

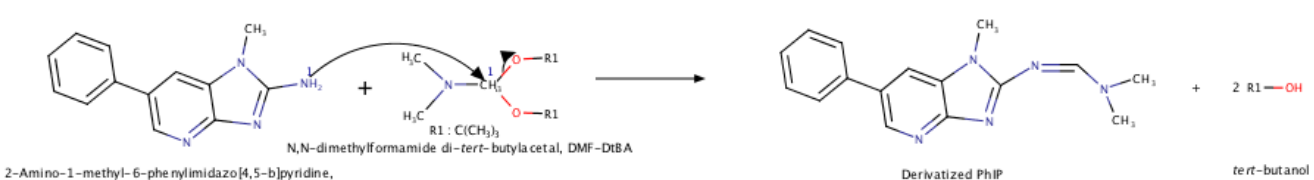
Molecular Mass of Potentially Carcinogenic Arginine-Based Heterocyclic Amines

Abstract

Investigation of cancer-inducing molecules in cooked foods has led to the discovery of mutagenic heterocyclic amines (HCAs) in meat heated to high temperatures. Amino acids creatin(in)e and phenylalanine form the precursor for these HCAs. Recent research, however, have found similar HCAs in plant-based proteins where arginine, rather than creatin(in)e, form molecules exhibiting similar mutagenic properties as those in meats¹. Presently, no papers have identified the structure of these HCAs due to the lack of information. This project attempts to contribute information regarding molecular mass and mass of side chains via various mass spectrometry analysis methods of novel arginine-based HCAs.

Methodology

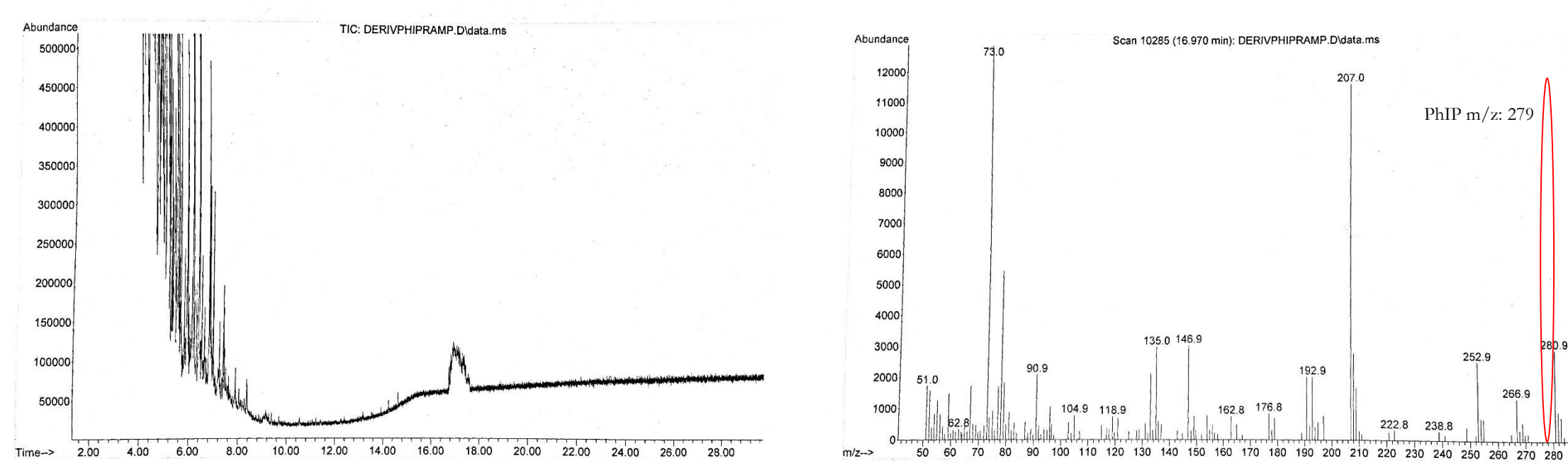
- Samples of PhIP (2-amino-1-methyl-6-phenylimidazo(4,5-b)pyrimidine) in methanol were used as test compound. PhIP, a carcinogen that shares the common starting amino acid phenylalanine as the HCA of interest, is hypothesized to be structurally alike.
- Gas chromatography mass spectrometry method (GCMS) development with Agilent Technologies 7890A GC System and 5975C Inert XL MSD took place. Derivatizing agent N, N-dimethylformamide di-*tert*-butylacetal (DMF-DtBA) used to enhance PhIP's ability to pass through separating column².



- Electrospray ionization mass spectrometry (ESI-MS) with Bruker micrOTOF-Q II at Notre Dame University also used to analyze PhIP samples.
- Large scale high pressure liquid chromatography (prep-HPLC) was used to separate and purify burned amino acid samples. Method refinement took place using Waters PrepLC 4000 system with PDA detector.
- Ames test conducted to assess mutagenicity for separated HCA fractions.

Results

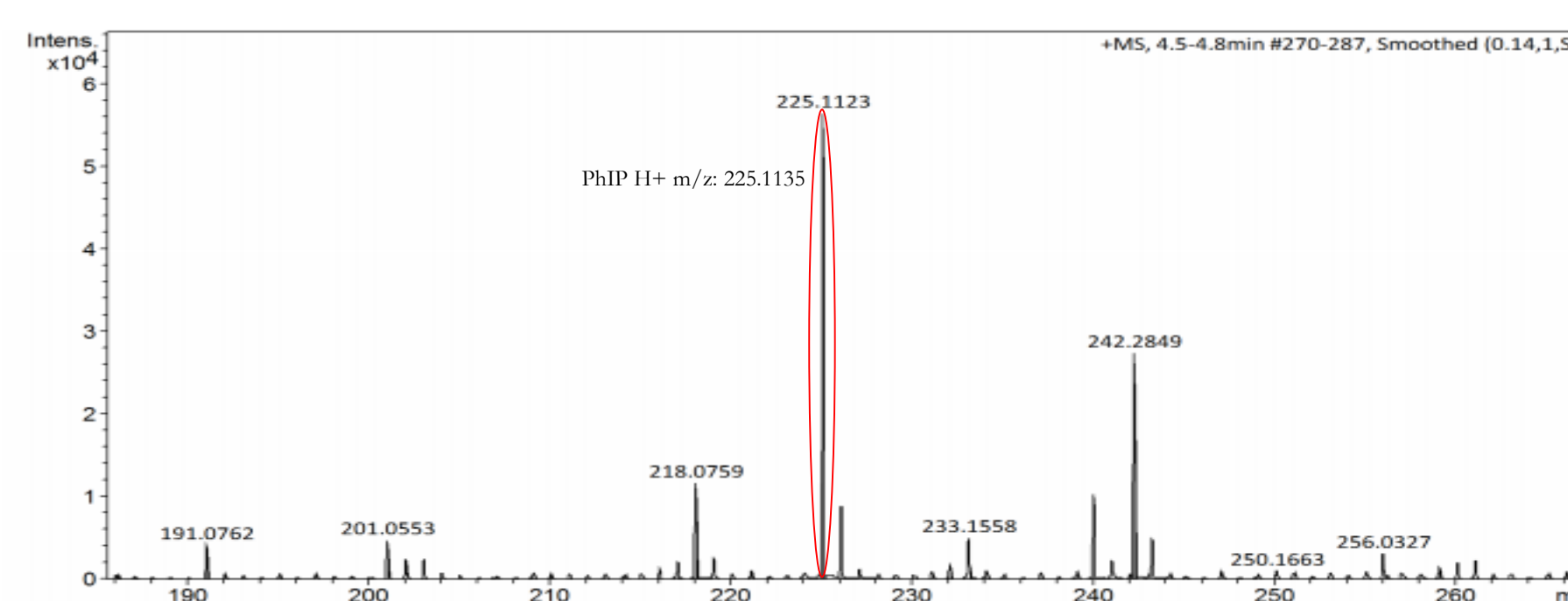
- Efforts to develop new method involving a derivatizing agent for mass spectrometry analysis of HCAs do not replicate results as described in peer-reviewed literature.



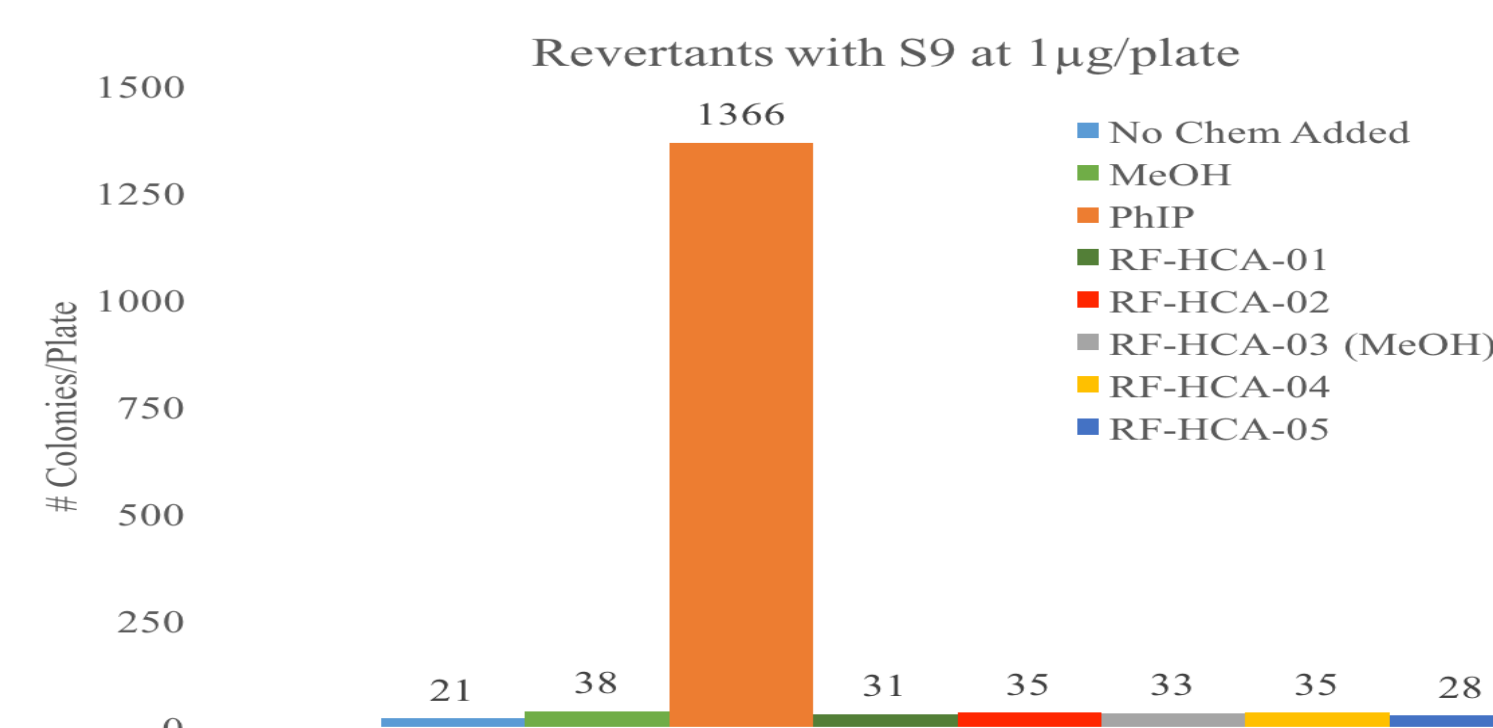
- Further development of the prep-HPLC methodology was determined where refined system preparation and minimum sample injection amount were established.

Sample	Injection Volume	Sample Concentration	Amount Injected	prep-HPLC Performance
PhIP	100 µL	10 µg/mL	1 µg	strong peak
HCA Extract	100 µL	10 µg/mL	1 µg	no peak
HCA Extract	100 µL	23 mg/mL	2.3 mg	weak peak
HCA Extract	500 µL	23 mg/mL	11.5 mg	medium peak

- Work with the ESI-MS was successful in separating out PhIP and correctly identifying its molecular mass.



- With the previously isolated HCA sample fractions, search for the mutagenic fraction using the Ames test gave inconsistent data from past results.



Discussion

- GC-MS was evaluated for its ability to identify molecular weights of HCAs. Changes in temperature, solvent mixture, type of column packing material, and sample injection method were investigated for best separation and detection of PhIP.
- Addition of derivatizing agent to polar amine group of PhIP was found insufficient to decrease its polar nature for clean separation. A more direct application of PhIP to mass spectrometer is required.
- A successful collaboration with Notre Dame University Mass Spectrometry and Proteomics Facility accurately separated and identified PhIP's molecular mass. This will pave the road for future molecular mass and structural analysis of isolated mutagenic HCAs.
- Previously isolated HCA fractions have revealed mutagenic characteristics even though current HCA fractions have not. More HCA content may be required.
- Future work will involve consolidation of all previous burned amino acid solutions with prep-HPLC for Ames test. Once mutagenic fraction has been found, ESI-MS analysis will be run to find molecular mass.

Acknowledgements

Rayford Alva and Dr. Brian Y. Y. Wong, Ph. D (Ames test)
Skyler Schell and Zachery Reichert (burning/prep-HPLC)
Ms. Nonka Sevova from Notre Dame University (ESI-MS)

Bibliography

1. Knize, M.G.; Cunningham, P.L.; Avila, Jones, A.L.; J.R.; Griffin Jr., E.A.; Felton, J.S. Formation of mutagenic activity from amino acids heated at cooking temperatures. *Food and Chemical Toxicology*. 1994, 32, 55-60.
2. Barceló-Carrachina, E., Santos, F., Puignou, L., & Garcera, M. (2005). Comparison of dimethylformamide dialkylacetal derivatization reagents for the analysis of heterocyclic amines in meat extracts by gas chromatography-mass spectrometry. *Analytica Chimica Acta*, 545(2), 209-217.