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Preparation of Schiff base Zinc Metal complex $(DMAPIMP)_2Zn$ and Development of HPLC Chromatographic method for its analysis

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ABSTRACT

A rapid, sensitive and specific RP-HPLC method involving UV detection was developed and validated for determination and quantification of $(DMAPIMP)_2Zn$ · Chromatography was carried out on Waters 2695 separation module HPLC system with Waters 2487 Dual wavelength Absorbance detector and Waters 2998 Photodiode Array Detector using a stainless steel column of dimension 15 cm x 4.6 mm , packed with octadecylsilane bonded to porous silica (Make : Phenomenex Prodigy 5 µ ODS 3 100A column is suitable) using filtered and degassed mixture of Buffer (Prepared by addition of 1 L purified water and 10 ml Acetic Acid and 5 ml Triethylamine) and Acetonitrile (60:40) as a mobile phase at a flow of 1.0 ml/min and effluent was monitored at 254 nm . The method was validated in terms of Specificity, Linearity and Range, Precision, Accuracy, Intermediate Precision, Solution Stability and Robustness. The assay was linear over the concentration range of $(DMAPIMP)_2Zn$ 0.05 mg/ml to 0.15 mg/ml respectively. Accuracy of the method was found to be 98.98 % - 101.22 % within precision RSD of 1.33. The system suitability parameters such as theoretical plates and tailing factor were found to be 6325 and 1.14 respectively. The method requires only 8 minutes as run time for analysis which prove the adoptability of the method for the routine quality control of the drug.

Key words : HPLC, Method Validation, Schiff base metal complex, $(DMAPIMP)_2Zn$

INTRODUCTION

A Schiff base is a special group of organic compounds also known as azomethine, anil or imine. Schiff bases are of the general formula $R_1HC=N-R_2$, where R_1, R_2 is an aryl or alkyl group that makes the Schiff base a stable imine. Schiff bases can be derived from a carbonyl compound and an aromatic amine by nucleophilic addition , followed by a dehydration to form an imine.[1-5]

A Schiff base, is named after the work of a German chemist Hugo Schiff [5], who condensed primary amines with carbonyl compounds for the first time leading to the formation of azomethine group (-C = N-)[6,7] .The Schiff base formation is really a sequence of two types of reactions, i.e. addition followed by elimination. A large number of aldehydes and ketones have been condensed with various amines to give Schiff bases.

Schiff bases act as good ligands forming complexes with various metal ions due to their proton donating ability and number of bonding sites which lead to different stereo chemical structures and can also give kinetic and thermodynamic stability to the metal complexes. Though monodentate Schiff base ligands have been synthesized and studied for the complexation with several metal ions, bi, tri and tetradentate Schiff base ligands, normally known as multidentate ligands, are of great importance because of the chelating property which gives extra stability to the metal complexes and interesting geometries have been observed.

Schiff bases form an important group of compounds in chemistry due to their useful physical and chemical properties and large number of reactions they undergo. They have wide use in industry due to their interesting pharmacological activity [8a-c]. A number of reviews on the Schiff bases have been published [8a-c] . Schiff bases derived from aromatic amines and aromatic aldehydes have a wide variety of applications in many fields, e.g., biological, inorganic and analytical chemistry[8d-h]. They are also used in optical and electrochemical sensors, as well as in various chromatographic methods, to enable detection due to enhanced selectivity and sensitivity[8i-k].

Schiff bases exhibit excellent characteristics and structural similarities with natural biological substances, relatively simple preparation procedures and the synthetic flexibility that enables design of suitable structural scaffolds[8 l-m].

Substituted aliphatic diamino compound such as N,N,Dimethyl propylene diamine is very active compound towards carbonyl compounds[9a-c]. These derivatives are useful in co-ordination chemistry due to presence of donor atoms such as N and O. Its derivatives also act as antibacterial agents[10]. Some hydroxyl derivatives of coumarins also show immunological & antitumor activities[11].

The condensation reaction between Salicyaldehyde and N,N,Dimethyl propylene diamine yields a new compound with mainly two donor sites suitable for the study of ligational behaviour attracted our attention to synthesize Schiff's base which is used for the preparation of the metal complexes of transition metals such as Co(II), Ni(II), Cu(II), and Zn(II)[12].

The bidentate ligand synthesized in the present work, has been characterized by elemental analyses, IR and NMR spectra. The newly synthesized complexes are coloured, nonhygroscopic and completely soluble in DMF and DMSO at room temperature.

The structures of these metal complexes have been investigated by various physicochemical techniques viz. elemental analysis, UV-Visible, IR, NMR, Mass, ESR spectral studies, XRD and thermal analysis.

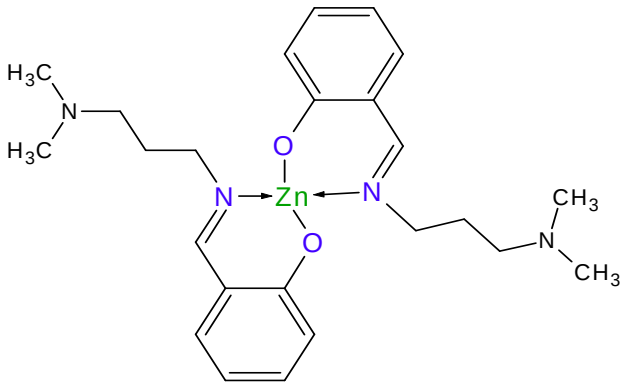
During the literature survey we found that there are very few analytical methods reported for Schiff's base metal complexes. With the advent of advanced analytical methods like HPLC, we thought of undertaking this initiative and study the HPLC method development parameters in detail as per ICH guidelines.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

EXPERIMENTAL SECTION

Synthesis of $(DMAPIMP)_2Zn$ complex [12-14]

The Schiff base compound was prepared *"in situ"* by the condensation of N,N-Dimethyl-propane-1,3-diamine(1.27 g, 0.0124 moles) with Salicylaldehyde (1.52 g, 0.0124 moles) in 20 ml ethanol solution and heated for 60 minutes at reflux temperature. $ZnCl_2$ (1.7 g, 0.125 moles) was added in above Schiff base solution with stirring. The mixture was stirred for 90 minutes at reflux temperature. Light yellow coloured precipitate was filtered while hot. Wet precipitate washed with ethanol 5 ml x 3 times, sucked dried and then dried in oven at 80-100°C with 96% 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 $(DMAPIMP)_2Zn$ complex is as follows. It is a square planar structure having diamagnetic nature.



Bis(2-[(*E*)-{[3-(dimethylamino)propyl]imino}methyl]phenol)Zinc, $(DMAPIMP)_2Zn$

In this paper we describe a simple, inexpensive, sensitive and validated HPLC method for the simultaneous determination of $(DMAPIMP)_2Zn$ complex.

HPLC Method Development and Method Validation:

In the present competitive world, the time and cost are most important factors in the Good Manufacturing Practices [15]**.** The main objective of method development[16] 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 (254 nm) of the $(DMAPIMP)_2Zn$ 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[17] 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. 2998 Photodiode Array detector
2) Ultraviolet spectrophotometer

b) **Column :** Phenomenex Prodigy 5µ ODS 3 100 A (Serial Number 440327-9)

Reagents and chemicals:

Methanol HPLC grade	: Apchem Ltd.
Acetonitrile HPLC grade	: Apchem Ltd.
Acetic Acid HPLC grade	: Apchem Ltd.
Triethylamine HPLC grade	: Apchem Ltd.
Purified Water (Type I)	: Millipore water purification system

Chromatographic Conditions *:*

Column type	: 150 mmx4.6mm, 5µm(Phenomenex Prodigy 5µ ODS 3 100 A is suitable)
Flow rate	: 1.0 ml/minute
Detector wavelength	: 254 nm
Injection volume	: 20 µl.
Diluent	: 50:50 (Water : Methanol)

Preparation of Buffer solution :
Transfer 800 ml of purified water to 1000 ml volumetric flask, add 10 ml of Acetic Acid, mix well and add 5 ml of Triethylamine in it with constant stirring, dilute upto the mark with purified water.

Preparation of Mobile phase :
A mixture of 60 volume of Buffer and 40 ml volumes of Acetonitrile. 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 $(DMAPIMP)_2Zn$ 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 upto the 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 $(DMAPIMP)_2Zn$ 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 upto the 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 $(DMAPIMP)_2Zn$ 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 upto the 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 % RSD for peak area response & retention time for the peak of $(DMAPIMP)_2Zn$ complex for replicate standard injections is not more than 2 % & 1.0 % respectively.
2) Tailing factor for the peak of $(DMAPIMP)_2Zn$ complex obtained in chromatogram of standard solution (A) is not more than 2.0.
3) The column efficiency for the peak of $(DMAPIMP)_2Zn$ complex obtained in chromatogram of standard solution (A) is not less than 5000 Theoretical plates.

RESULTS AND DISCUSSION

Analytical method used for Assay of $(DMAPIMP)_2Zn$ 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.

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 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). The % RSD of peak area response for Linearity Level 1 and Level 7 should not be more than 2.0 %. The % RSD of peak retention time for Linearity Level 1 and Level 7 should not be more than 1.0 %.

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

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 $(DMAPIMP)_2Zn$ complex was analyzed in six times for assay.
The assay of the $(DMAPIMP)_2Zn$ 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.

Reproducibility:
A batch of $(DMAPIMP)_2Zn$ 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 $(DMAPIMP)_2Zn$ complex Sample of working level concentration, Standard of $(DMAPIMP)_2Zn$ 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.

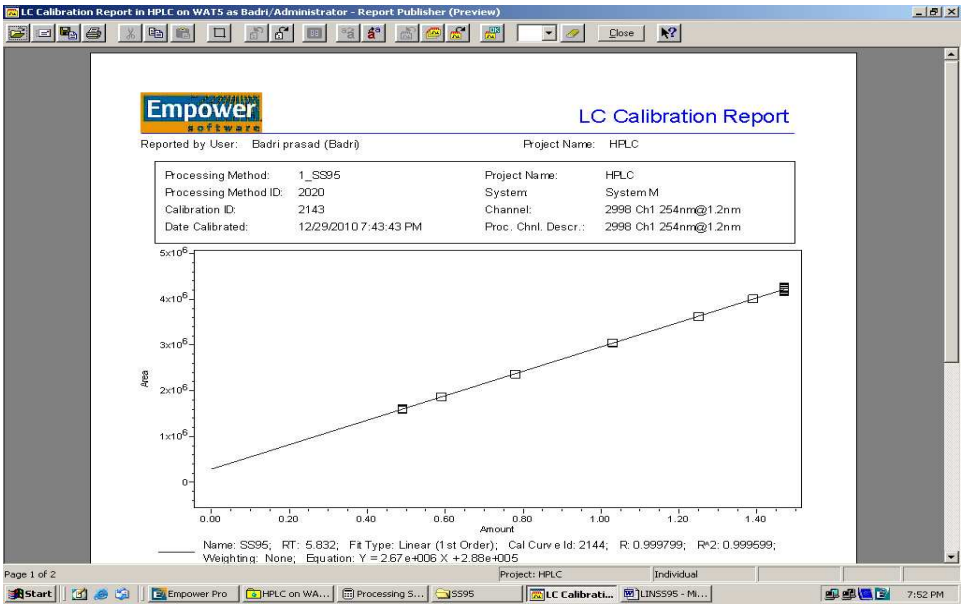
Robustness :

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

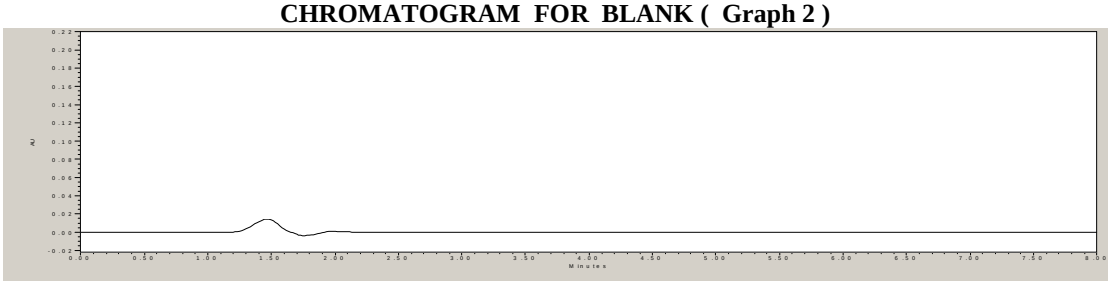
I) Decreasing the flow rate from 1.0 ml/min to 0.8 ml/min.
II) Increasing the flow rate from 1.0 ml/min to 1.2 ml/min
III) Decreasing lower component concentration in mobile phase by 5 %.
IV) Increasing lower component concentration in mobile phase by 5 %.

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.

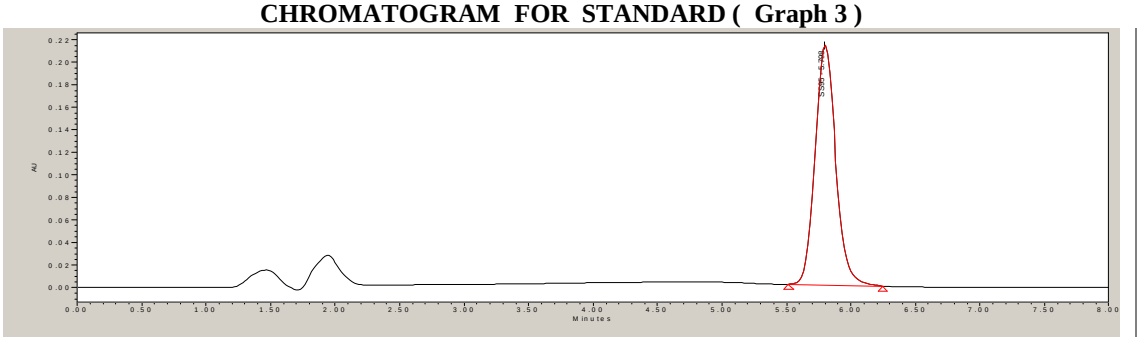
Graphs and Observation : Graph 1



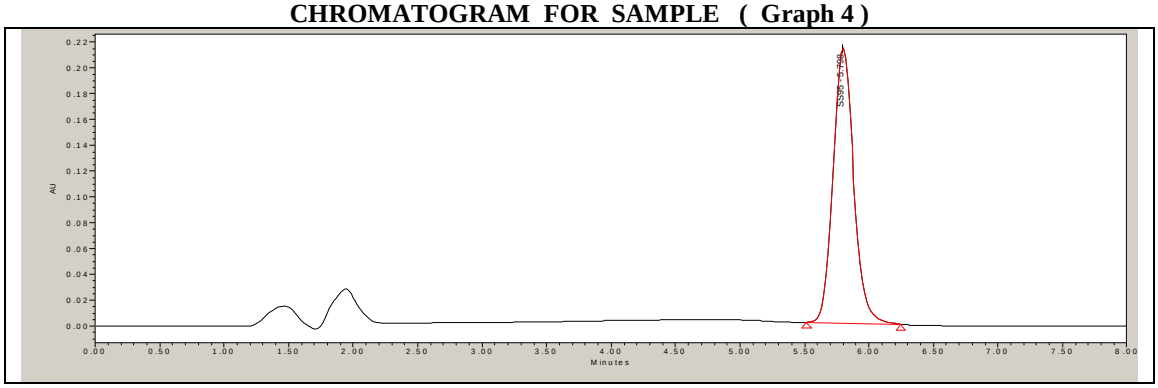
CHROMATOGRAM FOR BLANK (Graph 2)



CHROMATOGRAM FOR STANDARD (Graph 3)



CHROMATOGRAM FOR SAMPLE (Graph 4)



LINEARITY AND RANGE : Table 1

Linearity Level	Conc. of $(DMAPIMP)_2Zn$ complex in ppm	Mean Area of $(DMAPIMP)_2Zn$ complex
Linearity 50%	50	1599125
Linearity 60%	60	1870062
Linearity 100%	80	3048751
Linearity 120%	100	3623380
Linearity 140%	120	4013756
Linearity 150%	140	42190672
% y-Intercept		0.7
Regression Co-efficient		0.9996

Solution Stability :

The solution stability is monitored to check the stability of solution. A sample solution was preserved over a period of Initial, 3,4,6 and 10 hours and analyzed after the specified time intervals. The results of initial analysis and the results of analysis after preservation for assay of $(DMAPIMP)_2Zn$ complex was compared and found to be well within the set limit for solution stability study for $(DMAPIMP)_2Zn$ complex for 10 hours.

As the results of initial analysis and analysis after preservation up to 10 hours are comparable hence the solution is stable up to 10 Hours.

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

System suitability parameters	Specificity/ Linearity and Range/Precision	Intermediate precision	Robustness at Flow rate 0.8 ml/min	Robustness at Flow rate 1.2 ml/min	Robustness at decreasing lower component concentration by 5 %	Robustness at increasing lower component concentration by 5 %	Solution stability (Till 10 Hrs)
Blank interference	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
Column efficiency	6325	8578	7031	8026	8423	8293	6325
Tailing factor	1.14	1.26	1.16	1.32	1.25	1.29	1.14
% RSD Peak area response	1.30	0.70	0.20	0.30	0.30	0.20	1.30
% RSD Retention time	0.16	0.19	0.13	0.06	0.10	0.19	0.16
% RSD Assay	0.68	0.58	0.93	1.22	0.92	0.68	----
Cumulative % RSD Assay	-------	0.66	0.71	0.88	0.93	0.64	0.29

Observation table for Accuracy (Recovery) : Table 3

Accuracy Study	Replicate	Sample Area of $(DMAPIMP)_2Zn$ complex	% Recovery	Average	Std. Dev.	% Recovery	Amount Added in mg	Amount recovered in mg
50 % Accuracy Level I	1	4148597	100.14	98.98	1.03	100.14	152.80	152.58
	2	4279035	98.61			98.61	155.20	157.38
	3	4225568	98.19			98.19	152.60	155.41
100 % Accuracy Level II	1	5380481	101.17	101.22	0.12	101.17	200.20	197.89
	2	5384204	101.35			101.35	200.70	198.02
	3	5406997	101.13			101.13	201.10	198.86
150 % Accuracy Level III	1	6760678	99.90	99.68	1.45	99.90	248.40	248.66
	2	6785672	98.73			98.73	246.40	249.57
	3	6640527	101.01			101.01	246.70	244.23
Mean			99.96					
STD			1.33					
RSD			1.33					

CONCLUSION

The analytical method used for determination of assay of $(DMAPIMP)_2Zn$ 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 regression value is found to be 0.9996 which shows the response, is linear from 0.05 to 0.15 mg/ml. Specificity parameters showed that there is no interference or overlapping of the peaks either due to mobile phase components or diluents with the main peak. Coefficient of correlation is 0.9996 . The percentage RSD for precision is <2 which confirms that method is sufficiently precise and the total run time required for the method is only 7 minutes.

The proposed method is simple, fast, accurate, and precise and can be used for routine analysis in quality control of $(DMAPIMP)_2Zn$ complex .

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