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Method Developments and Validation of Atorvastatin and Clopidogrel in Tablet dosage form by Miceller Liquid Chromatography

Manoj Kumar[1,2] & Subhalaxmi Pradhan[1]*

[1]Division of Chemistry, School of Basic Sciences, Galgotias University, Greater Noida, Uttar Pradesh 203 201, India

[2]Vorick Analytical Research Pvt. Ltd, Bawana, Narela, Delhi 110 040, India

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To avoid heart attacks and strokes, a combination of atorvastatin and clopidogrel is used. A cholesterol-producing enzyme is inhibited by the lipid-lowering medication atorvastatin. Atorvastatin helps in reducing the level of bad cholesterol, Low Density Lipoprotein (LDL) and increasing good cholesterol level, High Density Lipoprotein (HDL) in our body. An antiplatelet drug called clopidogrel reduces the risk of dangerous blood clots by preventing platelets from adhering to one another. A novel, straightforward, and precise approach was used to estimate atorvastatin and clopidogrel quantitatively in tablet formulation by Micellar Liquid Chromatography (MLC). The MLC is a green and eco-friendly technique in Chromatography where surfactant solutions played the role as the eluent above the Critical Miceller Concentration (CMC).The separation of drug formulation was carried out by Reversed Phase Liquid Chromatography (RP-HPLC) using a Deoxyprobe C18 column (4.6 × 250 mm, 5 µm) with a mobile phase that contained 0.12M SDS and 10% Propanol-2 at a pH 3.7 maintained by o-Phosphoric Acid. The separation was performed at room temperature with an elution rate of 1.1mL/minat 220 nm. The retention time of atorvastatin is 2.3 while that of clopidogrel is 3.9 min. According to the schedule V of the drug and Cosmetic Act, the percentage composition of the sample under analysis ranged from 90 to 110%. The suggested procedure was in agreement with ICH requirements. In addition, the method was easy to use, affordable, safe, and non-harmful to the environment. It can be used for repetitive measurable estimation of atorvastatin and clopidogrel in tablets.

Keywords: Cardiac drugs, Critical micellar concentration, Reversed phase liquid chromatography, SDS, Surfactants

Introduction

Micellar Liquid Chromatography (MLC) is a familiar and simple-to-use technique of high-performance liquid chromatography. This chromatographic technique is increasingly being used to determine a wide range of chemicals used for pharma research, biological research, food industry & beverages and environmental materials.[1] MLC has many other advantages than other techniques like low cost, low toxicity and ease of working.[2] The ability to elute chemicals in a wide range of polarities (neutral and ionic) utilising isocratic elution, as well as the possibility of direct infusion of physiological fluids, are the most important aspects of MLC. Cardiovascular Drugs (CVDs) are medications that regulate how the heart and blood vessels work fine. One of the most significant and widely used pharmacological classes in the cardiovascular medications are Clopdegril bisulphate and Atorvastatin calcium.

Clopidogrel is a salt of Methyl-2-chlorophenyl tetrahydro thienopyridinium acetate bisulphate, used as prodrug of platelet inhibitor. This medicine is generally used for diminishing the risk of myocardial infarction and stroke.[3] The antiplatelet medication clopidogrel bisulphate prevents Adenosine Diphosphate (ADP) from connecting with its receptor and thereby activating the glycoprotein complex.[4,5] This medicine is considered as more effective than aspirin for lowering cardiovascular infections in people suffering with cardiovascular disease and offers an extra advantage to those taking aspirin for acute coronary syndromes. Clopidogrel is approved by Food and drug Administration (FDA) on November 17, 1997 and its chemical structure is given in Fig. 1.

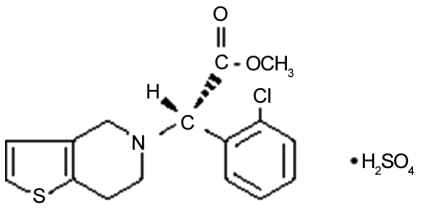

Fig. 1 — Structure of Clopidogrel

*Author for Correspondence
E-mail: subhalaxmipradhan@gmail.com

Atorvastatin calcium is the trihydrate calcium salt of (3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl -4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid, which lowers plasma cholesterol.[6] These drugs reduce LDL cholesterol and lipid levels and ultimately reduce the risk of cardiovascular disease.[3] The empirical formula for atorvastatin calcium is $(C_{33}H_{34}\ FN_2O_5)_2Ca{\bullet}3H_2O$, and its structure is shown in Fig. 2

According to literature review, the pharmaceutical use of combination of Atorvastatin and Clopidogrel is continuously increasing, so quantitative estimation of these two compounds in their pharmaceutical dosage is required. RP-HPLC method and concurrent analysis of Atorvastatin and Clopidogrel in pharmaceutical combination is reported by some researchers[3,7] but MLC analysis is not yet reported. Molecules are separated in MLC employing surfactant solutions in mobile phase, which has a high CMC. For MLC analysis, many anionic, cationic, and non-ionic surfactants, including Sodium Dodecyl Sulphate (SDS), Cetyltrimethyl Ammonium Bromide (CTAB), and Polyoxyethylene 23 lauryl ether, are frequently utilised. MLC outperforms reversed-phase liquid chromatography in terms of peak performance and well-resoluted peaks at shorter retention times. The key focus of current research work is to generate and verify an isocratic, quick, sensitive, fast, and simple MLC technique for measuring both atorvastatin and clopidogrel in tablet formulation using Sodium Dodecyl Sulphate in mobile phase. MLC is a novel method validated in this study first time for estimation of cardiovascular drugs using green solvents as mobile phase during chromatographic analysis.

Materials and Methodology

Aztolet tablet whose composition is Atovastatin 10 mg and Clopidogrel 75 mg of Sun Pharma were procured form local market. Standard solution of Atorvastatin and Clopidogrel used was Indian Pharamacopial Reference Standard (IPRS) of Atorvastatin and Clopidogrel supplied from Indian pharmacopeia Commission. The chemical and reagents used in the experiment are Sodium Dodecyl Sulphate (SDS), Propanol-2, HPLC grade water, and o-phosphoric acid.

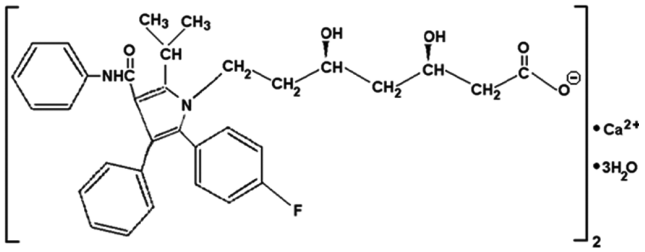

Fig. 2 — Structure of Atorvastatin calcium

Preparation of Stock solutions of Atorvastatin and Clopidogrel

The reference solutions of Atorvastatin and Clopidogrel were prepared by dissolving 1mg of each drug in 1mL of methanol separately. Then each stock solution is diluted for further preparation of mixture of reference solution. The combination of reference solution was prepared by mixing atorvastatin (50 $\mu g{\cdot}mL^{-1}$) and clopidogrel (75 $\mu g.mL^{-1}$) taking above diluted stored solution. The mobile phase was prepared by adding 0.12M Sodium Dodecyl Sulphate (SDS) solution with 10% Propanol-2 and the pH of the solution was accustomed to 3.5 by adding o-Phosphoric acid.

Instruments

The Agilent 1260 HPLC with ultraviolet detector and C18 column (2.5 cm × 0.46 cm), 5 µm particle size, was used to carry out the MLC analysis at 220nm wavelength. All solvents and the eluent were degasified by a sonicator and then filtered using 0.45 µm membrane filters. Analysis was carried out in isocratic conditions with elution rate of 1.0 $mL{\cdot}min^{-1}$.

The peak area was automatically measured and integrated by using DS Chrome Elite software and all calculations relevant to the qualitative study were carried out with standardization.

Linearity Study

Five standard solutions of atorvastatin with concentration varying from 5–25 $\mu g{\cdot}mL^{-1}$and similarly five solutions for clopidogrel in the range of 65–105 $\mu g{\cdot}mL^{-1}$ was examined to determine the linearity of the proposed study. The solutions were injected thrice and on the basis of calibration curves, the standard concentrations of atorvastatin and clopidogrel were determined. Additionally, system suitability tests were performed, and they met the requirements for theoretical plate, RSD, tailing factor, and resolution.

Preparation of Sample Tablets Solution

Twenty tablets were precisely weighed to determine the average weight and crushed to create fine powder. A tablet powder containing precisely 75 mg of clopidogrel and 100 mg of atorvastatin was dissolved in 25 mL of methyl alcohol in a volumetric flask with

a volume of 100 mL. After 15 minutes of sonication, the mixture produced a clear solution. Then dilution was done up to the mark of volumetric flask by adding membrane-filtered methanol. About 10 ml of the above prepared sample was diluted to 100 mL with methanol to obtain a concentration of 10 $\mu g \cdot mL^{-1}$ of atorvastatin, and 75 $\mu g \cdot mL^{-1}$ of clopidogrel, which is considered as the sample solution for injection.

Accuracy (Recovery Study)

About 1mg of standard Atorvastatin was dissolved in 1mL of methanol, then diluted to 10 mL and from that solution 8, 10 and 12 µL was injected in HPLC. Similarly 6 mg of Clopidogrel was dissolved in 10 mL of methanol, from that 10, 12.5 and 15 µL was injected in HPLC. Atorvastatin and Clopidogrel standard drug solutions were injected at 3 distinct measured amounts like 80, 100, and 120% for each drug to test the method's accuracy.

Result and Discussion

Method optimization

MLC analysis is carried out in a C18 column using 0.12 M SDS in 10% Propanol-2 and ortho-phosphoric acid (pH = 3.5) as mobile phase with a elution rate of 1 mL/min without changing solvent polarity at 25℃. The injection volume of sample is 20 µL and the chromatograms detected at 224 nm. The retention times (r_t) for atorvastatin and clopidogrel were 2.3 and 3.97 min, respectively using this mobile phase. The chromatograms obtained for the standard atorvastatin and clopidogrel solution and test solution of atorvastatin and clopidogrel tablet powder is represented in Figs 3 and 4 respectively.

The peak areas and retention periods of the Atorvastatin and Clopidogrel powdered tablet were found comparable to the standard Atorvastatin and Clopidogrel solution. Croitoru *et al*., 2015 developed a process for instantaneous measurement of

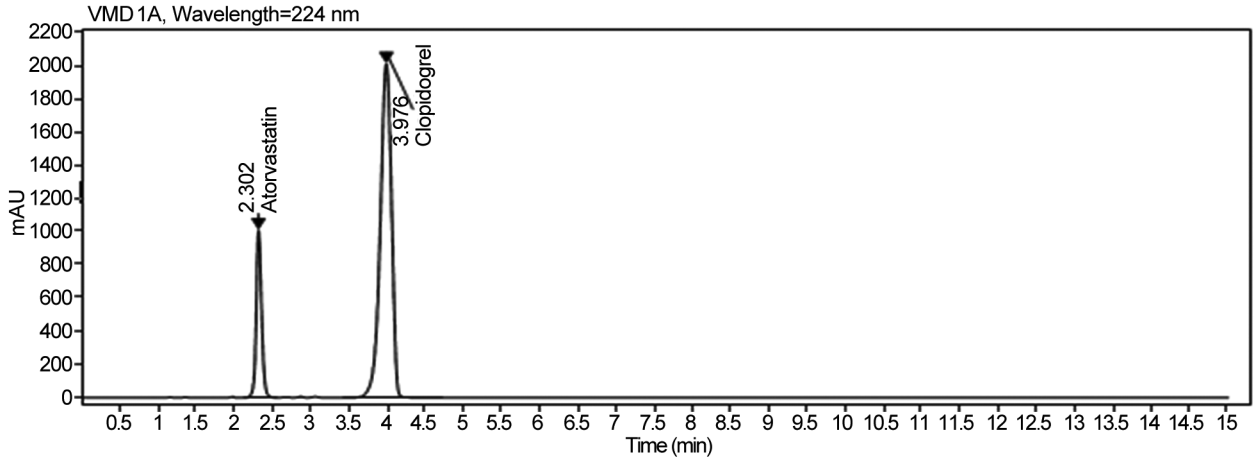

Fig. 3 — Chromatogram of Clopidogrel and Atorvastatin standard solution

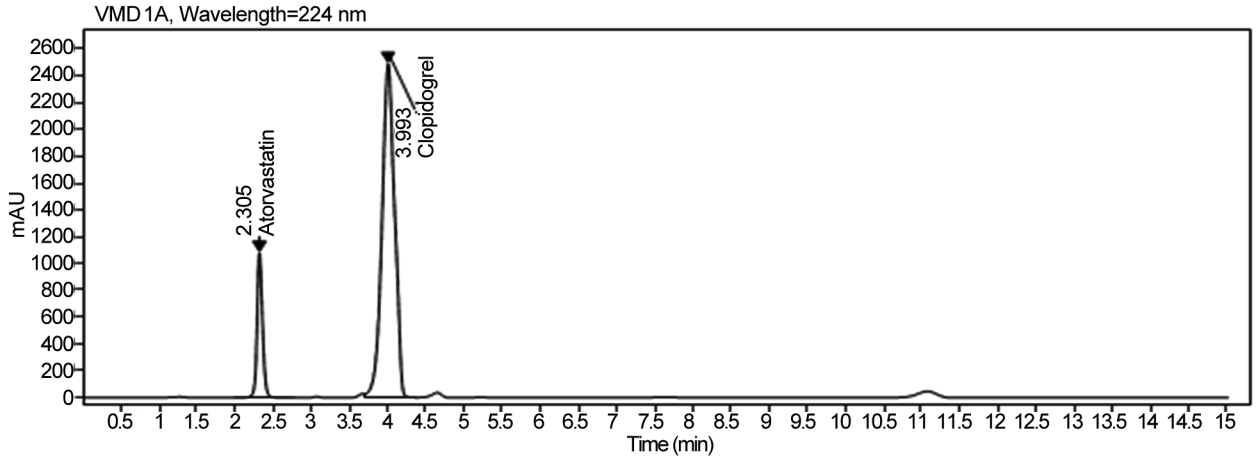

Fig. 4 — Chromatogram of Clopidogrel and Atorvastatin tablet powder solution

concentration of Clopidogrel bisulphate, its acid derivative, and atorvastatin in plasma of human being by HPLC.[8] They had used a BDS Hypersil C18 column (250 × 4.6 mm; 5 µm) to separate the chromatograms and had used changed concentration of eluent with an elution rate of 1 $mL{\cdot}min^{-1}$. They used three solvents, solvent A; 10 mM Sodium phosphoric acid buffer solution with pH 2.6 by adding 85% o-H_3PO_4, solvent B; acetonitrile and solvent C; methanol and there was a gradient run during the analysis. Atorvastatin and Clopidogrel bisulphate eluted at 11.03 and 12.96 min retention time respectively. Dangre *et al*., (2012) developed a method for synchronised analysis of above two cardiac medicine formulation in a capsule by using a Eurosphere C18 column (4.6 × 250 mm, 5 µm) and a mixture of acetonitrile and 0.01 M potassium dihydrogen phosphate (75:25 v/v, pH 6.1) as eluent at 240 nm.[3] It was observed that Atorvastatin and Clopidogrel chromatograms appeared at 3.5 and 10.75 min retention time respectively. The current study found that adapting MLC techniques and using 0.12M Sodium DodedcylSulphate (SDS) solution with 10% Propanol-2 and o-Phosphoric acid (pH=3.5) as mobile phase atorvastatin and clopidogrel eluted quickly at low retention time 2.3 and 3.97 min respectively. The obtained result is comparable with other researchers, who has analysed a combination of three drugs, aspirin, atorvastatin and ramiprilin MLC.[9] They have used C_8 column and aqueous solution of 0.12 M SDS solution: n-propyl alcohol (9:1, v/v) and 0.3% of triethyl amine by adding orthophosphoric acid (pH of 2.5) as mobile phase and the peak of atorvastatin observed at 2.3 min. So MLC method of separation is more preferable than RP-HPLC as separation occurs at less time using cheap and non-toxic mobile phase.

Method Validation

The process is optimised and validated for the instantaneous estimation of atovastatin and clopigogrel and suitability of system, specificity, precision, accuracy, linearity, Limit of Detection (LOD), Limit of Quantitation (LOQ), and robustness is evaluated as per ICH (International Conference of Harmonisation) guidelines.[10]

Linearity

An analytical method is said to be linear, if the test findings obtained are directly related to the concentration of analyte present in the sample within acertain range. The range of an anlytical method is determined from the higher and lower concentration of material in the test solution, which represents that the analytical technique has a satisfactory level of correctness, accurateness, and linearity. Five concentrations of 5–25 $\mu g{\cdot}mL^{-1}$ for atorvastatin and 65–105 $\mu g{\cdot}mL^{-1}$ for clopidogrel were used to assess the method's linearity. The response was plotted against the standard doses of atorvastatin and clopidogrel to create the calibration curve and the regression obtained for both is 0.9998, which is represented in Fig. 5.

System Suitability Study

The system suitability test findings are represented in Table 1 and the measured data are plate count, relative standard deviation, tailing factor, and resolution. The above parameters examined are within the permitted range, according to the findings of six replicate injections. Atorvastatin and Clopidogrel were consistently appeared and clearly distinguished at 2.3 min and 3.9 min, respectively, with RSD% of the reported retention times 0.2, suggesting high resolution among both peaks. This indicates good repeatability of six replicate injections during MLC analysis. Both the atorvastatin and clopidogrel peaks' tailing factors are under 2, which show that all of the peaks have good peak symmetry. Theoretical plate count (N) is calculated from the equation given below.

$$N = 16(t_R/W)^2$$

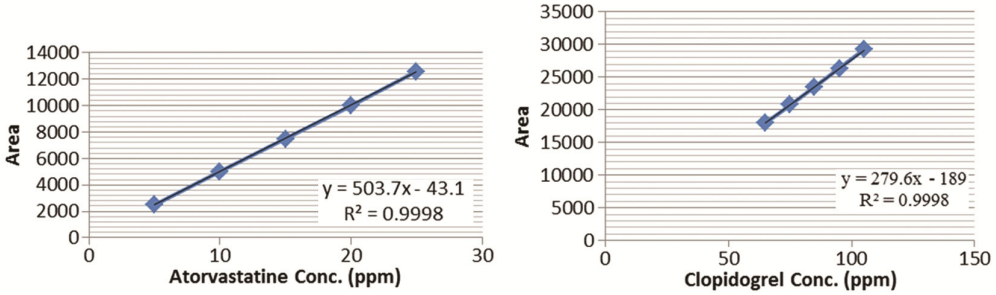

Fig. 5 — Linearity curve of standard doses of atorvastatin and clopidogrel in MLC analysis

where, N= Theoretical Plate count, t_R= retention time and W= peak width.

The theoretical plate count throughout the run is consistently greater than 2000, which shows column efficiency is good throughout the analytical measurement.[11]

Accuracy

The accuracy of an analytical method is measured by how closely its test results match its actual value. Atorvastatin and Clopidogrel standard drug solutions were spiked in a known concentration solution at three distinct amounts such as 80, 100, and 120% for each drug to test the method's accuracy. The amount of recovered percentage of Atorvastatin and Clopidogrel is used to measure accuracy of the method, which is represented in Table 2. In this analysis recovery ranged from 98.0 to 102.0%.

Precision

Precision is the degree to which measurements taken from different samples of the same homogeneous sample taken under identical conditions agree with one another. Six separate injections of the combination of Atorvastatin and Clopidogrel samples yielded peak areas that were reproducible and accurate for 3 days. The peak area data obtained from six replicate injections to determine intra-day and inter-day precision is shown in Table 3. Results of intraday and interday analysis confirm that the developed approach possess remarkable repeatability and precision. It is observed that all data articulated in percentage of relative standard deviationis within recognised limit and not exceeding 0.25% (recognised limit RSD% <2).

The maximum threshold value was set at 2.0%, with the RSD of the standard and test solutions being 0.6 and 0.8%, respectively. Intermediate precision

Table 1 — Appropriateness and precision findings of MLC Analysis (RSD % < 2)

S.No.	Retention Time (r_t)		Tailing Factor		Theoretical Plate count		Resolution factor
	Atorvastatin	Clopidogrel	Atorvastatin	Clopidogrel	Atorvastatin	Clopidogrel	
1	2.301	3.965	1.54	0.76	2161.59	2201.22	6.1
2	*2.304*	*3.973*	1.53	0.78	2170.75	2213.55	6.2
3	2.306	3.968	1.52	0.78	2180.47	2227.02	6.1
4	2.302	3.976	1.54	0.77	2175.11	2208.34	6.2
5	2.306	3.965	1.55	0.77	2169.89	2203.63	6.2
6	2.299	3.969	1.55	0.78	2179.03	2224.78	6.1
Mean	2.303	3.969	1.54	0.77	2172.81	2213.09	6.2
St. Dev.	0.00258	0.0044	0.11	0.0081	6.95	10.81	0.054
RSD%	0.12	0.11	0.76	1.06	0.32	0.49	0.89

Table 2 — Recovery results for Atorvastatin and Clopidogrel (Recovery %limit = 98–102%)

Sample No.	Theoretical conc. In µg mL^{-1}	The concentration found in µg mL^{-1}	Recovery%	Statistical data
		Atorvastatin		
S1 80%	80	79.61	99.51	Mean=99.77
S2 80%		79.34	99.12	Standard Deviation (SD) =0.812
S3 80%		80.54	100.68	RSD= 0.81%
S1 100%	100	99.22	99.22	Mean= 100.07
S2 100%		101.51	101.51	Standard Deviation=1.25
S3 100%		99.49	99.49	RSD=1.25%
S1 120%	120	120.62	100.52	Mean= 99.38
S2 120%		118.79	98.99	Standard Deviation=1.00
S3 120%		118.35	98.63	RSD= 1.01%
		Clopidogrel		
S1 80%	600	592.27	98.71	Mean=99.63
S2 80%		594.43	99.00	Standard Deviation=1.34
S3 80%		607.78	101.17	RSD=1.35%
S1 100%	750	758.21	101.09	Mean=99.93
S2 100%		742.07	98.94	Standard Deviation= 1.09
S3 100%		748.11	99.75	RSD=1.35%
S1 120%	900	884.54	98.28	Mean=99.06
S2 120%		902.88	100.32	Standard Deviation=1.10
S3 120%		887.12	98.57	RSD=1.1%

was investigated by different analyser on different day. The RSD was measured to be 0.9%.

LOD and LOQ

The limit of detection was determined by using low concentration of standard solution. In this case it was measured to be 1.0 $\mu g \cdot mL^{-1}$. By employing a modest concentration of standard solution, the limit of quantification was established. In this instance, it was measured to be 15 $\mu g \cdot mL^{-1}$ for Clopidogrel and 5 $\mu g \cdot mL^{-1}$ for Atorvastatin.

Range and Specificity

The range of an analytical technique is the variation between the higher and lower amounts of analytes in the standard solution for which the investigative technique possesses sufficient level of precision, accurateness, and linearity. It has been thoroughly observed that this MLC analysis has a sufficient level of exactness, accuracy, and linearity within the array of 80–120%. The RSD was calculated to be 0.4, 0.8, and 1.1% at various ranges. The capacity to reliably access the analyte with the existence of potential co-existing constituents is referred to as specificity. None of the excipients interfered with the Atorvastatin and Clopidogrel peak or the result in this method, where Clopidogrel and Atorvastatin were determined to be between 80 and 120%.

Robustness

The robustness of the procedure was confirmed via injecting standard and sample solution on multiple days and obtained result is presented in Table 4. The robustness of the procedure is indicated by the high degree of reproducibility of detection response and retention time.

Table 3 — Precision findings as the area of chromatogram of several analysis on three separate days (Atorvastatin 1mg/mL, Clopidogrel 7.5 mg/mL) (n=6), acceptance limit RSD % < 2)

	1st Day		2nd Day		3rd Day	
	Atorvastatin	Clopidogrel	Atorvastatin	Clopidogrel	Atorvastatin	Clopidogrel
1	4983.5250	20766.3701	4997.0987	20754.8709	4976.9094	20693.0912
2	4998.0564	20785.0087	4989.9054	20766.0089	4961.8098	20738.5139
3	4989.5648	20767.0124	4981.7098	20781.9844	4971.8923	20744.6387
4	4993.7654	20797.3264	4996.8137	20790.0768	4986.7601	20734.0078
5	4988.9782	20788.0512	4986.7862	20781.7421	4998.2006	20728.7109
6	4999.6547	20774.5871	4993.6508	20774.5197	4982.0051	20758.0041
Mean	4992.2574	20779.7979	4990.9941	20774.8671	4979.5962	20732.8277
St. Dev.	6.0807	12.4372	6.0544	12.7047	12.5393	21.9266
RSD%	0.12	0.06	0.12	0.06	0.25	0.11

Table 4 — Results for both Clopidogrel and Atorvastatin's robustness

Parameters	Time of retention		Tailing factor		Theoretical plate counts		Resolution
Flow rate (ml/min)	Atorvastatin	Clopidogrel	Atorvastatin	Clopidogrel	Atorvastatin	Clopidogrel	
0.9	2.300	3.965	1.54	0.78	2161.59	2201.22	6.1
1.0	2.295	3.942	1.55	0.78	2148.50	2221.72	6.1
1.1	2.292	3.935	1.54	0.76	2152.76	2211.22	6.2
Mean	2.295	3.947	1.54	0.77	2154.28	2211.38	6.13
St. Dev.	0.0040	0.015	0.0058	0.0112	6.602	10.2510	0.058
RSD%	0.18	0.40	0.37	1.49	0.31	0.46	0.94
SDS0.52M	2.314	3.975	1.54	0.77	2171.67	2225.56	6.1
SDS 0.50M	2.303	3.965	1.53	0.78	2161.59	2201.22	6.1
SDS 0.54M	2.296	3.955	1.54	0.79	2187.08	2196.87	6.0
Mean	2.304	3.965	1.536	0.78	2173.44	2207.88	6.06
St. Dev.	0.0074	0.01	0.0058	0.01	12.8374	15.4621	0.057
RSD%	0.39	0.25	0.38	1.28	0.59	0.70	0.95
pH 3.45	2.346	3.970	1.53	0.78	2179.09	2189.90	6.0
pH 3.50	2.306	3.965	1.54	0.77	2161.59	2201.22	6.1
pH 3.55	2.293	3.958	1.54	0.78	2150.88	2231.61	6.1
Mean	2.315	3.964	1.54	0.776	2163.85	2207.57	6.06
St. Dev.	0.0276	0.006	0.0058	0.0057	14.2245	21.56	0.057
RSD%	1.19	0.15	0.38	0.74	0.66	0.98	0.98

The method is resilient to minor intentional adjustments made to the flow rate, the buffer's pH, or the variable concentration of the mobile phase because no substantial variations were observed after making slight adjustments to the chromatographic conditions. The peaks of atorvastatin and clopidogrel were consistently symmetrical, tailing factor within 2, having resolution less than 2 and had standard deviations of atorvastatin and clopidogrel retention times of 0.1, demonstrating the analytical method's tolerance to slight variations. The result obtained is in agreement with other researchers who developed eco-friendly MLC method for determining a ternary combination of formulation having atorvastatin and clopidogrel.[9,12]

Conclusions

Atorvastatin and Clopidogrel can be analysed and estimated using micellar liquid chromatography in the pharmaceutical business. The analysis was completed quickly and accurately according to the MLC approach. MLC can be used to analyse a variety of medications, including antipyretic and anti-cardiac ones. Atorvastatin and Clopidogrel in dose formulation can be assayed (quantitatively analysed) using this established method. This method is green due to use of non-toxic mobile phase, less time taking and accurate in terms of accuracy, precision and specificity. This technique was determined to be authentic in accordance with ICH guidelines and can be used on a regular basis for quality control analysis of atorvastatin and clopidogrel in pharmaceutical industry.

References

1 Ibrahim F A, El-Brashy A M, El-Awady M I & Abdallah N A, Assessment of the greenness of spectrophotometric and micellar liquid chromatographic methods using two approaches: application to pharmaceutical analysis of hydrochlorothiazide and telmisartan, *Microchem J*, **148(1)** (2019) 197–205.

2 Ibrahim F A, El-Brashy A M, El-Awady M I & Abdallah N A, Fast simultaneous quantitation of valsartan and amlodipine besylate using an eco-friendly micellar HPLC-UV method: application to spiked human plasma and content uniformity testing for amlodipine, *Anal Method*s, **10(43)** (2018) 5227–5235.

3 Dangre S C, Shah S K, Suruse P B & Bhusari K P, Development of RP-HPLC method for simultaneous estimation of atorvastatin calcium and clopidogrel bisulphate in a pharmaceutical capsule dosage form, *Res J Pharm Technol*, **5(5)** (2012) 641–644.

4 Jarvis B & Simpson K, Clopidogrel: a review of its use in the prevention of atherothrom-bosis, *Drugs*,**60** (2000) 347–377.

5 Gurbel P A, Connor C M O, Cummings C C & Serebruany V L, Clopidogrel: the future choice for preventing platelet activation during coronary stenting, *Pharmacol Res*, **40** (1999) 107–111.

6 Andrew P L & McTavish D, Atorvastatin: a review of its pharmacology and therapeutic potential in the management of hyperlipidemias, *J Food Drug Anal*, **53** (2007) 828–847.

7 Yadav P, Taleuzzaman M & Kumar P, Simultaneous estimation of aspirin, atorvastatin calcium and clopidogrel bisulphate in a combined dosage form by RP-HPLC, *Asian J Chem Sci*, **6(4)** (2019) 1–10.

8 Croitoru O, Spiridon A M, Belu I, Turcu-Ftiolics A & Neamuu J, Development and validation of an HPLC method for simultaneous quantification of clopidogrelbisulfate, its carboxylic acid metabolite, and atorvastatin in human plasma: Application to a pharmacokinetic study, *J Anal Methods Chem*, **2015** (2015) 892470.

9 Abdallah N A, El-Brashy A M, Ibrahim F A & El-Awady M I, Micellar eco-friendly HPLC method for simultaneous analysis of ternary combination of aspirin, atorvastatin and ramipril: Application to content uniformity testing, *BMC Chemistry*, **17** (2023) 19–30.

10 ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Q2 (R1). [(https://www.ich.org/page/quality-guidelines, 08 March 2023)].

11 Gedawy A , Al-Salami H & Dass C R, Development and validation of a new analytical HPLC method for simultaneous determination of the antidiabetic drugs, metformin and Gliclazide, *J Food Drug Anal*, **27** (2019) 315–322.

12 Issa M M, Nejem R M, Shanab A A& Stefan-van Staden R I, Resolution of ternary mixture of aspirin, atorvastatin, and clopidogrel by chemometric-assisted uv spectroscopic and liquid chromatography methods, *Int J Spectrosc*, **2013** (2013) 1–8.