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应用纪要

Application of Intentionally Attenuated MRM Transitions for the Analysis of Natural Products

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates to develop intentionally attenuated MRM transitions in order to allow the quantitative analysis of natural product constituents of disparate abundance within a single acquisition.

Benefits

MRM acquisitions can be tailored to meet the specificity, sensitivity, and dynamic range requirements of natural products.

Introduction

Developing MRM transitions commonly involves fragmenting a precursor ion to determine the most abundant product ion fragments that do not result from non-specific losses such as loss of water or CO_2 from the precursor. However, when using MRM for the quantitative analysis of natural products comprised of components of widely disparate abundance, it can be beneficial to adapt MRM development and implementation in order to better accommodate specificity, sensitivity and dynamic range challenges presented by these samples. In cannabis and hemp, cannabinoids such as cannabidiol and Δ9-THC are often orders of magnitude more abundant than individual terpenoids. Using standard MRM transitions for the terpenoids combined with intentionally attenuated MRM transitions for cannabinoids, specificity is improved for individual cannabinoids and the relative response for the different analyte classes is made comparable despite their naturally occurring abundance differences.

Results and Discussion

Ethyl acetate extracts of cannabis and hemp were pooled to create a single aliquot with wide representation of terpenoids and cannabinoids encountered in the analysis of either type of cultivar. The combination of liquid extraction with liquid injection allows both cannabinoids and terpenes to

be observed in the same analysis. The use of headspace sample introduction or derivatization of samples for determining individual forms of THC would not be compatible with analyzing both terpenoids and cannabinoids in a single acquisition.

As a first stage in reducing the signal of the cannabinoids, the 13C isotope peak of the molecular ion was chosen as the precursor for these compounds which share the chemical formula of $C_{21}H_{30}O_2$. This reduces the response for these analytes to 23% of the intensity obtained when using the 12C isotope due to the lower naturally occurring abundance of the 13C isotope.

Next, product ion spectra of cannabidiol and Δ9-THC were acquired at multiple collision energies. Spectra were compared and extracted ion chromatograms (XIC) for various fragments were used to make a preliminary evaluation of sensitivity and specificity. Figure 1 shows the spectra obtained for the two cannabinoids with annotations on some abundant and selective fragments. Figure 2 shows a subset of the XICs being evaluated for sensitivity and selectivity. The more selective the fragment the higher the area % is for the given analyte. The uppermost chromatogram in this figure is an XIC for the 217 Da fragment from a 315 Da precursor demonstrating significantly increased selectivity for the detection of Δ9-THC (93.9%) at 19.28 minutes over the detection of cannabidiol (6.1%) at 19.09 minutes. Subsequently, an MRM acquisition method combining standard MRM transitions for the measurement of terpenoids and attenuated MRM transitions for cannabinoids was created and applied to the same sample aliquot. In Figure 3, one of the terpenoids is demonstrated to give similar response to the two cannabinoids despite being present in the sample at significantly lower abundance.

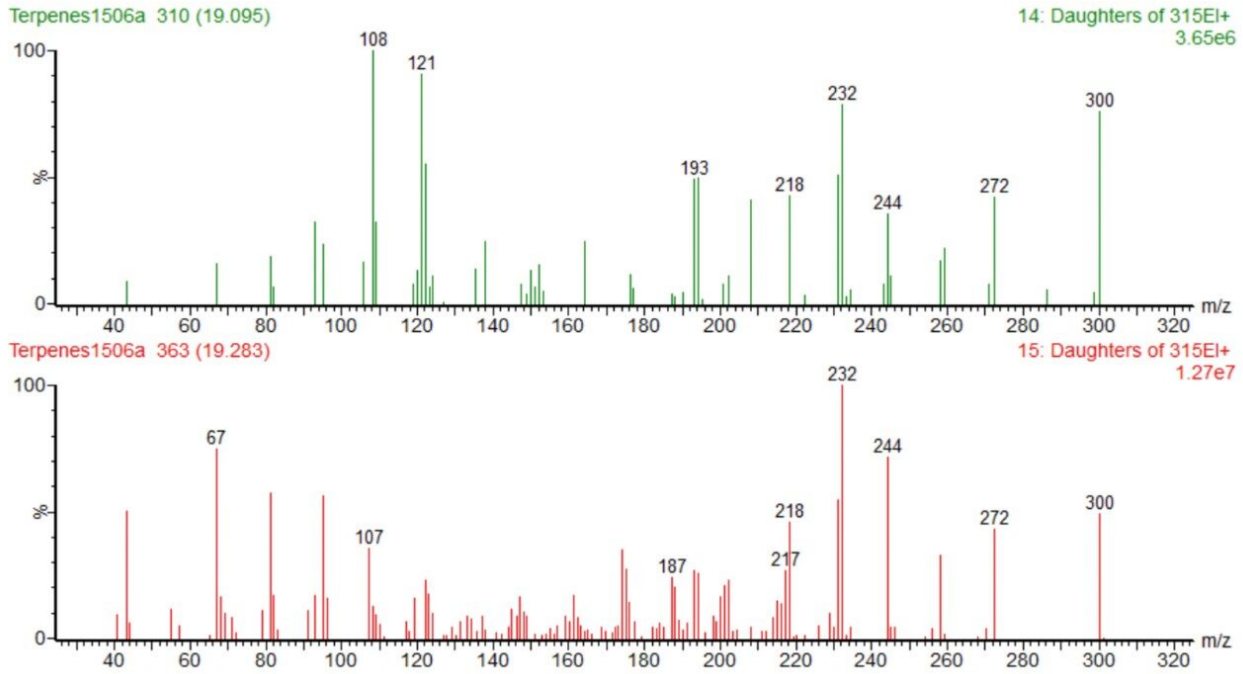

Figure 1. Example product ion spectra of Δ9-THC (lower spectrum) and cannabidiol (upper spectrum) from 13C precursor ion at 315 Da.

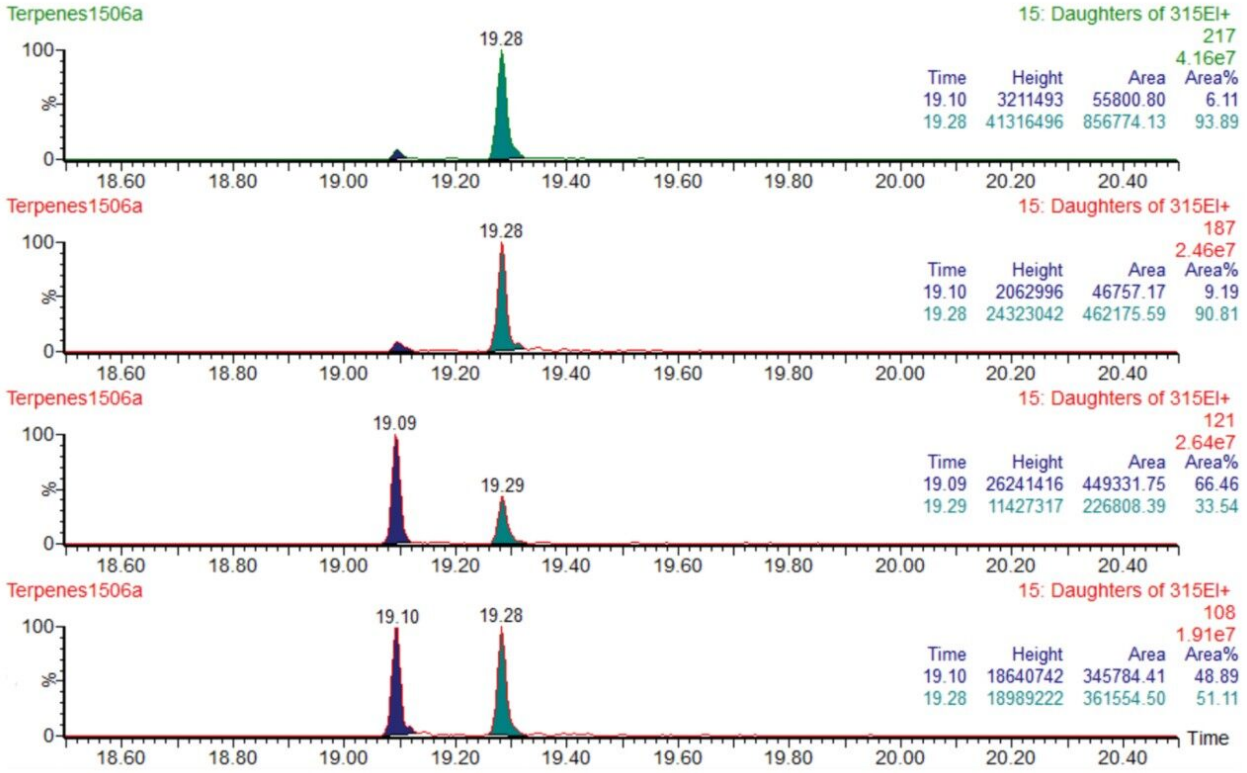

Figure 2. Product ion XIC's for cannabidiol at 19.09 minutes and Δ9-THC at 19.28 minutes.

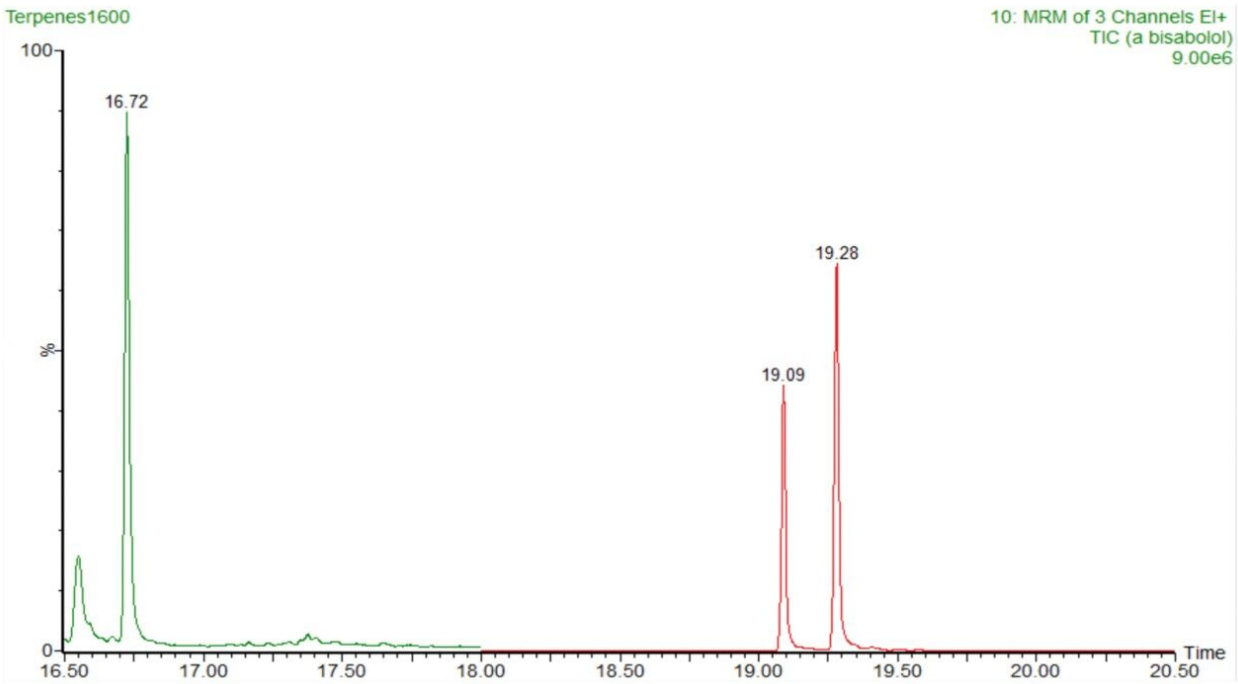

Figure 3. Terpene (bisabolol at 16.72 min) and cannabinoid MRM data overlaid.

Conclusion

The combined use of the 13C isotope peak for the precursor and lower abundance, higher specificity fragments for intentionally attenuated MRM transitions demonstrated the ability to reduce the response of high abundance compounds, cannabinoids in cannabis and hemp, in order to have their relative response made comparable to other low abundance components, terpenoids, of the homogenized biomass extracted for this study. This makes quantitation of compound classes of disparate relative abundance more readily compatible with the EI GC-MS/MS technique often applied to the analysis of natural products.

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