

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

Extraction of NSAIDs 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 non-steroidal anti-inflammatory drugs (NSAIDs) were extracted from pooled stripped plasma using a supported liquid extraction procedure. ISOLUTE® SLE+ 400 µL sample volume plates, part number 820-0400-P01 were used for extraction.

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

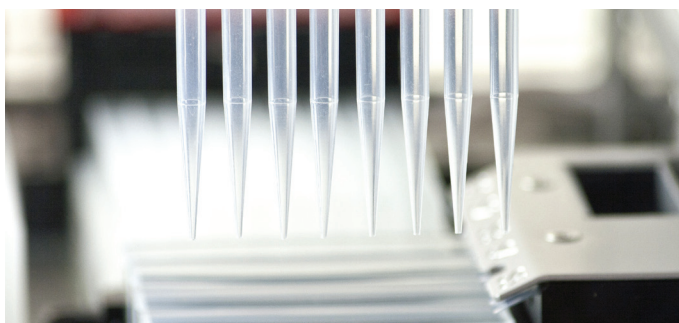


Procedure

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 Sulindac, Ketoprofen and Indomethacin at a concentration of 25 ng/mL respectively.

From this pooled plasma sample 250 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 a calibrated air-displacement pipette.



The pooled plasma sample was pre-treated 1:1 (v/v) with 1 % formic acid (250 µL). This sample dilution results in approximate loading pH of 3.2.

After pre-wetting the pipette tips via aspirate/dispense cycling and to mix the samples; 400 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 2 x 0.9 mL aliquots of methyl tertiary-butyl ether (MTBE) 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 200 µL of 60:40 (v/v) water/methanol solution.

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

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
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MRM Conditions

Analyte	Transition	RT (min)	Dwell (sec)	Cone (V)	Col Energy (V)
Indomethacin	358.1 to 139.0	2.5	0.1	35	18
Sulindac	357.1 to 233.1	0.9	0.1	35	26
Ketoprofen	255.1 to 209.1	1.2	0.1	35	13

Results

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

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

	Extrahera Sulindac Peak Area Ratio Summary	Manual Sulindac Peak Area Ratio Summary
Average Sulindac Peak Area	24622	22847
Improvement (%) vs. Manual Method	7.8	-
Average IS Peak Area	35199	32683
Improvement (%) vs. Manual Method	7.7	-
Average Peak Area Ratio	0.701	0.705
% RSD of Extrahera Extraction	4.01	6.43
Improvement (%) vs. Manual Method	37.6	-

	Extrahera Indomethacin Peak Area Ratio Summary	Manual Indomethacin Peak Area Ratio Summary
Average Indomethacin Peak Area	34395	27965
Improvement (%) vs. Manual Method	23.0	-
Average IS Peak Area	35199	32683
Improvement (%) vs. Manual Method	7.7	-
Average Peak Area Ratio	0.977	0.857
% RSD of Extrahera Extraction	5.46	8.56
Improvement (%) vs. Manual Method	36.2	-

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)
Sulindac	6.43	4.01
Indomethacin	8.56	5.46

For Sulindac the % RSD improved by 37%, for Indomethacin there was also an improvement in the % RSD by 36 % to 5.46%

The results also suggest that methods performed on the Extrahera could give higher recoveries due to increases in the absolute average peak areas.

Average Peak Area	Manual Procedure (n=96)	Extrahera (n=96)
Sulindac	22847	24622
Indomethacin	27965	34395

Both compounds returned average peak areas that were higher when sample extraction was performed using Extrahera than the manual method with an average peak area increase of 15.4%. The greatest improvement was measured with Indomethacin where the average peak area was increased by 23%.

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