Tryptophan end-tagging confers antifungal activity on a tick-derived peptide by triggering reactive oxygen species production

Court K. Chiramba[a], Dalton S. Möller[a], Christian D. Lorenz[b], Rumbidzai R. Chirombo[c], A. James Mason[d], Megan J. Bester[c], and Anabella R. M. Gaspar[a]*

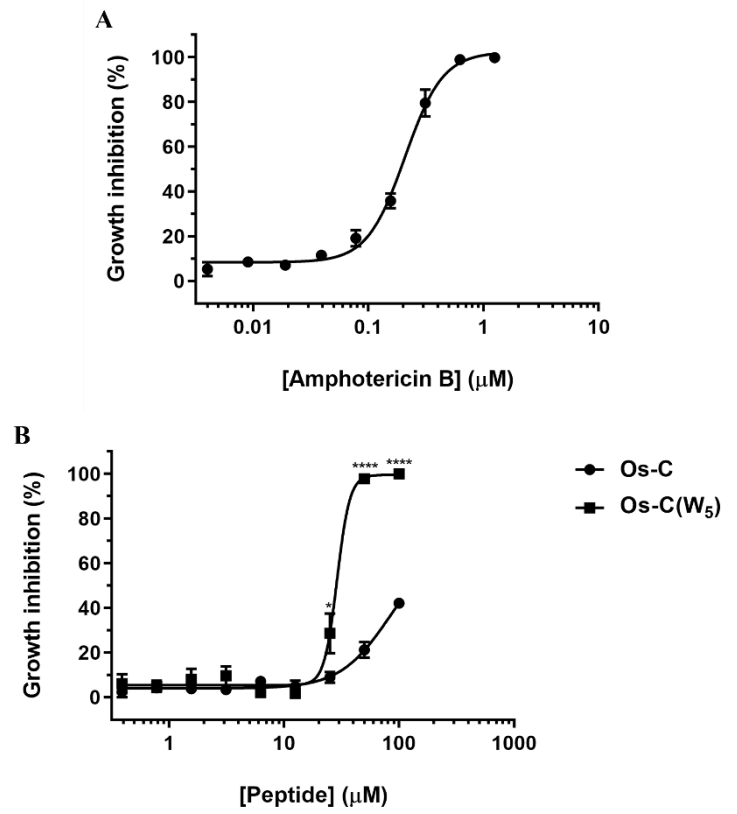
[a] *Department of Biochemistry, Genetics and Microbiology, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, 0002, South Africa.*

[b] *Department of Physics, King's College London, London, WC2R 2LS, United Kingdom.*

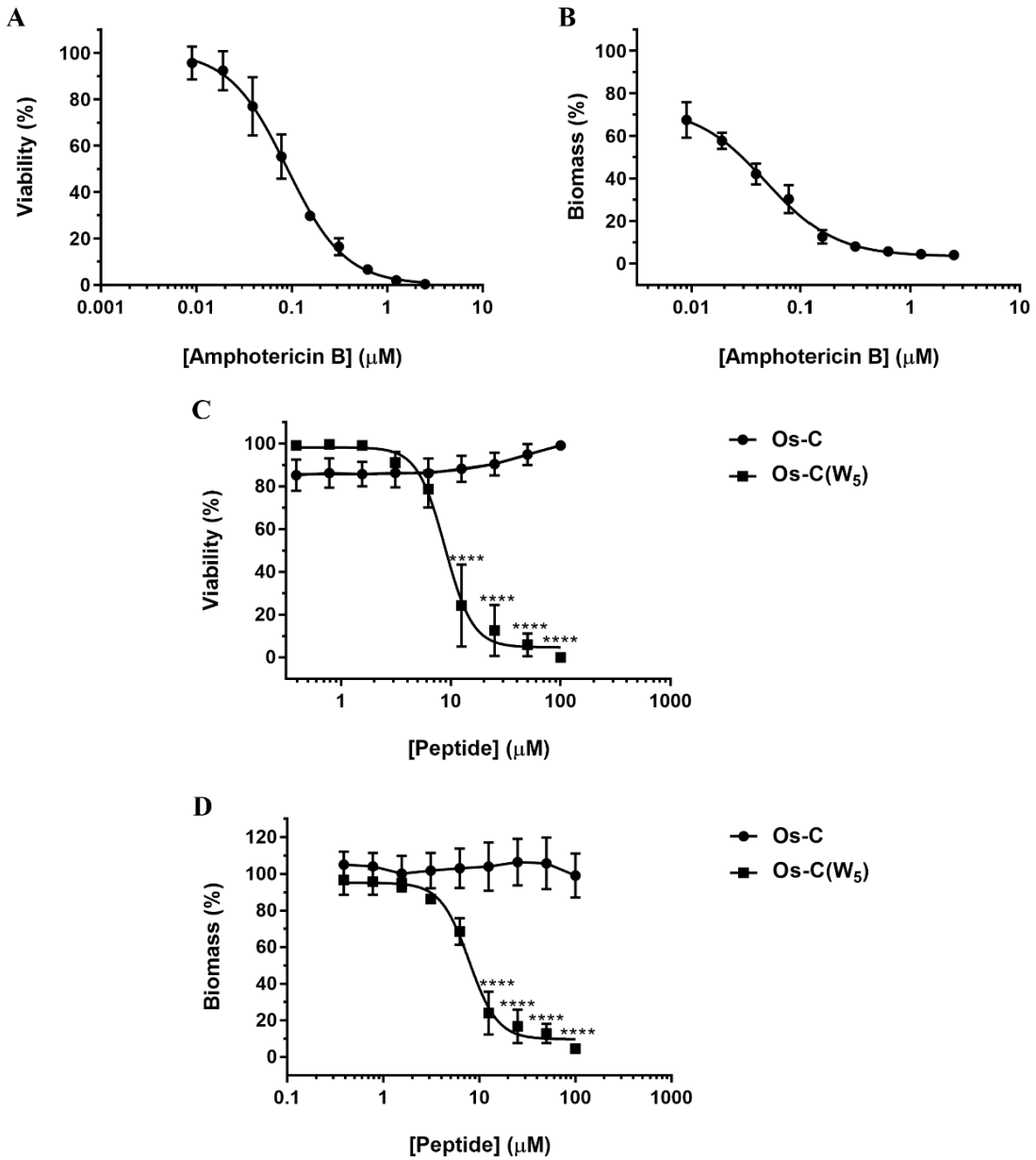
[c] *Department of Anatomy, Faculty of Health Sciences, University of Pretoria, Pretoria, 0002, South Africa.*

[d] *Institute of Pharmaceutical Science, School of Cancer & Pharmaceutical Science, King's College London, London, SE1 9NH, United Kingdom.*

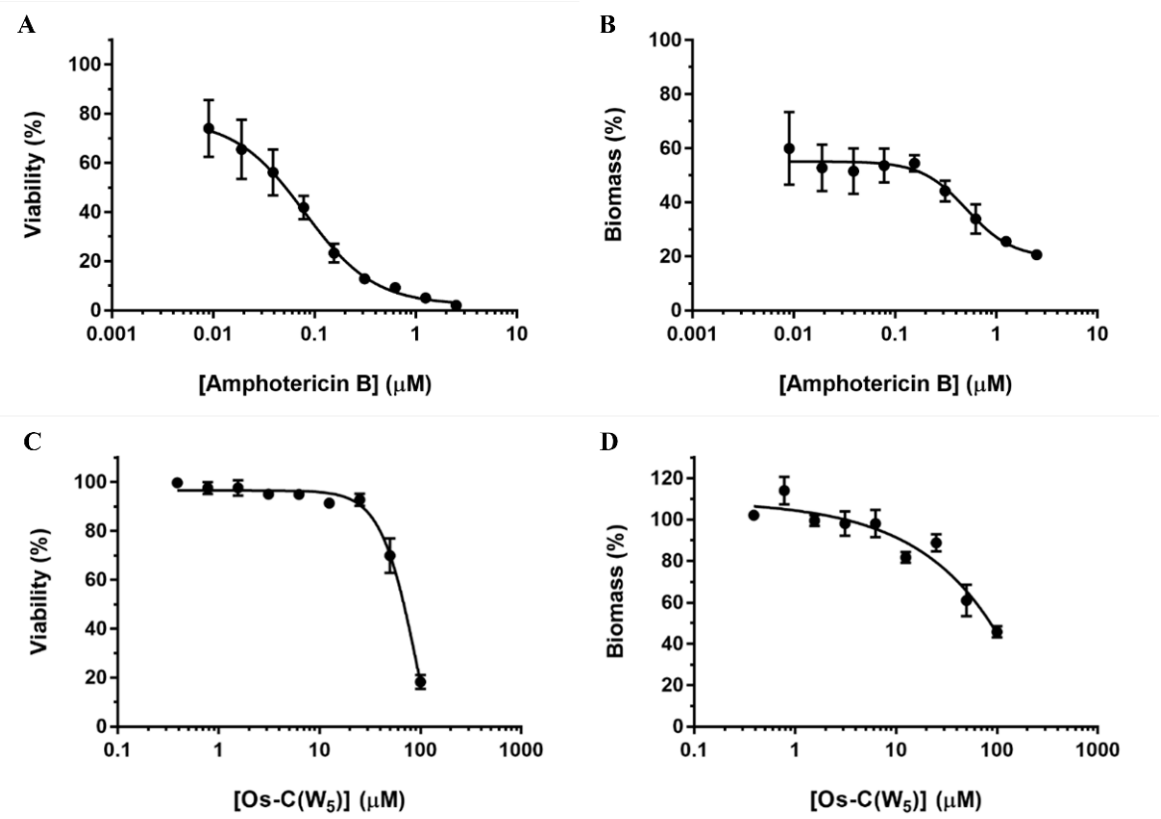
*Email: anabella.gaspar@up.ac.za



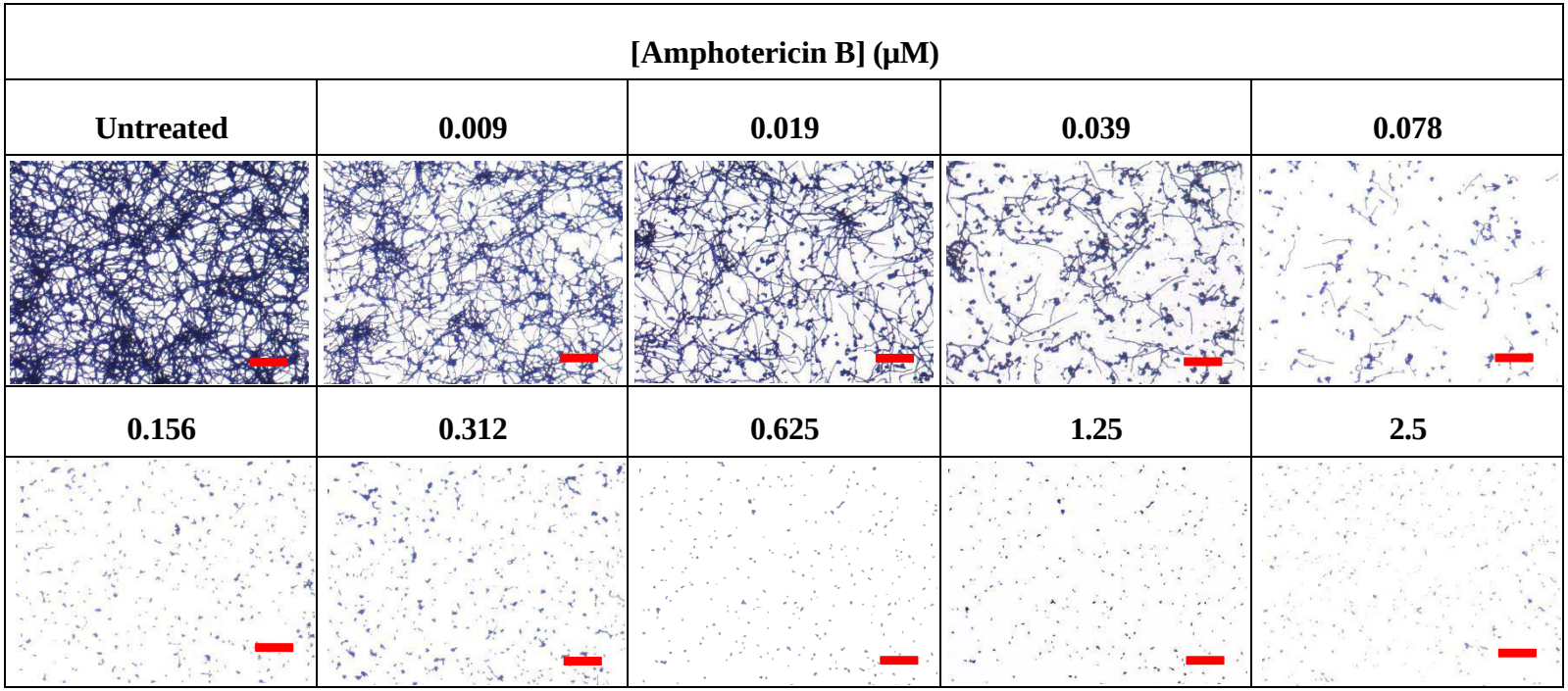
Supplementary Figure 1: Effect of antifungal agents on cell growth. (A and B) Planktonic *C. albicans* cells were exposed to concentrations of (A) Amphotericin B (0.004 – 1.25 μM) and (B) Os-C and Os-C(W_5) (both 0.39 – 100 μM) for 24 hours and antifungal activity was quantified by measuring the optical density at 530 nm. Data represents the mean ± SEM of three independent experiments. A two-way ANOVA was performed followed by a posthoc Tukey's multiple comparisons test. Asterisks (*$p < 0.05$; ****$p < 0.0001$) represent a significant difference between the same concentrations of Os-C and Os-C(W_5).



Supplementary Figure 2: Biofilm preventing activity of amphotericin B, Os-C and Os-C(W_5). *C. albicans* cells were grown in the presence of amphotericin B (0.009 – 2.5 μM) and Os-C and Os-C(W_5) (0.19 – 100 μM) for 24 hours. (A and C) Biofilm viability was determined using the CellTiter Blue cell viability assay. (B and D) Biofilm biomass was determined by solubilizing crystal violet bound to biofilms with 30% acetic acid. For (C) and (D), a two-way ANOVA was performed followed by a posthoc Bonferroni's multiple comparisons test. Data represents the mean ± SEM of three independent experiments. Asterisks (****$p < 0.0001$) represent a significant difference between the same concentrations of Os-C and Os-C(W_5), respectively.



Supplementary Figure 3: Biofilm eradicating activity of amphotericin B and Os-C(W_5). Preformed biofilms were treated with either (A and B) amphotericin B (0.009 – 2.5 μM) or (C and D) Os-C(W_5) (0.19 – 100 μM) for 24 hours then (A and C) viability was measured using CellTiter Blue cell viability assay. (B and D) Biofilm biomass was quantified by solubilizing bound CV with 30% acetic acid and measuring the absorbance at 550 nm. Data represents the mean ± SEM of three independent experiments.



Supplementary Figure 4: Microscopy images of cells exposed to amphotericin B. Cells were exposed to amphotericin B for 24 hours then stained with 0.1% crystal violet. Images are representative of three independent experiments and taken at 10× magnification. Scale bar = 100 µm.

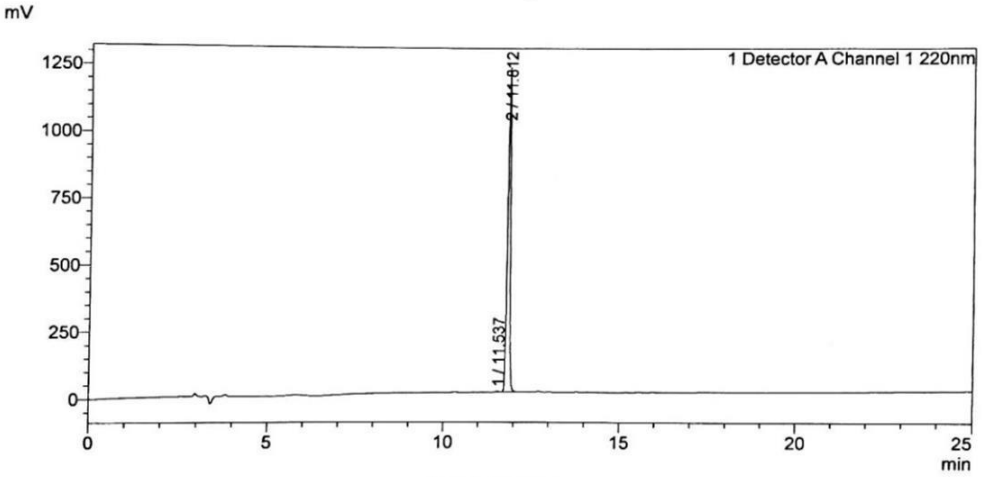
Sample Name :Os-C
Sample ID :U7284ED080-3
Time Processed :16:55:01
Month-Day-Year Processed :04/23/2019

Pump A : 0.065% trifluoroacetic in 100% water (v/v)
Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)
Total Flow:1 ml/min
Wavelength:220 nm
<<LC Time Program>>

Time	Module	Command	Value
0.01	Pumps	Pump A B.Conc	5
25.00	Pumps	Pump A B.Conc	65
25.01	Pumps	Pump A B.Conc	95
32.00	Pumps	Pump A B.Conc	95
32.01	Pumps	Pump A B.Conc	5
40.00	Pumps	Pump A B.Conc	5
40.00	Controller	Stop	

<<Column Performance>>
<Detector A>
Column : AlltimaTM C18 4.6 x 250 mm
Equipment:ZJ17010508

<Chromatogram>

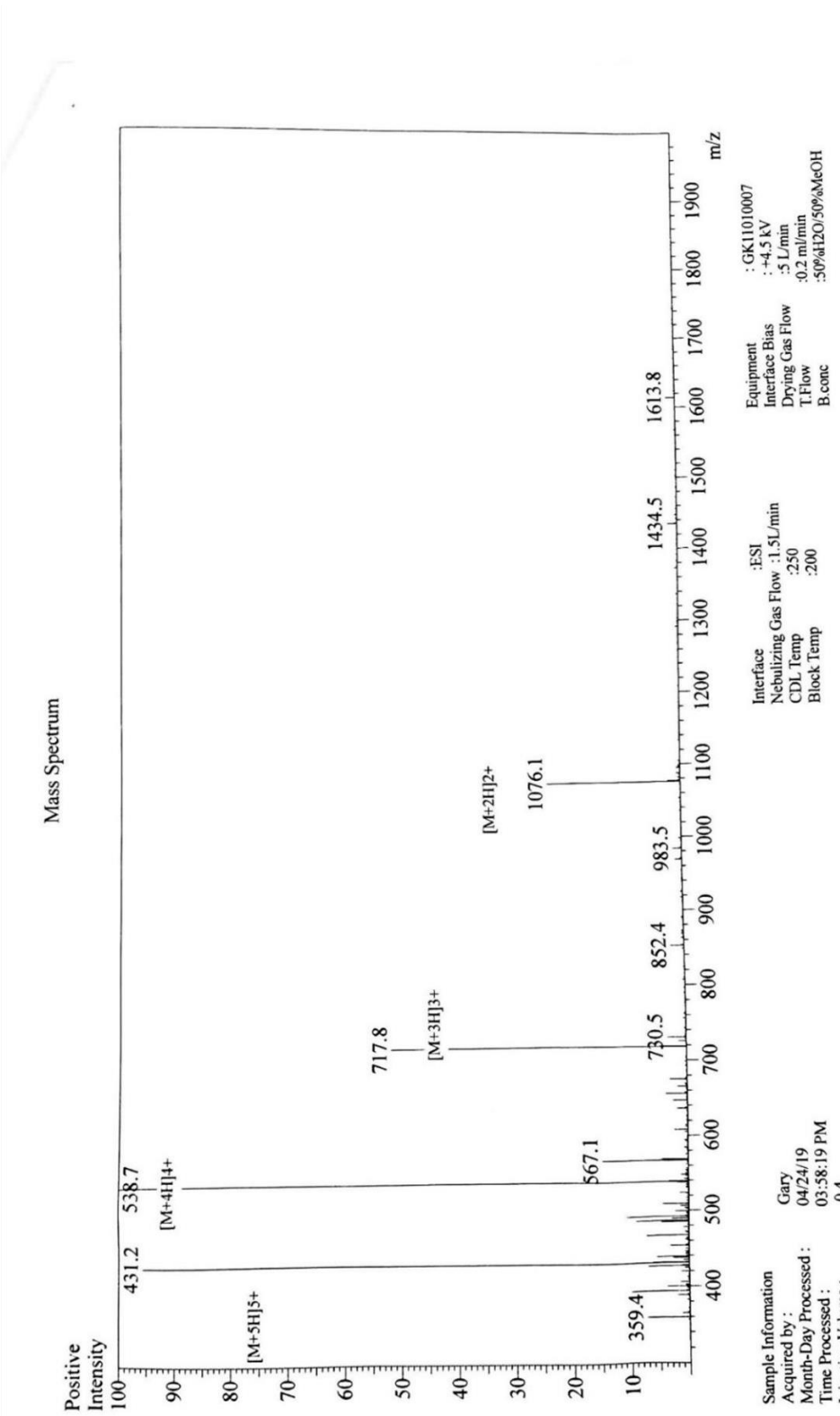


<Peak Table>

Detector A Channel 1 220nm

Peak#	Ret. Time	Area	Height	Area%
1	11.537	15935	3440	0.243
2	11.812	6554556	1226601	99.757
Total		6570491	1230041	100.000

Supplementary Figure 5: HPLC analysis of Os-C.



Supplementary Figure 6: Mass spectrometry analysis of Os-C.

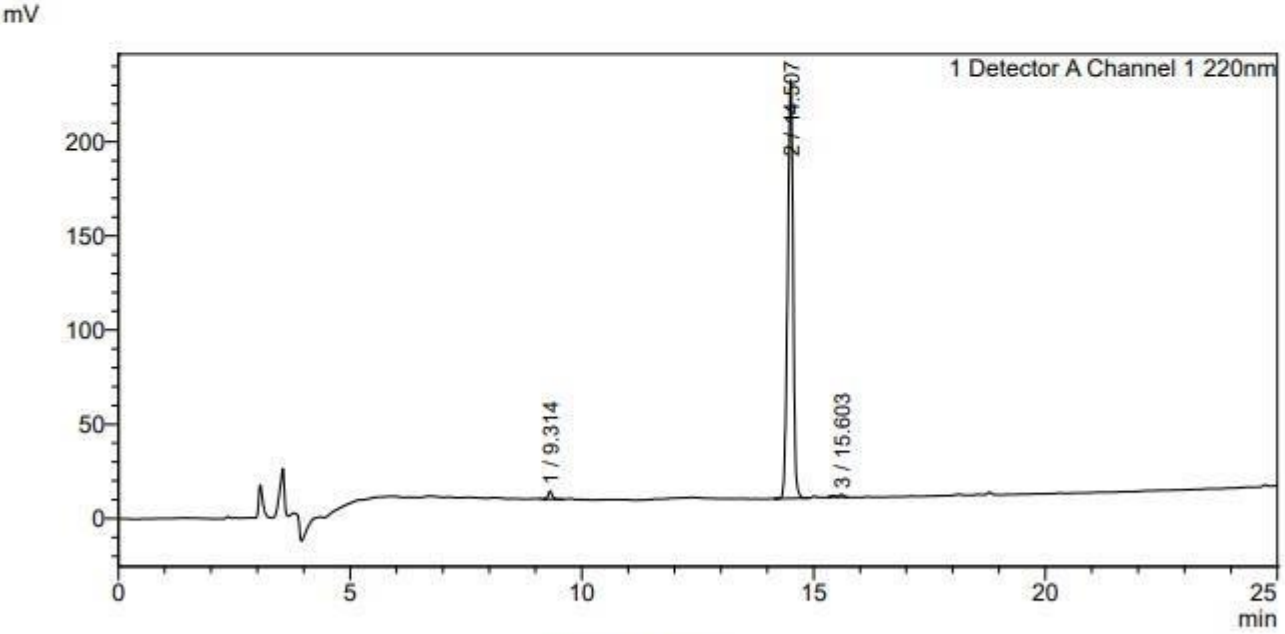
Sample Name :Os-C(W5)
Sample ID :U732ZHI190-1
Time Processed :15:38:55
Month-Day-Year Processed :12/03/2022

Pump A : 0.065% trifluoroacetic in 100% water (v/v)
Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)
Total Flow:1 ml/min
Wavelength:220 nm
<<LC Time Program>>

Time	Module	Command	Value
0.01	Pumps	B.Conc	5
25.00	Pumps	B.Conc	65
25.01	Pumps	B.Conc	95
27.00	Pumps	B.Conc	95
27.01	Pumps	B.Conc	5
35.00	Pumps	B.Conc	5
35.01	Controller	Stop	

<<Column Performance>>
<Detector A>
Column :Inertsil ODS-SP 4.6 x 250 mm
Equipment: GR11010440

<Chromatogram>



<Peak Table>

Detector A Channel 1 220nm

Peak#	Ret. Time	Area	Height	Area%
1	9.314	23880	4323	1.284
2	14.507	1823444	222154	98.013
3	15.603	13085	1481	0.703
Total		1860410	227957	100.000

Supplementary Figure 7: HPLC analysis of Os-C(W_5).

Supplementary Figure 8: Mass spectrometry analysis of Os-C(W_5).