The Comparative Analysis of Risperidone and it 9-hydroxy-metabolite using High Resolution Quadrupole Mass Spectrometry with Accurate Mass and Time-of-Flight Mass Spectrometry.

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Introduction: Risperidone and 9-hydroxyrisperidone are antipsychotic agents currently in use in the treatment of numerous disorders such as schizophrenia, attention deficit disorder and autism. The activity of these drugs involve dopamine and serotonin antagonism. The major metabolite, 9-hydroxyrisperidone, is mediated by CYP-450 enzyme isoforms CYP2D6 and CYP3A4. Various quantitative and qualitative studies have been developed for these analytes including LC/UV, GC/ECD, electrochemical detection and LC/MS/MS. Time of flight (TOF) mass spectrometry is currently the method of choice for the determination of structural information and empirical formula. Presently, to our knowledge there are no reports on the accurate mass determination of these analytes specifically determined by high resolution, triple stage quadrupole mass spectrometry. The present study compares and contrasts these two techniques and highlights potential benefits using a triple stage quadrupole MS capable of high resolution, accurate mass in determination for metabolite identification.

Experimental:

Sample Preparation. Risperidone (25 µM) was incubated with human liver microsomes (20mg/mL protein, 0 and 60 min). The quenched incubations consisted of equal volumes of drug solution and cell suspension stopped with methanol which were vortexed and centrifuged (13,000 rpm, 15 min, 5°C). Heat inactivated microsomes were used as blanks. 200µL of supernatant was transferred to HPLC vials.

LC Analytical. Injections (10 µL) were first loaded at 1 ml/min for 1.0 min on a Zorbax SB-C18 (4.6 x 12.5mm, 5µm d_p) guard column with a mobile phase of 5/95 acetonitrile/0.2% acetic acid in 10mM NH_4OAc (pH 3.5). An Agilent HP1090 loading pump, a Waters Surveyor HPLC system and a Valco 6-port switch valve were used. To chromatographically resolve the analytes, gradient elution (5 to 40% acetonitrile, 9 min, hold at 40%, 1 min; 40 to 90%, 0.5 min, hold 90%, 1.5 min, re-equilibrate 1.5 min) on a Zorbax SB-C18 analytical column min was used. Total runtime was 15 min. For the accurate mass determination by infusion, fractions were collected at 0.5 min intervals pooling replicates.

MS/MS. A Finnigan Quantum AM triple quadrupole mass spectrometer and a Waters MicroMass Q-ToF 1 time of flight mass spectrometer equipped with electrospray sources were operated in positive ion mode. MS Parameters (1) Quantum AM; Q1 full scan MS (*m/z* 150-700), Q1 and Q3 Instrument and High Resolution calibration was performed by infusion usinf a solution containg polytyrosine 1,3,6. Accurate mass was calibrated with 50 pmol/mL PEG. Q1 SIM accurate mass spectra was obtained by infusion with a scan rate of 10 µ/sec. Bufuralol ($C_{16}H_{23}NO_2H^+$) (*m/z* 262.1802) and Riserpine ($C_{33}H_{40}N_2O_9H^+$) (*m/z* 609.2807) were used as lock masses. Capillary temperature 325°C, Sheath gas 25 psi, Tube Lens 142 V, L0 –1.3 V. (2) Q-ToF 1;

calibrated with a solution containing sodium iodide (2 µg/mL) at a rate of 1 µL/min over the mass range 100 to 1000 u. Des gas 300, Des Temp 20 C, cone 30, Rf Lens 0.90, Extractor 0.

Results: Risperidone (RIS), the major 9-hydroxy risperidone (M1) metabolite and a second hydroxy metabolite (M2) formed in human liver microsomes incubations were chromatographically resolved in under 10 min. The empirical formula and accurate mass for the protonated molecules RIS ($C_{23}H_{27}FN_4O_2H^+$) (411.2203) (2.92 ppm) and M1 ($C_{23}H_{27}FN_4O_3H^+$) (427.2153) (3.04 ppm) were determined directly from the chromatographiclly separated incubations. Accurate mass confirmation for RIS, M1 and M2 ($C_{23}H_{27}FN_4O_3H^+$) (427.2156) (3.74 ppm) was determined by infusion of the separate fractions. The data compares favorably with those results obtained on the Q-ToF, RIS (411.2192) (0.2 ppm), M1 (427.2147) (1.60 ppm), M2 (427.2137) (0.70 ppm).

Conclusion: Mass measurement errors of within 5 ppm of calculated can be achieved 'on the fly' under gradient elution conditions using quadrupole MS. The formula and mass assignment for the $[M+H]^+$ of Risperidone ($C_{23}H_{27}FN_4O_2H^+$) (411.2191) and its 9-OH metabolite ($C_{23}H_{27}FN_4O_3H^+$) were determined. The mass assignment for a second hydroxy metabolite (M2) was within 5 ppm. Spectra acquired within a narrow mass window (SIM mode) using a quadrupole MS achieved error limits that were within the range of those achieved by quadrupole-TOF instrumentation. Instrument calibration for high resolution and accurate mass in simple and stable. The on-line column switching approach resulted in maximum sample pre-concentration and minimal sample preparation.

Q1 MS SIM scan accurate mass mode mass chromatogram and mass spectrum for RIS, 9OH-RIS (M1) and M2