Cyanide-trapped reactive metabolite screening: QTrap vs QTOF

Joanna E. Barbara*, Seema Muranjan, Phyllis Yerino, Mark J. Horrigan, Paul C. Toren and Andrew Parkinson
XenoTech LLC, 16835 W. 116th St., Lenexa, KS 66219, USA

Triple quadrupole linear ion trap (QTrap) detection (AB Sciex 4000QTrap), with and without ion trapping, and quadrupole time-of-flight (QTOF) detection (Waters Synapt G2) were directly compared for screening iminium and nitrenium ion reactive metabolites. Eight compounds incubated with NADPH- and NaCN-fortified human liver microsomes were analyzed by UHPLC/MS/MS with generic LC conditions and electrospray ionization parameters. For QTrap analysis, a neutral loss 27 (NL27) scan alone (QqQ mode) or coupled with an enhanced resolution scan and information-dependent acquisition of product ion spectra (QTrap IDA) mode) was employed. For QTOF analysis, the MS^E wide-band transmission scan type was employed for data-independent acquisition of precursor and product ion spectra. All conjugates detected with QTrap IDA mode were also observed with QqQ mode with the exception of two prochlorperazine (PR) conjugates associated with weak signals. In addition to the benefit of acquiring product ion spectra for detected components without reinjection, employing the ion trap function yielded ~50% increased sensitivity for some detected conjugates. Unexpectedly, several conjugates were detected with lower signal intensity in QTrap IDA mode. QTrap and QTOF-detected conjugates were consistent for six test compounds. However multiple nefazodone (NE) and PR conjugates detected by the QTrap with weak signal intensity were not detected by the QTOF. Fewer than 50% of the NE and PR conjugates were detected by the QTOF in MS^E mode. As expected, TOF detection was not optimal for detection of cyanide conjugates in terms of sensitivity. However, the accurate mass data proved invaluable for determining that multiple detected conjugates were not truly reactive metabolites but metabonates associated with methylenation of primary and secondary free amine groups by formaldehyde inherent to the incubation matrix. We have observed and reported this for experiments with diltiazem, but the phenomenon was not known to extend to multiple substrates. Conjugates associated with a nominal mass shift of +39 (+14+25) amu from the parent drug may commonly be misinterpreted as cyanide trapped carbonyl or nitroso compounds. Therefore, despite the lower sensitivity of the QTOF instrument, the data support the need for accurate mass analysis in reactive iminium and nitrenium ion metabolite screening.