

Liquid Chromatography/
Mass Spectrometry

Authors

LC/MS Applications Team

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Quantitative Analysis of Immunosuppressant Drugs in Serum Using a QSight 110 Triple Quadrupole Mass Spectrometer

Introduction

This note describes a sensitive, fast and accurate LC/MS/MS quantitation method utilizing a QSight™ 110 Triple Quadrupole Mass Spectrometer for the simultaneous analysis of the immunosuppressant drugs tacrolimus, sirolimus and cyclosporine A in serum, using everolimus as an internal standard.

Immunosuppressant drugs are used in allogeneic organ transplant patients to reduce the activity of the patient's immune system and lower the risk of organ rejection.

It is important to monitor levels of immunosuppressant drugs to ensure the success of organ transplantation. By monitoring immunosuppressant levels in human blood for cyclosporine, tacrolimus and sirolimus, doctors can better ensure that each individual is receiving the right amount and formulation of drug needed to treat the particular case. Tests are routinely ordered to measure the amount of drug in the blood to determine whether cyclosporine concentrations fall within the therapeutic or toxic ranges.

QSight 110 provides high levels of accuracy, linearity and robustness while providing the quantitative capability to measure immunosuppressant drugs with ease.

Experimental

Tacrolimus, sirolimus, cyclosporine A and everolimus standards obtained from Sigma Aldrich® were injected into a reconstituted serum, also obtained from Sigma Aldrich. Protein precipitation was achieved utilizing centrifugation in 100% MeOH.

Table 1. QSight 110 settings for this method.

ESI Voltage (Volts)	5000
HSID™ Temp. (°C)	150
Nebulizer Gas Setting	450
Drying Gas Setting	100
Heating Gas Setting	350
Source Temp. (°C)	350
Dwell Time (ms)	100
Pause Time (ms)	5

Table 2. Mass-to-charge values for the quantifier and qualifier transitions, along with their collision energy.

Compound	Parent Ion	Quantifier Ion	CE	Qualifier Ion	CE
Tacrolimus	821.6	768.5	24	786.5	22
Sirolimus	931.7	864.5	22	882.5	18
Everolimus	975.7	908.3	22	926.3	18
Cyclosporin	1219.8	199.0	83	1184.8	37

UHPLC analysis was performed using an Altus™ UPLC® system. 10 µL of each of the samples were injected into a Brownlee™ C18 column, (50 x 2.1 mm, 2.7 µm) running a gradient from 10 to 100% methanol in 10 mM NH₄OAc and 0.1% acetic acid at a flow rate of 0.6 mL/min.

Results

Figure 1 displays the extracted ion chromatograms (EICs) for the mixture of tacrolimus and sirolimus at a concentration of 0.1 ng/mL as well as cyclosporine A at a concentration of 1 ng/mL. No ion suppression or enhancement was observed.

Excellent linearity ($R^2=0.99999$) was found for tacrolimus and sirolimus within the concentration range of 0.1 to 200 ng/mL. For cyclosporine A, an R^2 value of 0.99999 was achieved by this method between 1 and 2000 ng/mL. A calibration curve of the linearity for tacrolimus is shown in Figure 2. The results shown for tacrolimus are typical of those found for each of the immunosuppressant drugs quantitated utilizing this method, as all possessed R^2 values of 0.99999 in the concentration ranges previously stated.

Conclusion

QSight 110 provides excellent quantitative data for immunosuppressant drugs achieving results that are well within the therapeutic range for tacrolimus (5-15 ng/mL) sirolimus (5-10 ng/mL) and cyclosporine A (120-240 ng/mL), respectively, while maintaining excellent linearity of over four orders of magnitude. Combining the sensitivity and linearity necessary to provide superior results at both high and low concentrations, the QSight 110 is an ideal platform for the quantitation of immunosuppressive drugs.

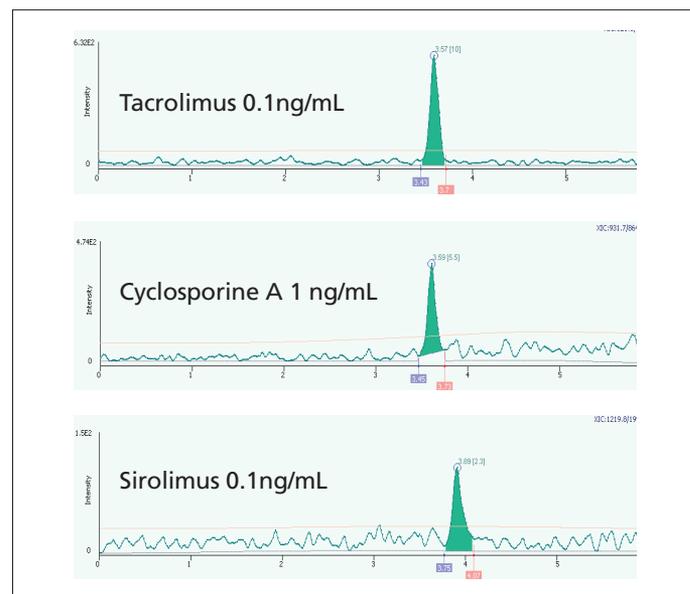


Figure 1. Quantitation of tacrolimus at 0.1 ng/mL, cyclosporine A at 1 ng/mL and sirolimus at 0.1 ng/mL.

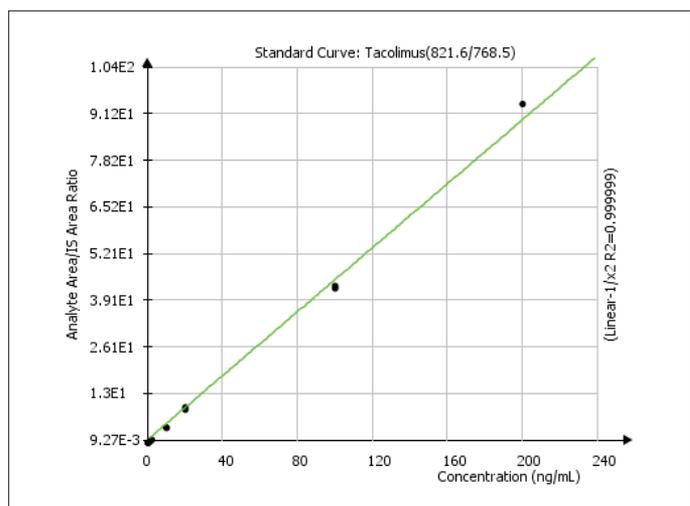


Figure 2. Tacrolimus calibration curve from 0.1 to 200 ng/mL.