Identification of sulfonamide-tethered *N*-((triazol-4-yl)methyl)isatin derivatives as inhibitors of SARS-CoV-2 Main Protease

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MTT Cytotoxicity Assay towards VERO-E6 Cells

To assess the half maximal cytotoxic concentration (CC50), stock solutions of the test compound **6b** were prepared in 10 % DMSO in ddH2O and diluted further to the working solutions with DMEM. The cytotoxic activity of the extracts was tested in VERO-E6 cells by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method with minor modifications. Briefly, the cells were seeded in 96 well-plates (100 µl/well at a density of 3×105 cells/ml) and incubated for 24 h at 37 °C in 5%CO2. After 24 h, cells were treated with various concentrations of the tested compound **6b** in triplicates. 24 h later, the supernatant was discarded, and cell monolayers were washed with sterile 1x phosphate buffer saline (PBS) 3 times and MTT solution (20 µl of 5 mg/ml stock solution) was add to each well and incubated at 37 °C for 4 h followed by medium aspiration. In each well, the formed formazan crystals were dissolved with 200 µl of acidified isopropanol (0.04 M HCl in absolute isopropanol = 0.073 ml HCL in 50 ml isopropanol). Absorbance of formazan solutions was measured at λ max 540 nm with 620 nm as a reference wavelength using a multi-well plate reader. The percentage of cytotoxicity compared to the untreated cells was determined with the following equation.

The plot of % cytotoxicity versus sample concentration was used to calculate the concentration which exhibited 50% cytotoxicity (CC50).

% cytotoxicity = ((absorbance of cells without treatment-absorbance of cells with treatment) /(absorbance of cells without treatment) X 100)

Cell-Based SARS-CoV-2 Inhibitory Assay

In 96-well tissue culture plates, 2.4×10^4 viral cells were distributed in each well and incubated overnight at a humidified 37°C incubator under 5%CO2 condition. The cell monolayers were then washed once with 1x PBS and subjected to virus adsorption (hCoV-19/Egypt/NRC-03/2020 (Accession Number on GSAID: EPI_ISL_430820)) for 1 h at room temperature (RT). The cell monolayers were further overlaid with 100µl of DMEM containing varying concentrations of the test compound **6b**. Following incubation at 37°C in 5% CO2 incubator for 72 h, the cells were fixed with 100 µl of 4% paraformaldehyde for 20 min and stained with 0.1% crystal violet in distilled water for 15 min at RT. The crystal violet dye was then dissolved using 100 µl absolute methanol per well and the optical density of the color is measured at 570 nm using Anthos Zenyth 200rt plate reader (Anthos Labtec Instruments, Heerhugowaard, Netherlands). The IC50 of the compound is that required to reduce the virus-induced cytopathic effect (CPE) by 50%, relative to the virus control.

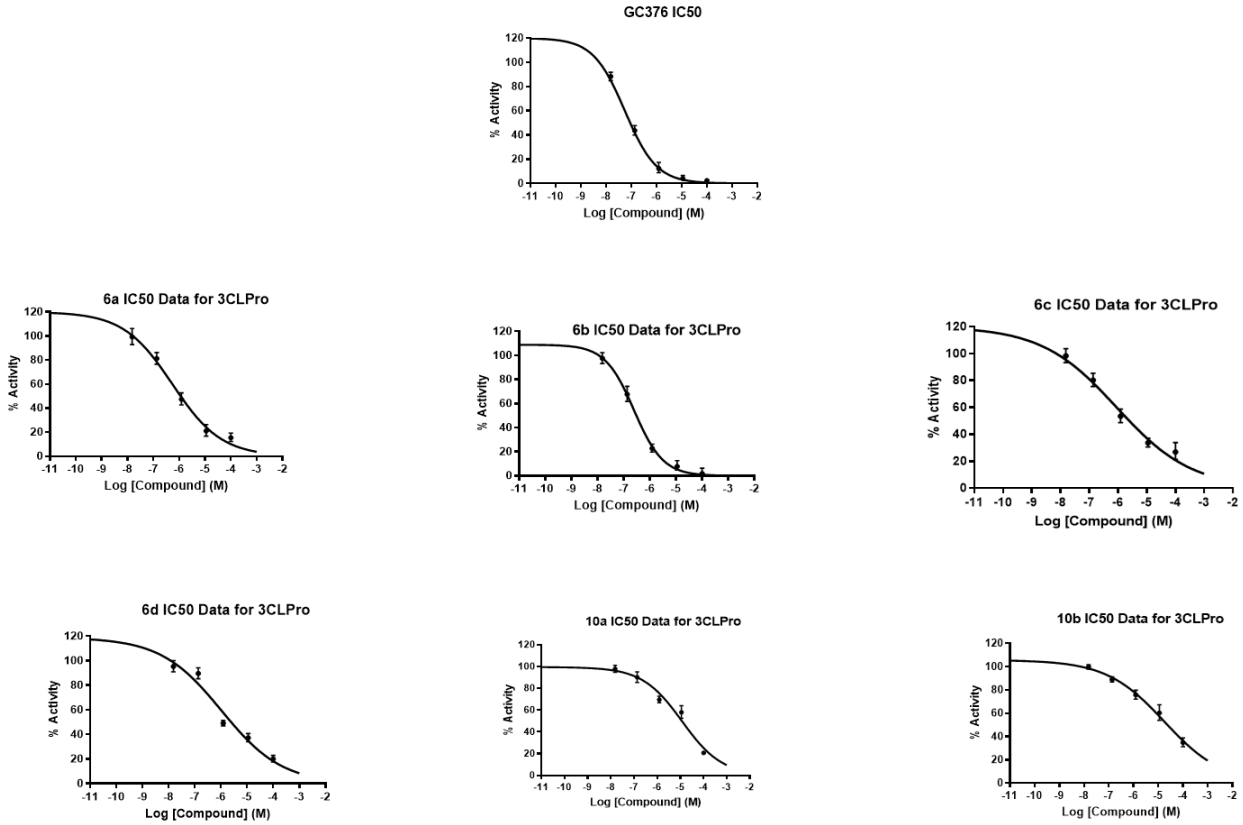


Figure S1. Curve fittings for Mpro enzyme inhibition assay against the main protease enzyme (Mpro) of SARS-COV2 showing the IC_{50} for GC376 and the investigated triazolo isatins (**6a-d** and **10a-b**)

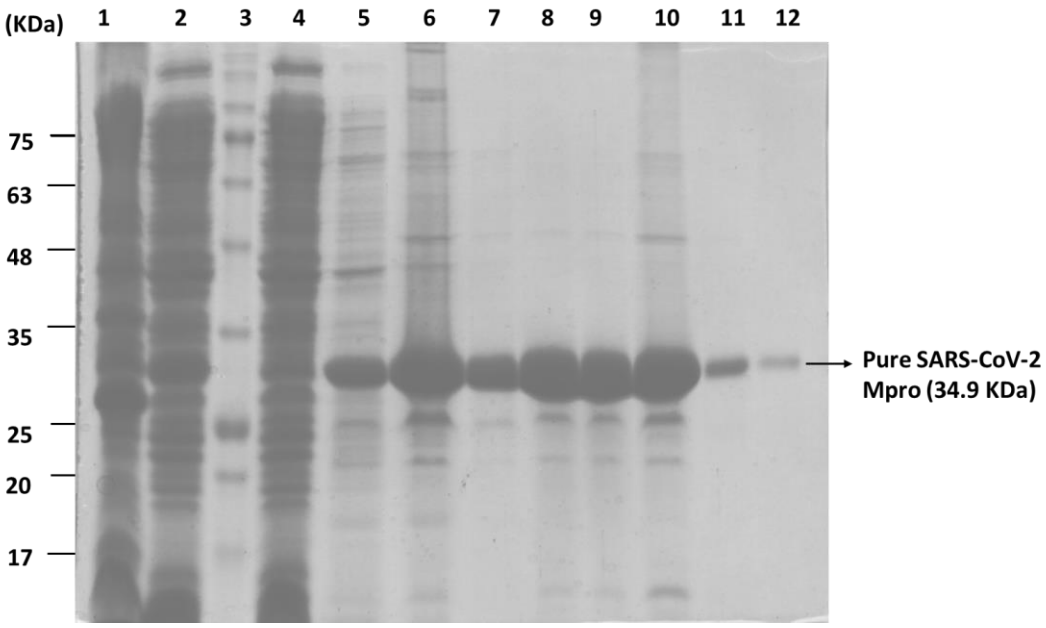
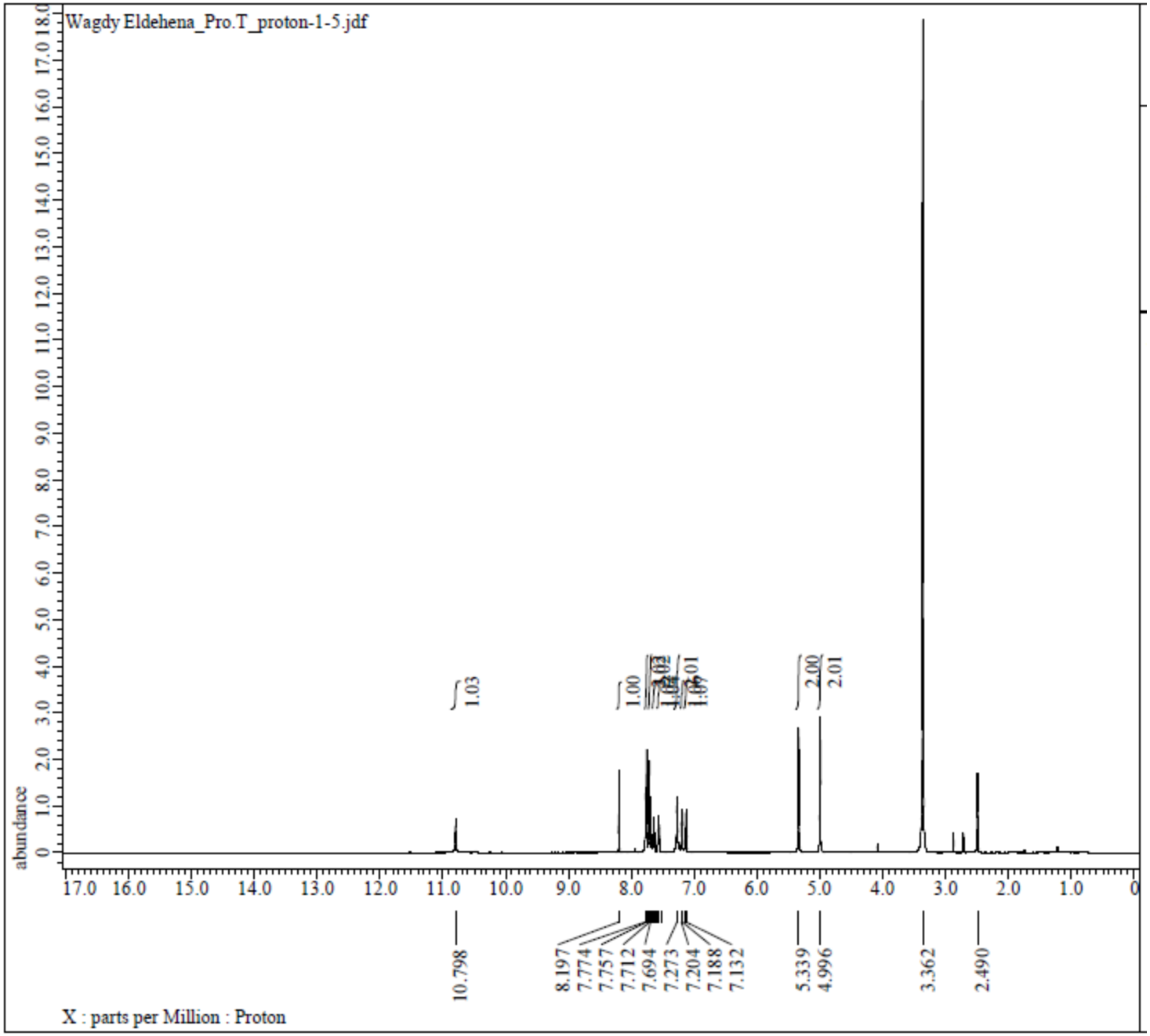


Figure S2. SDS-PAGE analysis for purification of SARS-CoV-2 Mpro. lane1: insoluble fraction of cell lysate; lane 2: soluble fraction of cell lysate; lane 3, protein molecular mass marker; lane 4: flow through of the soluble fraction of the cell lysate; lane 5: fraction obtained using the wash buffer, lanes 6-12: fractions containing Mpro protein eluted using the elution buffer.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2.01
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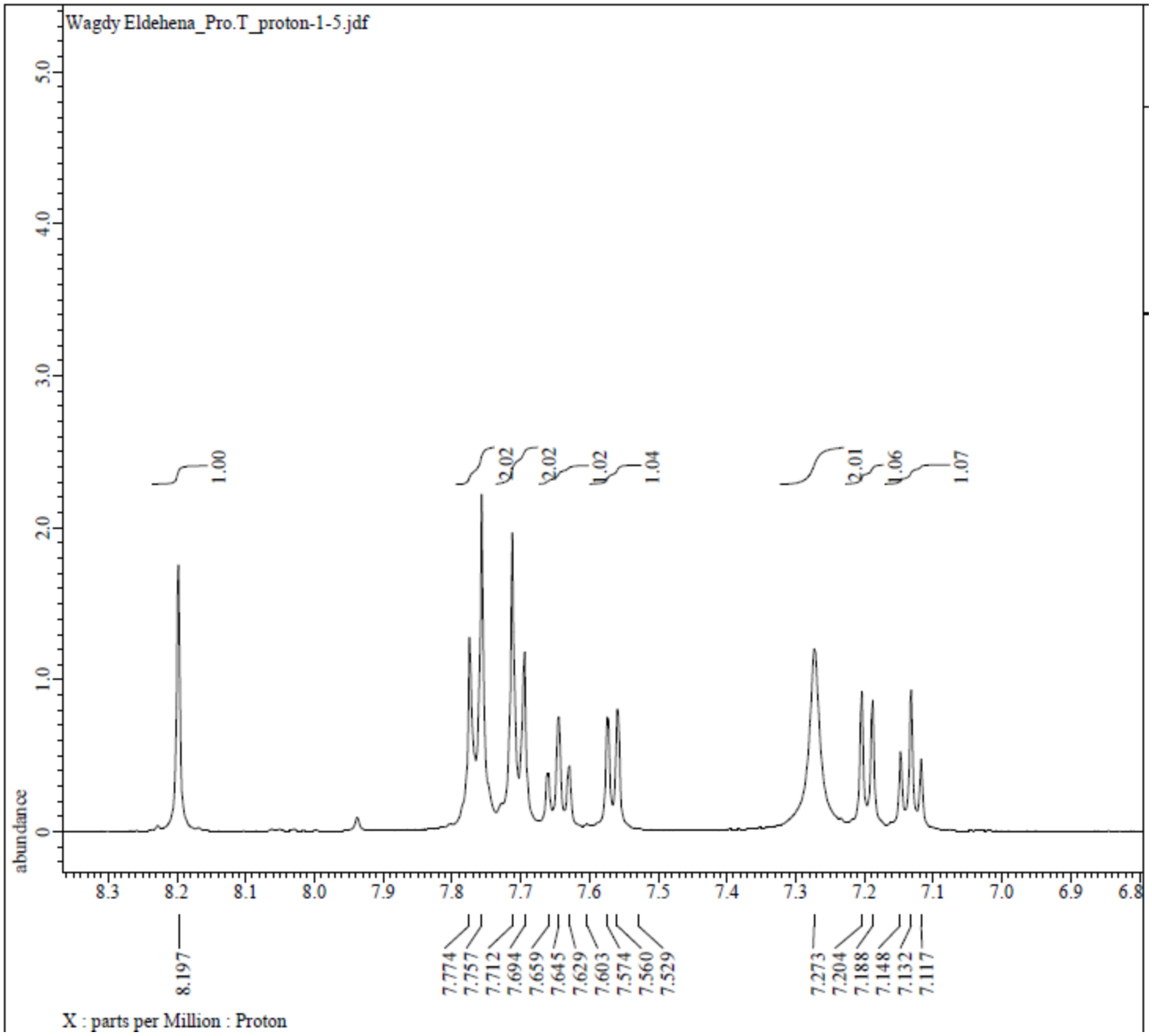
7.774
7.757
7.712
7.694
7.273
7.204
7.188
7.132
5.339
4.996
3.362
2.490
X : parts per Million : Proton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1.07
8.197
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7.757
7.712
7.694
7.659
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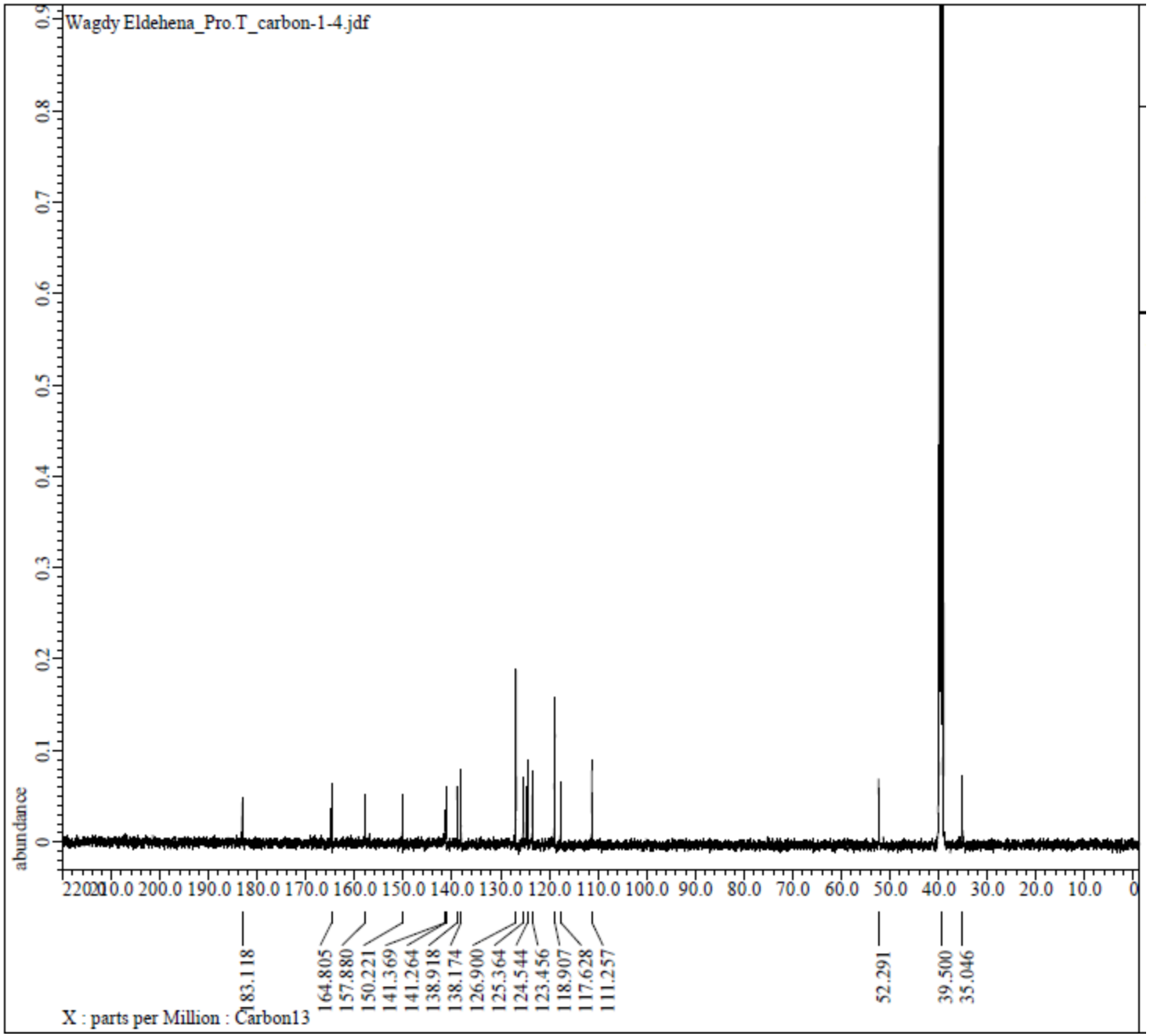
7.148
7.132
7.117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183.118
164.805
157.880
150.221
141.369
141.264
138.918
138.174
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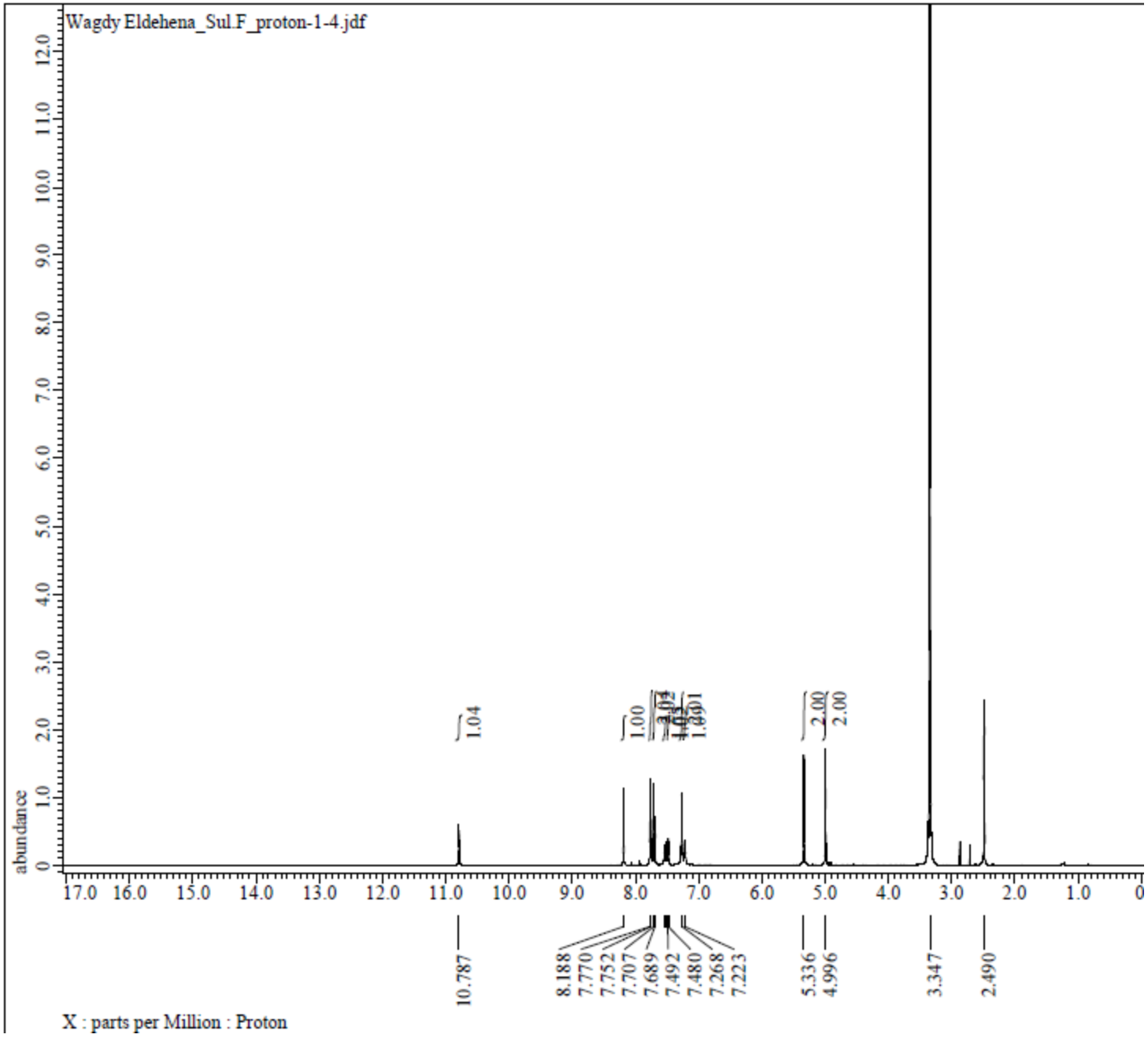
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7.752
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4.996
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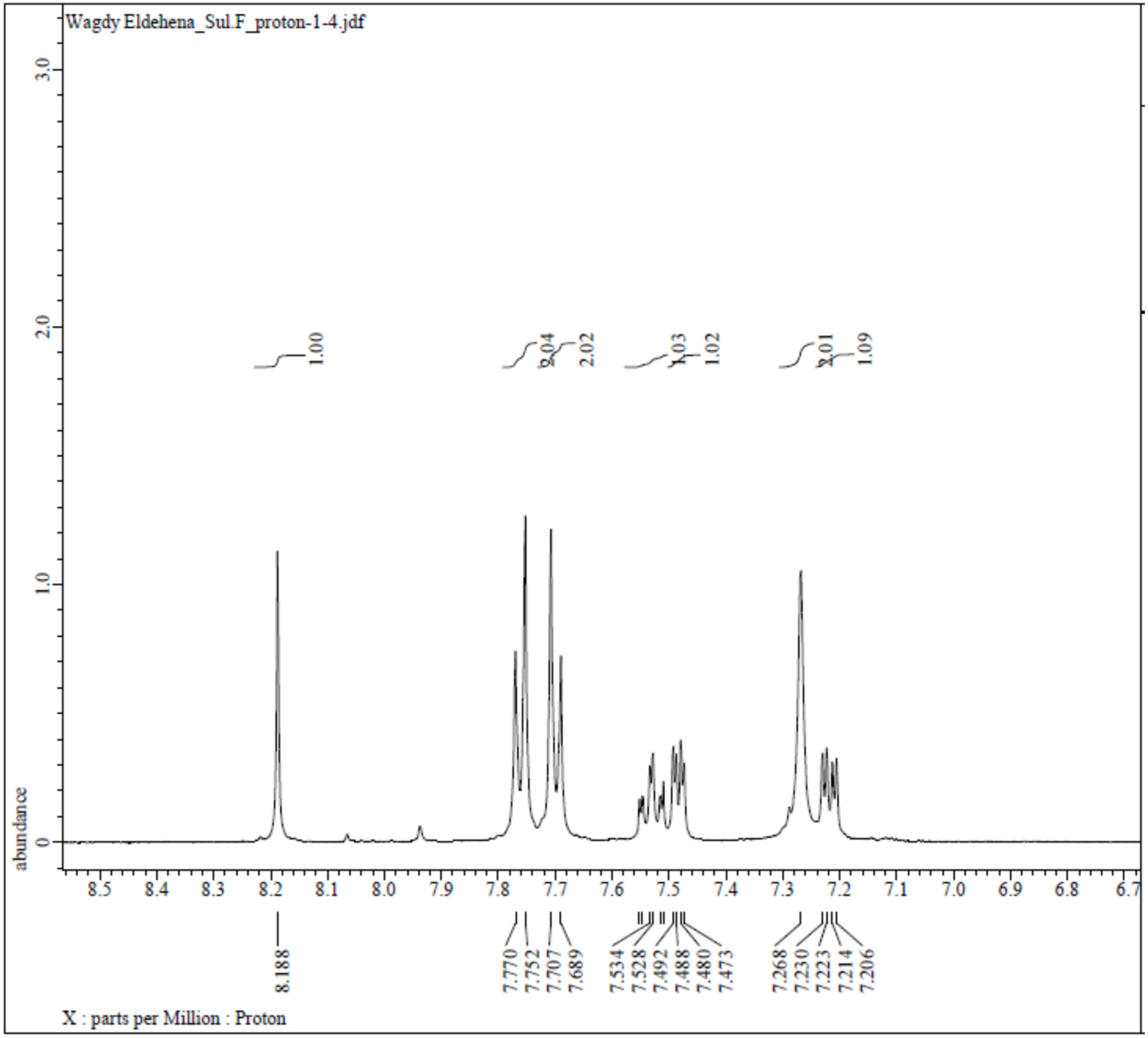
X : parts per Million : Proton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1.02
2.01
1.09
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8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
8.188
7.770
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7.707
7.689
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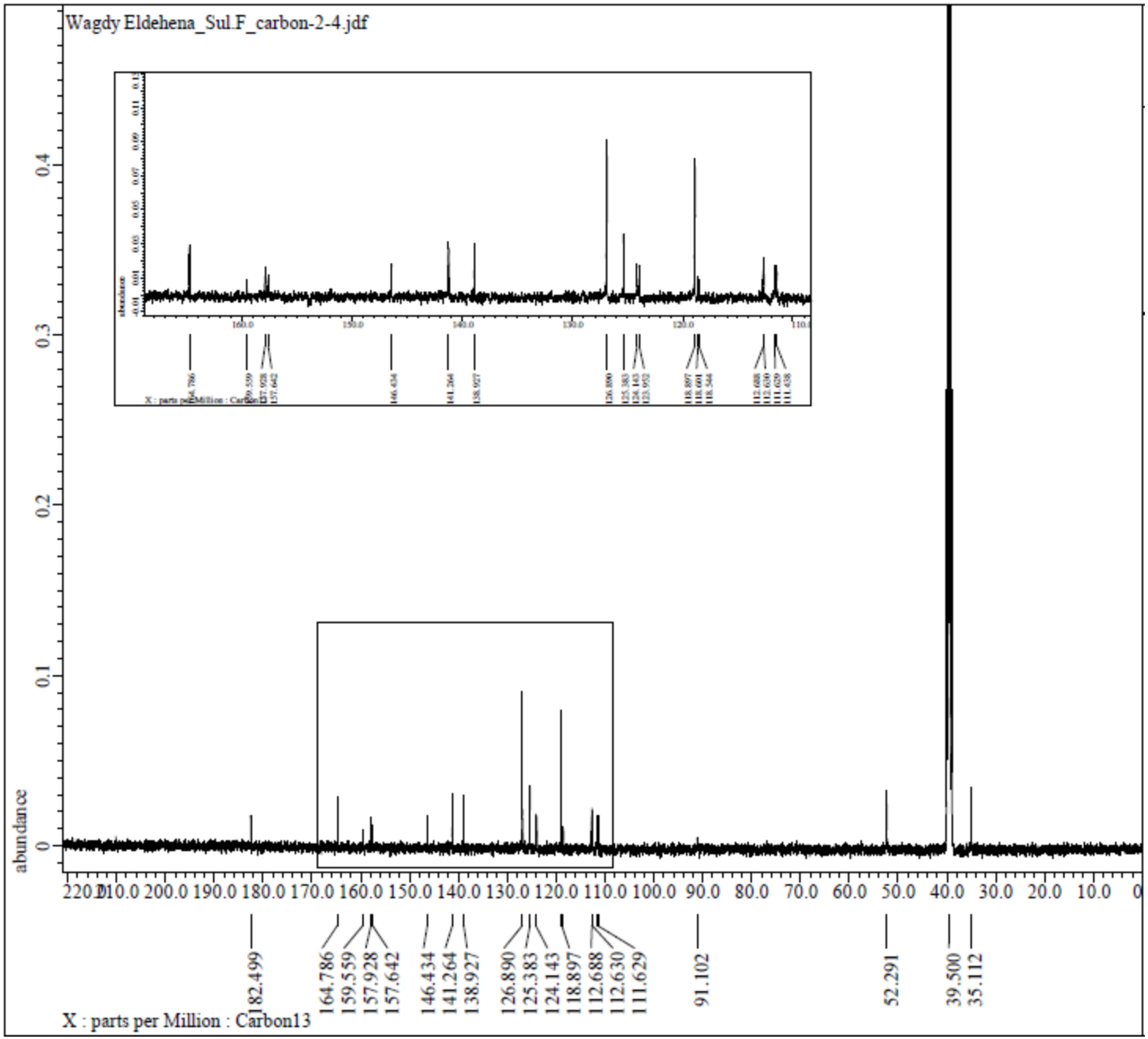
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abundance
X : parts per Million : Carbon13
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157.642
146.434
141.264
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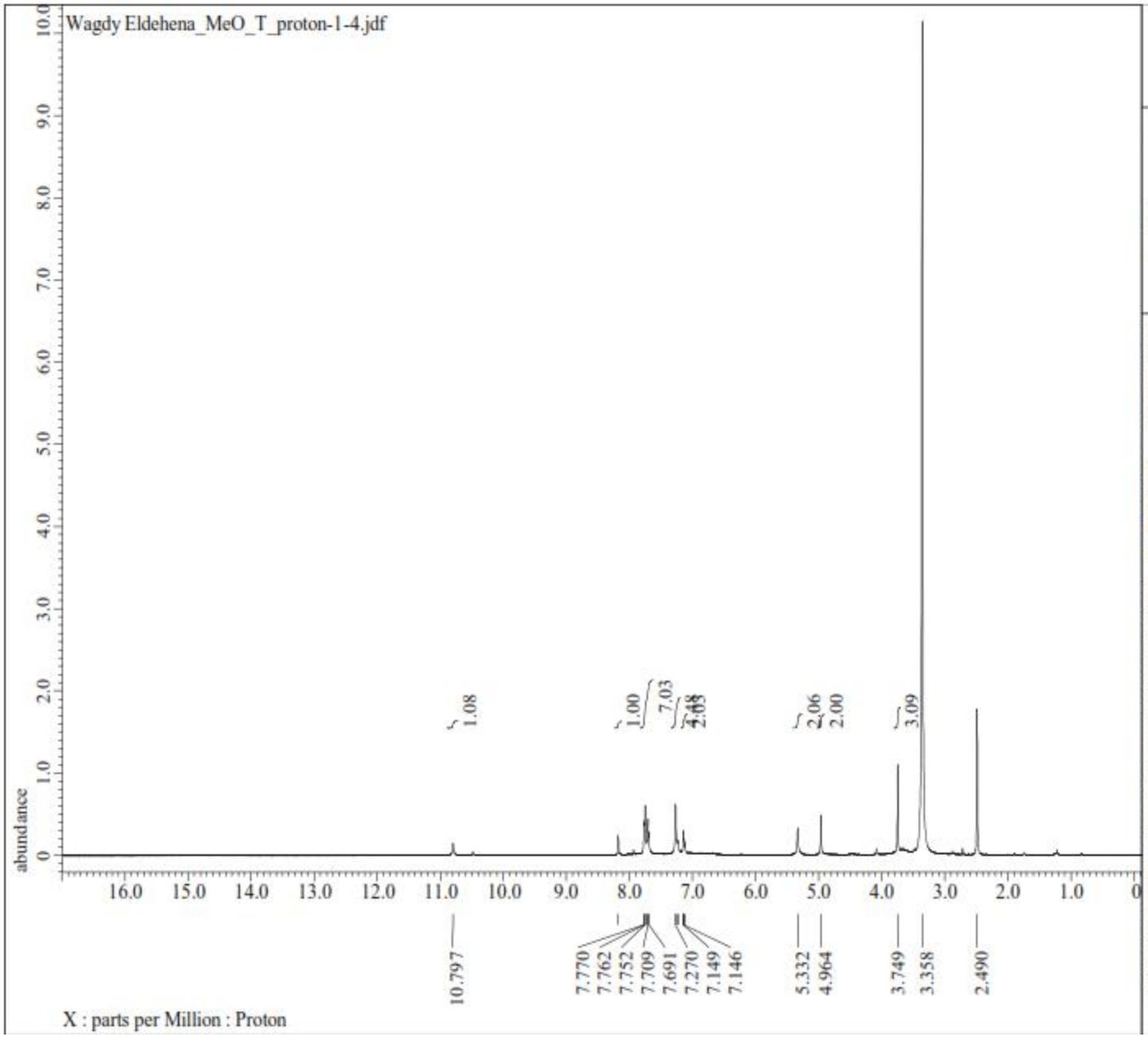

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JEOL ECA-500 II

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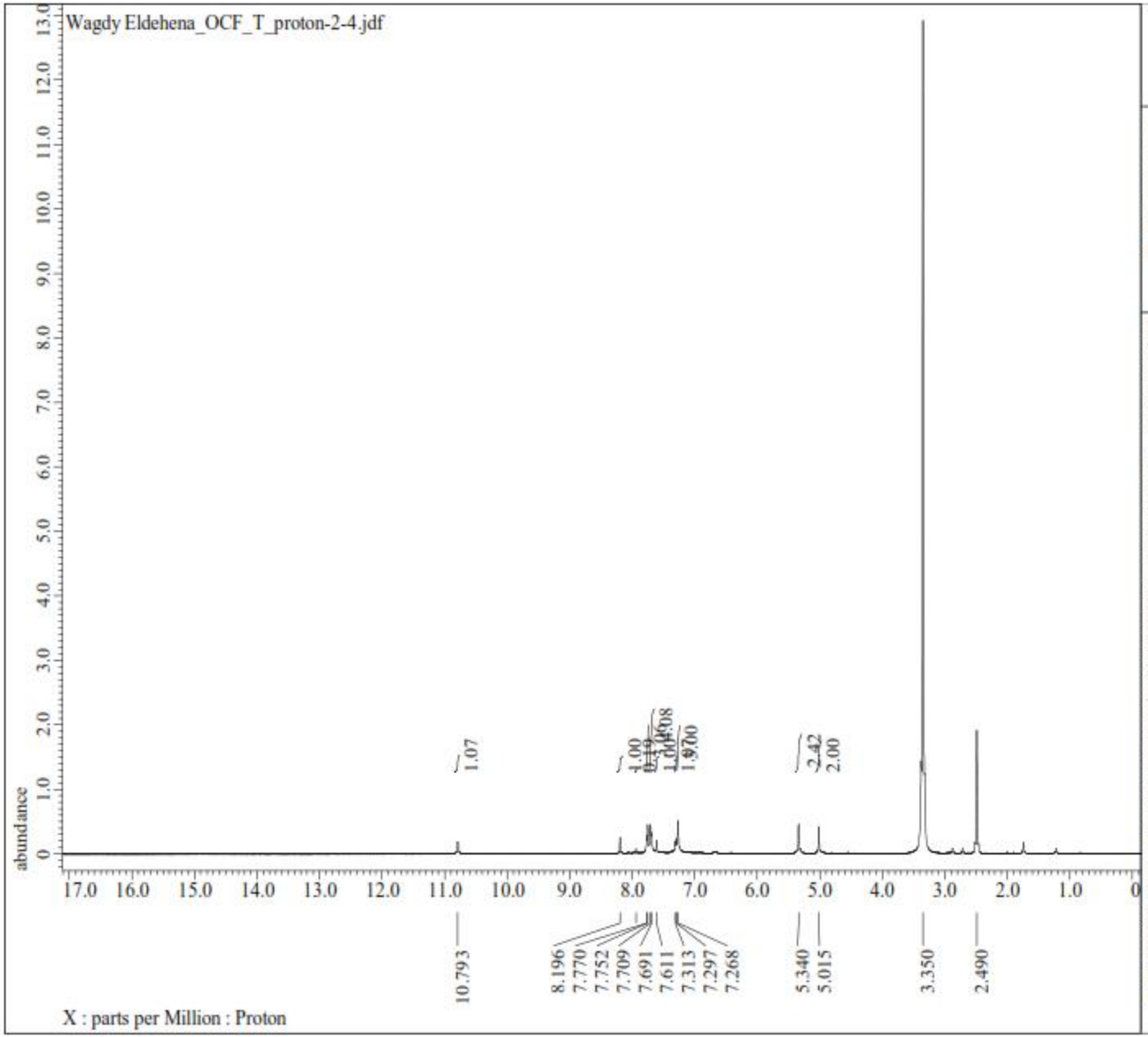

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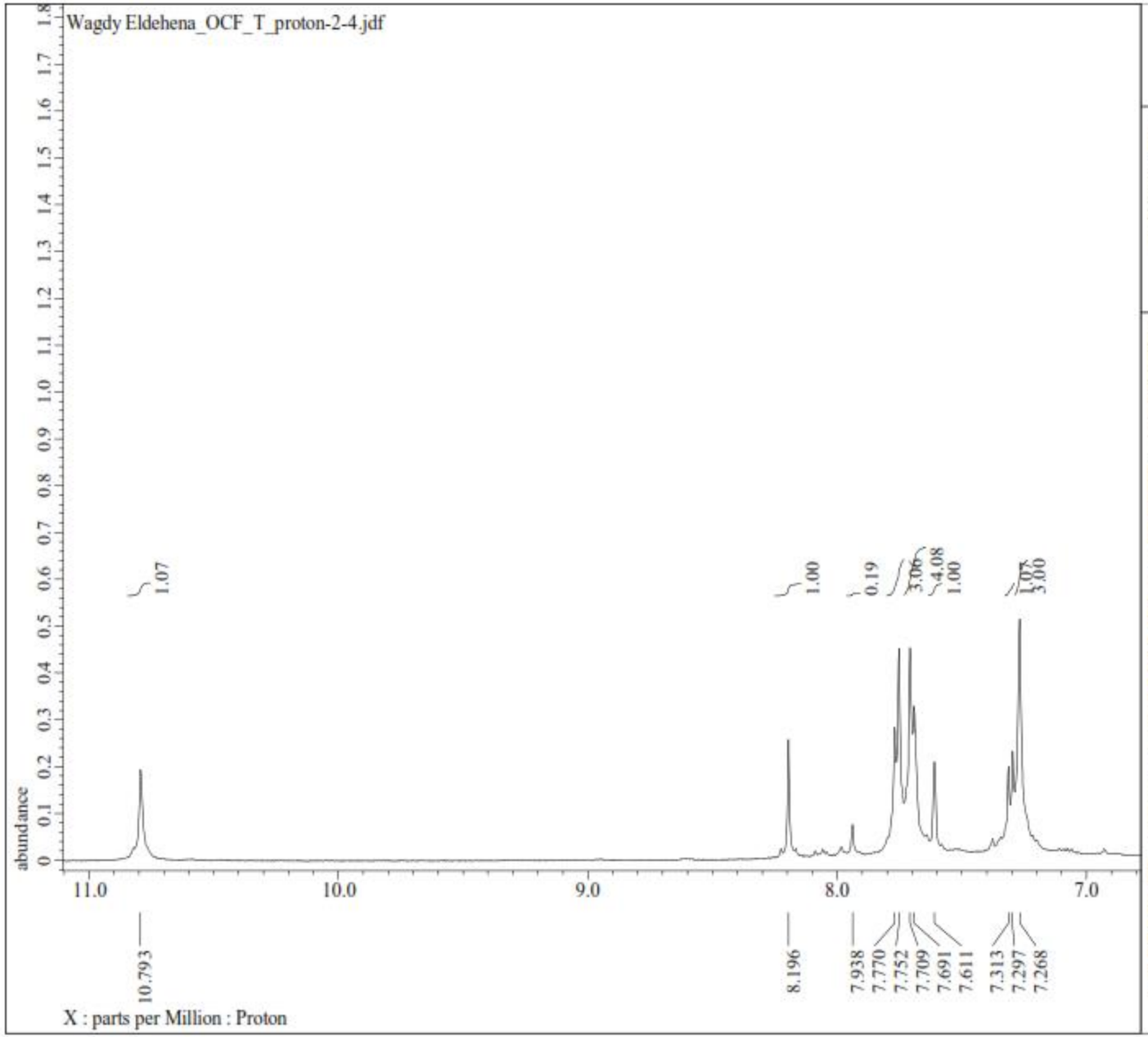

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X_Sweep_Clipped   = 10.50420168[kHz]
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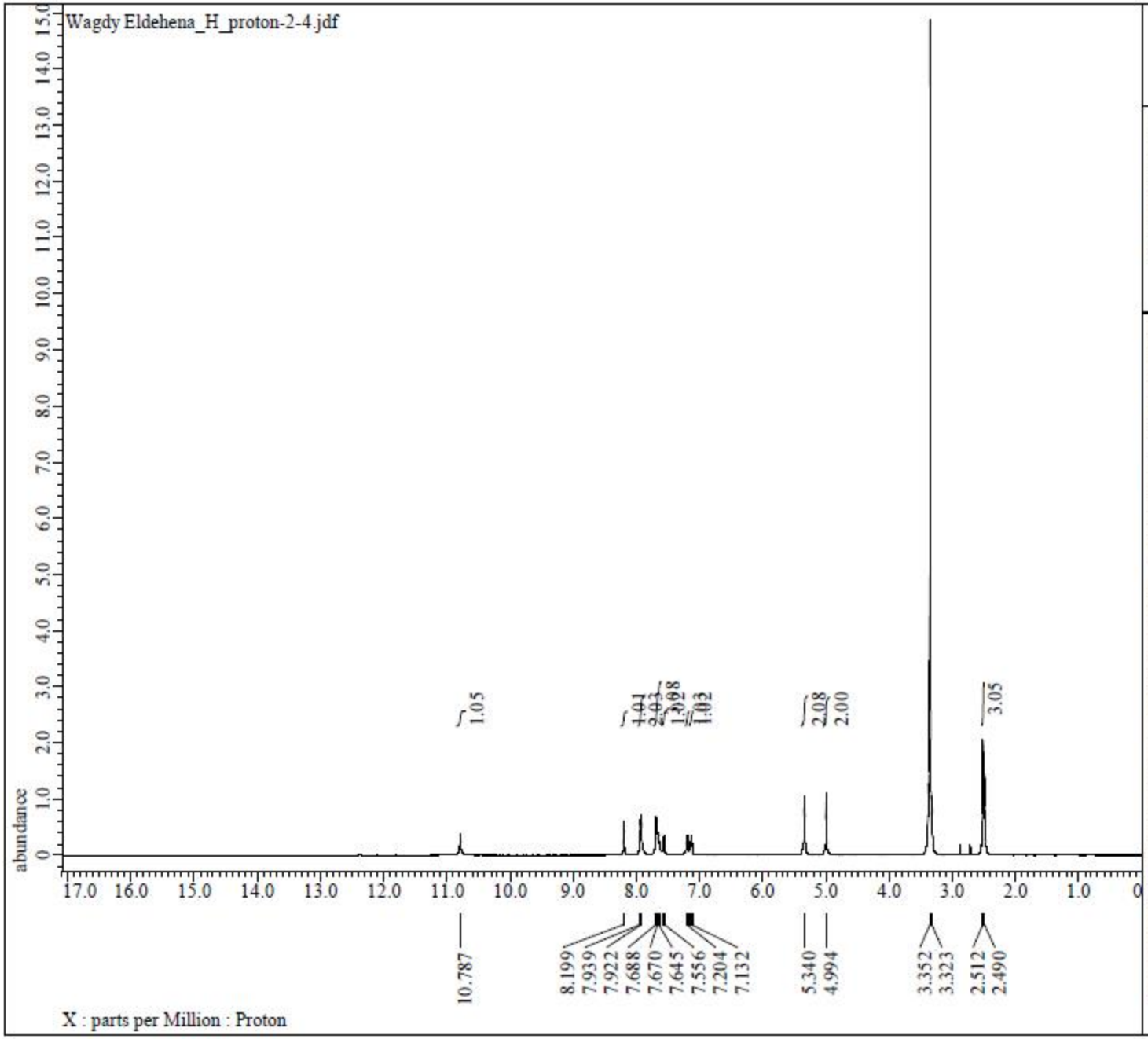

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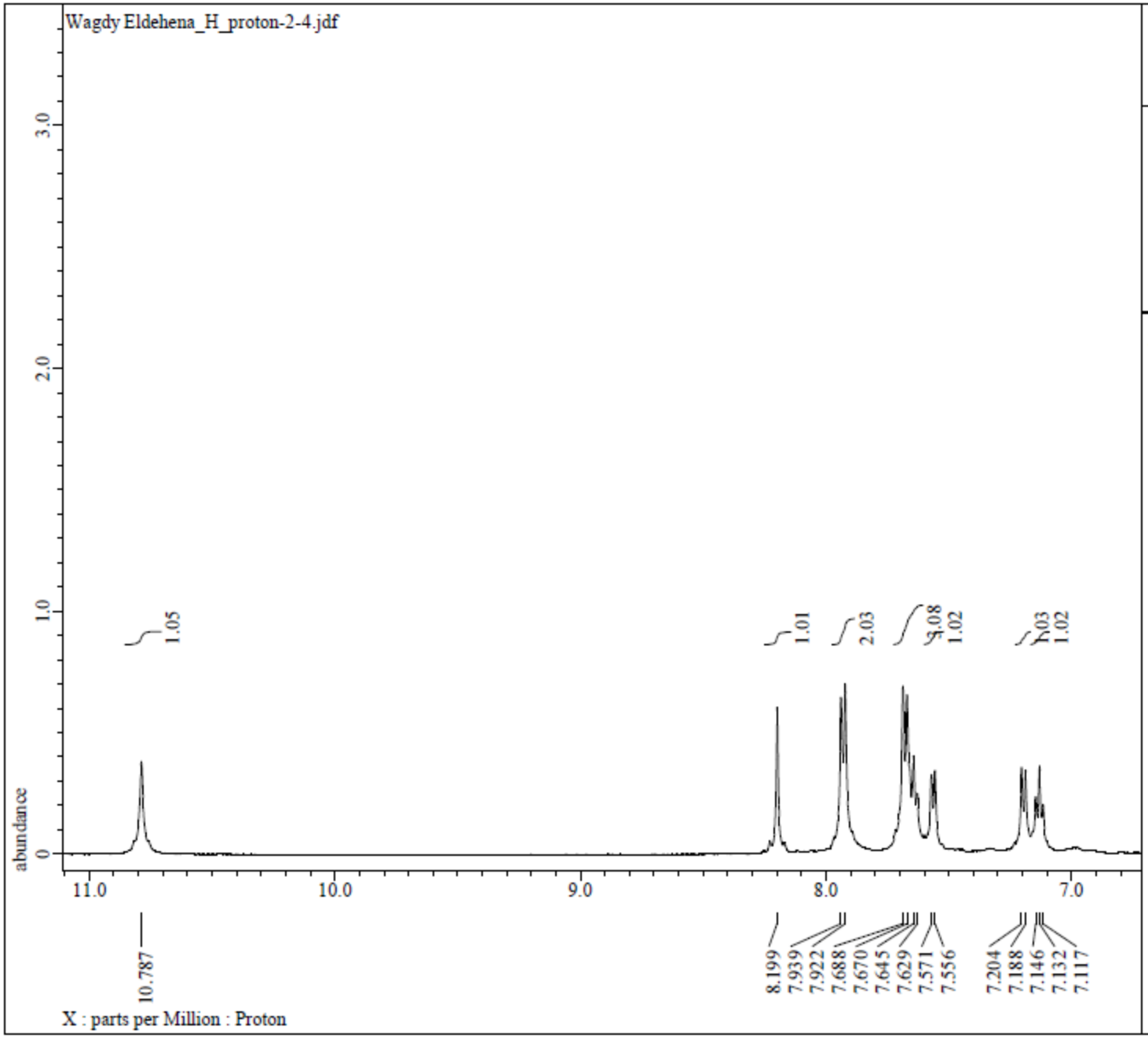

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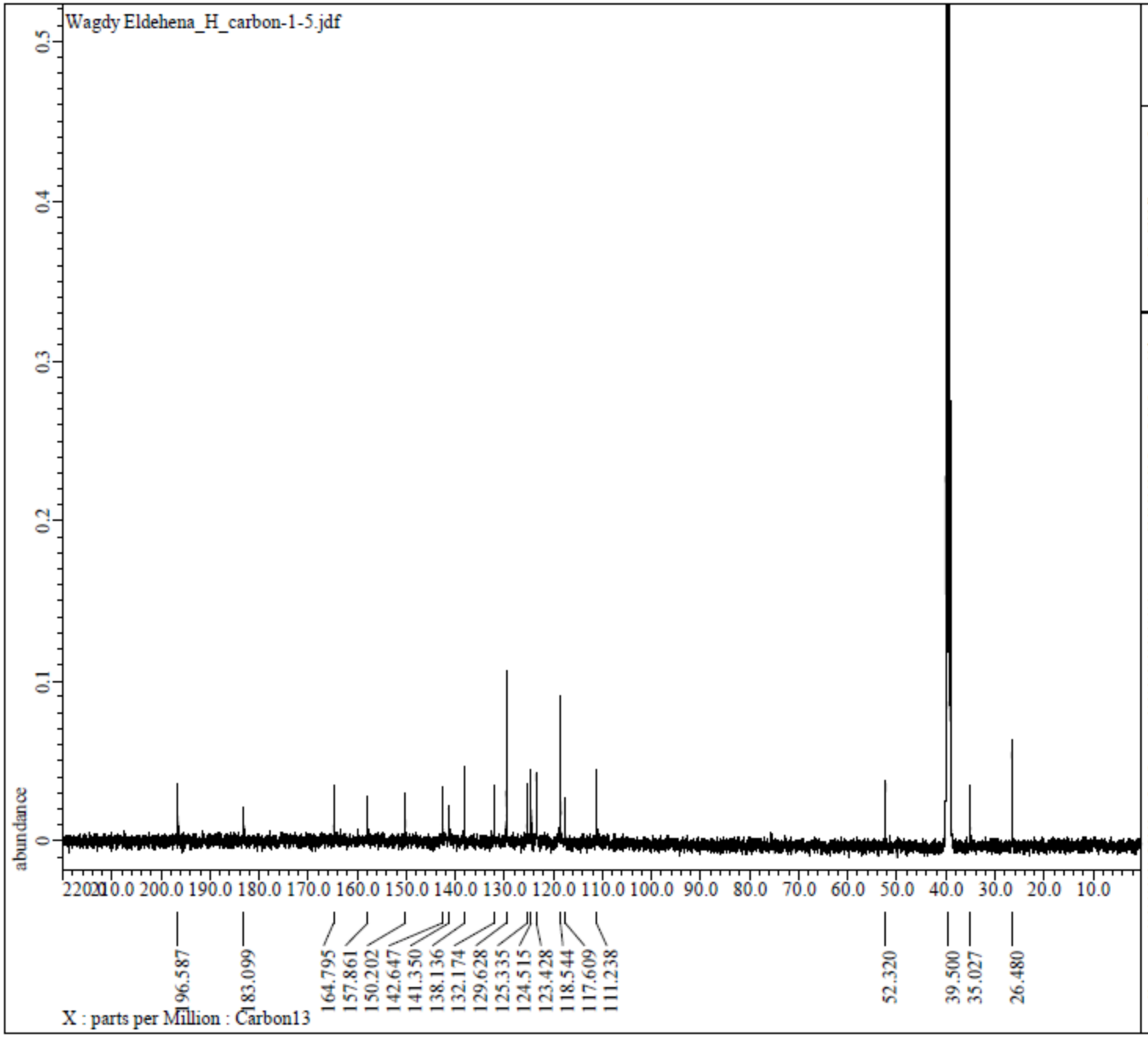

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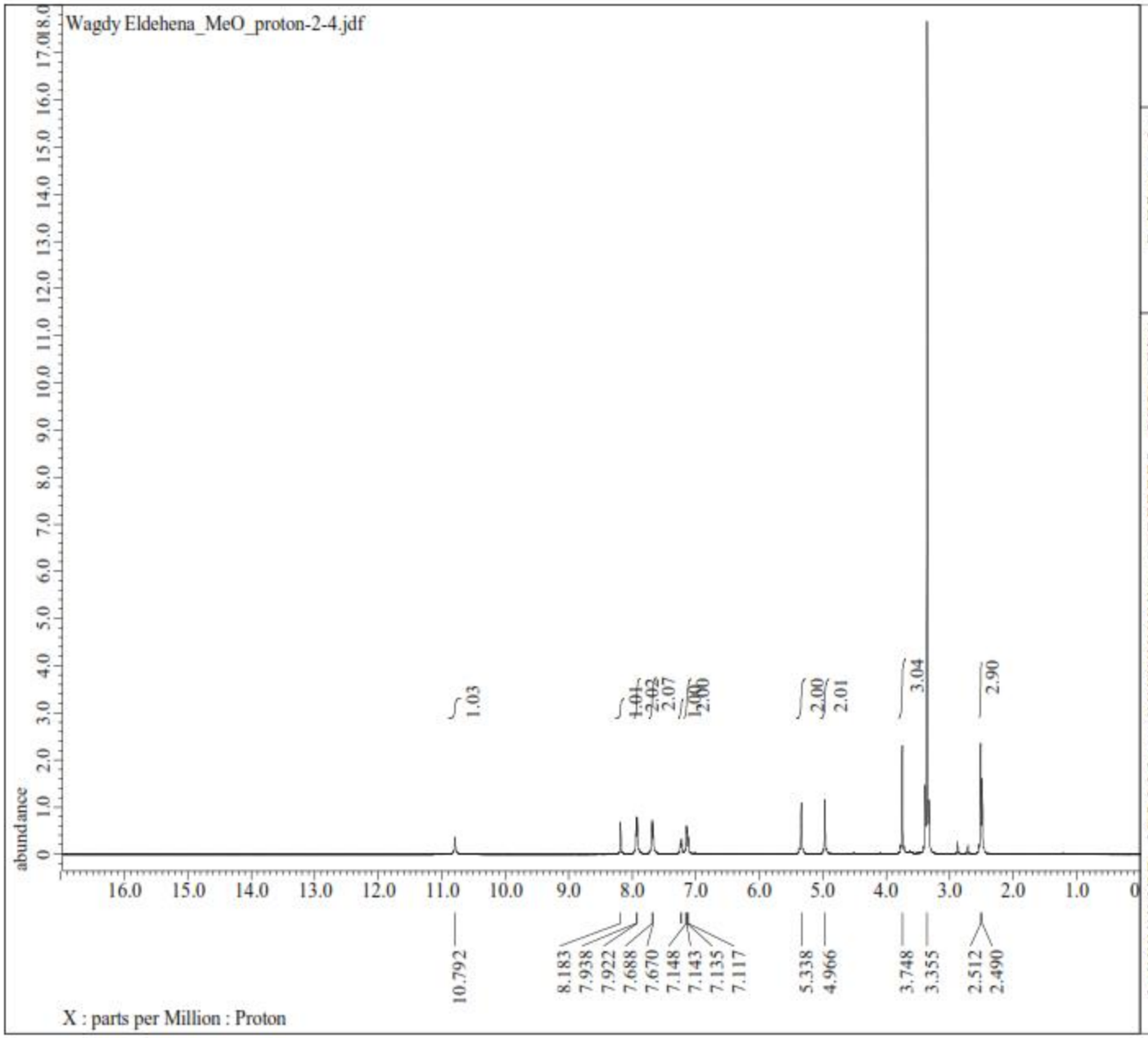
Dimensions        = X
Site              = JNM-ECA500II
Spectrometer      = DELTA2_NMR

Field_Strength    = 11.7473579[T] (500[MHz]
X_Acq_Duration    = 0.83361792[s]
X_Domain          = 13C
X_Freq            = 125.76529768[MHz]
X_Offset          = 100[ppm]
X_Points          = 32768
X_Prescans        = 4
X_Resolution      = 1.19959034[Hz]
X_Sweep           = 39.3081761[kHz]
X_Sweep_Clipped   = 31.44654088[kHz]
Irr_Domain        = Proton
Irr_Freq          = 500.15991521[MHz]
Irr_Offset        = 5.0[ppm]
Clipped           = FALSE
Scans             = 1024
Total_Scans       = 1024

Relaxation_Delay  = 2[s]
Recvr_Gain        = 60
Temp_Get          = 21.9[dC]
X_90_Width        = 48[us]
X_Acq_Time        = 0.83361792[s]
X_Angle           = 30[deg]
X_Atn             = 10[dB]
X_Pulse           = 16[us]
Irr_Atn_Dec       = 19.992[dB]
Irr_Atn_Noe       = 19.992[dB]
Irr_Noise         = WALTZ
Irr_Pwidth        = 92[us]
Decoupling        = TRUE
```

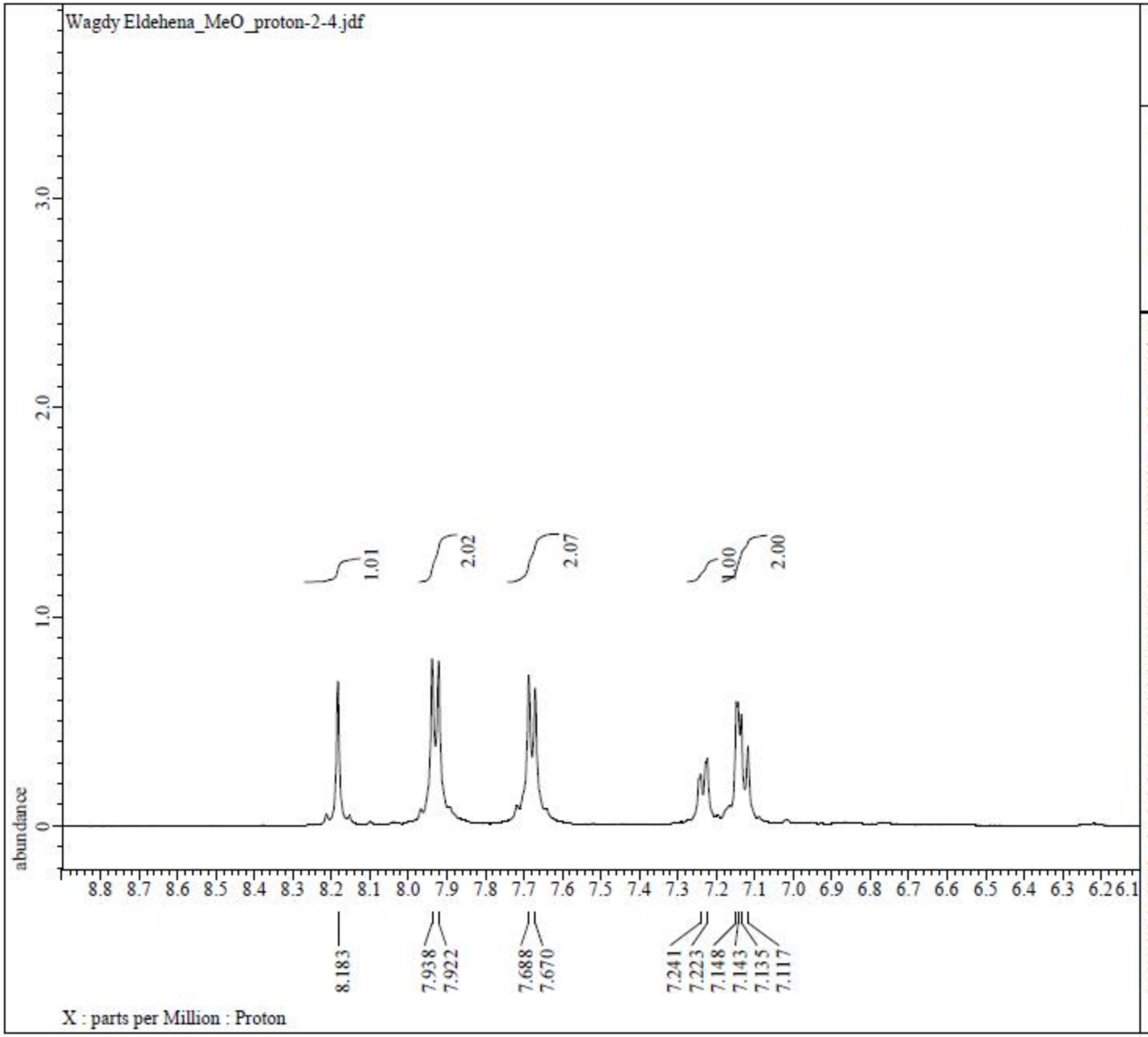


Mansoura University
JEOL ECA- 500 II
NMR Unit

```
---- PROCESSING PARAMETERS ----
dc_balance( 0, FALSE )
sexp( 0.2[Hz], 0.0[s] )
trapezoid( 0[%], 0[%], 80[%], 100[%] )
zerofill( 1 )
fft( 1, TRUE, TRUE )
machinephase
ppm

Derived from: Wagdy Eldehena_MeO_proton-2-1
```

Filename	= Wagdy Eldehena_MeO_proto
Author	= delta
Experiment	= proton.jxp
Sample_Id	= Wagdy Eldehena_MeO
Solvent	= DMSO-D6
Creation_Time	= 31-DEC-2022 15:35:26
Revision_Time	= 31-DEC-2022 15:43:23
Current_Time	= 31-DEC-2022 15:43:30
Comment	= single_pulse
Data_Format	= 1D COMPLEX
Dim_Size	= 13107
Dim_Title	= Proton
Dim_Units	= [ppm]
Dimensions	= X
Site	= JNM-ECA500II
Spectrometer	= DELTA2_NMR
Field_Strength	= 11.7473579[T] (500[MHz])
X_Acq_Duration	= 1.24780544[s]
X_Domain	= 1H
X_Freq	= 500.15991521[MHz]
X_Offset	= 7.0[ppm]
X_Points	= 16384
X_Prescans	= 1
X_Resolution	= 0.80140699[Hz]
X_Sweep	= 13.1302521[kHz]
X_Sweep_Clipped	= 10.50420168[kHz]
Irr_Domain	= Proton
Irr_Freq	= 500.15991521[MHz]
Irr_Offset	= 5.0[ppm]
Tri_Domain	= Proton
Tri_Freq	= 500.15991521[MHz]
Tri_Offset	= 5.0[ppm]
Clipped	= FALSE
Scans	= 40
Total_Scans	= 40
Relaxation_Delay	= 5[s]
Recvr_Gain	= 46
Temp_Get	= 21.5[dC]
X_90_Width	= 12.20[us]
X_Acq_Time	= 1.24780544[s]
X_Angle	= 45[deg]
X_Atn	= 2.5[dB]
X_Pulse	= 6.14[us]
Irr_Mode	= Off
Tri_Mode	= Off



Mansoura University
JEOL ECA-500 II
NMR Unit

```
---- PROCESSING PARAMETERS ----
dc_balance( 0, FALSE )
sexp( 0.2[Hz], 0.0[s] )
trapezoid( 0[%], 0[%], 80[%], 100[%] )
zerofill( 1 )
fft( 1, TRUE, TRUE )
machinephase
ppm

Derived from: Wagdy Eldehena_MeO_proton-2-1
```

```
Filename          = Wagdy Eldehena_MeO_proto
Author            = delta
Experiment        = proton.jxp
Sample_Id         = Wagdy Eldehena_MeO
Solvent           = DMSO-D6
Creation_Time     = 31-DEC-2022 15:35:26
Revision_Time     = 31-DEC-2022 15:44:52
Current_Time      = 31-DEC-2022 15:44:56

Comment           = single_pulse
Data_Format       = 1D COMPLEX
Dim_Size          = 13107
Dim_Title         = Proton
Dim_Units         = [ppm]
Dimensions        = X
Site              = JNM-ECA500II
Spectrometer      = DELTA2_NMR

Field_Strength    = 11.7473579[T] (500[MHz])
X_Acq_Duration    = 1.24780544[s]
X_Domain          = 1H
X_Freq            = 500.15991521[MHz]
X_Offset          = 7.0[ppm]
X_Points          = 16384
X_Prescans        = 1
X_Resolution      = 0.80140699[Hz]
X_Sweep           = 13.1302521[kHz]
X_Sweep_Clipped   = 10.50420168[kHz]
Irr_Domain        = Proton
Irr_Freq          = 500.15991521[MHz]
Irr_Offset        = 5.0[ppm]
Tri_Domain        = Proton
Tri_Freq          = 500.15991521[MHz]
Tri_Offset        = 5.0[ppm]
Clipped           = FALSE
Scans             = 40
Total_Scans       = 40

Relaxation_Delay  = 5[s]
Recvr_Gain        = 46
Temp_Get          = 21.5[dC]
X_90_Width        = 12.28[us]
X_Acq_Time        = 1.24780544[s]
X_Angle           = 45[deg]
X_Atn             = 2.5[dB]
X_Pulse           = 6.14[us]
Irr_Mode          = Off
Tri_Mode          = Off
```