구연 11

A Metabolomic Profiling and Quantification of Tacrolimus (FK506) and O-demethylated Metabolite in Human using Electrospray Tandem Mass Spectrometry (ESI/MS/MS) and Ion Trap Mass Spectrometry (MS/MSn)

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Background: Tacrolimus (FK506), a potent immunosuppressive agent, is a neutral lipophilic 23-membered macrolide structure and commonly used to prevent rejection of organ transplants. It is extensively hepatic metabolized by the CYP450 3A subfamily. We aimed here to develop a simple, fast, sensitive and more specific method, which can be used for the metabolomic profiling as well as pharmacokinetic studies of both Tacrolimus and its metabolites simultaneously.

Method: We applied two relative mass spectrometric techniques, ESI/MS/MS and MS/MSn for the quantification, metabolic identification and metabolomic profiling of tacrolimus. We used liquid-liquid extraction as a sample preparation method for whole blood, plasma and urine samples and gradient elution on an RP C18 column. Detection of tacrolimus and metabolites were achieved using ESI positive mode and quantification was performed using MRM mode from m/z 821.7>768.6 and 809.5>756.4 as internal standards. We have also used full scan MS mode for complete profiling and identification of additional metabolites, amino acids, and organic acids using different sample extraction and derivatization methods.

Results: The method was validated for the analysis of samples; the limit of detection was 0.01 ng/mL. The calibration curve was linear up to 1000ng/mL(r = 0.998). Also, based on the MS/MSn results, major metabolites have been identified (e.g. de-methylated). The pharmacokinetic parameters of both the parent drug and its major metabolite were measured in kidney transplant patients after oral administration using ascomycin (I.S). Also, there is good correlation between ESI/MS/MS and MS/MSn results were obtained and found to be the powerful tools for identification and quantitative analysis of metabolites of tacrolimus.

Conclusion: A Specificity, sensitivity and speed of the method with simultaneous quantification of tacrolimus and its metabolites in MRM mode allows its use for complete PK studies, therapeutic drug monitoring and evaluation of toxic effects of metabolites. We are also looking forward for metab-

olomic studies by comparison of metabolic profiles between healthy subjects and kidney transplant patients.

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