Detection and Quantitation of Exemestane, Letrozole and Anastrozole in Human Serum by LC-MS/MS and Atmospheric Pressure Chemical Ionization

Julia D. Addiss, Mildred M. Redor-Goldman, Scott M. Goldman, Nigel J. Clarke, Ph.D., Research and Development, Mass Spectrometry Department

Quest Diagnostics Inc., 33608 Ortega Hwy, San Juan Capistrano, CA 92675

Carcinomas that express estrogen receptors are sensitive to the proliferation of estrogens, whose biosynthesis is catalyzed by the aromatase enzyme. An effective therapeutic option for the treatment of these estrogen-responsive cancers in postmenopausal women involves the use of aromatase inhibitors such as exemestane, letrozole, and anastrozole. Exemestane is a steroidal irreversible inhibitor, whereas letrozole and anastrozole are non-steroidal competitive inhibitors. These selective drugs interfere in the synthesis of estrogen in peripheral tissues by inactivating aromatase. They do so by binding to the active site of the enzyme involved in the conversion of androgens to estrogens. The resulting decrease in circulating estrogen levels can slow, and even stop, the growth of breast cancer cells. Monitoring the serum concentrations of exemestane, letrozole, and anastrozole can help determine the patient's adherence to the drugs, as well as provide useful information regarding concentration-dependent side effects and therapeutic efficacy.

Measurement of exemestane, letrozole, and anastrozole was completed using an on-line extraction procedure with high-turbulent flow liquid chromatography and detection by tandem mass spectrometry. A minimum sample volume of 100 µL was utilized, spiked with internal standard, and diluted. After mixing, the serum samples were extracted on-line before separation by HPLC using a reverse-phase column on an Aria TLX4 HTLC system from ThermoFisher. Analysis was accomplished by positive APCI MRM mode on a triple quadrupole tandem Ultra mass spectrometer from ThermoFisher. Total run time was 5.4 minutes per sample with a one minute collection window permitting the use of four channels, resulting in a high throughput assay.

The calibration curve showed consistency in reproducibility and linearity. The method gave linear results over a range of 1-200 ng/mL for exemestane and 5-500 ng/mL for letrozole and anastrozole. The lower limits of quantitation were 0.4 ng/mL for exemestane, 0.8 ng/mL for anastrozole, and 2.2 ng/mL for letrozole. The inter- and intra-assay coefficients of variation for this assay were 2.1% to 5.9% at 15-80 ng/mL for exemestane, 2.9% to 5% at 10-300 ng/mL for anastrozole, and 2.9% to 5.6% at 25-300 ng/mL for letrozole.

Conclusion:

Monitoring the serum concentrations of these drugs in women with estrogen-responsive breast cancer allows for better dosing and determination of efficacy. This selective and sensitive assay provides a means of doing so with quick turn-around time and minimal sample preparation that can easily be automated if necessary.