

Automated Sample Extraction Technique for LC-MS/MS Analysis of the Pharmaceutical Drug Zolmitriptan in Human Plasma

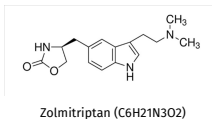
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Introduction

Typical bioanalysis workflows for small molecules use organic solvents to precipitate proteins and extract analyte(s). Sometimes, extra clean-up, including liquid-liquid extraction (LLE) or solid phase extraction (SPE), is needed to further remove the matrix to satisfy sensitivity, accuracy, and precision requirements. However, method development for bioanalysis is rather challenging. Some operating procedures are tedious and difficult to scale up.

We provide an automated workflow using Biotage ISOLUTE® SLE+ Supported Liquid Extraction (SLE) technology to extract analyte(s) from the biomatrix. This method offers better matrix cleanup than protein precipitation, is much easier to scale up and automate than LLE and is less complicated and time-consuming than SPE.

In this study, we spiked Zolmitriptan, a selective serotonin receptor agonist, into plasma to mimic the biological samples for PK/PD studies and used it to evaluate the performance of the workflow regarding matrix effect, recovery, phospholipid removal, linearity, accuracy, and precision.



Supported Liquid Extraction (SLE)

SLE is analogous to LLE but uses the diatomaceous earth as physical support to allow the aqueous samples to spread over the surface in a thin layer. When the non-water miscible solvent passes through the bed, the analytes partition into the organic solvent, while the aqueous matrix components remain in the aqueous layer on the surface and are removed from the fraction collection (Figure 1).

After vortexing, 50 µL of the sample (with or without Zolmitriptan) was aliquoted to a 96-well plate and transferred to the Biotage® Extrahera™ workstation with the following extraction procedures completed by automation:

- 1) Pretreated samples with 150 µL 0.5M ammonium hydroxide in water and mixed thoroughly
- 2) Loaded 190 µL of pretreated mixtures onto a 200µL ISOLUTE® SLE+ plate. Applied positive pressure (5 bar) for 5 seconds to allow liquid to adsorb into the ISOLUTE® SLE+ sorbent.
- 3) Waited 5 minutes.
- 4) Elute with 500 µL MTBE and allowed flow through by gravity for 2 minutes. Applied positive pressure (2.5 bar) for 60 seconds to push all the solvent into the collection plate.

After the SLE extraction, the elution plate was transferred to the TurboVap® 96 Dual evaporator and used the following parameters for evaporation: *N₂ flow, 40L/min; temperature, 25°C (gas) and 40°C (plate); plate height, 64 mm.* Dried extracts were reconstituted using 100 µL acetonitrile/water for LC-MS/MS analysis.



Figure 2. Biotage ISOLUTE® SLE+ automation workflow for sample preparation in Bioanalysis

LC-MS/MS Conditions

Instrument: Shimadzu Nexera X₂
Column: Restek Ultra AQ C18 3 µm 100 x 2.1 mm
Mobile Phase:
A: 0.1% Formic Acid in Water
B: 0.1% Formic Acid in Acetonitrile
Column Temp: 40°C
Flow Rate: 0.4 ml/min
Instrument: Sciex 5500 MSD
Ion Source Temperature: 550°C
Ion pair for MRM acquisition: 288.1 (Q1 mass, Da)/58.1 (Q3 mass, Da)
Acquisition Parameters: DP: 70 V, EP: 10 V, CE: 45 eV, CXP: 10 V, Dwell time: 50 msec.

Results and Discussion

Selection of pretreatment and Elution Solvent

Plasma samples were with 0.5 M NH₃OH to keep the analyte in a non-ionized state. Both MTBE and EtOAc showed comparable extraction performance. MTBE yielded lower overall matrix effects (Table 1) and therefore was selected as the elution solvent.

Table 1. Selection of Elution Solvent: MTBE vs. EtOAc

Level (n=3)	Experiment	MTBE	EtOAc
QC low (8 ng/mL)	Recovery (%)	77	81
	Matrix effect (%)	101	112
QC mid (60 ng/mL)	Recovery (%)	85	84
	Matrix effect (%)	99	119
QC high (85 ng/mL)	Recovery (%)	82	87
	Matrix effect (%)	102	114

Phospholipid Removal

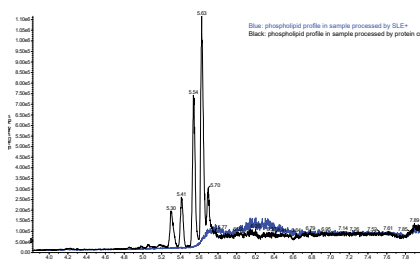


Figure 4. Phospholipid removal comparing ISOLUTE® SLE+ and traditional protein precipitation. The phospholipid profile was monitored by the MRM transition Q1, 184, Da/Q3, 184, Da using the same LC condition as

Linearity

Linearity was evaluated from 1-100 ng/mL based on plasma samples spiked with different analyte concentrations. Calibration curves ran on 3 plates of different batches on different days showed good reproducibility with satisfactory linearity.

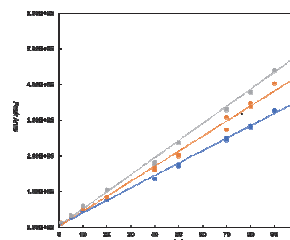


Figure 3. Zolmitriptan Linear curves, Plate1(blue): $y=42173x + 27830$ ($R=0.9982$), Plate2 (orange): $y=47984x + 37269$ ($R=0.9989$), Plate3 (gray): $y=34855x + 58662$ ($R=0.9993$)

Accuracy, Precision, and Phospholipid Removal

Excellent precision and accuracy were received within and between the ISOLUTE® SLE+ plates (Table 2). The ISOLUTE® SLE+ workflow offers comparable results to the traditional protein precipitation method (Table 3). However, SLE was more effective in removing the biomatrix interference, especially the phospholipids (Figure 4).

Table 2. Accuracy and Precision

Level	Experiment	Intra plate	Inter plate
QC low (8 ng/mL)	Accuracy (%)	102.0	101.3
	RSD (%)	4.8	6.0
QC mid (60 ng/mL)	Accuracy (%)	105.6	105.8
	RSD (%)	3.4	6.0
QC high (85 ng/mL)	Accuracy (%)	105.8	105.8
	RSD (%)	3.3	4.0

Table 3. Biotage ISOLUTE® SLE+ Workflow vs Traditional Protein Precipitation

	Biotage ISOLUTE® SLE+	Protein precipitation
Precision, RSD (%)	3.1	1
Accuracy (%)	101.4	95.9

Conclusion

The automated ISOLUTE® SLE+ workflow demonstrated excellent performance in matrix effect, recovery, linearity, accuracy, and precision for the extraction of zolmitriptan from plasma samples.

Utilization of Biotage automated workstation,® Extrahera™ Classic, and TurboVap® 96 Dual evaporator, greatly saved the analyst operation time spent on the bench.

In addition to plasma, this workflow can be adapted to analyze most hydrophobic compounds in other biomatrices. For non-liquid bio-samples (e.g., stool, cell, tissue), a homogenization step (using Biotage® Lysera) is needed before the SLE extraction.

References

1. Vishwanathan, Karthick, et al. Rapid Communications in Mass Spectrometry 14.3 (2000): 168-172.
2. Little JL, et al. Journal of Chromatography B. 2006 Apr 3;833(2):219-30.