Determination of ibotenic acid and muscimol in *Amanita* mushrooms and rat plasma by HPLC-UV and LC/MS

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AIMS: *Amanita muscaria* and *Amanita pantherina* are toxic mushrooms containing ibotenic acid (IBO) and muscimol (MUS) as the active constituents. In recent years, it has been reported that young persons intentionally ate *Amanita* mushrooms to evoke hallucination in several countries. We developed analytical methods for identification and quantification of IBO and MUS in Amanita mushrooms. Moreover, we developed a quantitative method for them in rat plasma.

METHODS: *Amanita mushrooms:* IBO and MUS were extracted from dried mushrooms (50 mg) with aqueous methanol and derivatized with dansyl chloride (DNS-Cl). After extraction with ethyl acetate and evaporation of the solvent, the residues were ethylated with 1.25 M hydrogen chloride in ethanol. The resulting derivatives were identified by LC-MS/MS using an ESI source in the positive mode and quantified by HPLC-UV. Calibration curves were constructed using IBO/MUS spiked blank mushrooms (Pleurotus ostreatus) extract by an external standard method. For MS/MS analysis, m/z 419 and 347 were selected as precursor ions for DNS-IBO ethyl ester and DNS-MUS, respectively.

Rat plasma: Rat plasma (0.1 ml) spiked with IBO, MUS and internal standard (diazepam) was deproteinized using methanol and derivatized with dansyl chloride. After extraction with ethyl acetate and evaporation of the solvent, the resulting derivatives were quantified by LC-MS under the SIM mode. DNS-IBO, DNS-MUS and IS were quantified by monitoring ions of m/z 391, 347 and 285, respectively.

RESULTS AND DISCUSSION: *Amanita mushrooms:* Identification of IBO and MUS were performed by monitoring of the following product ions: m/z 355, 235, 183 (DNS-IBO ethyl ester) and m/z 317, 276, 226, 183 (DNS-MUS). The recoveries of IBO/MUS from mushrooms by aqueous methanol extraction were more than 98%. Calibration curves were linear in the range of 40-2500 ppm (IBO) and 25-2500 ppm (MUS). Under the UV detection, LOD/LOQ of MUS and IBO were 1.4 ppm/4.6 ppm and 7.8 ppm/25.9 ppm, respectively. The presicion and accuracy (for the intra- and inter-assay) were 1.8~8.7% and -5.0~11.1% at three concentrations for two analytes, respectively. IBO/MUS contents in the caps of Amanita mushrooms were in the ranges of ND-1277 ppm/40-1318 ppm for samples circulated in the drug market and 182-1839 ppm/46-1203 ppm for samples naturally grown, respectively.

Rat plasma: The recoveries from rat plasma by methanol deproteination using were 83.5% (IBO) and 91.4% (MUS). Calibration curves were linear in the range of 25-1000 ng/ml (IBO) and 5-500 ng/ml (MUS). LOQ was 25 ng/ml (IBO) and 5 ng/ml (MUS). The precision and accuracy (for the intra- and inter-assay) were 4.2~11.7% and -8.1~15.6% at four concentrations for two analytes, respectively. This method was successfully applied to pharmacokinetic study of MUS in a rat following a single 2 mg/kg oral dose of MUS.

KEYWORDS: *Amanita muscaria, Amanita pantherina, Rat plasma, Ibotenic acid, Muscimol, Dansylation, HPLC-UV, LC/MS*

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