# Ratiometric fluorescence determination of Gd(III) using plumbagin: method validation and analysis of real samples

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***Table 1S.*** *Optimized operating parameters of the ICP-MS (PerkinElmer NexION 300X)*

| **Operating Parameter** | **Measured Value** |
|---|---|
| Nebulizer gas flow STD/KED (L/min) | 0.85 |
| Auxiliary gas flow (L/min) | 1.40 |
| Plasma gas flow (L/min) | 11.0 |
| Deflector voltage (V) | -9.50 |
| ICP RF power (W) | 1350 |
| Analog stage voltage (V) | -1700 |
| Pulse stage voltage (V) | 900 |
| KED cell gas A (L/min) | 0.42 |
| DRC cell gas A (L/min) | 0.60 |

***Table 2S.*** *Selected isotopes of rare earth elements for ICP-MS analysis*

| No. | Element | Symbol | Isotope |
|---|---|---|---|
| 1 | Scandium | Sc | 45 |
| 2 | Yttrium | Y | 89 |
| 3 | Lanthanum | La | 139 |
| 4 | Cerium | Ce | 140 |
| 5 | Praseodymium | Pr | 141 |
| 6 | Neodymium | Nd | 142 |
| 7 | Samarium | Sm | 152 |
| 8 | Europium | Eu | 153 |
| 9 | Gadolinium | Gd | 157 |
| 10 | Terbium | Tb | 159 |
| 11 | Dysprosium | Dy | 164 |
| 12 | Holmium | Ho | 165 |
| 13 | Erbium | Er | 166 |
| 14 | Thulium | Tm | 169 |
| 15 | Ytterbium | Yb | 174 |
| 16 | Lutetium | Lu | 175 |

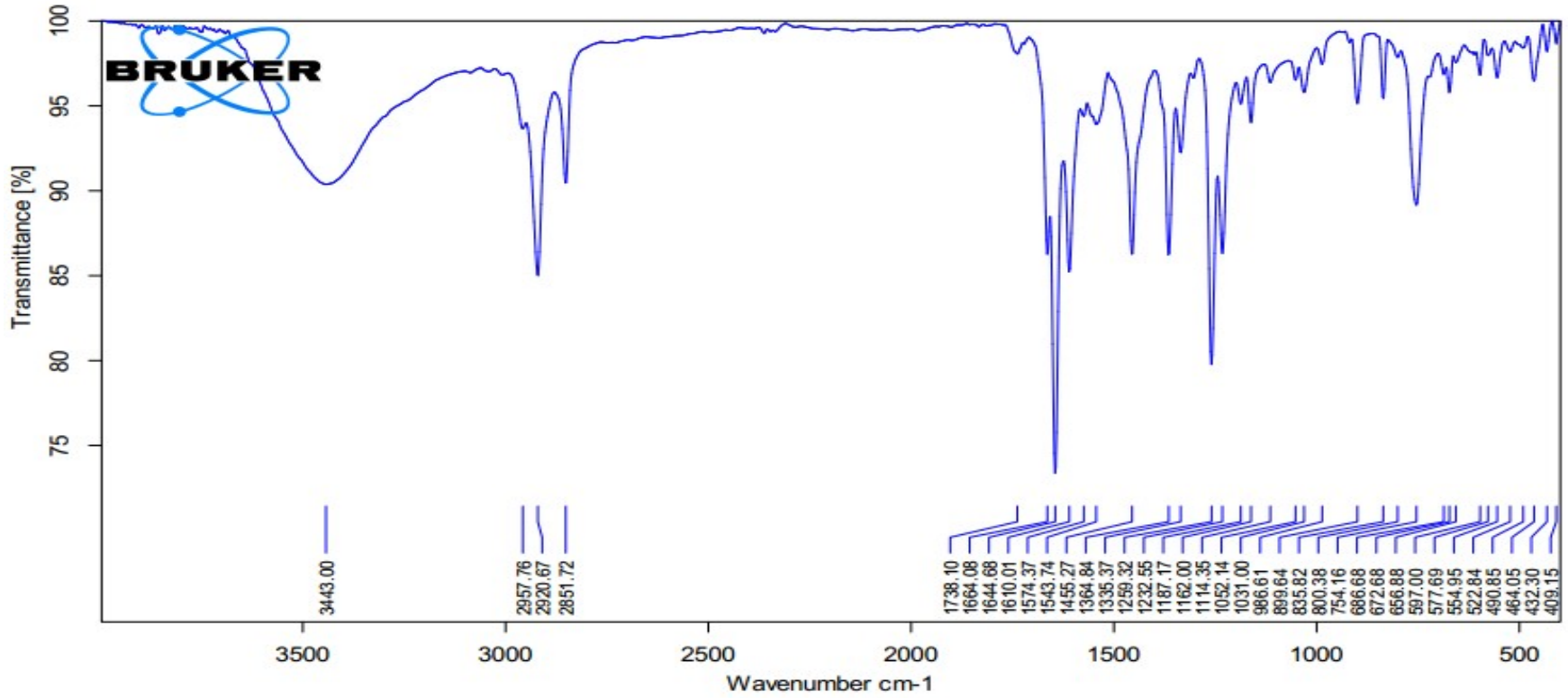


***Figure 1S.*** *The FT-IR spectrum of the Gd-PLB complex*

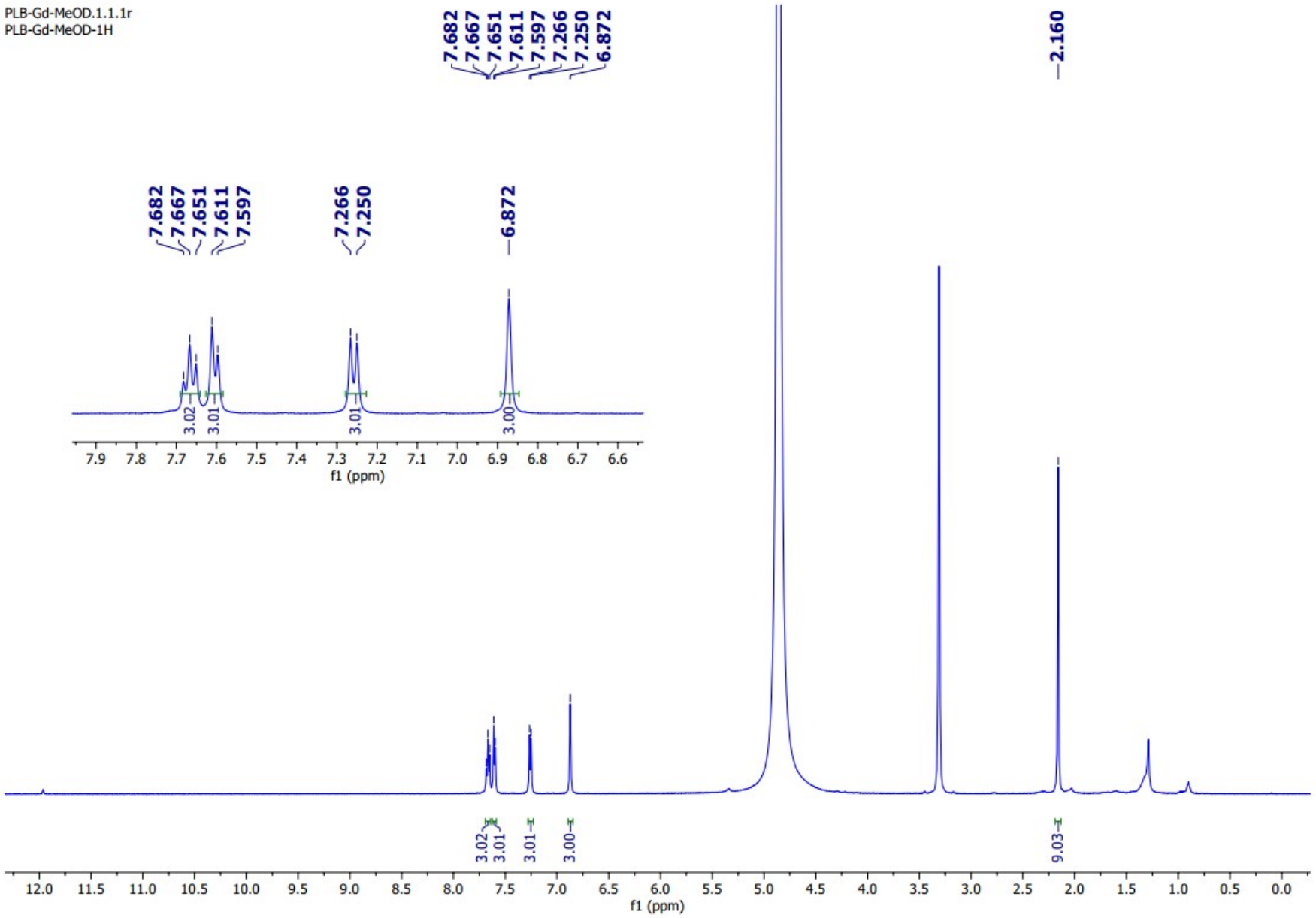


***Figure 2S.*** *The [1]H-NMR spectrum of Gd-PLB complex in solid phase*

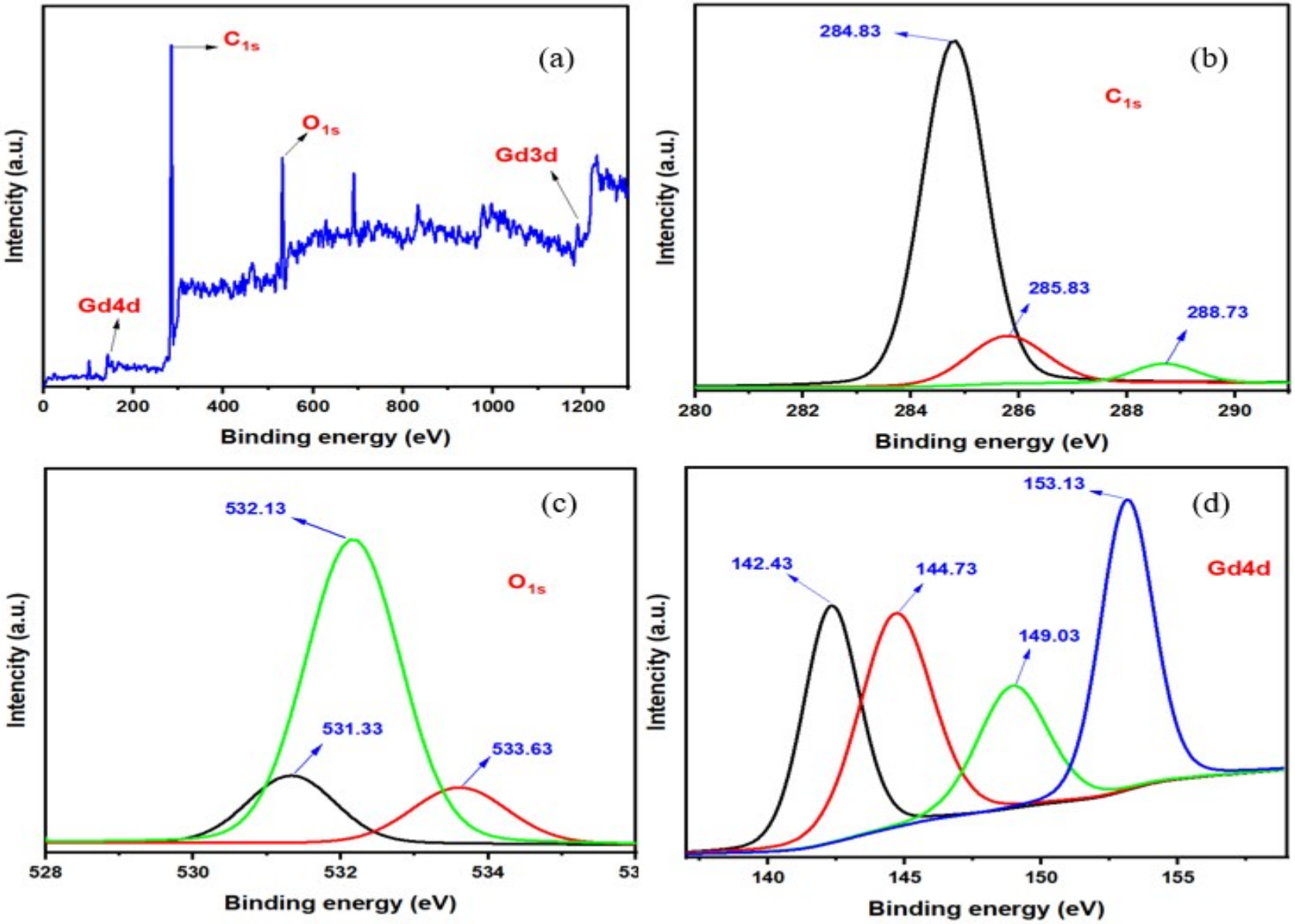


***Figure 3S.*** *The XPS spectrum of Gd-PLB complex in solid phase*

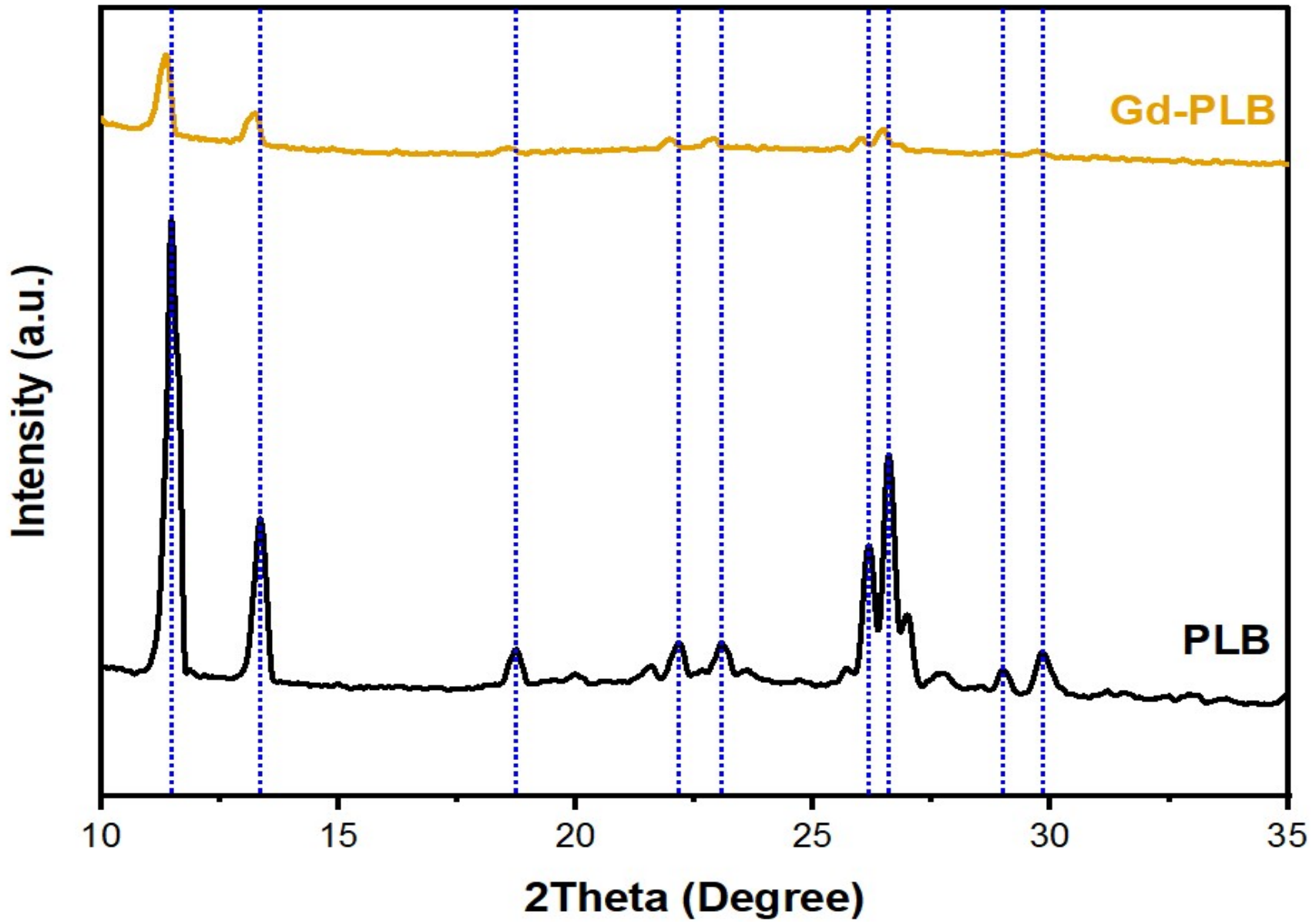


***Figure 4S.*** *The XRD spectrum of Gd-PLB complex in solid phase*

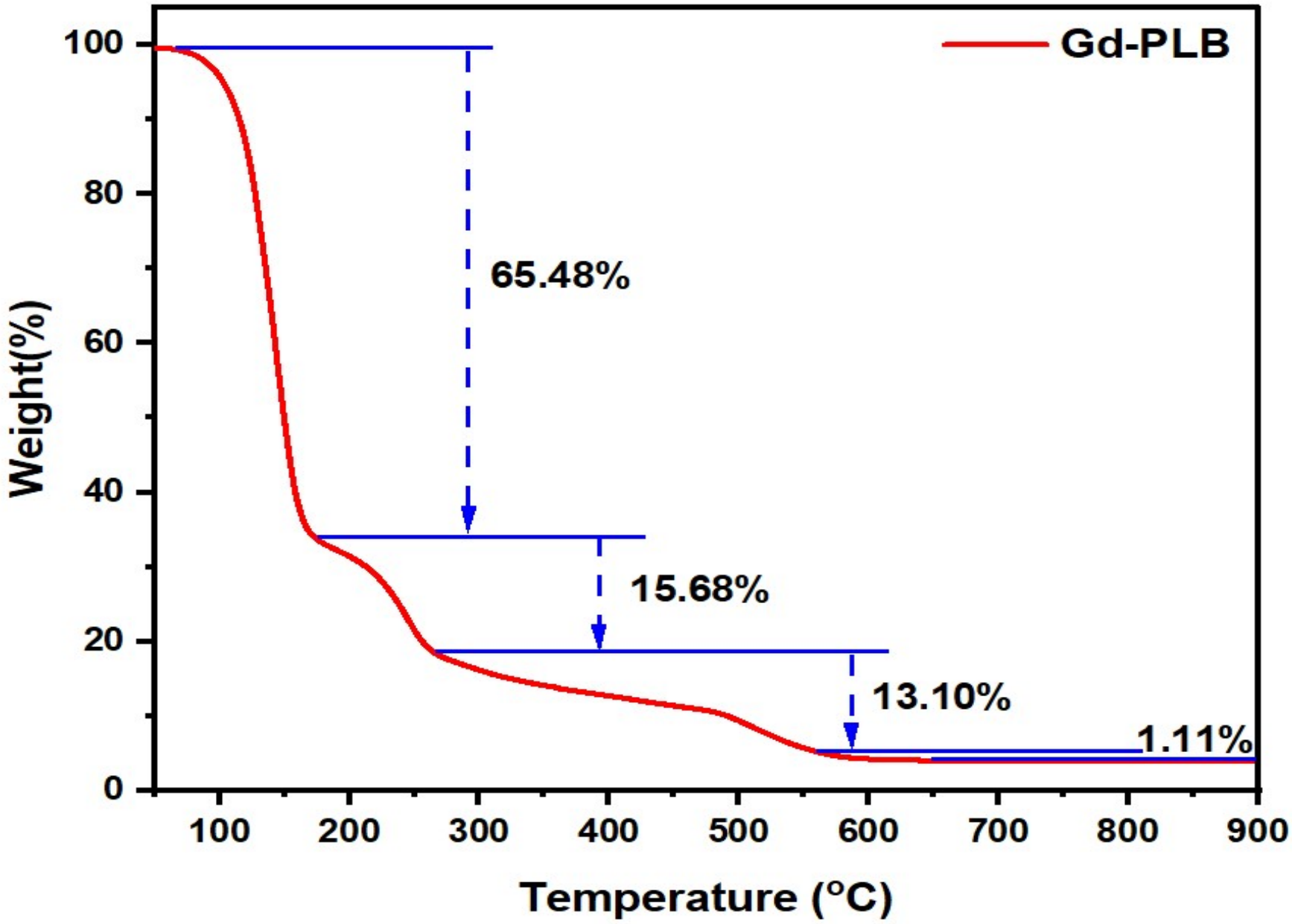


***Figure 5S.*** *The TGA spectrum of Gd-PLB complex in solid phase*


| Element | Line | Mass% | Atom% |
|---|---|---|---|
| C | K | 76.72 ± 0.31 | 83.9 ± 0.34 |
| O | K | 19.2 ± 0.45 | 15.76 ± 0.37 |
| Gd | K | 4.08 ± 0.32 | 0.34 ± 0.03 |
| Total | | 100.00 | 100.00 |

***Figure 6S.*** *SEM-EDS of Gd-PLB complex in solid phase*

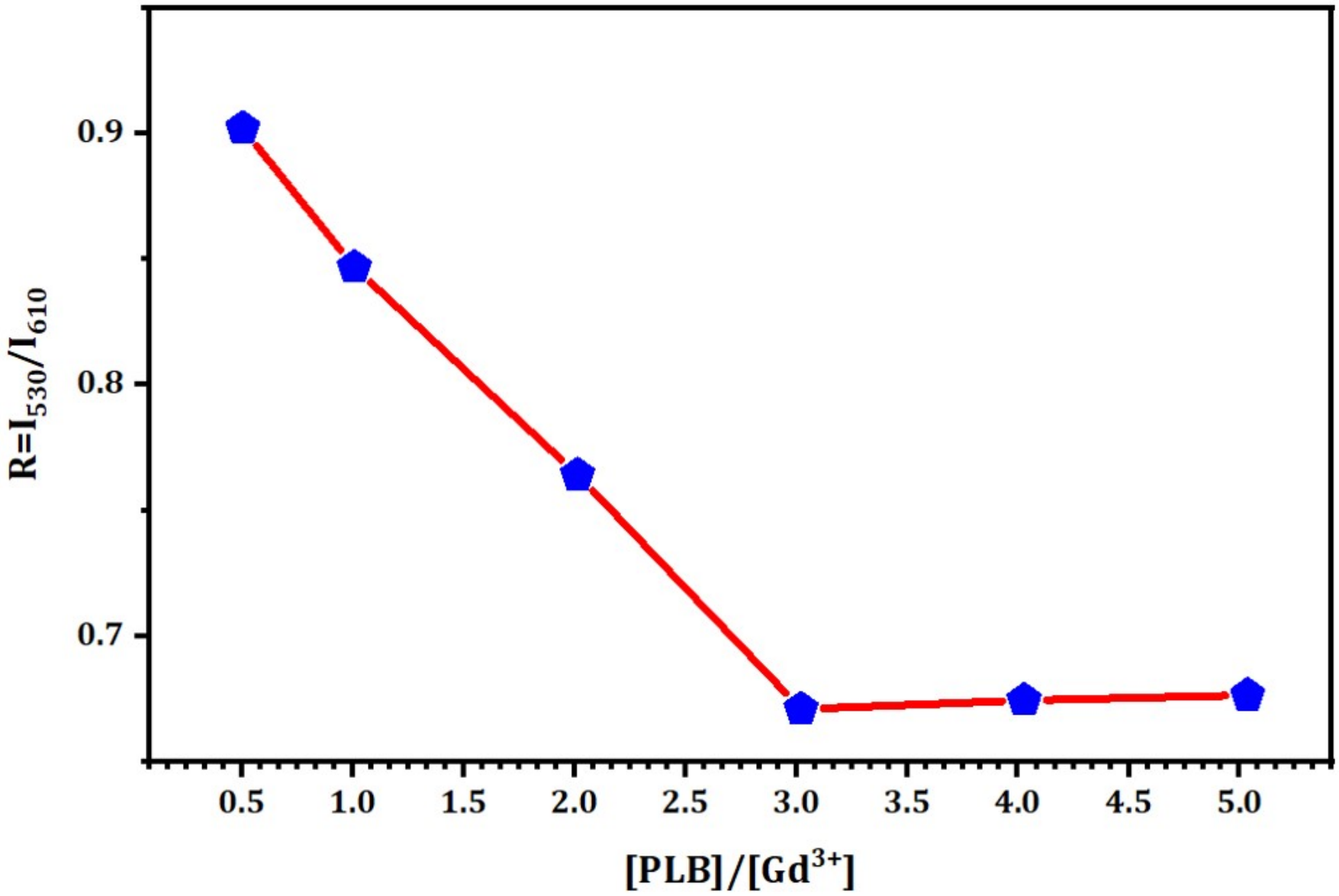


*Figure 7S. The molar ratio plot*

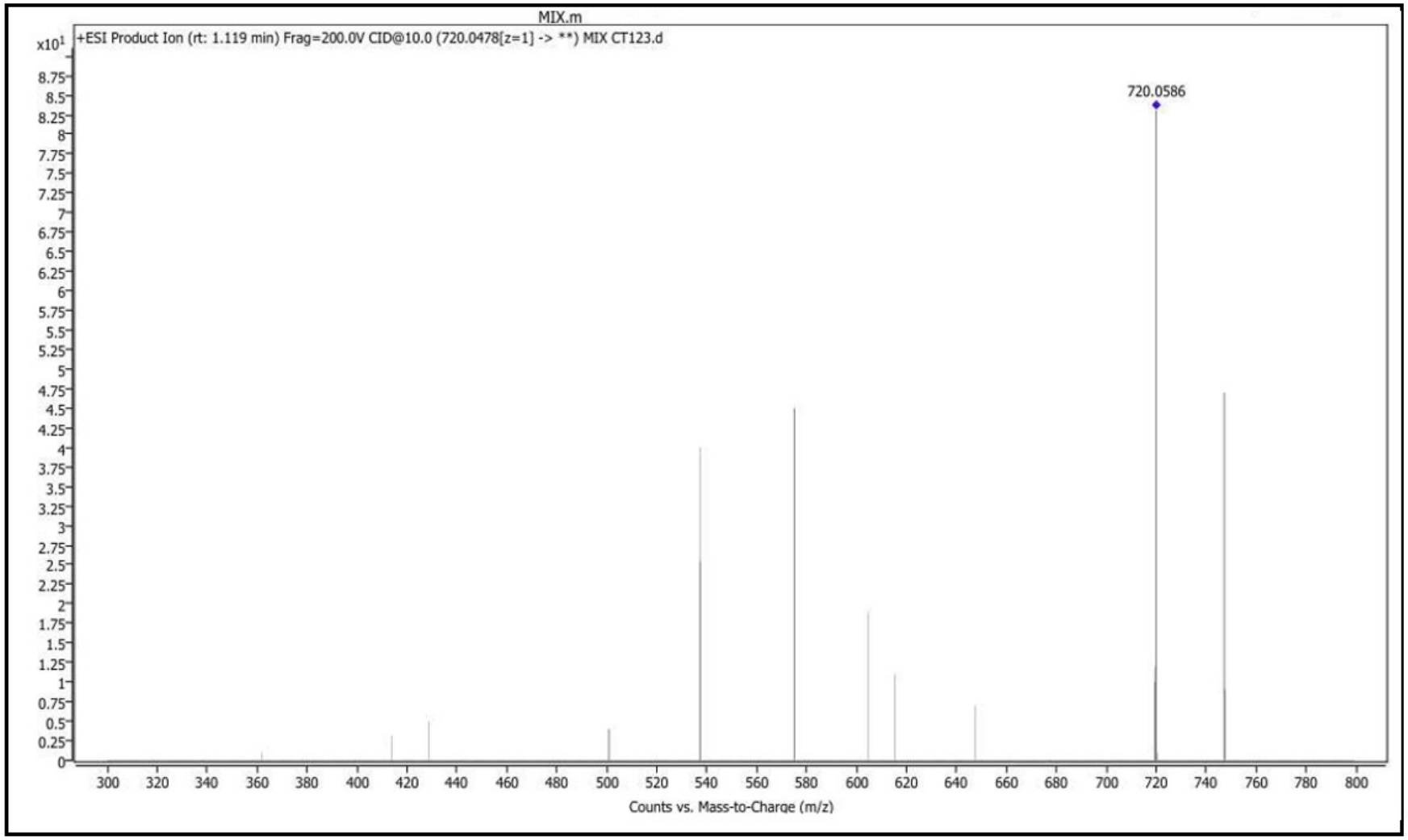


*Figure 8S. The ESI-MS spectrum of $Gd(PLB)_3$ complex in solid phase*

***Table 3S.*** *GY-AGREE assessment for the Gd–PLB fluorescence method*

| **Criterion (12 Principles of Green Analytical Chemistry** | **Brief description** | **Evaluation for the Gd-PLB** | **Score** |
|---|---|---|---|
| Sample preparation | Minimal sample preparation | Simple dissolution and direct fluorescence measurement | 0.7 |
| Sample size | Low sample consumption | Only ~3 mL per measurement, no digestion required | 0.8 |
| Reagent consumption | Reduced use of hazardous reagents/solvents | Mixed solvent ACN:$H_2O$ (2:3 v/v); no strong acids or bases used | 0.5 |
| Waste generation | Minimal waste generation | No solid waste; aqueous waste easily diluted and disposed | 0.7 |
| Energy consumption | Low energy consumption | Performed at room temperature without heating or intense light source | 0.85 |
| Automation and miniaturization | Use of eco-friendly materials | Standard glassware and quartz cuvette; no disposable plastics | 0.6 |
| Operator safety | Operator safety | No toxic gases or corrosive or flammable reagents involved | 0.85 |
| Instrumental energy efficiency | Non-toxic reagents | PLB is a natural compound; $Gd^{3+}$ used at low concentration | 0.8 |
| Method greenness in terms of reagents and solvents | High selectivity minimizing pretreatment | Highly selective toward $Gd^{3+}$ with negligible interference from common ions | 0.85 |
| Waste treatment and recyclability | Adequate sensitivity and accuracy | Low LOD/LOQ; excellent agreement with ICP-MS results | 0.78 |
| Analytical throughput | Low-energy instrumentation | Fluorometer with low power consumption | 0.7 |
| Overall environmental impact | Potential for automation or reusability | Compatible with flow-injection systems or reusable sensors | 0.8 |

Average score: 0.74 - Overall assessment: good level of analytical greenness

***Table 4S.*** *Eco-Scale Scores for the Assessment of the Greenness of the Gd-PLB Fluorescence Method*

| **Parameter** | **Penalty Points** | **Justification/Notes** |
|---|---|---|
| Consumables (reagents, tips) | 2 | Small consumption of consumables |
| Buffer/salts hazard | 1 | Moderate hazard from buffers and salts |
| Energy consumption | 1 | Low-energy operation |
| Organic waste volume | 5 | Volume of organic waste generated |
| Acetonitrile usage | 10 | Use of acetonitrile (high hazard) |
| Total penalty Points | 19 | |
| Eco-Scale Score | 81 | Calculated as 100 - total penalty points |

Eco-Scale Score = 100 – Total Penalty Points.

Scores $\geq 75$ are considered “excellent” in terms of green analytical chemistry.

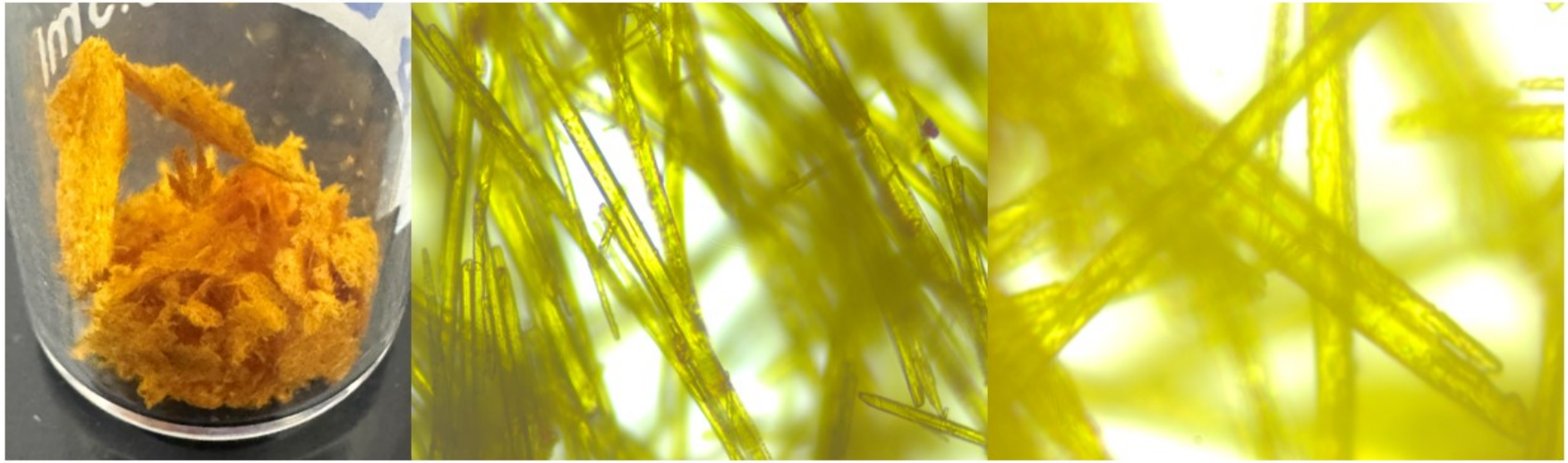

***Figure 9S. Gd**-PLB complex was synthesized and crystal micrographed*

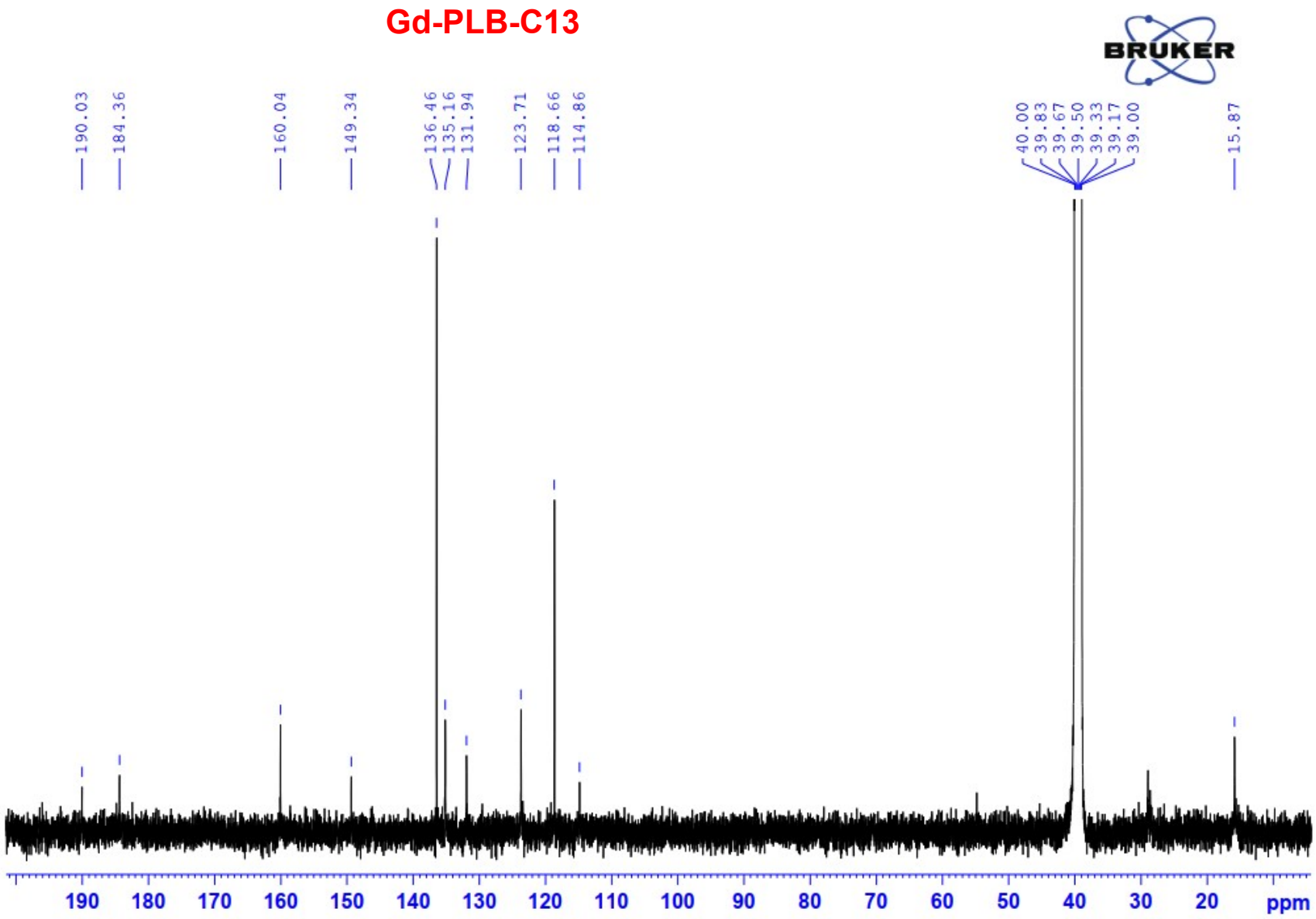


***Figure 10S.*** *The $^{13}$C-NMR spectrum of Gd-PLB complex in solid phase*

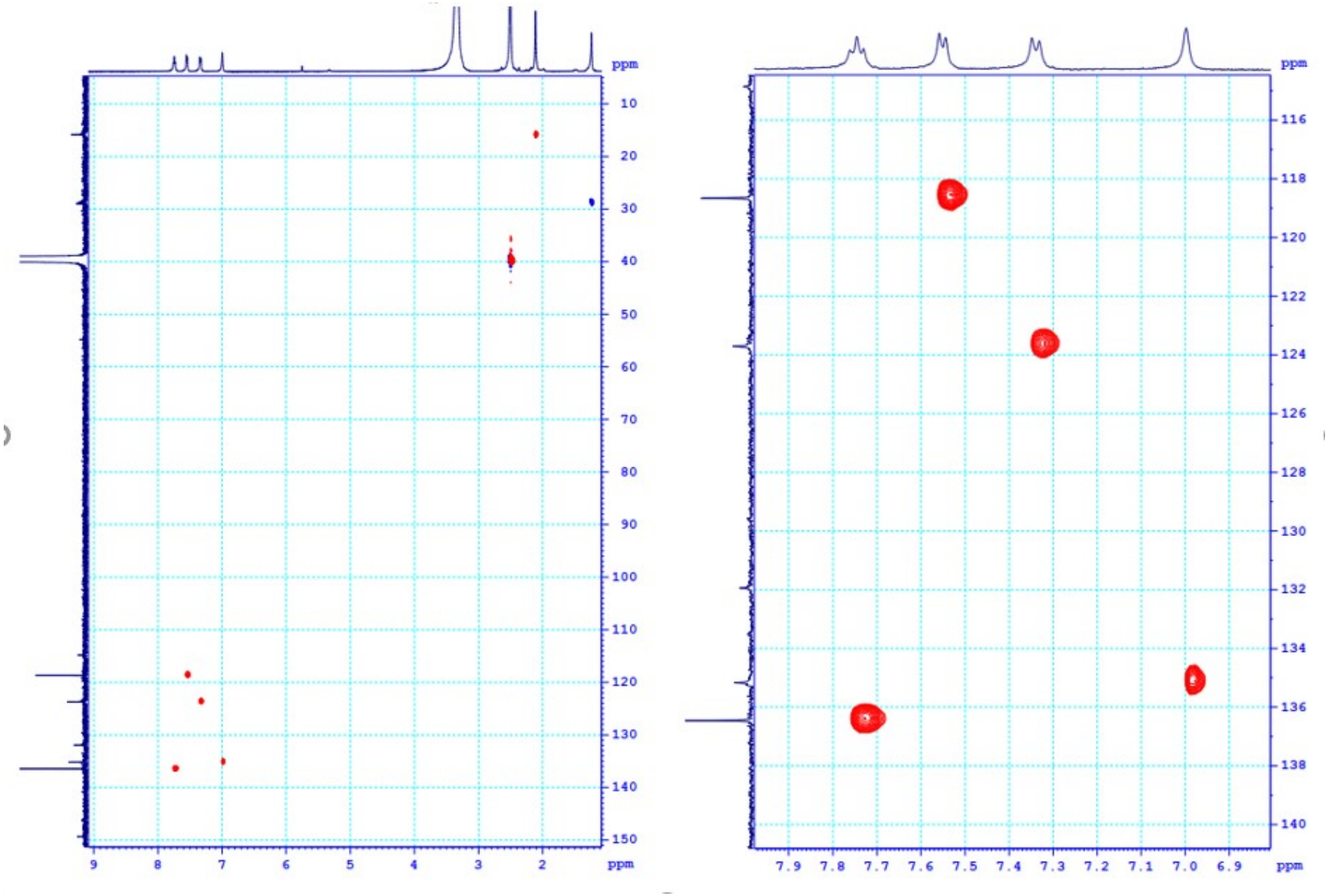


***Figure 11S.*** *The HSQC spectrum of Gd-PLB complex in solid phase*

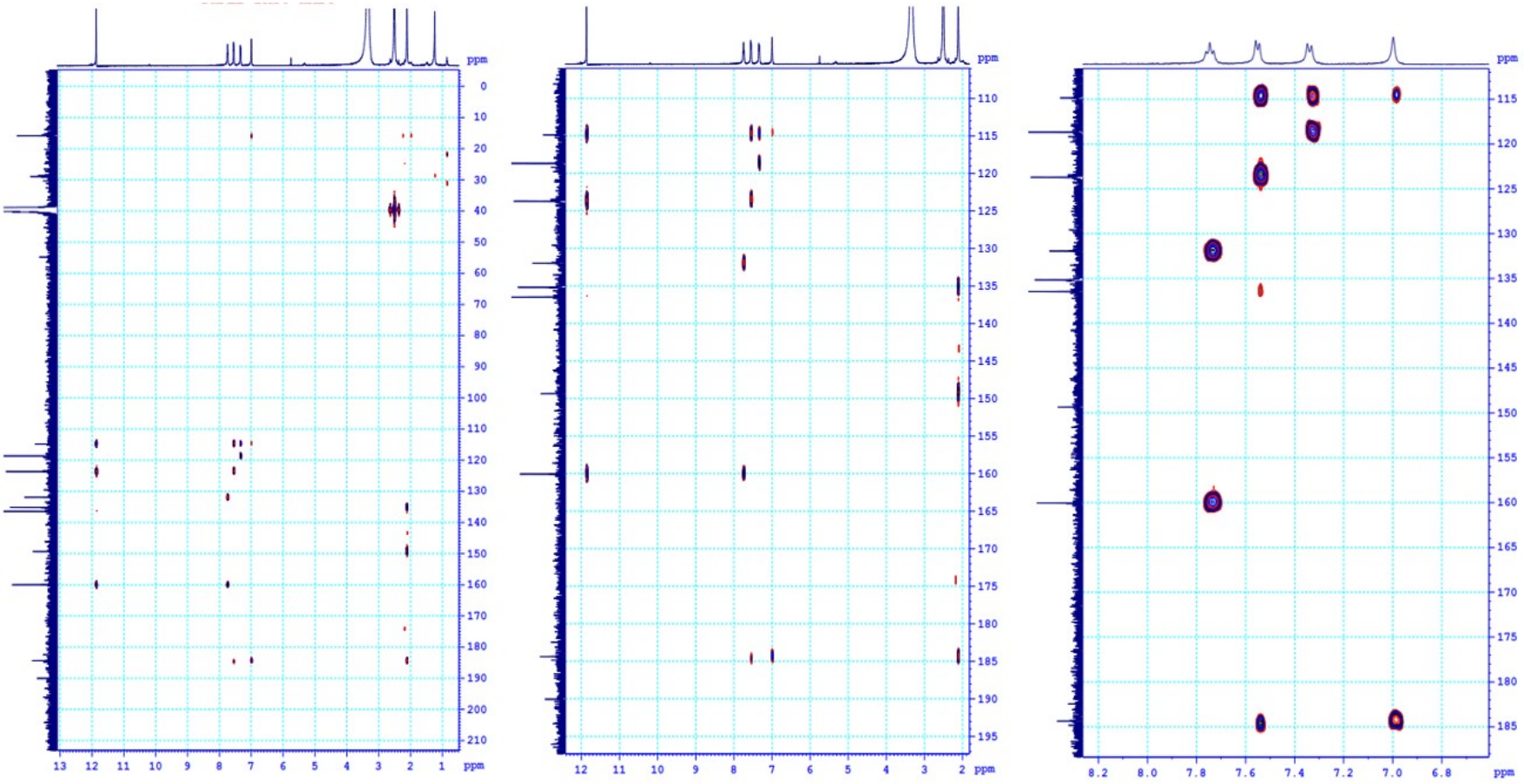

***Figure 12S.*** *The HMBC spectrum of Gd-PLB complex in solid phase*

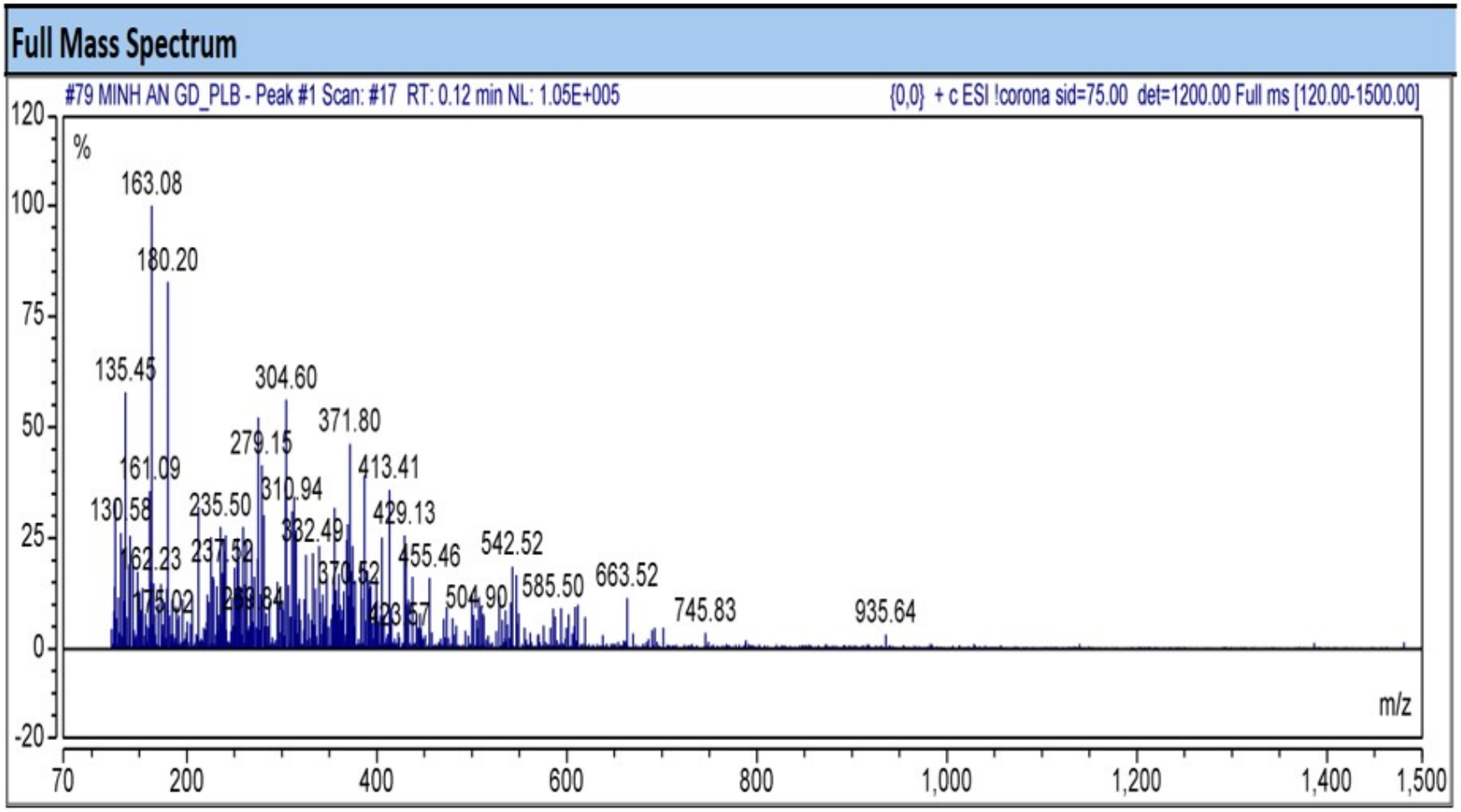


*Figure 13S. The ESI-MS spectrum of $Gd(PLB)_3$ complex in solution*

***Table 5S.*** *Excitation energy and MOs involved in excitation for $Gd(PLB)_3$ complex at theoretical level B3PW91/def2-SVP (Gd, C, O, H)*

| MOs | Electron transition MO | $E_{HOMO}$, eV | $E_{LUMO}$, eV | $DE_{gap}$, eV | Wavelength, l(nm) | Energy, kcal.mol$^{-1}$ |
|---|---|---|---|---|---|---|
| 164 → 165 | HOMO → LUMO | -4.041 | -3.549 | 0.492 | 2520.000 | 11.346 |
| 163 → 165 | HOMO-1 → LUMO | -4.576 | -3.549 | 1.026 | 1208.421 | 23.660 |
| 162 → 165 | HOMO-2 → LUMO | -6.118 | -3.549 | 2.569 | 482.616 | 59.243 |
| 161 → 165 | HOMO-3 → LUMO | -6.183 | -3.549 | 2.634 | 470.706 | 60.741 |
| 164 → 167 | HOMO → LUMO+2 | -4.041 | -1.197 | 2.844 | 435.949 | 65.584 |
| 164 → 168 | HOMO → LUMO+3 | -4.041 | -1.114 | 2.927 | 423.587 | 67.498 |

***Table 6S.*** *Determination of detection limit and quantification limit of the method*

| PBL.VC2 | Symbol | C-Gd (ppm) | H (%) |
|---|---|---|---|
| $Gd_{spike}$ 1.0 ppm | M1 | 4.44 | 102.4 |
| | M2 | 4.43 | 100.7 |
| | M3 | 4.41 | 98.7 |
| | M4 | 4.34 | 91.6 |
| | M5 | 4.48 | 105.8 |
| | M6 | 4.31 | 89.3 |
| | M7 | 4.38 | 96.0 |
| | Average | 4.40 | **97.8** |
| Calculate and check results | SD | 0.059 | |
| | MDL = 3.143*SD | 0.19 | |
| | LOQ = 3*MDL | 0.56 | |

MDL assessment is calculated through the following 3 conditions

| Condition | Result | Evaluate |
|---|---|---|
| MDL < Spike < 10 x MDL | 0.19 < 1.0 < 1.9 | Obtain |
| 80% < H% < 110 % | 80% ≤97.8 ≤110 % | Obtain |

***Table 7S.*** *The results of re-confirm the LOQ*

| **PBL.VC2** | **Symbol** | **C-Gd (ppm)** | **H%** |
|---|---|---|---|
| $Gd_{spike}$ 1.0 ppm | S1 | 4.44 | 102.1 |
| | S2 | 4.34 | 91.9 |
| | S3 | 4.42 | 100.4 |
| | S4 | 4.39 | 96.6 |
| | S5 | 4.47 | 105.2 |
| | S6 | 4.35 | 92.6 |
| | S7 | 4.41 | 98.7 |
| | S8 | 4.42 | 99.9 |
| | S9 | 4.40 | 98.1 |
| | S10 | 4.44 | 102.2 |
| Calculate and check results | Average | 4.41 | 98.8 |
| | SD | 0.042 | |
| | $RSD_r$ (%) | 0.95 | |

LOQ assessment was calculated through the following 2 conditions

| **Condition** | **Result** | **Evaluate** |
|---|---|---|
| $RSD_r \leq C^{-0.15}$ | **0.95** < 1.00 | Obtain |
| 80% < H% < 110 % | 80%≤ 98.8 ≤110 % | Obtain |

***Table 8S.*** *Repeatability assessment results table*

| **PBL.VC2** | 1 | 2 | 3 | 4 | 5 | 6 | $RSD_r$ % | Acceptance level |
|---|---|---|---|---|---|---|---|---|
| $C_{sample}$ | 3.42 ppm | | | | | | | |
| $C_{spike}$ 1.0 ppm | 4.41 | 4.44 | 4.38 | 4.37 | 4.41 | 4.41 | 0.58 | 7.94 |
| $C_{spike}$ 5.0 ppm | 8.46 | 8.32 | 8.40 | 8.43 | 8.46 | 8.43 | 0.64 | 6.24 |
| $C_{spike}$ 10 ppm | 13.30 | 13.11 | 12.84 | 12.81 | 12.87 | 13.13 | 1.53 | 5.62 |

***Table 9S.*** *Table of results of method repeatability assessment*

| **PBL.VC2** | **Low (ppm)** | | **Average (ppm)** | | **High (ppm)** | |
|---|---|---|---|---|---|---|
| Analytical value | 4.41 | 4.41 | 8.46 | 8.44 | 13.30 | 13.37 |
| | 4.44 | 4.45 | 8.32 | 8.32 | 13.11 | 13.15 |
| | 4.38 | 4.40 | 8.40 | 8.37 | 12.84 | 13.46 |
| | 4.37 | 4.40 | 8.43 | 8.37 | 12.81 | 13.42 |
| | 4.41 | 4.48 | 8.46 | 8.44 | 12.87 | 12.97 |
| | 4.41 | 4.41 | 8.43 | 8.45 | 13.13 | 13.11 |
| Average per employee, $\bar{y}_{ij}$ | 4.40 | 4.43 | 8.41 | 8.40 | 13.01 | 13.25 |
| Standard deviation of each employee, $S_{ij}$ | 0.026 | 0.034 | 0.054 | 0.053 | 0.20 | 0.20 |
| Overall average, $\bar{\bar{y}}_{ij}$ | 4.42 | | 8.41 | | 13.13 | |
| Repeatability variance, $S_r^2$ | 0.001 | | 0.003 | | 0.039 | |
| $S_d^2$ | 0.001 | | 0.001 | | 0.169 | |
| $\bar{\bar{n}}$ | 6 | | 6 | | 6 | |
| Variance among employees, $S_L^2$ | 0.000 | | 0.000 | | 0.022 | |
| Recurrence variance, $S_R^2$ | 0.001 | | 0.003 | | 0.061 | |

| | | | |
|---|---|---|---|
| Repeatability standard deviation, $S_R$ | 0.032 | 0.06 | 0.25 |
| Repeatability $RSD_R$ | 0.72 | 0.67 | 1.88 |
| Reproducibility assessment according to AOAC Acceptance level | 15.89 | 12.48 | 11.25 |

***Table 10S.*** *Recovery assessment results table*

| **PBL.VC2** | 1 | 2 | 3 | 4 | 5 | 6 | %Rtb |
|---|---|---|---|---|---|---|---|
| $C_{sample}$ | 3,42 ppm | | | | | | |
| $C_{spike}$ 1.0 ppm | 4.41 | 4.44 | 4.38 | 4.37 | 4.41 | 4.41 | 98.5 |
| R% | 98.6 | 102.3 | 96.5 | 94.9 | 99.3 | 99.4 | |
| $C_{spike}$ 5.0 ppm | 8.46 | 8.32 | 8.40 | 8.43 | 8.46 | 8.4 | 99.7 |
| R% | 100.8 | 97.9 | 99.5 | 100.1 | 100.8 | 100.2 | |
| $C_{spike}$ 10ppm | 13.30 | 13.11 | 12.84 | 12.81 | 12.87 | 13.13 | 95.3 |
| R% | 98.8 | 96.9 | 94.2 | 93.9 | 94.5 | 97.1 | |

***Table 11S.*** *Results for determining measurement uncertainty*

| No | Analysis time | Analysis results (ppm) |
|---|---|---|
| 1 | | 4.44 |
| 2 | | 4.43 |
| 3 | Phase 1 | 4.41 |
| 4 | | 4.34 |
| 5 | | 4.48 |
| 6 | | 4.44 |
| 7 | | 4.34 |
| 8 | Phase 2 | 4.42 |
| 9 | | 4.39 |
| 10 | | 4.47 |
| 11 | | 4.35 |
| 12 | | 4.41 |
| 13 | Phase 3 | 4.42 |
| 14 | | 4.40 |
| 15 | | 4.44 |
| 16 | | 4.44 |
| 17 | Phase 4 | 4.38 |
| 18 | | 4.37 |

| | |
|---|---|
| 19 | 4.41 |
| 20 | 4.41 |
| Sample mean (Xtb) | 4.41 |
| Standard deviation (SD) | 0.040 |
| Repeatability (CV%) | 0.92 |
| Error 10mL (mL) | 0.025 |
| Volume error due to temperature V10mL (mL) | 0.0048 |
| u(Volume 10) (mL) | 0.0113 |
| u(average 10) (%) | 0.11 |
| Wavelength error of HITACHI F-2700 machine (nm) | 3.0 |
| u(HITACHI F-2700) (nm) | 1.5 |
| u(HITACHI F-2700) (%) | 0.30 |
| u (Parameters) (%) | 0.32 |
| Total condition (%) | 0.97 |
| tα; k | 2 |
| **Extended OD (%)** | **1.94** |