Supporting Information

Influence of reactive species on the modification of biomolecules generated from the soft plasma

Pankaj Attri[1], Naresh Kumar[1], Ji Hoon Park[1], Dharmendra Kumar Yadav[2], Sooho Choi[3], Han S. Uhm[1], In Tae Kim[4], Eun Ha Choi[1*], Weontae Lee[3*]

[1]*Plasma Bioscience Research Center / Department of Electrical and Biological Physics, Kwangwoon University, Seoul, Korea 139-701.*
[2]*Laboratory of Nanoscale Characterization & Environmental Chemistry, Department of Chemistry, College of Natural Sciences, Hanyang University, Seoul 133-791,Korea*
[3]*Department of Biochemistry, College of Life Science & Biotechnology, Yonsei University, Seoul, 120-749, Korea*
[4]*Department of Chemistry, Kwangwoon University, Seoul, Korea 139-701*

Calculation for generation of OH radicals in N_2 plasma.

In order to understand the reason for more generation of H_2O_2, we have to understand the generation of OH radicals in N_2 plasma. One of the most important reactive species in the N_2 plasma are the excited nitrogen molecules in a metastable level of $N_2(A_3\Sigma_u^+)$, which dissociate water molecules generating hydroxyl radicals and hydrogen atoms. The excitation energy $\varepsilon*$ of the metastable level is $\varepsilon* = 6.8$ eV and the excitation coefficient α_{N2*} of nitrogen molecules by the plasma electrons is given[1] as:

$$\alpha_{N2*}(T_e) = \frac{2}{\sqrt{\pi}} q v_{th} (\varepsilon * + 2T_e) \exp\left(-\frac{\varepsilon *}{T_e}\right) \quad (1)$$

Where v_{th} is the electron thermal speed. For the electron temperature $T_e = 1$ eV, the excitation coefficient in Eq. (1) is calculated as $\alpha_{N2*} = 6.4\times10^{-12}$cm^3/s. The nitrogen molecules in the excited metastable state will dissociate the water molecules represented as $N_2(A_3\Sigma_u^+) + H_2O \rightarrow OH + H + N_2$ with dissociation coefficient[2] of $\alpha_{HO} = 5\times10^{-14}$ cm^3/s and then returning back to the ground state of N_2. This quenching mechanism of excited molecules is dominant in the atmospheric nitrogen in vicinity of abundant water molecules. Therefore, the rate equation of the nitrogen molecules in the excited metastable state is

$$\frac{dn_{N2*}}{dt} = \alpha_{N2*} n_{N2} n_p - \alpha_{HO} n_{N2*} n_{H2O}, \quad (2)$$

Where n_{N2} and n_{H2O} are nitrogen and water molecular densities, respectively, and n_p is the plasma density. The nitrogen molecules in the metastable state can be estimated in steady-state with $dn/dt = 0$ given by $n_{N2*} = \alpha_{N2*}n_{N2}n_p/\alpha_{HO}n_{H2O} = (6.4\times10^{-13}\text{cm}^3/\text{s})(2.6\times10^{19}/\text{cm}^3)(10^{12}/\text{cm}^3)/(5\times10^{-14}\text{cm}^3/\text{s})/(1\times10^{17}/\text{cm}^3) = 3.3\times10^{15}/\text{cm}^3$ for the plasma density of $n_p = 10^{12}/\text{cm}^3$. This is the estimated number of the nitrogen molecular density in the metastable state. There are many ways to eliminate the hydrogen atoms including OH + H + M $\rightarrow$ H_2O + M with its rate coefficient[3] of $\alpha_{H2O} = 4.38\times10^{-30}(T_r/T)^2$ $\text{cm}^6/\text{mole}^2/\text{s} = 1.14\times10^{-10}$ $\text{cm}^3/\text{mole/s}$ in air at T= 300 K, which is the most dominant reaction in the N_2 gas. Therefore, in the steady-state case, assuming that the hydroxyl density is about $n_{OH} = 5\times10^{15}/\text{cm}^3$, the hydrogen density can be calculated to be

$$n_H = \alpha_{HO}n_{N2*}n_{H2O}/\alpha_{H2O}/n_{OH} = (5\times10^{-14}\text{cm}^3/\text{s})(3.3\times10^{15}/\text{cm}^3)(1\times10^{17}/\text{cm}^3)/(1.14\times10^{-10}\text{cm}^3/\text{s})/(5\times10^{15}/\text{cm}^3)=3\times10^{13}/\text{cm}^3$$

which gives the estimate of the hydrogen density in the nitrogen plasma. Meanwhile, the OH radicals may get disappeared by many ways. The leading reactions of OH eliminations are as: OH + OH + M $\rightarrow$ H_2O_2 + M with its rate coefficient[4] of $\alpha_{H2O2} = 6.83\times10^{-31}(T_r/T)^{0.8}$ $\text{cm}^6/\text{mole}^2/\text{s} = 1.78\times10^{-11}\text{cm}^3/\text{mole/s}$ in gas at T= 300K. In order to find the OH radical density in the steady-state solution, we considered OH generation in N_2 plasma using $\alpha_{N2*}n_{N2}n_p = \alpha_{HO}n_{N2*}n_{H2O} = \alpha_{H2O2}n_{OH}^2$, which is expressed as $n_{OH}^2 = \alpha_{N2*}n_{N2}n_p/\alpha_{H2O2}$. Therefore, the hydroxyl density estimation is $n_{OH}\approx 3.06\times10^{15}/\text{cm}^3$. Therefore, the hydroxyl density in the nitrogen plasma is estimated to be several times of $10^{15}/\text{cm}^3$. It is important to investigate the generation of NH radicals. The hydrogen atom density is estimated as $n_H = 3\times10^{13}/\text{cm}^3$. The dissociation coefficient of nitrogen molecules by N_2 + e $\rightarrow$ N + N + e due to the electron impact is given by

$$k_N(T_e) = 4.26\times10^{-10}\sqrt{T_e}(10+2T_e)\exp\left(-\frac{10}{T_e}\right), \quad (3)$$

which is $k_N = 1\times10^{-12}$ cm^3/s at T_e = 1eV. Atomic nitrogen may disappear by forming NH which can be represented as, N + H $\rightarrow$ NH with its reaction coefficient[5] of $\alpha_{NH} = 1.3\times10^{-12}\text{cm}^3/\text{s}$. But, the most dominant reaction may be N + OH $\rightarrow$ NO + H with its reaction coefficient[6] of $\alpha_{NO} = 4.7\times10^{-11}\text{cm}^3/\text{s}$.

The rate equation of nitrogen atom is given by

$$\frac{dn_N}{dt} = k_N n_{N2} n_P - \alpha_{NO} n_N n_{OH}, \tag{4}$$

whose steady-state solution is $n_N = k_N n_{N2} n_p / \alpha_{NO} n_{OH} = 1.1\times10^{14}/\text{cm}^3$. This is the approximated number of atomic nitrogen for $n_{OH} = 5 \times10^{15}/\text{cm}^3$. We can see a considerably high atomic nitrogen density. These atomic nitrogen and hydrogen atoms combine to form NH radical. which may disappear through the reaction of NH + OH forming NH_2, or HNO, or H_2O with its reaction coefficient[3] of $\alpha_M = 8\times10^{-11}\text{cm}^3/\text{s}$. The rate equation of NH radical is expressed as

$$\frac{dn_{NH}}{dt} = \alpha_{NH} n_H n_N - \alpha_M n_{NH} n_{OH} \tag{5}$$

whose steady-state solution with $dn_{NH}/dt = 0$ is given by $n_{NH} = \alpha_{NH} n_H n_N / \alpha_M n_{OH} = 1.07\times10^{10}/\text{cm}^3$. The density of NH radical in comparison with the hydroxyl density was found to be very low. However, discharge plasma in the nitrogen gas mixed with water molecules generates the NH radicals, but the density of NH radicals is 5 orders in magnitude less than the hydroxyl density. This suggests that hydroxyl radicals are the dominating species in the nitrogen plasma and which in turn react with each other to form hydrogen peroxides.

References

1. Uhm, H. S. *et al.* Dissociation and excitation coefficients of nitrogen molecules and nitrogen monoxide generation. *Phys Plasmas* **29**, 083502-083506 (2013).

2. Herron, J. T. Evaluated chemical kinetics data for reactions of N(2D), N(2P), and $N_2(A_3\Sigma_u^+)$ in the gas phase. *J Phys Chem Ref Data* **28**, 1453–1483 (1999).

3. Baulch, D. L. *et al.* Evaluated Kinetic Data for Combustion Modeling: Supplement II. *J Phys Chem Ref Data* **21**, 411–429 (1992).

4. Forster, R. *et al.* High pressure range of the addition of HO to HO, NO, NO2, and CO. I. Saturated laser induced fluorescence measurements at 298 K. *J Chem Phys* **103**, 2949 (1995).

5. Brown, R. L. A Measurement of the Rate of the Reaction N + H + M → NH + M. *Int J Chem Kinet* **5**, 663-668 (1973).

6. Baulch, D. L. *et al.* Evaluated kinetic data for combustion modeling. Supplement I. *J Phys Chem Ref Data* **23**, 847– 1033 (1994).

Figure Captions

Fig. S1. Conversion of TA to HTA using different feeding gases plasma for 3 min using fluorescence spectroscopy.

Fig. S2. The emission spectra of atmospheric-pressure plasma jet (APPJ) with Air as feeding gas.

Fig. S3. The emission spectra of atmospheric-pressure plasma jet (APPJ) with N_2 as feeding gas.

Fig. S4. The emission spectra of atmospheric-pressure plasma jet (APPJ) with Ar as feeding gas.

Fig. S5. The emission spectra of atmospheric-pressure plasma jet (APPJ) for NO/N_2 at 337 nm in all feeding gases plasma.

Fig. S6. (a) pH and (b) Temperature for all feeding gases plasma for 3 min treatment. All values are expressed as ± SD in triplicates. Students't-test was performed to control (* denotes $P<0.05$, ** denotes $P<0.01$).

Fig. S7. Far-UV CD spectra analysis of (a) Hb and (b) Mb at different concentration of H_2O_2. The data points are average values of at least six determinations, the error bars indicating ± mean deviation.

Fig. S8. Melting temperature determination of Hb using the differential scanning calorimetry (DSC). The data points are average values of at least three determinations.

Fig. S9. Melting temperature determination of Mb using the differential scanning calorimetry (DSC). The data points are average values of at least three determinations.

Fig. S10. Melting temperature determination of Hb using the circular dichroism (CD). The data points are average values of at least three determinations.

Fig. S11. Melting temperature determination of Mb using the circular dichroism (CD). The data points are average values of at least three determinations.

Fig. S12. Fluorescence analysis of (a) Hb and (b) Mb at different concentration of H_2O_2. The data points are average values of at least six determinations.

Fig. S13. Gel electrophoresis of Hb and Mb**.**

Fig. S14. UV-vis spectroscopy of (a) Hb in different feeding gases; (b) Mb in different feeding gases; (c) Hb in different concentration of H_2O_2 and (d) Mb in different concentration of H_2O_2. The data points are average values of at least three determinations.

Fig. S15: ^{1}H NMR of Hb (blue) and Hb with 60 μM of H_2O_2 (red).

Fig. S16: ^{1}H NMR of Mb (blue) and Mb with 60 μM of H_2O_2 (red).

Fig. S17. Liquid Chromatograph /Capillary Electrophoresis- Mass Spectrometer (LC/CE-MS) based qualitative bioanalysis of Glycine, Glycine + Ar, Glycine + Air and Glycine + N_2.

Fig. S18. Liquid Chromatograph /Capillary Electrophoresis- Mass Spectrometer (LC/CE-MS) based qualitative bioanalysis of Glutamic acid, Glutamic acid + Ar, Glutamic acid + Air and Glutamic acid + N_2.

Fig. S19. Liquid Chromatograph /Capillary Electrophoresis- Mass Spectrometer (LC/CE-MS) based qualitative bioanalysis of Asparagine, Asparagine + Ar, Asparagine + Air and Asparagine + N_2.

Fig. S20. Liquid Chromatograph /Capillary Electrophoresis- Mass Spectrometer (LC/CE-MS) based qualitative bioanalysis of Arginine, Arginine + Ar, Arginine + Air and Arginine + N_2.

Fig. S21. Liquid Chromatograph /Capillary Electrophoresis- Mass Spectrometer (LC/CE-MS) based qualitative bioanalysis of Alanine, Alanine + Ar, Alanine + Air and Alanine + N_2.

Fig. S22. Liquid Chromatograph /Capillary Electrophoresis- Mass Spectrometer (LC/CE-MS) based qualitative bioanalysis of Threonine, Threonine + Ar, Threonine + Air and Threonine + N_2.

Fig. S23. Liquid Chromatograph /Capillary Electrophoresis- Mass Spectrometer (LC/CE-MS) based qualitative bioanalysis of Proline, Proline + Ar, Proline + Air and Proline + N_2.

Fig. S24. Liquid Chromatograph /Capillary Electrophoresis- Mass Spectrometer (LC/CE-MS) based qualitative bioanalysis of Lysine, Lysine + Ar, Lysine + Air and Lysine + N_2

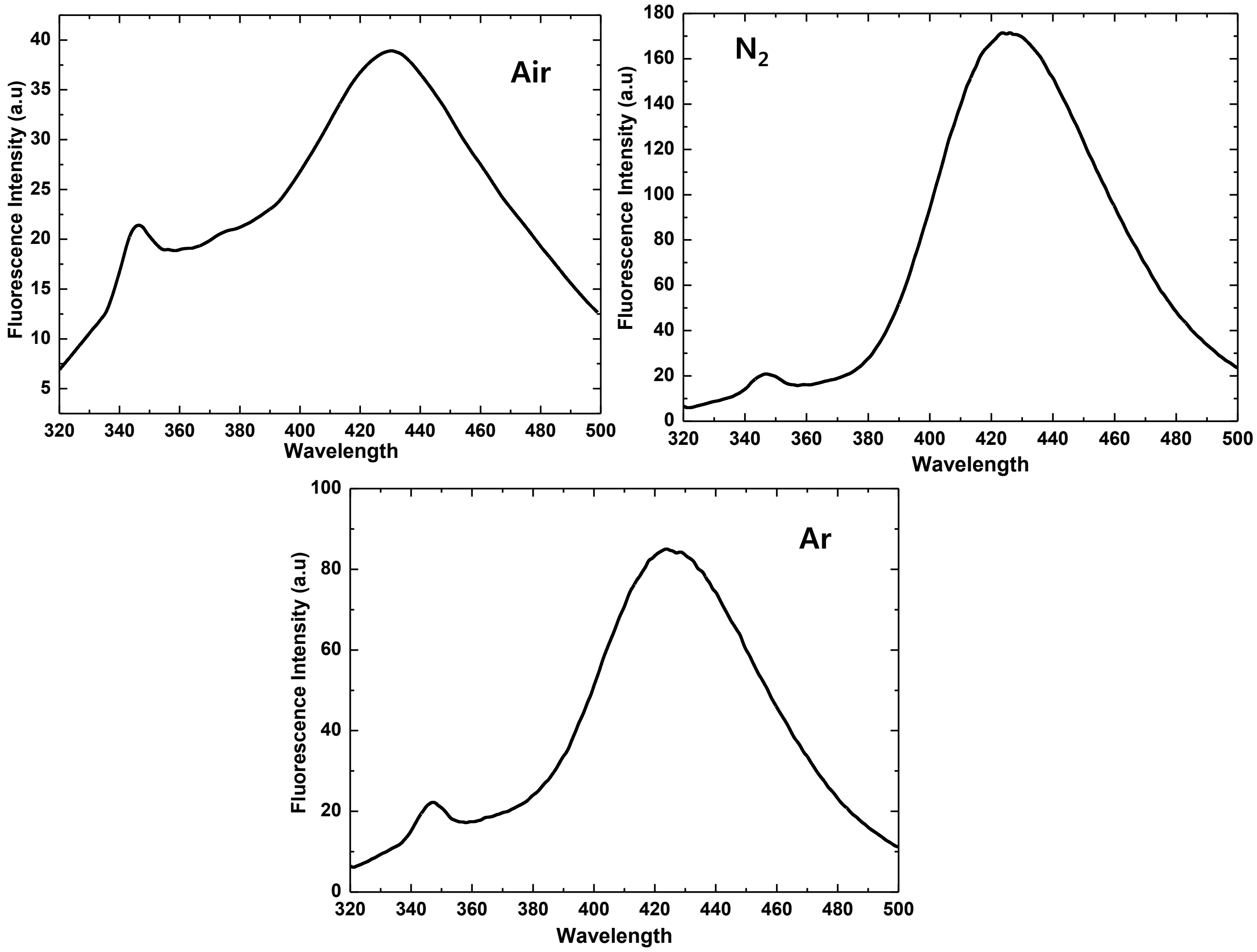

Air
Fluorescence Intensity (a.u)
Wavelength
N2
Fluorescence Intensity (a.u)
Wavelength
Ar
Fluorescence Intensity (a.u)
Wavelength

Fig. S1.

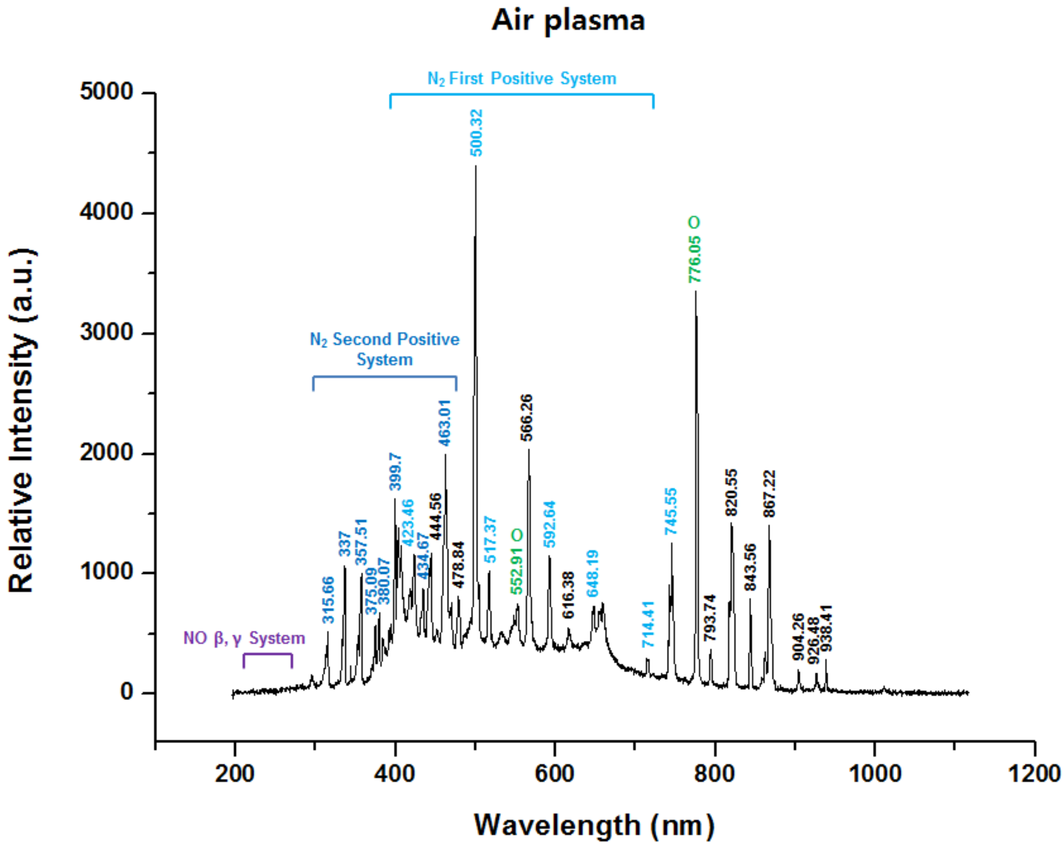
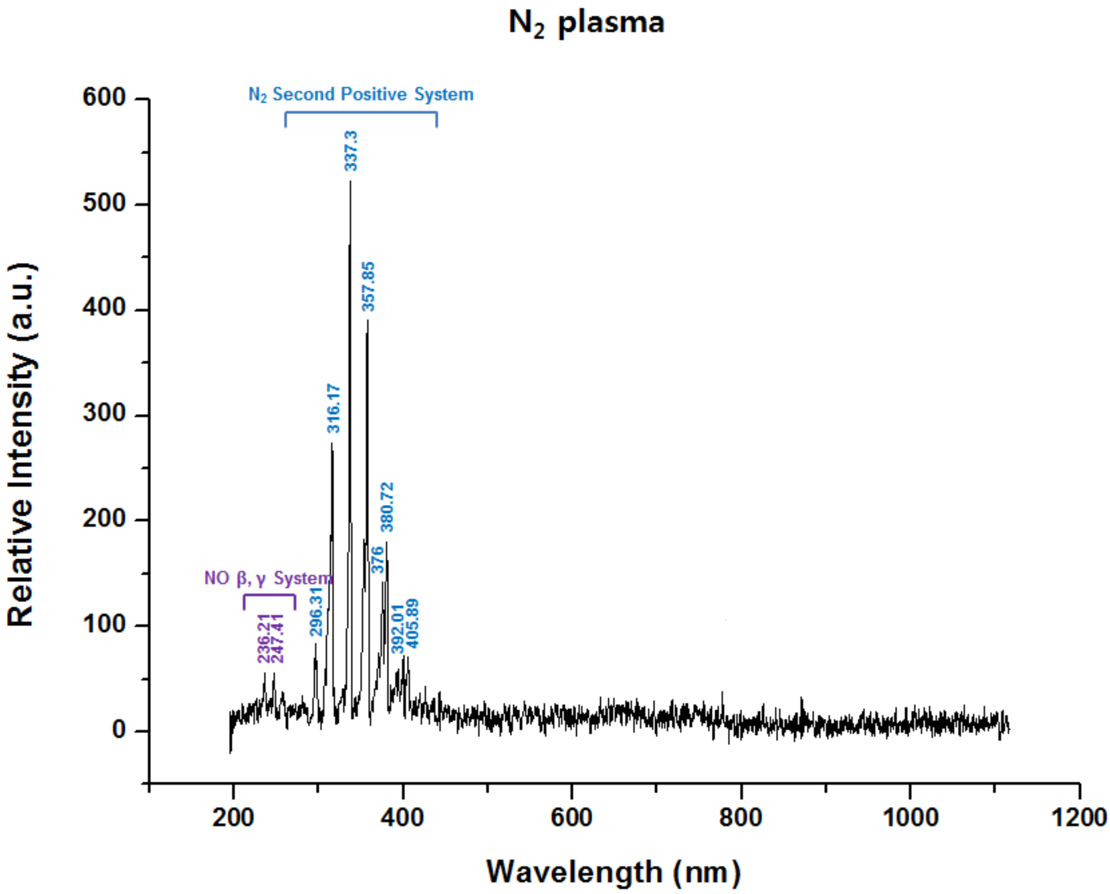

Air plasma
N2 First Positive System
N2 Second Positive System
NO β, γ System
Relative Intensity (a.u.)
Wavelength (nm)
500.32
776.05
O
463.01
566.26
399.7
820.55
867.22
745.55
444.56
423.46
337
357.51
434.67
592.64
517.37
O
552.91
648.19
478.84
616.38
315.66
375.09
380.07
714.41
793.74
843.56
904.26
926.48
938.41
0
1000
2000
3000
4000
5000
200
400
600
800
1000
1200

Fig. S2



N2 plasma
N2 Second Positive System
NO β, γ System
337.3
357.85
316.17
380.72
376
296.31
392.01
405.89
236.21
247.41
Relative Intensity (a.u.)
Wavelength (nm)
600
500
400
300
200
100
0
200
400
600
800
1000
1200

Fig. S3

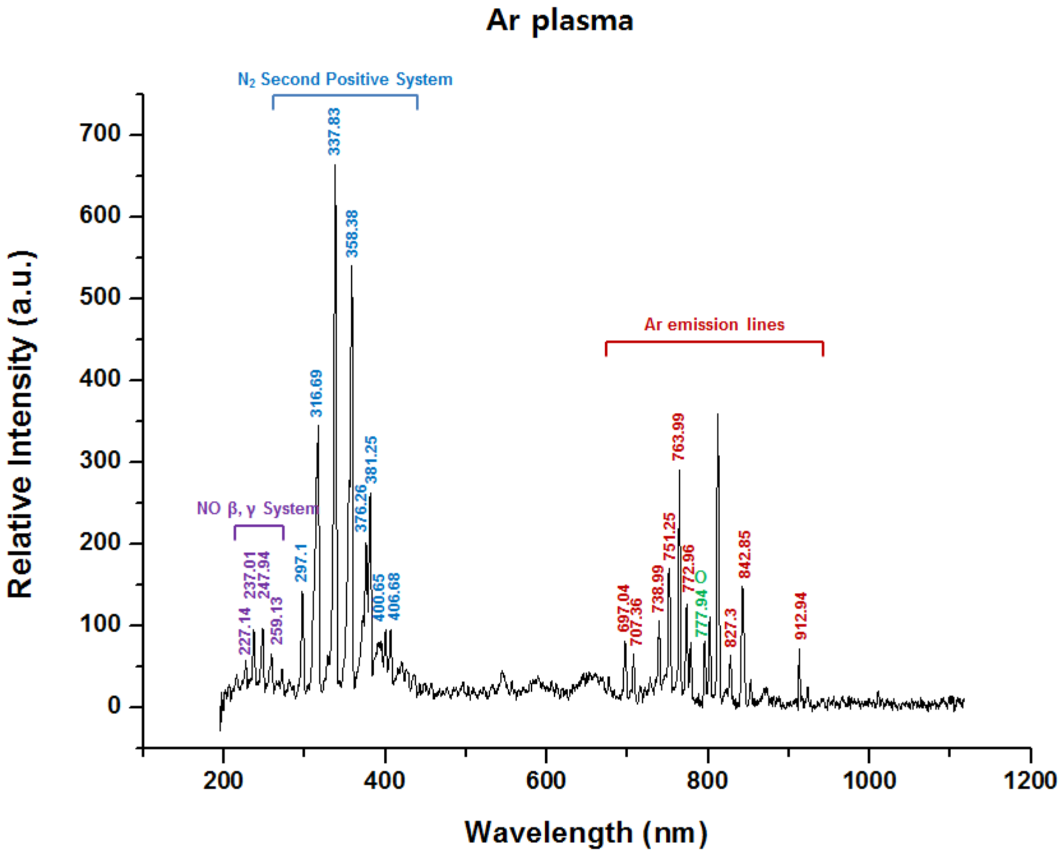

Ar plasma
N2 Second Positive System
NO β, γ System
Ar emission lines
Relative Intensity (a.u.)
Wavelength (nm)
227.14
237.01
247.94
259.13
297.1
316.69
337.83
358.38
376.26
381.25
400.65
406.68
697.04
707.36
738.99
751.25
763.99
772.96
777.94
O
827.3
842.85
912.94
0
100
200
300
400
500
600
700
200
400
600
800
1000
1200

Fig. S4

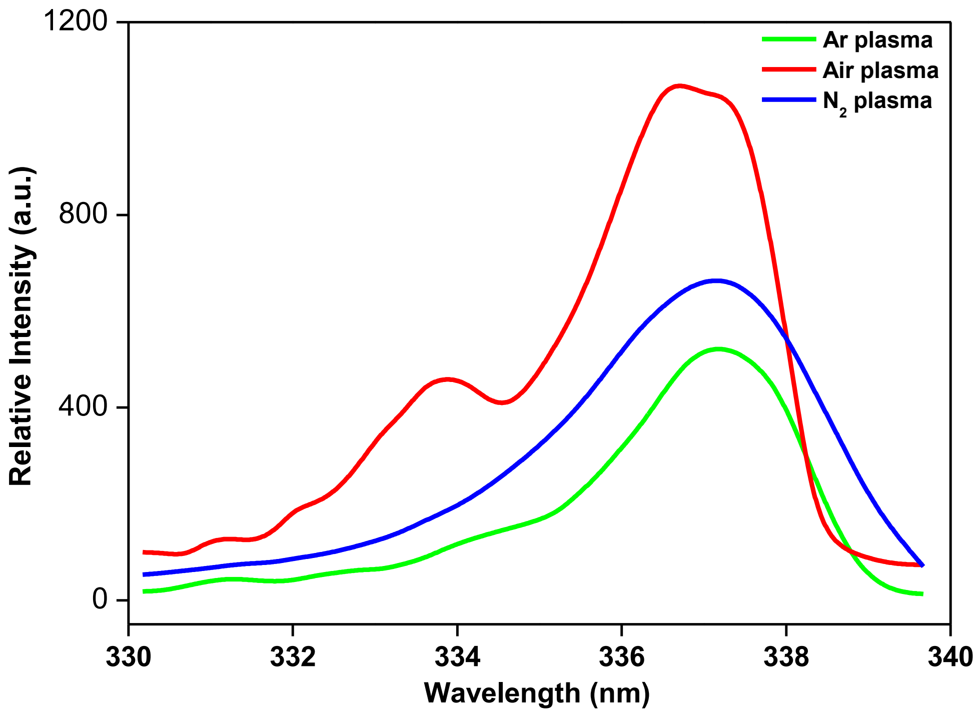

1200
800
400
0
Relative Intensity (a.u.)
330
332
334
336
338
340
Wavelength (nm)
Ar plasma
Air plasma
N2 plasma

Fig. S5

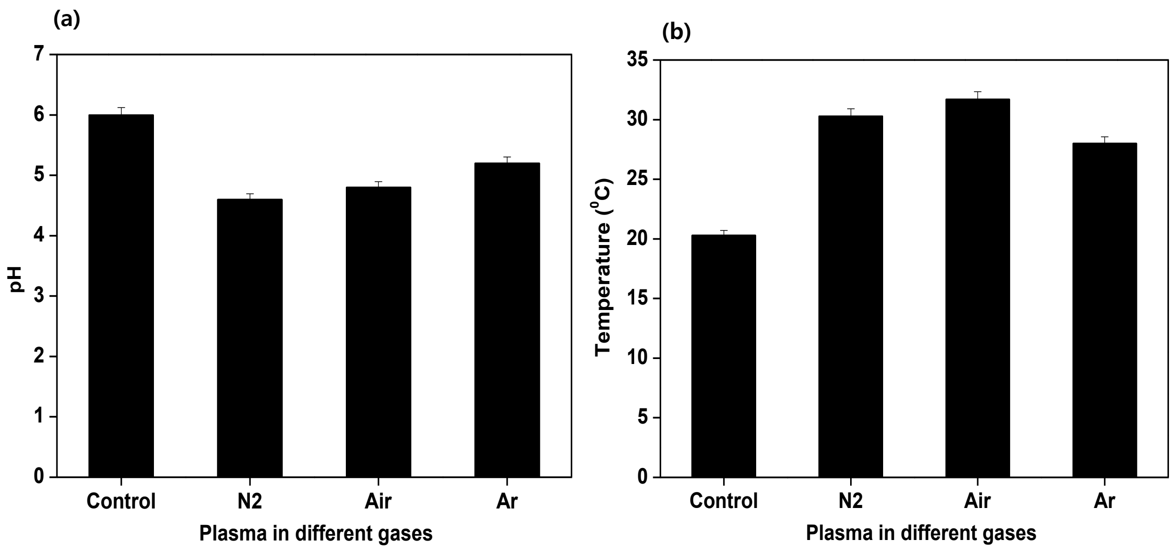

(a)
pH
7
6
5
4
3
2
1
0
Control
N2
Air
Ar
Plasma in different gases
(b)
Temperature (°C)
35
30
25
20
15
10
5
0
Control
N2
Air
Ar
Plasma in different gases

Fig. S6

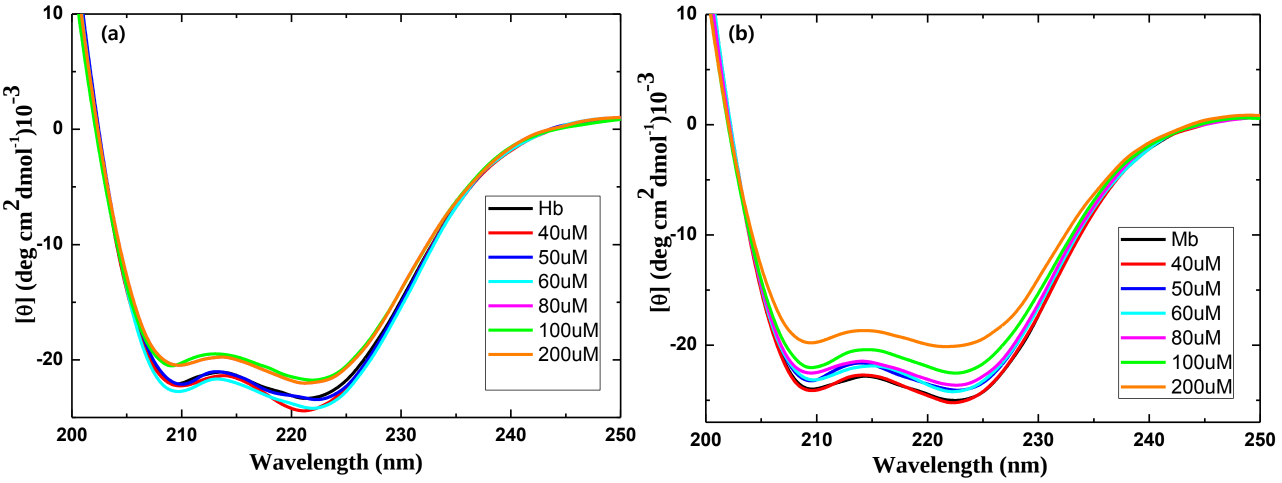

(a)
[θ] (deg cm2dmol-1)10-3
Wavelength (nm)
Hb
40uM
50uM
60uM
80uM
100uM
200uM
(b)
Mb
40uM
50uM
60uM
80uM
100uM
200uM

Fig. S7

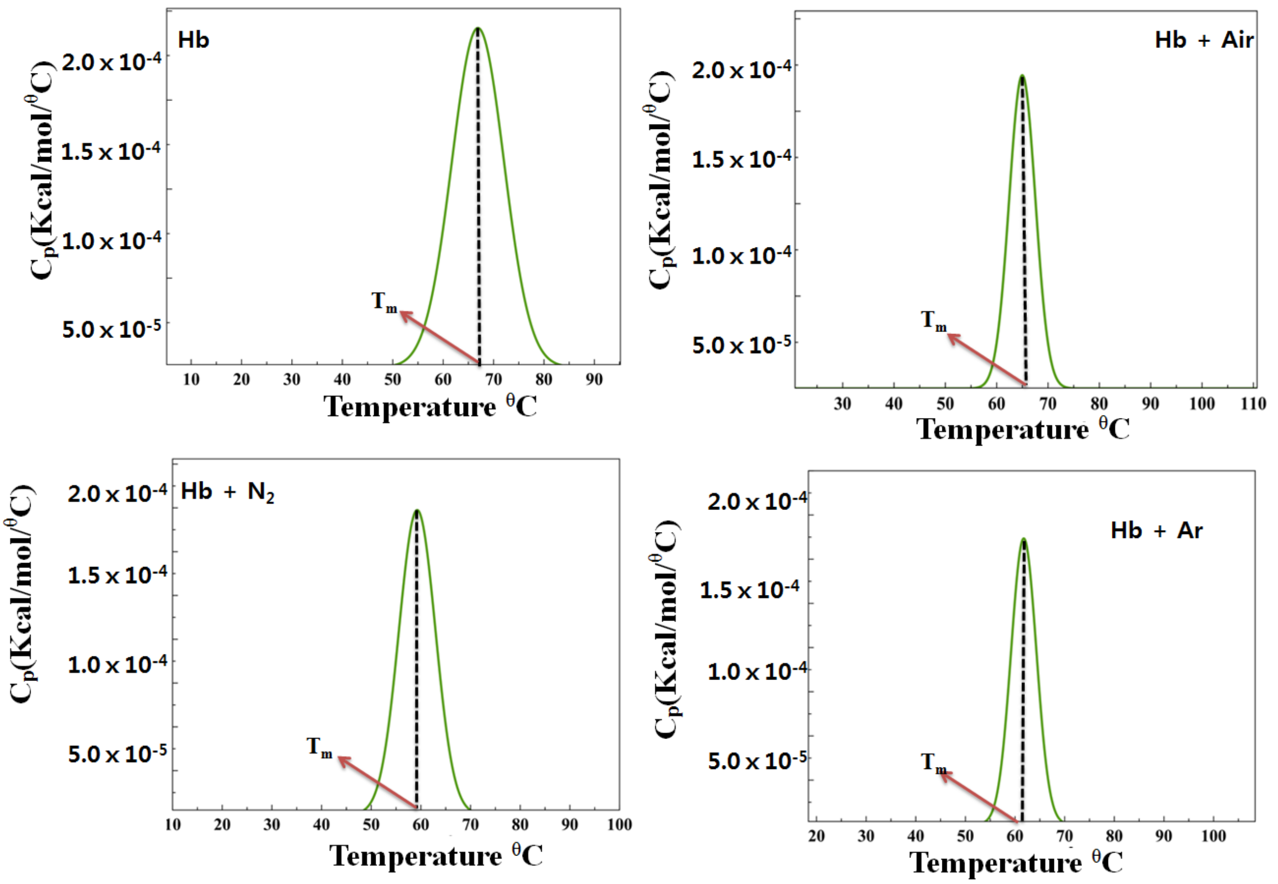

Hb
Hb + Air
Hb + N2
Hb + Ar
Cp(Kcal/mol/θC)
Temperature θC
Tm

Fig. S8

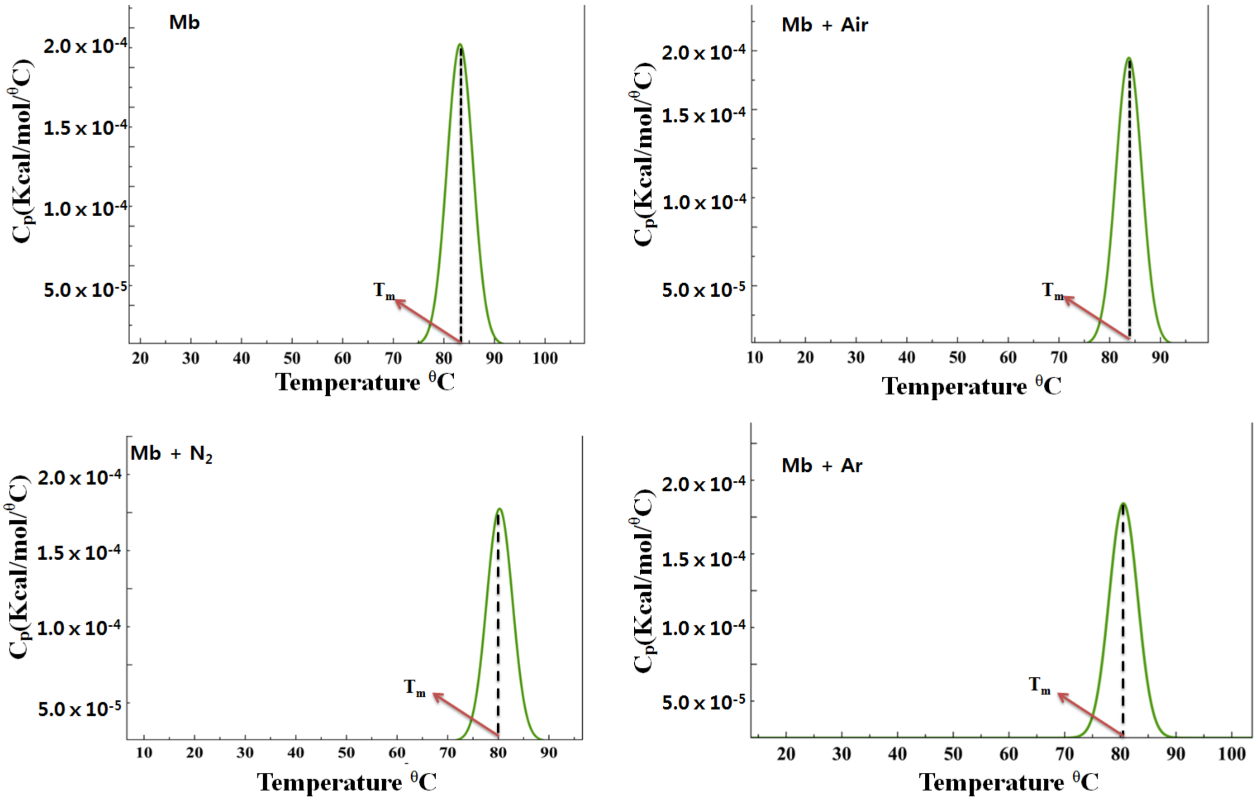

Mb
Mb + Air
Mb + N2
Mb + Ar
Tm
Cp(Kcal/mol/θC)
Temperature θC
2.0 x 10-4
1.5 x 10-4
1.0 x 10-4
5.0 x 10-5
10
20
30
40
50
60
70
80
90
100

Fig. S9

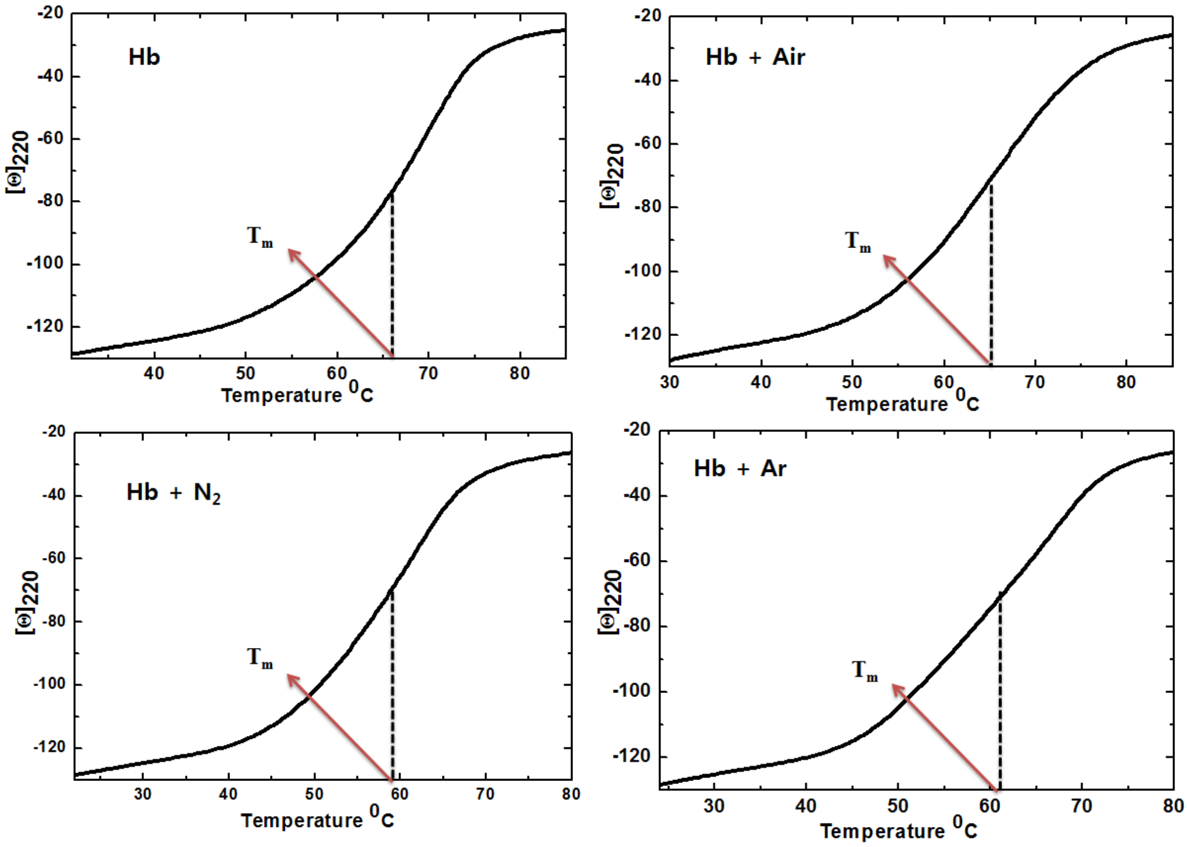

Hb
Hb + Air
Hb + N2
Hb + Ar
T m
[Θ]220
Temperature 0C
-20
-40
-60
-80
-100
-120
30
40
50
60
70
80

Fig. S10

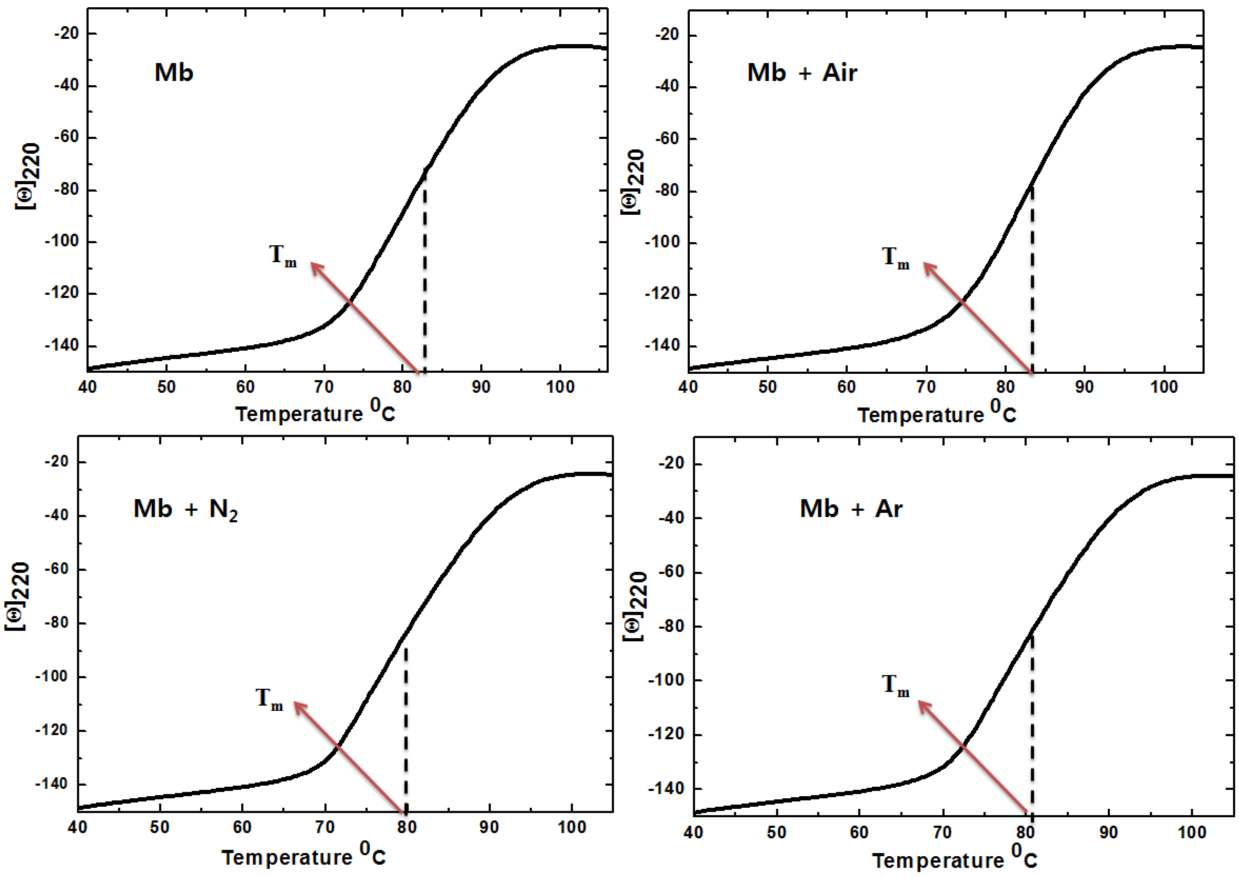


Fig. S11

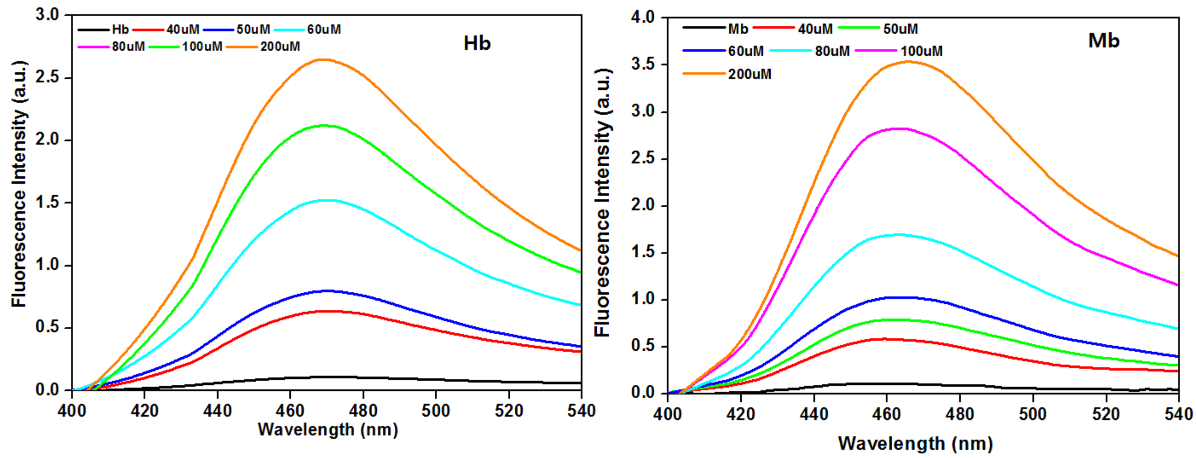


Fig. S12

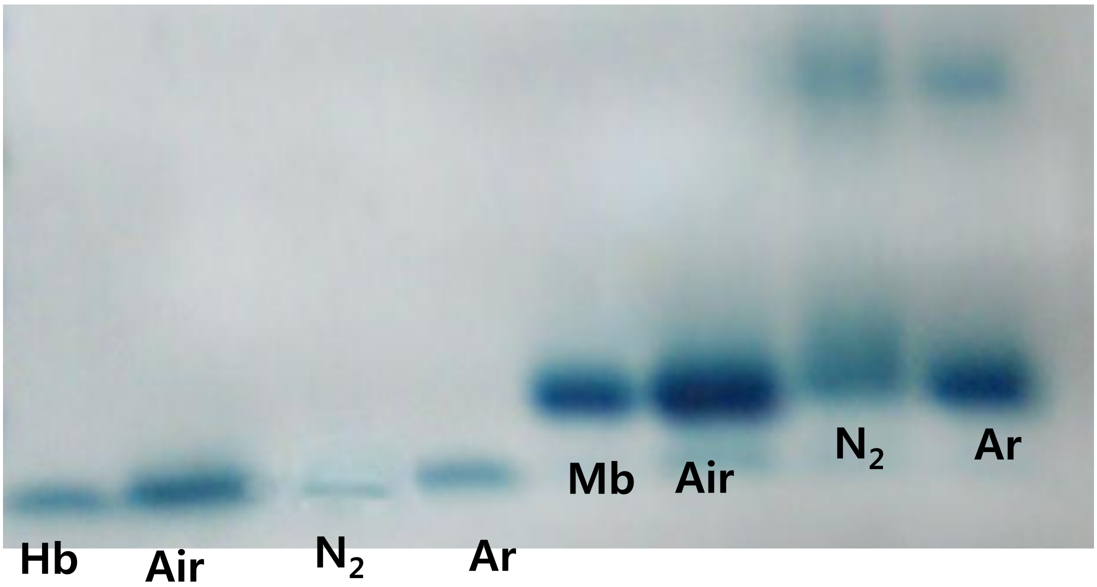


Fig. S13

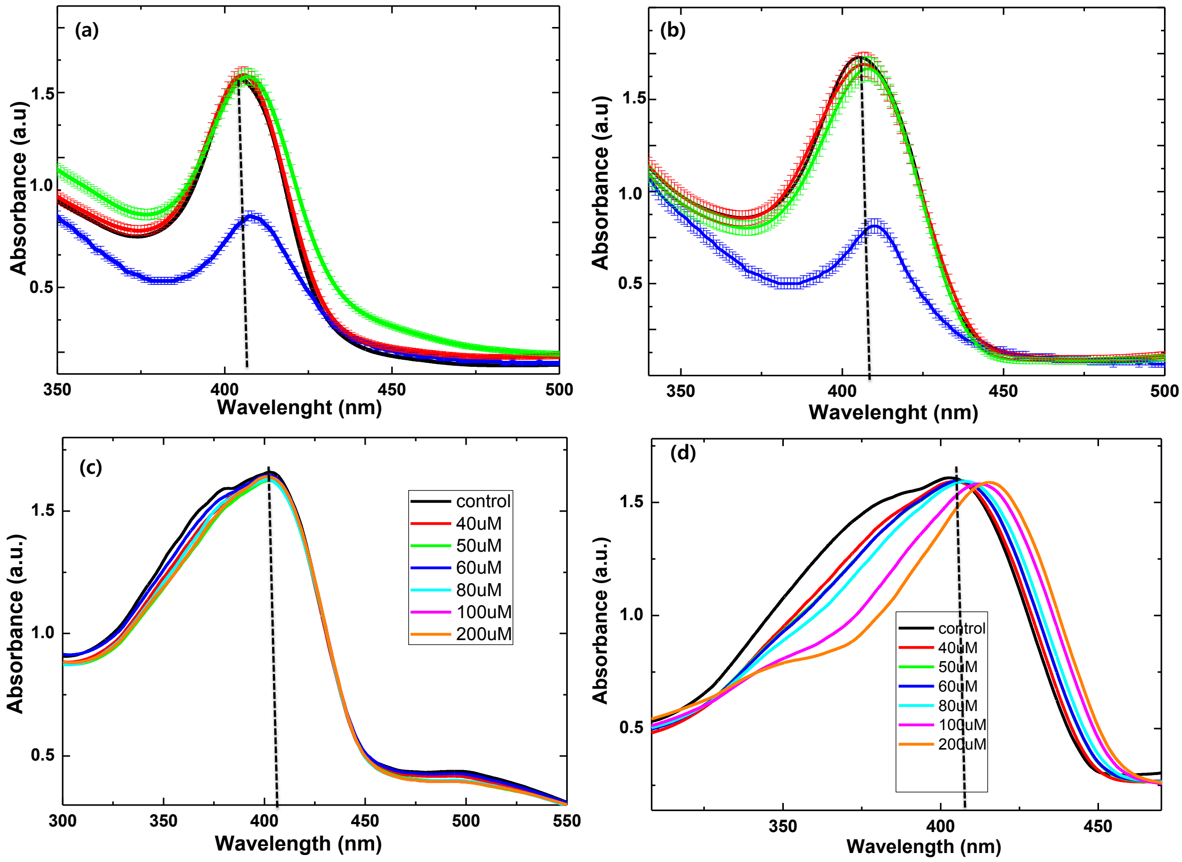


Fig. S14

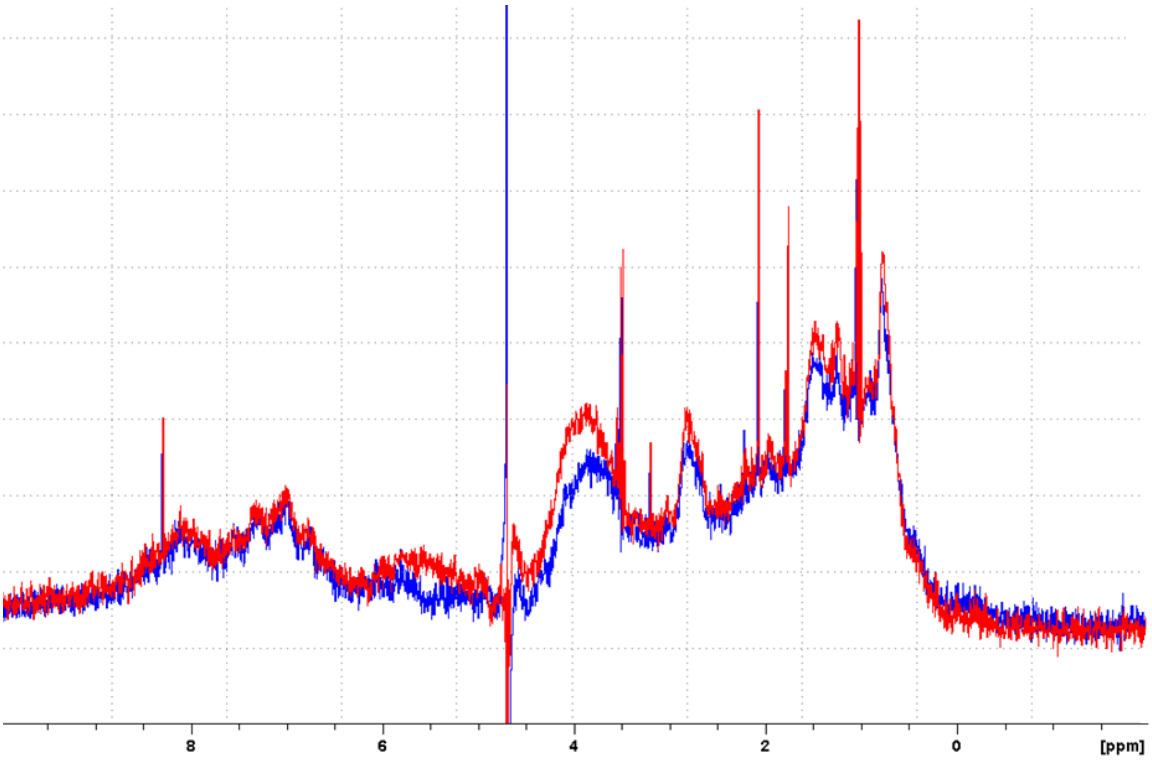


Fig. S15

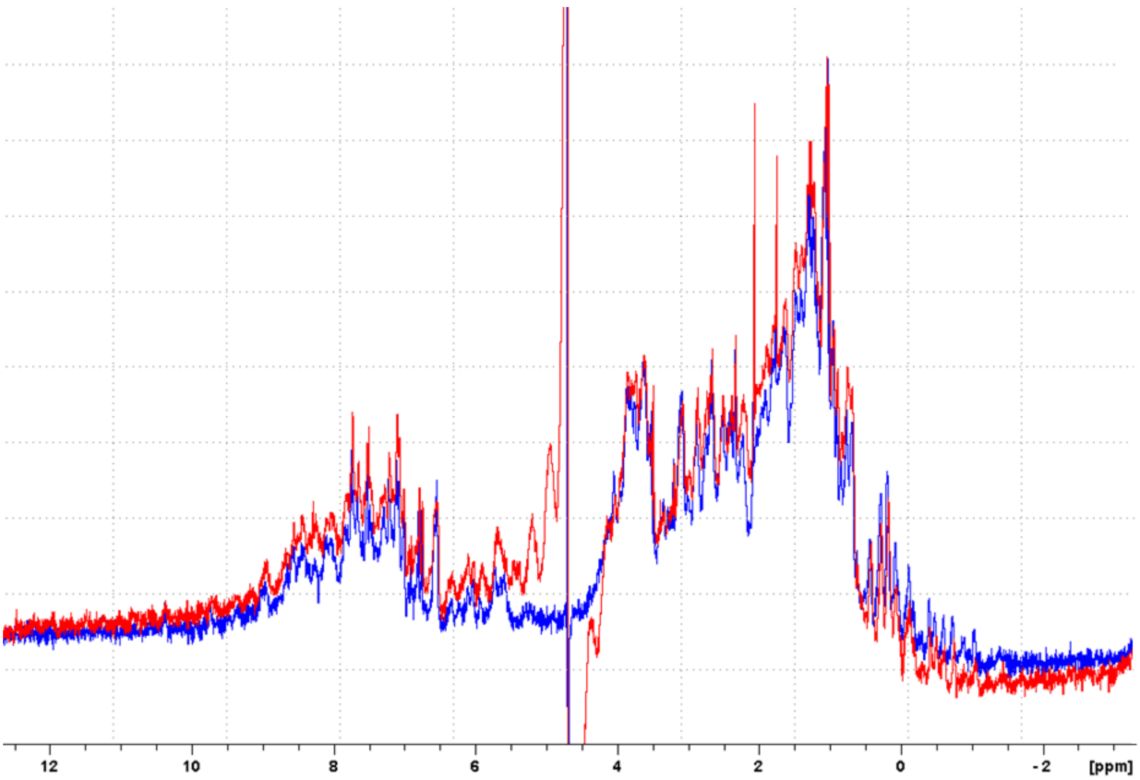
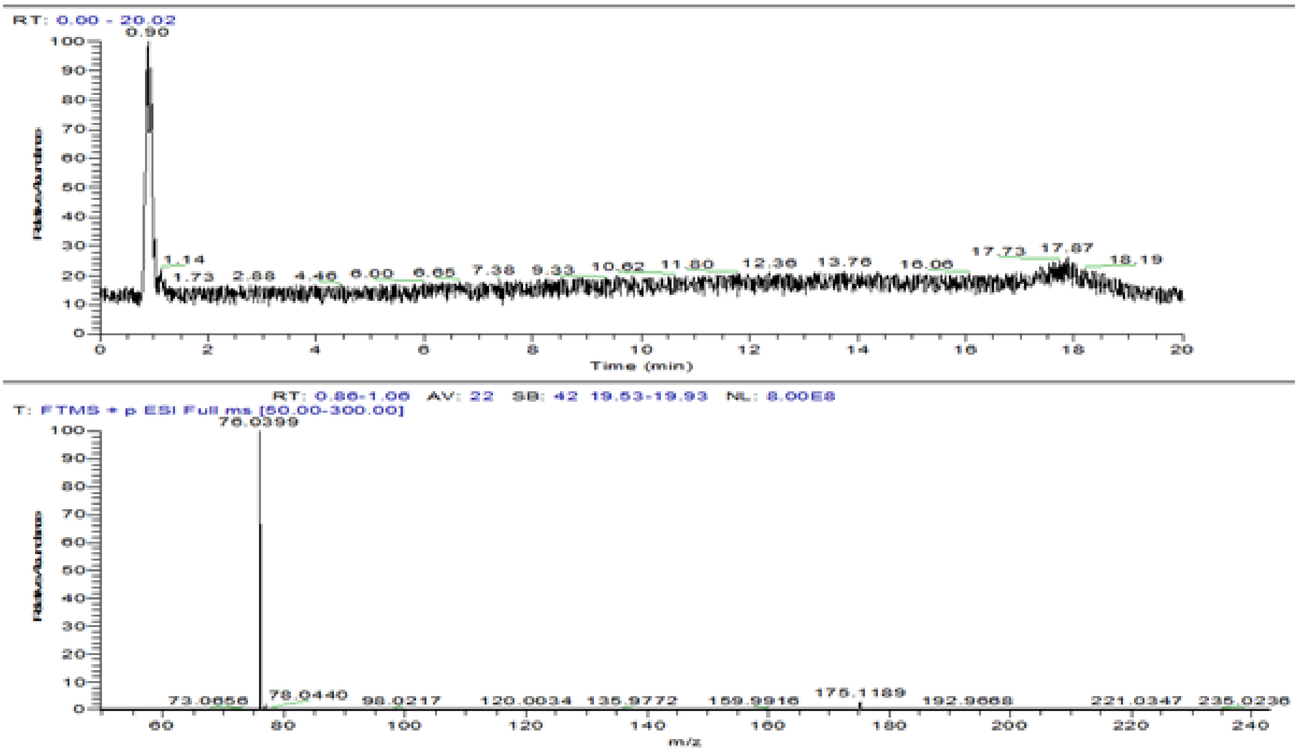
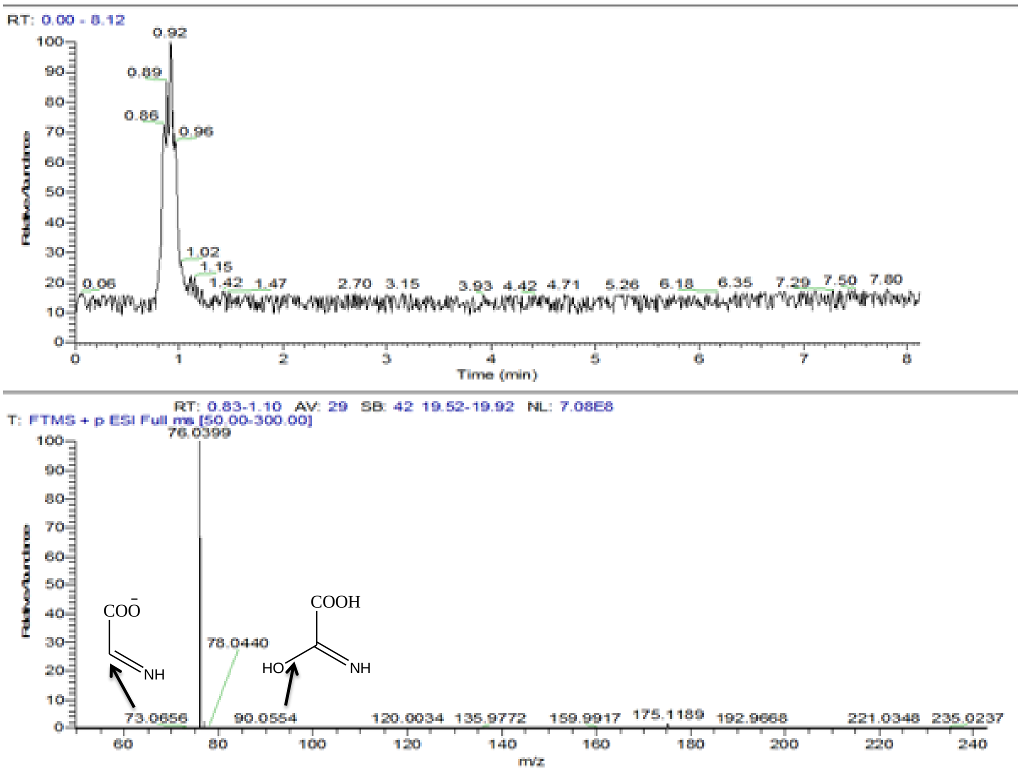


Fig. S16

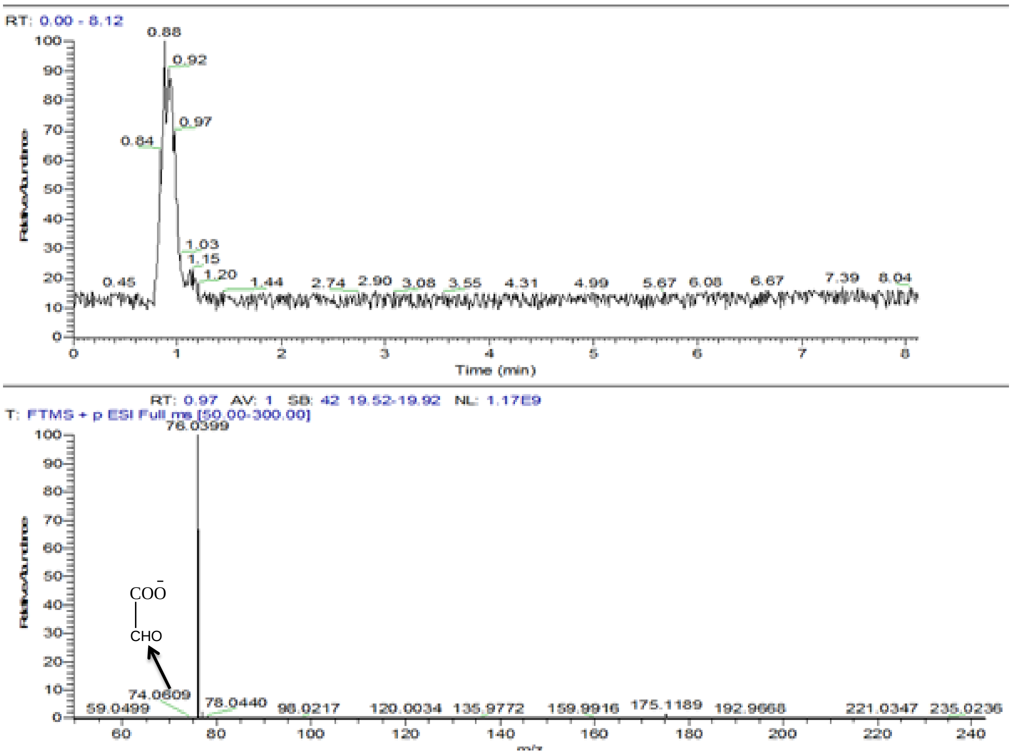
Glycine Control



Glycine + Ar



Glycine + N_2



Glycine + Air

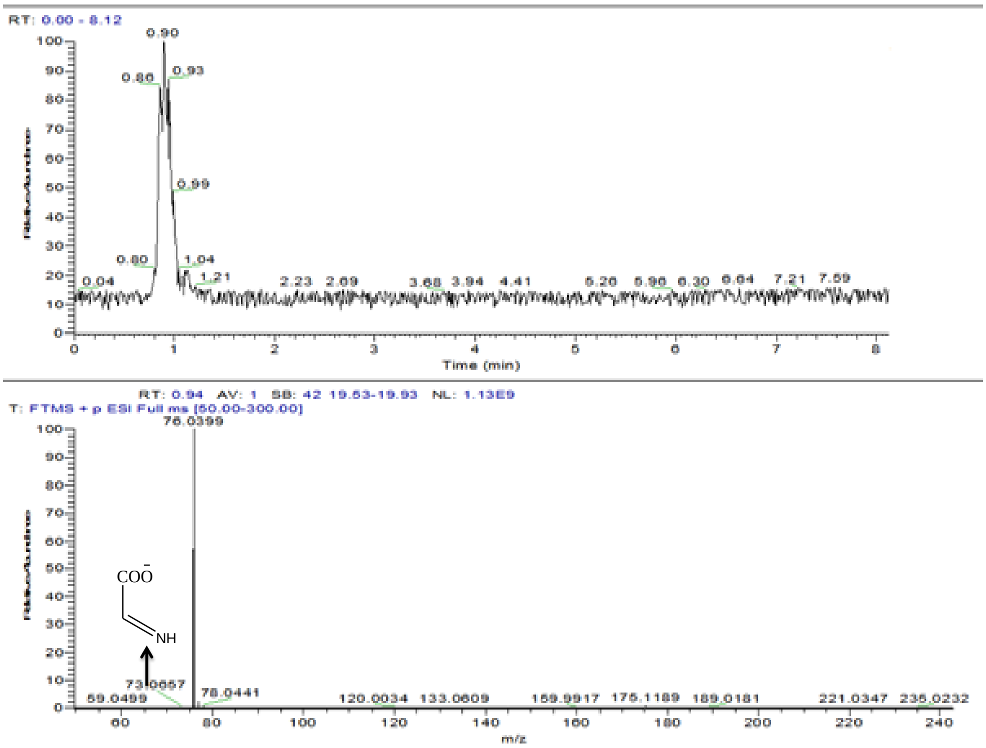
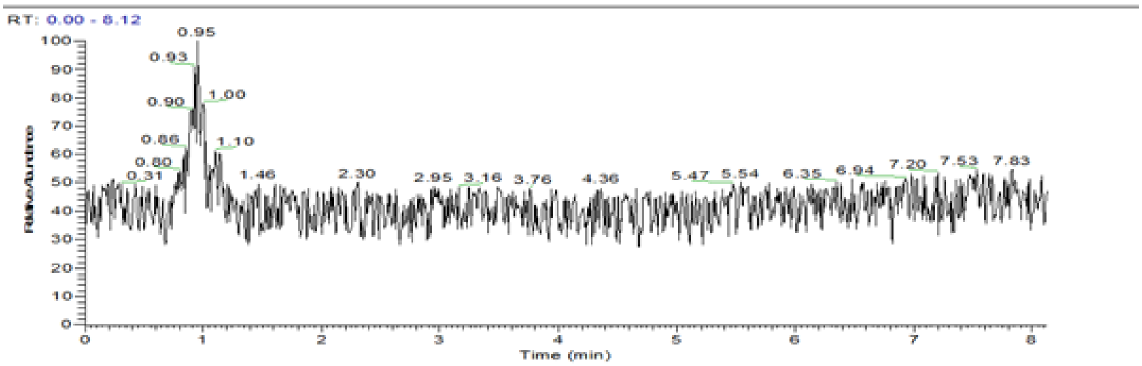
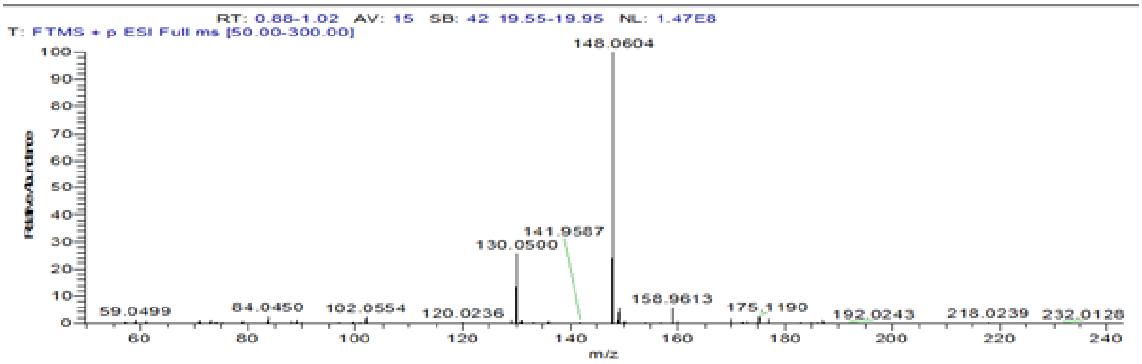


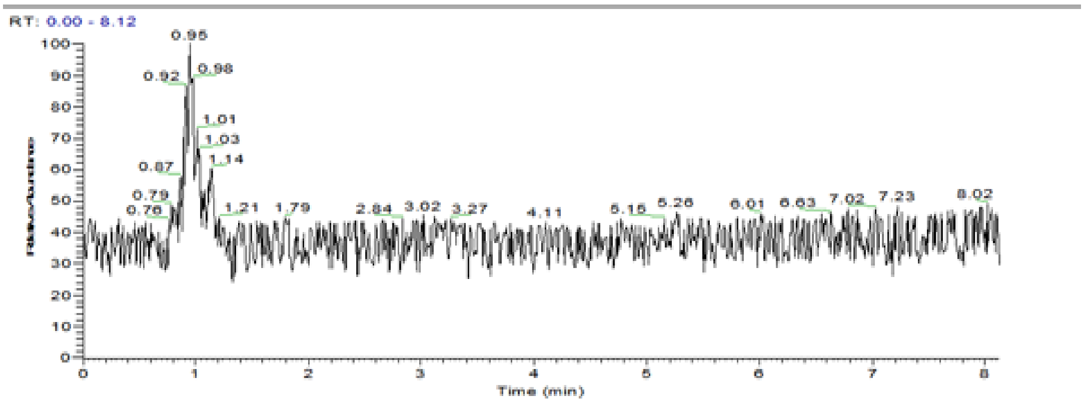
Fig. S17

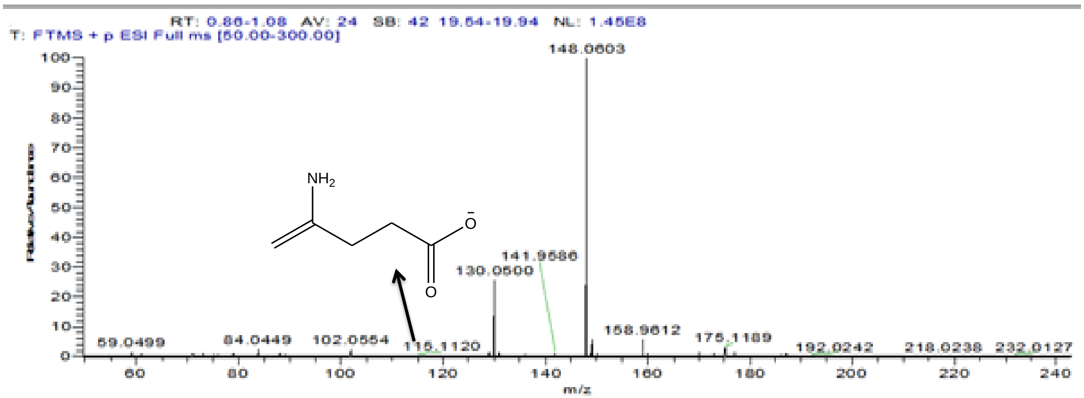
Glutamic acid (Glu)



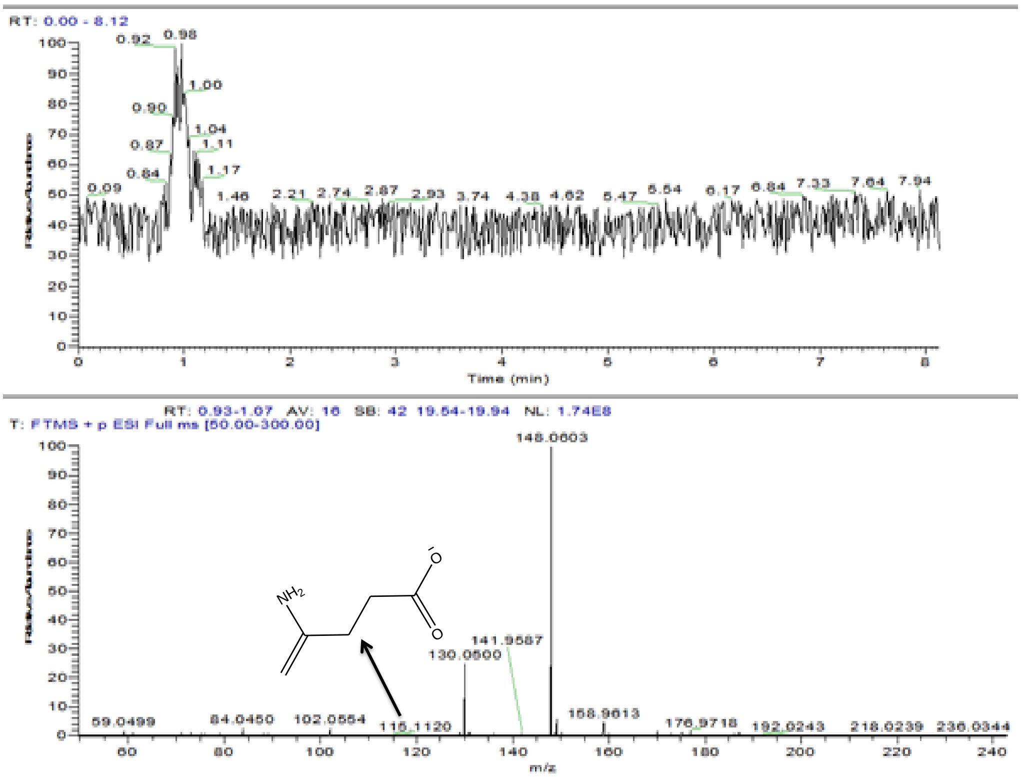


Glutamic acid + Ar





Glutamic acid + Air



Glutamic acid + N_2

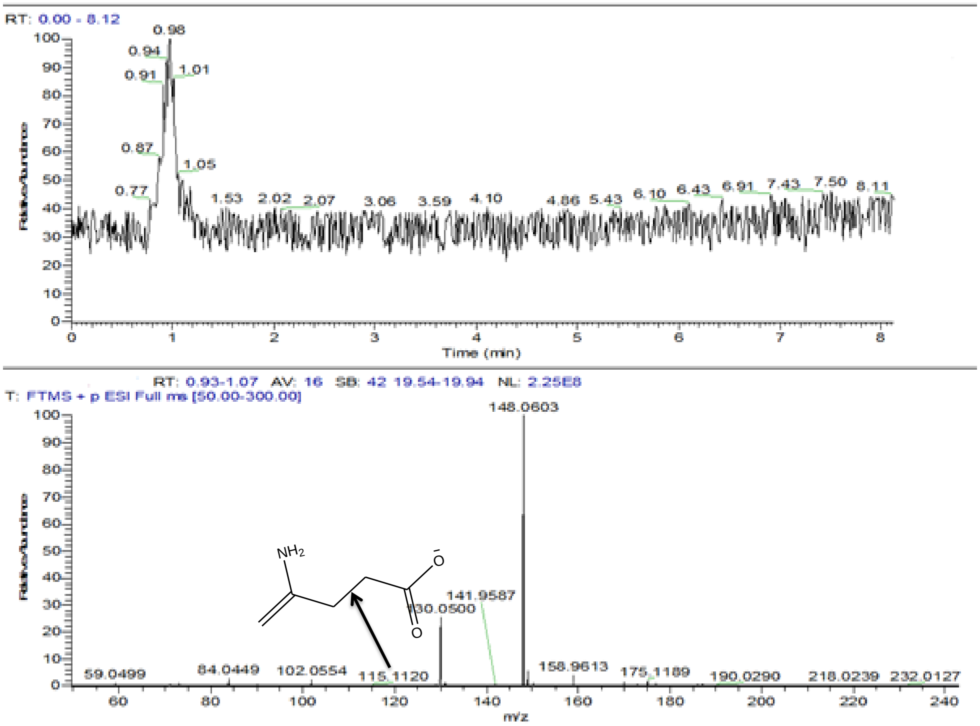
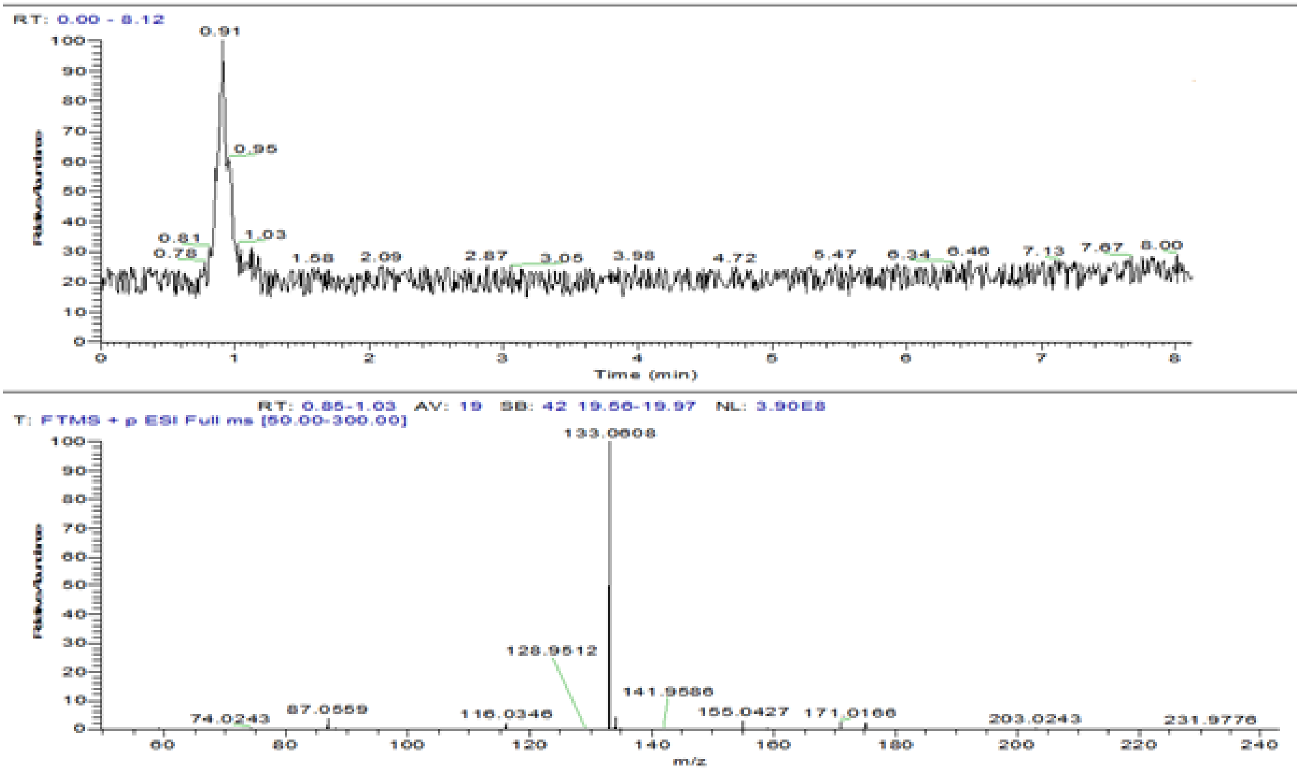
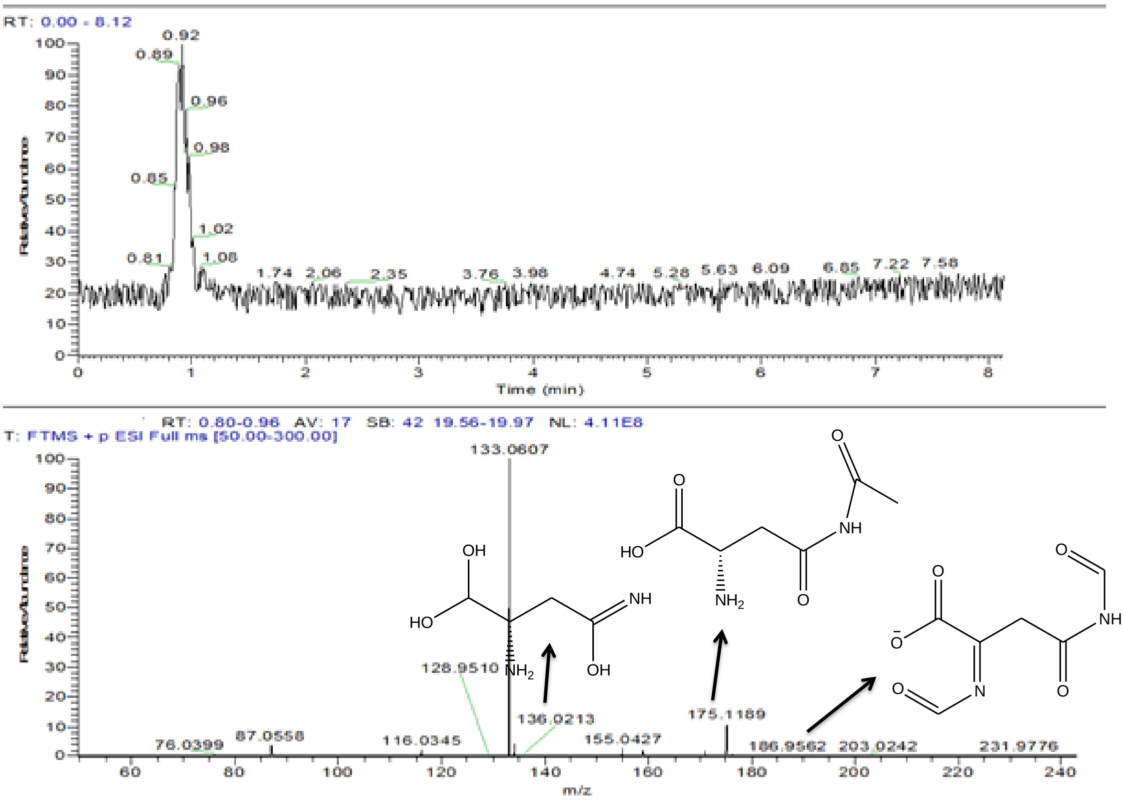


Fig. S18

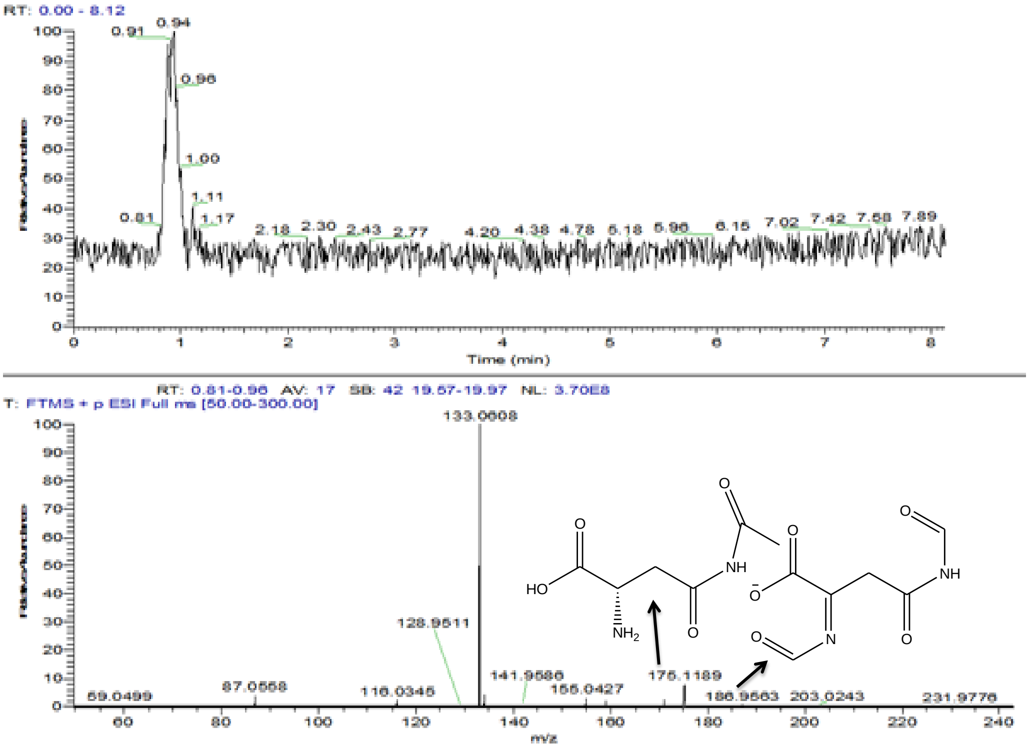
Asparagine control



Asparagine + Ar



Asparagine + N_2



Asparagine + Air

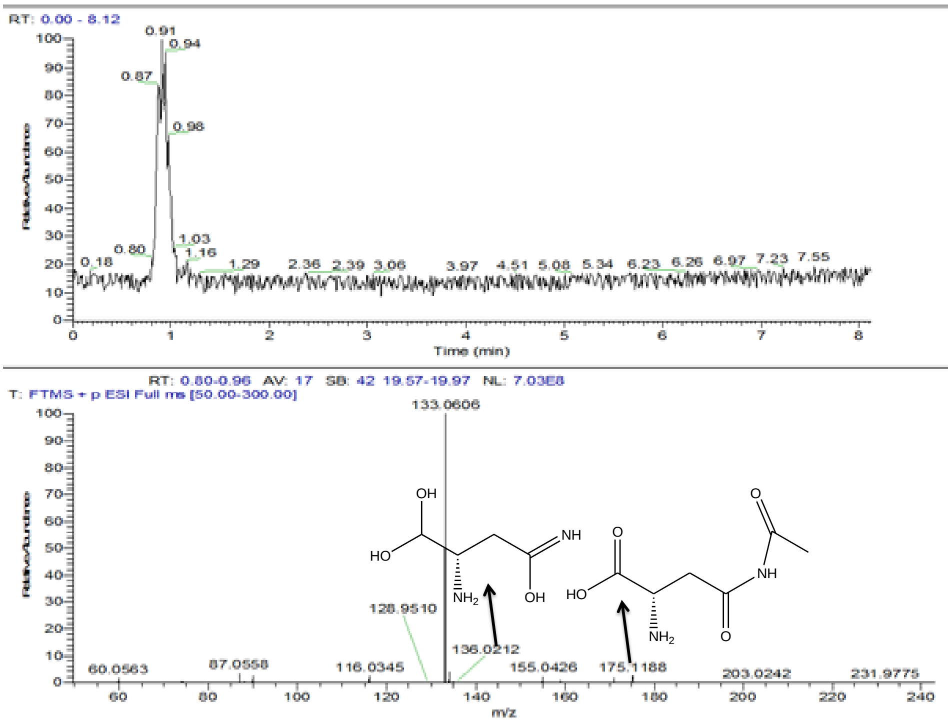
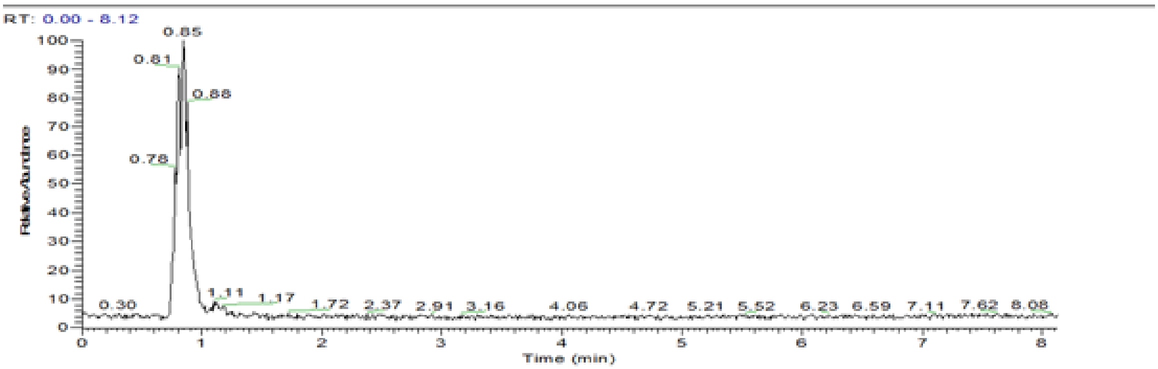
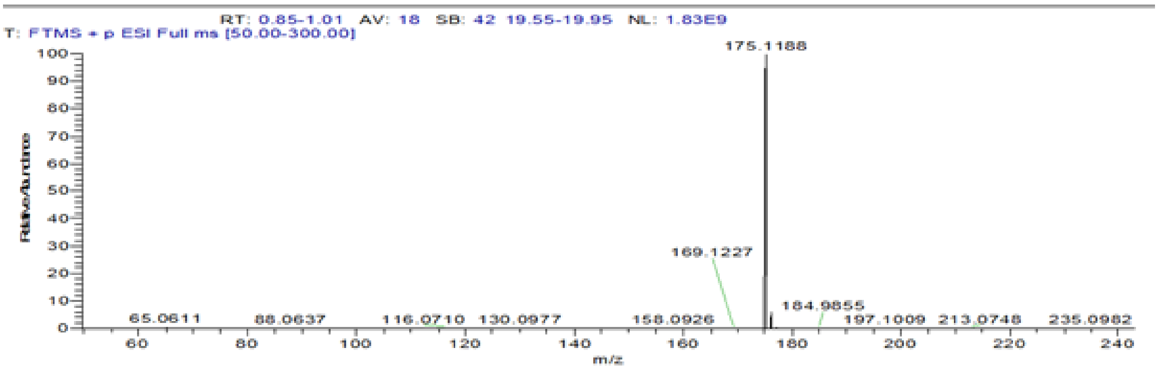


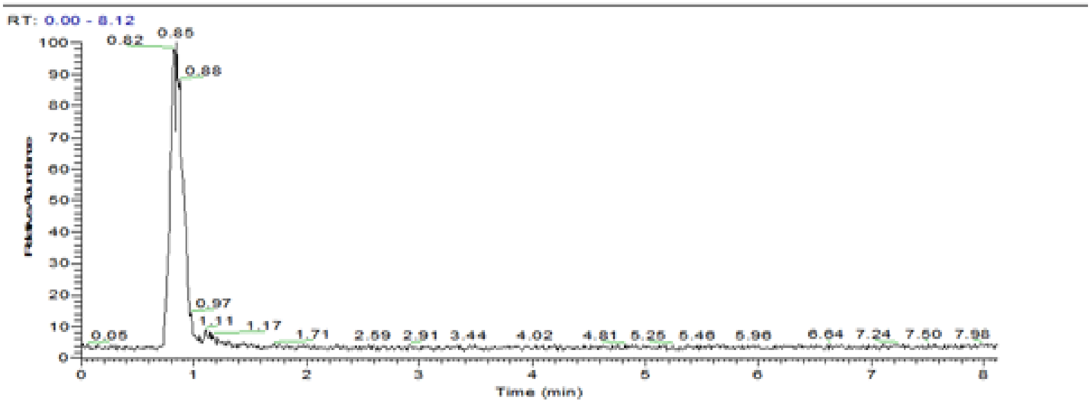
Fig. S19

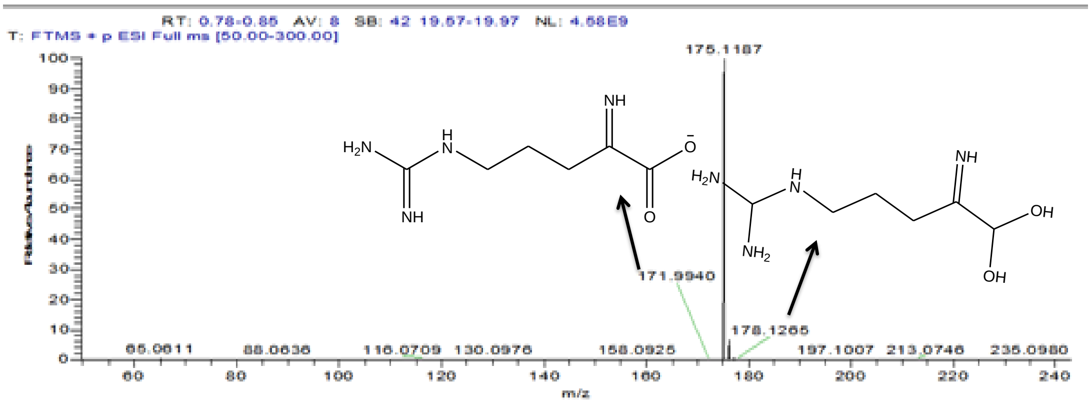
Arginine control



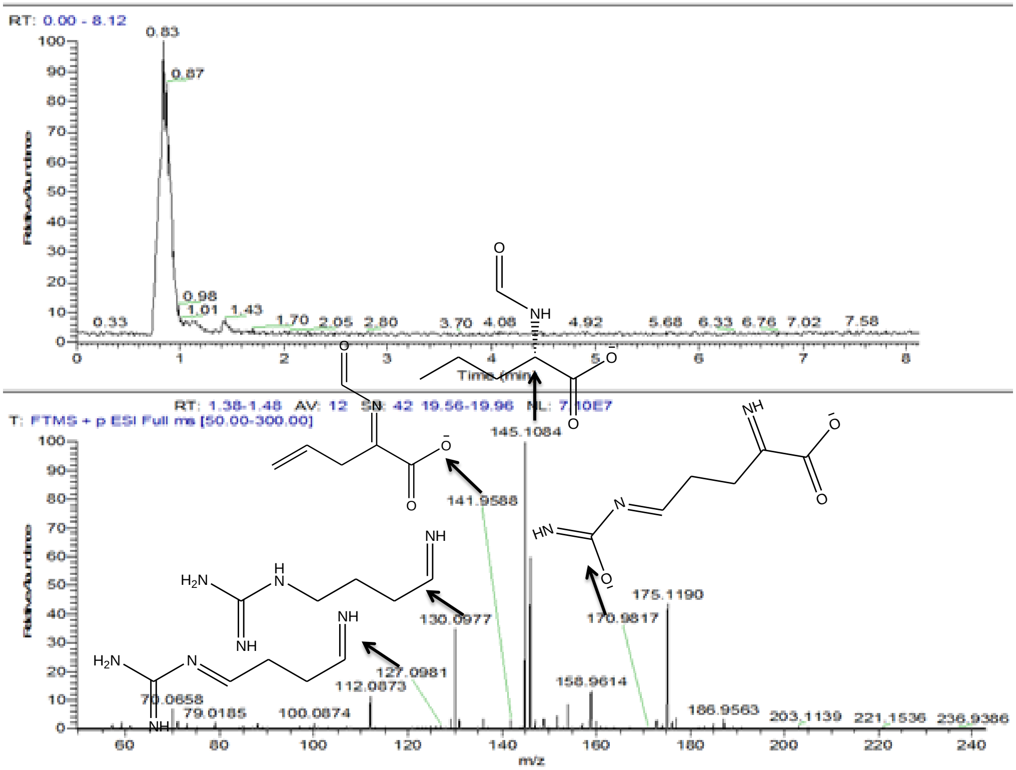


Arginine + Ar





Arginine + N_2



Arginine + Air

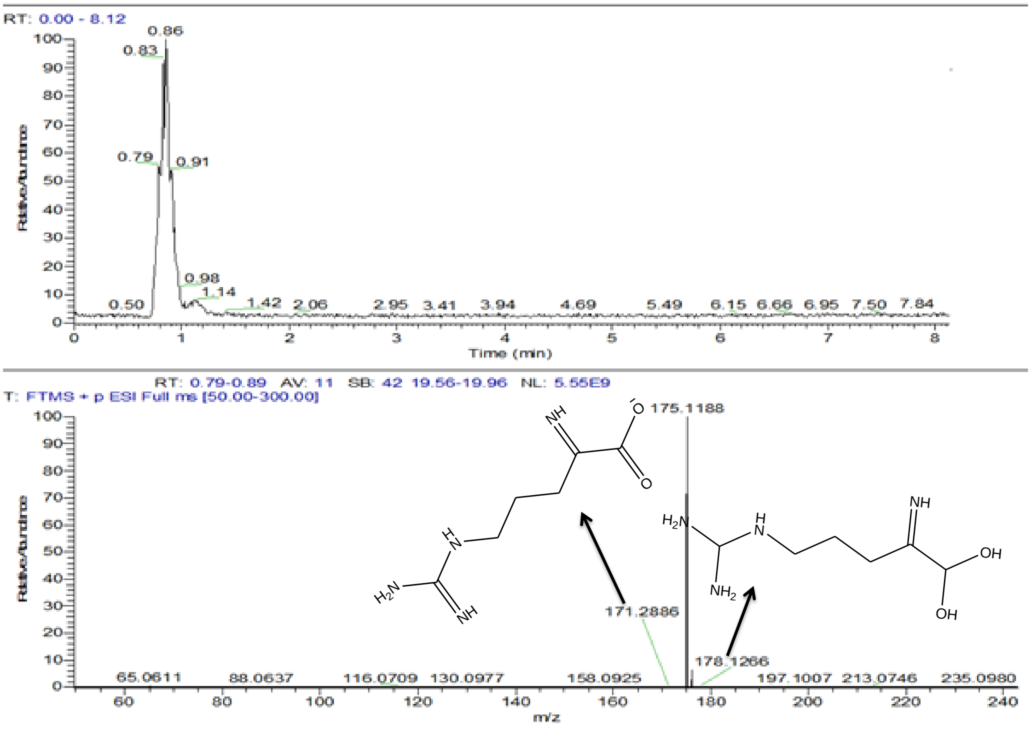
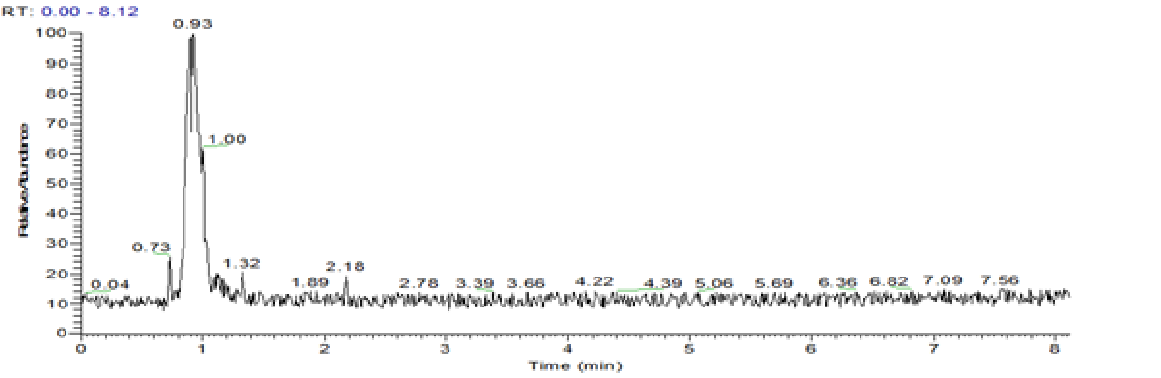
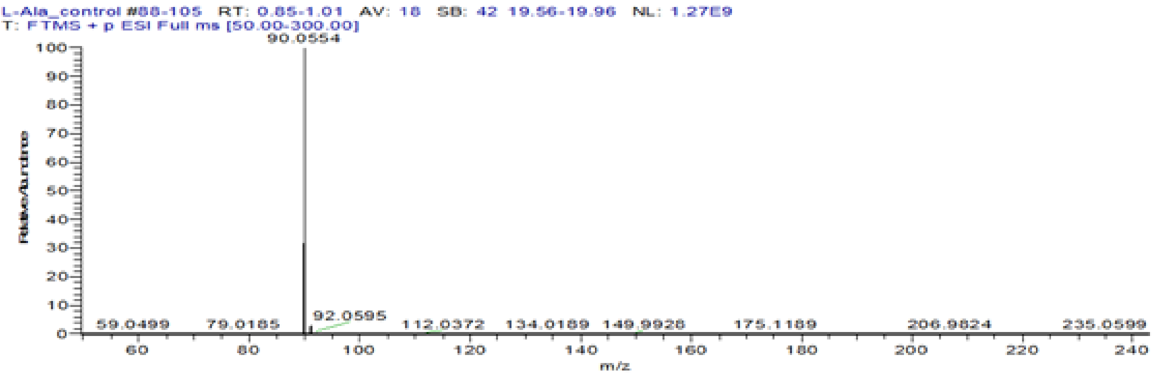


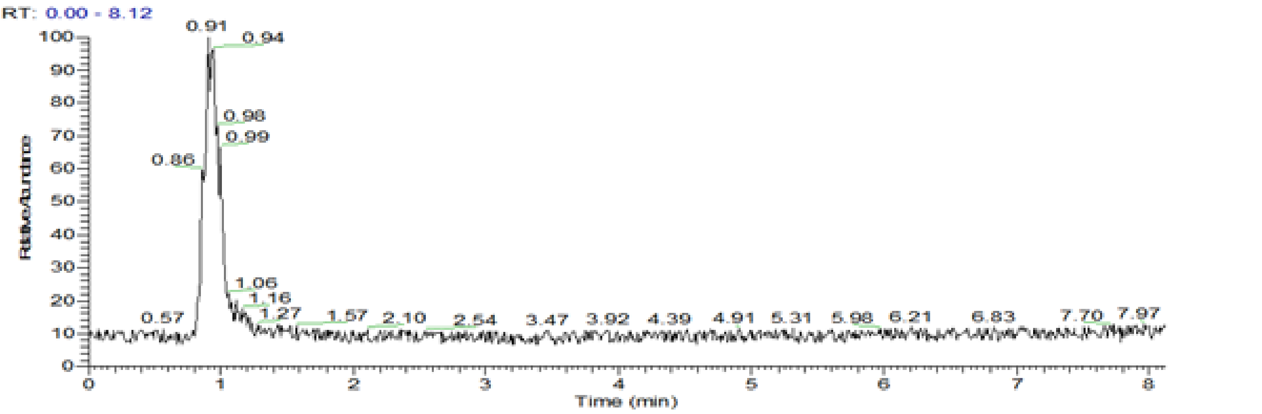
Fig. S20

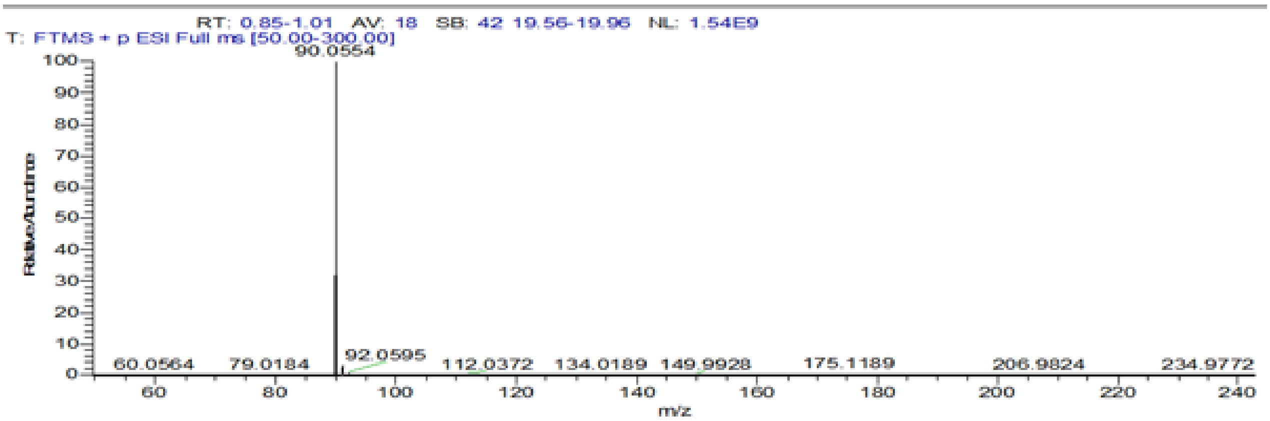
Alanine control



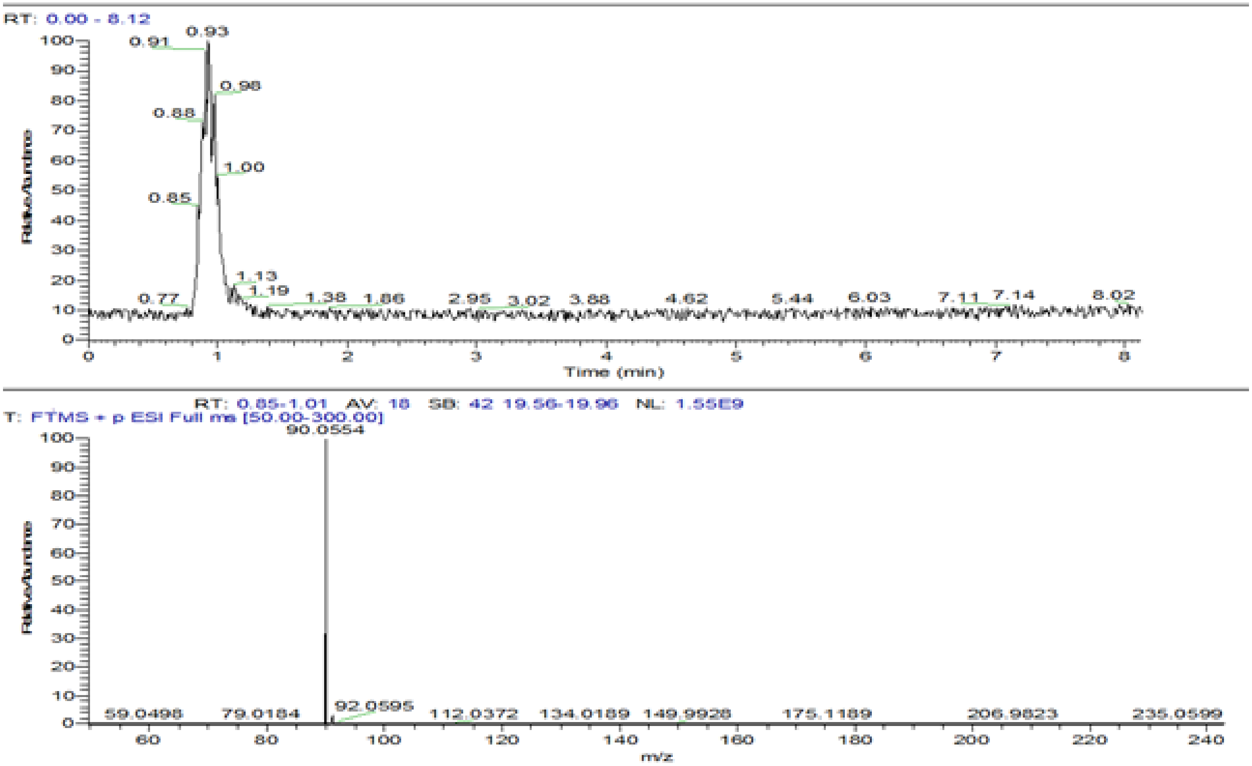


Alanine + Ar





Alanine + N_2



Alanine + Air

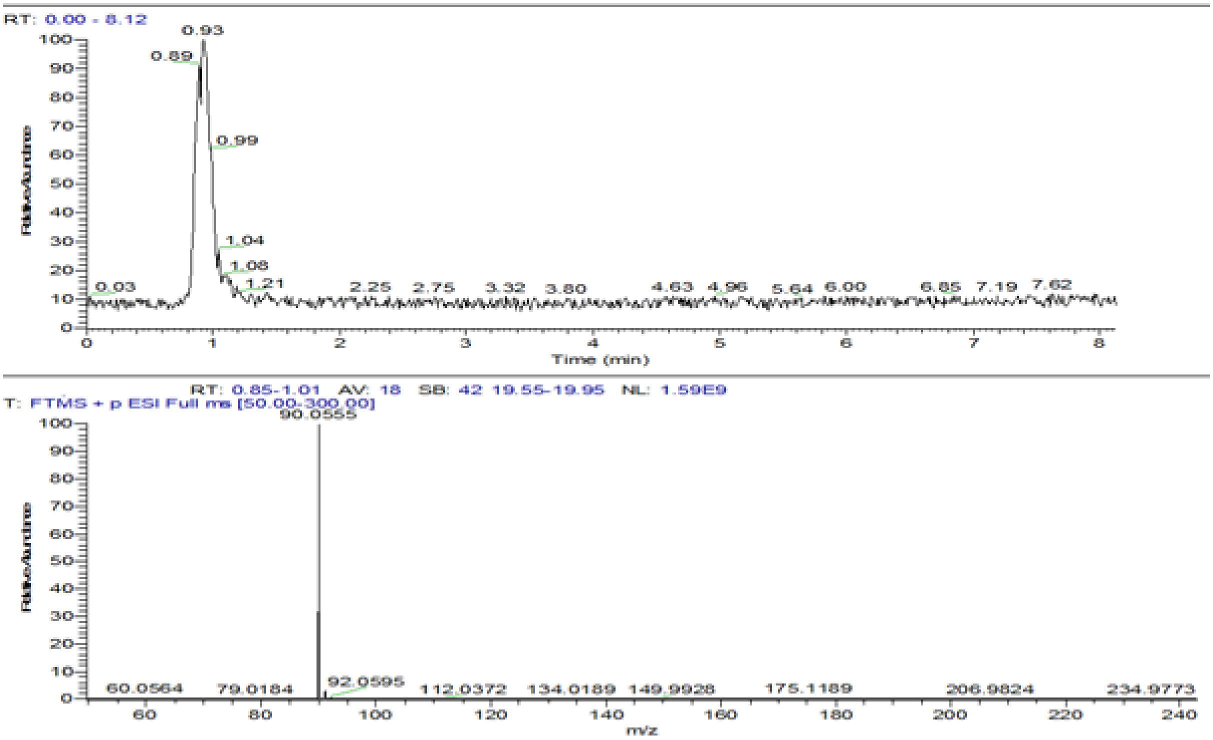
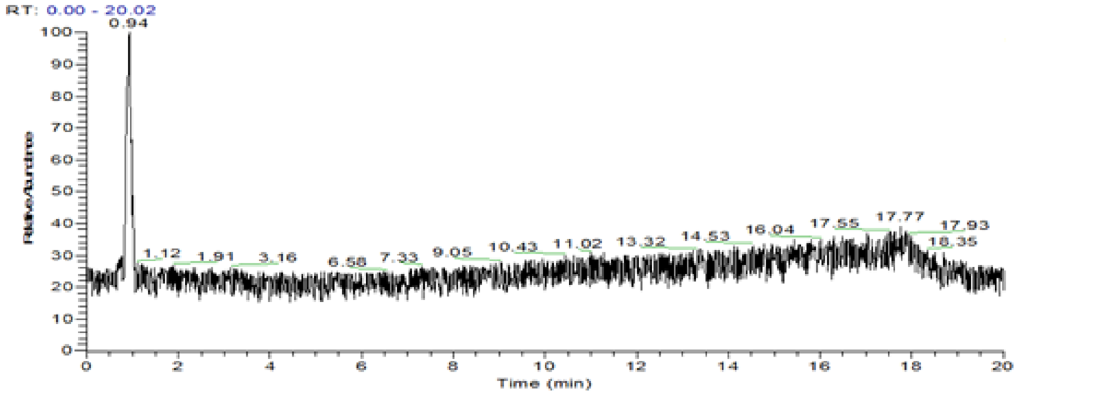
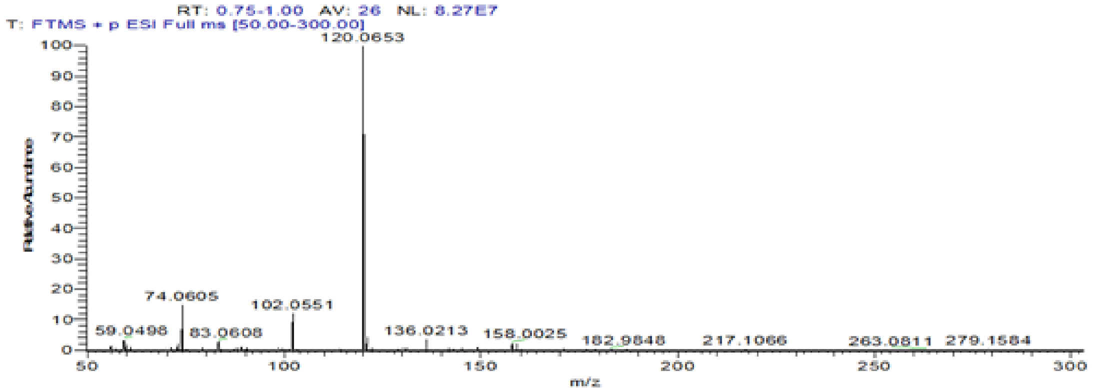


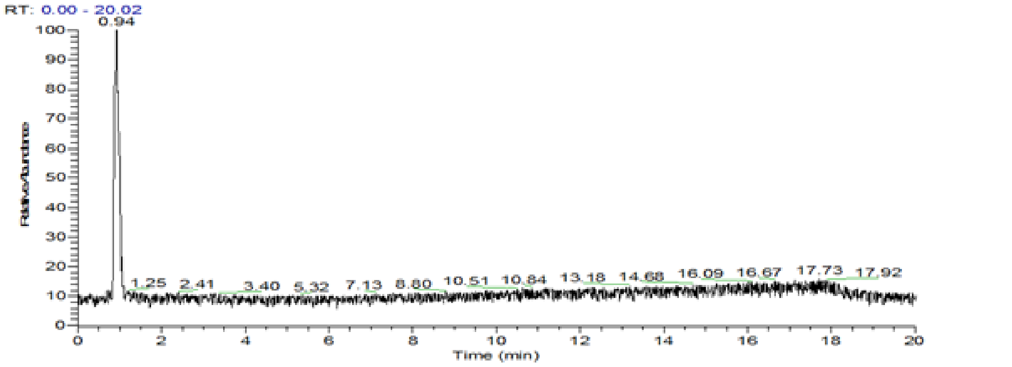
Fig. S21

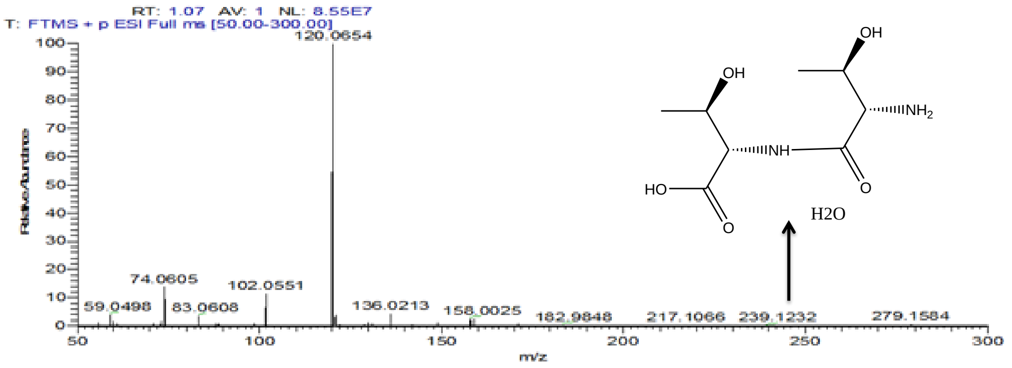
Threonine control



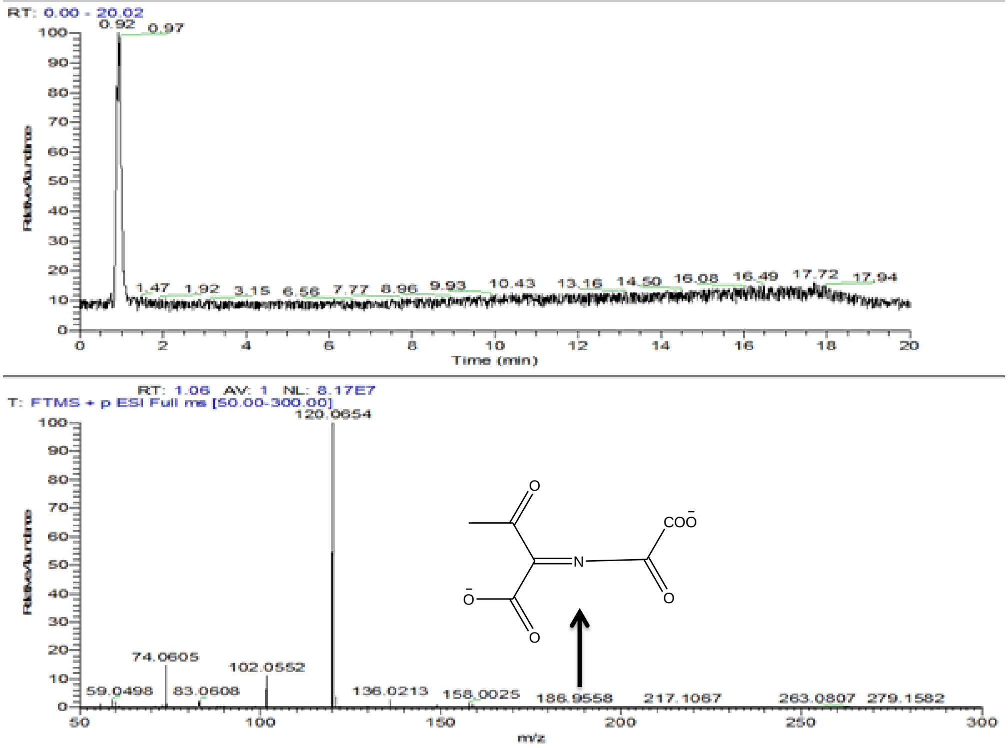


Threonine + Ar





Threonine + Air



Threonine + N_2

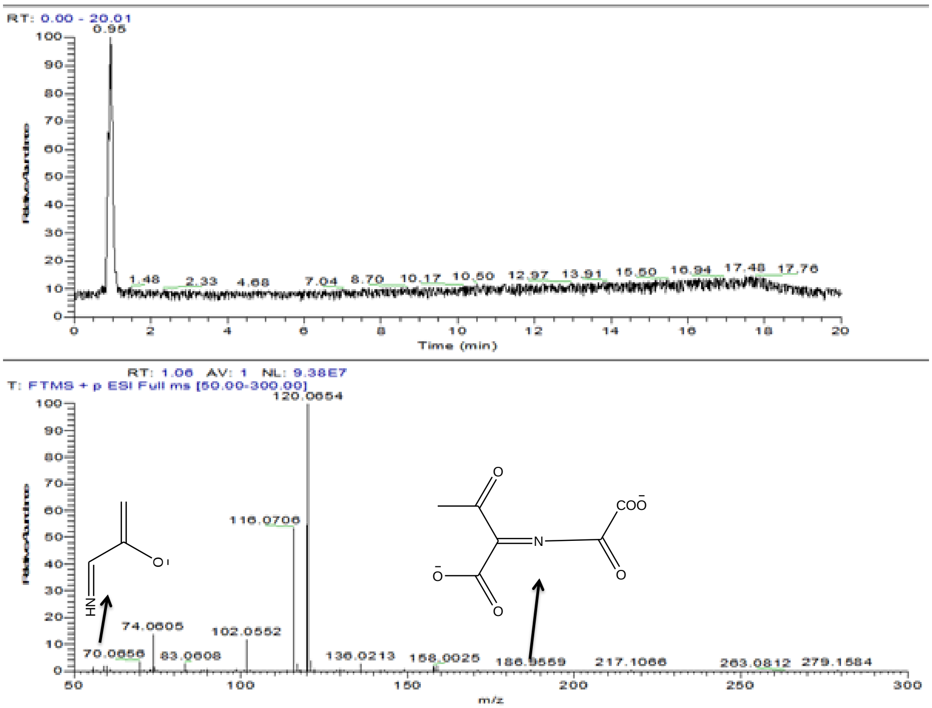
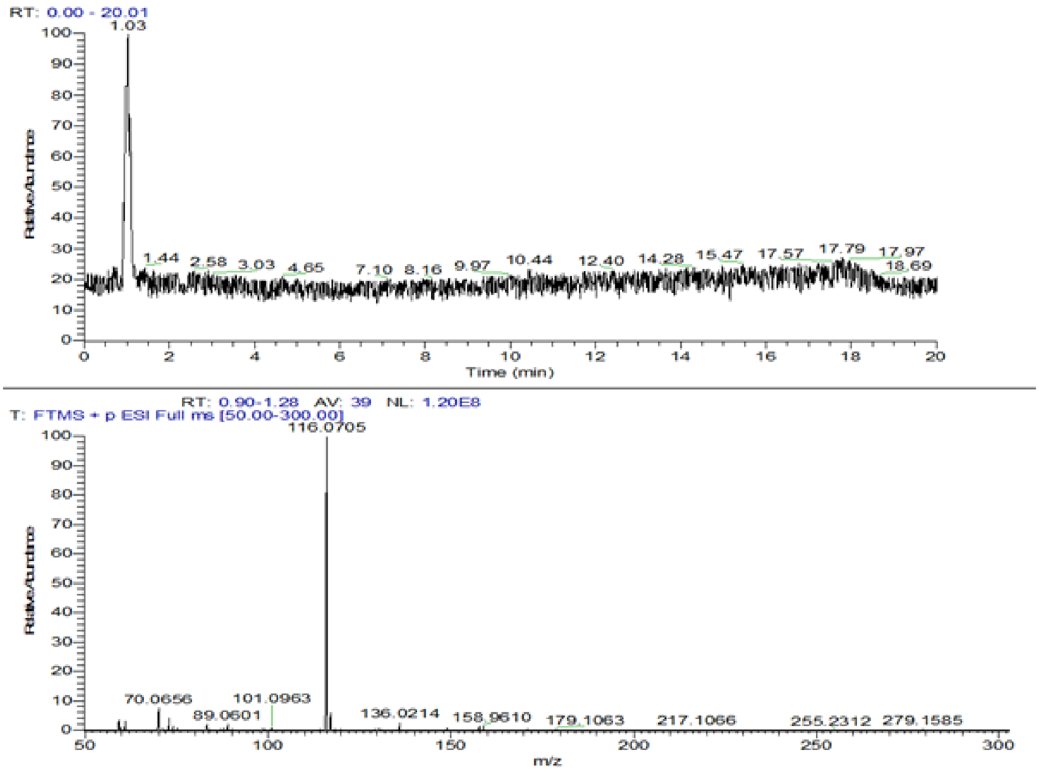
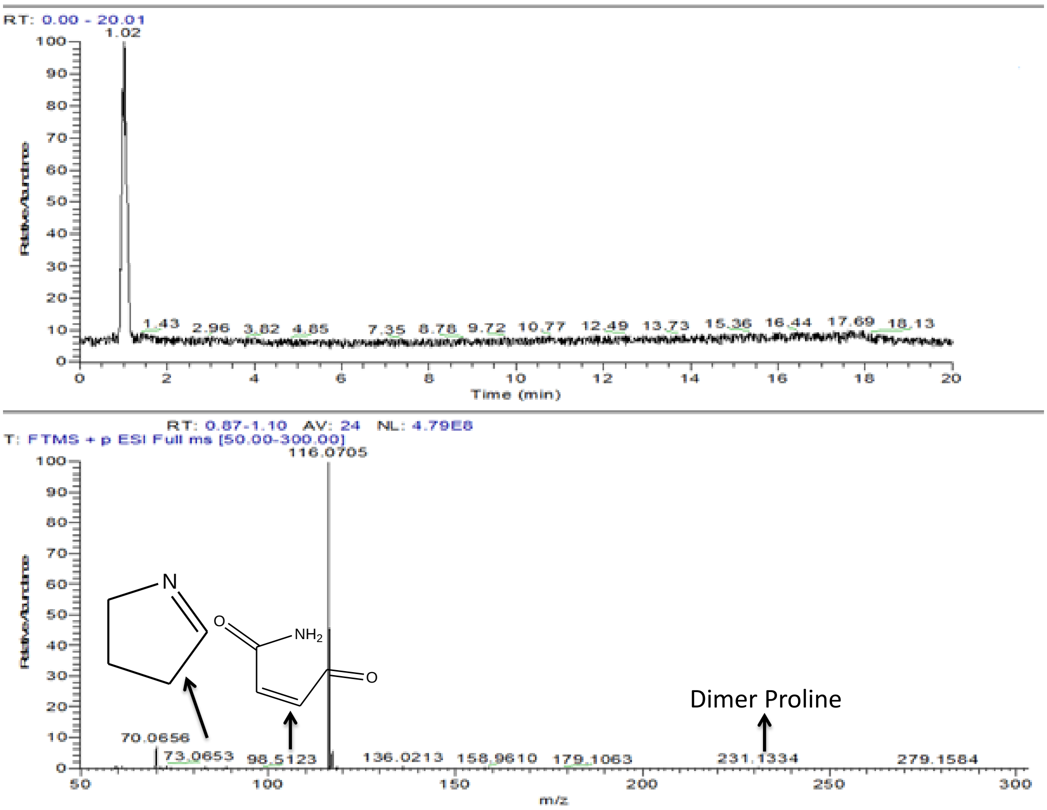


Fig. S22

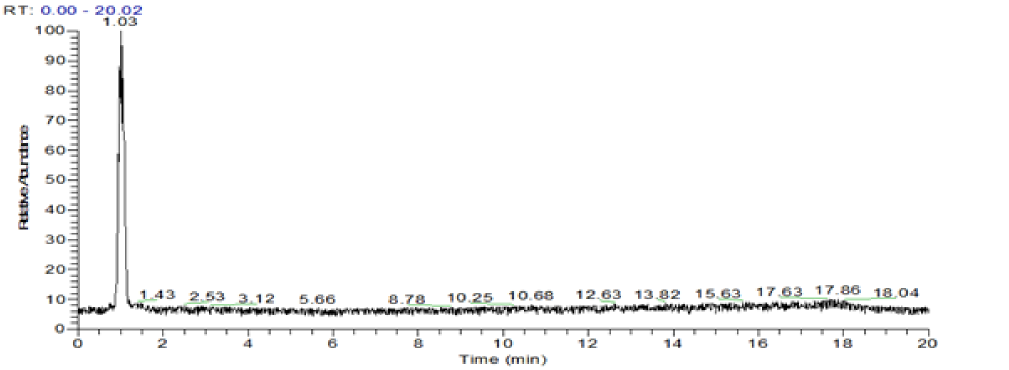
Proline Control

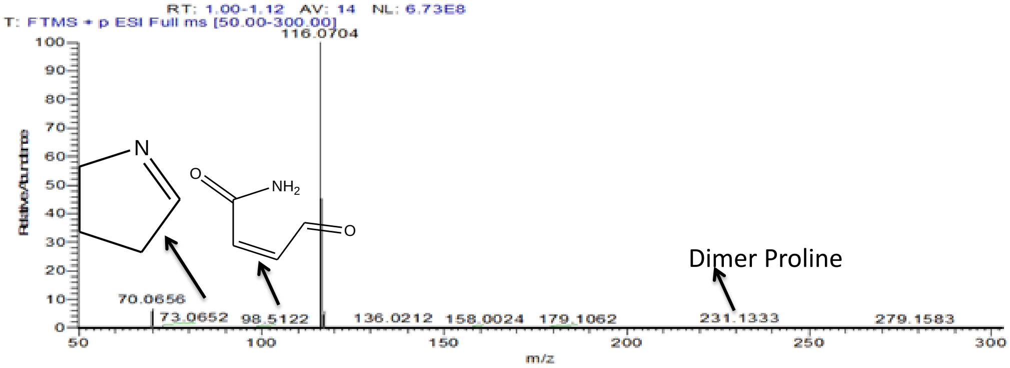


Proline + Ar

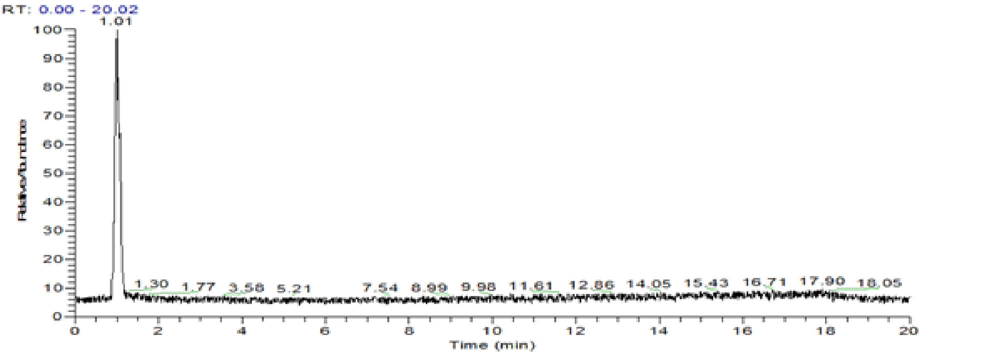


Proline + Air





Proline + N_2



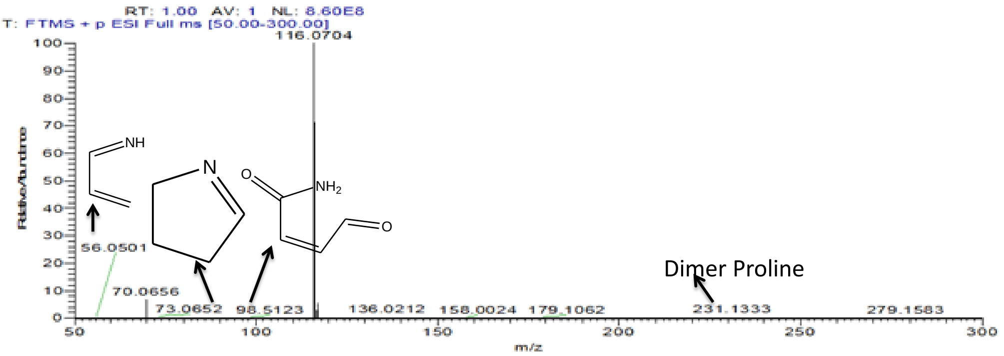
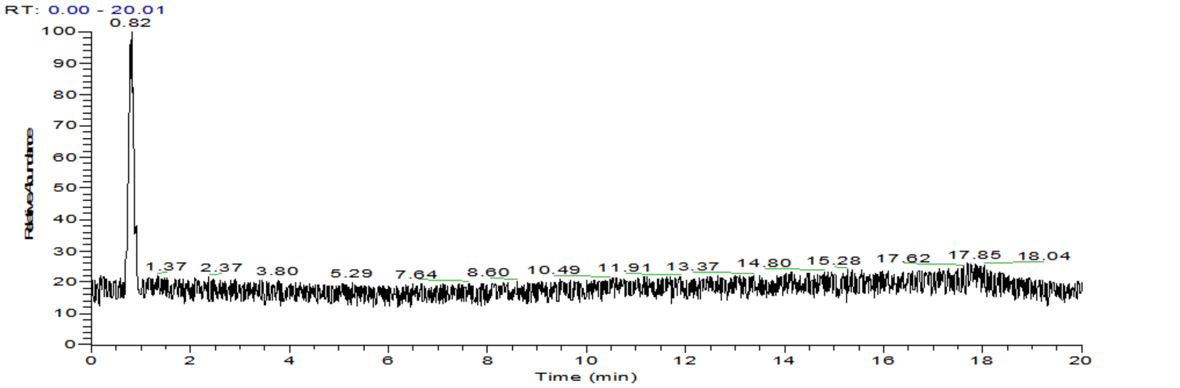
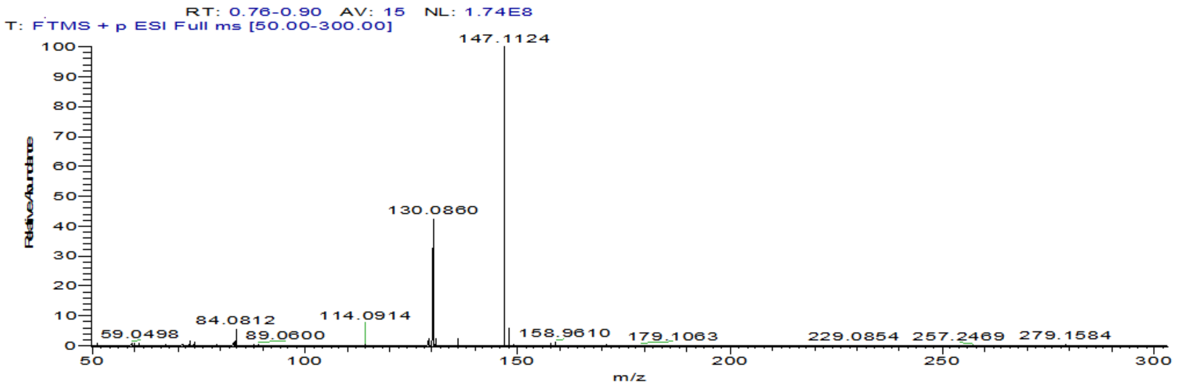


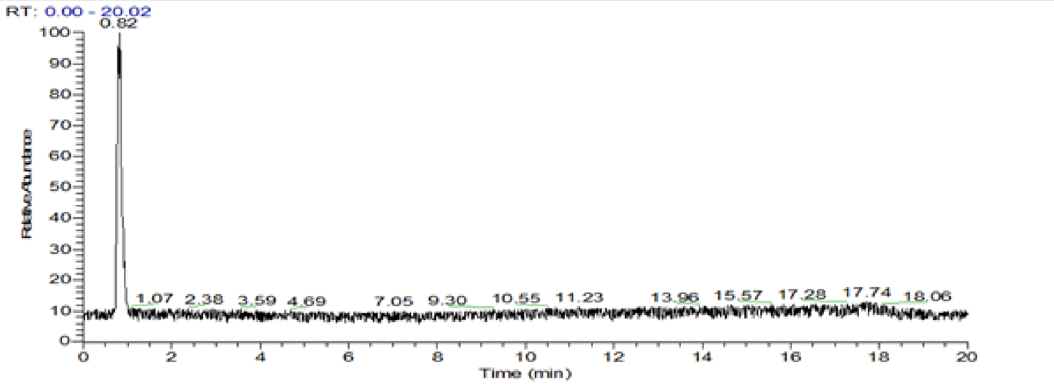
Fig. S23

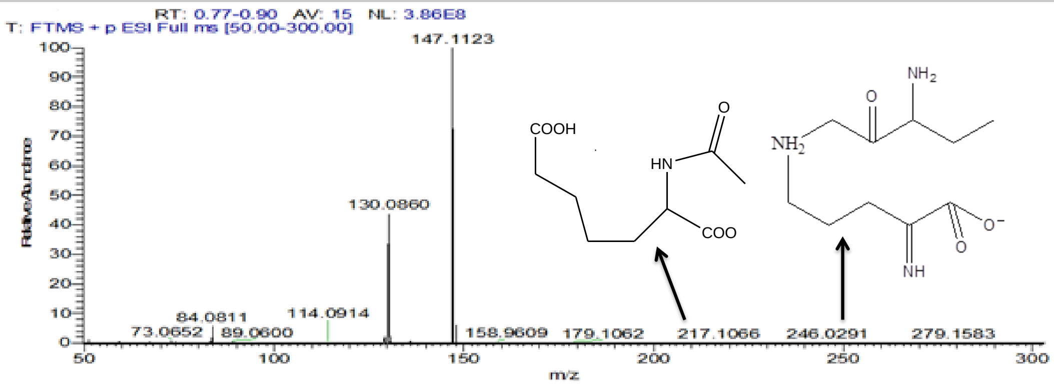
Lysine control



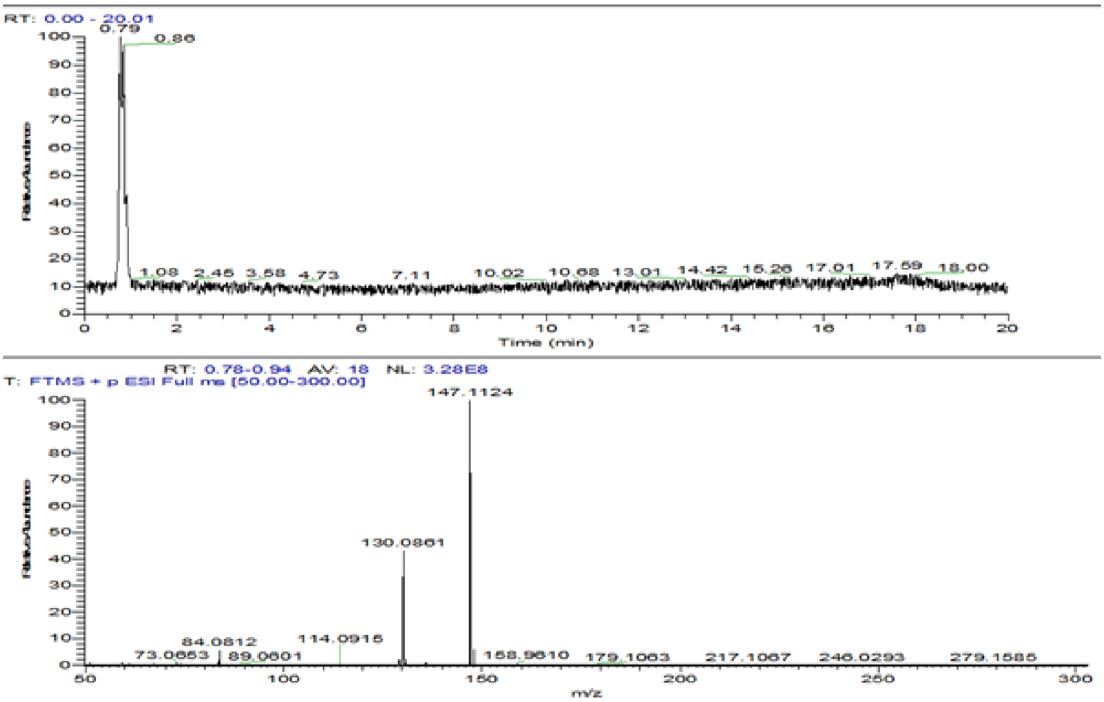


Lysine + Ar





Lysine + Air



Lysine + N_2

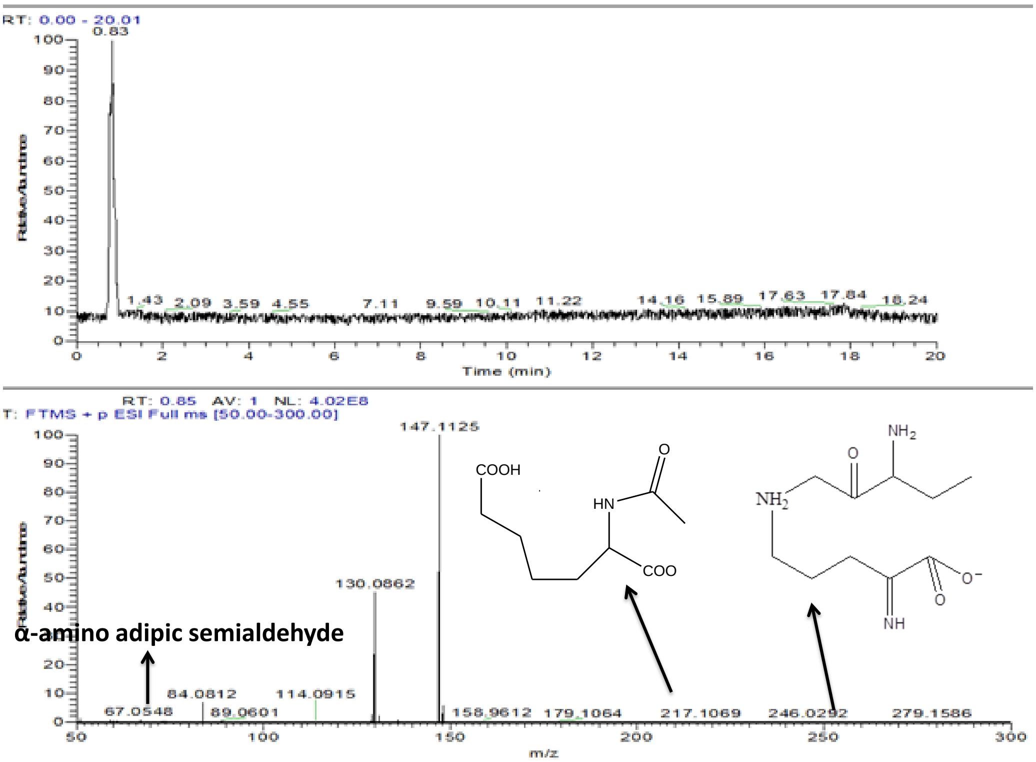


Fig. S24

Table S1: Comparison of the melting temperature between DSC and CD of Hb and Mb.

Sample	DSC T_m/ (^{0}C)	CD T_m/ (^{0}C)
Hb	66.37	66.20
Hb + Air plasma	65.10	65.01
Hb + N_2 plasma	59.25	59.10
Hb + Ar plasma	61.75	61.59
Mb	84.10	84.20
Mb + Air plasma	83.70	83.10
Mb + N_2 plasma	80.01	79.60
Mb + Ar plasma	80.98	81.50

Table S2. The variation in the particle size of proteins (Hb and Mb) after the treatment with soft plasma jet using different feeding gases.

Sample	**Hydrodynamic Diameter (*d*H) (nm)**
Hb	9.7 ± 5
Hb + Air plasma	5.8 ± 6
Hb + N_2 plasma	59.2 ± 3
Hb + Ar plasma	25.4 ± 4
Mb	1.7 ± 10
Mb + Air plasma	0.8 ± 9
Mb + N_2 plasma	43.2 ± 4
Mb + Ar plasma	37.6 ± 3