Increasing the selectivity of clenbuterol detection in urine samples by using LC/MS/MS in MRM3 mode

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The selectivity of MSMS in residue analysis has enabled users to decrease significantly the analysis time. In the majority of cases, single chromatographic peaks will be observed with triple quadrupole operated a unit resolution. However, as is the case with clenbuterol urine samples, the presence of endogenous species leads to interferences which negatively affect the LOQ. To improve selectivity, several solutions have been proposed; 1) improve chromatographic separation, 2) increase resolution to improve parent mass selection, and 3) using LC-FAIMS-MSMS. Here we propose an alternative to improve specificity in quantitative applications; using MRM3 on a hybrid quadrupole-linear ion trap (QqLIT). The concept is to rely on the selectivity of the fragmentation pathway. Clenbuterol generates 3 majors fragments that can be monitored in quantitative analysis; 203, 168 and 132. However, in the majority of the urine samples analyzed in this study, interferences were observed in each one of the MRM transitions. However, fragment 168 and 132 are both formed via fragment ion 203 as an intermediate. Therefore MS3 was used to increase the selectivity of clenbuterol detection with MS3 without changes in the LC condition or addition of hardware to the LC-MSMS system. The detection limit was improved by 4x and single LC peaks were detected (improved selectivity). Details on the operational consideration for such analysis as well as validation results for this study will be presented.

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