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Simultaneous determination of four immunosuppressants in whole blood – A fast method on a Shimadzu tandem LCMS-8030 mass analyser

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1: Introduction

The amount of different kind of organ transplantations is increasing steadily since the improvement of this technology in the past century. The European organization Eurotransplant counts several thousand surgical interventions in Europe every year. To avoid rejection of the transplanted organ by the immune system, a complex therapy of immunosuppressants in combination with other drugs is necessary. A therapeutic concentration range in blood minimizes the risk of unwanted side effects. Immunoassays which are an alternative method for monitoring immunosuppressants, often lead to insufficient results due to their cross reactivity to drug metabolites. In this study we established a fast, kit-based screening method for routine use of immunosuppressants based on LCMS/MS technology.

2: Materials and Methods

The four immunosuppressants Cyclosporine A, Tacrolimus, Sirolimus and Everolimus are often used in therapy followed by autologous organ transplantation. These drugs were analyzed in whole blood samples using a commercially available kit supplied by Chromsystems®2, München, Germany. The samples are first prepared by a short manual protein precipitation, injected into the Shimadzu LCMS-8030 triple-quad system and afterwards analyzed by LabSolution software. An online trapping system separates the analytes from matrix signals and eliminates unwanted effects like ion suppression.

LC/MS/MS:

Key feature of LCMS-8030 triple quadrupole mass spectrometer

- Ultra fast polarity switching of 15msec
- Ultra fast scan speed of up *to 15,000 u/sec*
- UFsweeper™ technology dramatically minimizes cross talk
- Excellent linearity with wide dynamic range

Figure 1: LCMS-8030 triple quadrupole mass spectrometer

3: Results

3-1 Method Validation

Linearity

Linearity was evaluated by analysis of everolimus standards over the concentration range of the calibration curve (Table 1). Correlation coefficient was 0,99956 for everolimus, 0,99916 for tacrolimus, 0,99944 for sirolimus and 0,99973 for cyclosporine a respectively.

Drug	Range (µg/l)	Lower limit of quantification (µg/l)
CsA	25-900	3.9
Everolimus	2-40	0.395
Tacrolimus	2-40	1.003
Sirolimus	2-50	0.997

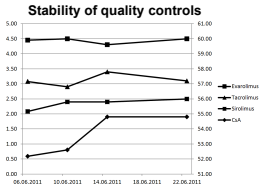
Table 1. Range and LOQ.

Precision

Interday- and Intraday-Precision were carried out by evaluation of quality control samples prepared from the same specimen(n=5) and measured at different times of the day(intraday) or different days (interday) with the same instrument parameters. Inter- and intraday precision were assessed by daily routine samples of hospital patients in replicates prepared for each method. Interday-precison for low-quality-control-samples are shown in table 2.Interday precision was also obtained by quality control samples of 4 different levels for all four immuno-suppressants supplied by Chromsystems. Stability of these control samples were monitored by measurement of the same batch/lot at different times during the course of 14 days (Table 3).

Drug	CV
CsA	1.4 %
Everolimus	6.4 %
Tacrolimus	5.2 %
Sirolimus	9.4 %

Table 2. CV for interday precision of QK1.



Drug	06.06. 2011	10.06. 2011	14.06. 2011	22.06. 2011
Everolimus	2.45	2.50	2.30	2.50
CsA	52.18	52.60	54.80	54.80
Tacrolimus	2.08	1.90	2.40	2.10
Sirolimus	2.08	2.40	2.40	2.50

Table 3. QC 1-stability during the course of 14 days.

Accuracy

Accuracy limits were set within the following criteria: 90-110% and imprecision CV of less than 10%. LC-MS/MS within run accuracy were determined by analyzing 4 quality controls at different levels (therapeutic range). The accuracy biases of the quality controls are shown in Table 4:

Drug	AB QC1	AB QC2	AB QC3	AB QC4
CsA	-0,5%	2.13%	1.8%	6.8%
Everolimus	3.01%	2.28%	2.33%	-1.11%
Tacrolimus	-5.62%	-2.72%	4.1%	9.3%
Sirolimus	-7.32%	2.77%	-0.37	4.43%

Table 4. accuracy bias of QC1-4.

3-2 Passing-Bablok analysis Method comparison

Comparison of methods is an important process in validation of a new analytical measurement brought into laboratory routine. In this case it was evaluated using Passing-Bablok-analysis to confirm a distinct relation[3] between either HPLC-MS vs. HPLC-MS/MS (everolimus) or Immunoassay vs. HPLC-MS/MS (csA, tacrolimus, sirolimus). All four regression curves show good correlation. Slope: 1,02 (sirolimus), 0,99 (tacrolimus), 0,97 (everolimus) and 1,03 for csA. 25-69 samples were examined under the same conditions over a course of 2 weeks to give a spectrum of repeatability under the given circumstances.

Drug	Method comparison
CsA	Immunoassay
Everolimus	HPLC-MS
Tacrolimus	Immunoassay
Sirolimus	Immunoassay

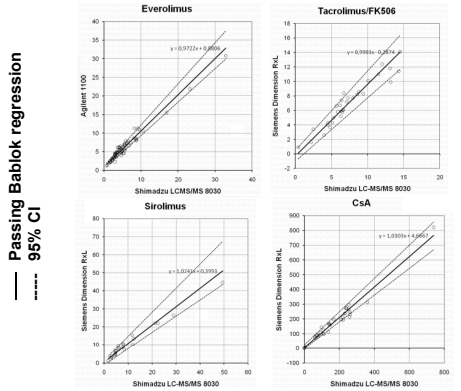


Figure 2. Passing-Bablok-Regressions.

4: Conclusion

Quantitative Assessment of immunosuppressant drug levels is, though extremely important to patients needs and sufficient organ survival in transplantation medicine, a challenging part of clinical laboratory procedure[4]. Although there have been many approaches to simplify HPLC-MS by immunoassay methods it is still the most reliable method and gold standard procedure in modern clinical monitoring of immuno-suppressant therapy.

5: References

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