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Synthesis of schiff base zinc metal complex $(MAPIMP)_2Zn$ and development of HPLC chromatographic method for its analysis

Ramesh Yamgar[1], Prasad Kamat[2] and Sudhir Sawant[3]*

[1,2]Department of Chemistry, Patkar College, Goregaon (w), Mumbai, INDIA
[3]Department of Chemistry, Ismail Yusuf of College, Gogeshwari (E), Mumbai, INDIA

ABSTRACT

A simple, fast and accurate method has been developed for the determination of $(MAPIMP)_2Zn$ by High Pressure Liquid Chromatography. The analysis was carried out on Waters 2695 separation module HPLC system with Waters 2487 Dual wavelength Absorbance detector. The column used was a stainless steel column of dimension 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (Make : Neosphere 120-5-C18 Altima column is suitable).The detector used was Ultraviolet (UV) detector. The validation of proposed method was also carried out.

Key words:HPLC, Schiff base metal complex, $(MAPIMP)_2Zn$.

INTRODUCTION

Schiff bases form an important group of compounds in chemistry not only because of their useful physical and chemical properties and large number of reactions they undergo but also because of their wide use in industry and their interesting pharmacological activity. Schiff bases derived from substituted aliphatic amines and aromatic aldehydes have a wide variety of applications in many fields, *e.g.* biological, inorganic and analytical chemistry [1-5].

Among the organic reagents generally used, Schiff bases possess excellent characteristics, structural similarities with natural biological substances, relatively simple preparation procedures and the synthetic flexibility that enables design of suitable structural scaffolds [6,7]. Many biologically important Schiff bases have been reported in the literature possessing, antibacterial [8,9,10], antifungal [11,12,13], antimicrobial [14,15,16], anticonvulsant [17], antiHIV [18], anti-inflammatory and antitumor activities.

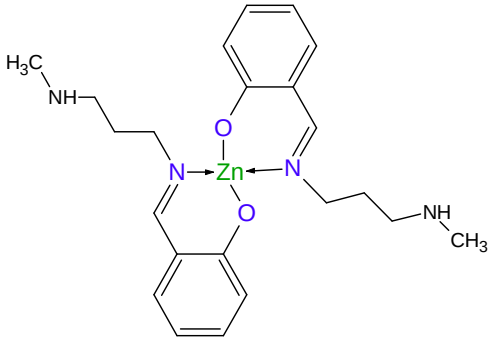
During the literature survey we found that there are very few non destructive analytical methods reported for schiffs base metal complexes. With the advent of advanced analytical methods like HPLC, we thought to undertake this initiative and study the HPLC method development parameters in detail as per ICH guidelines. Such methodology would help research and development activity in exploring further the estimation of Schiff base metal complexes by HPLC method.

EXPERIMENTAL SECTION

Preparation of $(MAPIMP)_2Zn$ complex [19-21]

The Schiff base compound was prepared in situ by the condensation of N-Methyl-propane-1,3-diamine(1.1 g, 0.0125 moles) with salicylaldehyde (1.52 g, 0.0125 moles) in 20 ml ethanol solution and heated for 30 minutes at reflux temperature. $ZnCl_2$ (1.6 g, 0.125 moles) was added in above Schiff base solution with stirring. The mixture was stirred for 2-3 hours at reflux temperature and filtered. Light yellow coloured crystalline material was recrystallized from Ethanol with 85% yield.

The compound was characterized by ^1H NMR, IR and Mass spectra. Zn Metal content was estimated by EDTA complexometric titration method. The proposed structure of the $(MAPIMP)_2Zn$ complex is as follows. It is a square planar structure having diamagnetic nature.



$(MAPIMP)_2Zn$

HPLC Method Development and Method Validation

In the present competitive world, the time and cost are most important factors in the Good Manufacturing Practices [22]**.** The main objective of method development[23] is user friendly approach without compromising the quality aspects. The use of solid buffer is avoided because it affects the column life. Several other methods are also tried but amongst that this method is chosen for the validation as it is more simple, fast and accurate. The wavelength maxima (220 nm) of the $(MAPIMP)_2Zn$ complex is first determined by using Ultraviolet Spectrophotometer. Also the solubility is determined in different solvents. The sample is freely soluble in 50:50 water : methanol. Also the peak purity is confirmed with Waters PDA system which proves that there is no coelusion.

Method Validation

The main objective of validation of an analytical procedure[24] is to demonstrate that the procedure is suitable for its intended purpose; this document describes the characteristics, with predefined acceptance criteria, that should be evaluated during the validation. Well-

characterized reference materials, with documented purity, should be used throughout the validation study. The degree of purity necessary depends on the intended use.

This document considers the various validation characteristics in distinct sections. The arrangement of these sections reflects the process by which an Analytical procedure may be developed and evaluated. In practice, it is usually possible to design the experimental work such that the appropriate validation characteristics can be considered simultaneously to provide a sound, overall knowledge of the capabilities of the analytical procedure. For instance: Specificity, Linearity, Range, Accuracy and Precision.

The following elements of validation shall be analyzed during the validation exercise

1. Specificity
2. Linearity and Range
3. Accuracy
4. Repeatability and Intermediate Precision
5. Robustness
6. System Suitability data
7. Solution Stability

Material and Instruments used

a) **Instruments used :** 1) Waters 2695 separation module HPLC system with waters 2487 Dual wavelength Absorbance detector.·
2) Ultraviolet spectrophotometer

b) **Column used** : Neosphere 120-5-C18 Altima (Batch number 2284)

Reagents and chemicals

Methanol HPLC grade	: Apchem Ltd.
Acetonitrile HPLC grade	: Apchem Ltd.
Orthophosporic acid HPLC grade	: Apchem Ltd.
Triethylamine HPLC grade	: Apchem Ltd.
Puirified Water (Type I)	: Millipore water purification system

Chromatographic Conditions

Column type	: 0.25 m x 4.6 mm, 5µm (Neosphere is suitable)
Flow rate	: 1.0 ml/minute
Detector wavelength	: 220 nm
Injection volume	: 10 µl.
Diluent	: 50:50 (Water : Methanol)

Preparation of Buffer solution

Transfer 500 ml of purified water to 1000 ml volumetric flask, add 7 ml of Triethylamine in it with constant stirring, dilute upto the mark with purified water. Mix and continue the stirring. Adjust the pH to 3.00 ± 0.05 with dilute orthophosphoric acid.

Preparation of Mobile phase

A mixture of 12 volume of acetonitrile , 38 volumes of Methanol and 55 volume of buffer solution (prepared as above). Filter through 0.45µm filter paper. and degas.

Preparation of Standard solution

Standard Solution A : (This conc. of solution is 100 ppm / 0.01 % / 0.1 mg/ml)

Weigh accurately about 100.0 mg of $(MAPIMP)_2Zn$ complex TS and transfer into a 100 ml volumetric flask, added sufficient amount of diluent, sonicate to dissolve, allow it cool at room temperature and dilute to upto mark with diluent. Further dilute 10 ml of this solution to 100 ml with diluent. Filter through Millipore Millex-HN Nylon 0.45 µm syringe filter.

Standard Solution B : (This conc. of solution is 100 ppm / 0.01 % / 0.1 mg/ml)

Weigh accurately about 100.0 mg of $(MAPIMP)_2Zn$ complex TS and transfer into a 100 ml volumetric flask, added sufficient amount of diluent, sonicate to dissolve, allow it cool at room temperature and dilute to upto mark with diluent. Further dilute 10 ml of this solution to 100 ml with diluent. Filter through Millipore Millex-HN Nylon 0.45 µm syringe filter.

Preparation of Test Solution

Weigh accurately about 100.0 mg of $(MAPIMP)_2Zn$ complex sample and transfer into a 100 ml volumetric flask, added sufficient amount of diluent, sonicate to dissolve, allow it cool at room temperature and dilute to upto mark with diluent. Further dilute 10 ml of this solution to 100 ml with diluent. Filter through Millipore Millex-HN Nylon 0.45 µm syringe filter.

Sequence of injections

Sequence I: 1) Blank, 2) Std sol. A, 3) Std. B1,

Sequence II: 1) Std. B1 ----- Std. B6, 2) Blank, 3) Sample -1, 4) Std-B.

Procedure

Inject separately equal volumes (10 µl) of the Blank solution (i.e. Diluent), standard solution A, Standard solution B (in six replicates) & sample solution into the chromatograph. Record the chromatograms & measure the responses for the major peaks.

Test is not valid unless following System Suitability requirements are achieved

1) The similarity factor between two replicate standards (separately prepared & injected) is between 0.98 & 1.02. Calculate similarity factor using formula.

$$\frac{\text{Peak area of } (MAPIMP)_2Zn \text{ complex TS in Std A}}{\text{Peak area of } (MAPIMP)_2Zn \text{ complex in Std B } (1^{st} \text{ Injection})} \times \frac{\text{Wt of } (MAPIMP)_2Zn \text{ complex TS in Std B (mg)}}{\text{Wt of } (MAPIMP)_2Zn \text{ complex TS in Std A (mg)}}$$

(If similarity factor does not fall within 0.98 & 1.02, prepare fresh standard solutions in duplicate, re-inject & calculate similarity factor again as above)

2) The % RSD for peak area response & retention time for the peak of $(MAPIMP)_2Zn$ complex for replicate standard injections is not more than 2 % & 1.0 % respectively.

3) Tailing factor for the peak of $(MAPIMP)_2Zn$ complex obtained in chromatogram of standard solution (A) is not more than 2.0.

4) The column efficiency for the peak of $(MAPIMP)_2Zn$ complex obtained in chromatogram of standard solution (A) is not less than 2000 Theoretical plates.

RESULTS AND DISCUSSION

Analytical method used for Assay of $(MAPIMP)_2Zn$ complex (in % w/w) on as is basis by using High performance liquid chromatography technique is validated.

Validation is carried out on Waters Alliance (quaternary gradient with VWD) HPLC system. The validation of the method was assessed by, establishing validation criteria's such as Specificity and System suitability, Linearity and Range, Precision, Accuracy, Reproducibility, Robustness and Solution stability study.

Specificity and System Suitability

Specificity was carried out to monitor interference from blank (diluent) to monitor system suitability (To check the number of theoretical plates and tailing factor)

The results are found to be well within the acceptance criteria set for the specificity and system suitability study, hence the method is specific.

Table 1: Linearity and Range

Linearity Level	Conc. of (MAPIMP)2Zn complex in ppm	Mean Area of (MAPIMP)2Zn complex
Linearity 50%	50	720042
Linearity 60%	60	859471
Linearity 100%	80	1125206
Linearity 120%	100	1405933
Linearity 140%	120	1722141
Linearity 150%	140	1972446
% y-Intercept	0.8	
Regression Co-efficient	0.9995	

Table 2: Observation table for Specificity / Precision / Intermediate precision / Robustness / Solution stability

System suitability parameters	Specificity/ Linearity and Range/Precision	Intermediate precision	Robustness at Buffer pH 2.8	Robustness at Buffer pH 3.2	Robustness at increasing lower component concentration by 5 %	Robustness at decreasing lower component concentration by 5 %	Solution stability (Till 3 Hrs)
Blank interference	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Similarity factor	1.00	0.99	1.01	1.01	1.00	1.00	0.99
Column efficiency	4345	5784	5877	5937	6203	6045	5784
Tailing factor	0.95	1.02	1.01	1.00	1.00	1.00	1.02
% RSD Peak area response	2.2	2.2	2.4	0.4	0.5	0.3	2.2
% RSD Retention time	0.1	0.1	0.3	0.1	0.4	0.1	0.1
% RSD Assay	0.92	0.86	0.89	0.44	0.89	0.89	----
Cumulative % RSD Assay	-------	0.93	0.90	0.79	0.91	0.83	0.14

Table 3: Observation table for Accuracy

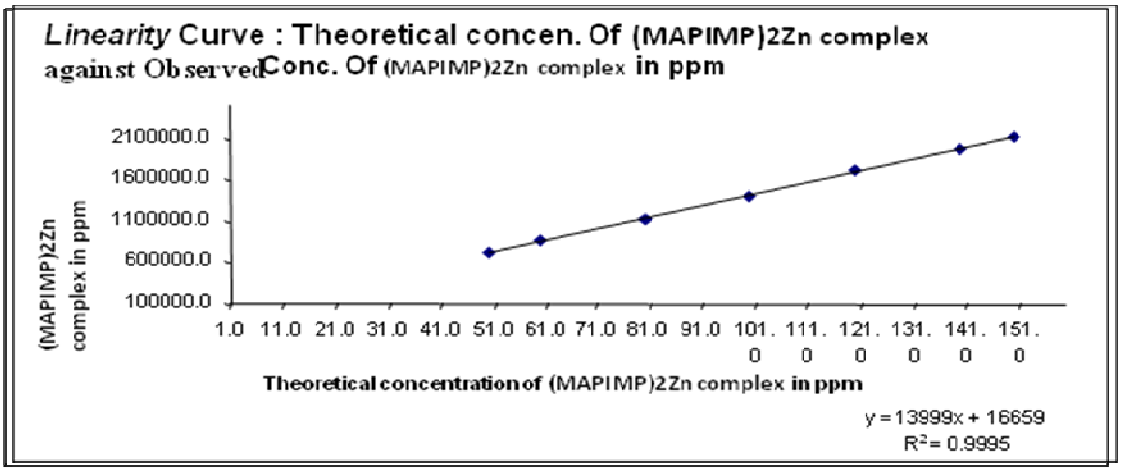
Accuracy level	Replicates	% Mean Recovery
	1	101.74
I	2	
	3	
	1	101.20
II	2	
	3	
	1	98.15
III	2	
	3	

Linearity and Range

Linearity and range was carried out over a range of 50 % to 150% of working level concentration. The Linearity regression correlation coefficient, % y intercept. The linearity regression correlation coefficient for the component was found to be within the limit (Not less than 0.999).The % y-intercept for the component was found to be within limit (Not more than ± 2.0).

As the results obtained are well within the criteria set for the linearity and range, Hence the method is linear.

Graph 1: Graphs and Observation



Precision

A) System precision

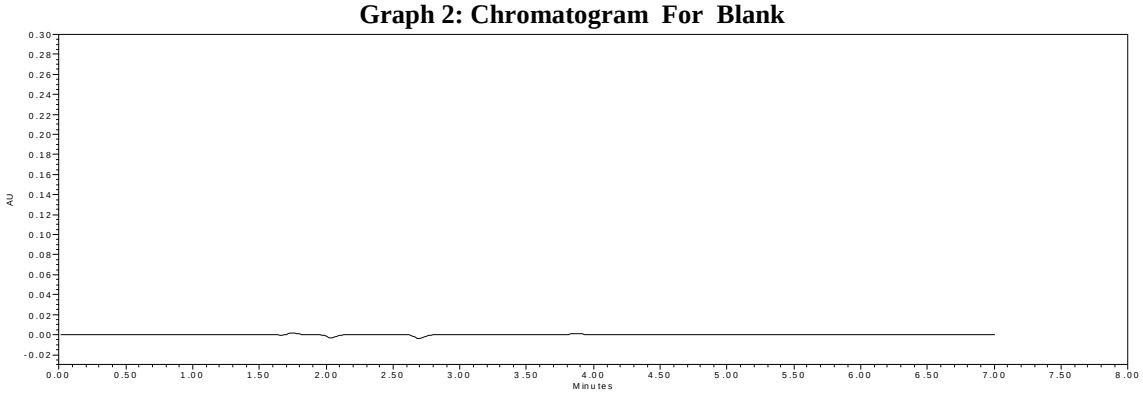
Standard solution of working concentration was injected into the chromatograph in six replicates. The % RSD for peak area response and retention time were found to be within the limit. (Not more than 2 % for peak area response and Not more than 1 % for retention time). The system suitability parameters like Theoretical plates and Tailing factor were found within the limits.

B) Method precision

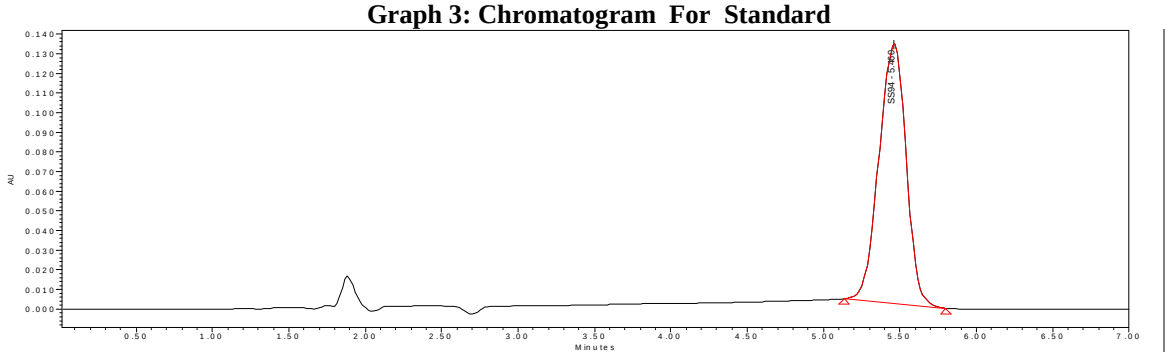
A batch of $(MAPIMP)_2Zn$ complex was analyzed in six times for assay. The assay of the $(MAPIMP)_2Zn$ complex (in % w/w) on as is basis was calculated. The % RSD of assay was found to be well within the limit set for precision.

As the results of system precision and method precision are well within the limit, hence the method is precise.

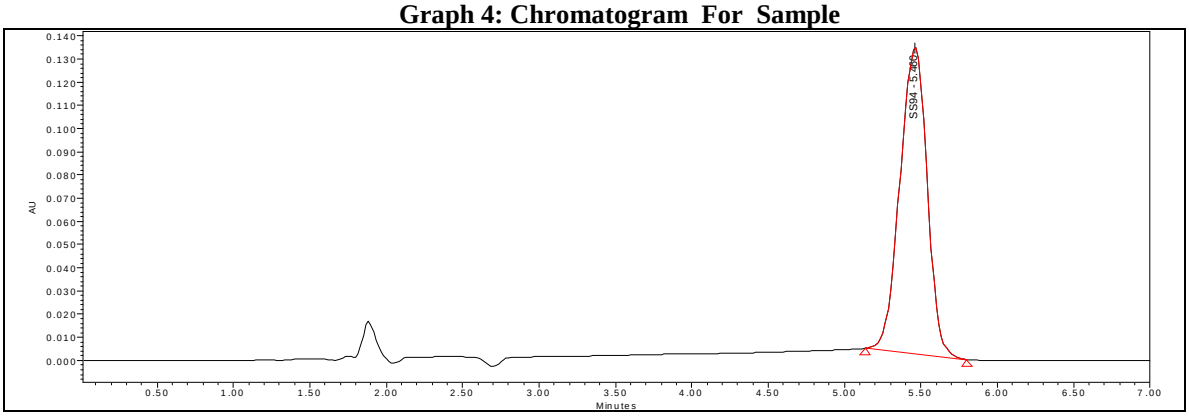
Graph 2: Chromatogram For Blank



Graph 3: Chromatogram For Standard



Graph 4: Chromatogram For Sample



Reproducibility

A batch of $(MAPIMP)_2Zn$ complex was reanalyzed by another analyst on another system for six times for assay. Results were calculated. The results of reproducibility study along with precision study were compared and found to be well within the limits set for reproducibility study.

As the results obtained by two different analysts are comparable, hence the method is reproducible.

Accuracy

To the $(MAPIMP)_2Zn$ complex Sample of working level concentration, Standard of $(MAPIMP)_2Zn$ complex is added (of 50 % ,100 % & 150 % i.e. 0.05 mg/ml , 0.10 mg/ml & 0.15 mg/ml) and recovery for individual levels were done in triplicate and calculated.

The % recoveries observed for the levels are found to be well within the limit set for the accuracy study (Not less than 98.0 % and not more than 102.0 %). This shows that the component is recoverable and hence method is accurate.

Robustness

The robustness of method was carried out by changing the different chromatographic conditions (one at a time) such as,

I) By Changing the pH of the Buffer from 3.00 to 2.80.
II) By Changing the pH of the Buffer from 3.00 to 3.20.
III) Increasing lower component concentration in mobile phase by 5 %.
IV) Decreasing lower component concentration in mobile phase by 5 %.

The results of robustness study along with precision study were compared and found to be well within the limits set for the robustness study. This shows that the method is robust.

Solution Stability

The solution stability is monitored to check the stability of solution. A sample solution was preserved over a period of 3 and 8 hours and analyzed after the specified time intervals. The results of initial analysis and the results of analysis after preservation for assay of $(MAPIMP)_2Zn$ complex was compared and found to be well within the set limit for solution stability study for $(MAPIMP)_2Zn$ complex for 3 hours.

As the results of initial analysis and analysis after preservation up to 3 hours are comparable hence the solution is stable up to 3 Hours.

CONCLUSION

The analytical method used for determination of assay of $(MAPIMP)_2Zn$ complex (in % w/w) on as is basis complies with the acceptance criteria of the analytical parameters such as Specificity and system suitability, Linearity and range, Precision, Accuracy, Reproducibility, Robustness and Solution stability study. Hence method stands validated. The method can be used for routine quality control and stability study analysis.

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