THE USE OF COLLISION ENERGY DEPENDENT ION RATIO (ColEnDIR) TO SUPPORT IDENTIFICATION

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Colander

ColEnDIR

Introduction

Triple quadrupole mass spectrometers (MS/MS) are heavily used in residue analysis. The parent ion selected in Q1 is fragmented with a specific collision energy (ColEn) in Q2. According to the SANTE guidelines [1], at least two mass-transitions (MRM) and the ratio of their signals (i.e. the ion-ratio) should be evaluated for compound identification. Further criteria include retention time (RT), RT-ratio against the internal standard (IS), peak shape and comparison of the latter parameters with those of the respective isotopically labelled internal standard (ILIS). The ColEn is optimized for each MRM of one compound with the signal intensity decreasing when moving away from the optimized value. Interfering isobaric ions have a different molecular structure and thus typically a different ColEn optimum. When shifting the ColEn, the ion-ratio patterns differ from compound to compound. The use of different ColEn values for one MRM can thus be used to support identification. This concept was recently implemented in the SANTE document [1]. This approach can be helpful in identifying analytes having only one suitable MRM (e.g. for molecules with a low mass, such as trifluoroacetic acid (TFA), or with a chemically resistant molecular structure, such as naphthylacetic acid) or in extreme cases, where no proper MRM is available at all (e.g. thiocyanate). For the latter analytes, a "pseudo MRM" (i.e. parent → parent) may be used setting the ColEn very low (**Fig.1** top-right). Such "pseudo MRMs" lack in specificity and are often interfered. In all these cases, measurement at different ColEn and monitoring the shifts in ion intensity is a suitable work-around for supporting analyte identification. The approach is easily implementable into existing methods.

Experimental

TFA, a compound of high actuality, was used as an example to demonstrate the benefits of this concept. Calibration standards prepared in both pure solvent and matrix were used. The approach was also tested on real samples containing TFA. The measurement system was equipped with a Waters APP column and a Sciex QTrap 5500 MS, see also PD-16 and [2].

Results and discussion

For the MRM 113→69 (*m/z*) a ColEn of -20V resulted in the highest signals. When shifting the ColEn to lower or higher values (i.e. to -5V, -10V, -30V), the signal intensity diminished, resulting in different signal ratios versus the signal at -20V (**Fig.1** and **2**). As interfering matrix compounds typically show different signal shifts and ion ratio patterns than the analytes, this effect can be utilized as an alternative or an additional criterion for identification. The approach is especially interesting for analytes where qualifier MRMs (in the case of TFA: the in-source fragment 69→19 and pseudo-MRM 113→113) show very poor sensitivity thus severely compromising low-level identification according to the SANTE criteria (see **Fig.1**) [1]. In the specific case, one MRM was acquired multiply at different ColEn (**Fig.1**) with the most intensive serving as quantifier and the others as qualifiers. Signal ratios of the resulting signals were calculated (**Fig.2**). The repeatability and reproducibility of the resulting ion ratios of TFA 113→69 was tested in spiked solvent, in spiked tomato extract and in samples with incurred TFA residues, i.e. kiwi fruit and grape (**Fig.2**).

The resulting ion ratios in tomato extract and in the samples with incurred residues corresponded well with the ion ratios in the solvent calibration and were highly reproducible with the relative standard deviations (RSDs) ranging from 0.7% (FV CE -5V/-20V in kiwi) to 4.7% (FV CE -20V/-30V in spiked tomato). **Fig.1** shows exemplarily the evaluation view for TFA that was contained at 0.03 mg/kg in a lemon sample from the local market.

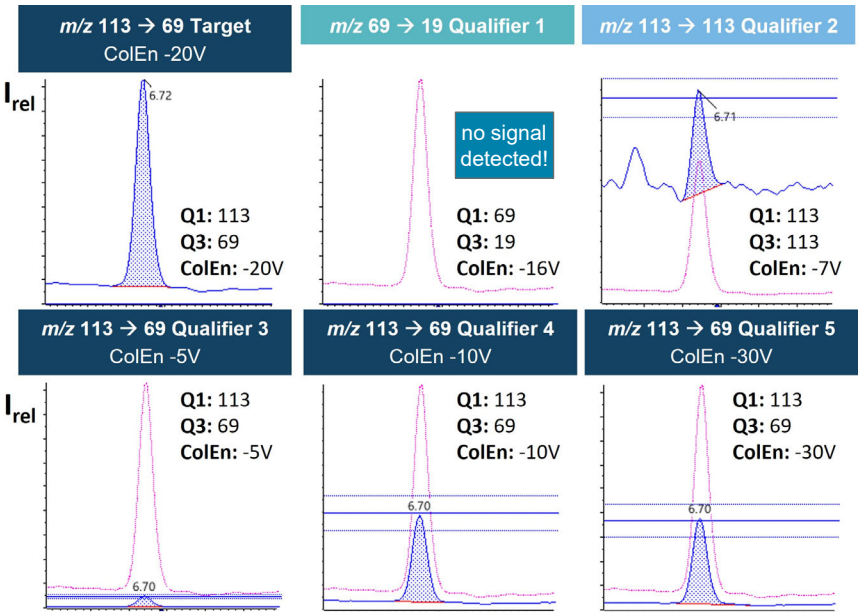

Fig.1: **Top**: Chromatograms of the TFA target MRM (113→69) at the optimum ColEn of -20V and chromatograms of the poorly sensitive qualifier MRMs 69→19 and 113→113. **Bottom**: Chromatograms of TFA for the MRM 113→69 at ColEn deviating from the optimum (i.e. -5V, -10V and -30V). Blue horizontal lines = ion ratio tolerance bands generated by Sciex OS; blue signal line = signal of respective MRM; red signal line = signal of target MRM overlaid. Sample: Lemon sample extract containing 0.03 mg/kg TFA. Measured with an APP column and a QTrap 5500.

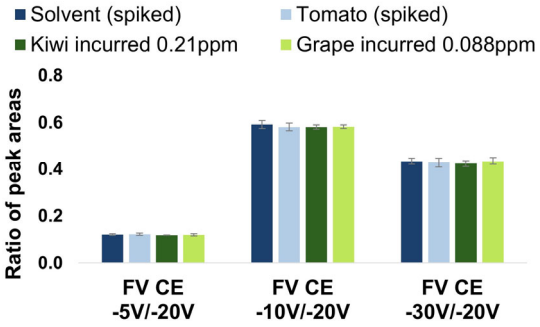

Fig.2: Ion ratios of TFA signals obtained for the MRM 113→69 at different ColEn (ColEn optimum of -20V set as reference) in spiked solvent compared to spiked tomato extract as well as kiwi and grape with incurred residues.

Summary

The measurement of MRMs with varying collision energies (ColEn) was shown to be reproducible in spiked standards and samples with incurred residues and was recently implemented in the quality control guidelines (SANTE v2026) [1]. The intention of the measurement of such MRMs is to complement other identification criteria and to provide a work-around in cases where qualifier MRMs are not sensitive enough. In the particular example it was possible to considerably lower the identification limit (and with it the LOQ) of TFA, as shown in PD-16.

LITERATURE:

[1] Analytical quality control and method validation procedures for pesticide residues analysis in food and feed – SANTE 11312/2021 v2026.

[2] Anastassiades *et al.* QuPPe-PO V12.3.

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