

Validation of a Method for Full Scan GC/MS Confirmation of Residues of Clenbuterol in Human Urine

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Mass spectrometry is a powerful and widely used technique for confirming the identity of target analytes in the presence of interferences in complex biological and environmental matrices. The detection of characteristic fragment ions in appropriate ratios and eluting at the correct retention time provides a high degree of certainty as to the compound responsible for an observed response. It is essential to minimise the uncertainty of identification when performing analyses that will have legal or financial ramifications, such as when testing for evidence of doping in sport or detecting residues of banned veterinary drugs in primary produce.

Criteria are well established for identity confirmation in residue analysis when using selected ion monitoring techniques and validation of such a method's fitness for purpose is relatively straightforward. However, identity confirmation using full scan spectra is highly desirable in some cases because this provides a continuum of data and many more comparison points. A comparison of full scan mass spectra can be easier to comprehend for reviewers with little experience in the field – for example in court. Unfortunately, operation in full scan mode leads to reduced sensitivity and increased problems with matrix interference. In many cases an additional more selective clean-up will be needed if confirmation by full scan MS is required. This is frequently a problem in analysis of urine samples for the β -agonist clenbuterol. Triclosan, a commonly-used chlorinated anti-bacterial is often present at high levels in samples and is not effectively removed in the clean-up provided by the general screening procedure for anabolic agents. Although this and other matrix interferences do not prevent detection of clenbuterol at sub-ppb levels by GC/HRMS in selected ion mode, the extracts are not clean enough to provide effective full scan confirmation at the necessary level of 2 ng/mL.

This presentation will outline an approach to verifying the ability of a newly developed selective clean-up to provide clean full scan mass spectra for identity confirmation of clenbuterol at sufficiently low concentrations for effective doping control. The validation was performed by determining the size of background peaks arising from interfering compounds at the same retention time as clenbuterol bis-TMS in a range of blank urine extracts. Criteria for two types of interference relevant to full scan confirmation were considered. To ensure ion ratios were acceptable, background peaks with the same unit m/z ratio as those of the analyte would be required to be less than 20% of the height of the relevant clenbuterol fragment at the lowest confirmable concentration. At the same time, peaks at other m/z would need to be less than 50% of the height of the base peak of the clenbuterol if a recognisable mass spectrum was to be obtained at the method confirmation limit. The data from analysis of blank urine samples was used to determine the lowest concentration of clenbuterol that could be confirmed with 95% confidence under these criteria.
