Full automated sensitive determination of immunosuppressant drugs in whole blood, using high quality internal standardization.

1. Introduction

Measurement of immunosuppressant drugs (Figure 1) is essential during organ transplantation. Under-dosing can lead to organ rejection, while over-dosing can cause serious toxicity. Traditional methods to measure immunosuppressant drugs in whole blood are based on either immunosuppressants or chromatography. Immunosuppressants, though, are affected by matrix interferences and lack of specificity. LC-MEMS has then become the gold standard due to its specificity, precision and sensitivity. However, it has still one major drawback: current LC-MEMS platforms demand personnel with experience, and for whole blood samples, tedious sample preparation. As a consequence, sample throughput is generally much lower than for immunoassays. We here report a fully automated procedure for the quantification of four major immunosuppressant in whole blood samples, using 13C labelled internal standards.

2. Methods and Materials

The quantitative analysis of immunosuppressant (Figure 2) was performed using reagents provided in Alphasys Dosimune® kit. The Immunosuppressant and internal standard were monitored using HPLC-MS/MS system (Nexera X2 and CLAM-8050, Shimadzu, Kyoto). Sample preparation was performed using extraction buffer and internal standard set provided in Alphasys Dosimune® kit. Analytical performance of the method was monitored using whole blood calibrators and whole blood QC. Automatic sample preparation was performed using CLAM-2000 module (Shimadzu, Kyoto).

3. Results

3.1. Method conditions

The method enables the quantification of tacrolimus, sirolimus, everolimus and cyclosporine A in whole blood samples. The established quantitation strategy for this compounds is to use internal calibrations using deuterium labeled standards. However, they generally suffer from poor isotopic enrichment, leading to overestimation of the unlabeled form. We have thus 13C labeled standards for tacrolimus, sirolimus and everolimus. This guarantees better isotopic enrichment, better precision of the results, long term stability of the standards and perfect co-elution with the analytes, leading to a better correction of matrix effects. Linearity was confirmed in the range 0.5 to 45 ng/mL for tacrolimus, sirolimus, everolimus, and in the range 5 to 1500 ng/mL for cyclosporine A (Figure 3). For all analytes, 2 points of linearity models where above 0.99, with S/N > 25 for LOD levels (Figure 4). Controls showed accuracies comprised in between 85 and 115% for all analytes (Table 3).

3.2. Calibration in whole blood

Linearity was confirmed, in whole blood, in the range 0.5-45 ng/mL for tacrolimus (Figure 3.a), sirolimus (Figure 3.b), everolimus (Figure 3.c), and 5-1500 ng/mL for cyclosporine A (Figure 3.d).

3.3. Limits of quantification in whole blood

The limits of quantification (LOQ), in whole blood, are 0.5 ng/mL for tacrolimus, sirolimus, and everolimus, and 5 ng/mL for cyclosporine A. The signal to noise ratio is above 25 at LOQ levels.

3.4. Performance Evaluation

Whole blood controls showed accuracies comprised in between 85% and 115% for all analytes.

Table 3. Whole blood controls sample accuracies.

4. Novel Aspect

Fully automated sensitive quantification of immunosuppressant drugs in whole blood, using high quality 13C labelled internal standards, increasing data quality, throughput and safety.

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