Volume 11, Issue 10, October– 2026 International Journal of Innovative Science and Research Technology
ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1016

Sensitive Level of Detection of Heavy Metals by Using PDA Detector in API and Formulation of Simvastatin

Dr. R. K. Sangeetha[1]; S. Shimna[2]; K. Hifathulla[3]; A. Rohith Kannan[3]

[1]Associate Professor, Department of Pharmaceutical Analysis, Sri Ramakrishna Institute of Paramedical Science, College of Pharmacy, Coimbatore-641044
[2;3]Master of Pharmacy, Department of Pharmaceutical Analysis, Sri Ramakrishna Institute of Paramedical Science, College of Pharmacy, Coimbatore-641044

Publication Date: 2026/10/27

Abstract: A simple, rapid and sensitive ultra-fast liquid chromatographic technique was developed for the determination of heavy metals in pharmaceutical dosage form. Lead and copper were detected and quantified at µg level in simvastatin, a pharmaceutical product used in the treatment of lowering cholesterol level in body. Calcium disodium edetate was used as the chelating agent. The chromatographic analysis was done on a C18 column (250 × 4.6 mm, 5 µm) with a mobile phase of water: organic solvents (50 % acetonitrile: 50 % methanol) in the ratio of 15:85 V/V, with a flow rate of 1.0 mL min-1 at detection wavelength of 237 nm and the retention time was found to be 8.423 minutes for simvastatin. Calibration curve was linear from 1-5 µg mL-1 with correlation coefficient of 0.998 respectively. The developed method was applied for the analysis of the spiked metal ions in formulation and satisfactory results were achieved.

Keywords: *UFLC-PDA, Method Validation, Simvastatin, Lead Nitrate, Copper Sulphate, ICH Guidelines.*

How to Cite: Dr. R. K. Sangeetha; S. Shimna; K. Hifathulla; A. Rohith Kannan (2026) Sensitive Level of Detection of Heavy Metals by Using PDA Detector in API and Formulation of Simvastatin. *International Journal of Innovative Science and Research Technology*, 11(10), 1864-1872. https://doi.org/10.38124/ijisrt/26oct1016

I. INTRODUCTION

Heavy metal toxicity is serious threat that carries numerous kinds of health risk factors. Their toxicity depends on several factors including the dose, route of exposure, and duration of exposure of heavy metals and it is naturally occurring elements that have a high atomic weight and density above 5 gm per cm3 and adversely affect the environment and living organism (1,2).

Lead is naturally occurring in the environment and also has wide application in industrial agricultural and domestic areas the exposure of lead mainly occurs through inhalation of lead contaminated dust particles or aerosols and ingestion of lead contaminated food, water. Lead poisoning is mostly related to the gastrointestinal tract and central nervous system by inhibiting the N-methyl-D-aspartate receptor (NMDAR) and can block neurotransmission by inhibit neurotransmitter release (3,4).

Copper is an essential nutrient that is incorporated into a number of metalloenzymes involved in haemoglobin formation, carbohydrate metabolism, catecholamine biosynthesis, and hair keratin. However, copper also makes it potentially toxic because the transition between Cu (II) and Cu(I) can result in the generation of superoxide and hydroxyl radicals. Copper has been linked to cellular damage leading to Wilson disease in humans (5,6).

These metals are essential to maintain various biochemical and physiological function in living organism when in very low concentration, However, they become noxious when they exceed certain threshold concentrations.

Calcium Disodium Versenate (edetate calcium disodium injections, USP) is a sterile, injectable, chelating agent in concentrated solution for intravenous infusion or intramuscular injection. Each 2.5 mL ampul contains 500 mg of edetate calcium disodium (equivalent to 200 mg mL-1) in water for injection. chemically [[N,N′-1,2-ethanediyl-bis[N-(carboxymethyl)-glycinato]](4-)N,N′, O,O′,ON,ON′]-,disodium,hydrate,(OC-6-21)-Calciate(2-) with a molecular formula of C10H12CaN2O8•xH2O and molecular weight 374.27 (anhydrous). The pharmacological effect of edetate calcium disodium is due

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1016

to the formation of chelates with divalent and trivalent metals used in treatment of lead poisoning7-9.

Simvastatin is one the well-known HMG-CoA reductase inhibitor belonging to the class of statins shown in Figure 1. It acts by inhibiting HMG-CoA reductase, a rate limiting enzyme in the synthesis of cholesterol in liver and used for the treatment of dyslipidemia and the prevention of cardiovascular diseases.

It is chemically known as (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxotetrahydro-2H-pyran-2yl] ethyl }-3,7-dimethyl-1,2,3,7,8,8a-hexahydro naphthaalene-1-yl-2,2-dimethylbutanoate (Fig.1.). A HMG-CoA reductase (3-hydroxy-3-methylglutarylcoenzyme), simvastatin is prodrug which is converted into its β-hydroxy form which inhibits HMG-CoA Reductase enzyme, a rate limiting enzyme in the synthesis of cholesterol in liver (10-11).

The literature survey reveals that established method for determination of simvastatin as individual and combination drugs but there is no specific method for the determination of heavy metal complexes in simvastatin and its formulations.

The aim of the work is heavy metal complex with the selected chelating agent (calcium disodium versenate) to determine the heavy metals in the drug simvastatin and its formulation by spiking the complexes of heavy metals. Hence the research work can be used to determine the simvastatin bulk drugs and detect the presence of heavy metal in the tablet formulation in the selected sensitive range of concentration12-15.

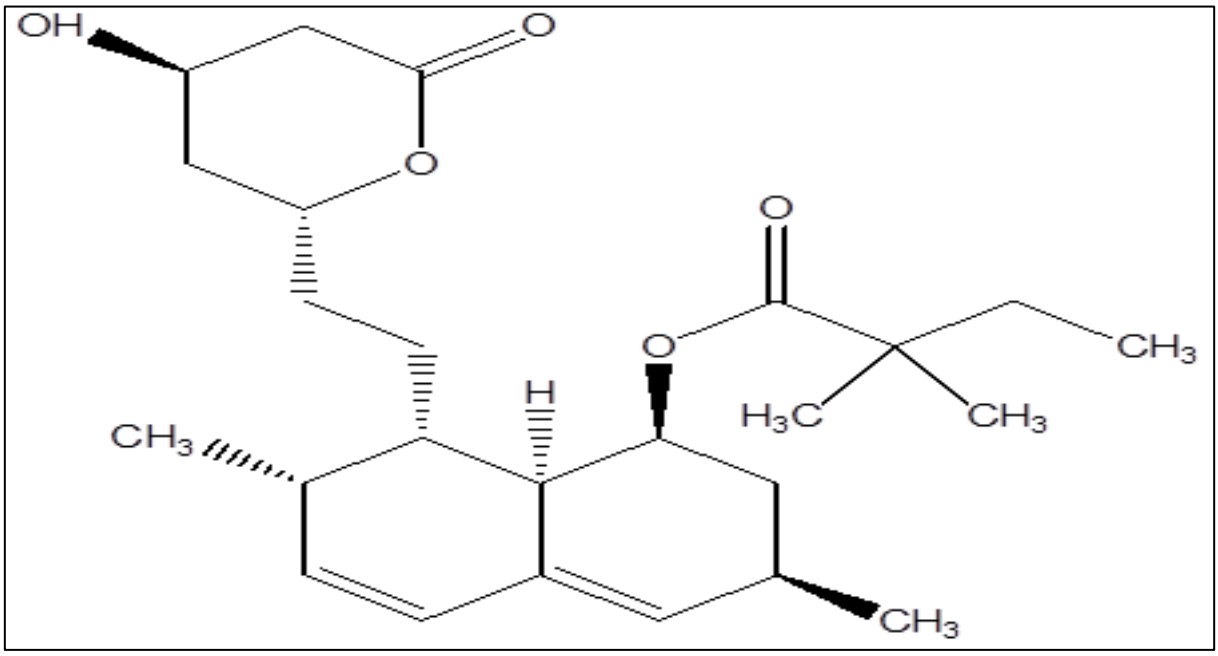


Fig 1 Chemical Structure of Simvastatin.

II. MATERIALS AND INSTRUMENTS

➢ *Materials*

Reference standard of simvastatin was purchased from Yarrowchem, Mumbai, India. The Pharmaceutical formulation Simvotin 10 and 20 mg sun pharmaceutical Ind. Ltd., was purchased from local market. UFLC grade solvents water was procured from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India and methanol was procured from S d. Fine chemical Ltd., India and Acetonitrile was procured from Himedia Laboratories Pvt. Ltd., India. Heavy metals lead nitrate and copper sulphate was purchased from S d. Fine chemicals Ltd., India.

- *Instrument*

The chromatographic separation attained by Shimadzu Prominence UFLC (shimadzu corporation, Kyoto, Japan) equipped with LC-20 AD pump, SPD-M40A diode array detector with stationary phase Shim-pack solar C18 (250 × 4.6 mm, 5µm particle size) Chromatograms were recorded and integrated on PC installed with Lab solution software, version 5.97 SP1. Spectrophotometric analysis was performed by using Jasco V-630 UV/Vis – spectrophotometer (Jasco corporation, Tokyo, Japan).

➢ *Methods*

- *Selection of Solvent*

The simvastatin was freely soluble in methanol and further dilution carried out by using the same. The chelating agent calcium disodium versenate and salts of heavy metals such as lead and copper completely soluble in water. Hence water was selected to prepare a stock solution and further dilution were carried out with methanol.

- *Selection of Wavelength*

The suitable wavelength for the UV analysis was determined by recording the spectrum of simvastatin in the range of 200-400 nm. The wavelength at which the

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1016

absorbance was found to be maximum was chosen for the study. The wavelength of 237 nm was selected for the study.

- *Preparation of Stock Solutions*

The stock solution of 1000 µg mL-1 of simvastatin and chelating agent was prepared separately by using methanol as solvent. The stock solution of lead nitrate and copper sulphate was prepared individually with water to get a concentration of 1000 µg mL-1.

- *Preparation of Complex for Lead Nitrate & Copper Sulphate with Calcium Disodium Versenate*

1 mL of calcium disodium versenate was pipetted out from the stock concentration of 100 µg mL-1 and 1 mL of lead nitrate & copper sulphate was pipetted out form the stock concentration of 10 µg mL-1 and finally made up with to 10 mL with methanol to get concentration of 10 µg mL-1 of chelating agent and 1 µg mL-1 of lead nitrate & copper sulphate to form complex for 30 minutes as separate solutions.

- *Method Validation 18*

After achieving the optimal chromatographic separation, the method was subjected to validation process in order to prove its reliability and suitability for its intended use. The proposed method was validated as per ICH guidelines Q2 (R1).

- *System Suitability*

System suitability performance like retention time, number of theoretical plates, tailing factor were calculated by injecting standard solution six times. The results were compared with the standard limits as per the guidelines.

- *Specificity*

Specificity is the ability of a method to discriminate between the analyte of interest and other components that are present in the sample. These studies are performed to check the interference in the optimized method.

- *Linearity*

From standard stock solution, a working standard solution was prepared and simvastatin were found to be linear in the range of 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5 µg mL-1 respectively. The calibration curve was plotted by using peak area Vs concentration of the standard solution. From the calibration curve, the slope and intercept were calculated.

- *Accuracy*

Accuracy of the method was determined by recovery experiments. The accuracy of the method was evaluated in triplicate at two levels (50 % and 100 %) and the recovery (%) was calculated the difference between the amount of analyte present in pre-analysed formulation to that of the simvastatin added to the formulation.

- *Precision*

Precision of the method was determined by performing repeatability studies the intraday and interday precision of the method was studied by analyzing the response of simvastatin at concentration level of 3 µg mL-1 during different day (interday precision) within the same day and (intraday precision) and their % RSD values were calculated.

- *Limit of Detection (LOD) and Limit of Quantification (LOQ)*

The LOD and LOQ of simvastatin was estimated by using statistical equation for LOD $3.3 \times \sigma/S$ and LOQ using $10 \times \sigma/S$, where σ is the standard deviation of intercept and S is the slope of the calibration curve.

- *Robustness*

The robustness of the RP-UFLC technique was assessed by examining sample under experimentally changed chromatographic circumstances. Like mobile phase ratio, flow rate, and wavelength are modified the deviation among the results obtained is well within the limits.

- *Assay of Marketed Formulation*

Twenty tablets weighed accurately and powdered. Powder equivalent to 10 mg of simvastatin was accurately weighed and transferred to a 25 mL volumetric flask, dissolving using methanol and sonicate for 20 minutes. The solution was filtered using whatmann (No.1) filter paper. from the filtered solution further dilution was carried out to get final concentration of 4 µg mL-1 of simvastatin using methanol as diluent.

➢ *Linearity of Heavy Metal Complexes*

- *Linearity of Lead Nitrate & Copper Sulphate*

Linearity of lead nitrate & copper sulphate was performed individually by taking from working standard solution which was found to be linear in the range of 0.5-3 µg mL-1 respectively. The calibration curve was plotted by using peak area versus concentration of standard solution. From the calibration curve, the slope and intercept were calculated.

- *Study of Spiked Heavy Metals in the Formulations*

The assay formulation of two dosage form of 10 and 20 mg tablets were checked for the presence of lead and copper in the selected range. There was no peak found for the heavy metals. So, the concentration of 1 µg mL-1 of lead complex & copper complex were spiked to a tablet solution in the concentration of 4 µg mL-1 and chromatogram was recorded. The peak eluted were compared with the standard solution peak area and calculated.

III. RESULTS AND DISCUSSION

➢ *Selection of Detection Wavelength*

The standard solution of 10 µg mL-1 simvastatin in methanol was scanned between 200-400 nm and based on the wavelength selected based on the maximum absorbance and good response of the drug in the detector. The wavelength selected for simvastatin was 237 nm is shown in Figure 2.

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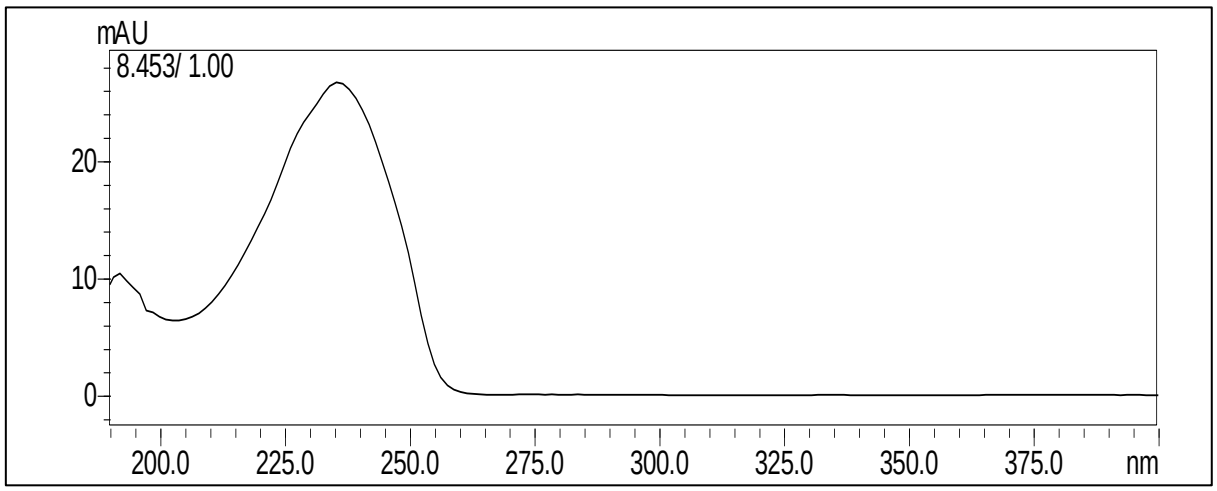


Fig 2 UV Spectrum of Simvastatin.

➢ *Optimized Chromatographic Conditions*

The results for the optimized chromatographic condition with representative chromatogram is shown in Fig. 3.

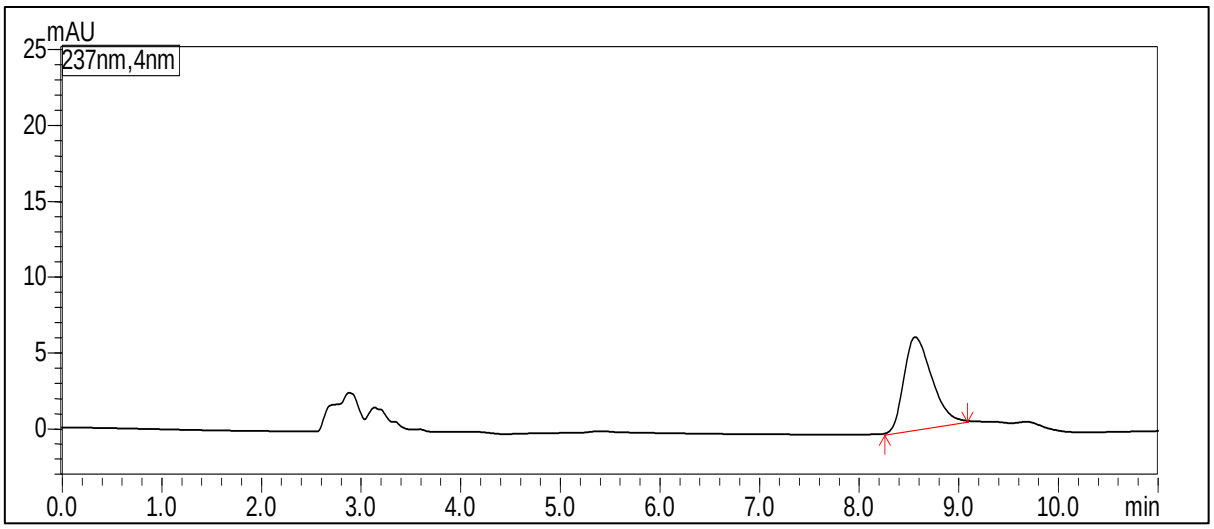


Fig 3 Representative Chromatogram of Simvastatin.

➢ *Method Validation*

- *System Suitability*

System suitability was performed by injecting six replicate injection of 100 % test concentration, number of theoretical plates, asymmetrical factor was satisfactory. The chromatograms confirm the presence of simvastatin at 8.4 minutes without any interference. The results of system suitability are shown in the Table 1.

Table 1 System Suitability Study Data.

Compound	% RSD of Peak area	No. of theoretical plates	Tailing factor
Simvastatin	0.45	4706	1.217

- *Specificity*

Specificity of proposed UFLC method was performed and we did not find any interfering peaks at the retention time of simvastatin which shows that the method is specific.

- *Linearity*

Simvastatin was found to be linear in the range of 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5 µg mL^{-1} respectively. The correlation coefficient of simvastatin was found to be 0.998 respectively. The peak area of each concentration is given in the Table 2 and calibration graph shown in Figure 4.

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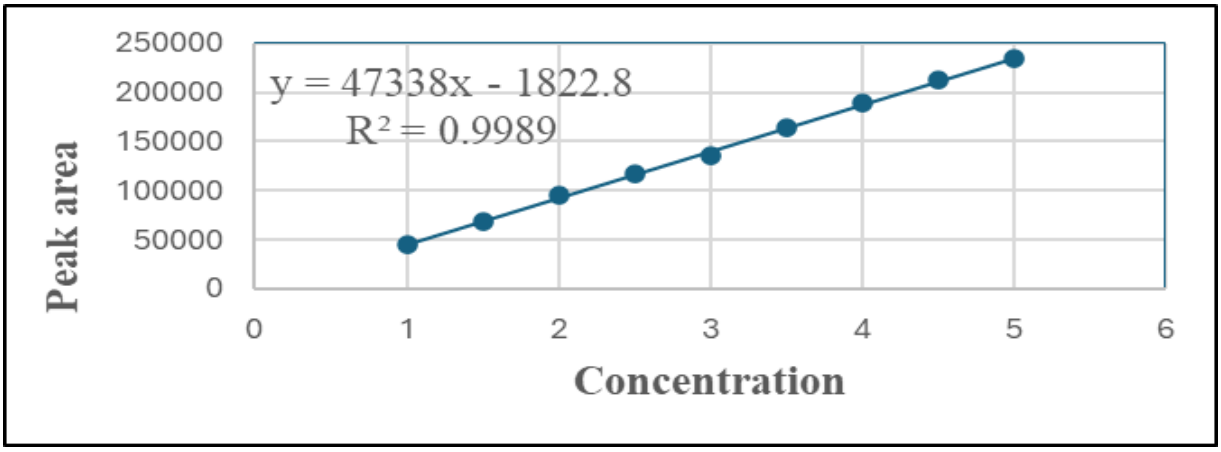


Fig 4 Calibration Graph of Simvastatin.

Table 2 Linearity of Simvastatin.

Concentration µg mL^{-1}	Peak area
1	45468
1.5	68794
2	95561
2.5	116852
3	135372
3.5	163533
4	189530
4.5	212654

- *Accuracy*

The accuracy of the method was determined by recovery experiments. A known quantity of the pure drug was added to the pre-analyzed sample formulation of 50 %, 100 % & 150 % levels. The recovery studies were carried out three time of each level and the percentage recovery and % RSD was calculated. The % recovery of simvastatin was found to be in the limit of 98-102 % as per the ICH guidelines. The % recovery values are given the Table 3.

Table 3 Accuracy Studies Data of 10 mg and 20 mg Simvastatin.

Drug	% Amount of standard drug added	% Drug recovered	% RSD
Simvotin 10 mg	50	99.5	1.32
	100	99.1	0.41
	150	100.2	0.26
Simvotin 20 mg	50	100.3	0.76
	100	99.4	0.38
	150	100.7	0.26

- *Precision*

The Precision of the method was determined by studying reproducibility and repeatability. The peak areas of drug and % RSD of intraday and interday was calculated and it was found to be within the limit of NMT 2 % as per the ICH guidelines. The intraday and interday precision study of simvastatin values are given in the Table 4 & 5.

Table 4 Intraday and Inter Precision Data of Simvastatin.

Concentration µg mL^{-1}	Peak area	
	Intra day	Inter day
3 µg mL^{-1} of simvastatin	137472	137472
	137633	137510
	137162	137238
	137389	137638
	137295	137391
	137578	137562
Mean	**137421.5**	**137468.5**
Standard deviation	**176.7334151**	**140.2451425**
% RSD	**0.12**	**0.10**

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- *Repeatability*

Table 5 Repeatability of Sample Injection.

Repeatability of injection	Simvastatin
RP – 1	137570
RP – 2	137121
RP – 3	137591
RP – 4	137440
RP – 5	137269
RP – 6	137728
Mean	**137453.16**
Standard deviation	**224.769**
% RSD	**0.16**

- *Limit of Detection and Limit of Quantification*

The LOD value of simvastatin was found to be 0.41 µg mL^{-1} and LOQ value of simvastatin were found to be 1 µg mL^{-1} respectively.

- *Robustness*

The robustness of the method was determined as per ICH guidelines under different conditions including change in Mobile phase, wavelength, and flow rate. The results of all the robustness parameters were verified and it was found that there was no significant impact of the method variations on the results. The results are summarized in Table 6.

Table 6 Robustness Data of Simvastatin.

Parameters	Factor	Simvastatin	
		Peak area	%RSD
Flow rate: (± 0.1)	0.9 mL/min	136985	**0.41**
	1.1mL/min	137499	**0.46**
Mobile phase Ratio (± 1)	16:84	137464	**0.37**
	14:86	139160	**0.46**
Detection wavelength (± 1 nm)	236 nm	131659	**0.28**
	238 nm	133557	**0.29**

- *Assay of Marketed Formulation*

Sample solution was injected, chromatogram was recorded and the quantification was done by regression equation and it was found that the assay results of Simvastatin are within the standard limits.

➢ *Determination of Heavy Metal Complexes*

- *UV Spectrum of Heavy Metal Complexes*

The solution of heavy metal complexes of lead nitrate and copper sulphate in the concentration of 10 µg mL^{-1} were prepared individually and scanned in the UV range of 200-400 nm. The wavelength of maximum absorbance for lead nitrate was found to be 241 nm and copper sulphate was found to be 247 nm. The corresponding spectrum of heavy metal complexes are shown in Fig. 5 and 6 respectively.

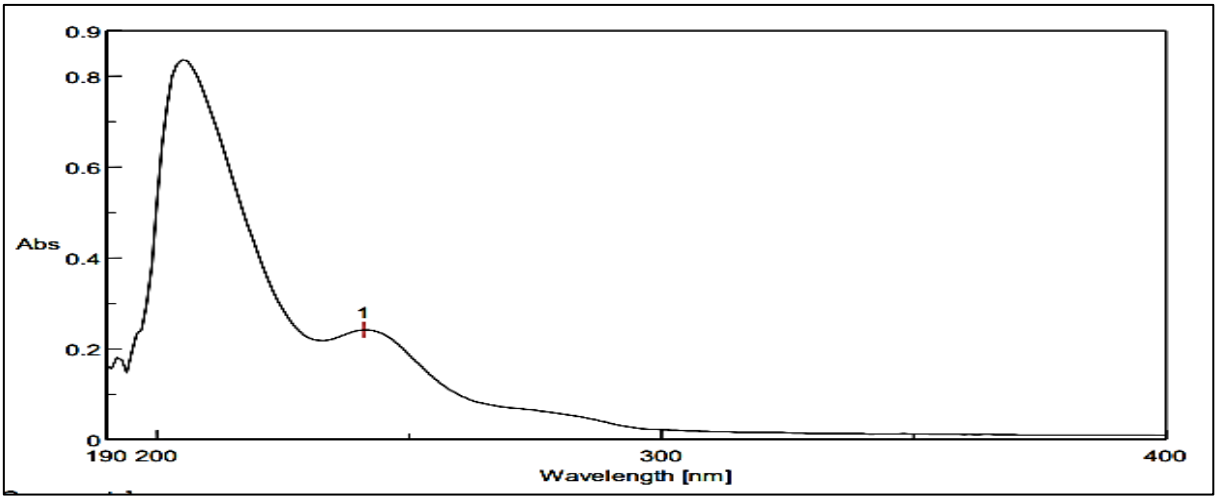


Fig 5 UV Spectrum of Lead Nitrate Complex.

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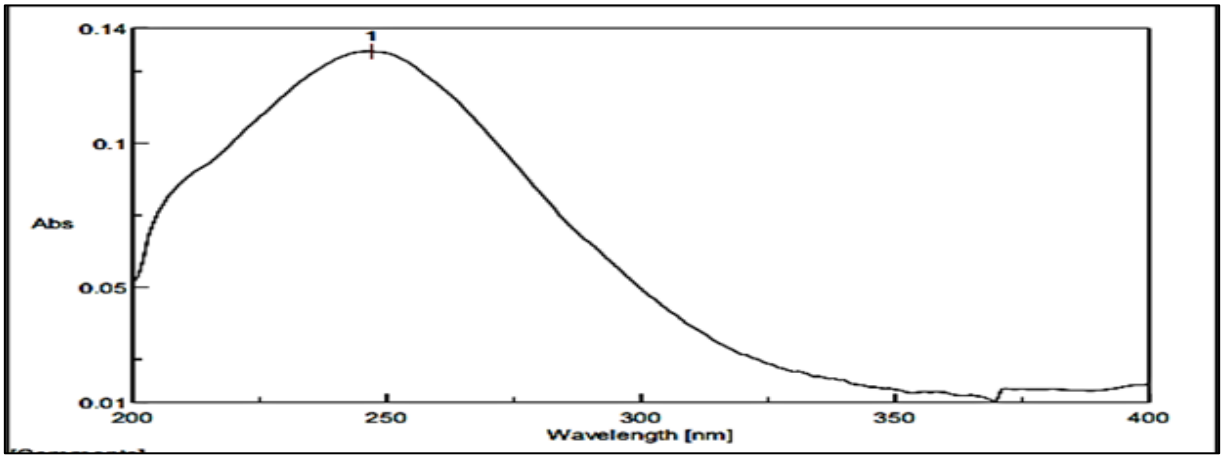


Fig 6 UV Spectrum of Copper Sulphate Complex.

- *Linearity of Lead Nitrate and Copper Sulphate Complex*

Lead nitrate & Copper sulphate complex were found to be linear in the range of 0.5-3 µg mL-1 respectively. The calibration curves were plotted as peak area versus concentration of the standard solution with correlation coefficient of 0.998 and 0.999. The respective chromatograms are shown in Fig. 7 & 8 and the results are summarized in Table 7 and 8.

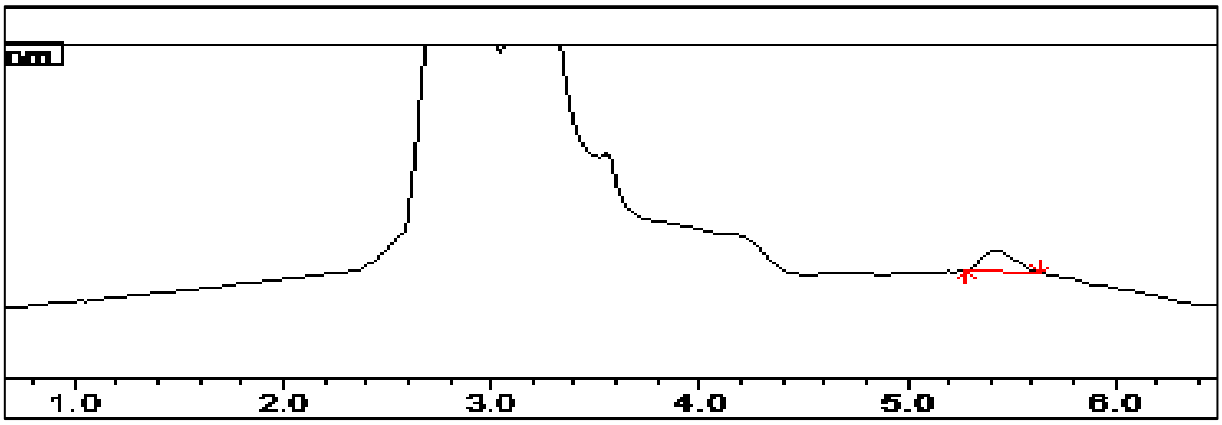


Fig 7 Representative Chromatogram of 0.5 µg mL 1 of Lead Nitrate Complex.

Table 7 Linearity of Lead Nitrate Complex.

Concentration µg mL^{-1}	Peak area
0.5	643
1	846
1.5	993
2	1168
2.5	1347
3	1525

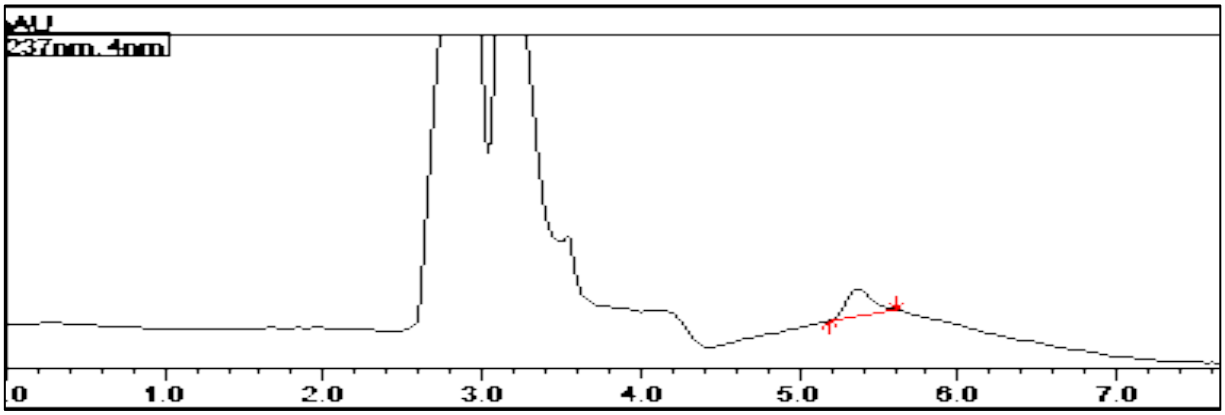


Fig 8 Representative Chromatogram of 0.5 µg mL 1 of Copper Sulphate Complex.

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Table 8 Linearity of Copper Sulphate Complex.

Concentration µg mL^{-1}	Peak area
0.5	821
1	1032
1.5	1257
2	1459
2.5	1623
3	1854

- *Simvastatin Spiked with Lead Nitrate Complex*

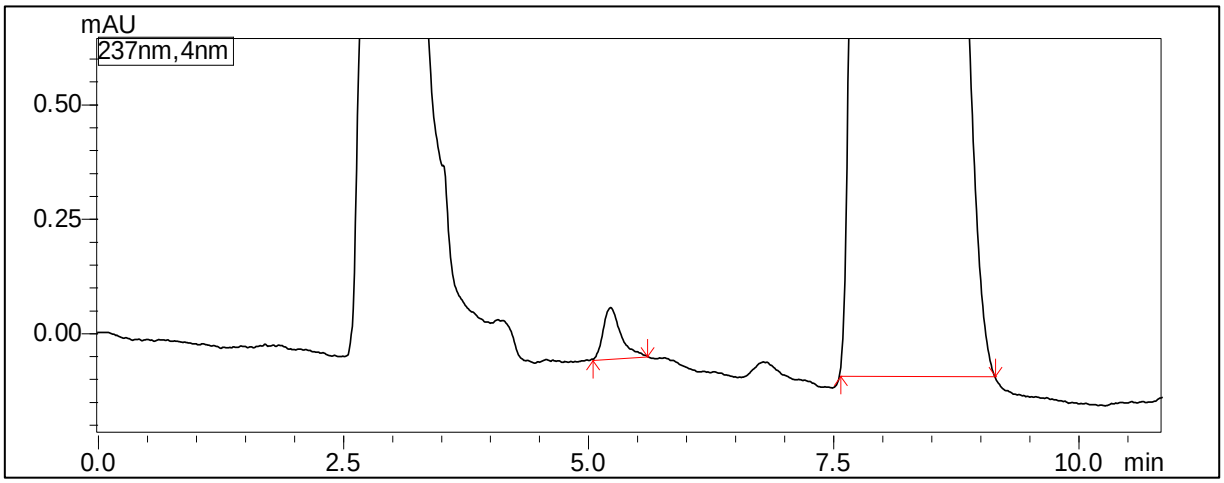


Fig 9 Chromatogram of Simvastatin Spiked with Lead Nitrate Complex.

- *Simvastatin Spiked with Copper Sulphate Complex*

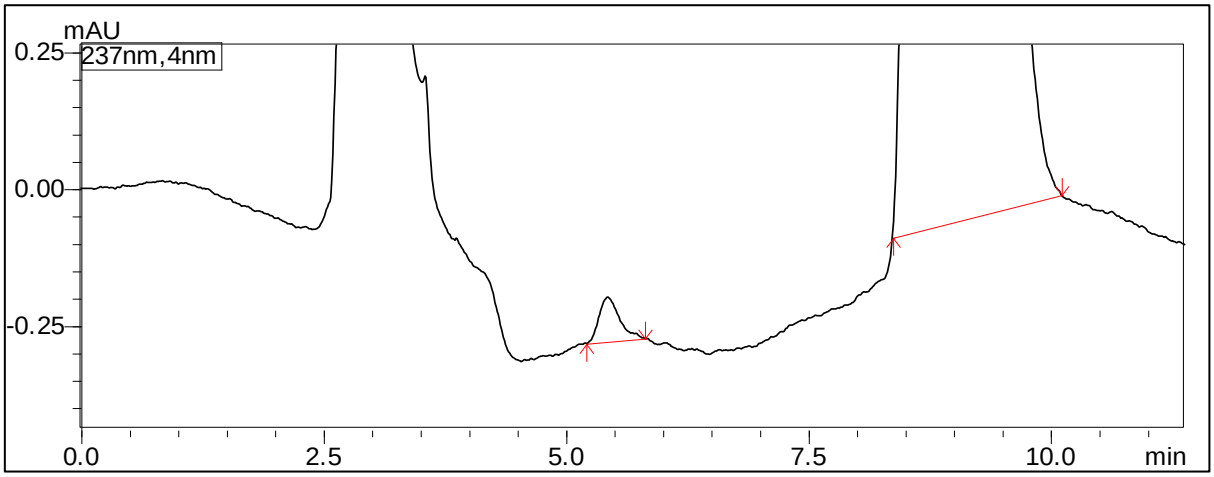


Fig 10 Chromatogram of Simvastatin Spiked with Copper Sulphate Complex.

➢ *System Suitability (Chromatographic Performance)*

USP defines parameters that can be used to determine system suitability before analysis. These parameters include plate number (N), tailing factor and relative standard deviation (RSD) of peak area or peak height for repetitive injection.

The Copper Sulphate and Lead Nitrate gave good peak shape within a run time of 10min. the number of theoretical plates and tailing factor were found to be within the limits using the developed chromatographic conditions.

IV. CONCLUSION

The present study was developed and validated to determine the heavy metal complexes in the simvastatin formulation. Study of heavy metal complex with the selected chelating agent (calcium disodium versenate) is not reported till date by UFLC method. It is sensitive technique for the determination of the heavy metal as complexes incorporated with PDA detector for the specificity and selectivity of selected heavy metals. The proposed method was found to be sensitive, accurate, precise, robust and specific. Hence, we have focused on the estimation of heavy metal in the drug simvastatin and its formulation by spiking the complex of

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26oct1016

heavy metals and it can be used to determine the simvastatin bulk drugs and detect the presence of heavy metal in the tablet formulation in the selected sensitive range of concentration.

ACKNOWLEDGEMENT

The authors are grateful to the SNR Sons Charitable Trust, Coimbatore for providing the necessary facilities to carry out the research work.

REFERENCES

[1]. Tchounwou PB., Yedjou CG., Patlolla AK., Sutton DJ.: Heavy metal toxicity and the environment. Exp Suppl. 2012, 101:133-64.
[2]. Engwa GA., Ferdinand PU., Nwalo FN., Unachukwu MN.: Mechanism and health effects of heavy metal toxicity in humans. Poisoning in the modern world-new tricks for an old dog. 2019, 19(6), 10:70-90.
[3]. U.S. National Library of Medicines, National center for biotechnology information, Available at https://pubchem.ncbi.nlm.nih.gov/compound/Lead-nitrate. Access date- 05 Apr 2023.
[4]. Balali-Mood M., Naseri K., Tahergorabi Z., Khazdair MR., Sadeghi M.: Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. Front Pharmacol. 2021,13(4), 12:643972.
[5]. U.S. National Library of Medicines, National center for biotechnology information, Available at https://pubchem.ncbi.nlm.nih.gov/compound/Copper-Sulfate. Access date-05 Apr 2023.
[6]. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012-. Copper. [Updated 2017 Oct 30]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK548090/
[7]. U.S. National Library of Medicines, National center for biotechnology information, Available at https://pubchem.ncbi.nlm.nih.gov/compound/Calcium-disodium-edetate. Access Date- 05 Apr 2023.
[8]. https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/008922s016lbl.pdf. Access date- 05 Apr 2023.
[9]. Flora SJ., Pachauri V.: Chelation in metal intoxication. Int. J. Environ. Res. Public Health., 2010,7(7), 2745-88.
[10]. U.S. National Library of Medicines, National center for biotechnology information, Available at https://pubchem.ncbi.nlm.nih.gov/compound/Simvastatin. Access date- 05 Apr 2023.
[11]. Talreja O., Kerndt CC., Cassagnol M.: Simvastatin, [Updated 2022 Jun 6], In: StatPearls [Internet], Treasure Island (FL), StatPearls Publishing, 2023 Jan, Available from: https://www.ncbi.nlm.nih.gov/books/NBK532919/
[12]. Birari AE.: Development and Validation of UV-Spectrophotometric Method for Estimation of Simvastatin in Bulk and Solid Dosage Form. Int. J. of Pharma Sci., 2015, 6(1), 185-89.
[13]. Munir A., Ahmad M., Malik MZ., Minhas MU.: Analysis of simvastatin using a simple and fast high-performance liquid chromatography-ultra violet method development, validation and application in solubility studies. Tropical Journal of Pharmaceutical Research., 2014, 13(2), 135-9.
[14]. Chavhan V., Ghante M., Sawant S.: Development and validation of RP-HPLC method for simultaneous estimation of sitagliptin phosphate and simvastatin in bulk and tablet dosage form. J App Pharm., 2014, 6(3), 327-38.
[15]. Sangeetha.R.K.: Study of Chelate Complex of Heavy Metals with Calcium Disodium Versenate and Methanolic Extract of Carica Papaya Leaves by UV Spectroscopy. IAR J Pharm., 2021, 30(4), 10-15.
[16]. Sethi PD., Sethi's HPLC: High-Performance Liquid Chromatography: Quantitative Analysis of Pharmaceutical Formulations. CBS; 2010. pp.17-19.
[17]. Chatwal G., and Anand S.: Instrumental method of Chemical Analysis: (analytical chemistry), 3rd edition; ISBN No.:978-93-5142-088-0, Himalaya Publishing House, New Delhi, 2016, pp.2624-2639.
[18]. Validation of Analytical Procedures: Text and methodology Q2 (R1), ICH Harmonized Tripartite Guidelines, 2005, 1-12.