Distinguishing 1- and 3-methylhistidine isomers based on combined chromatographic and ion abundance characteristics

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Introduction

The compound 3-methylhistidine (3-MH) has been widely employed as a muscle protein turnover marker in a broad range of human and animal studies and together with its isomer, 1-methylhistidine (1-MH) (a product of anserine breakdown), are both widely present in biological matrices of animal origin. These isomers exhibit almost identical retention times in reverse phase high pressure liquid chromatography (HPLC) and poor gas chromatography behaviour making their individual determination cumbersome without employing derivatisation or ion-pairing methods. Here we demonstrate that by taking into account their mass spectra and hydrophilic interaction chromatography (HILIC) behaviour, individual and fast determination of both isomers is possible.

Methods

Individual, 1μg/mL solutions of 1- and 3-MH in H_2O:MeOH 1:1 were infused in the mass spectrometer using a syringe pump and the experimental conditions detailed below. The mass spectrometer was set in product scan mode and 50 scans were taken in 5V collision energy (CE) increments (5-50V) in profile mode. Scans were averaged and centered while plots of the most selective and abundant ion fragments were prepared using both absolute and relative intensity.

Once optimal detection conditions were determined, a series of preliminary method development experiments were performed in order to investigate conditions for a possible separation of the two isomers in HILIC mode base on their slightly different physicochemical properties. Mobile phase composition and column temperature were optimised in order to achieve chromatographic separation between the isomers.

Experimental Conditions

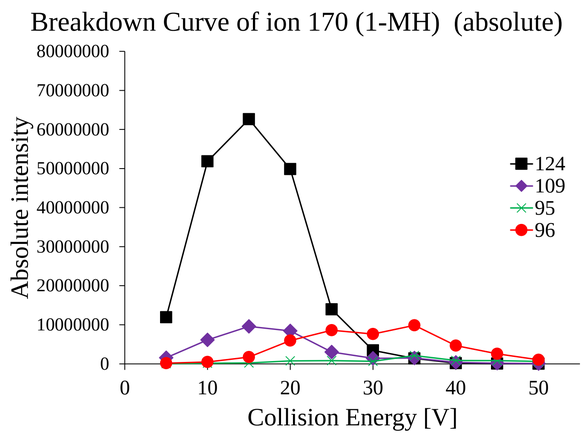
Thermo Scientific TSQ Quantum Access MAX coupled to an Accela™ 1250 UHPLC pump and Accela™ Autosampler.
ACQUITY UPLC BEH Amide, 1.7μm, 2.1x150mm

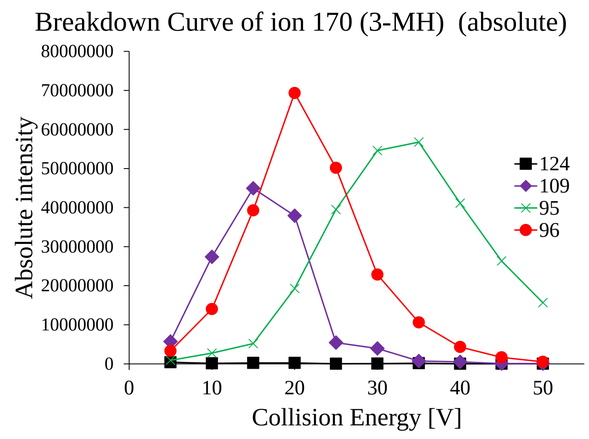
Direct infusion:
Flow rate: 10uL/min
Scan settings: Parent 170 to 10-172 m/z (profile mode)
Spray Voltage: 3500V
Vaporizer temp: 50°C, Capillary temp: 240°C

Chromatography:
Flow rate: 350uL/min, column temperature 60°C
Solvent A: MeCN 0.1%HCOOH
Solvent B: H_2O 0.1% HCOOH
65%A (hold 3min) then 40%A (over 2min)
Equilibration time: 10min
Spray Voltage: 3500V
Vaporizer temp: 250°C, Capillary temp: 320°C

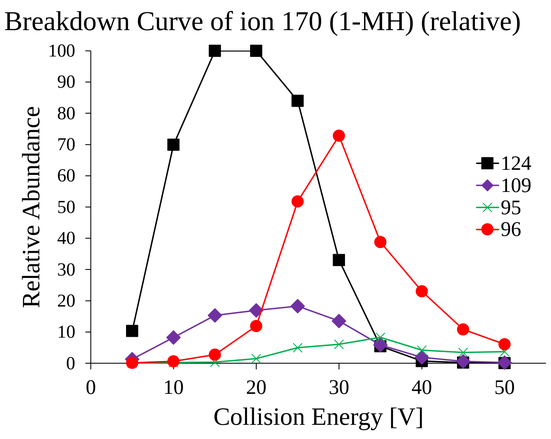
Results

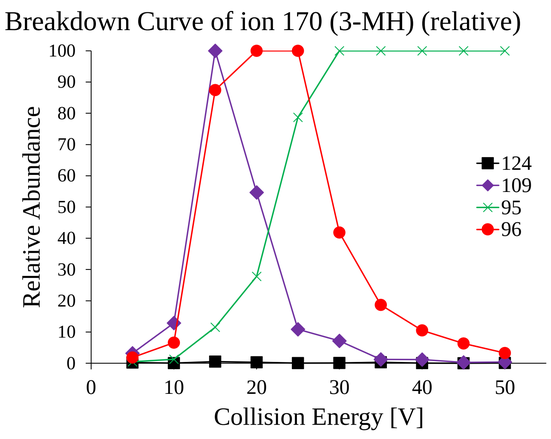
Ion breakdown curves for 1- and 3-methylhistidine



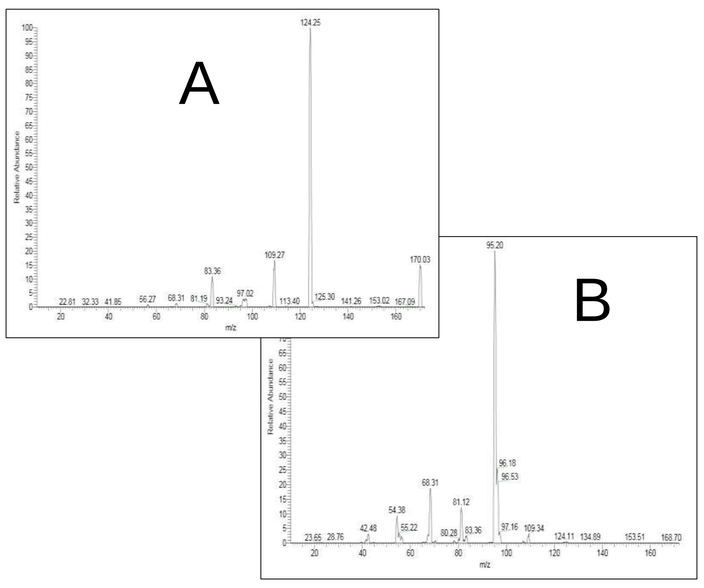


Ion breakdown curves of the most selective and intense fragment ions for 1- and 3-MH presented in absolute intensities. Each value corresponds to an average of 50 scans. It is demonstrated that the most intense ion fragments are 124 in the case of 1-MH and 96 and 95 in the case of 3-MH. The intensity of ion 124 is significantly higher in the mass spectrum of 1-MH, while that of ion 95 is significantly higher in the mass spectrum of 3-MH. Fragment ions 109 and 96 are present in significant intensities in both isomer breakdown curves.





Ion breakdown curves for 1- and 3-MH presented in relative abundances (relative intensities). Each value corresponds to an average of 50 scans. Fragment ion 124 appears to be selective for 1-MH and the base ion for CE of 15-20V. Ion 109 appears to be present in significant intensities in both 1- and 3-MH spectra and so is ion 96. Ion 95 is the base ion for CE above 30V in the spectrum of 3-MH and is present in relatively low intensities in the spectrum of 1-MH at the same CE range. Therefore, in combination with chromatographic separation this ion can be used selectively for 3-MH.



A: Mass spectrum at a CE of 15V for 1-MH. Ion 124 is the most intense peak in the spectrum.
B: Mass spectrum at a CE of 35V for 3-MH. Ion 95 is the most intense peak in the spectrum.
C: Chromatogram of both 1- and 3-MH at a concentration of 0.56μg/mL. Adequate chromatographic separation was achieved that, combined with the optimised ion transitions, can provide the means to perform a fast and individual determination of both isomers.

Conclusions

As shown, 1- and 3- methylhistidine exhibit unique ion abundance characteristics in their mass spectra that may allow for their selective detection, provided the two isomers are present in the sample in similar concentrations. However, this is not the case in most biological matrices, so a degree of chromatographic separation is required in order to achieve both selective and sensitive determination. Matrix effects from unknown sample components are expected due to the relative short retention times, hence, rigorous sample clean-up is suggested in order to minimise such effects.

In the present study, both ion abundance and chromatographic selectivity is achieved in combination with short retention times, thus paving the way for the development of a high throughput method that can be employed for the analysis of a large number samples (hundreds or even thousands) in muscle catabolism studies in animals and humans.

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