Effectiveness of Multiple Internal Standards: Deuterated Analogues of Methylenedioxymethamphetamine, Methylenedioxyamphetamine, Methamphetamine, and Amphetamine

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Abstract

With the increasing abuse of methylenedioxymethamphetamine (MDMA) thereby requiring analysis, we have undertaken a systematic evaluation on parameters associated with the analysis of MDMA and related compounds, including methylenedioxyamphetamine (MDA), methamphetamine (MA), and amphetamine (AM). Parameters studied included three solid-phase adsorbents, five derivatization reagents, and four deuterated internal standards (IS). This report examines whether differences in quantitation data derived from the use of four ISs (one for each analyte) and two ISs (one for AM and MA, one for MDA and MDMA) are statistically significant. Two types of samples were included in this study. The first type (Type I) included four replicate sets of standard solutions prepared in urine matrix. All analytes (AM, MA, MDA, and MDMA) were included in all samples, and these analytes' concentrations in each set were at five levels (100, 250, 500, 1000, and 2000 ng/mL). Four deuterated analogues (MA-d_8, AM-d_8, MDMA-d_5, and MDA-d_5) at 500 ng/mL were also included in all solutions. The second type of samples (Type II) included 25 case urine specimens. Most of these specimens contained MA/AM and/or MDMA/MDA. The specific objective of this study is to determine whether the 4-IS approach can indeed generate better quantitative data than a less-costly 2-IS. For Type I samples, where the true concentrations of the analytes are known, two-sample *t*-test is adapted to examine whether the two sets of prediction errors (i.e., known concentration minus calculated concentration) resulting from the 4-IS and the 2-IS approaches are statistically different. For Type II samples, where the analytes' true concentrations are unknown, one-sample *t*-test was adapted to determine whether the difference of the quantitation results derived from the 4-IS and the 2-IS approaches is statistically significant. Statistical analysis of quantitation data derived from Types I and II samples indicates that differences in MDA and MDMA concentrations resulting from the use of one (MDA-d_5 or MDMA-d_5) or two (MDA-d_5 and MDMA-d_5) are statistically nonsignificant. On the other hand, similar analysis on data derived from Type I samples indicate the use of the analytes' respective deuterated analogues as the ISs appear to generate better quantitative data for AM and MA.

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Introduction

With the increase in the abuse of methylenemethamphetamine (MDMA, ecstasy) and the resultant analysis requirements, we have undertaken a systematic evaluation on parameters associated with the analysis of MDMA and related compounds, including methylenedioxyamphetamine (MDA), methamphetamine (MA), and amphetamine (AM). Parameters studied included liquid–liquid and solid-phase (three adsorbents) extraction, five derivatization groups, and four deuterated internal standards (ISs). This report examines whether differences in quantitation data derived from the use of four ISs (one for each analyte) and two ISs (one for AM and MA, one for MDA and MDMA) are statistically significant.

Internal standard method using isotopic analogues (mainly deuterated) of the analytes in conjunction with selected ion monitoring (SIM) gas chromatography–mass spectrometry (GC–MS) protocol are routinely used for quantitative analysis of drugs and their metabolites in biological matrices (1,2). For multi-component analysis, multiple ISs (one deuterated analogue for each analyte) are commonly adapted in the analytical protocol. In an earlier study (3), in which pentobarbital-d_5 was used as the sole IS using one-point calibration approach for the quantitation of four barbiturates (butabital, amobarbital, pentobarbital, and secobarbital), we have observed that quantitation results for pentobarbital were not necessarily better than those for the other analytes.