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アプリケーションノート

Utility of UPLC-MS/MS and SPE for High Throughput Quantitative Bioanalysis

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Abstract

The use of 30 mm UPLC columns coupled with Oasis SPE in Elution format was investigated to increase the

speed of quantitative bioanalytical methods while maintaining sensitivity and resolution of closely related analytes.

Benefits

- Rapid development of robust bioanalytical methods
- Shorten run times without sacrificing separations of closely related analytes

Introduction

Bioanalytical laboratories are required to develop fast LC methods which still separate analytes, closely related compounds, and metabolites from endogenous components. Many of the challenges of developing a bioanalytical method revolve around meeting the rigorous criteria set forth in the FDA Guidance for Industry for Bioanalytical Method Validation, including the investigation of matrix effects. Both good chromatography and sample preparation are necessary for ensuring rugged bioanalytical methods. Two representative mixtures of closely related analytes were chosen. Mixture 1 contains imipramine, D4-imipramine, and clomipramine. Mixture 2 contains risperidone, 9-OH risperidone (the major circulating metabolite), and clozapine. This work demonstrates the utility of sub-2-m chromatographic particles packed in short columns coupled to highly selective mixed-mode sample preparation for the development of quantitative bioanalytical methods.

Experimental

ACQUITY UPLC Conditions

Column:	ACQUITY UPLC BEH C_{18}, 2.1 × 30 mm, 1.7 m
Part number:	186002349
Mobile phase A:	10 mM CH_3NH_4COOH, pH 9
Mobile phase B:	MeOH

Injection volume:	8.0 L
Column temp.:	50 °C
Sample temp.:	15 °C
Sample Diluent:	50:50 H_2O:MeOH
Strong needle wash:	60:40 ACN:IPA × 0.5% HCOOH (500 L)
Weak needle wash:	95:5 H_2O:MeOH (500 L)

Gradient

Time (min)	%A	%B	Curve	Flow rate (mL/min)
0.00	60	40	6	1.0
0.50	5	95	6	1.0
0.55	5	95	6	1.5
0.85	5	95	6	1.5
0.90	60	40	6	1.0
1.00	60	40	6	1.0

Waters Quattro Premier Conditions

Desolvation temp.:	350 °C
Cone gas flow:	50 L/Hr
Desolvation gas flow:	700 L/Hr

Collision cell pressure:	$2.6 \times 10^{(-3)}$ mbar

Oasis MCX µElution 96-well plate (2 mg/well of sorbent), p/n: 186001830BA < https://www.waters.com/nextgen/us/en/shop/sample-preparation--filtration/186001830ba-oasis-mcx-96-well--elution-plate-2-mg-sorbent-per-well-30--m-1-p.html> :

- Condition with 200 L MeOH
- Equilibrate with 200 L water
- Load 400 L diluted plasma sample (200 L plasma + 200 µL 4% phosphoric acid)
- Wash with 200 L 2% HCOOH in water
- Wash with 200 L MeOH
- Elute with 2 × 25 L 5% NH_4OH in 90:10 MeOH:H_2O for the risperidone mixture or 2 × 25 L 5% NH_4OH in ACN for the imipramine mixture
- Dilute with 50 L water

Results and Discussion

To selectively separate analytes from matrix components, mixed mode SPE was utilized due to its retention by both reversed-phase and ion-exchange mechanisms. SPE recoveries for all six analytes were 90% for extracted human plasma samples. A representative chromatogram of imipramine, the closely related compound clomipramine, and the internal standard D4-imipramine at 0.1 ng/mL in extracted human plasma is shown in Figure 1. A representative chromatogram of risperidone, its hydroxylated metabolite, and the internal standard clozapine at 0.1 ng/mL in extracted human plasma is shown in Figure 2. Sample preparation was demonstrated to be selective for the analytes of interest, as average matrix effects were 15% for the six compounds evaluated, even under these rapid gradient conditions.

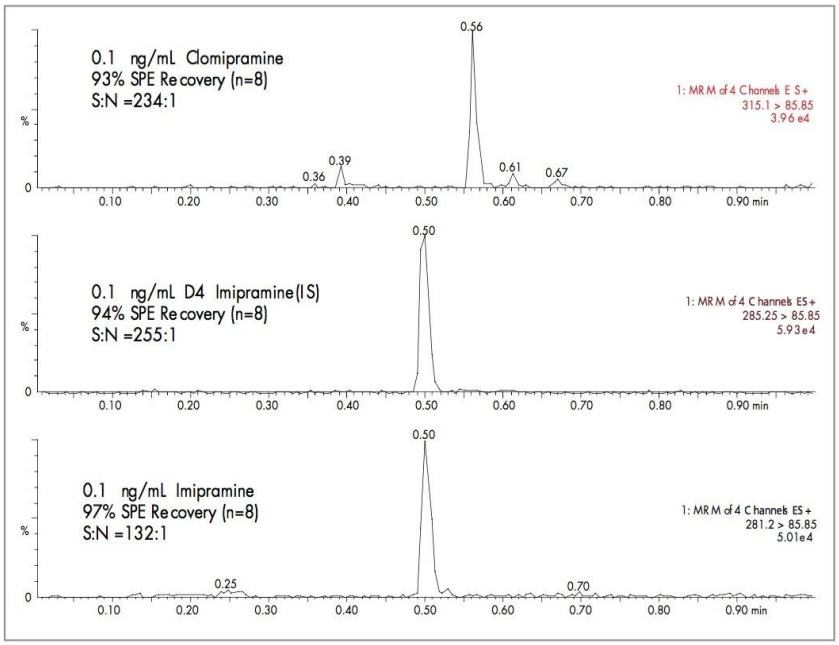

Figure 1. UPLC-MS/MS chromatogram of a 0.1 ng/mL sample of imipramine, D4-imipramine, and clomipramine in human plasma, prepared with Oasis MCX µElution plate SPE.

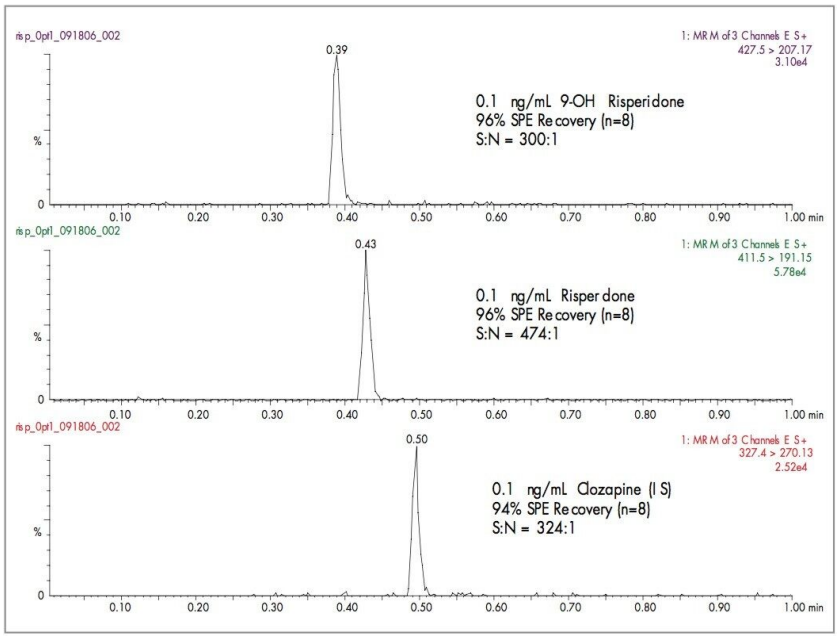

Figure 2. UPLC-MS/MS chromatogram of a 0.1 ng/mL sample of risperidone, 9-OH risperidone, and clozapine in human plasma, prepared with Oasis MCX µElution plate SPE.

To obtain a cleaner final extract for analysis of the risperidone mixture, the elution solvent was modified from 5% NH_4OH in 100% MeOH to 5% NH_4OH in 90:10 MeOH:H_20. Using less organic solvent in the final eluate more selectively elutes the analytes while retaining very hydrophobic interferences on the SPE sorbent. For the imipramine mixture, the elution solvent was modified from 5% NH_4OH in 100% MeOH to 5% NH_4OH in 100% ACN. Imipramine and the related compounds are very hydrophobic and require a strong elution solvent to fully desorb from the SPE sorbent. Previous work (1) has shown that plasma phospholipids, some of the compounds most heavily implicated in matrix effects, are more soluble in protic solvents such as methanol and less soluble in aprotic solvents such as acetonitrile.

For this reason, acetonitrile was substituted for methanol in the final elution, resulting in a cleaner final extract for LC–MS-MS analysis. SPE was performed on a 96-well µElution plate format which enables elution in volumes as low as 10–25 L. This provides sample concentration without drying down and reconstituting the extract, resulting in improved sample throughput and increased sensitivity.

Chromatography was performed on a 30 mm UPLC column to reduce cycle time. A total cycle time of only 1 minute adequately separated analytes from each other and any endogenous interferences that may have been present.

Conclusion

The combination of UPLC technology, short columns, and Oasis SPE in µElution plate format offers significant advantages to bioanalytical scientists. The sub-2-m particles used in UPLC allow for increased resolution at higher linear velocities, which enables method development scientists to shorten run times without sacrificing separations of closely related analytes. The added resolution of UPLC also allows the use of 30 mm columns instead of 50 or 100 mm to further shorten LC run times. As demonstrated in this work, closely related analytes were separated in under a minute with this combination. Such short LC run times increase the need for effective sample preparation.

The use of mixed-mode Oasis SPE in µElution plate format achieves both sample pre-concentration and cleaner extracts, which minimizes matrix effects, even under very rapid gradient conditions. The combination of selective sample preparation and the speed and sensitivity of the UPLC/Quattro Premier platform facilitate rapid development of robust bioanalytical methods.

References

1. E. Chambers, D. Wagrowski-Diehl, Z. Lu, and J. Mazzeo, *J. Chromatogr. B*, 852 (1-2), 22–34 (2007).

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ACQUITY UPLC System <https://www.waters.com/514207>

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