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VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LOVASTATIN AND OLEANOLIC ACID IN ANTILIPIDEMIC TABLETS

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ABSTRACT

In the modern era, rapid high-performance liquid chromatography (HPLC) is the most effective system for the separation of active pharmaceutical ingredients for pharmaceutical analysis due to its extremely effective separation with high sensitivity and accuracy. Establishing and validating an analytical technique using HPLC is essential for upholding the quality of products, as it gives data on accuracy, linearity, precision, and detection. This study focused on developing and validating an HPLC technique for effective evaluation of lovastatin (LV) and oleanolic acid (OA) in core and coat antilipidemic tablets formulated. RP-HPLC process for concurrent assessment of lovastatin also oleanolic acid was performed by means of a Jasco HPLC scheme in addition SunQSil C18 4.6 × 250 mm column (5 µm). The mobile phase utilized for the proposed method was Methanol as well as Water (90:10 v/v), the mobile phase was adjusted to pH 3 using orthophosphoric acid, with a flow rate of 1 mL per min, also finding of both drugs was performed at 210 nm. The validation of the proposed method was carried out in accordance with ICH Q2 (R1) guidelines. Retention time of Lovastatin, in addition to Oleanolic acid, was identified as 4.41 also 6.43 min, respectively. Linearity for Lovastatin also Oleanolic acid was measured in the interval of 2-12 µg/mL with mean percent recovery of 99.99% and 99.82%, respectively. The method demonstrated satisfactory precision, as the percentage relative standard deviation values for both intra- and inter-day precision were below 2%. The proposed simultaneous estimation technique presented exceptional precision, accuracy, linearity, as well as robustness. This simple technique may effectively be applied for the simultaneous estimation of LV as well as OA in therapeutic dosage form. This contributes significantly to ensuring the safety and efficacy of antilipidemic drugs, supporting broader public health goals.

Keywords: Lovastatin, Oleanolic Acid, RP-HPLC, Simultaneous Estimation, Validation, Antilipidemic Tablets.
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INTRODUCTION

With over 18 million deaths each year, cardiovascular diseases are the leading cause of mortality worldwide. Lipid-lowering agents, such as statins and natural compounds, play a vital role in prevention and management. Their therapeutic success relies heavily on accurate dosing and purity, highlighting the need for reliable analytical methods to ensure quality control and patient safety.[1] This study supports Sustainable Development Goal 3, which focuses on promoting health and well-being for people of all ages. By developing and validating a robust RP-HPLC method for the simultaneous estimation of lovastatin and oleanolic acid, this research contributes to the safe, effective, and affordable use of essential medicines in the fight against non-communicable diseases. Lovastatin (LV), a natural statin, is a secondary metabolite produced by Aspergillus terreus and Monascus ruber in the 1970s.[2] Lovastatin (LV) is chemically "(1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a hexahydronaphthalen-1-yl (2S)-2-methylbutanoate" as shown in Fig.-1a. LV decreases lipid levels by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase; thus, it acts as a potent cholesterol-lowering drug. The[3] Key regulatory steps in cholesterol biosynthesis are catalyzed by this enzyme in the liver (Mevalonate pathway). Also, LV reduces low-density lipoprotein and triglyceride levels and raises the level of high-density lipoprotein.[4] It has been confirmed that in its therapeutic use, LV is also valuable in the treatment of vital diseases such as arterial sclerosis, septicemia, peripheral arterial disease, strokes, and bone fractures.[5] As a biologically active compound, oleanolic acid (OA) is a pentacyclic triterpenoid of the oleanane type, generally present in nearly 200 different varieties of plants.[6]

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As per the research, oleanolic acid has various pharmacological actions like hepatoprotective, antioxidation, lipid balance, anticancer, and anti-inflammatory.[7] Chemically Oleanolic acid is "(4aS,6aS,6bR,8aR,10S,12aR,12bR,14bS)-10-hydroxy-2,2,6a,6b,9,9,12a-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-4a-carboxylic acid" (Fig.-1b).[8] OA is a specific inhibitor of Intestinal acyl-CoA:cholesterolacyl transferase (ACAT), is intracellular enzyme involved in the cholesterol absorption process from the small intestine and converts into cholesteryl esters, which are transferred to the liver. The accumulation of cholesteryl ester in macrophages leads to the formation of foamy cells and which is nothing but the hallmark of the initial stage of atherosclerosis.[9,10] Cholesterol absorption at the small intestine is reduced by OA, also it reduces plasma cholesterol and triglyceride levels by retarding absorption and retention of metabolic fatty acid, decreases VLDL synthesis in liver that can block deposition of lipids in blood vessels, thus reducing the possibility of heart attacks.[11] Elevated levels of cholesterol and triglycerides raise the risk of cardiac diseases, such as cardiovascular disease, as well as myocardial infarction. By inhibiting ACAT, oleanolic acid acts as an effective and safe agent in the treatment of diseases caused due to increased levels of lipids.[12] Co-administration of LV and OA in fixed doses, respectively, reduces the cholesterol levels that are synthesized by the liver and absorbed from the diet.[13,14]

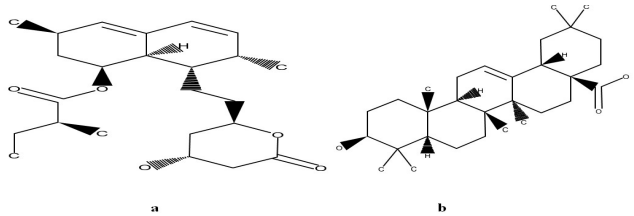

Fig.-1: Chemical Structures of (a) Lovastatin, (b) Oleanolic Acid

This study aims to develop and validate a straightforward, reliable, and precise RP-HPLC method for the concurrent estimation of lovastatin and oleanolic acid in combined antilipidemic tablet formulations. Despite their pharmacological significance, no validated method currently exists for their simultaneous quantification in a single dosage form, highlighting a crucial gap in analytical methodology for combination therapies.

EXPERIMENTAL

Materials and Methods

Pharmaceutically pure gift sample of Lovastatin was provided by Lupin Pharmaceutical Ltd, Mumbai, and Oleanolic acid was procured from Shri Samartha Enterprises, Pune. Chemicals, as well as solvents used, were of analytical and HPLC grade. The HPLC setup included a mobile phase delivery pump (PU 2080 Plus Intelligent), a JASCO UV-2075 UV-VIS detector, a Rheodyne sample injection port with a 20 µl loop, also Borwin chromatography software (Version 1.50). A C-18 reverse-phase column (SunQSil250 × 4.6, 5 µm) was used for evaluation and separation of Lovastatin (LV) and Oleanolic acid (OA) in LV-OA mixture. For the proposed method, the mobile phase comprised methanol in addition to water (90:10 v/v), with the adjustment of pH to 3. The system operated with a maintained flow rate of 1 mL/min also the finding of both drugs occurred at 210 nm, with the column temperature set to 30°C.

Preparations of Standard Stock Solutions

A Lovastatin stock solution (1000 µg/mL) was prepared by dissolving 10 mg of the drug in 10 mL of methanol, yielding a final concentration of 1000 µg per mL. Similarly, to obtain a final concentration of 1000 µg per mL, 10 mL of methanol was used to dissolve 10 mg of Oleanolic acid to prepare a standard stock solution (1000 µg per mL). A 1 mL aliquot of the prepared standard stock solution was transferred and diluted with methanol to a total volume of 10 mL, resulting in final concentrations of 100 µg/mL for both Oleanolic acid and Lovastatin.

Preparation of Working Stock Solution

For the construction of a linearity curve, starting with the standard stock solution (100 µg/mL), the stock solutions were formulated by adding a suitable volume of mobile phase to form a linearity range of 2-12 µg per mL for Oleanolic acid as well as Lovastatin, each.

Analytical Discussion

The analytical method was designed and validated in line with ICH guidelines, evaluating critical parameters including specificity, system suitability, linearity, accuracy, precision, robustness, as well as the limits of detection (LOD) and quantification (LOQ). Specificity was confirmed by injecting standard, sample, placebo, and blank solutions to ensure clear separation of analyte peaks from other matrix components. System suitability tests demonstrated that the entire analytical setup, including equipment and reagents, was appropriate for the analysis. The linearity of the method was assessed within the concentration range of 2 to 12 µg/mL for both LV and OA, showing a strong correlation between concentration and response. Accuracy was assessed using spike recovery at 50%, 100%, and 150% levels of a 4 µg/mL sample, with results expressed as mean %RSD. Precision studies included intraday and interday analyses of samples at 4, 6, and 8 µg/mL, confirming the method's reproducibility and repeatability. Robustness was tested by varying flow rate, mobile phase composition, and detection wavelength, with minimal impact on recovery, peak area, and retention time. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the signal-to-noise ratio, applying the equation $A = K(\sigma/S)$, where K is 3.3 for LOD and 10 for LOQ, and σ represents the standard deviation and S is the slope of the calibration curve. Overall, the method proved reliable and suitable for routine analysis of the drug combination.[15]

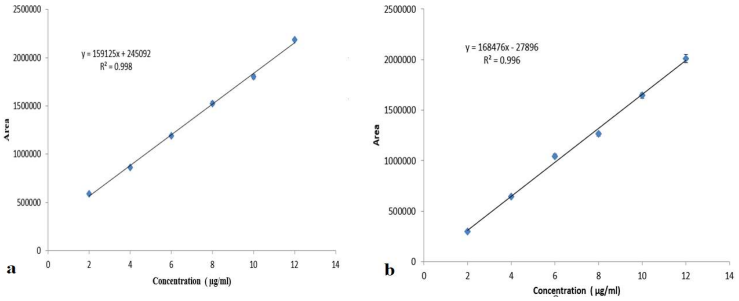

Fig.-2: Calibration Curve of a) Lovastatin, b) Oleanolic Acid

RESULTS AND DISCUSSION

Simultaneous Estimation of LV and OA: Selection of Mobile Phase

Various mobile phases were tested for the establishment of a technique for the simultaneous estimation of LV as well as OA to find the best solution for the differentiation of both active pharmaceutical ingredients by altering mobile phase composition (Fig.-3).

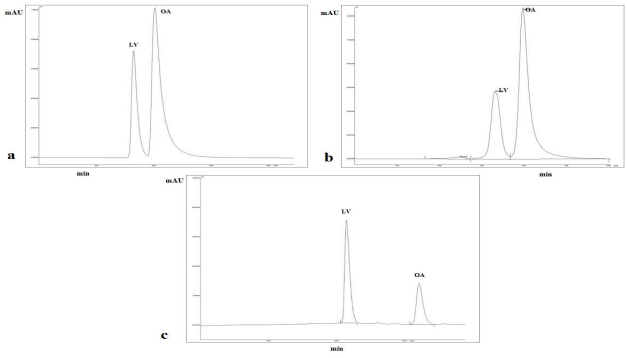

Fig.-3: Chromatogram of a mixture of (a) Lovastatin and Oleanolic Acid with ACN: MEOH (50:50 v/v), (b) Lovastatin as well as Oleanolic Acid with ACN: MEOH (20:80 v/v), (c) Lovastatin as well as Oleanolic Acid MeOH: Water pH 3 Adjusted with OPA (90:10 v/v)

The Acetonitrile (ACN): Methanol (50:50 v/v) was initially used, but a considerable number of theoretical plates were not observed in the result. The methanol was subsequently tried with a high proportion. Methanol: Water in a 90:10 v/v, was adjusted to a pH of 3 using Ortho Phosphoric Acid (OPA), produced a sufficient number of theoretical plates and well-defined peaks. For LV and OA, the retention times observed were 4.41 min., in addition to 6.43 min., correspondingly (Fig.-3). The

optimized mobile phase, methanol: water (90:10 v/v, pH 3 adjusted with OPA), achieved well-defined peaks with retention times of 4.41 minutes for LV and 6.43 minutes for OA.

Specificity

It was estimated by inserting a placebo as well as a blank solution into the analytical system and analyzing the peaks observed in the sample solution with the standard solution. There was no interference between the two peaks. The detected peaks in the sample solution aligned with the standard peaks of Oleanolic acid and Lovastatin, confirming the specificity of the method. The method demonstrated specificity, with clear alignment of sample and standard peaks without interference.

System Suitability

The assessment of system suitability performance involved the examination of factors, including theoretical plates, retention time, asymmetric factor, as well as resolution. The results indicated satisfactory performance of the method within the system (Table-1) as the column efficiency determined was found to be more than 2000 USP plate count; the asymmetric factor for the same peak is not more than 2.0. The method demonstrated good system suitability with high column efficiency and acceptable asymmetry, ensuring reliable performance.

Table-1: System Suitability Parameters for Oleanolic Acid and Lovastatin

Drugs	Concentration µg/ml	RT (min)	Area	Plates	Asymmetry	Resolution
Lovastatin	4	4.417	865950.68	2871.08	1.19	--
Oleanolic acid	4	6.433	648344.75	2970.43	1.32	6.26

Linearity

The constructed calibration curves displayed linearity in the concentration interval of 2-12µg/ml for Oleanolic acid as well as Lovastatin, each (Tables-2 and 3). The correlation coefficient (R^2) values for LV along with OA were observed to be 0.998 and 0.996, respectively (Fig.-2). Also, the technique was verified with the help of ANOVA analysis, and it demonstrates linear regression with no deviation from linearity ($P < 0.05$). The method exhibited strong linearity for both Oleanolic acid and Lovastatin, with high correlation coefficients and no significant deviations confirmed by ANOVA.

Table-2: Linearity Study of Oleanolic Acid

Conc.				Area			Average	SD	%
µg/mL	1	2	3	4	5	6			RSD
2	297455.80	297757.76	297656.80	291964.90	297581.10	297667.88	296680.70	2312.47	0.77
4	648344.75	649876.66	642343.65	634563.66	633456.65	662321.98	645151.22	10808.37	1.67
6	1051637.30	1043454.76	1054538.00	1004245.65	1054325.80	1066893.98	1045849.24	20727.04	1.98
8	1262222.93	1287678.76	1276568.90	1235434.78	1243543.99	1278978.66	1264071.33	20883.33	1.65
10	1620619.86	1667876.77	1612345.70	1632147.50	1670543.66	1667493.70	1645171.19	26485.83	1.61
12	1996902.52	1965459.90	2076789.98	1995432.77	1990245.55	2045279.80	2011685.08	40100.69	1.99

Accuracy

It is also expressed as % recovery, which measures the correctness of the analytical method. The mean percentage recovery of LV and OA of all three levels was within the normal limit of 98-102%. It is verified by a % RSD of less than 2% (Table-4). The proposed method demonstrated high accuracy, with mean percentage recovery for LV and OA within 98–102% and a percentage relative standard deviation below 2%.

Table-3: Linearity Study of Lovastatin

Conc.	Area						Average	SD	%
mg/ml	1	2	3	4	5	6			RSD
2	570769.19	595514.63	595630.33	599999.6	585489.96	592885.26	590048.16	10588.47	1.79
4	865950.68	858863.56	855251.627	866262.2	866965.93	858848.66	862023.77	4974.73	0.57
6	1194078.44	1158965.30	1189114.32	1198928.60	1195886.29	1192196.63	1188194.93	14698.39	1.23
8	1504230.82	1521911.48	1507804.29	1533262.25	1518125.66	1562566.26	1524650.13	21272.52	1.39
10	1787201.48	1795998.62	1792302.867	1798189.26	1787846.63	1855847.96	1802897.80	26300.77	1.45
12	2190175.96	2193188.29	2192783.26	2199659.63	2163225.26	2176854.92	2185981.22	13446.39	0.61

Table-4: Accuracy Study for LV and OA

Sample	Recovery Rate	Quantity of Sample (µg per mL)	Added Quantity (µg per mL)	Peak Area	Recovered quantity (µg per mL)	Recovery (%)	Average% Recovery ± % RSD	Mean Recovery (%)
LV	50%	4	2	1195769.23	5.963	99.386	100.089 ± 0.627	99.99
		4	2	1207308.55	6.036	100.592		
		4	2	1204389.62	6.017	100.287		
	100%	4	4	1522347.8	8.012	100.146	99.988 ± 0.179	
		4	4	1520790.99	8.002	100.023		
		4	4	1517860.67	7.983	99.794		
	150%	4	6	1841758.9	10.015	100.152	99.918 ± 0.206	
		4	6	1835587.87	9.976	99.765		
		4	6	1836759.45	9.984	99.838		
OA	50%	4	2	1038349.75	5.998	99.960	100.310 ± 0.693	99.82
		4	2	1037324.77	5.992	99.859		
		4	2	1049976.12	6.067	101.110		
	100%	4	4	1368709.5	7.958	99.481	99.616 ± 0.140	
		4	4	1370435.77	7.969	99.609		
		4	4	1372453.25	7.981	99.759		
	150%	4	6	1719654.76	10.042	100.415	99.555 ± 0.759	
		4	6	1700048.99	9.925	99.252		
		4	6	1695767.8	9.900	98.998		

Precision

For both Lovastatin and Oleanolic acid, RSD results, in the precision studies of intra as well as inter-day, were under 2.0% (Tables-5 and 6). Each drug shows an acceptable range of % RSD values, which indicates the proposed method was satisfactorily précised, having outstanding repeatability and reproducibility.

Table-5: Intra-Day Precision Study for Lovastatin and Oleanolic Acid

Sample	Concentration (µg/ml)	Area	% Recovery	Average % Recovery ± % RSD
LV	4	879284.6	99.450	100.478 ± 1.220
		894486.6	101.834	
		883744.7	100.149	
	6	1207605	100.623	100.178 ± 0.890
		1193523	99.151	
		1208911	100.760	
	8	1554631	102.677	101.569 ± 0.955
		1531639	100.874	
		1535243	101.157	
OA	4	650544	100.673	100.245 ± 0.551
		648976.67	100.441	
		643452.21	99.621	
	6	996204.9	101.310	100.893 ± 0.528
		993819.19	101.074	
		985924.05	100.293	
	8	1309467.94	99.225	99.994 ± 1.125
		1312790.24	99.472	
		1337234.89	101.285	

Table-6: Inter-day Precision Study for Lovastatin and Oleanolic Acid

Sample	Concentration (µg/ml)	Area	% Recovery	Average % Recovery ± % RSD
LV	4	883086.01	100.046	99.937 ± 0.339
		879966.66	99.557	
		884115.96	100.208	

	6	1218557.3	101.768	100.452
		1199641.15	99.791	±
		1199710.78	99.798	1.135
	8	1537564.22	101.339	100.578
		1533129.96	100.991	±
		1512898.71	99.405	1.025
OA	4	651349.07	100.793	100.740
		648967.12	100.439	±
		652677.83	100.990	0.277
	6	1000729	101.758	100.824
		993782.12	101.071	±
		979346.22	99.643	1.070
	8	1314562.36	99.603	99.745
		1309238.89	99.208	±
		1325621.44	100.424	0.622

Robustness

It was studied via small variations for flow rate, mobile phase ratio, and wavelength. In a robustness study, the percentage relative deviation for LV and OA was found to be below 2%, demonstrating the satisfactory robustness of the proposed method. (Tables-7 and 8).

Table-7: Robustness Study for LV

Variables	Value	Lovastatin				
		Concentration (µg per mL)	Average peak area (N=3)	SD	Percent RSD	Mean percent RSD
Mobile phase ratio (A: B) v/v	92:8	4	888304.58	3489.502	0.393	0.570
	90:10	4	880998.2	4965.326	0.564	
	88:12	4	885857.78	6672.563	0.753	
Flow rate (ml/min)	0.95	4	884758.453	3475.567	0.393	0.443
	1	4	885040.653	2174.034	0.246	
	1.05	4	887311.653	6129.858	0.691	
Wavelength (nm)	209	4	883723.790	4890.543	0.553	0.558
	210	4	880002.107	4774.939	0.543	
	211	4	883707.823	5113.734	0.579	

Table-8: Robustness Study for OA

Variables	Value	Oleanolic acid				
		Concentration (µg per mL)	Average peak area (N=3)	SD	percent RSD	Mean percent RSD
Mobile phase ratio (A: B) v/v	92:8	4	641062.177	2616.313	0.408	0.369
	90:10	4	642037.213	2352.470	0.366	
	88:12	4	640069.620	2125.532	0.332	
Flow rate (ml/min)	0.95	4	645340.287	7417.739	1.149	1.026
	1	4	643721.713	9795.305	1.522	
	1.05	4	643628.993	2626.785	0.408	
Wavelength (nm)	209	4	637847.787	2463.044	0.386	0.376
	210	4	644593.493	2573.058	0.399	
	211	4	646319.543	2211.619	0.342	

LOD and LOQ

These parameters were estimated with formulas 3.3 σ/S as well as 10 σ/S, respectively. Here, 'σ' signifies the response's Standard Deviation for the lowest concentration within the range, as well as S denotes the calibration curve's slope. The LOD values for Lovastatin (LV) and Oleanolic acid (OA) were observed to be 0.169 µg per mL as well as 0.250 µg per mL, respectively, with the corresponding LOQ calculated as 0.512 µg per mL for LV and 0.758 µg per mL for OA. (Table-9). The method demonstrated good

sensitivity with low LOD as well as LOQ values for both Lovastatin and Oleanolic acid, ensuring reliable detection at low concentrations.

Table-9: LOD and LOQ of LV and OA

Parameter	LV (μg/mL)	OA (μg/mL)
Limit of Detection	0.169	0.250
Limit of Quantification	0.512	0.758

Assay

Twenty tablets (comprising Core and Coat Tablets prepared in the laboratory), every tablet holding 50 mg of Oleanolic Acid, as well as 10 mg of Lovastatin, were individually balanced and pulverized. A 10 mg equivalent of Oleanolic Acid (Lovastatin 2 milligrams) was placed into a 10 milliliter graduated flask, also subsequently dilutions were made using methanol, reaching a total volume of 10 milliliters. The resultant solution was strained, and subsequent dilutions were performed using methanol to attain 4 µg/ml as the final concentration for Oleanolic Acid as well as 4 µg/ml for Lovastatin separately. This process was repeated 6 times, and each solution was injected under optimized conditions with the recorded area for each drug. The entire procedure was repeated for accuracy, and concentrations along with percent purity were determined using the linear equation (Table-10).

Table-10: Assay of Tablet Formulation

SN	Oleanolic acid			Lovastatin		
	Area of peak	Recovered Quantity (µg/ml)	Recovered percentage	Area of peak	Recovered Quantity (µg/ml)	Recovered percentage
1	654834.90	4.052	101.310	886632.33	4.020	100.491
2	640342.67	3.966	99.159	889962.55	4.024	100.602
3	644723.10	3.992	99.809	882999.67	4.045	101.124
4	640045.87	3.965	99.115	876632.3	4.001	100.033
5	639987.43	3.964	99.107	882563.64	3.961	99.034
6	636342.81	3.943	98.566	884119.41	3.999	99.964
Mean	642712.797	3.98	99.51	4152.35	4.008	100.208
SD	5940.273	3.980	99.511	0.47	0.026	0.651
% RSD	0.924	0.035	0.881	885925.99	0.650	0.650

Present research work developed a simple and validated HPLC method for analyzing Lovastatin (LV) and Oleanolic acid (OA), using a 90:10 methanol-water mobile phase (pH 3 with OPA) and UV detection at 210 nm. The method showed linearity (2-12 µg/mL), precision (% RSD < 2%), and accuracy (99.99% recovery for LV, 99.82% for OA). The LOD in addition to LOQ values were determined to be 0.16 as well as 0.51 µg/mL for Lovastatin (LV), also 0.25 as well as 0.75 µg/mL for Oleanolic acid (OA), correspondingly. The method met ICH Q2 (R1) guidelines, ensuring reliable and precise results. By enabling accurate and cost-effective quality control of pharmaceutical formulations, this work contributes to improving access to safe and effective medications, ultimately enhancing overall public health and well-being.

CONCLUSION

The developed method for the simultaneous estimation of Lovastatin and Oleanolic acid was thoroughly validated for precision, accuracy, robustness, and linearity. The results confirm that the RP-HPLC technique is simple, reliable, accurate, precise, and robust, offering a cost-effective solution for routine quality control. By enabling efficient and dependable analysis of pharmaceutical formulations, this method directly supports SDG 3 - Good Health and Well-Being, by promoting access to safe, effective, and quality-assured medicines. Accurate analytical methods are essential for ensuring the therapeutic efficacy and safety of pharmaceutical products. Thus, the proposed method contributes to strengthening healthcare systems by supporting consistent drug quality monitoring.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. 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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