.5	-

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	Extrahera Alprenolol Peak Area Ratio Summary	Manual Alprenolol Peak Area Ratio Summary
Average Metropolol Peak Area	279380	284395
Improvement (%) vs. Manual Method	-1.8	-
Average IS Peak Area	548257	564335
Improvement (%) vs. Manual Method	-2.8	-
Average Peak Area Ratio	0.512	0.505
% RSD of Extrahera Extraction	4.3	4.9
Improvement (%) vs. Manual Method	12.2	-

Conclusion

A significant reduction to the % RSD was measured when using the Biotage® Extrahera™ for both compounds compared to the manual processing approach.

% RSD	Manual Procedure (n=96)	Extrahera (n=96)
Metoprolol	3.2	2.5
Propanolol	5.6	5.1
Alprenolol	4.9	4.3

For Metoprolol the % RSD improved by 19 %, for Propranolol there was also an improvement in the % RSD of 8 % compared to the manual method. For Alprenolol using Extrahera an improvement in the %RSD of 12 % was also observed.

For all analytes when processing using Extrahera there was a slight reduction in the average peak areas for all compounds.

Average Peak Area	Manual Procedure (n=96)	Extrahera (n=96)
Metoprolol	189717	186759
Propanolol	120435	117460
Alprenolol	284395	279380
Pindolol	564335	548247

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