Simultaneous Determination of Amphetamine Related Compounds in Human Plasma by HPLC with Peroxyoxalate Chemiluminescence Detection

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AIMS: Abuse of amphetamine related compounds (APs) such as methamphetamine (MP), amphetamine (AP), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA) and p-methoxymethamphetamine (PMMA) has been spreading and causing serious social problems worldwide. Recently, it was found that illegal MDMA tablets contain several abused drugs, and also multi-drug use become popular. Thus the adverse reactions may be caused due to the unexpected interaction among the concomitant drugs. To protect human health from these risks, a sensitive and selective analytical method for simultaneous determination of drugs of abuse is requisite.

In this study, an HPLC-peroxyoxalate chemiluminescence (PO-CL) detection method for above APs including hydroxylated metabolites, p-hydroxymethamphetamine (p-HMP) and p-hydroxyamphetamine (p-HAP), in human plasma was examined. Sensitive determination of APs could be achieved by a fluorescence labeling with 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F).

METHODS: APs in human plasma were extracted with ethyl acetate. The extracts were evaporated under nitrogen gas, and labeled with 20 mM DBD-F in borate buffer (pH 8.5) for 20 min at 80°C. The DBD-derivatives were isocratically separated within 45 min by an ODS column (150 x 4.6 mm, 3 μm) with a mixture of 10 mM imidazole-HNO₃ buffer (pH 6.5) : acetonitrile : tetrahydrofuran (52:44:4, v/v/v%) at a flow rate of 0.7 mL/min. The post-column PO-CL reagent solution use was a mixture of 2 mM bis (2,4,5-trichloro- 6-carbopentoxyphenyl) oxalate and 15 mM hydrogen peroxide in acetonitrile.

RESULTS: The calibration curves of APs and hydroxylated metabolites were linear with more than 0.995 of correlation coefficients. Detection limits (S/N=3) of MDMA, MDA, MP, AP, PMMA, p-HMP and p-HAP were 0.10, 0.75, 0.08, 1.10, 0.06, 0.14 and 0.75 ng/mL, respectively.

CONCLUSIONS: The proposed method is sensitive enough to determine lower concentrations of APs in human plasma, and thus it might be applicable for their forensic or toxicological study.

Keywords: Amphetamines, HPLC-peroxyoxalate chemiluminescence, DBD-F