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# SYNTHESIS OF THE NOVEL COUMARIN PROBES AND SENSITIVE DETECTION AND SEPARATION OF CATIONS USING RP-LC AND THEORETICAL INVESTIGATIONS

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**ABSTRACT**

Three new UV-visible probes have been synthesized under a Schiff-based reaction by derivatizing the coumarin molecule for sensitive detection of metal ions. All the probes were characterized using different spectroscopic methods. The probes demonstrated exceptional UV-visible absorption properties, with their $\lambda_{maxes}$ at 308, 318, and 345 nm. The probes showed high UV-visible sensitivity for metal ions, particularly $Zn^{2+}$, $Al^{3+}$, $Fe^{3+}$, $Mn^{2+}$, and $Co^{2+}$ ions. The ability of the probes was investigated by testing different concentration samples at the nanomolar scale. The effect of various functional groups in detection was investigated in RP-HPLC analysis. The probe's lowest energy structures and HOMO-LUMO orbitals were developed using DFT calculations. The interactions of metal ions with probe (**P1-P3**) were investigated by developing 3D structures of complexes using DFT.

**Keywords:** Coumarin, Naphthalene, Derivatives, Probes, Schiff Base, RP-HPLC.

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## INTRODUCTION

In recent years, the analysis of metal ions and their concentration in drinking water, food, soil, rocks, industrial waste, and agriculture has drawn much interest because of their direct impact on human and environmental health.[1,2] A small amount of heavy metals in the diet is essential because of their critical role in biological reactions. For example, lower concentrations of $Al^{3+}$ aid in plant growth, $Zn^{2+}$ is a necessary component of human blood plasma (12–6 mM),[3,4] magnesium and iron metal ions are used to prepare hemoglobin and chlorophyll, respectively, and calcium ions (Ca, Cu, Fe, Mn, Co, and Cd) participate in vital biological catalytic reactions. However, excessive concentrations of metal ions have adverse effects on humans. For example, higher concentrations of $Al^{3+}$ (above 3–10 mg, according to the WHO) cause osteoporosis and Alzheimer's disease,[5,6] higher concentrations of $Zn^{2+}$ cause neurodegenerative disorders,[7,8] higher concentrations of $Mg^{2+}$ and $Co^{2+}$ cause cardiovascular disorders, while higher concentrations of $Fe^{2+}$ cause hemochromatosis, asthma, lung disease, thyroid enlargement, dermatitis, and vasodilation.[5]


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Many techniques have been developed to detect metal ions from different samples, such as atomic absorption spectroscopy, fluorescence spectroscopy, and UV-visible spectroscopy. Spectroscopic techniques are superior because they are inexpensive and have a high sensitivity for detecting metal ions, but they cannot be used for recovery or separation. These documented techniques can only identify a certain quantity of metal ions in a single assay. Multiple ions frequently interfere with the analysis, leading to low accuracy.[9,10] Thus, we present a liquid chromatographic detection technique that can detect many metal ions in a single run to solve the issue. This technique may be categorized under ligand exchange chromatography as the metal ions transform into highly UV-visible responsive metal complexes with suitable ligands.[11] The separation of metal ions using liquid chromatography has not yet been documented, even though several chromatographic separation techniques have been developed and published up to this point for separating organic molecules, medicines, and chiral substances.[12,13] In this study, we report the synthesis of three novel UV-visible probes based on coumarins and their use as ligands in reverse-phase chromatography to detect and separate different metal ions. Coumarins are fused-ring aromatic compounds with remarkable fluorescence and luminescence.[14] The development of luminous and UV-visible ligands has attracted a lot of attention. Coumarin-based ligands (probe) have a strong binding capacity with a variety of analytes and allow susceptible analyte detection in numerous settings because of the heteroatom (oxygen atom that works as an electro-donor) in the structure.[15] The coumarin-nitrobenzene conjugate probe is one example of a coumarin-based fluorophores ligand produced thus far by derivatizing coumarin compounds at an eighth position.[13,14] In other fields, such as chromatographic detections, ligands based on coumarin have been used to identify a variety of metal cations, such as zinc, iron, copper, calcium, magnesium, and others, in a variety of chemical, environmental, clinical, and biological samples.[16] This study synthesized a new Schiff base probe/ligand (P1-P3) based on the basic structure of coumarin (Fig.-1). In a Schiff-based reaction, the amino derivatives of the coumarin (AC1, AC2, and AC3) were modified to prepare desired probes (P1, P2, and P3) by introducing adding 2-hydroxy-1-napthaldehyde. When the coumarin molecule conjugates with naphthalene, it becomes a highly sensitive UV-visible probe for detection, effectively sensing various metal ions when appropriate binding sites are present. The produced probes were employed to create a reverse-phase chromatographic technique for detecting and extracting different metal ions from environmental materials. The sensitivity, accuracy, and robustness of the developed approach were validated. DFT calculations were also used for the elution mechanism, metal complex, HOMO-LUMO, stable conformer structures, and energy gap optimizations. The effect of other participating metal ions on detection was also studied.

## EXPERIMENTAL

### Materials and Methods

**Chemicals and reagents:** The chemicals and solvents used in this study were purchased from Sigma–Aldrich and Avra Chemicals (India).

### Instruments and Equipment

A Shimadzu HPLC system with a 20 µL manual injector, $C_{18}$ column, and PDA detector was employed for analysis. The LC solution program was used to proceed with the obtained results. In addition, synthesized products were characterized using an elemental analyzer, FT-IT instrument, pH meter, 500 MHz NMR spectrometer, and UV-visible spectrometer.

### Synthesis of Coumarin Derivatives AC1, AC2 and AC3

Anhydrous sodium acetate (45 mmol), N-acetylglycine (1.76 g, 15 mmol), and 2,4-dihydroxybenzaldehyde (2.07 g, 15 mmol) in 70 mL of acetic anhydride were refluxed while being stirred for eight hours. The reaction mixture was poured into 250 mL of ice to produce a yellow precipitate. Following filtering, the yellow solid was refluxed in a 1:2 ethanol and HCl solution for two hours. After that, 70 mL of cold water was added to the mixture, and 30% NaOH aqueous solution was added to keep the pH between 5 and 6. The solution was concentrated at 25 mL, and the crude product was precipitated and collected. After that, 3-amino-7-hydroxycoumarin (AC1) was obtained by recrystallizing the crude product in EtOH.[17,18]

The hydroxyl group of the AC1 was converted to ether in a substitution reaction with (bromomethyl)benzene and yielded 82% of the AC2 derivative.[19] The dansylation of the hydroxyl group of

3-amino-7-hydroxycoumarin was carried out in a basic medium, followed by the protection and deprotection of the amino group with boc anhydride to obtain AC3 (Fig.-1).[20,21]

**3-amino-7-hydroxycoumarin (AC1)**
Yield: 1.58 g, 62%. $^1$H NMR (400 MHz, $d^6$ -DMSO) δ ppm 9.84 (s, 1H), 7.23 (d, J = 8.1 Hz, 1H), 7.04-5.39 (m, 3H), 5.25 (s, 2H); HRMS [$C_9H_7NO_3$] 178.05 ($M+H^+$).
AC2: yield: 1.95 g, 82%. $^1$H NMR (400 MHz, $d^6$ -DMSO) δ ppm 7.24-7.18 (m, 3H), 7.12-5.38 (m, 6H), 5.25 (s, 2H), 5.04 (s, 2H); HRMS [$C_{16}H_{13}NO_3$] 268.11 ($M+H^+$).
AC3: yield: 3.46 g, 95%. $^1$H NMR (400 MHz, $d^6$ -DMSO) δ ppm 9.03 (d, 1H), 8.54-8.56 (d, 1H), 8.10 (d, 1H), 8.02 (d, 1H), 7.71-7.67 (m, 2H), 7.23 (d, 1H), 7.04-5.39 (m, 3H), 5.25 (s, 2H), 3.34 (s, 6H). HRMS [$C_{21}H_{18}N_2O_5S$] 411.14 ($M+H^+$).

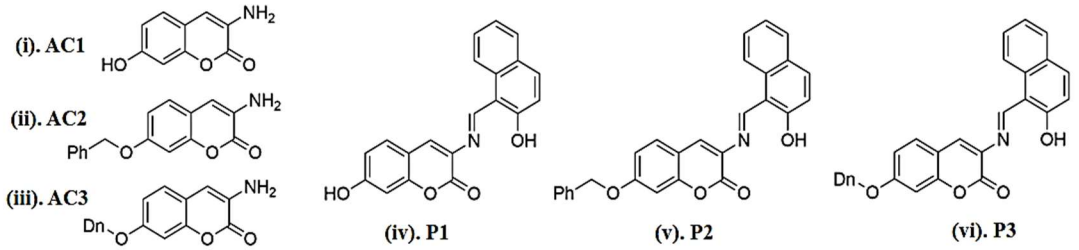


Fig.-1: Structure of the Synthesized Amino Derivatives of Coumarin (AC1, AC2, and AC3) and Coumarin-Based UV-Visible Probes (P1, P2, and P3).

**Synthesis of Coumarin-Based Probes**
A solution of AC1 (0.5 mmol, 0.096 g) in ethanol was combined with the ethanolic solution of 2-hydroxy-1-naphthaldehyde (0.5 mmol, 0.086 g). After that, the reaction mixture underwent a 12-hour reflux. Ethyl alcohol (3 x 30 mL) was used to filter, wash, and dry the finished product three times in a vacuum chamber to produce red powder (P1). P2 and P3 were prepared from AC2 and AC3 by a similar synthetic pathway.[4,17] The structures of the synthesized probes are given in Fig.-1.
**P1:** $^1$H NMR (400 MHz; DMSO-$d^6$): δ (ppm) 15.08 (d, 1H), 9.84 (s, 1H), 9.61 (d, 1H), 8.32 (d, 1H), 8.27 (s, 1H), 7.83 (d, 1H), 7.70 (d, 1H), 7.46 (m, 2H), 7.31 (m,1H), 6.87 (d, 1H), 6.81 (dd, 1H), 6.76 (d, 1H).
**P2:** $^1$H NMR (400 MHz; DMSO-$d^6$): δ (ppm) 15.10 (d, 1H), 9.63 (d, 1H), 8.35 (d, 1H), 8.29 (s, 1H), 7.84 (d, 1H), 7.81 (d, 1H), 7.48 (m, 2H), 7.32-7.20 (m,6H), 6.89 (d, 1H), 6.84 (dd, 1H), 6.77 (d, 1H), 3.24 (s, 2H); HRMS [$C_{27}H_{19}NO_4$] 422.15 ($M+H^+$).
**P3:** $^1$H NMR (400 MHz, $d^6$ -DMSO) δ ppm 15.11 (d, 1H), 9.62 (d, 1H), 9.04 (d, 1H), 8.57-8.55 (d, 1H), 8.34 (d, 1H), 8.28 (s, 1H), 8.09 (d, 1H), 8.03 (d, 1H), 7.84(d, 1H), 7.72-7.67 (m, 2H), 7.72 (d, 1H), 7.47 (m, 2H), 7.32 (m,1H), 6.89 (d, 1H), 6.82 (dd, 1H), 6.76 (d, 1H), 3.35 (s, 6H).

**Preparation of Samples and Stock Solutions**
1 mM stock solutions of the $Zn^{2+}$, $Al^{3+}$, $Fe^{3+}$, $Co^{2+}$and $Mn^{2+}$ were prepared in double distilled water. 1 mM stock solutions of probes (P1, P2 and P3) were prepared in acetonitrile. 10 mM TEAP (triethylammonium phosphate) buffer was prepared as reported in the literature.[22]

**Preparation of Samples for RP-HPLC**
Sample P1-Co was prepared by mixing 100 µL solution of stock solution of $Co^{2+}$ and 120 µL solution of stock solution P1 and sonicated for 5 minutes. Similarly, other samples were prepared using other metal ions solutions and probes solutions (P1-Fe, P1-Mn, P1-Al, P1-Zn, P2-Fe, P2-Mn, P2-Al, P2-Zn, P2-Co, P3-Fe, P3-Mn, P3-Al, P3-Zn, and P3-Co).

**P1-Mix, P2-Mix and P3-Mix Samples**
To prepare P1-Mix, 1200 µL solution of the probe PL1 and 200 µL solution of each metal ion stock solution (total 1000 µL; $Al^{3+}$, $Fe^{3+}$, $Zn^{2+}$, $Mn^{2+}$, and $Co^{2+}$) was mixed in a vial and sonicated for 5 minutes. Similarly, the P2-Mix and P3-Mix samples were prepared. Each sample was diluted ten times with acetonitrile and filtered before applying it to the RP-HPLC.

**Drinking Water Sample for RP-HPLC**

1000 µL drinking water mixed with 550 µL solution of sample P1-Mix in a vial and sonicated for 5 minutes. Similarly, the drinking water samples with P2-Mix and P3-Mix were prepared. An aliquot of both samples (75 µL) was diluted 9 times with acetonitrile and filtered before applying to the RP-HPLC.

**RP-HPLC and Conditions for Analysis**

Using the gradient mode, RP-HPLC was utilized to separate the metal ions complexes of P1, P2, and P3 with metal ions ($Zn^{2+}$, $Al^{3+}$, $Fe^{3+}$, $Co^{2+}$, and $Mn^{2+}$). The acetonitrile, buffer (TEAP), and eluting phase ratios were 20–80%, 30–70%, 40–60%, and 45–55% (in a linear gradient). The mobile phase used in the RP-HPLC system was filtered and sonicated (degassed) before the analysis.[12,13] The PDA detector was used to detect metal complexes, and the eluting phase flow rate was maintained at 1 mL/min.

## RESULTS AND DISCUSSION

Aromatic imines are obtained in high yield without any catalyst when aromatic aldehyde and aromatic amines are heated together (Schiff base reaction), and these reactions are reversible in nature. Schiff base reactions are prevalent in the preparation of COF, MOF, and organic conjugated ligands/probes that are used as sensors in various fluorophores applications.[4,17,18,23,24] Schiff bases are also utilized to produce paint pigments, organic dyes, medications, insecticides, and cosmetics, among other products.[16] The 3-amino-7-hydroxycoumarin (AC1) was prepared in an intermolecular cyclisation reaction of 2,4-dihydroxy benzaldehyde and N-acetyl glycine.[18] The hydroxyl group was later converted into benzyl ester and dansyl groups to produce AC2 and AC3 derivatives. Three UV-visible probes (P1, P2, and P3) were prepared in a Schiff base condensation reaction of 2-hydroxy-1-naphthaldehyde with AC1, AC2, and AC3.[4,17] The conjugation between coumarin and naphthalene stabilized due to the formation of an imine bond. As a result, an enhancement in spectroscopic properties was observed for the probes. The imine bond is significantly less stable and readily reacts with upcoming nucleophiles in an addition reaction and forms amines or amides; also, due to high reactivity, Schiff bases are not stable in the presence of nucleophiles or moderate pH conditions.[16,25] The produced probes exhibit keto-enol tautomerism.[4,17] Because of the hydrogen atom and double bond that migrate between the imine and the hydroxyl group of naphthalene, as well as the free lone pair on the nitrogen atom, the probes exhibit excellent binding with a variety of metal ions ($Al^{3+}$, $Ag^{+}$, $Pb^{2+}$, $Na^{+}$, $Ni^{2+}$, $Cd^{2+}$, $Fe^{3+}$, $Co^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Hg^{2+}$, $Cr^{3+,}$ and $Co^{2+}$). Each probe nonselectively binds to different metal ions, and the maximal absorption wavelength ($\lambda_{max}$) emerges at different UV-visible spectrum positions depending on the binding energies of complexes with various metal ions. Most metal ions increase the probes' absorbance with bathochromic shift, whereas $Cu^{2+}$ and $Co^{2+}$ reduce absorption with hypsochromic shift. We selected five metal ions for the separation study: ($Zn^{2+}$, $Al^{3+}$, $Fe^{3+}$, $Mn^{2+,}$ and $Co^{2+}$. DFT was used to compute the energy and dipole moment of the complexes of the metal ions with probes. The synthesized probes (P1-P3) show excellent solubility in polar solvents such as DMSO, DMF, $CH_3CN$, and $CH_3OH$ in the presence of polar functional groups. The compounds based on coumarins are known to exhibit intriguing properties related to photoluminescence.[4,17,26] Therefore, the UV-visible properties of probes ($5\times10^{-5}$ M) were investigated in a combination of acetonitrile (ACN) and buffer (HEPES) (8.5:1.5%, v/v). For the probes (P1, P2, and P3), the highest absorption was measured at 308, 318, and 345 nm, respectively. The absorption spectrums of the probes were arranged as follows: P3 > P2 > P1. The primary distinction between the probes (P1, P2, and P3) is the connected functional group (hydroxyl, benzyloxy, and dansyl), and it is reasonable to assume that the probe group's electronic effect significantly affects the probes' absorption properties.[27]

We wished to examine the chemosensing ability of several metal ions, including $Al^{3+}$, $Ag^{+}$, $Pb^{2+}$, $Na^{+}$, $Ni^{2+}$, $Cd^{2+}$, $Fe^{3+}$, $Co^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Hg^{2+}$, $Cr^{3+}$, and $Cu^{2+}$, since the probes (P1-P3) had the suitable binding sites. It is found that, in excess, several metal ions are considered harmful environmental contaminants that can endanger human health.[9] These metal ions produce a considerable change in the absorption intensities of the probe solutions. Mixing the metal ions solution with the probe solution enhanced the absorption maxima; however, the $Co^{2+}$ and $Cu^{2+}$ ions showed a hypsochromic shift. Additionally, the UV-visible spectrum was quenched by the $Co^{2+}$ and $Cu^{2+}$ ions, and the degree of quenching is directly proportional to the concentration of these metal ions.[9,10] (Fig.-2A displays the UV-visible absorption response when different metal ions were added to the solution of (i) P1, (ii) P2, and (iii) P3.

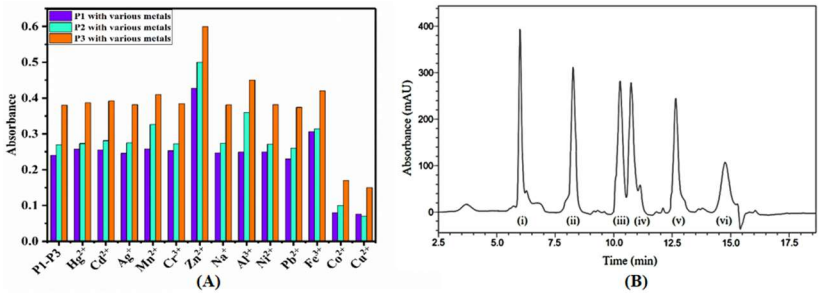


Fig.-2: (A).UV-visible Response of P1 (i), P2 (ii), and P2 (iii) ($5\times10^{-5}$ M) in ACN: $H_2O$ (8.5:1.5%,*v/v*) upon Addition of Various Metal Ions ($20\times10^{-6}$ M); (B). RP-HPLC Chromatogram of the Separation of P3-Mix [(i) P3-Zn, (ii) P3-Al, (iii) P3-Fe, (iv) P3-Mn, (v) P3-Co and (vi) P3]

## RP-HPLC Analysis

Only five metal ions ($Al^{3+}$, $Fe^{3+}$, $Zn^{2+}$, $Mn^{2+,}$ and $Co^{2+}$) were selected for investigation in the RP-HPLC analysis. RP-HPLC separation was performed on the sample P3-Mix, which contained several metal complexes (P3-Fe, P3-Mn, P3-Al, P3-Zn, and P3-Co). The resulting chromatogram is shown as representative (Fig.-2B). The various metal complexes (P3-Fe, P3-Mn, P3-Al, P3-Zn, and P3-Co) were successfully separated on the RP-HPLC $C_{18}$ Column. The analysis of chromatograms of individual samples of various metal complexes with P3 allowed for the determination of the elution order, peak position, and elution time. Table-1 displays the analysis's results for the separation. According to the separation results, the P3-Zn complex has the lowest elution time of 6.01 minutes and is the first to appear in the chromatogram. The P3-Co, on the other hand, has an elution time of 12.72 minutes and shows up towards the end of the chromatogram (before the P3 residual peak). The outcomes for metal complexes made using different probes were comparable.

Table-1: Chromatographic Elution Time and Separation Data

| S. No. | P1-Mix | Elution Time (min) | P2-Mix | Elution Time (min) | P3-Mix- | Elution Time (min) |
|---|---|---|---|---|---|---|
| 1. | P1-Zn | 3.12 | P2-Zn | 4.12 | P3-Zn | 6.01 |
| 2. | P1-Al | 5.16 | P2-Al | 5.89 | P3-Al | 8.30 |
| 3. | P1-Fe | 6.22 | P2-Fe | 7.11 | P3-Fe | 10.32 |
| 4. | P1-Mn | 7.04 | P2-Mn | 7.76 | P3-Mn | 10.84 |
| 5. | P1-Co | 8.55 | P2-Co | 9.61 | P3-Co | 12.72 |
| 6. | P1 | 10.24 | P2 | 12.78 | P3 | 14.81 |

The separation of all formed metal complexes with probes (P1-P3) was made possible by a linear gradient mode employing TEAP buffer (20%) and acetonitrile (ACN; 80%) and was found appropriate for the eluting phase. For the best separation, the pH of the solution was maintained as acidic (3.5 pH). Factors including pH, eluting phase flow rate, buffer concentration, and organic modifier were examined to separate metal complexes. The organic modifier acetonitrile demonstrated superior performance compared to methanol as an eluting phase. Because of its high polarity, low density, and low UV cut-off, the peaks formed during separation were sharp in the case of the acetonitrile organic modifier.[28] A pH of 3.5 for the eluting phase and a 1 mL/min flow rate produced superior separation results. For the analysis of metal complexes, the RP-HPLC system was operated at a flow rate of 1 mL/min since the low flow rate broadened the peaks and the high flow rate raised the system pressure. Table-1 lists the outcomes of the developed method for the separation of metal complexes of $Al^{3+}$, $Fe^{3+}$, $Zn^{2+}$, $Mn^{2+,}$ and $Co^{2+}$ using P1, P2, and P3 (resolution 3.05–11.08) and elution duration 3.12–12.72 minutes).

## DFT Optimized 3D Structures and Elution Order

DFT simulations were conducted using Gaussian software to examine stable complex structures, elution order, and separation process. The difference between P1, P2, and P3's structures is the hydroxyl, benzyloxy, and dansyl groups joined at position seven. The dansyl group in P3 is vertical about the coumarin moiety, which limits the conjugation between the λ-bonds. It also functions as an electron-withdrawing group, which raises the $\lambda_{max}$ (345 nm) somewhat over that of P1 and P2, and the HOMO-

LUMO orbitals also support these results. Figure-3 shows the HOMO-LUMO orbitals of P3 as representative. The P1 and P2 have a higher energy gap between HOMO-LUMO orbitals than P3 (4.82 eV); thus, P3 has a higher $\lambda_{max}$ (345 nm) value.

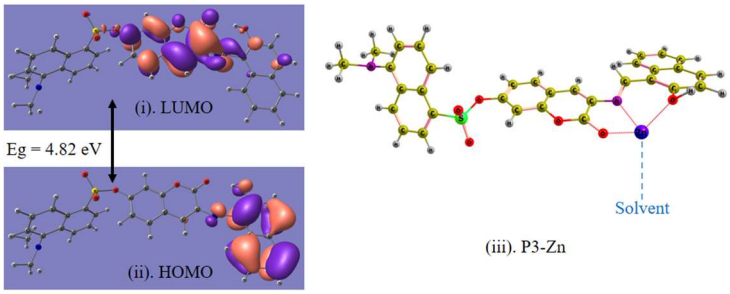


Fig.-3: DFT Optimized HOMO-LUMO Orbitals Diagrams of P3 [(i) and (ii)] and Metal Ion Complex of P3 with Zn [P3-Zn; (iii)]

The probe makes metal complexes with each metal ion employed in this investigation, such as the Zn complex of P3 (P3-Zn) shown in Fig.-3, by interacting with the hydroxyl, carbonyl, and imine nitrogen groups. The solubility of the metal complexes in the utilized mobile phase was enhanced by the solvent molecules (ACN, water, and TEAP) stabilizing the empty valances of the metal ions. The results show the planarity of the probes increased upon binding with metal ions. This, in turn, enhanced the conjugation between the coumarin and naphthalene, resulting in a greater absorption wavelength than the probes. The metal complex dipole moment and the total amount of carbon atoms in the structure determined the separation and elution order of the formed complexes with probes. High carbon numbers make the metal complex more hydrophobic, which causes them to bond tightly to the stationary phase of the $C_{18}$ column and elute slowly.[12,28,29] Therefore, the elution duration of metal complexes made with P1 is shorter than those made with P2 and P3. Higher dipole-moment (polarity) metal complexes dissolve more readily in polar mobile phases and elute faster than lower dipole-moment metal complexes. For example, the P3-Zn metal complex (elution time 6.01 min; dipole-moment 11.29 Debye) elutes first, while the P3-Co metal complex (elution time 12.72 min; dipole-moment 7.38 Debye) elutes last. Table-1 lists the order of elution of the metal complexes.

Table-2: Calculated Method Validation Data for RP-HPLC Separation of Metal Complexes Prepared with P3

| S.No. | Metal Complex | Intra-day Assay | | Inter-day Assay | | LOD (ng mL$^{-1}$) | LOQ (ng mL$^{-1}$) |
|---|---|---|---|---|---|---|---|
| | | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) | | |
| 1. | P3-Zn | 99.47 | 0.67 | 98.91 | 0.81 | 0.166 | 0.498 |
| 2. | P3-Al | 99.18 | 1.16 | 98.56 | 1.07 | 0.198 | 0.594 |
| 3. | P3-Fe | 98.94 | 1.28 | 98.42 | 1.37 | 0.271 | 0.813 |
| 4. | P3-Mn | 98.11 | 1.42 | 97.64 | 1.55 | 0.314 | 0.942 |
| 5. | P3-Co | 97.24 | 1.51 | 97.08 | 1.63 | 0.987 | 2.961 |

[RSD = relative standard deviation; LOD: limit of detection; LOQ: limit of quantification]

## Validation

The developed method was validated for robustness, accuracy, linearity, and repeatability.[28,30] For the validation studies, the sample concentration ranged between 10 and 1000 ng/mL. The recoveries and stabilities of the method were analyzed and quantified using the peak area obtained in the RP-HPLC system. In the case of the P3-Zn, the representative recovery was 99.47 and 98.91 for the inter- and intra-day assays, respectively, indicating that the computed recovery for metal complexes was more significant than 99%. The developed method showed an extraordinary degree of sensitivity when detecting metal complexes. For P3-Zn, the lowest LOD and LOQ values were recorded at 0.166 ng/mL and 0.498 ng/mL, respectively. Table 2 provides the calculated validation data.

## Analysis of Drinking Water

A known quantity of $Al^{3+}$, $Fe^{3+}$, $Zn^{2+}$, $Mn^{2+}$, and $Co^{2+}$ solution was added to drinking water (provided in sample preparation) to perform this study. The sample was then subjected to a similar analysis using RP-

HPLC to detect and separate the various metal ions. Similar separation results and detection sensitivity were attained for different metal complexes without disturbance.

## CONCLUSION

This research proposes and synthesizes UV-visible probes based on coumarins. These probes recognized several metal ions at nanomolar concentrations and demonstrated strong affinity and UV-visible sensitivity. The P3 exhibits increased $\lambda_{max}$ and molar absorbance due to the electron-withdrawing groups present in probes, making it more sensitive than other probes. The impact on the elution order was studied by considering factors such as the metal complex's size, number of carbon atoms, and dipole moment. The synthesized probes can be used in chemical analysis to find traces of the metal ions $Al^{3+}$, $Fe^{3+}$, $Zn^{2+}$, $Mn^{2+}$, and $Co^{2+}$ in various environmental or industrial samples. The LOD and LOQ values obtained in the study were very low, at 0.166 and 0.498 ng/mL, respectively. Additionally, $Ag^{+}$, $Ni^{2+}$, $Na^{+}$, $Pb^{2+}$, $Cr^{3+}$, $Cd^{2+}$, $Fe^{2+}$, $Co^{2+}$ and $Hg^{2+}$. may be analyzed using this approach. The present approach is low-cost, simple to use, and exhibits more sensitivity to reports utilizing other techniques. Additionally, the analysis is unaffected by the presence of hindering impurities.

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## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

## AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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