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THE ANALYSIS OF MYCOPHENOLIC ACID USING LC/MS/MS

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Introduction

Mycophenolic acid (MPA) suppresses the immune response by blocking pathways that are complimentary to the effects of Cyclosporin A. Unlike azathioprine and methotrexate, which have a non-selective effect on DNA synthesis in all cell types, MPA has a selective effect on lymphocyte proliferation. In combination with cyclosporin A, MPA is more effective than azathioprine for the prophylaxis of kidney, heart and lung transplant rejection[1,2]. MPA has also been used in the treatment of acute kidney transplant rejection, rheumatoid arthritis and graft-versus-host disease.

Recent reports based on retrospective studies of renal and heart transplant patients have shown that there is a significant relationship between the dose-interval MPA AUC and risk for acute rejection[3,4]. Among these patients there was a natural 10-fold variation in the dose-interval MPA AUC. Other factors (eg disease state) were also found to cause pharmacokinetic variability for MPA by altering the ratio of protein-bound (inactive) to free (active) drug. Because of these inter-individual differences, monitoring of MPA plasma concentrations is becoming standard practice to guide individualisation of MMF therapy[4].

Immunoassays for MPA have a positive bias of 20% - 30% when compared to measurements made by HPLC-UV methods and this has been attributed to the presence of high concentrations of MPA metabolites in the plasma[5,6]. Although HPLC-UV methods have adequate sensitivity for MPA and are able to distinguish the drug from it's metabolites, throughput is very low (3 samples per hour) and other therapeutic drugs may interfere with the assay[7].

Methodology

A Quattro LC tandem mass spectrometer fitted with a Z Spray ion source was used for all analyses. The instrument was operated in electrospray ionisation mode and was coupled to a Waters 2790 Alliance HT HPLC system. All aspects of system control and data acquisition were controlled using MassLynx NT v3.4 software.

Chromatography

Samples (10 µL) were analysed using a Waters Spherisob 5 µm CN column (4.6 mm x 30 mm) eluted at a flow rate of 0.5 mL/min with 65% aqueous acetonitrile containing 2 mM ammonium acetate and 0.1% formic acid.
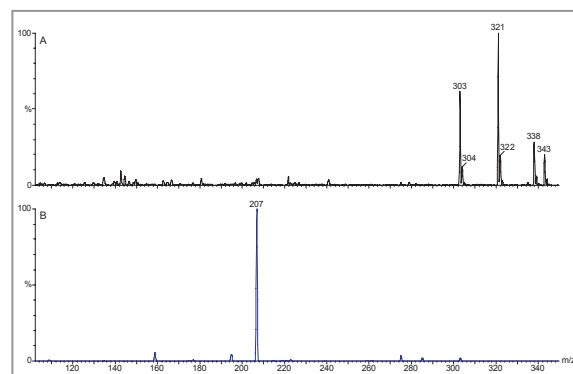

Figure 1. Positive ionisation electrospray mass (A) and CID (B) spectra of MPA dissolved at a concentration of 10 mM in 65% aqueous acetonitrile containing 2 mM ammonium acetate and 0.1% formic acid. Cone voltage 28 V (A & B), collision energy 22eV (B).
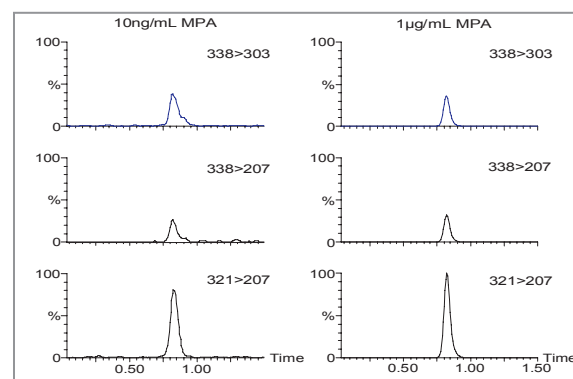

Figure 2. The relative sensitivities of three MRM transitions for MPA. 10 mL injections of standard applied to a Sphersiorb CN column (30 mm x 4.6 mm) eluted isocratically with 65% aqueous acetonitrile containing 2 mM ammonium acetate and 0.1% formic acid at a flow rate 0f 0.5 mL/min.

Results

In the presence of ammonium acetate and at low cone voltage MPA predominantly forms an ammoniated ion (*m/z* 338) whilst at higher cone voltage, the protonated molecule becomes more intense. The ammoniated ion can be fragmented to the dehydrated parent (*m/z* 303) or to *m/z* 207, but fragmentation to the protonated molecule is inefficient. Fragmentation of the protonated molecule again generates a single intense product ion (Figure 1).

The published HPLC procedures for the analysis of MPA are based on the analysis of protein-precipitated plasma and sample processing is therefore simple and rapid[6,7].

However, the UV detection method employed is non-specific and requires relatively long HPLC run times (20 min) to separate MPA from the major interferences. Even under these conditions, the assay is subject to interference from unidentified sources (presumed to be therapeutic drugs)[6].

In contrast, MS/MS provides a very selective detection mechanism such that even in complex mixtures, compound-specific mass chromatograms will give a single peak (*See Waters* Application Brief *LC/MS/MS Analysis of Amino Acids Using AccQTag Derivatisation*). This allows the use of high throughput "generic" HPLC methods designed to retain the compounds of interest for a short time so that they elute after the void volume. The method described here has a cycle time of 2 min, injection to injection, and can easily detect 10 pg of MPA on column (S:N 16:1) compared to 300 pg on column (S:N 3:1) reported as the limit of detection for the HPLC method. The true limit of detection for the LC/MS/MS method will be established when suitable plasma calibrators are available.

MPA is used in relatively high dose with a therapeutic range from 1 - 3.5 µg/mL in plasma. The increased sensitivity of LC/MS/MS will allow processing of smaller volumes of plasma than are currently used for HPLC-UV methods so that high throughput can be achieved. This may be of particular importance if microfiltration is adopted as a standard procedure to remove protein-bound MPA prior to analysis.

Summary

- With positive electrospray ionisation, mycophenolic acid forms strong protonated and ammoniated molecules that readily fragment.

- MPA can be analysed using the same chromatographic conditions used for the analysis of Cyclosprin A, Tacrolimus and Sirolimus.

- The LC/MS/MS assay is rapid (approximately 2 minute cycle time) and should offer a limit of detection below 1 ng/mL (<10 pg on column).

- The greater sensitivity and selectivity of the LC/MS/MS assay over current LC-UV methods may reduce the time required for sample preparation.
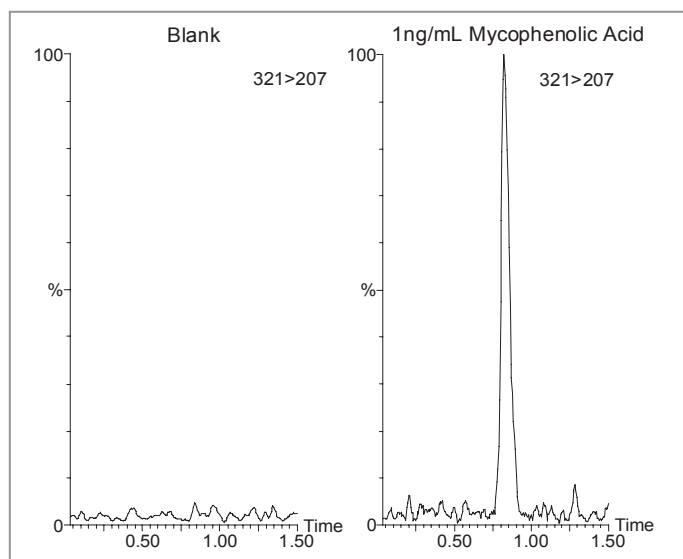

Figure 3. Mass chromatograms from the calibration curve for Sirolimus. (A) solvent blank, (B) 0.2 µg/L Sirolimus, raw data and (C) 0.2 µg/L Sirolimus, smoothed, integrated data.

References

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