Conference abstract SL-03

How to Deal with Ion Suppression – the Foe of HPLC-MS/MS

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Sci Pharm. 2009; 77: 170 doi:10.3797/scipharm.oephg.21.SL-03

It is well accepted, that a HPLC-MS/MS assay can outperform immunoassays in therapeutic drug monitoring (TDM) due to its increased accuracy, sensitivity, and precision. However, the occurrence of sudden and unpredictable ion yield attenuation, can hamper assay performance. Such “ion suppression effects” are considered a central analytical problem if using an MS/MS detector in bio-analysis. Since pathological patient specimen compositions or individual co-medications may be major causes for ion yield fluctuations, measures have to be taken to evaluate these interferences prior to transferring an HPLC-MS/MS assay to routine use.

A fully validated online SPE-HPLC-MS/MS assay setup (MS: API4000QTrap, SPE column: Oasis HLB, HPLC column: XDB-C-18, 1:20 sample dilution, cycle time 3.5min) designed for fast routine immunosuppressant TDM of cyclosporine A, tacrolimus, sirolimus, and everolimus [1] was evaluated. Post column infusion or drug spiking experiments were performed. A total of 54 drugs as well as icteric, haemolytic, and lipaemic blank matrices have been screened.

Infusion experiments showed only little influence of the spiked drugs on the analyte ion profiles, whereas spiking experiments unveiled strong signal suppression or enhancement in many cases. For cyclosporine A and tacrolimus the respective internal standards cyclosporine D and ascomycin balanced the effects, whereas some significant changes were observed for sirolimus and everolimus using either 32-desmethoxyrapamycin or ascomycin as co-eluting internal standards. Pathologically altered matrices did not influence quantitative results.

We do conclude, that even if using highly diluted samples and modern 2D chromatography, co-medication does cause ion yield attenuations in HPLC-MS/MS. Optimal internal standards are needed to balance these effects.

[1] Seger C, Tentschert K, Stöggl W, Griesmacher A, Ramsay S. A rapid HPLC MS/MS method for the simultaneous quantification of cyclosporine A, tacrolimus, sirolimus and everolimus in human blood samples. Nat Protoc; in press.