DRUG ANALYTICAL RESEARCH
ISSN: 2527-2616 *www.seer.ufrgs.br/dar*

New HPLC method for determination of fluvastatin anti-hyperlipidemic drug in bulk and pharmaceutical dosage using simvastatin as internal standard

Safwan Ashour[a,b,*], Mouhammed Khateeb[c]

[a]*Department of Materials Science, Faculty of Engineering, University of Gaziantep, Gaziantep, Türkiye*
[b]*Department of Chemistry, Faculty of Sciences, University of Aleppo, Aleppo, Syria*
[c]*Department of Basic Sciences, Faculty of Engineering, University of Al-Baath, Homs, Syria*
**Corresponding author: sashour@gantep.edu.tr*

A new, and reliable RP–HPLC method has been investigated and validated for the assay of fluvastatin sodium (FVS) in pharmaceutical capsules. The chromatographic method was carried out using an ODS Hypersil C18 column (250×4.6 mm i.d., 5 µm particle size); a mixture containing methanol and 0.10 M ammonium acetate (70:30, *v/v*), a flow rate 1.0 mL/min and UV detection at 305 nm. The retention time for fluvastatin and the internal standard simvastatin (SVS) was found to be 3.2 and 6.4 minutes, respectively. Despite the wide range of the analyte concentration, the method showed good linearity (r>0.9998) in the range 2.0–320.0 µg mL^{-1}. The proposed method was validated with respect to linearity, accuracy, precision, and robustness. The limit of detection was 0.54 µg mL^{-1}. The successful application of the new HPLC method indicates its routine use to quantify of FVS in pharmaceuticals.

Keywords: Fluvastatin, high–performance liquid chromatography, pharmaceuticals.

Article received at 02/17/2023 and accepted at 03/28/2023.
https://doi.org/10.22456/2527-2616.130255

Introduction

Fluvastatin sodium (FVS), (3R,5S,6E)-rel-7-[3-(4-fluorophenyl)- 1-(1-methylethyl)-1H-indol-2-yl]-3,5-dihyroxy-6-heptenoicacid, monosodium salt, is a competitive inhibitor of HMG-CoA reductase, which is responsible for the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate, a precursor of sterols including cholesterol. It is used to reduce triglycerides, LDL-cholesterol, apoliporotein B and to increase HDL-cholesterol, in the treatment of hyperlipidaemias including hypercholesterolaemias and combined hyperlipidaemia. FVS is metabolized in the liver, primarily via hydroxylation of the indole ring at the 5 and 6-positions. N-dealkylation and beta-oxidation of the side-chain also occurs. The hydroxyl metabolites have some pharmacological activity, but do not circulate in the blood. FVS has two optical enantiomers, an active 3R, 5S and an inactive 3S, 5R form. FVS is 98% bound to plasma proteins [1-3]. The official method reported in BP [4] describes a non-aqueous titration for the assay of fluvastatin. Several analytical methods have been described for the quantitative determination of fluvastatin sodium in pharmaceutical dosage forms by voltammetry [5-8], derivative spectrophotometry and spectrofluorimetry [9], reversed-phase high-performance liquid chromatography (RP–HPLC) with ultraviolet detection [9-15], high-performance thin-layer chromatography (HPTLC) [9,16], kinetic spectrophotometry [17], spectrophotometry based on oxidative coupling reactions with 3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate (MBTH) [18], UV-spectrophotometry [19,20], atomic absorption spectroscopy [21], capillary electrophoresis [22] and gas chromatography-FID [23]. Fluvastatin has been determined with other drugs in combined pharmaceutical formulations with different derivative spectrophotometric methods [24-26]. As revealed from the literature, few methods determine FVS in formulations using HPLC. Additionally, the reported methods depended on used heating [10] or hazardous solvents such as acetonitrile and multiple and expensive salts [9–15]. Therefore, the aim of the current study was to develop a precise, accurate, reproducible cheap and validated HPLC–UV method for the estimation of fluvastatin in bulk and in commercial dosage forms. In the proposed method, the separation and elution of fluvastatin and internal standard were during 7min run time. The precision of the described method has been checked regarding F-test using a pharmacopeia method as reference. The developed method can be serves as an alternative to the methods described in pharmacopeias.

Experimental

Instrumentation

The L–2000 HPLC system composed of L–2130 binary pump (flow rate range of 0.000-9.999 mL/min), degasser, L–2350 column oven (temperature range of 1-85^{o}C), L-2200autosampler (injection volume of 0.1-100 µL) and L-2455 photodiode array (PDA) detector (190-850 nm) containing a quartz flow cell (10mm path and 17 µL volume), all from Hitachi (Japan). Chromatograms were analyzed and integrated automatically using the EzChrom Elite Hitachi Software.

Metrohm compact titrator, Sartorius analytical balance, WTW pH meter and Dihan sonicator were used for all preparations.

Materials and chemicals

Analytical reagent grade ammonium acetate (NH_4OAc), HPLC grade methanol and water were purchased from Merck (Darmstadt, Germany). Working reference standard of fluvastatin sodium (FVS, $C_{24}H_{25}FNO_4Na$, 433.46 g/mole) was supplied by ALPHARM Chemical Co (China). We found its purity to be 99.6% after analyzed according to the compendial method. Simvastatin (SVS) was used as the internal standard, and supplied by Cipal Ltd, India, and we found that its purity is as high as 99.8%. The following commercial formulations were subjected to the analytical procedure: Fluvastatin 20 (Kimi, Syria), labeled to contain 20 mg fluvastatin sodium/capsule, Almastatin 40 (Alma company, Syria), labeled to contain 40 mg fluvastatin sodium/capsule, and Fluvastat 40 (BPI, Syria), labeled to contain 40 mg fluvastatin sodium/capsule.

Chromatographic conditions

Chromatographic separation and quantitative analysis were performed on ODS Hypersil C18 column (250×4.6 mm, 5 µm particle size, Macherey–Nagel Germany). Mobile phase consisting of a mixture of MeOH and 0.10 M NH_4OAc buffer pH 4.8 (70:30%*v/v*). 0.45 µm nylon membrane filter was used for mobile phase filtering and ultrasonic agitation to remove gases before use. Mobile phase flow rate set at 1.0 ml/min, detection was at 305 nm and an ambient column oven temperature of 25.0±0.1 °C.

Standard solutions of FVS and SVS

Standard stock solutions of FVS and SVS were produced at a 1.0 mg mL^{-1} concentration by dissolving the appropriate weigh of drug in MeOH. Working standard solutions were then prepared by diluting the stock standard solution with MeOH to the desired concentration. The solutions showed to be stable for three days within 2–8 °C.

Calibration curve

Aliquots of standard FVS solutions (2.0–320.0 µg mL^{-1}) were placed into a set of calibrated volumetric flasks. 1.0 mL of SVS was added (100 µg mL^{-1} in the final volume) to each sample. The volume was then adjusted with MeOH and thoroughly shaken.10 µL of each solution was injected into the column (five replicates) immediately after preparation. The peak area of the chromatograms versus FVS concentrations was plotted to construct the calibration graphs.

Capsule samples preparation

The entire content of twenty capsules containing FVS was weighed and the average weight determined for the content of one capsule. The combined content was mixed well. Into 100 mL separated calibrated flasks, five weighed quantities of the powder, each equivalent to 20 mg FVS, were transferred and dissolved in about 90 mL of MeOH. Swirled the flask's content for 5 minutes before being filled with MeOH. The sample solutions were filtered and a suitable concentration was prepared in 10 mL volumetric flasks containing 1 mL of the internal standard SVS. The recommended producers were used in the range of concentration achieved above.

Results and discussion

Optimum chromatographic conditions for the presented method

To overcome the shortages of the reported methods, and to develop a suitable assay for the reliable selective analysis of the FVS, an isocratic LC method with DA detection was investigated. SVS was used as internal standard to reduce the problem of the many-fold dilution required in the classical batch procedures, and also control undetermined changes in active pharmaceutical ingredient concentration and instrument response fluctuations.

Achieving sufficient accuracy with peak symmetry in an acceptable analysis time is a very important aspect in the development of the LC method. To come up with this, Numerous experiments have been conducted to improve the stationary and mobile phases. In order to test the stationary phase, the following reversed-phase columns are studied: ODS Hypersil C18 (4.6×250 mm), Nucleodur C8 (4.6×250 mm), Nucleodur C18 (4.6×250 mm), Supelcosil C8 (4.6×250 mm) and Hichrom 5 C8 (4.6×250 mm). All of them gave clear separation between FVS and SVS. While the use of ODS Hypersil C18 column (4.6×250 mm, particle size 5 µm) achieved the most favorable resolution between the FVS and SVS peaks, and thus it was selected in this work.

Several components of the mobile phase were tested using different aqueous phases and organic modifiers with various proportions. The aqueous phase ammonium acetate was chosen and the effect of its concentration value in the mobile phase on the chromatographic elution of the drugs was investigated in the range from 0.01 to 0.50 M. The experimental showed that the mobile phase consisting of 0.10 M NH_4OAc (pH 4.8) and MeOH (30:70, *v/v*) gave the best resolution of the cited drug and the internal standard from the related substances within acceptable analysis time. Increasing the percentage of methanol to more than 75% led to elute the FVS peak with the solvent front, whereas decreasing the proportion of methanol to lower than 65% led to broad peaks and prolonged retention times. 70% was found to be the optimum MeOH percentage (Figure 1).

The mobile phase flow rate effect on the chromatographic elution of FVS was investigated. For all experimental, the optimum separation between FVS

and SVS and peak symmetry for the drug was obtained when isocratic elution was used at a flow rate 1.0 mL min^{-1} and 25 ºC (Figure. 2).

Diode array detection was used to achieve the quantification based on peak area measurement. The wavelength of 305 nm was found the most suitable for the determination of the analyte and the internal standard because it corresponds to a high absorption of both. Figure 3 shows a chromatogram obtained by the cited method with retention time about 3.207 and 6.403 min for FSV and SVS, respectively.

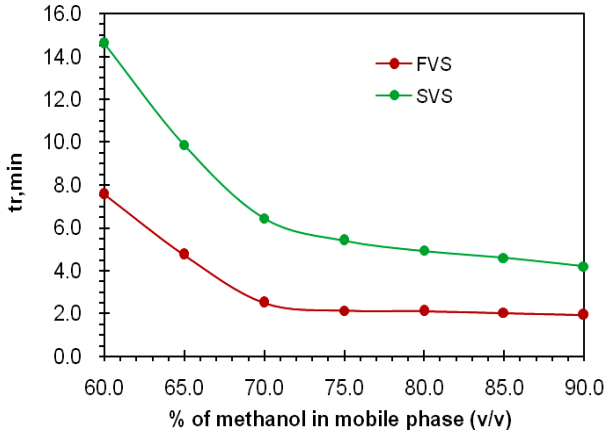

Figure 1. Effect of change of % MeOH in the mobile phase on the retention time of FVS and SVS.

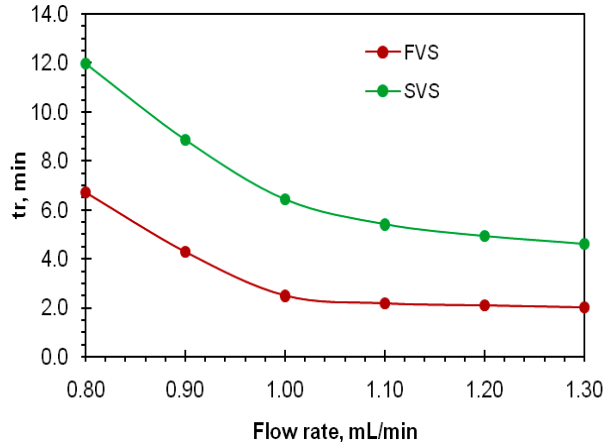

Figure 2. Change of retention time of FVS and SVS as a function of mobile phase flow rate.

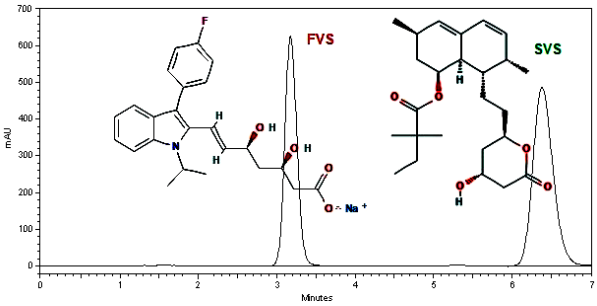

Figure 3. HPLC chromatogram of a mixture 120 µg mL^{-1} FVS and 100 µg mL^{-1} SVS at 305 nm.

Method validation

The validation of the proposed method was investigated according to ICH guidelines [27].

Linearity

To investigate the linearity of the developed HPLC method, two series of different analyte concentrations (n=5) were analyzed. By least squares processing of the calibration data [28], for the first series, a linear regression equation for the mean peak area of FVS (A_{FVS}) versus the concentration of FVS was constructed, while for the second series, a linear regression equation for the mean peak area ratio of FVS to that of SVS ($R_{FVS/SVS}$) versus the concentration of FVS was generated. At optimal conditions for the proposed method, the results showed that the peak areas measured at 305 nm are linearly proportional to the analyte concentration. Table 1 shows the calibration data and statistical parameters including linearity limit, linear regression equations, correlation coefficients (r), standard deviations of the intercept (S_a) and standard deviation of slope (S_b). The correlation coefficient values (>0.9998) indicates good linearity.

Table 1. Linearity and sensitivity results for analysis of fluvastatin using the developed method.

Parameters	**FVS**
Retention time (min)	3.208
linearity limit (µg mL^{-1})	2.0-320.0
Regression equation	$A_{FVS} = 0.1835C_{FVS}+0.1186$
Correlation coefficient (r)	0.9999
S_a	0.00843
S_b	0.00391
Regression equation for the ratio of peak areas	$R_{FVS/SVS} = 0.0141C_{FVS}+0.0091$
Correlation coefficient (r)	0.9999
S_a	0.00231
S_b	0.00078
LOQ (µg mL^{-1})	1.638
LOD (µg mL^{-1})	0.540

Sensitivity

Limit of detection (LOD) and limit of quantification (LOQ) of the proposed method were calculated experimentally after getting the regression equation. LOD was expressed as the concentration of drug that generated a response to three times of the signal to-noise (S/N) ratio LOD=$3.3\times S_b/m$ and LOQ was 10 times of the S/N ratio LOQ=$10\times S_b/m$ (where S_b is the standard deviation of the intercept and m is the slope of the calibration curve) [29].

System suitability

120 µg mL^{-1} standard solution of FVS in the presence of 100 µg mL^{-1} of SVS was prepared and injected six times in to the HPLC system. System suitability parameters were checked to demonstrate that the LC system to be used was suitable for the intended application. The Results were within the preferable limits and represented in Table 2.

Table 2. System suitability parameters.

Parameters	FVS	SVS	Preferable levels [4]
Capacity factor (k')	3.35	6.03	2–10
Selectivity (α)	–	1.80	1.0–2.0
Resolution (R_s)	–	16.15	> 1.5
No. of theoretical plates (N)	35723	36025	> 2500
Tailing factor (T)	1.07	1.08	< 1.5
% RSD for six injections	1.09%	1.18%	<5%

Precision and accuracy

The precision (%RSD and standard analytical error (SAE)) was determined at two levels; intra- and inter-day precision. Different concentration levels of FVS and SVS (100 µg mL^{-1}) were examined five times within one day and at three succeeding days. The values were under the limit of 5% proving the adequate precision of the method (Table 3). For accuracy, the same concentrations of FVS and SVS was assayed by the developed method and then calculating the % recovery which is indicated by the excellent recovery as shown in Table 3.

Specificity

The resolution factor of peak purity of FVS was studied from the nearest resolving peak in order to assess the specificity of the method. The comparison of chromatogram scanned for standard and sample showed no peaks within a range of retention time. Therefore, the peak of FVS was pure and thus the presented HPLC method has specificity.

Table 3. Intra- and inter-day accuracy and precision results of FVS in pure samples by the presented HPLC method.

	Amount Taken (µg mL^{-1})	Found*±SD (µg mL^{-1})	RSD %	Recovery %	SAE
Intra–day	5.00	5.05±0.08	1.64	101.00	0.036
	25.00	25.23±0.29	1.14	100.92	0.129
	100.00	100.39±0.77	0.94	100.39	0.344
	200.00	206.17±1.25	0.59	103.08	0.559
Inter–day	5.00	5.03±0.09	1.79	100.60	0.040
	25.00	25.14±0.28	1.11	100.56	0.125
	100.00	100.09±0.81	0.81	100.09	0.362
	200.00	202.08±1.30	0.64	101.04	0.581

*Average of five determinations ± SD.

Robustness

Robustness was determined by evaluating the effect of a slight variation of certain parameters using five replicates of FVS standard (100 µg mL^{-1}). Robustness was found to be within the limits. The results shown in Table 4 indicated that the slight change in mobile phase composition and flow rate did not notably affect the system suitability. Thus, the method is robust.

Table 4. Results of robustness study of FVS.

Parameter variation	FVS			
	Assay %	RSD %	t_r	T
MeOH 65%	100.83	1.34	5.053	1.07
MeOH 70%	100.62	1.29	3.207	1.05
MeOH 75%	100.39	1.45	3.103	1.04
pH 4.6	101.09	1.48	3.236	1.08
pH 4.8	100.39	1.27	3.207	1.04
pH 5.0	100.93	1.37	3.174	1.05
flow rate 0.9 mL/min	100.82	1.47	4.178	1.09
flow rate 1.0 mL/min	100.98	1.26	3.207	1.06
flow rate 1.1 mL/min	100.62	1.21	2.750	1.03

Application to capsules

The improved method is sensitive and specific for the quantification of FVS and it is also subject to validation of various criteria, and thus has been applied for FVS assay in capsules. Each sample was analyzed in five replicates after drug extraction as mentioned above in the experimental section. FVS and SVS were eluted at their specific retention times and no interfering peaks were observed from the capsule excipients (Figure 4).
When data for recovery were compared statistically by Student's t-test (for accuracy) and F-test (for precision) using the pharmacopoeial method [4], the results of the new method showed satisfactory accuracy and precision. The calculated mean values were less than the theoretical ones at the 95% confidence level for five degrees of freedom [28] for both tests, which indicating no significant differences between the recoveries obtained from the two compared methods (Table 5).

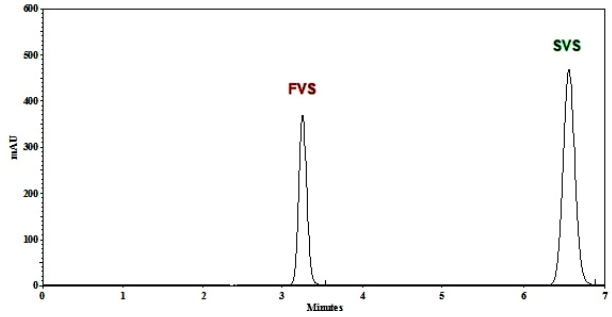

Figure 4. HPLC chromatogram of a mixture of 100 µg mL^{-1} FVS and 100 µg mL^{-1} SVS prepared from fluvastat 40 mg capsules at 305 nm.

Table 5. Assay of formulation of FVS by RP-HPLC.

Sample	HPLC Proposed method			Pharmaco. method
	Taken µg mL^{-1}	Found µg mL^{-1}	% Found	% Found
Fluvastatin 20	5.00	5.06	101.20	101.03
	100.00	100.28	100.28	100.95
	200.00	201.85	100.92	101.04
Mean[a]±SD			100.80±0.47	101.00±0.05
t			1.70	1.88
F			1.22	
Almastatin 40	5.00	5.04	100.80	100.83
	100.00	100.83	100.83	100.45
	200.00	202.33	101.16	101.01
Mean[a]±SD			100.93±0.20	100.76±0.28
t			1.91	2.08
F			1.18	
Fluvastate 40	5.00	5.10	102.00	101.67
	100.00	101.02	101.02	100.98
	200.00	200.27	100.13	100.04
Mean[a]±SD			101.05±0.93	100.80±0.82
t			1.88	2.16
F			1.32	

[a]Average of five determinations. At 95% confidence limit the theoretical *t* and *F*-values at five degree of freedom are *t* = 2.776 and *F* =6.26.

Conclusions

The proposed procedure is simple, inexpensive, reproducible, can be reliably used by almost every drug laboratory for routine analysis in drug quality control laboratories and more sensitive (LOD= 0.540 µg mL^{-1}) than the official method. The excellent recoveries indicate the absence of interference from frequently encountered excipients or additives. Also, when the presented method is compared with the advanced spectrophotometric techniques and similar methods reported for analysis FVS, it has a higher sensitivity and has a wider range of linearity. In addition, it is an environmentally friendly chromatographic procedure due to its low solvent consumption and short analytical runtime.

Conflict of interest

The author declares no conflicts of interest.

References

1. Walsh P. Physicians' Desk Reference (PDR). In: Montvale NJ, ed. Medical Economics Company Co.; 59th ed. 2005, p. 2326–2331.
2. Martindale, The Complete Drug Reference. Sweetman SC (editor). The Pharmaceutical Press, London, UK: 2005, p. 918.
3. Budavari S, eds. The Merck Index. 13th ed. New Jersey: Merck and Co. Inc.; 2001. p. 747–748.
4. British Pharmacopoeia. Her Majesty Stationery Office; London, UK, 2013.
5. Neves MMPS, Nouws HPA, Delerue-Matos C. Direct electroanalytical determination of fluvastatin in a pharmaceutical dosage form: batch and flow analysis. Anal Lett. 2008; 41(15): 2794–2804.
6. Dogan B, Tuncel S, Uslu B, Özkan SA. Selective electrochemical behavior of highly conductive boron-doped diamond electrodes for fluvastatin sodium oxidation. Diamond Relat Mat. 2007; 16(9): 1695–1704.
7. Özkan SA, Uslu B. Electrochemical study of fluvastatin sodium analytical application to pharmaceutical dosage forms, human serum, and simulated gastric juice. Anal Bioanal Chem. 2002; 372(4): 582–658.
8. Jin-long YAN, Xiu-ping LU. Determination of fluvastatin sodium by differential pulse voltammetry. J Chin Clin Med. 2006; 1(1): 33–36.
9. Moussa BA, Abadi AH, Abou-Youssef HE, Mahrouse MA. Spectroscopic and chromatographic determination of fluvastatin sodium in presence of its acid degradate. Int J PharmTech Res. 2010; 2(1): 875–898.
10. Assassi AL, Roy CE, Perovitch P, Auzerie J, Hamon T, Gaudin K. Green analytical method development for statin analysis. J Chromatogr A. 2015; 1380: 104–111.
11. Abdallah OM. RP-HPLC determination of three anti-hyperlipidemic drugs in spiked human plasma and in dosage forms. E-J Chem. 2011; 8(2): 753–761.
12. Akabari AH, Suhagia BN, Saralai MG, Sutariya VA. Development and validation of stability indicating RP-HPLC method for estimation of fluvastatin sodium in bulk and capsule dosage form. Eurasian J Anal Chem. 2017; 12(2): 87–105.
13. Gomes FP, García PL, Alves JMP, Singh AK, Kedor–Hackmann ERM, Santoro MIRM. Development and validation of stability-indicating HPLC methods for quantitative determination of pravastatin, fluvastatin, atorvastatin and rosuvastatin in pharmaceuticals. Anal Lett. 2009; 42(12): 1784–1804.
14. Saminathan J, Sankar ASK, Anandakumar K, Srinivasan S, Vetrichelvan T. Validated RP-HPLC

method for fluvastatin sodium in bulk and its dosage form. J Pharm Sci Res. 2009; 1(3): 90–96.
15. Maree S, Du Preez JL, Du Plessis LH, Du Plessis J, Gerber M. A novel HPLC method developed and validated for the detection and quantification of atorvastatin, fluvastatin, pitavastatin and pravastatin during transdermal delivery studies. Pharmazie 2020; 75: 164–166.
16. Akabari AH, Shah U, Solanki S, Patel MB, Suhagia BN. Development and validation of sensitive HPTLC method for quantitative analysis of fluvastatin sodium in bulk and pharmaceutical dosage form. Int J Pharm Res. 2014; 6(2): 73–78.
17. Ashour S, Bahbouh M, Khateeb M. Kinetic spectrophotometric determination of fluvastatin in pharmaceutical preparations. Int J Biomed Sci. 2010; 6(1): 19–26.
18. Ashour S, Khateeb M. A novel use of oxidative coupling reactions for determination of some statins (cholesterol–lowering drugs) in pharmaceutical formulations. Spectrochim Acta Part A. 2011; 78: 913–917.
19. Saminathan J, Sankar ASK, Anandakumar K, Vetrichelvan T. Simple UV spectrophotometric method for the determination of fluvastatin sodium in bulk and pharmaceutical formulations. E-J Chem. 2009; 6(4): 1233–1239.
20. Hotkar K, Bhosale D, Khanapure M, Kalyani N. Simple UV spectrophotometric method for the determination of fluvastatin. Int J Creat Res Thoughts. 2020; 8(3): 2023–2027.
21. Kamal MF, Morshedy S, Saad DA, Moneeb MS. Green atomic absorption spectroscopic methods for the determination of rabeprazole sodium and fluvastatin sodium in pure and pharmaceutical dosage forms. Int Res J Pure Appl Chem. 2021; 22(6): 8–14.
22. Dogrukol AKD, Kircali K, Tuncel M, Aboul–Enein HY. Validated analysis of fluvastatin in a pharmaceutical formulation and serum by capillary electrophoresis. Biomed Chromatogr. 2001; 15(6): 389–392.
23. Aslan SS, Sagirli O, Ersoy L. Development and validation of a GC-FID assay for determination of fluvastatin in pharmaceutical preparations. Quimica Nova. 2009; 32(9): 2347–2350.
24. Erk N. Rapid spectrophotometric method for quantitative determination of simvastatin and fluvastatin in human serum and pharmaceutical formulations. Die Pharmazie. 2002; 57(12): 817–819.
25. Stolarczyk M, Maślanka A, Apola A, Rybak W, Krzek J. Derivative spectrophotometric method for simultaneous determination of zofenopril and fluvastatin in mixtures and pharmaceutical dosage forms. Spectrochim Acta Part A. 2015; 148: 66–71.
26. Stolarczyk M, Apola A, Maślanka A, Kwiecień A, Opoka W. Spectrophotometric method for simultaneous determination of valsartan and substances from the group of statins in binary mixtures. Acta Pharm. 2017; 67(4): 463–478.
27. ICH, Q2 (R1). Validation of analytical procedures: text and methodology, Int. Conf. harm. Geneva; 2005.
28. Miller JN, Miller JC. Statistics and Chemometrics for Analytical Chemistry.Chichester, UK: Ellis Horwood; 2010.
29. Long GL, Winefordner JD. Limit of Detection. A Closer Look at the IUPAC Definition. Anal Chem. 1983; 55(7): 712A–721A.