The benefits and pitfalls of using MRM^3 detection for the analysis of plasma free metanephrines by liquid chromatography-tandem mass spectrometry

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Abstract

Background:

Liquid chromatography coupled to tandem mass spectrometry using multiple reaction monitoring (MRM) is a powerful tool for the quantitation of target analytes in complex matrices. However, this technique lacks specificity when plasma free metanephrines are measured. In this presentation we demonstrate the use of multistage fragmentation (MRM^3) to improve the analytical selectivity of plasma free metanephrine service whilst also outlining other considerations that need to be taken into account before introducing this technology to a routine clinical chemistry laboratory.

Methods:

The metanephrines were extracted from plasma using weak cation exchange solid phase extraction before being separated by hydrophilic interaction liquid chromatography. Normetanephrine and metanephrine were quantitated by either MRM transitions or by MRM3 transitions m/z 166→134→79 and m/z 180→149→121, respectively.

Results:

Over a six month period, approximately 1% of patient samples (n=21) presented with uncharacterised co-eluting substances that interfered with the routine assay, resulting in an inability to report results. Quantitation using MRM^3 removed these interferences and enabled measurement of the target compounds.

Deming regression analysis demonstrated a good correlation between MRM^3 and MRM methods when using patient samples unaffected by interferences (y=1.00x -0.00nmol/L for normetanephrine and y=0.99x +0.03nmol/L for metanephrine) and between the MRM^3 method and the median of all liquid chromatography tandem mass spectrometry laboratories enrolled in a quality assurance program (y=0.97x +0.03nmol/L for normetanephrine and y=1.03x –0.04nmol/L for metanephrine).

In addition to an increased requirement for preventative maintenance, other drawbacks of using MRM^3 detection included an increase of imprecision and a decrease in sensitivity compared to MRM detection. However, imprecision for the MRM^3 method was within acceptable limits (CV=6.2-7.0% for normetanephrine and 6.1-9.9% for metanephrine; n=10) and the lower limits of quantitation (0.20nmol/L for normetanephrine and 0.16nmol/L for metanephrine) were lower than the upper reference intervals for normal healthy patients and thus sufficient for clinical diagnostic analysis.

Conclusions:

The use of MRM^3 technology improves the analytical selectivity of plasma free metanephrine quantitation by liquid chromatography tandem mass spectrometry whilst demonstrating sufficient levels of analytical sensitivity and precision to provide a robust clinical diagnostic service.