Supporting Information

A Benzimidazole based chemodosimeter for the Fluorometric detection of Zn and Cu via 1, 5 proton shifts and C-N Bond Cleavage.

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CONTENTS

¹H NMR spectrum of Compound APBHN

Fig S1: ¹H NMR spectrum of APBHN.

¹³C NMR spectrum of Compound APBHN

193.17

148.45
145.11
144.02
138.71
133.26
132.82
132.28
131.92
129.55
129.06
129.00
127.42
126.65
125.14
123.40
123.11
122.04
118.64
118.12
115.36
114.58
111.30
110.38

63.53

56.29
40.10
40.01
39.93
39.84
39.76
39.67
39.51
39.34
39.17
39.01

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm
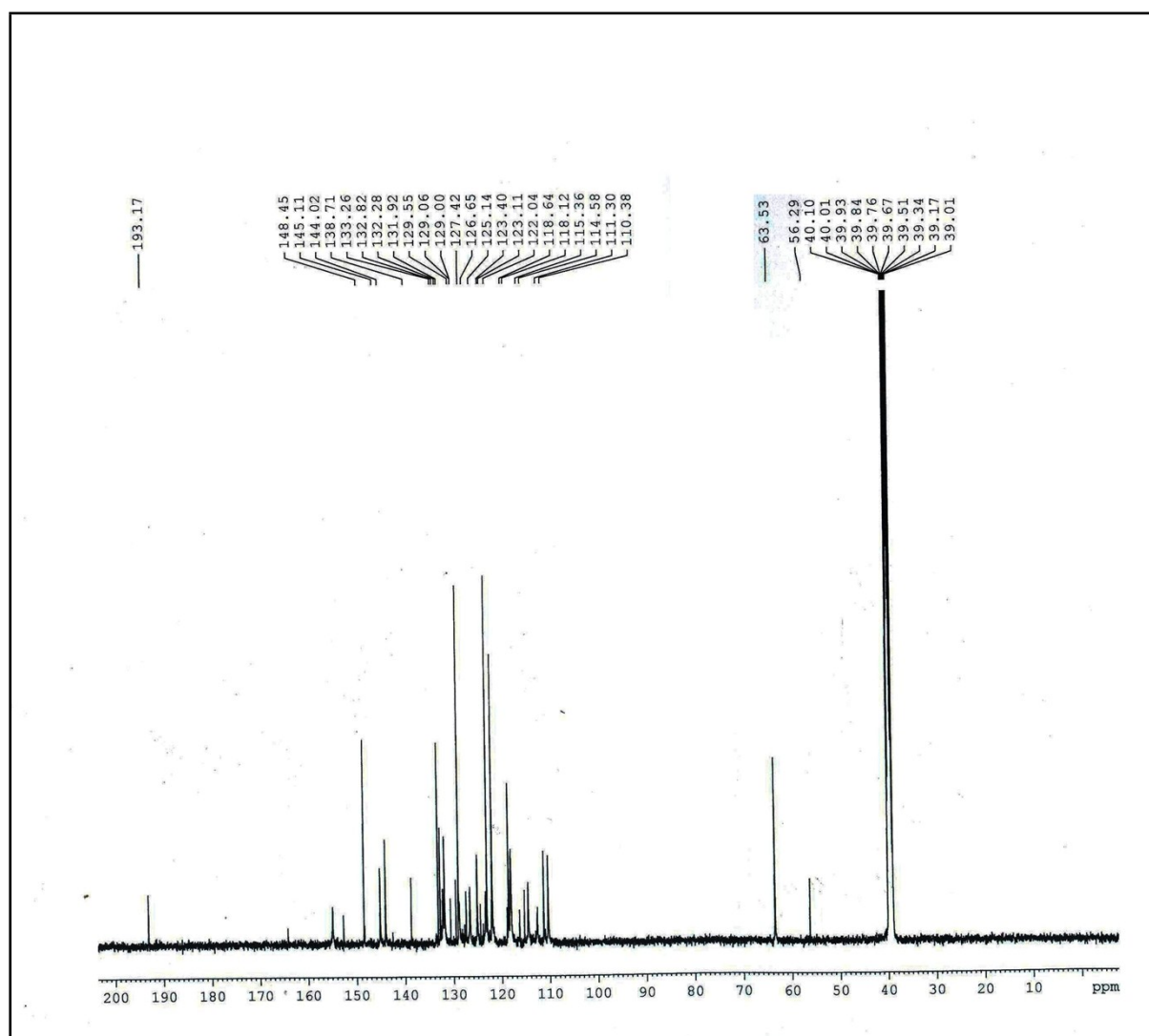
FigS2:¹³C NMR spectrum of Compound APBHN.
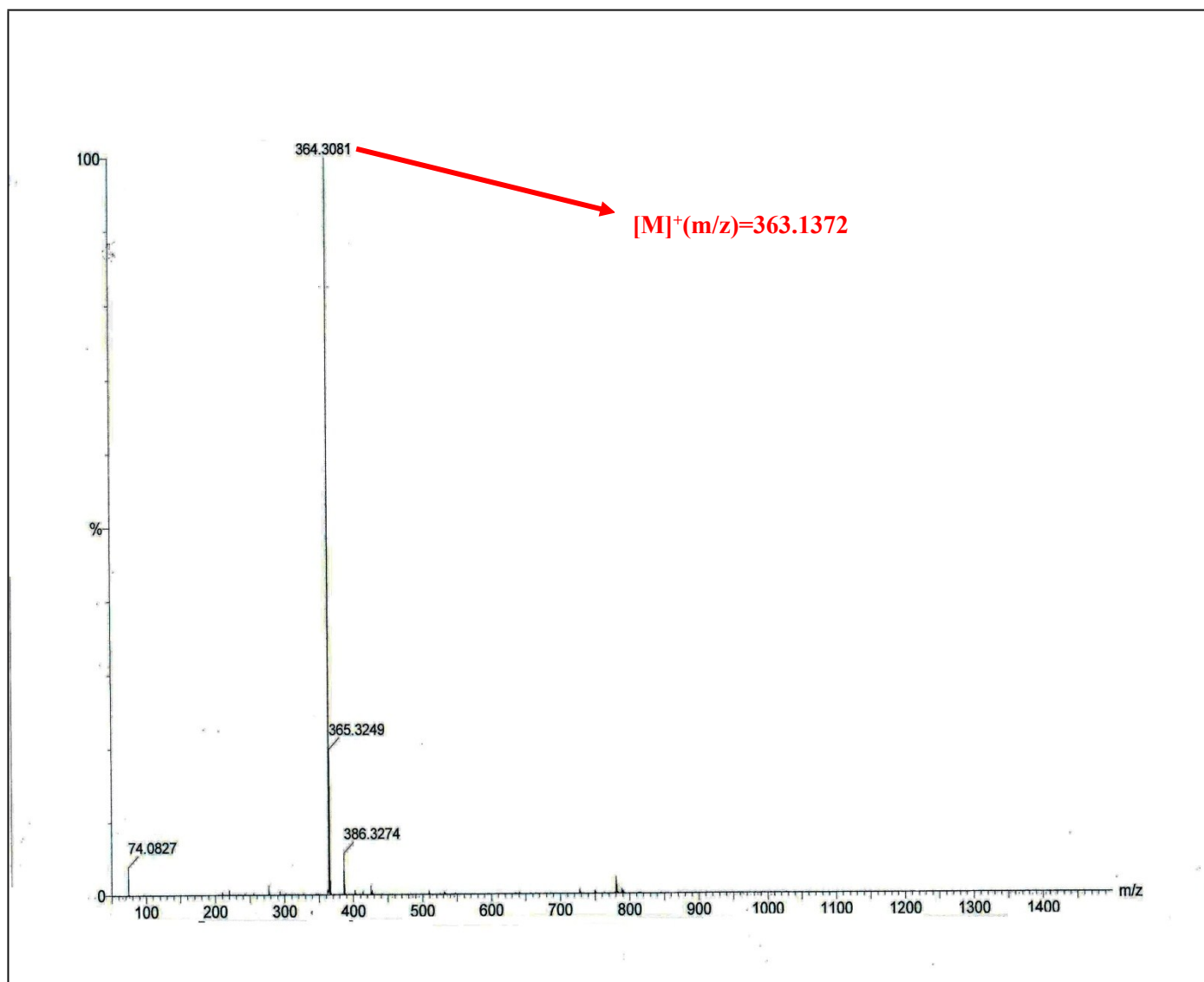
Mass spectrum of compound APBHN

Fig S3: Mass spectrum of APBHN.

IR Spectra of the APBHN

Fig S4: IR Spectra of APBHN.

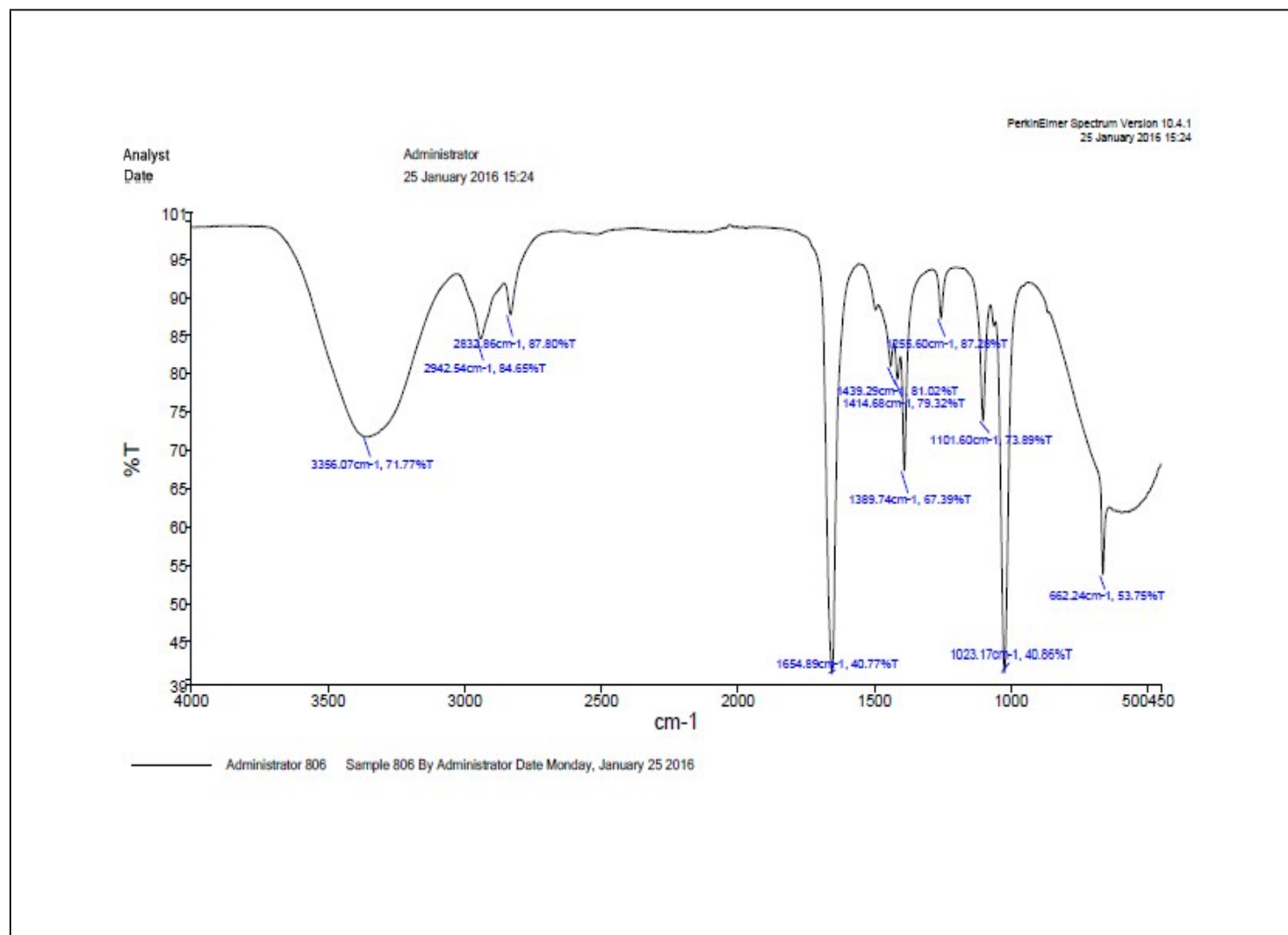**Mass spectrum of the Cu complex (1:1)**

Fig S5. Mass spectrum of Cu-APBHN (1:1)complex.

Mass spectrum of the Cu complex (1:2)
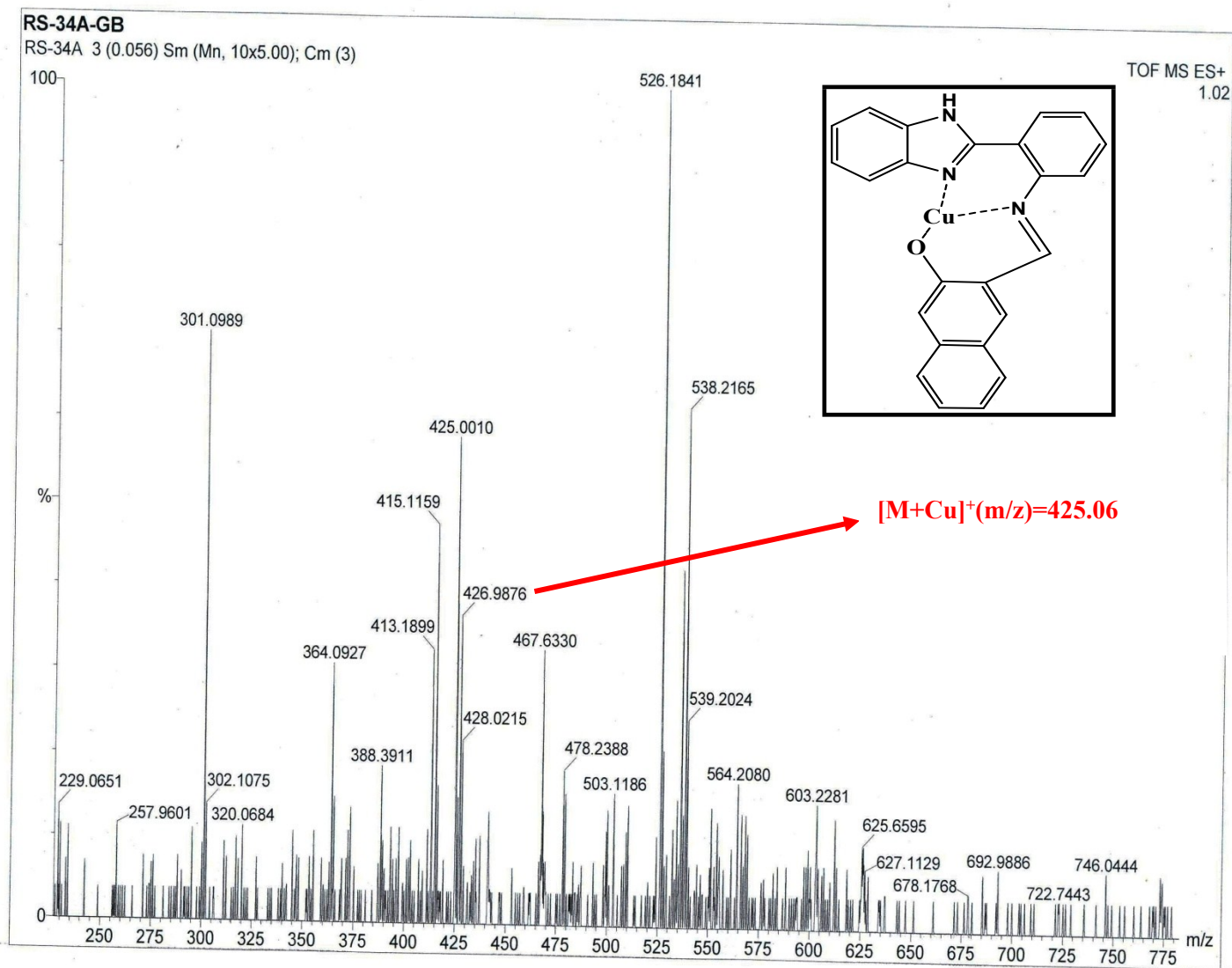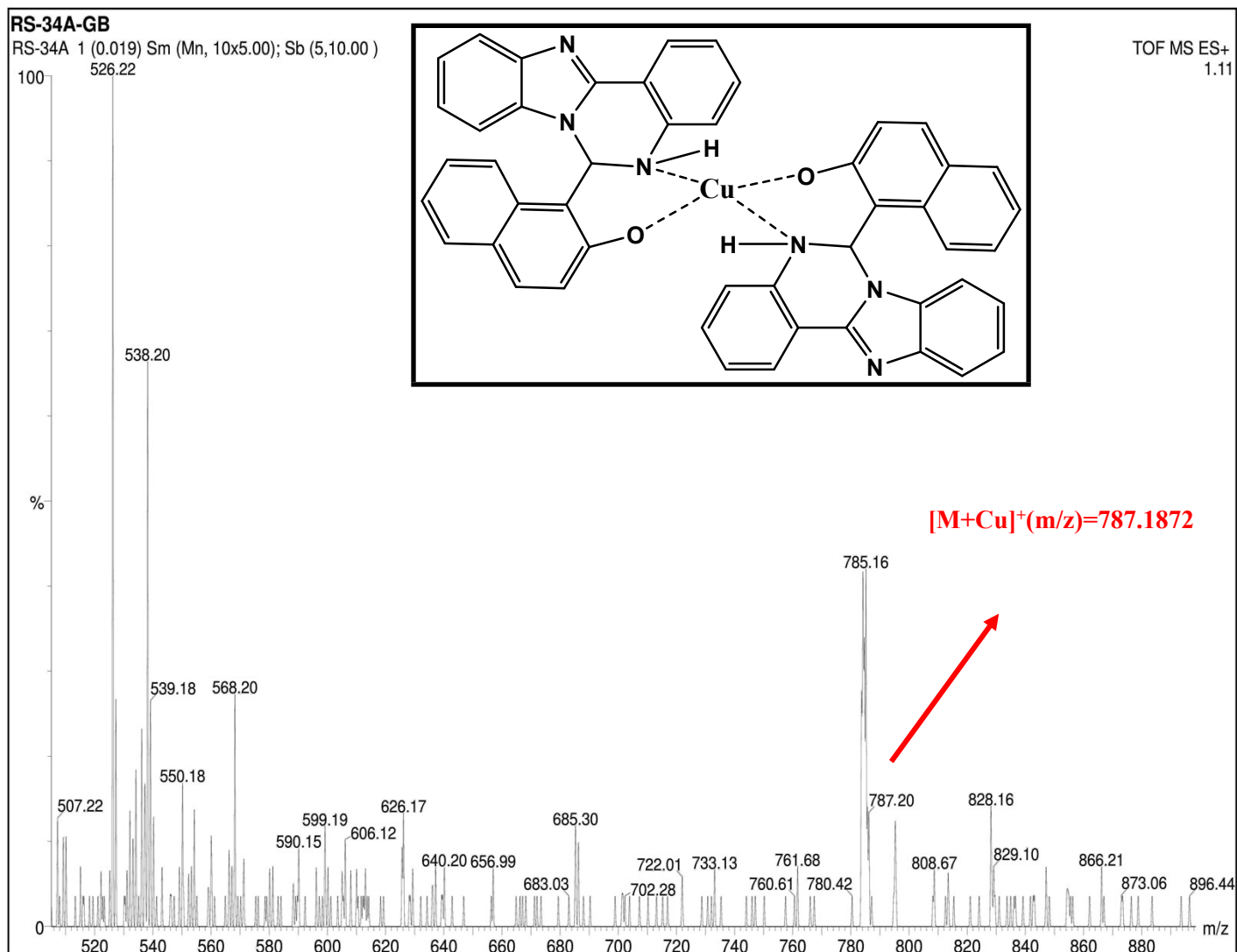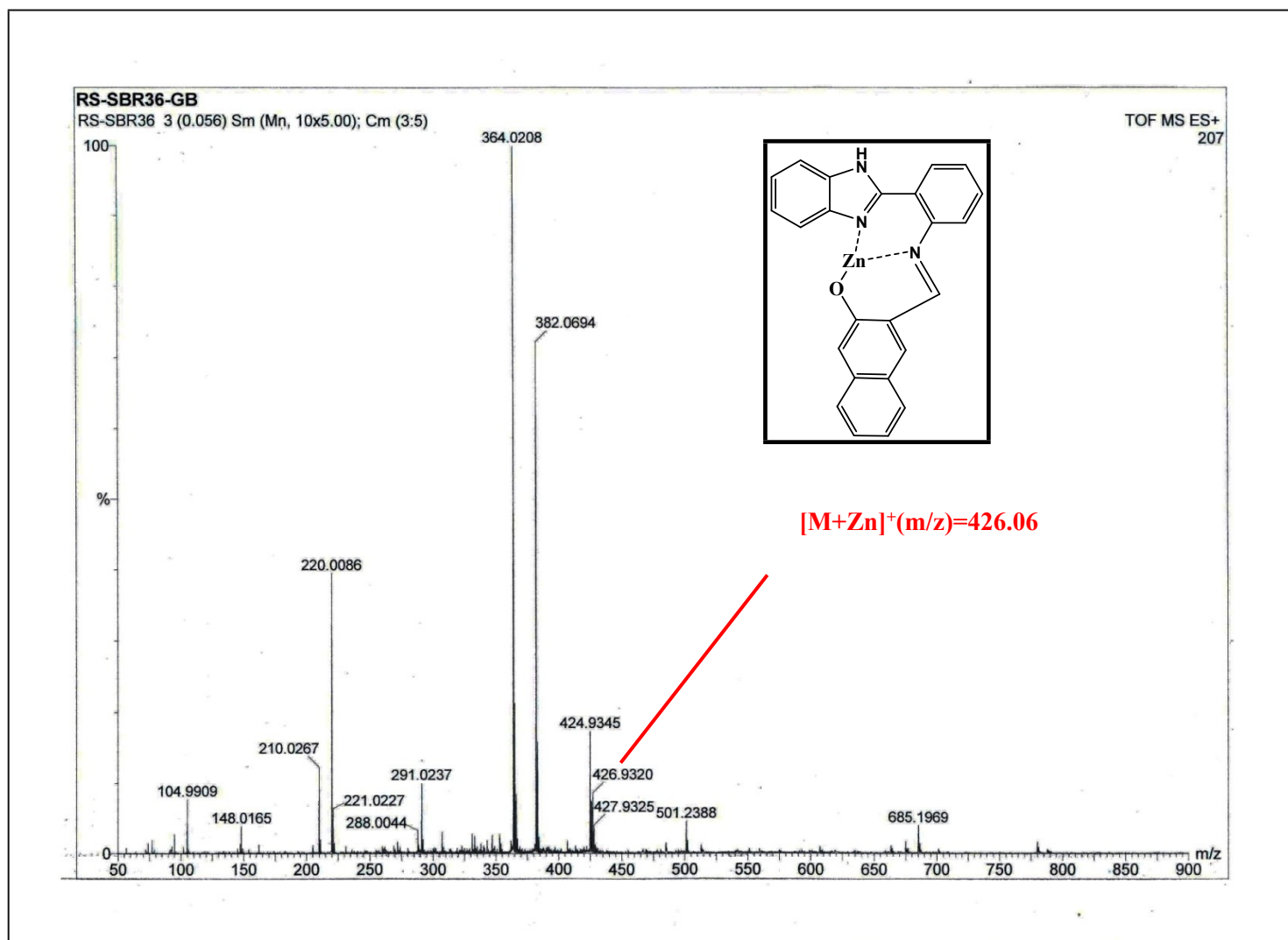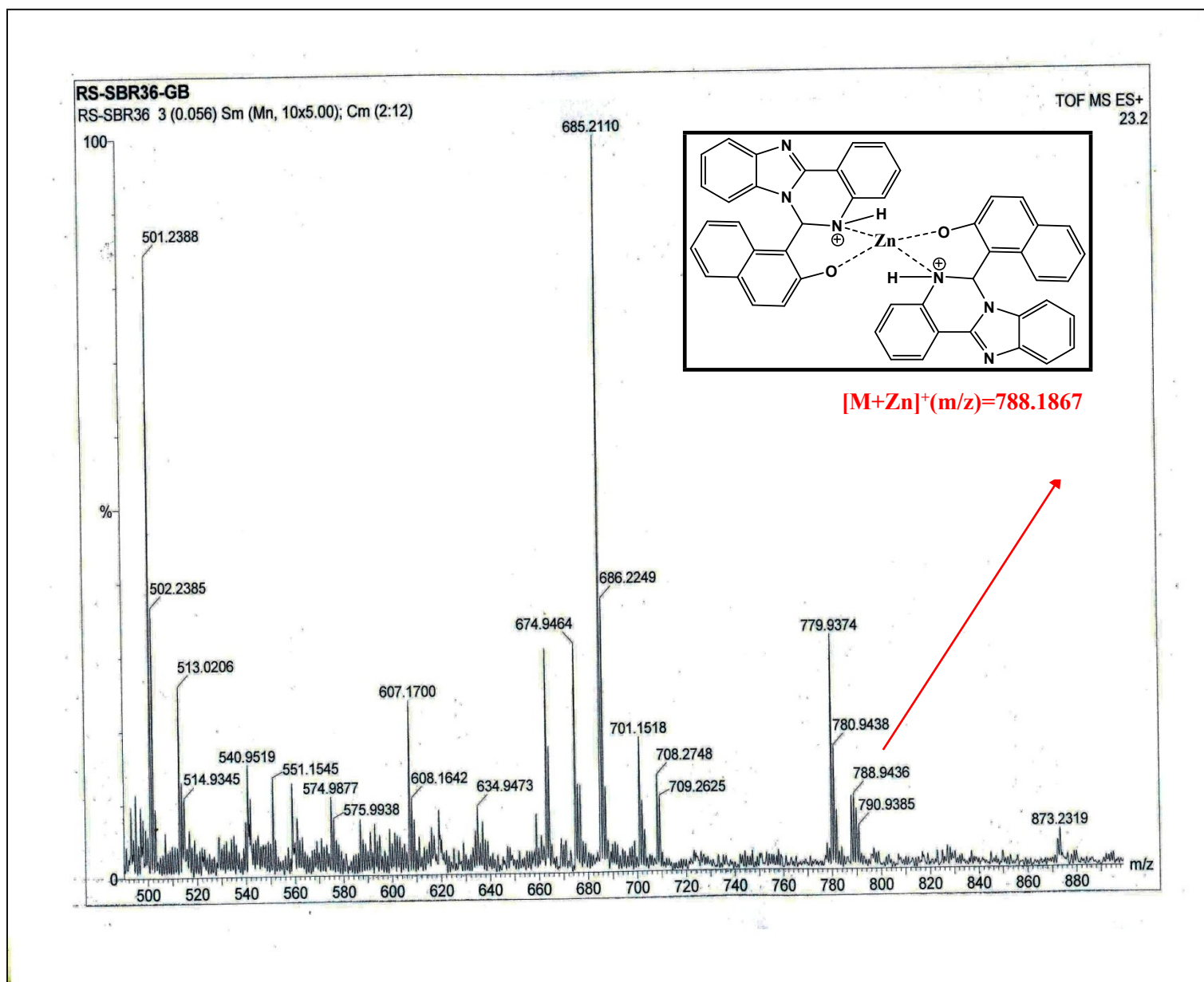

Fig S6. Mass spectrum of Cu-APBHN(1:2) complex.

Mass spectrum of the Zinc Complex(1:1).

FigS7: Mass spectrum of Zn-APBHN(1:1) complex.

Mass spectrum of the Zinc complex (1:2).

FigS8. Mass spectrum of the Zn-APBHN(1:2) complex.
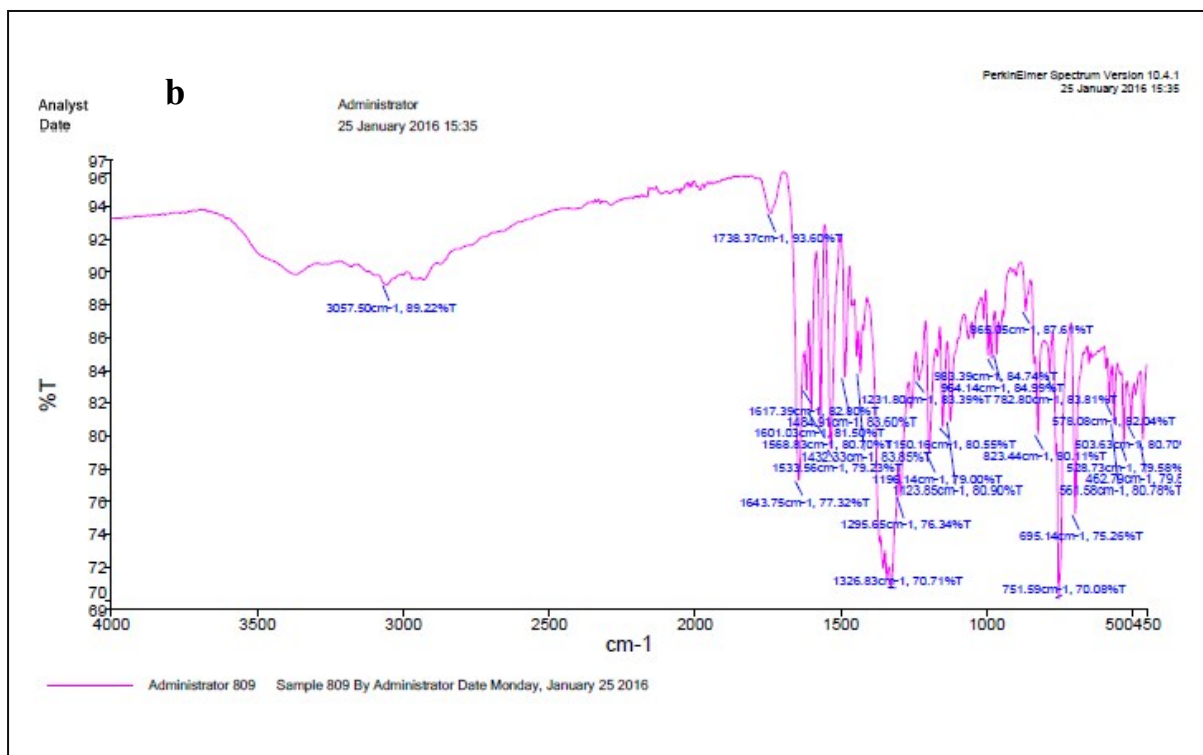
IR Spectrum of Copper and Zinc complex:
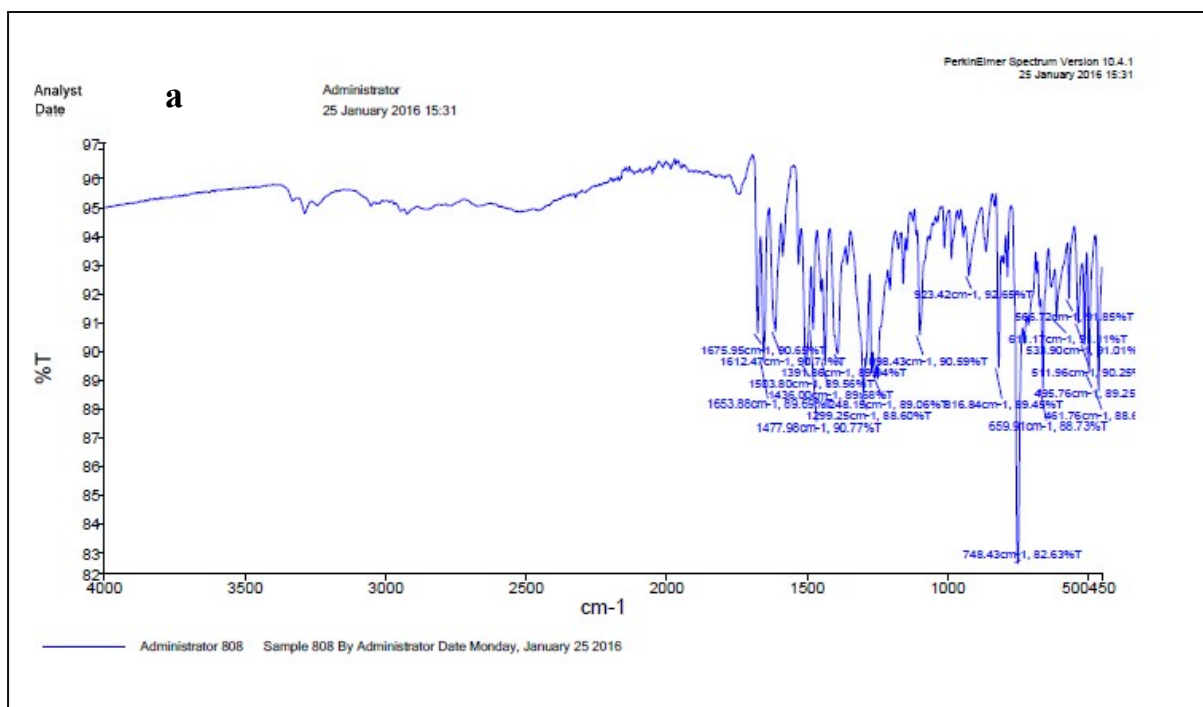
Fig S9.a)IR spectra of the Cu-APBHN complex. **b**) IR spectra of the Zn-APBHN complex.

Determination of Association Constant (Ka):

By UV-vis method:

Association constant was calculated according to the Benesi-Hildebrand equation. Ka was calculated following the equation stated below.

$1/(A-A_o) = 1/\{K(A_{max}-A_o) [M^{x+}]^n\} + 1/[A_{max}-A_o]$

Here Ao is the absorbance of receptor in the absence of guest, A is the absorbance recorded in the presence of added guest, Amax is absorbance in presence of added $[M^{x+}]$max and Ka is the association constant, where $[M^{X+}]$ is $[Zn^{2+}]$ and $[Cu^{2+}]$. The association constant (Ka) could be determined from the slope of the straight line of the plot of $1/(A-Ao)$ against $1/[M^{x+}]$ and is found to be 1.34×10^4 M^{-1} for Zn^{2+} and 6.4×10^4 M^{-1} for Cu^{2+}.
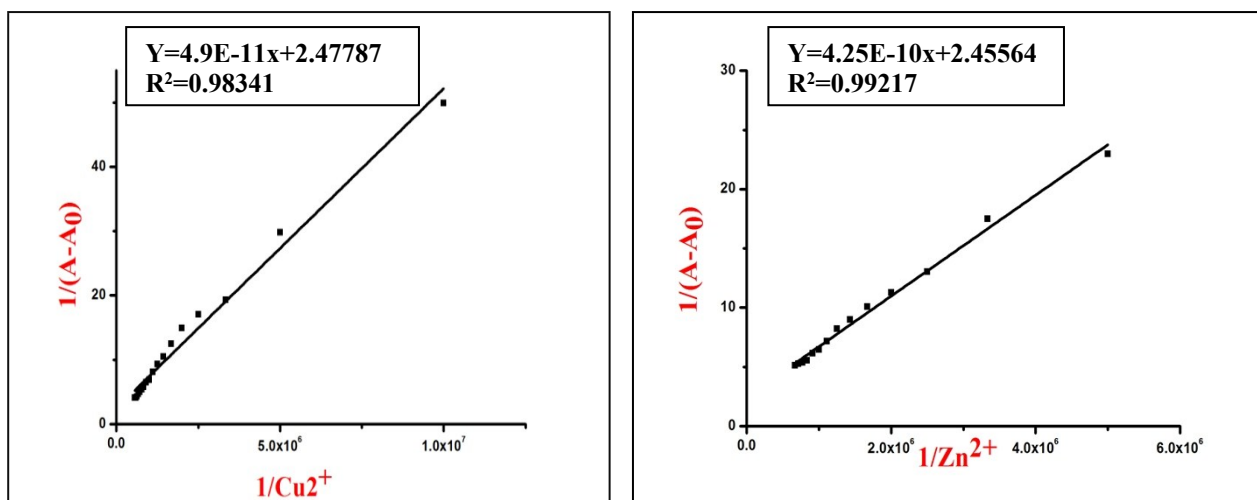
FigS10. Benesi-Hildebrand plot from absorption titration data of receptor (10 μM) with Cu^{2+} and Zn^{2+}.

Calculation of the detection limit

The detection limits DL of APBHN for Zn^{2+} and Cu^{2+} were determined from the following equation1:

DL = K* Sb1/S

Where K = 2 or 3 (we take 3 in this case); Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve.

From the graph (a) we get slope = 1.4×10^{10}, and Sb1 value is 26100 and from graph (b) we get slope = 2.43×10^9 and Sb1 value is 11988.

Thus using the formula we get the Detection Limit for Zn^{2+} is 5.59 µM i.e. APBHN can detect Zn^{2+} ion in this minimum concentration and Detection Limit for Cu^{2+} is 0.148 µM.
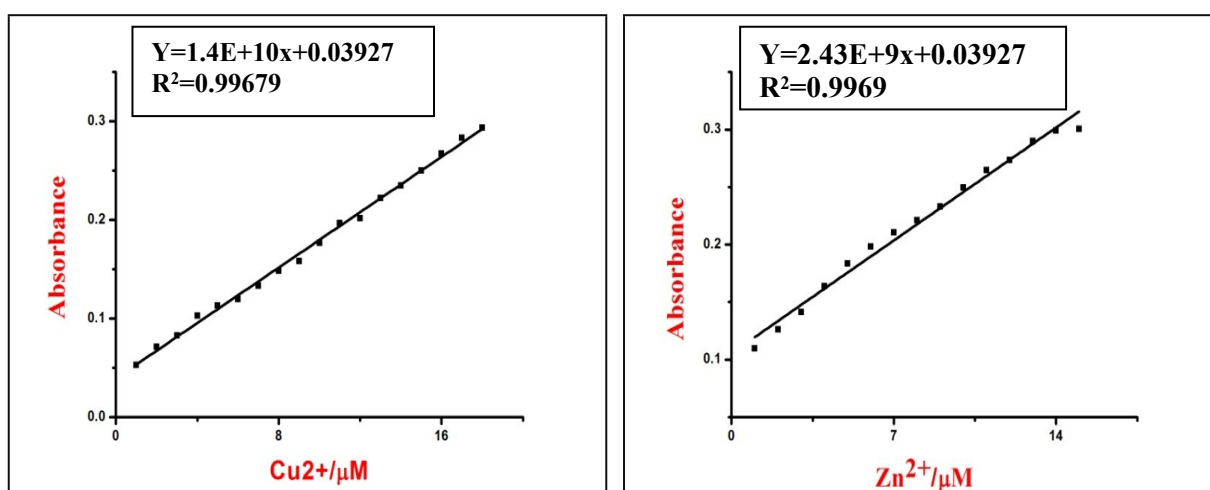
FigS11: The linear change of fluorescence intensity as a function of (a) $[Cu^{2+}]$ at 435nm and (b) $[Zn^{2+}]$ at 430 nm.

Fluorescence Spectra:

FigS12.Fluoresence Intensity plot of a) Amino-phenyl Benzaimidazole and b) hydroxy napthaldehyde.

Job's plot by absorbance method:

Stock solution of same concentration of sensor and Zn^{2+} and Cu^{2+} was prepared in the order of 10 μM in (at 25 °C) at pH 7.4 in HEPES buffer. The emission spectrum in each case with different host–guest ratio but equal in volume was recorded. Job's plots were drawn by plotting ΔI.Xhost vs Xhost (ΔI = change of intensity of the emission spectrum during titration and Xhost is the mole fraction of the host in each case, respectively).
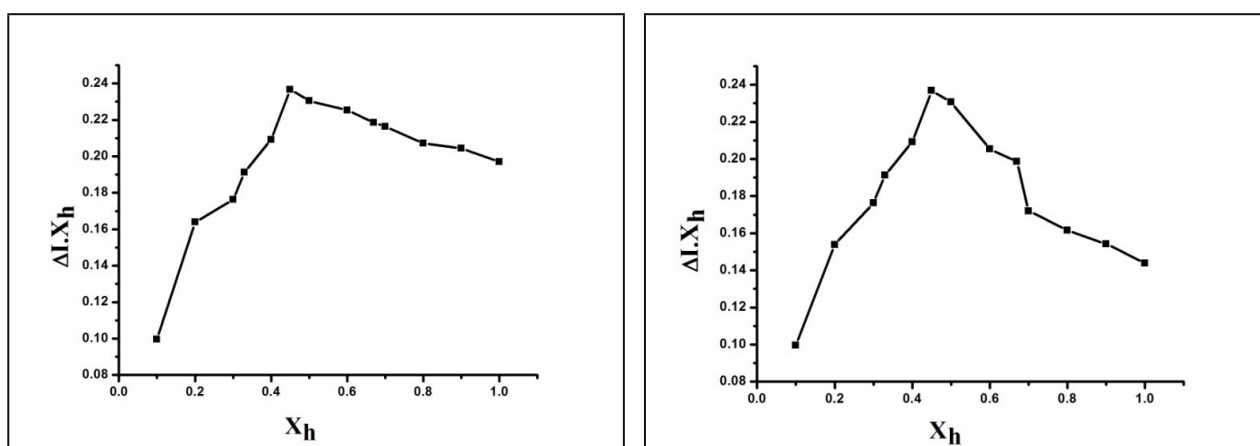
FigS13. Jobs plot diagram of HAFPA for a) Cu^{2+} and b) Zn^{2+}(where X_h is the mole fraction of host and ΔI indicates the change of the intensity).

The highest peak at 0.45 indicates the formation of both 1:1 and 1:2 complexes.

Determination of the fluorescence quantum yield

Here, the quantum yield φ was measured using the following equation:

$$\Phi_X = \Phi_S \times (I_x/I_s) \times (A_x/A_s) \times (n_x/n_s)^2$$

where X and S indicate the unknown and standard solution respectively, φ = quantum yield, I = area under the emission curve, A = absorbance at the excitation wavelength, and n =index of refraction of the solvent. Here φ measurements were performed using quinine sulphate in ethanol as a standard [φ =0.54] (error ~ 10%).

Photophysical parameters of the complexes in Methanol solution at room temperature. Table (S1):

| Sample	$\lambda_{max,}$ nm (ε, M^{-1} cm^{-1})	$\lambda_{emi,}$ nm	Φ ($\times 10^{-3}$)	k_r, s^{-1} ($\times 10^5$)	k_{nr}, s^{-1} ($\times 10^8$)	τ_1, ns	τ_2, ns
Ligand	398(12612818.3), 480(12713784)	385,480	0.76	7.10(385) 7.18(480)	1.77(385) 1.77(480)	1.069(385) 1.058(480)	4.27(385) 4.23(480)
Cu Complex	385(1510855440) 430(1298335905) 480(1510855440)	385,430, 480	0.64(385) 0.74(430) 0.64(480)	7.55(385) 7.12(430) 2.16(480)	2.94(385) 3.19(430) 8.46(480)	8.47(385) 1.95(430) 2.96(480)	3.39(385) 7.82(430) 1.18(480)
Zn Complex	385(1346850575) 430(1254777430) 480(1510855440	385,430, 480	0.72(385) 0.74(430) 0.72(480)	8.15(385) 6.04(430) 2.80(480)	7.21(385) 4.24(430) 2.61(480)	9.44(385) 1.29(430) 2.60(480)	3.77(385) 5.18(430) 1.04(480)

Photoluminescence Decay of ligand APBHN
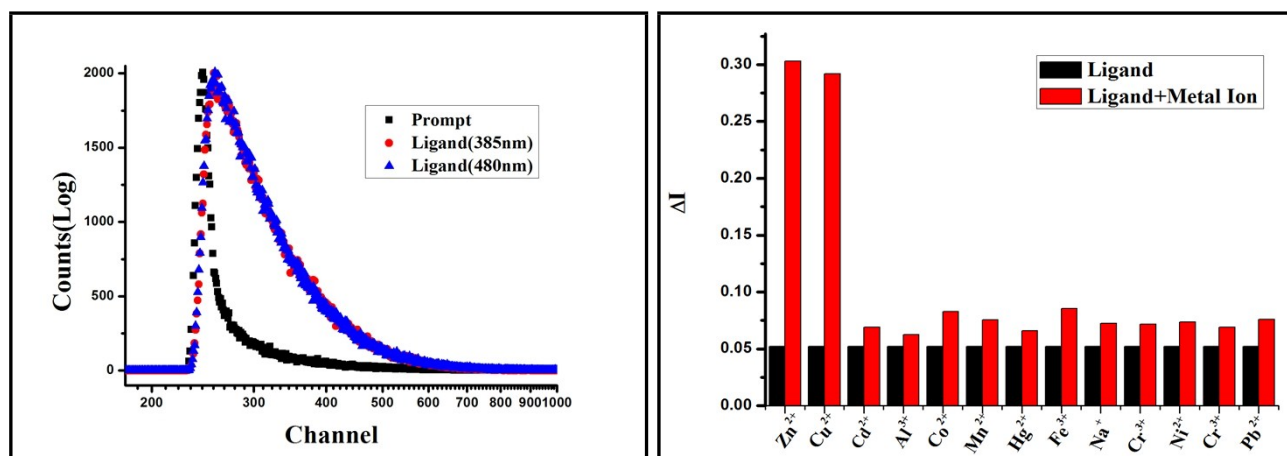
Fig.S14 :(a) Changes in the time-resolved photoluminescence decay of Ligand APBHN in methanol at room temperature obtained with 300 nm excitation. The emission at 385 and 480 nm was monitored. **(b)** Change in the absorbance as bar representation of APBHN ($c = 1.0 \times 10^{-5}$ M) after addition of 1.0 equiv. of each of the guest cations ($c = 2.0 \times 10^{-4}$ M) to CH_3OH–10 mM aqueous HEPES buffer (8/2, v/v, 25 °C) at 430 nm.

Computational Method

Full geometry optimizations were carried out using the density functional theory (DFT) method at the Becke-3-Lee-Yang-Parr(B3LYP) 24 level for the ligand APBHN and its Zn^{2+} and Cu^{2+} complexes. The 6-31+G (d,p) basis set was assigned for all the elements. All calculations were performed with Gaussian03program with the aid of the Gauss View visualization program.

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Table (S2). **Vertical electronic excitations of APBHN,Zn²⁺-APBHN and Cu²⁺- APBHN calculated by TDDFT/B3LYP/CPCM method**.

Compoud	Excitation	Theoretical Wavelength(nm)	CI	Λexpt. (nm)
APBHN	HOMO-1 →LUMO+3 HOMO → LUMO+2 HOMO-2 → LUMO HOMO-1 → LUMO	228 229 288 339	0.6004 0.6325 0.6932 0.6081	225 290 350
Cu-APBHN (1:2)	HOMO-1 → LUMO+3 HOMO-2 → LUMO+1	360 436	0.8680 0.5825	360 435
Cu-APBHN (1:1)	HOMO-1 → LUMO HOMO → LUMO+1 HOMO-1 → LUMO	433 366	0.3417 0.3769 0.1169	435 360
Zn-APBHN (1:2)	HOMO-3 →LUMO+2 HOMO-1 →LUMO	340 422	0.16673 0.23471	330 425
Zn-APBHN (1:1)	HOMO→ LUMO+2 HOMO → LUMO+2 HOMO → LUMO+3	420 333 291	0.2929 0.2209 0.2697	425 330 290

Crystal Data

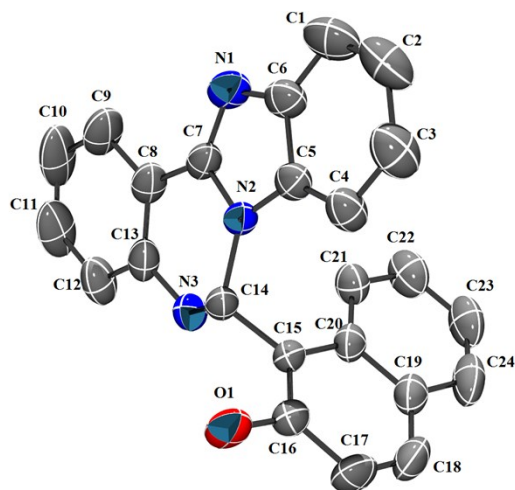| 2APBHN,2C₃H₇NO	
Formula	$C_{54}H_{48}N_8O_4$
M_r	872
Crystal system	Monoclinic
Space group	Cc
a / Å	33.1101 (12)
b / Å	8.5492 (3)
c / Å	16.6530(6)
α /°	90.00 (1)
β /°	99.906 (1)
γ /°	90.00 (1)
V /Å³	4643.6(3) (2)
Z	8
D_{calcd} /mg m⁻³	1.246
μ /mm⁻¹	0.081
θ /°	2.28-32.3
T /K	293(2)
Reflns collected	8079
R1,[a] wR2[b] [$I > 2\sigma(I)$]	0.0798, 0.2489
GOF on F^2	1.029
CCDC no	1430234

Table S3: (a) Crystal data and structure refinement parameters for ligand APBHN. **b)** ORTEP plot and atom labelling scheme of **1** at 50% ellipsoid probability. Hydrogen atoms are omitted for clarity
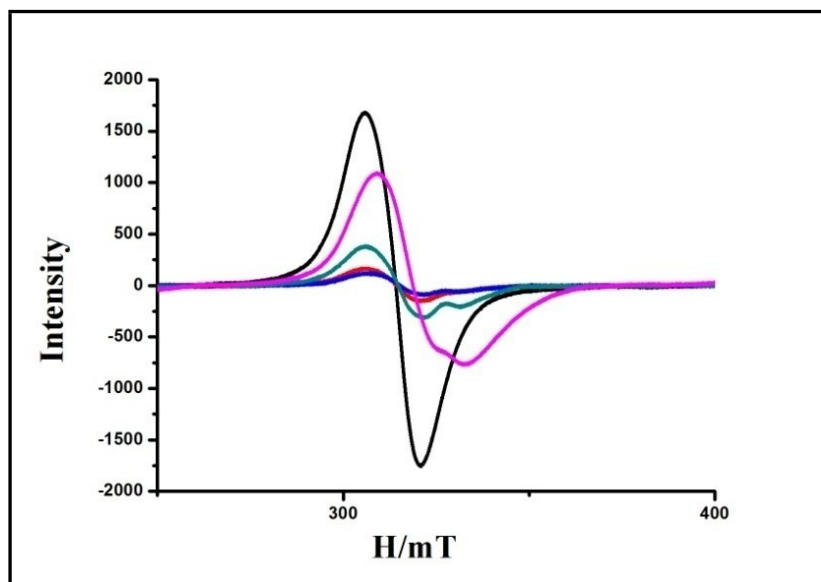
EPR Study

Fig.S15. EPR spectrum Cu^{2+} with increasing concentration of Ligand APBHN in toluene/ methanol (4:1) at 298 K (instrument settings: microwave frequency, 9.4304 GHz; microwave power, 0.998 mW; modulation frequency, 100 kHz).