Application Note CA-1822322

LC-MS/MS Analysis in Immunosuppressant Drug Research using EVOQ™ Elite LC-MS/MS System

Abstract

An LC-MS/MS research method has been developed for analysis of four immunosuppressant drugs, namely, tacrolimus, sirolimus, everolimus, and cyclosporine A, in the plasma. The method is sensitive to detect 0.5 ng/mL level of tacrolimus, sirolimus, everolimus, and 10 ng/mL of cyclosporine A. The linear calibration range of 0.5 to 50 ngmL was achieved for tacrolimus, sirolimus, and everolimus, 10 to 1000 ng/ml for cyclosporine A.

Introduction

Immunosuppressant drugs inhibit a body's immune system and are used in organ transplant patients to prevent organ rejection. These drugs have narrow therapeutic indices and highly variable pharmacokinetics. Because of this, it is necessary to continue research on these drugs to improve the scientific understanding of dosage requirements following use.

Until recently, quantitative analyses of these drugs has been performed primarily using immunoassays. The use of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has emerged as a new analytical technique for the determination of immunosuppressant drugs. The LC-MS/MS technique offers distinct advantages of

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sensitivity, selectivity, and accuracy by avoiding the cross-reactivity with metabolites in immunoassay and, when used in high-throughput, can offset initial high costs of LC-MS/MS instrumentation and a skilled analytical chemist. This application note describes a fast, sensitive, and robust quantitative research method using LC-MS/MS for the simultaneous analysis of tacrolimus, sirolimus, everolimus, and cyclosporine A in plasma.

Experimental

Sample Preparation

Stock Solutions: Standard solutions of tacrolimus, sirolimus, everolimus, and cyclosporine A and blank plasma were purchased from Sigma-Aldrich (St. Louis, MO) and diluted with MeOH to prepare primary stock solutions that were spiked in matrix blank to prepare calibration solutions.

The plasma matrix blank was prepared by adding 9 ml of cold acetonitrile to 3 ml of plasma (3:1 v/v). The resulting solution was vortexed for 30 seconds and allowed to stand for 15 minutes at room temperature, followed by 15 minutes of centrifugation at 15,000 rpm. The supernatant was collected as matrix blank and used to prepare the matrix spiked calibration solutions.

Chromatography (Advance UHPLC)

- Column: YMC Triart C18 (50 mm x 2.1 mm x 1.9 µm)
- Column temperature: 80 °C
- Flow rate: 0.5mL/min
- Mobile phase A: Water with 0.1% Formic acid + 10 mM Ammonium Format
- Mobile phase B: Acetonitrile with 0.1% Formic Acid
- Gradient conditions:

0.00 min	60% B
1.25 min	60% B
1.40 min	98% B
3.00 min	98% B
3.01 min	60% B
4.00 min	60% B

Injection volume: 5 µL

Mass Spectrometer (EVOQ Elite)

- HESI: +4500 V
- Probe Temperature: 450 °C
- Probe gas: 45 units
- Nebulizer gas: 40 units
- Cone gas temp: 350 °C
- Cone gas: 20 units
- Active exhaust: On
- Collision gas: Argon 1.25 mTorr

Optimized MRM Transitions:

Compound	Parent Ion	Product Ion	Collision Energy (V)
Tacrolimus	821.5	768.3	16
Sirolimus	931.5	864.5	16
Everolimus	975.7	908.4	16
Ascomycin (IS)	809.5	756.5	16
Cyclosporine	1220.1	1203.2	22
Cyclosporine D (IS)	1234.2	1217.3	21

Results and Discussion

With the sensitivity and selectivity of the LC-MS/MS, the sample preparation is simplified with only protein precipitation and the supernatant can be directly injected for analysis. The LC-MS/MS system has proved to be sensitive to detect the concentration levels of 0.5 ng/ml for tacrolimus, sirolimus, and everolimus, and 10 ng/ml for cyclosporine A in plasma. Figure 1 shows the representative chromatograms at these levels in plasma.

The Bruker MSWS software controlling the EVOQ LC-MS/MS system was equipped with the optional PACER™ software, which enables "exception based data-review," a revolutionary feature that significantly reduces the error rate of peak integration for quantitative analysis. Figure 2a-d show four different calibration curves (7-levels each with three replicates) processed by PACER™ from 0.5 ng/mL to 50 ng/mL for tacrolimus, sirolimus, and everolimus with linearity of $R^2 > 0.997$.and from 10 ng/mL to 1000 ng/ml for cyclosporine A with linearity of $R^2 > 0.995$.

To test the system robustness, a sequence of 7 level calibrations (each three replicates) and 3 level QC standards in matrix (each six replicates) were repeated 5 times. The R^2 of the calibration curves were found still be >0.994 and RSD for each QC level variation are generally 3 – 8% across over 200 injections of matrix samples.

Conclusion

Four immunosuppressant drugs are analyzed in plasma on EVOQ Elite LC-MS/MS system following a simple protein precipitation of the plasma sample. The method is fast, sensitive, and robust with a low limit of quantitation.

Figure 1: MRM Chromatograms of Four Imminosuppressents in Plasma Matrix

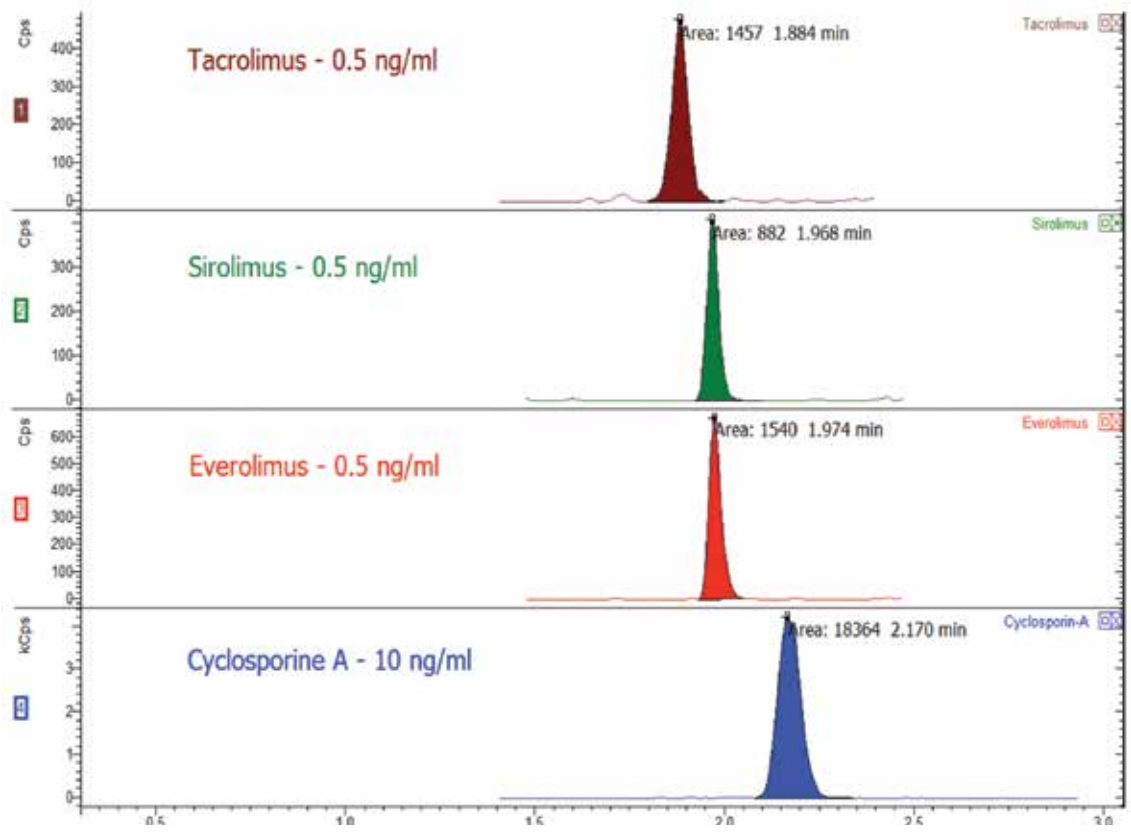
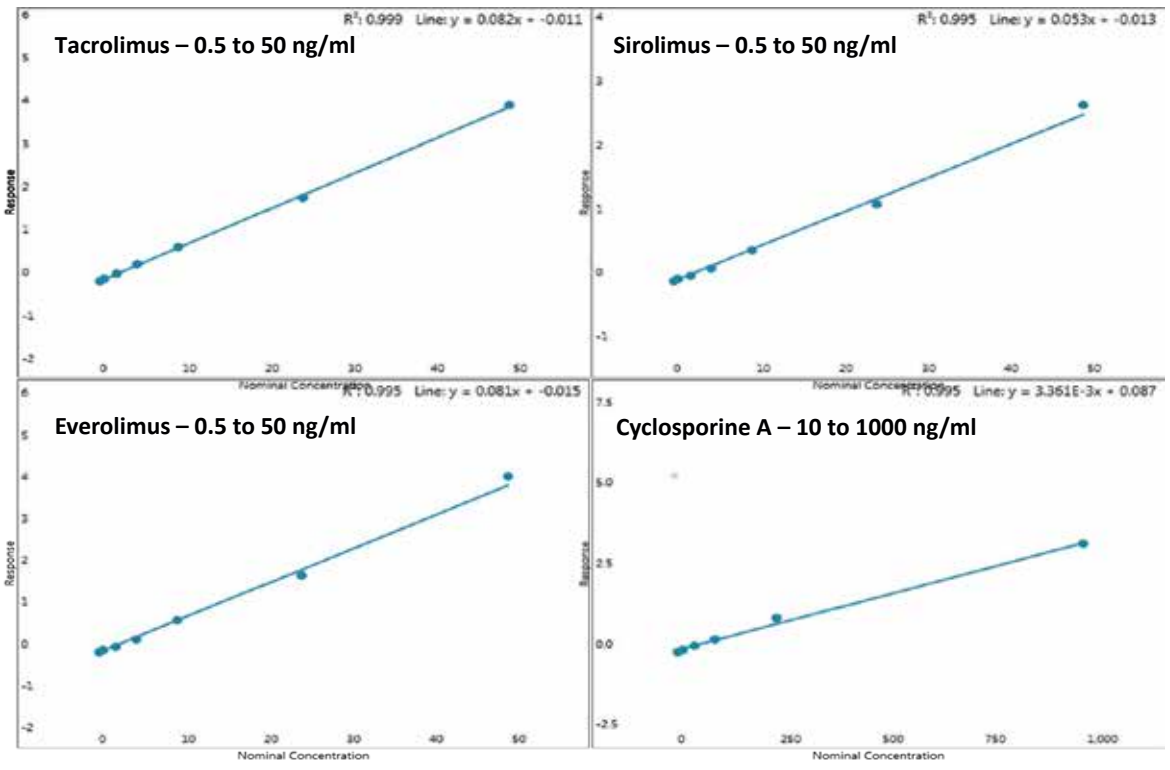


Figure 2: Calibration Curves of Four Imminosuppressents in Plasma Matrix



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