K6 Quantitative Determination of SSRI Drugs and Metabolites in Human Plasma by SPE-LC-MS/MS

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After attending this presentation, attendees will obtain valuable information about an improved, accurate, sensititve, and specific method for the quantitative analysis of the non-tricyclic class of anti-depressant drugs in human biological samples obtained from suicide decedents.

The presentation will impact the forensic community by significantly advancing our knowledge regarding the prior use of SSRI drugs in suicide decedents. This information will be a critical element in building a community-based treatment approach to preventing suicides.

Selective serotonin re-uptake inhibitors (SSRIs), a class of non- tricyclic antidepressants, are marketed as safe and effective in treating depression, anxiety disorders, and some personality disorders. Although, questions related to their safety were raised, with studies reporting a possible association with suicidal tendencies, inferences regarding the validity and strength of such an association have been divergent. The goal of this study was to develop a rapid and sensitive HPLC- MS/MS/ESI method for simultaneous determination and screening of the most commonly prescribed SSRIs in human plasma samples from suicide decedents.

A solid phase extraction (SPE) method coupled to LC-MS/MS was developed for the simultaneous analysis of 5 SSRIs, Fluoxetine (Fluox), Paroxetine (Parox), Fluvoxamine (Fluvox), Sertraline (Sert), and Citalopram (Citalo), and three of their pharmacologically active N- demethylated metabolites, Norfluoxetine (Norfluox), Norsertraline (Norsert), and N-desmethylcitalopram (Descitalo), using Waters Oasis HLB SPE cartridges. Stock solutions of the individual drugs, as well as the internal standards (I.S.), Fluox-d_6, Norfluox-d_6, Parox-d_6, Sert-d_3, Norsert-d_4, and Citalo-d_6, for calibration standards and QC were prepared in MeOH and stored at -20°C.

An LC system consisting of Agilent HP 1100 series and a Thermo/Finnigan Quest TSQ triple-stage quadrupole MS, equipped with Xcalibur (v 1.1) operating software was used for data analysis. Ionization was achieved using electrospray in the positive ionization mode. Chromatographic separation of all the compounds was achieved within 15 mins using a Waters YMC ODS-AQ C18 (150×2 mm, 3 µm) analytical column, and a mobile phase gradient consisting of 0.1% formic acid in water and MeOH at 10%, 30%, 40%, and 10% for 1, 1, 4, and 9 min, respectively. Identification and quantification were based on selected reaction monitoring.

Fluox, Parox, Fluvox, Sert, Citalo, Norfluox, Norsert, Descitalo, Fluox-d_6, Norfluox-d_6, Parox-d_6, Sert-d_3, Norsert-d_4, and Citalo-d_6 were detected by measuring transitions of m/z 310→ 148, m/z 330→ 192, m/z 319→ 200, m/z 306→ 159, m/z 325→ 262, m/z 296→ 134, m/z 292→ 159, m/z 311→ 262, m/z 316→ 154, m/z 302→ 140, m/z 336→ 198, m/z 309→ 159, m/z 296→ 160, and m/z 331→ 262, respectively. To evaluate linearity, three calibration curves over a concentration range of 1–1000 ng/mL for each of the compounds, were tested separately. A $1/X^2$ weighted quadratic curve was used for quantification. The method was fully validated, including inter- and intra-run accuracy (within 15% of target concentration) and precision (CVs <15%) for QC samples at 5, 50 and 300 ng/mL. The mean recovery for all SSRI drugs ranged from 32-74%. Stability testing showed no evidence of degradation in processed plasma samples during 3 successive freeze/thaw cycles or after storage at -20°C for at least four weeks or at 4°C after at least 24-48 hrs.

The method described herein is accurate, sensitive, highly specific, and can be used for routine therapeutic drug monitoring, toxicological screening, as well as for the study of the pharmacokinetics and metabolism of the SSRI drugs in biological specimens from normal subjects, as well as from suicide decedents.

Anti-Depressants, SSRI Drugs, LC-MS/MS

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