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ANALYSIS OF IN-VIVO SAMPLES FOR METABOLITE IDENTIFICATION WITH A MODIFIED Q-TOF MASS SPECTROMETER WITH ENHANCED DYNAMIC RANGE AND SPECTRAL RESOLUTION
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[Purpose] With the current publication from the FDA on the guidance of metabolites in safety testing (MIST), there is added emphasis on the need to have adequate LC/MS platforms to be able to accommodate these new challenges. In order to address these challenges, we will present an LC/MS workflow that consisted of a modified QToF mass spectrometer (SYNAPT G2 HDMS) with enhanced dynamic range and improved spectral resolution. [Methods] Rat plasma, bile and urine samples taken at different time points after administration of Ritonavir were used for these analyses. UPLC was used for chromatographic separation coupled with an MS2 strategy that consist low and high collision energy function to obtain the all precursor and fragment ion information during a single LC injection. Data was automatically processed with chemical intelligent application software (MetaboLynx XS). [Results and Discussion] By this approach, the results revealed all metabolites of Ritonavir in the three different biological matrices. The MS2 strategy allowed for an easy –to-review data analysis of fragment ions and corresponding alignment with the low energy data so that structural information could be derived from a single injection. During data processing, the software first identifies the major dealkylated fragments from the structure of the parent drug and uses this information to generate mass defect filters to eliminate false positives. [Conclusions] Combination of UPLC, MS2 strategy and chemical intelligent data processing software increase throughput and exhaustiveness of metabolite identification.

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PLASMA AND URINE PROFILING OF INTACT ISOFlavONE METABOLITES
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[Purpose] A number of epidemiological studies and animal experiments have indicated that the isoflavones, daidzein (Dein) and genistein (Gein), could be benefit to prevent of hormone-dependent cancer such as breast cancer and prostate cancer, and osteoporosis. In the body, isoflavones mainly are present as the phase II metabolites such as glucuronides and sulfates, and intact aglycones are also detected in small proportions. Recently, it has been reported that several conjugates as well as aglycones may work as the biological active compounds or the sources at the target cells. It is also possible that the overall activity of the conjugated compounds in the cells should result from the enzymatic hydrolysis of the conjugates to its aglycones by tissue sulfatase and β-glucuronidase. The objective of this study is to clarify the disposition of aglycones and their conjugated metabolites in humans.
[Methods] After two healthy volunteers (1 male and 1 female) orally received 50 g of kinako, blood and urine were collected. Sixteen isoflavone metabolites in plasma or urine were simultaneously measured by the HPLC-UV-DAD (diode-array detector) method combined with solid-phase extraction using an Oasis HLB cartridge.
[Results and Discussion] Dein-7-glucuronide-4'-sulfate, Gein-7-glucuronide-4'-sulfate, and Gein-7,4'-diglucuronide were identified as the main metabolites of Dein and Gein in plasma, while the major urinary metabolites were their monoglucuronides. Only trace amounts of sulfoglucuronides of Dein and Gein are detected in urine.
[Conclusions] The plasma and urine profiles of 16 metabolites of daidzein and genistein demonstrate the involvement of desulfation or deglucuronidation of the conjugated metabolites in the process of renal excretion.