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CONFERENCE

57th ASMS Conference on Mass Spectrometry

TITLE

Determination of Acetylsalicylic Acid and Salicylic Acid in Human Plasma by LC-MS/MS

AUTHORS

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ABSTRACT

Novel Aspect: A very challenging assay for two polar analytes having cross-talk issue and instability in plasma

Introduction

Acetylsalicylic acid (Aspirin, ASA) is one of the most frequently used drugs in the treatment of mild to moderate pain, including that of migraines and fever. ASA also has an antiplatelet, or "anti-clotting", effect and is used in long-term, low doses to prevent heart attacks, strokes and blood clot formation in people at high risk for developing blood clots. ASA can be easily hydrolyzed to salicylic acid (SA) in human plasma. “Cross-talk” was observed resulting from in-source fragmentation. The stability of ASA and HPLC separation between ASA and SA pose a great challenge to develop a chromatographic assay. We report here the development and validation of an LC/MS/MS assay for acetylsalicylic acid and salicylic acid in human Plasma.

Method

Thirty mL of fortified human plasma samples (human plasma: [100mM PMSF:Acetic Acid/50:50 (v:v)]/1000:20 [v:v]) were spiked with internal standard and immediately processed by protein precipitation extraction with 240 mL of acetonitrile. One hundred mL of supernatant was transferred and mixed with 200 mL of solvent (acetonitrile:water:formic acid/25:75:0.15) for LC/MS/MS analysis. The extracted samples of ASA, SA and their internal standards were delivered by Shimadzu pump and CTC autosampler through a LC program with a flow rate of 0.4 mL/minute, and detected on a Sciex API 4000 tandem mass spectrometer in negative TurboIonSpray mode.

Preliminary results

Because ASA can easily convert to SA in biological matrix, the plasma samples were fortified with PMSA and acetic acid to stabilize the ASA. A protein precipitation method was developed to obtain consistent extraction recovery. Through a reverse-phase HPLC column and binary mobile phases, ASA and SA were successfully separated with retention times of 1 min difference, to avoid the effects of mass spec in-source conversion and cross-talking. The total run time was ~6 minutes. The precursor/product ion transitions under multiple reaction monitoring (MRM) were: 179.0/136.9 (for ASA), and 136.9/93.0 (for SA). The LC/MS/MS assay was validated over a range of 20 to 10,000 ng/mL for ASA, 100 to 50,000 ng/mL for SA in human plasma using 30 µL sample volume. Quality control samples at 4 concentrations were used to determine precision and accuracy. Intra-assay precision ranged from 10.1 to 2.8% for ASA, 0.9 to 9.6% for SA, while Inter-assay precision ranged from 4.7 to 8.9 % for ASA, 3.7 to 8.5% for SA. The Intra-assay accuracy (defined as % error from nominal) ranged from -10.7 to 6.3% for ASA, -9.8 to 2.2% for SA, and the Inter-assay accuracy ranged from -7.1 to 2.3% for ASA, -6.8 to -1.6% for SA. The analytes are stable in fortified plasma for 8 hours on ice bath, for at least 39 days of storage at -70°C and -20°C and after 4 freeze/thaw cycles. The analytes are also stable in processed sample for 189 hours at 4°C. In addition, the selectivity, dilution reproducibility, matrix effect and recovery were also demonstrated. The assay was shown to be rugged, sensitive, selective, accurate and reproducible. It

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was successfully applied to support a bioequivalence study.