EVALUATION FOR SAFETY TESTING OF DRUG METABOLITES WITH LC-MSn SYSTEM USING RADIOISOTOPE (2)
HIGH PERFORMANCE APPROACH FOR IDENTIFICATION OF IN VIVO METABOLITES IN BIOLOGICAL SAMPLE
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The recently fixed FDA guidance “Safety Testing of Drug Metabolites” encourages the identification of differences in drug metabolism between animals used in nonclinical safety assessments and humans as early as possible during the drug development process.
The high resolution U-HPLC, micro volume cell mounted Radio detector and accurate mass LC-MSn system was applied for searching expected and/or non-expected metabolites followed by structural determination of the metabolites. This system may provide more sensitive and robust qualitative and quantitative data to identify metabolic profiles of in vitro and in vivo samples. The aim of this study was to establish a comprehensive method to find metabolites using our system. Tolbutamide, which is known as a traditional sulfonylurea oral hypoglycemic drug, was selected as a model substrate. In the urine, feces and bile samples, almost all metabolites reported before, e.g., p-hydroxymethyl-, p-carboxy-tolbutamide, p-tolyl sulfonylurea and p-tolyl sulfonylamide were identified. Additionally, some new metabolites were also identified, e.g., S-(p-tolylcarbamoyl) glutathione.
We will illustrate the utility of our approach in metabolites identification in several different matrices. Sensitivity and accuracy in the present system will also be discussed.

EVALUATION FOR SAFETY TESTING OF DRUG METABOLITES WITH LC-MSn SYSTEM USING RADIOISOTOPE (3)
NEW STRATEGY TO QUANTIFY THE RELATIVE CONCENTRATION OF UNKNOWN METABOLITES TO UNCHANGED
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FDA issued the Guidance of Safety Testing of Drug Metabolites (STDM) in February 2008. This guidance may request to evaluate whether metabolites either are identified only in humans or are present at disproportionately higher levels in humans than in any of the animal species used during standard nonclinical toxicology testing.
Radioisotope is useful for quantification of unknown metabolite without standard samples, although the sensitivity is too low to qualify for clinical testing like microdosing. We investigate the relative concentration of unknown metabolites to unchanged by both high sensitive LC-MS/MS and radioactivity, after administration of 14C-Tolbutamide (1mg/kg) to male rats. Plasma was collected at 1, 2, 4, 8 and 24 and urine was collected for 0-24 hours after dosing. The concentration ratio of major hydroxyl metabolites to unchanged tolbutamide in plasma was less than 2% at every time points by both LC-MS/MS and radio activity, which cleared the 10% rule recommended by the Guidance of STDM about the AUC ratio of metabolites to unchanged.
We searched the metabolites comprehensively using the high sensitive and high resolution MS (LTQ Orbitrap XL), and found a new hydroxyl metabolite of tolbutamide in both plasma and urine.
We will show the various methodology to characterize the metabolites comprehensively, and new strategy of STDM to quantify the relative concentration of unknown metabolites to unchanged.