INTRODUCTION

Simple LC/MS/MS methods have been widely adopted in clinical chemistry laboratories for the therapeutic drug monitoring (TDM) of single immunosuppressant drugs including Tacrolimus, Cyclosporin A and Sirolimus1,2. For newer immunosuppressants, such as Everolimus, this may be the only method currently available to the clinical chemist. A demand for multi-analyte methods has arisen from the use of combination therapy and the need to streamline laboratory workflow. Typically, these methods use on-line SPE and are unattractive to some laboratories because they tend to reduce sample throughput. We have therefore investigated the use of more advanced MS/MS technology in combination with simple chromatography to deliver a rapid multi-analyte immunosuppressant TDM method.

METHODS

Mass Spectrometry

A Waters Acquity Tandem Quadrupole Detector (TQD) coupled to an Acquity UPLC™ (Waters Corporation, Manchester, UK) was used for all analyses. The full system configuration is shown in Figure 1. The instrument was operated in positive electrospray ionisation mode. All data acquisition was performed using MassLynx™ 4.1 software with auto data processing using the QuanLynx™ Application Manager.

The compound-dependent cone voltage was optimised to maximise the abundance of the precursor ion entering the source and selected to pass through the first quadrupole to the collision cell. Collision-induced dissociation was facilitated by argon and collision energy to produce characteristic product ions. Using this information a specific Multiple Reaction Monitor (MRM) experiment was created and is shown in Table 1.

RESULTS

A chromatogram of the lowest extracted whole blood immunosuppressants chromatogram is shown in Figure 2. Each chromatogram is annotated with the compound name, the ions selected for monitoring and the collision energy to produce characteristic product ions. All acquisitions were performed using MassLynx™ 4.1 software with auto data processing using the QuanLynx™ Application Manager.

The Passing-Bablok linear regression analysis between the patient samples run using the single and multi-analyte LC/MS/MS methods give good agreement with statistical processing using Passing-Bablok linear regression analysis.

Table 2: Summary of the intra-day precision (n=5) determined using Chromsystems controls.

Table 1: The tuning parameters used when monitoring the four immunosuppressants and their internal standards.

Table 2: Summary of the intra-day precision (n=5) determined using Chromsystems controls.

CONCLUSIONS

1. The Acquity-TQD with its fast scanning capabilities has been demonstrated to be able to quantify multiple immunosuppressants in a single analytical run with simple sample preparation.

2. The analytes are detectable at the required limit of detection, linear, with good intra and inter-day precision.

3. The analytes elute away from significant ion suppression regions.

4. Comparison of patient samples by a single analyte LC/MS/MS method give good agreement with statistical processing using Passing-Bablok linear regression analysis.

References


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