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Dedicated to the memory of
Professor Ioan Silaghi-Dumitrescu (1950 – 2009)

BIOGENIC AMINES FINGERPRINTS EVIDENCED BY PERFORMANT MS ANALYSIS

Michaela Dina STĂNESCU,[*a] Florian HARJA,[a] Cristina MOSOARCA[b] and Alina D. ZAMFIR[a,b]

[a]Chemical and Biological Sciences Department, Faculty of Food Engineering, Tourism and Environmental Protection, "Aurel Vlaicu" University, Elena Dragoi 2-4, Arad 310330, Roumania
[b]Mass Spectrometry Laboratory, National Institute for Research and Development in Electrochemistry and Condensed Matter, Plautius Andronescu 1, Timişoara 300224, Roumania

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Biogenic amines are one of the compounds to be monitored in food due to their influence on human health. Lately a number of analytical methods have been developed in order to determine accurately the content of biogenic amines like: *Histamine*, *Cadaverine*, *Putresceine*, *Tyramine*, *Tryptamine*, etc. in food (fish, meat, cheese, wine, beer, etc.). The HPLC methods, mainly used, are rigorously exact but very tedious and need supplementary workup of the sample. Thus, a method based on MS analysis using an accurate procedure like Chip-based Nanoelectrospray Ionization Tandem seems more appropriate for a different approach to biogenic amine identification. The identification of the most common biogenic amines from wine through their MS spectra is presented.

INTRODUCTION

The biogenic amines have been lately studied in connection with food safety.[1,2] Such interest has been generated by the fact that these compounds play important roles in organisms. Some of them are necessary in small quantities due to their metabolic role, as neurotransmitters (*Histamine*) or as precursors for neurotransmitters (*Tyramine*, *Phenylethylamine*). These monoamines are the key factors for motor control, emotion and cognition. In higher quantities, than metabolic necessary, these compounds may generate neurological and psychiatric disorders like: depression, schizophrenia and Parkinson's.[3]

Digestion disorders have been also observed in cows eating food reach in biogenic amines.[4]

Usually, biogenic amines presence reflects the food spoilage for fish, meat, cheese and even wine.[5-7] Their presence in excess leads to sickness like: allergies, hypertension, tachycardia, nausea, vomiting etc.[8]

Biogenic amines may combine with nitrites giving nitrosamines, which are carcinogenic compounds.[9]

The biogenic amines are formed in wine during malo-lactic fermentation by the decarboxylation of the corresponding aminoacids.[10,11] The most frequent monoamines encountered in wine are: *Histamine*, *Tyramine*, *Cadaverine*, *Putresceine* and *Phenylethylamine*. Being in small quantities, very accurate analytical methods are needed for their detection.[12]

Most of the isolation and detection methods are based on chromatography: HPLC, GC or TLC. Usually chromatographic procedures involved derivatization with different reagents for improving detection of biogenic amines.[13-22] These methods are laborious and time consuming, as well

* Corresponding author: stanescu@uav.ro (smichaeladina@yahoo.com)

as costly, due to special devices necessary for a good separation. Other methods are based on enzyme sensors, monitoring amine transformations. Most of these relay on the *Mono-* or *Di-amineoxidase* conversion of biogenic amines into the corresponding aldehydes with monitoring of the secondary product, hydrogen peroxide:[23,24]

$$RCH_2NH_2 + O_2 \rightarrow RCHO + H_2O_2 + NH_3$$

Also transfer reaction catalyzed by *Transglutaminase* with detection of the released ammonia was used for biogenic amine detection.[25]

$$RCH_2NH_2 + R'CONH_2 \rightarrow RCH_2NHCO\ R' + NH_3$$

Capillary electrophoresis is another method for analyzing biogenic amines being a fast method with good detection sensitivity. [26-28]

The tremendous progress of mass spectroscopic methods made possible a qualitative and quantitative determination of trace compounds in food. A number of papers dedicated to biogenic amines analysis in food including wine have been published. [29-31] These studies combine MS analysis with other procedures (HPLC, CE).

This paper presents the spectral MS data for some of the biogenic amines, most frequently found in wine, in a positive ionization mass-spectroscopy, as well as their possible identification, based on MS fingerprint, in a commercial red wine.

RESULTS AND DISCUSSION

In literature there is no analysis concerning the chemical structure of the ions generated by fragmentations from biogenic amines during the MS process. Thus, for an easy identification by MS, the fragmentation pattern in the experimental conditions, of three frequently encounter compounds, was studied. For each of the amines: *Histamine*, *Cadaverine* and *Tyramine*, the molecular ion has been evidenced. The further fragmentation of the amine depends on the chemical structure of the parent ion and the stability of the fragmentation products.

Histamine was characterized by a first peak at 112 representing the molecular peak of the protonated amine, as seen in Fig. 1.

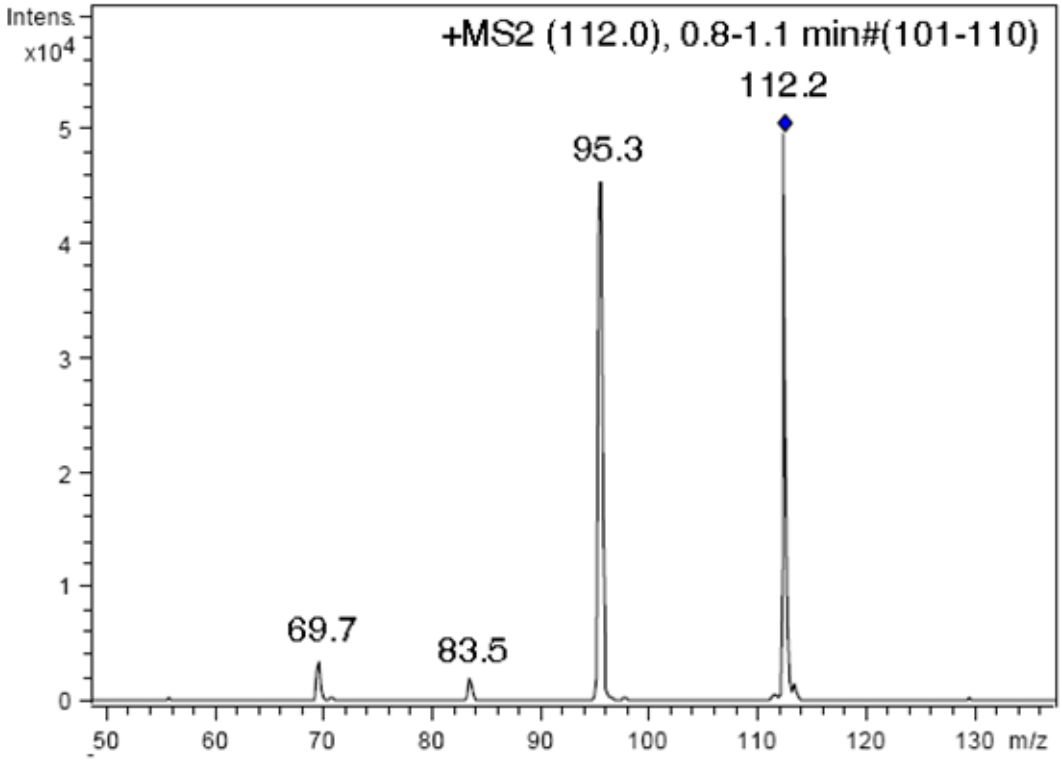
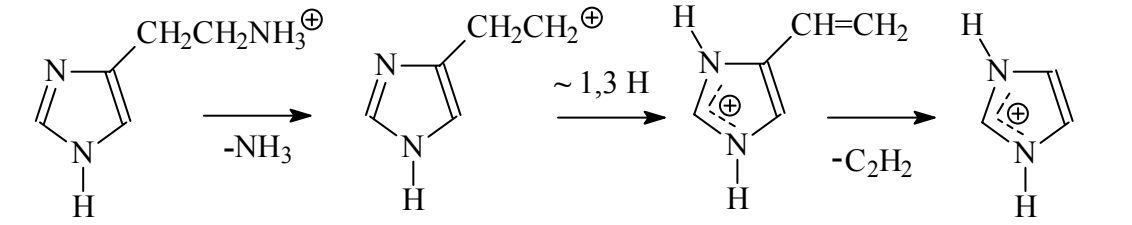


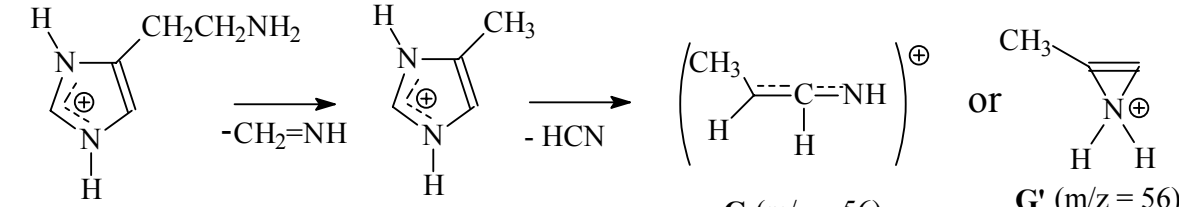
Fig. 1 – Fragmentation of protonated *Histamine* (m/z=112).

The fragmentation of the parent ion has two possible routes. In the major pathway (see Figure 1) the following fragmentations have been observed:



A (m/z = 112) **B** (m/z = 95) **C** (m/z = 95) **D** (m/z = 69)

The molecular ion **A** (m/z = 112) eliminates an ammonia molecule leading to the unstable primary cation **B** (*m/z* = 95) which by a rearrangement gives the more stable, conjugated, vinylimidazole ion **C** (*m/z* = 95). A further fragmentation of vinylimidazole ion **C** (*m/z* = 95), by acetylene elimination lead to the very stable protonated imidazole **D** (*m/z* =69). Another fragmentation is observed from the other possible protonated histamine **E** (m/z = 112) by a loss of a CH_2NH fragment with formation of ion **F** (m/z = 83):



E (m/z = 112) **F** (m/z = 83) **G** (m/z = 56) **G'** (m/z = 56)

The further transformation, into **G** or **G'**, described in literature[32] for compounds such as **F**, has not been evidenced in our experimental conditions.

Cadaverine generates in positive ionization conditions, the protonated molecular ion **H** (m/z = 103) which loose an ammonia with the cyclization to the protonated piperidine **I** (*m/z* = 86) (see Figure 2).

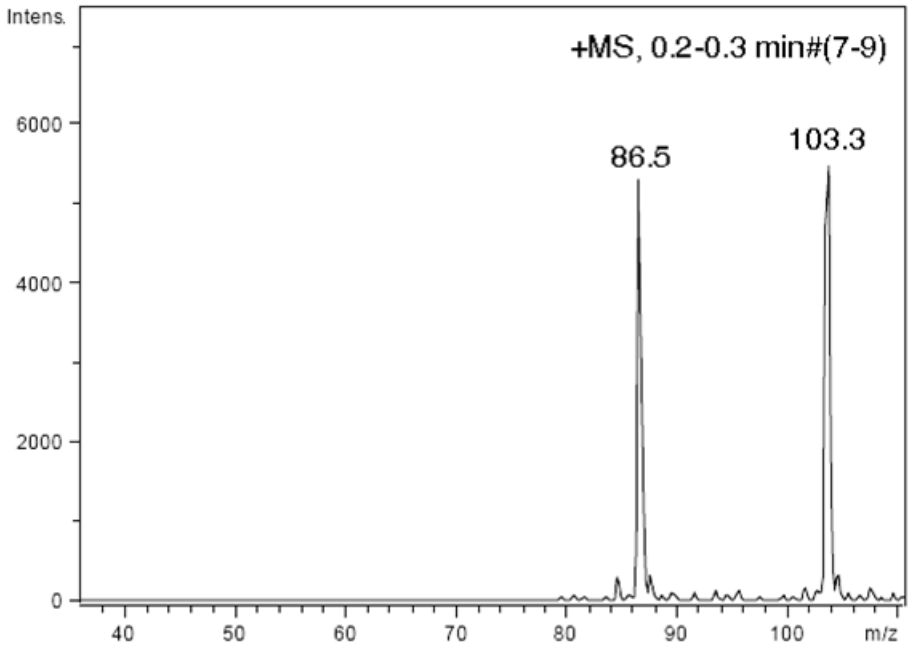
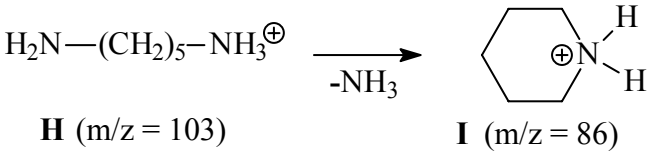


Fig. 2 – Fragmentation of protonated *Cadaverine* (m/z = 103).

The ion **I** is quite stable (6 atom ring) in the experimental applied conditions:



H (m/z = 103) **I** (m/z = 86)

Tyramine could be also identified by the protonated molecular peak **J** (*m/z* = 138).

By the usual ammonia molecule elimination, a very stable bicyclic ion **K** (*m/z* = 121) is formed (Figure 3), intermediate evidenced by Cram in the solvolysis of 2-arylethyl derivatives.[33]

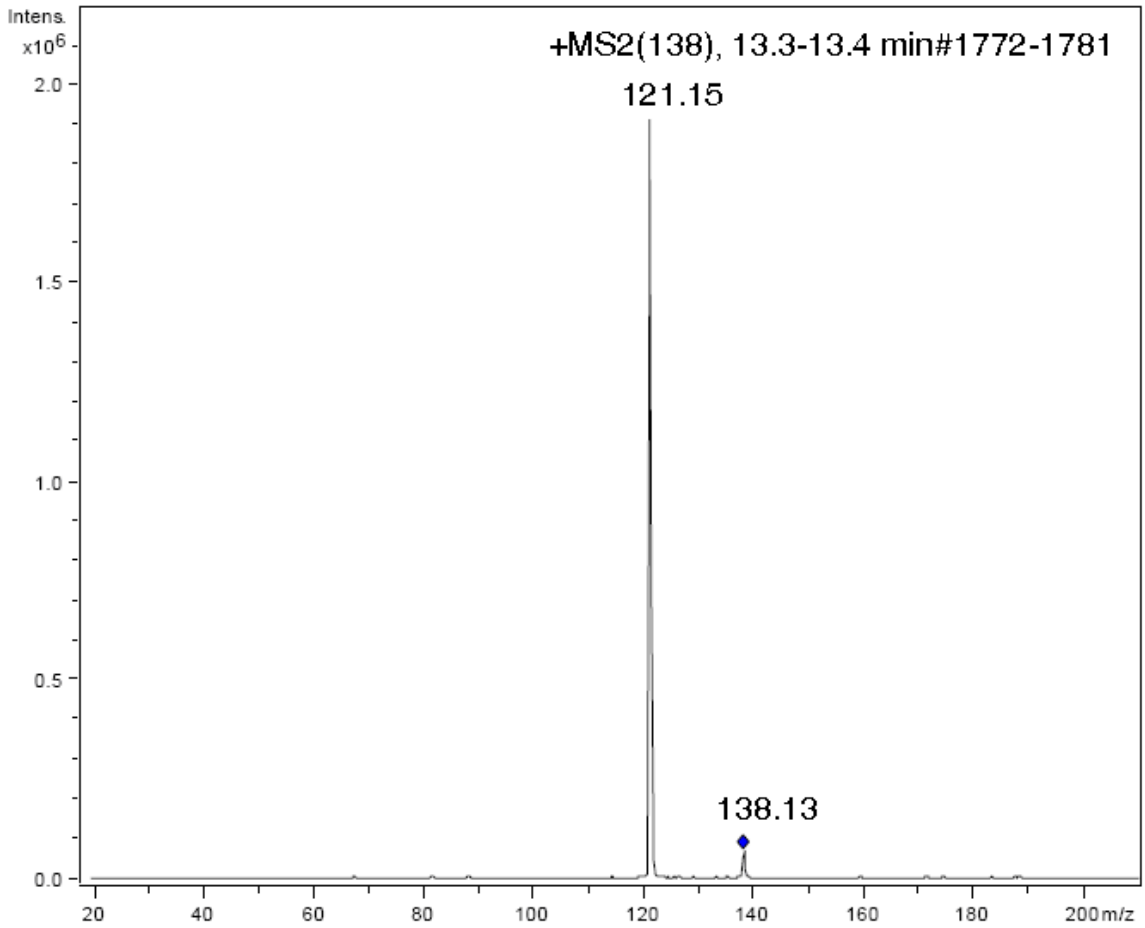
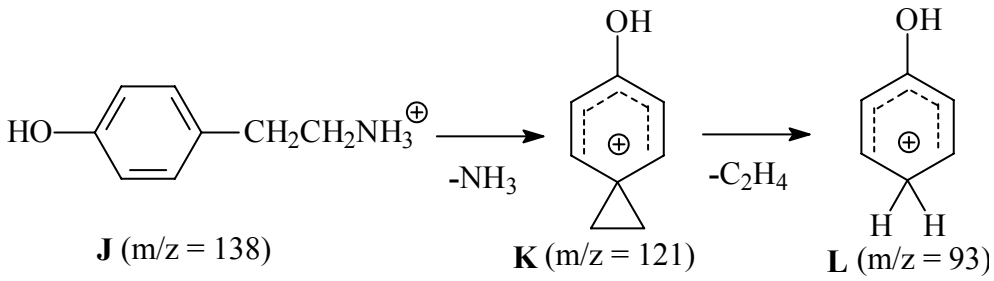


Fig. 3 – Fragmentation of protonated *Tyramine.*



J (m/z = 138) **K** (m/z = 121) **L** (m/z = 93)

By the next fragmentation, *via* C_2H_4 elimination, the simple phenonium **L** (*m/z* = 93) (Figure 4) resulted:

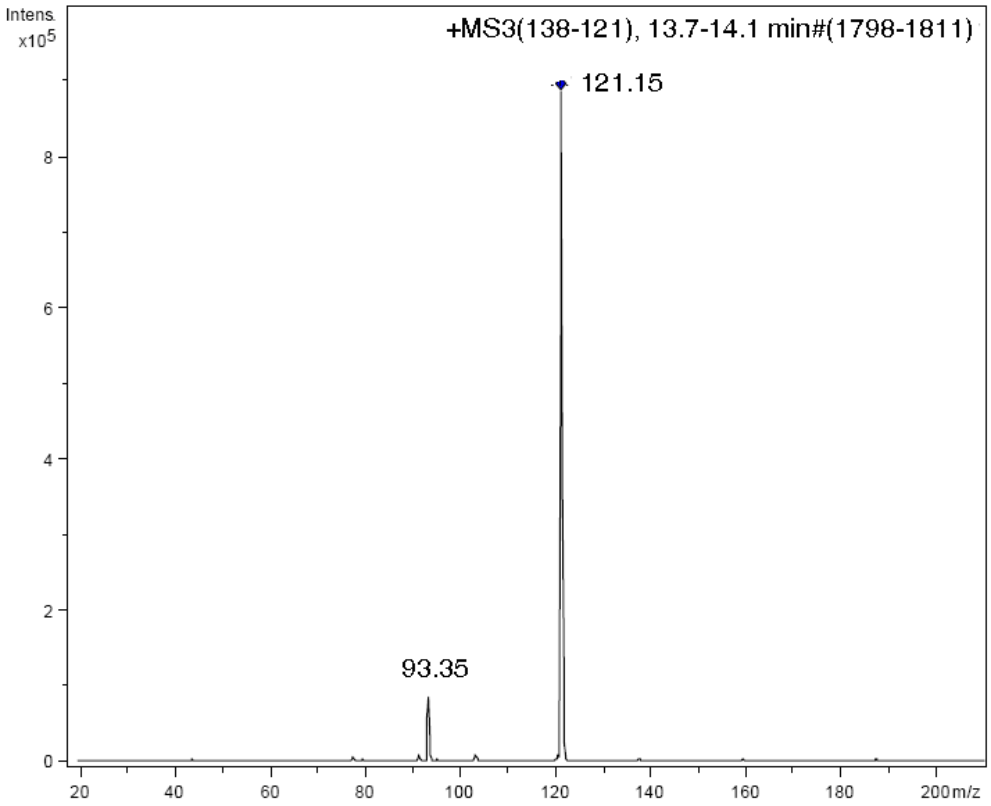


Fig. 4 – Fragmentation of phenonium ion **K.**

The easy identification of these compounds in a MS spectrum of a wine sample is illustrated in Figure 5, where a sample of red wine with an added content (10 mg L^{-1}) of *Cadaverine* is presented:

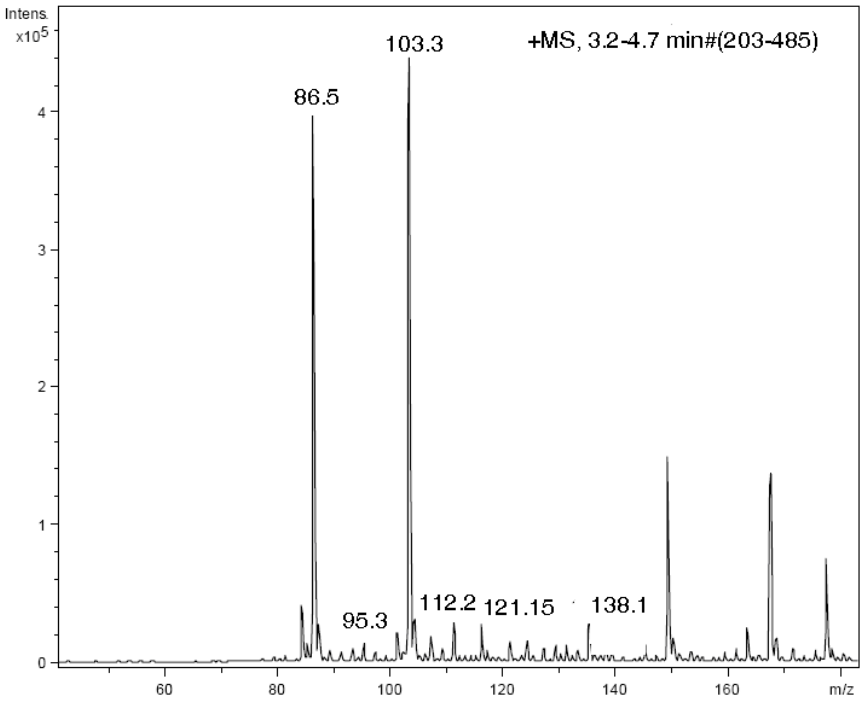


Fig. 5 – Segment of the MS fragmentation for a red wine with added *Cadaverine.*

Beside *Cadaverine* (peaks 86-MS2 and 103-MS1), *Histamine* (peaks 95-MS2 and 112-MS1) as well as *Tyramine* (peaks 121-MS2 and 138-MS1) may be identified, based on the sensitivity of the measuring procedure. Based on chromatographic data the analyzed wine has less than 10 mg L^{-1} content of all biogenic amines.[34] New experiments regarding a quantitative determination are in progress.

EXPERIMENTAL

Materials

All the reagents are analytical grade. Methanol and formic acid were obtained from Merck (Darmstadt, Germany). The biogenic amines: *Histamine*, *Tyramine*, *Cadaverine* are purchased from Sigma-Aldrich (Germany).

Sample preparation

Qualitative analysis of the biogenic amines were performed on samples prepared by solving 5 mg of pure biogenic amine (BA) into 1 mL solvent (water:methanol, 1:1), then 10 µL of BA sample was mixed with 30 µL water and 10 µL formic acid and 10 µL of the mix was introduced in the sample plate of the NanoMate robot.

The wine sample was left to warm up to room temperature then 1.5 mL of wine was centrifuged. After centrifugation 0.1 mL of wine was siphoned, added 0.9 mL solvent water:methanol (1:1) and 0.03 mL formic acid. After this, 0.07 mL pure *Cadaverine* (0.870 g/mL – density) was added to spike the sample. 10 µL of the prepared sample was then introduced in the sample plate of the NanoMate robot.

Mass spectrometry analyses

Mass spectrometry was conducted on a High Capacity Ion Trap Ultra (HCT Ultra, PTM discovery) mass spectrometer from Bruker Daltonics, Bremen, Germany. All mass spectra were acquired in the mass range 15-300 *m/z*, with a scan speed of 8000 *m/z* per second. Tandem mass spectrometry was carried out by collision-induced dissociation (CID) using He as the collision gas. For MS/MS sequencing the precursor ions were selected within an isolation width of 2 u. Each fragmentation spectrum was obtained by accumulating scans at variable rf signal amplitudes within 0.6-0.8 V.

Fully automated chip-based nanoelectrospray

Fully automated chip-based nanoelectrospray was performed on a NanoMate robot incorporating ESI 400 Chip technology (Advion BioSciences, Ithaca, USA) controlled and manipulated by ChipSoft 7.1.1 software operating under Windows system. The robot was coupled to the HCT Ultra mass spectrometer via an in laboratory made mounting system, which allows robot O-xyz positioning with respect to HCT counterelectrode as described before.[35]

10 µL aliquots of the working sample solutions were loaded onto a NanoMate 96-well plate. The robot was programmed to aspirate the entire volume of the sample, followed by 2 µL of air into the pipette tip and afterwards deliver the sample onto the inlet side of the 400 microchip. NanoMate HCT MS system was tuned to operate in the positive ion mode. Electrospray was initiated by applying a voltage of 1.5 kV on the pipette tip, and 0.30-psi nitrogen backpressure. In order to prevent possible *in-source* fragmentation, HCT capillary exit was set to 20 V. The source block maintained at the constant temperature of 200ºC provided an optimal desolvation of the generated droplets without the need of desolvation gas. Following sample infusion and MS analysis, the pipette tip was ejected and a new tip and nozzle were used for each sample, thus preventing any cross-contamination or carry-over. Each chip nozzle had an internal diameter of 2.5µm, which under the given conditions delivered a working flow rate around the value of 100nL/min.

Data analysis

All mass spectra were processed by Data Analysis 3.4.software from Bruker Daltonik (Bremen, Germany).

CONCLUSIONS

Based on experimental results, one may say that most important biogenic amines found in food, namely: *Histamine*, *Tyramine* and *Cadaverine*, have distinct and easy to identify peaks, in performant MS measurements. Intermediate fragmentations were highlighted, in agreement with the literature in regards to ion stability and fragmentation, thus making biogenic amines fingerprinting accurate. This approach makes possible their quick identification in food and fermented beverages, like wine.

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