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Efficient Extraction of Quetiapine in Plasma Using Oasis PRiME MCX

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APPLICATION BENEFITS

- Faster, simplified sample preparation workflow
- Elimination of at least 98% of phospholipids compared to protein precipitation
- No evaporation or reconstitution necessary

WATERS SOLUTIONS

Oasis® PRiME MCX µElution Plate

ACQUITY® UPLC® I-Class System (FTN)

ACQUITY CSH C_{18} Column

Xevo® TQD Mass Spectrometer

MassLynx® Software

Oasis MCX µElution Plate

KEYWORDS

Oasis PRiME MCX, SPE, quetiapine, phospholipid removal, LC-MS/MS

INTRODUCTION

Bioanalysis plays an essential role in drug discovery and development, of which sample preparation is a critical step in order to achieve reliable results.[1] Speed, method robustness and cost are important factors to consider when developing an analytical method for a high-throughput laboratory.

Oasis PRiME MCX, a mixed-mode/strong cation-eXchange Solid-Phase Extraction (SPE) product, has been developed to generate cleaner extracts for basic compounds compared to other sample preparation techniques, by the removal of phospholipids. In this application, a fast 3-step protocol, that eliminates the conditioning and equilibration steps compared to conventional Solid Phase Extraction (SPE), was used to extract quetiapine from human plasma in a 96-well plate µElution format.

The structure of quetiapine is shown in Figure 1. Quetiapine is a dibenzothiazepine derivative and antipsychotic agent used for the treatment of a wide variety of psychotic disorders. This basic drug (pKa 7.06 of the amine) is ideal for sample extraction using a cation exchange sorbent.

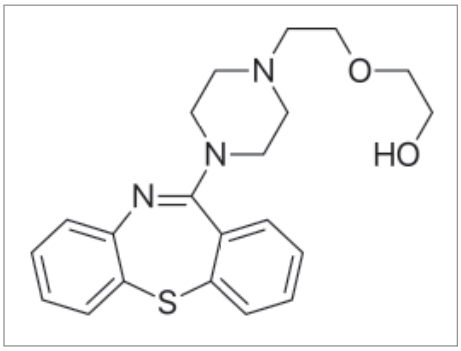

Figure 1. Structure of quetiapine.

This application note describes the analysis of quetiapine using three sample extraction protocols; protein precipitation, Oasis MCX and Oasis PRiME MCX to compare analyte recovery and matrix effects including phospholipid removal.

EXPERIMENTAL

Sample preparation

In-house calibrators were prepared by spiking quetiapine (Sigma Aldrich, Dorset, England) into pooled human plasma (Sera Laboratories, West Sussex, United Kingdom) over the concentration range 2.5–100 ng/mL. QC samples were also prepared in-house at 7.5, 30, and 75 ng/mL.

Sample extraction

1. Protein precipitation

100 µL of plasma calibrator/QC was transferred to a graduated microfuge tube.

The proteins were precipitated by the addition of 300 µL of methanol. Samples were capped and vortexed for 30 seconds prior to centrifugation for 5 minutes at 13000 rpm.

2. Oasis MCX

100 µL of plasma calibrator/QC was transferred to a graduated microfuge tube. To disrupt protein binding and protonate the analyte to enable ion exchange interactions, 100 µL of 4% phosphoric acid was added to each sample. The samples were capped and vortexed for 30 seconds prior to centrifugation for 5 minutes at 13000 rpm. The supernatant was transferred to a Oasis MCX 96-well µElution Plate that had been conditioned with methanol and equilibrated with water. The plate was washed with 2% formic acid (aqueous) followed by 100% methanol. Samples were eluted with 2 x 25 µL of 5% ammonium hydroxide in methanol followed by 50 µL of water. The plate was sealed and lightly vortexed for 30 seconds before analysis.

3. Oasis PRiME MCX

100 µL of plasma calibrator/QC was transferred to a graduated microfuge tube. To disrupt protein binding and protonate the analyte to enable ion exchange interactions, 100 µL of 200 mM ammonium formate with 4% phosphoric acid was added to each sample. The samples were capped and vortexed for 30 seconds prior to centrifugation for 5 minutes at 13000 rpm. The supernatant was transferred to a Oasis PRiME MCX 96-well µElution plate and washed with 100% methanol. Samples were eluted with 2 x 25 µL of 5% ammonium hydroxide in methanol followed by 50 µL of water. The plate was sealed and lightly vortexed for 30 seconds before analysis.

The Solid-Phase extraction procedures are summarized in Figure 2.

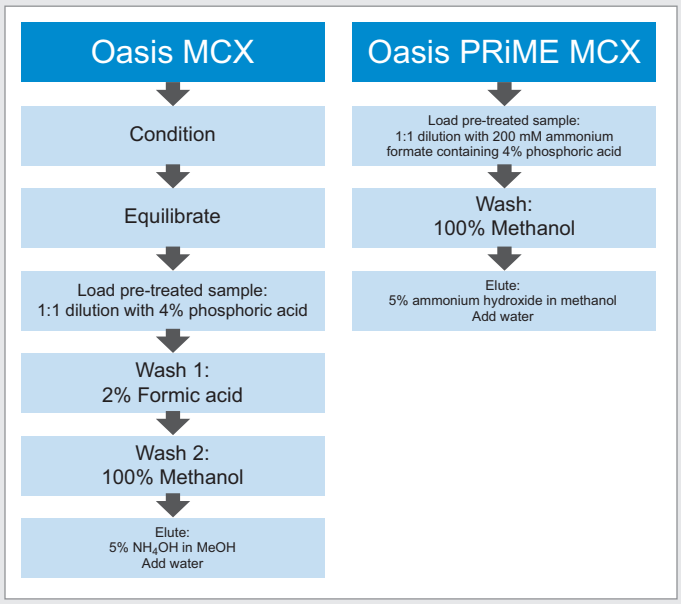

Figure 2. Oasis PRiME MCX comparison with traditional SPE extraction methodology, with no conditioning and equilibration, sample extraction is simplified to just 3 steps.

LC conditions

LC system:	ACQUITY UPLC I-Class System (FTN)
Column:	ACQUITY UPLC CSH C_{18} 1.7 µm, 2.1 x 100 mm (p/n 186005297)
Mobile phase A:	Water with 0.1% formic acid
Mobile phase B:	Methanol
Wash solvent:	80% methanol(aq) and 0.1% formic acid
Purge solvent:	20% methanol(aq) and 0.1% formic acid
Seal wash:	20% methanol(aq)
Column temp.:	45 °C
Sample temp.:	10 °C
Injection vol.:	5 µL
Flow rate:	0.4 mL/min
Gradient:	See table 1
Run time:	4.3 minutes (approximately 5.0 minutes injection to injection)

Table 1. Gradient table for the separation of quetiapine.

Time (min)	Flow rate (mL/min)	%A	%B	Curve
Initial	0.4	90	10	Initial
3.00	0.4	5	95	6
3.50	0.4	90	10	11

MS conditions

System:	Xevo TQD
Resolution:	MS1 and MS2 (0.75 FWHM)
Acquisition mode:	Multiple Reaction Monitoring (MRM) (see table 2 for details)
Polarity:	ESI positive
Capillary voltage:	0.5 kV
Cone voltage:	See table 2
Source temp.:	150 °C
Desolvation temp.:	600 °C
Dwell time:	0.05 seconds
Inter-scan delay:	0.02 seconds
Inter-channel delay:	0.01 seconds

Table 2. Guideline MRM parameters for quetiapine qualifier and quantifier.

Analyte	Precursor ion (*m/z*)	Product ion (*m/z*)	Cone voltage	Collision energy
Quetiapine (Quan)	384.1	253.05	40	30
Quetiapine (Qual)	384.1	221.1	40	45

Data management

MassLynx v4.1 with TargetLynx™ Application Manager

RESULTS AND DISCUSSION

RECOVERY

The extraction recovery for protein precipitation, Oasis MCX, and Oasis PRiME MCX sample extraction were determined by the analysis of three samples across the concentration range in replicates of 6. The extraction recovery was calculated using the following equation:

$$\%\text{Recovery} = \left(\frac{\text{Area A}}{\text{Area B}}\right) \times 100$$

Where A is the peak area of an extracted sample and B is the peak area of an extracted matrix sample in which quetiapine was added post-extraction. Excellent extraction recovery and precision was observed across the concentration range for quetiapine using Oasis MCX (mean 94.5% recovery) and Oasis PRiME MCX (mean 94.0% recovery), however, very poor recovery (mean 27.7% recovery) was observed for the protein precipitation method (Table 3).

Table 3. Precision performance (%RSD) and extraction recovery (%Recovery) for protein precipitation (PPT), Oasis MCX, and Oasis PRiME MCX Protocols.

	PPT		Oasis MCX		Oasis PRiME MCX	
	Recovery	RSD	Recovery	RSD	Recovery	RSD
QC low	25.6%	2.0%	97.8%	3.5%	96.7%	8.5%
QC mid	27.8%	1.2%	94.2 %	5.3%	94.6%	4.6%
QC high	29.6%	0.8%	91.3 %	3.0%	90.8%	4.4%

MATRIX EFFECTS AND PHOSPHOLIPIDS REMOVAL

The matrix effects for protein precipitation, Oasis MCX, and Oasis PRiME MCX sample extraction were determined by the analysis of three samples across the concentration range in replicates of 6. The matrix effects were calculated using the following equation:

$$\text{Matrix effects} = \left(\left(\frac{\text{Peak area in the presence of matrix}}{\text{Peak area in the absence of matrix}}\right)_{-1}\right) \times 100$$

The peak area in the presence of matrix refers to an extracted matrix sample in which quetiapine was added post-extraction. The peak area in the absence of matrix refers to a solvent solution of quetiapine.

There was significant matrix effects observed when using protein precipitation; the mean matrix effect was -58.4% (range -56.4% to -59.6%) indicating severe ion suppression. Using mixed-mode/strong cation-eXchange Solid-Phase extraction the matrix effects observed were negligible, Oasis MCX mean matrix effect was -2.7% (range 0 to -4.3%) and Oasis PRiME MCX mean matrix effect was -0.1% (range -1.9% to 1.2%).

To assess phospholipid removal a parent ion scan experiment was conducted monitoring parent ions of *m/z* 184 in plasma sample extracts using the three extraction protocols. The phospholipid peak areas were normalized to the protein precipitation extract, which contained the highest levels. Figure 3 shows the comparative phospholipid levels in plasma sample extracts from protein precipitation, Oasis MCX and the 3-step Oasis PRiME MCX Protocol demonstrating phospholipid removal of up to 98% for the 3-step Oasis PRiME MCX Protocol when compared to protein precipitation.

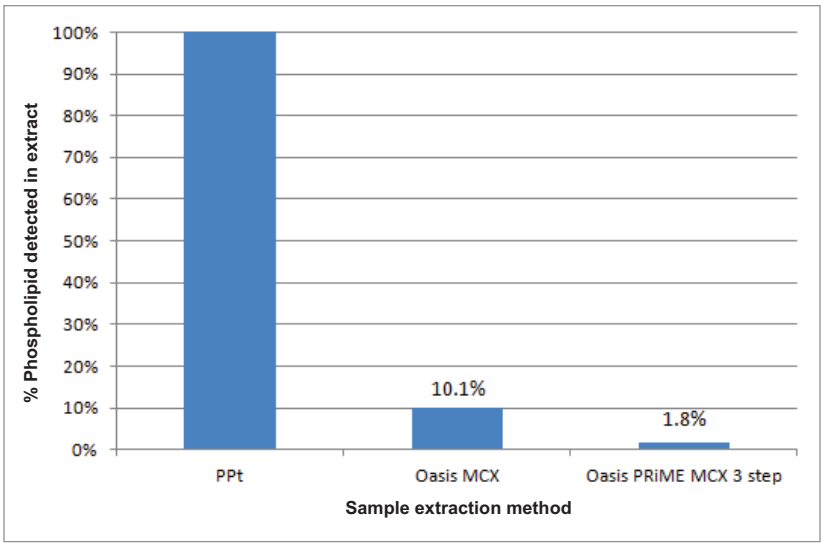

Figure 3. Bar chart to demonstrate the levels of phospholipid present in the extracted plasma sample when using protein precipitation, Oasis MCX and Oasis PRiME MCX.

CONCLUSIONS

Oasis PRiME MCX and the 3-step protocol removes 98% of phospholipids compared to protein precipitation, whilst, providing reproducible results and cleaner extracts with negligible matrix effects.

Even though protein precipitation is an inexpensive form of sample preparation, the matrix effects indicate that inefficiencies of the sample clean-up which may lead to poor robustness of the bioanalytical method.

The µElution format and 3-step protocol enables a faster workflow, through the use of a simplified protocol and direct injection of the plasma extract without the need for evaporation or reconstitution. This SPE format and extraction protocol can be easily automated using a liquid handling robot to improve laboratory workflow, eliminate transcription errors and allow for sample tracking capabilities.

References

1. Mohammad MaHDI Moeina, Aziza El Beqqalib, Mohamed Abdel-Rehimb. Bioanalytical method development and validation: Critical concepts and strategies. *J Chromatography B*. 2017; 1043:3–11.

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