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Highly sensitive and specific detection of histamine via the formation of self-assembled magic number cluster with thymine by mass spectrometry

Jiamu Sun, Zhen Qin, Jia Liu, Chengsen Zhang and Hai Luo*

Beijing National Laboratory for Molecular Sciences, College of Chemistry and Molecular Engineering, Peking University, Beijing, 100871, China.

E-mail: hluo@pku.edu.cn Tel: +86-10-62751499 Fax: +86-10-62751708

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Abstract

A novel method for the detection of histamine (HIM) via the formation of self-assembled magic number cluster with thymine (T) by electrospray ionization tandem mass spectrometry (ESI-MS/MS) is described. The formation of the magic number cluster $[T_{17} + HIM + 2H]^{2+}$ shifts the MS signal of histamine to the interference-free higher mass range and the signal intensity is increased by four orders of magnitude. In addition, the formation of $[T_{17} + HIM + 2H]^{2+}$ is highly specific to histamine compared with its metabolite and other similar biogenic amines, which may be attributed to both of its amino and imidazole groups. The linear dynamic range of the method is in the range of 1 nM - 20 μM, and the limit of detection can be as low as 0.1 nM. The feasibility of this method is further demonstrated by the quantitative analysis of histamine in a red wine sample. Since little sample preparation or separation is required before the analysis, this method provides a rapid new way for the sensitive and specific detection of histamine by MS.

Introduction

Histamine (HIM), plays an essential role in the normal metabolism,[1] and it also occurs in many foods and beverages to various degrees as a result of the decarboxylation process of histidine.[2] A high level of histamine in foods and wines is an important indicator of unsanitary conditions during the making and storage process.[3, 4] Thus, the rapid detection and accurate quantitation of histamine is critical in food safety testing. Due to its low levels in the complex matrices, the analysis of histamine are typically carried out by a separation technique combined with a selective detection.[5-8] However, in these methods, sample preparations such as pre-concentration and derivatization are essential, which are time-consuming and laborious.

Mass spectrometry (MS) is increasingly applied in the analysis of histamine and related biogenic amines because of its significant advantages: sensitivity, selectivity and rapid analysis.[9-12] Chen and coworkers have used neutral desorption extractive electrospray ionization mass spectrometry for the rapid in-vivo analysis of histamine and other biologic amines in frozen meat without sample preparation and separation.[10, 11] However, such direct MS detection of small molecules in complex matrices often suffers from large background noise in the low mass range (< 150 Da) which is caused by the species and their fragments of the matrices. An alternative for the detection of small molecules by MS is the adoption of mass tags. These mass tags are added to the target molecules, which allow them to be detected in an interference-free high mass range via the mass-shift. [13, 14]

Various means of adding the mass tags to the analytes have been reported in the analysis of small molecules by MS. For example, Zhang and coworkers have introduced a new tag reagent with positive charge to let the small analytes be efficiently ionized and detected in the higher mass range by forming covalent matrix-analyte adduct in MALDI-MS.[14] Nyadong et al. have used alkyl amines to directly quantify active ingredients such as artesunate in medical tablets by forming non-covalent complexes using reactive DESI-MS.[15, 16] We have shown that β-cyclodextrin can be used as a “mass shift” reagent to improve the signals of

carboxylic acids via the formation of inclusion complexes in negative HALDI-MS and developed a method to determine the amount of lipoic acid in Riviva eye serum by standard addition.[17]

However, to the best of our knowledge, there have been no reports utilizing the self-assembled magic number clusters in the qualitative and quantitative analysis of small analytes. In fact, many studies have been reported on the self-clustering of nucleobases.[18-26] In these previous reports, thymine can aggregate to form the magic number clusters in the presence of alkali metal ions or ammonium cation through non-covalent interactions.[22-24] In other words, these magic number clusters enable the small target ions such as Na^+, K^+ and NH_4^+ to be detected by MS through "mass-shift" to the higher mass range. Moreover, the formation and stability of the magic number clusters of thymine are highly correlated to the size, proton affinity and other properties of the central cation,[21, 24, 26] which inspire us to utilize the magic number clusters to analyze the target ions with high specificity.

Herein, we demonstrate a novel method to sensitively and selectively determine histamine by adding thymine (T) as the clustering agent using ESI-MS/MS. This method allows the histamine (HIM) to be detected in a higher and interference-free mass range by forming the magic number cluster $[T_{17} + HIM + 2H]^{2+}$ at *m/z* 1127. The high signal intensity and high specificity of $[T_{17} + HIM + 2H]^{2+}$ offer an excellent candidate of non-covalent mass tag for the detection of histamine. This method was sensitive enough to be applied to the analysis of histamine present in a red wine sample, without tedious sample pretreatment and LC separation.

Experimental

Chemicals and Reagents

HPLC grade methanol was purchased from Fisher Scientific (Pittsburgh, PA, USA). Thymine, histamine·2HCl, 2-phenethylamine, dopamine hydrochloride, tryptamine, 4-imidazoleacetic acid hydrochloride were obtained from Sigma-Aldrich (St. Louis, MO, USA). Deuterated histamine·2HCl [A,A,B,B,-D_4] was purchased from Cambridge Isotope Laboratories, Inc (Andover, MA, USA). A 10 mM stock solution of each analyte was prepared in solution of methanol/water (1:1, v:v) and kept at 4 ℃ before its use. The red wine was purchased from a local market and diluted 1000 times by water/methanol solution (1:1, v:v) before the analysis.

ESI-MS

All the experiments were performed in positive ion mode on a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer (San Jose, CA, USA) with a home-built ESI source.[22] The relative abundance of cluster $[T_{17} + HIM + 2H]^{2+}$ was optimized by selectively tuning several instrumental parameters of MS to minimize dissociation of the non-covalent clusters during desolvation. The main experimental parameters were optimized as follows: sample flow rate 5 μL/min, spray voltage 4.5 kV, nebulizing gas pressure 0.4 MPa, capillary voltage 3.0 V, heated capillary temperature 150 °C, tube lens offset -70.0 V. In the tandem MS experiments for the quantitation of histamine, the cluster $[T_{17} + HIM + 2H]^{2+}$ was isolated in the ion trap with an isolation window of 10 *m/z* units and activated by using the collision energy at 11 % (the manufacturer's unit). The resonant excitation occurs at a q_z of 0.25 for 30 ms. The mass range was all set as 50 – 2000 m/z and each mass spectrum was the average result over about 3 min. All data were processed using the instrument software (Xcalibur version 1.4 SR1).

Results and discussion

The magic number cluster of thymine induced by protonated histamine

Fig. 1 displays the positive ESI mass spectrum of the solution containing histamine and, for comparison, the mixture of histamine and thymine in methanol/water (1:1, v:v). In Fig. 1(a), the protonated histamine $[HIM + H]^+$ was detected with poor MS signal response. Interfering ions in the low mass range caused serious signal suppression. The most significant interfering ions were observed at *m/z* 279 and *m/z* 149, corresponding to dibutyl phthalate and its characteristic fragment ion.[27] Both the poor MS signal response and high background noise in the low mass range result in the low sensitivity of histamine in this detection mode.

Fig. 1(b) shows the mass spectrum of histamine upon addition of thymine. The magic number cluster $[T_{17} + HIM + 2H]^{2+}$ at *m/z* 1127 was clearly observed in the high mass range with good signal-to-noise (S/N). More importantly, the signal intensity of $[T_{17} + HIM + 2H]^{2+}$ was significantly higher than that of $[HIM + H]^+$ without the addition of thymine (in Fig. 1(a)), and the relative abundance of interfering ions was less than 1%. The magnification effect brought by the clustering agent will be further discussed below. The existence of $[T_5 + NH_4]^+$ at *m/z* 648 has been previously reported owing to the presence of about 0.02% ammonium ion in the thymine sample.[22, 25] Some other low abundant clusters of thymine induced by histamine $[T_{11} + HIM + 2H]^{2+}$ at *m/z* 749 and $[T_{12} + HIM + 2H]^{2+}$ at *m/z* 812 were also observed. Collision-induced dissociation (CID) was performed to identify these clusters (see Fig. S1). In the MS/MS spectrum of $[T_{17} + HIM + 2H]^{2+}$, the parent ion underwent an interesting fragmentation process by the loss of a thymine pentamer, producing the characteristic fragment ion $[T_{12} + HIM + 2H]^{2+}$ at *m/z* 812 (see the inset of Fig. 1(b)).

Optimization of the formation of $[T_{17} + HIM + 2H]^{2+}$

The formation of $[T_{17} + HIM + 2H]^{2+}$ is influenced by many factors, including MS instrumental parameters and the molar ratio of thymine and histamine. The relative abundance of $[T_{17} + HIM + 2H]^{2+}$ was optimized by selectively tuning the MS

instrumental parameters (see Fig. S2-S3).

The molar ratio of thymine and histamine was optimized to increase the "yield of derivatization". The signal intensity of $[T_{17} + HIM + 2H]^{2+}$ as the function of the concentration of thymine was shown in Fig. 2. The intensity of $[T_{17} + HIM + 2H]^{2+}$ experienced a large increase as the amount of thymine increased from 10^{-6} M to 10^{-4} M. This is consistent with the previous reports that clusters are commonly formed in ESI from the concentrated solutions, e.g., $\geq 10^{-4}$ M.[28, 29] When the concentration of thymine was above 10^{-4} M, the intensity of $[T_{17} + HIM + 2H]^{2+}$ increased gradually and then dropped again slightly. Finally, the concentration of thymine was optimized as 3.0×10^{-4} M, which was used throughout the following experiments.

The high specificity in forming the magic number cluster toward histamine and enhanced detection of histamine

The richness of clustering and the propensity towards particular magic numbers are the result of particular structure features.[19] The strong preference of clustering of thymine and histamine towards doubly charged 17-mer indicates that the high specificity is correlated with both the amino and the imidazole group of histamine.

The specificity of the magic number cluster was examined using a few of biological amines similar to histamine including phenethylamine (PEA), dopamine (DOPA), and tryptamine (TRY), as well as histamine's metabolite imidazole-4-acetic acid (IAA). Solutions containing thymine and the respective analyte were analyzed by ESI-MS. The results show that phenethylamine, dopamine, and tryptamine can induce thymine to form pentameric clusters (see Fig. S4), which is in accordance with the previously reported quintet cluster of thymine induced by the protonated amines.[26] But remarkably, no high-order magic number clusters of thymine ($n \geq 15$) are observed. This is probably due to the fact that the high-order magic number clusters need more than one positive charge center to stabilize the structure, in accordance with our previous report that the doubly charged 15-mer ($[T_{15} + 2M]^{2+}$) are stabilized by two metal ions.[24] Besides, the formation of the magic number clusters also depends on the size and the charge of the charge center, so the high-order cluster of uracil

induced by calcium ion $[U_{18} + Ca]^{2+}$ and $[U_{24} + Ca]^{2+}$ (E. L. Zins and coworkers had reported) are different from that induced by protonated amines.[20] For phenethylamine, dopamine, and tryptamine, the low proton affinity[30-33] and big size of the phenyl or indazole group relative to the imidazole group of histamine make them inappropriate to be the positive charge centers to induce the high-order magic number clusters of thymine. In fact, our recent study shows that imidazolium ion can induce thymine to form hexameric and high-order magic number clusters (see Fig. S5), confirming the inevitable role of imidazole group in clustering with thymine. Moreover, we further validate the role of protonated amino group of histamine in forming $[T_{17} + HIM + 2H]^{2+}$ with thymine by choosing imidazole-4- acetic acid as a comparison. The result shows that without the amino group, no doubly charged 17-mer of thymine but the hexameric clusters $[T_6 + IAA + H]^+$ at *m/z* 882 were observed. Thus both of the amino group and imidazole group of histamine are indispensable for the formation of $[T_{17} + HIM + 2H]^{2+}$, resulting this derivatization is highly specific to histamine.

The high specificity in forming the magic number cluster toward histamine is also embodied in the selectively enhanced detection of histamine. The enhancement of MS signal intensity for histamine by clustering with thymine (T) was significant and the analytes discussed above were further compared with histamine. To verify the magnification effect by the magic number clusters, the factor *R* for each analyte (A) in the following equation can be used:

$$R = \frac{I_{[Tm + A + nH]^{n+}}}{I_{[A + H]^{+}}}$$

Where $I_{[Tm + A + nH]^{n+}}$ is the MS signal intensity of the magic number cluster $[T_m + A + nH]^{n+}$ obtained from a solution containing both A and T; $I_{[A + H]^{+}}$ is the signal intensity of the protonated analyte $[A + H]^+$ with A at the same concentration and in the absence of T. Owning to their low molecule weights, histamine, phenethylamine, and imidazole-4-acetic acid all have rather poor MS signal responses partly due to the poor transmission efficiency of the ion trap mass spectrometer in the low mass range. After the addition of T, these small analytes were mass-shifted to the higher mass range via the formation of the magic number clusters $[T_{17} + HIM + 2H]^{2+}$, $[T_5 + PEA$

+ H]$^+$, [T_6 + IAA + H]$^+$ and their MS signal intensities were improved by 2 ~ 4 orders of magnitude (see Fig. 3). In particular, the magnification factor R for histamine was up to 2.20 × 10^4 fold, about 100-times higher than that of the phenethylamine and imidazole-4-acetic acid. We believe the significant S/N enhancement for histamine is attributed to both the effect of the mass-shift towards the "noise-free" higher mass range and the unique high MS signal response of the magic number cluster [T_{17} + HIM + 2H]$^{2+}$. Note that the signal magnification effect by forming magic number clusters of thymine was not observed for other analytes. For example, dopamine and tryptamine could induce the pentameric clusters of thymine, but the formation of the clusters had no positive effect on the detection of them (the magnification factor R < 1, see the insert table in Fig. 3). The almost exclusive formation of [T_{17} + HIM + 2H]$^{2+}$ and its amazing MS signal response prompted us to develop a highly sensitive and specific method for the quantitation of histamine in a real complex sample.

Sensitivity and dynamic range

The quantitative experiments employed tandem MS to detect histamine on account of sensitivity (with better S/N) and pecific identification. The characteristic fragment ion [T_{12} + HIM + 2H]$^{2+}$ at *m/z* 812 generated from [T_{17} + HIM + 2H]$^{2+}$ at *m/z* 1127 was used for the quantification. The relationship between the measured signal intensity at *m/z* 812 and the concentration of histamine was drawn in Fig. 4, showing a linear regression equation lg y = 0.958 ▪ lg x + 12.30 (linearity coefficient R^2 = 0.9835) and a dynamic range of four orders of magnitude from 1 nM to 20 μM. The LOD of histamine was 0.1 nM, which is far lower than those for a previously reported LC-ESI-MS/MS method without derivatization.[9] The relative standard deviation (RSD) varied from 3 % to 11 % for six replicates at different histamine concentrations in the linearity response range.

The method validation for the real sample analysis by ESI-MS/MS

To demonstrate the feasibility of this method for real sample analysis, we chose a red wine for the test. The matrix of the red wine is complex, in which the alkaline

metal ions such as K^+, Na^+ can also induce the formation of thymine clusters. The effects of salt and acidification on the signal intensity of $[T_{17} + HIM + 2H]^{2+}$ were discussed in the supporting information, and the results showed that the addition of the salt or acid resulted in the decrease of the signal intensity of $[T_{17} + HIM + 2H]^{2+}$ (see Fig. S6-S7). Thus, sample dilution is required before the real sample analysis.

The matrix effect of the red wine was evaluated by comparing the MS signal response between the standard solutions and the 100-fold, 500-fold, 1000-fold diluted red wine samples spiked with the standard at the same level. To avoid the disturbance and uncertainty brought by the histamine originally present in the red wine, its isotope labeling reagent, histamine·2HCl [A,A,B,B,-D_4] (short as HIM (D4)), was chosen as the standard. The signal intensities of the fragment ion $[T_{12} + HIM (D4) + 2H]^{2+}$ at *m/z* 814 generated from the isotope internal standard cluster $[T_{17} + HIM (D4) + 2H]^{2+}$ at *m/z* 1129 were compared in Fig. 5. When the red wine was diluted 100 times, the signal of *m/z* 814 was seriously suppressed by the matrix effect to the extent that it could be observed only when the concentration of the internal standard was high up to 1000 nM. However, the inhibitory effect attenuated obviously as the dilution multiple increased. When the red wine sample was diluted by 1000-fold, the signal intensity of *m/z* 814 was in the same order of magnitude with the standard solution at three concentration levels of 10 nM, 100 nM and 1000 nM. It is noted here that the signal response at 10 nM is a little bit higher than the standard solution because of the contribution from the isotopic peaks (See Fig. S8) of histamine originally existed in the red wine. These results demonstrate that the matrix effect of red wine is minor after 1000-fold dilution and the rapid semi-quantitative of histamine in red wine can still be achieved with sufficient sensitivity. The level of the histamine in the diluted red wine was found to be about 10^{-8} mol/L which was comparable with the level obtained by HPLC-MS.[9]

Various approaches have been utilized to overcome the matrix effect.[34-36] In this study, we have used the internal standard to control the variations in the analysis of real samples. The standard solutions containing histamine, the internal standard HIM (D4) (kept as 3.0×10^{-8} M) and thymine were investigated by ESI-MS/MS. The

averaged signal intensities of *m/z* 812 and *m/z* 814 in MS/MS spectra, corresponding to the specific fragments of the doubly charged 17-mer cluster ions at *m/z* 1127 and *m/z* 1129 respectively, were compared. The analytical calibration curve (see Fig. 6) shows a linear response between the analyte-to-internal standard signal intensity ratio (I_{812} / I_{814}) and the concentration of histamine, with a linear regression equation y = 3.057E7 ▪ x + 0.03086 (linearity coefficient R^2 = 0.9992) and a linear range from 1 nM to 80 nM. The precisions were in the 4 % ~ 10 % RSD range for six replicates at different concentrations**.**

The 1000-fold diluted red wine was measured to contain 1.64 × 10^{-8} M of histamine (RSD is 4 % for six replicates). In order to confirm these measured values, various amounts of histamine were added into the diluted red wine on the basis of the measured histamine levels. The recoveries were in the range of 97.8% ~ 104% in all cases (see Table 1.) The good reproducibility and recovery of this method are comparable to the traditional LC methods, indicating that this method can be developed into a routine analysis.

Conclusions

We have developed a novel method using self-assembled magic number cluster of thymine as a "non-covalent mass tag" to determine histamine by ESI-MS/MS. This method has an excellent sensitivity with the limit of detection down to 0.1 nM, because of the high MS signal response of $[T_{17} + HIM + 2H]^{2+}$ and the ensuing mass-shift of histamine to the interference-free higher mass range. Furthermore, this method shows a very good selectivity towards histamine over other biologically relevant analytes, because both amino and imidazole groups of histamine are central in the formation of the magic number cluster $[T_{17} + HIM + 2H]^{2+}$. The application of this method was demonstrated by the analysis of histamine in a red wine. Admittedly, one problem of this method is that the presence of salt or acid could suppress the signal intensity of $[T_{17} + HIM + 2H]^{2+}$, thus sample dilution is necessary to reduce the matrix effect of real samples. However, compared with the traditional LC methods, this method has the advantages of simplicity and speed, because it does not need

tedious sample-preparation, time-consuming covalent derivatization or separation before the analysis.

This work is the first time to use self-assembled magic number clusters of thymine for the specific detection of a target analyte. We believe that it can be a supplement to the existing studies on the quantification of an analyte via the formation of clusters.[29, 37, 38] Our previous work on the magic number clusters of uracil and its homologues using DESI-MS and LDSPI-dual spray MS suggested that the formation of thymine clusters could be on the millisecond time scale. [22, 25, 39] The rapid clustering reactivity of thymine in the gas-phase and the high selectivity for a particular analyte make it possible to directly analyze the analyte present in complex real samples by using such ambient MS techniques. These types of ambient MS experiments are now being explored in our laboratory.

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Table 1. Recovery study of histamine (HIM) added to the 1000-fold diluted red wine

Sample	HIM added (mol/L)	HIM found (mol/L)	RSD (n = 6)	Recovery (%)
1	0(red wine)	1.64×10^{-8}	4%	—
2	10^{-8}	2.62×10^{-8}	8%	99.2%
3	2×10^{-8}	3.56×10^{-8}	3%	97.8%
4	4×10^{-8}	5.88×10^{-8}	5%	104%

RSD: relative standard deviation

FIGURE LEGENDS

Fig. 1 The ESI-MS spectra of (a) 1.0×10^{-5} mol/L histamine (HIM) and (b) the mixture of 1.0×10^{-5} mol/L histamine and 3.0×10^{-4} mol/L thymine (T) in methanol/water (1:1, v: v). The inset in (b) shows the MS/MS spectrum of $[T_{17} + HIM + 2H]^{2+}$ at *m/z* 1127.

Fig. 2 The effect of thymine's concentration on the ESI-MS signal intensity of $[T_{17} + HIM + 2H]^{2+}$ at *m/z* 1127 in single logarithmic scale. The concentration of histamine (HIM) was kept as 1.0×10^{-5} mol/L. Each data point is the average of 4 measurements.

Fig. 3 The ESI-MS signal enhancement effects for histamine (HIM), phenethylamine (PEA), dopamine (DOPA), tryptamine (TRY) and imidazole-4-acetic acid (IAA) (each with the concentration of 1.0×10^{-5} mol/L) after the addition of the clustering agent thymine (T) (3.0×10^{-4} mol/L). Each column represents the average result of 4 replicate measurements; the error bar represents one standard deviation of the data. The break region is from 100 to 200. The inset table shows the detected magic number cluster $[T_m + A + nH]^{n+}$ and the calculated signal magnification factor R ($R = I_{[Tm + A + nH]^{n+}}/I_{[A + H]^{+}}$) for the analyte (A). All the experimental parameters were kept the same during the analysis.

Fig. 4 The correlation of the signal response of the fragment ion $[T_{12} + HIM + 2H]^{2+}$ at *m/z* 812 generated from $[T_{17} + HIM + 2H]^{2+}$ at *m/z* 1127 by using a collision energy of 11 % (the manufacturer's unit) with the concentration of histamine in double logarithmic scales. Each data point designates 6 measurements.

Fig. 5 The matrix effect of the diluted red wine sample. The comparison of signal intensity of the fragment ion $[T_{12} + HIM(D4) + 2H]^{2+}$ at *m/z* 814 generated from $[T_{17} + HIM(D4) + 2H]^{2+}$ at *m/z* 1129 between the standard solutions of

deuterated histamine (HIM(D4)) and the 1000-, 500-, 100-fold diluted red wine samples spiked with 10 nM, 100 nM, 1000 nM HIM(D4) at the same level. Each column represents the average of 4 measurements.

Fig. 6 The analyte-to-internal standard ratio (I_{812} / I_{814}) versus the concentration of histamine. I_{812} and I_{814} represent the signal intensities of $[T_{12} + HIM + 2H]^{2+}$ and $[T_{12} + HIM(D4) + 2H]^{2+}$ in the MS/MS spectra of $[T_{17} + HIM + 2H]^{2+}$ and $[T_{17} + HIM(D4) + 2H]^{2+}$ at *m/z* 1127 and *m/z* 1129 respectively. Each data point designates 6 measurements.

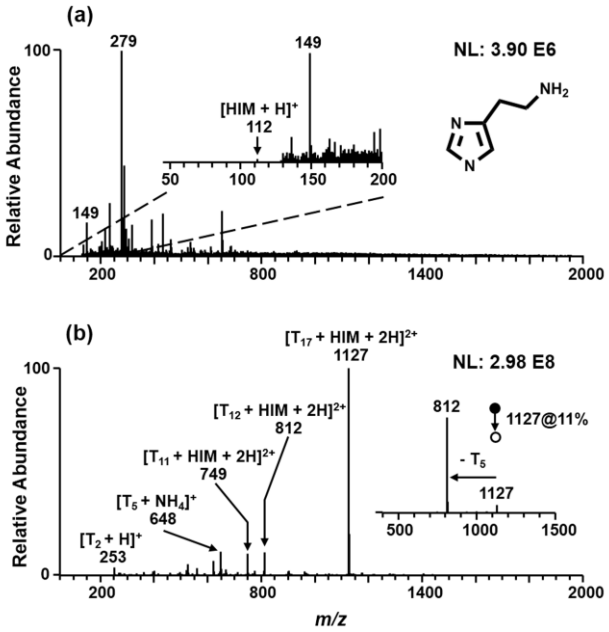


Fig. 1 The ESI-MS spectra of (a) 1.0×10^{-5} mol/L histamine (HIM) and (b) the mixture of 1.0×10^{-5} mol/L histamine and 3.0×10^{-4} mol/L thymine (T) in methanol/water (1:1, v: v). The inset in (b) shows the MS/MS spectrum of $[T_{17} + HIM + 2H]^{2+}$ at *m/z* 1127.

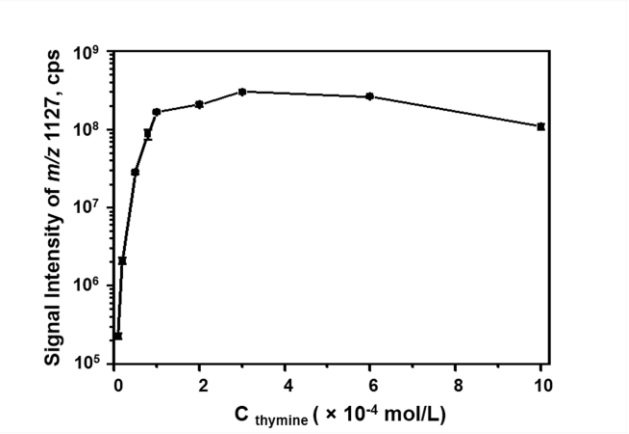


Fig. 2 The effect of thymine's concentration on the ESI-MS signal intensity of $[T_{17}$ + HIM + 2H$]^{2+}$ at *m/z* 1127 in single logarithmic scale. The concentration of histamine (HIM) was kept as 1.0×10^{-5} mol/L. Each data point is the average of 4 measurements.

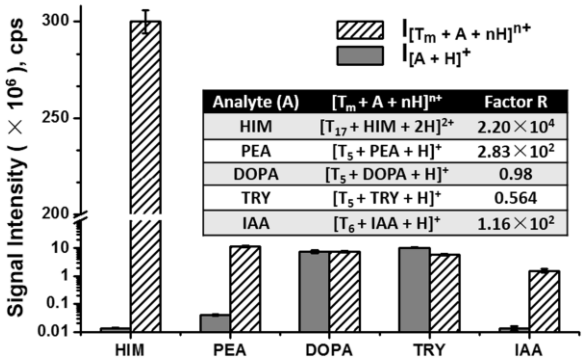


Fig. 3 The ESI-MS signal enhancement effects for histamine (HIM), phenethylamine (PEA), dopamine (DOPA), tryptamine (TRY) and imidazole-4-acetic acid (IAA) (each with the concentration of 1.0×10^{-5} mol/L) after the addition of the clustering agent thymine (T) (3.0×10^{-4} mol/L). Each column represents the average result of 4 replicate measurements; the error bar represents one standard deviation of the data. The break region is from 100 to 200. The inset table shows the detected magic number cluster $[T_m + A + nH]^{n+}$ and the calculated signal magnification factor R ($R = I_{[Tm + A + nH]^{n+}}/I_{[A + H]^{+}}$) for the analyte (A). All the experimental parameters were kept the same during the analysis.

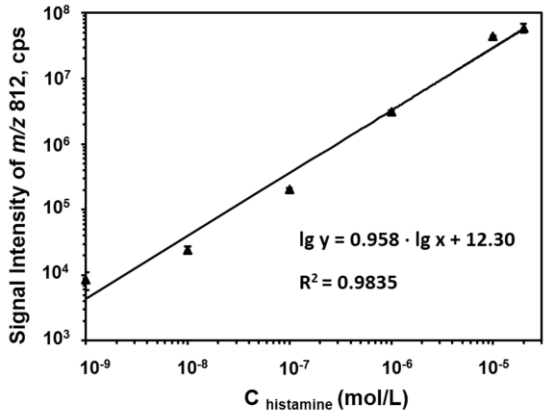


Fig. 4 The correlation of the signal response of the fragment ion $[T_{12} + HIM + 2H]^{2+}$ at *m/z* 812 generated from $[T_{17} + HIM + 2H]^{2+}$ at *m/z* 1127 by using a collision energy of 11 % (the manufacturer's unit) with the concentration of histamine in double logarithmic scales. Each data point designates 6 measurements.

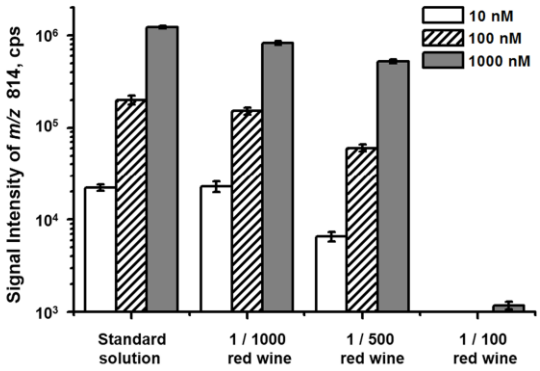


Fig. 5 The matrix effect of the diluted red wine sample. The comparison of signal intensity of the fragment ion $[T_{12} + HIM(D4) + 2H]^{2+}$ at *m/z* 814 generated from $[T_{17} + HIM(D4) + 2H]^{2+}$ at *m/z* 1129 between the standard solutions of deuterated histamine (HIM(D4)) and the 1000-, 500-, 100-fold diluted red wine samples spiked with 10 nM, 100 nM, 1000 nM HIM(D4) at the same level. Each column represents the average of 4 measurements.

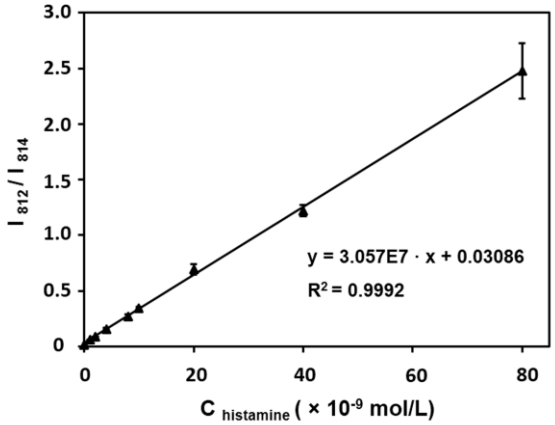
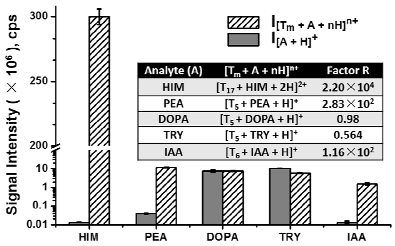


Fig. 6 The analyte-to-internal standard ratio (I_{812} / I_{814}) versus the concentration of histamine. I_{812} and I_{814} represent the signal intensities of $[T_{12} + HIM + 2H]^{2+}$ and $[T_{12} + HIM(D4) + 2H]^{2+}$ in the MS/MS spectra of $[T_{17} + HIM + 2H]^{2+}$ and $[T_{17} + HIM(D4) + 2H]^{2+}$ at *m/z* 1127 and *m/z* 1129 respectively. Each data point designates 6 measurements.

Graphical abstract:



The self-assembled magic number cluster of thymine (T) significantly enhanced the MS signal of histamine with high specificity.