RESEARCH ARTICLE

# Estimation of Atorvastatin Calcium and Vinpocetine in a Pharmaceutical Dosage form using Validated Simultaneous UV Spectroscopic and HPLC Methods

Rita R. Lala*, Amol S. Shinde

*Prin. K. M. Kundnani College of Pharmacy, Pharmaceutics Department, Mumbai: 400005, Maharashtra, India.*

***Received: 10th January, 2020; Revised: 21st Fabruary, 2020; Accepted: 10th March, 2020; Available Online: 25th March, 2021***

## ABSTRACT

The study aims to validate the Simultaneous equation (SE) method by UV spectroscopic method and by high-performance liquid chromatography (HPLC) method for the atorvastatin calcium (ATS) and vinpocetine (VIN) pharmaceutical formulations. SE method was developed and absorbance was measured at 246 and 273 nm for ATS and VIN, respectively. ATS and VIN's linearity range were 2-20 µg/ mL and 1-24 µg/mL, respectively. HPLC method was developed simultaneously to determine the ATS and VIN in tablet as a dosage form. The analysis has been done by using Agilent ZORBAX -C18 (250 X 4.6 mm, 5 um) and mobile phase containing acetonitrile: 0.2% orthophosphoric acid (70:30 v/v) at pH 5 (adjusted with Sodium hydroxide). The 1.0 mL/min flow rate was maintained, and detection was carried out at 238 nm (isosbestic point). The retention time of ATS and VIN was found to be 4.16 and 8.20 minutes, respectively. The calibration curves were linear in the concentration range 20–120 µg/ mL and 5–30 µg/ mL for ATS and VIN, respectively. The recoveries were found in the range by both methods. The proposed methods were validated successfully, which was found to be very precise, specific, and accurate; also suitable for ATS and VIN pharmaceutical tablet dosage form, and any dosage form where these two drugs will be present.

**Keywords:** Absorbance, Atorvastatin Calcium, Chromatogram, Isobestic point, Simultaneous equation method, Vinpocetine.

International Journal of Pharmaceutical Quality Assurance (2021); DOI: 10.25258/ijpqa.12.1.4

**How to cite this article:** Lala RR, Shinde AS. Estimation of Atorvastatin Calcium and Vinpocetine in a Pharmaceutical Dosage form using Validated Simultaneous UV Spectroscopic and HPLC Methods. International Journal of Pharmaceutical Quality Assurance. 2021;12(1):26-30.

**Source of support:** Nil.

**Conflict of interest:** None

## INTRODUCTION

Atorvastatin calcium is acting as a cholesterol-lowering agent. Atorvastatin is a reversible competitor of the 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMG-CoA), which catalyzes the conversion of HMG-CoA to mevalonate, an early and a rate-limiting step in cholesterol biosynthesis. It is also used in the prevention of cardiovascular events.[1] VIN is a vasoactive vinca alkaloid and synthetically derived from the *Vinca minor*. *L* plant. It is used in the treatment of cerebrovascular circulatory disorder and cognitive disorders.[2] Atorvastatin is used in the treatment of hyperlipidemia which showed some side effects when administered. The lipophilicity of ATS, which allows them to cross the blood-brain barrier, might be conferred to the protection of memory or adversely influence memory due to statin, which may inhibit the problem in myelin production. The after discontinuation of treatment, replenishment of myelin recovered. Hence, during the treatment, to avoid the side effect of ATS, we tried to use the VIN along with ATS, which may overcome problems associated with therapy.[3-6]

The UV and HPLC method is developed to validate for both drugs in a single dosage form and in combination with both drugs. UV Spectroscopic SE method analysis, HPLC method is not available as per literature and not reported for both drugs together. There are several methods are reported and developed for both drugs individually. Pharmacokinetic methods was developed for the determination of ATS loaded in nano lipid carriers.[7] HPLC method developed for the determination of Atorvastatin and its impurities in bulk drugs and tablets.[8] Reverse phase high performance liquid chromatography (RP-HPLC) method developed and validated for assessment of Atorvastatin calcium and Nicotinic acid in combined tablet dosage form Sangshetti JN, *et al*.[9] Pharmacokinetic HPLC method developed for the solid lipid nanoparticles (SLNs) to determine the vinpocetine in plasma samples.[10]

Hence, the aim and objective of the present research was to validate the simple, accurate, specific, and precise SE and HPLC method for the estimation of ATS and VIN in a combinational pharmaceutical dosage form.

**Author for Correspondence:* rr.lala@kmkcp.edu.in

## MATERIALS AND METHODS

SRS Pharmaceuticals, Mumbai gifted ATS calcium and VIN. Methanol, Acetonitrile (ACN), HPLC grade, orthophosphoric acid (OPA), Sodium hydroxide pellets (NaOH) were purchased from Merk, India. HPLC-grade water was used during the experiment.

### Instrument/Apparatus

UV- Vis Jasco 530 Double Beam Spectrophotometer was used with 1 cm quartz cell for UV Spectrometric analysis. JASCO HPLC LC-2000 Plus series, Pump PU-2080, detector UV-2075 plus, Chrompass software with Agilent ZORBAX C18 (250 × 4.6 mm, 5µm size) column was used. Mettler electronic balance was used for weighing.

### Chromatographic Conditions

The chromatographic conditions are shown in following Table 1.

### Preparation of Standard Stock Solutions for SE Method

10 mg ATS and VIN were weighed and transferred in a separate 10 mL volumetric flask. Both drugs were dissolved by using methanol and sonicated for 2 minutes. One mL solution was withdrawn and again diluted up to 10 mL to get 100 µg/ mL stock solutions of ATS and VIN, respectively.

The serial dilutions of stock solutions were prepared in the range of 4, 8, 12, 16, and 20 µg/mL and 1, 2, 3, 4, and

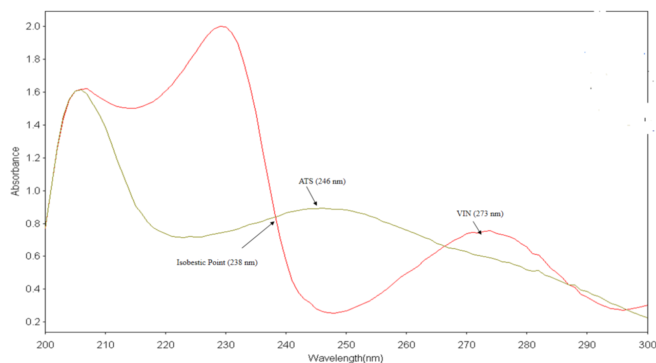


**Figure 1:** Overlay UV absorbance spectra of ATS and VIN

**Table 1:** Chromatographic conditions for HPLC method development

| *Parameters* | *Specification* |
|---|---|
| HPLC Pump | Jasco Pump PU-2089 |
| HPLC Detector | Jasco UV-2070 Plus UV Detector |
| Column | Agilent Zorbax $C_{18}$ |
| Elution | Isocratic |
| Dimension of column | 250 × 4.6 mm, 5 µm Size |
| Flow rate | 1 mL/min |
| Mobile phase | ACN :( 0.2%) OPA [70:30]<br>NaOH was used to maintain pH 5 |
| Inject volume | 10 µL |
| Absorbance maxima | 238 nm |
| Run time | 20 mins |
| Retention time of ATS and VIN | 4.16 and 8.20 mins |

5 µg/mL for ATS and VIN respectively by using methanol. The working standards were scanned in the range of 200-400 nm to determine the ʎ max of drugs. The absorbances were measured at 246 nm and 273 nm for ATS and VIN. The overlay and individual ʎ max of both drugs were shown in Figure 1. The standard curves were plotted of different concentrations measured at ʎ max wavelengths. The concentrations of ATS (X) and VIN (Y) were determined in the sample solution and marketed formulation by using the following formula

In the Simultaneous equation method, absorbance's of both drugs were measured at two selected ʎ max wavelengths of both drugs i.e., 246 and 273 nm, respectively.

$$C_X = A_2ay_1 - A_1ay_2/ax_2ay_1 - ax_1ay_2 \quad ...1$$

$$C_Y = A_1ax_2 - A_2ax_1/ax_2ay_1 - ax_1ay_2 \quad ...2$$

CX and CY are the concentrations of ATS and VIN, A1 and A2 are the concentration of sample solution at 246 and 273 nm, respectively. $ax_1$, $ax_2$ is the absorptivity of ATS at 246 nm and $ay_1$ and $ay_2$ are the absorptivities of VIN at 273 nm, respectively.[11] The method was developed and validated as per the International Conference on Harmonization Guidelines [12].

The concentration range for ATS was 4, 8, 12, 16, and 20µg/mL and 1, 2, 3, 4, 5 µg/ mL for VIN. Accuracy and recovery of the proposed method were carried out by the standard spiking (API) method at three different levels, i.e., 50%, 100% and 150%. Precision was carried out at three different time intervals on same day (Intraday) and on three different days (Interday) for ATS and VIN. Both drugs were analyzed in triplicate at three concentrations, i.e., 50, 100, and 150%. Limit of detection (LoD) and limit of quantification (LoQ) was calculated by using the formula 3.3S.D/S and 10S.D/S, where SD is the standard deviation of Y-intercept and S is the slope value from the calibration graph (Table 2).

### Preparation of Sample Solution (Marketed Tablets) for SE Method

Twenty tablets of ATS (Lipvas tablet) and VIN (Cognitol Tablet) were weighed accurately and finely powdered separately. Powder equivalent to ATS 10 mg and 10 mg VIN was accurately weighed into a 10 volumetric flask, dissolved in methanol and sonicated for 10 minutes. The individual solutions of ATS and VIN were filtered through 0.45 µm nylon membrane filter. 0.8 mL from ATS solution and 0.2 mL from VIN solution were added to 100 mL volumetric flask. The sample solution was analyzed at 246 and 273 nm by using UV Vis Spectrophotometer. The amount of ATS and VIN was calculated by using a linear regression equation.

### Preparation of Standard Stock Solutions for HPLC Method

The standard stock solutions of 1000 µg/mL ATS and VIN were prepared by dissolving 10 mg of each drug in a separate 10 mL volumetric flask using methanol. The further dilutions were prepared by using Mobile phase as a diluent. The concentration range was used for calibration, i.e. 20–120 µg/mL and 5-30 µg/mL for ATS and VIN.

**Table 2:** Validation of ATS and VIN by SE method and HPLC method (n=3)

| | *SE method* | | | *HPLC method* | | |
|---|---|---|---|---|---|---|
| *Parameters* | *ATS* | *VIN* | | *ATS* | *VIN* | |
| Wavelength (nm) | 246 | 273 | | 238 | | |
| Linearity range (µg/ mL) | 2-20 | 1-24 | | 20-120 | 5-30 | |
| Slope and Y intercept | 0.041, 0.0032 | 0.0339, 0.0347 | | 479.62, 1172.9 | 284.09, 108.18 | |
| Regression Coefficient ($r^2$) | 0.999 | 0.997 | | 0.9979 | 0.997 | |
| *Accuracy Levels* | *% Recovery* | | | | | |
| 50% | 101.403 | 99.85 | | 100.99 | 100.825 | |
| 100% | 101.075 | 98.988 | | 100.589 | 99.30 | |
| 150% | 100.873 | 101.15 | | 99.972 | 99.71 | |
| *SE method* | *ATS % RSD* | | | *VIN % RSD* | | |
| Precision Levels | 50% | 100% | 150% | 50% | 100% | 150% |
| Inter day | 0.593 | 0.253 | 0.175 | 1.683 | 1.024 | 1.237 |
| Intra day | 0.638 | 0.457 | 0.466 | 1.704 | 1.039 | 1.512 |
| *HPLC method* | *ATS % RSD* | | | *VIN % RSD* | | |
| Precision Levels | 50% | 100% | 150% | 50% | 100% | 150% |
| Inter day | 0.801 | 0.806 | 0.418 | 1.074 | 1.265 | 0.777 |
| Intra day | 0.816 | 0.811 | 0.437 | 1.119 | 1.473 | 0.803 |
| *SE Method* | *ATS* | *VIN* | | *HPLC Method* | *ATS* | *VIN* |
| LOD (µg/ mL) | 0.063 | 0.073 | | LOD (µg/ mL) | 0.112 | 0.0125 |
| LOQ (µg/ mL) | 0.191 | 0.224 | | LOQ (µg/ mL) | 0.356 | 0.078 |

**Table 3:** System suitability parameters of HPLC method

| *Parameter* | *ATS (Mean ± % RSD)* | *VIN (Mean ± % RSD)* |
|---|---|---|
| Number of theoretical plates | 3774.67 ± 1.24 | 6215.12 ± 1.44 |
| Asymmetry factor | 1.5 ± 0.66 | 1.7 ± 0.89 |
| Resolution factor | 5.53 ± 1.0 | 9.84 ± 1.32 |
| Retention time (mins) | 4.16 ± 1.31 | 8.20 ± 0.86 |

Each value represents mean ± SD, n=3.

### Preparation of Sample Solution (Marketed Tablets) for HPLC

Twenty tablets of ATS (Lipvas Tablet) and VIN (Cognitol Tablet) were weighed accurately and finely powdered separately. Powder equivalent to ATS 10 mg and 10 mg VIN was accurately weighed into a 10 volumetric flask, dissolved in methanol and sonicated for 10 minutes. The individual solutions of ATS and VIN were filtered through 0.45 µm nylon membrane filter. 0.8 mL from ATS solution and 0.2 mL from VIN solution were added to 10 mL volumetric flask. The mobile phase was used for volume makeup. The amount of ATS and VIN was calculated from linear regression equation.

### Analytical Validation

*System Suitability*

System Suitability is played an important parameter to develop the method. This is mainly performed to check the resolution and reproducibility of the method also, the suitability of method is also confirmed for analysis. It includes the following parameter, which is mentioned in Table 3. 80 µg/mL and 20 µg/mL standard solution concentration of ATS and VIN was injected into the system (n = 6). Results of all the parameters with mean and % RSD was stated in Table 3.

*Specificity*

The developed method was validated as per ICH guidelines.[12] To assess the method specificity and interference of any mobile phase composition to the retention time of both drugs during analysis. Thus, the blank mobile phase was injected into the HPLC system at the following system. Suitable conditions in Table 1 and chromatogram were observed, and the peaks' responses were measured if any.

*Linearity and Range*

Linearity was done by plotting the calibration curve of ATS and VIN. The calibration curve for ATS and VIN was obtained by plotting the peak area versus concentration of ATS in the range of 20–120µg/mL and peak area versus VIN concentration in the range of 5–30 µg/mL.

*Accuracy and Precision*

Accuracy and recovery of the proposed method were carried out by standard spiking (API) method in known concentration at three different recovery levels i.e., 50, 100, and 150%. Precision was carried out by measuring the responses at three different times on the same day (Intraday) and on three different days (Interday) for three different levels (50, 100 and 150%) of ATS and VIN in a triplicate.

*Robustness*

Robustness of the developed method studied for to check the effects of operating conditions on the results. The standard concentration of ATS and VIN, i.e., 80: 20 µg/mL, was robust.

The operating analytical conditions were changed such as flow rate, pH, and composition of mobile phase. The %RSD was calculated for all the varied conditions to assess robustness.

*Limit of Detection and Limit of Quantitation*

LOD and LOQ were calculated by using the formula 3.3S.D/S and 10S.D/S where SD is the standard deviation of response and S is the slope value from the calibration curve.

## RESULTS AND DISCUSSION

### SE Method

The calibration curves were plotted by absorbance vs concentration. The concentrations were followed the Beers Lamberts law, and Regression ($r^2$) values (coefficient of correlation) were found for ATS and VIN are 0.997 and 0.999 respectively. The UV and HPLC validation of all the parameters as per ICH guidelines are shown in Table 2.

Recovery studies for both drugs are 101.403, 101.075, 100.873% for ATS and 99.85, 98.988 and 101.15% for VIN. The results revealed that the proposed method was highly accurate and reproducible. The precision study was done at three different levels by measuring the sample solution's absorbance without changing any procedure. The %RSD was found to be in the range i.e., less than 2 for ATS and VIN respectively at interday and intraday. Hence we can conclude that the results of precisions are acceptable.

The minimum amount of concentration, i.e., LoD, was detected and estimated for ATS was found to be 0.063 µg/ mL, and LOQ was 0.191µg/ mL. The LoD and LoD for VIN were found to be 0.073 µg/ mL and 0.224 µg/mL. From the results we can conclude that the minimal concentration can be determined precisely by the proposed developed and validated method.

### HPLC Method

The different types of solvents like ACN, methanol, water, and ratios were used for the development of the HPLC method.

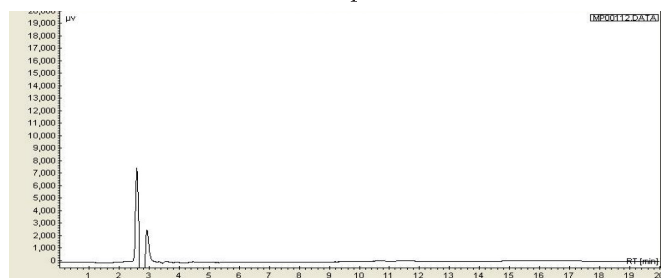

**Figure 2:** Chromatogram of blank mobile phase

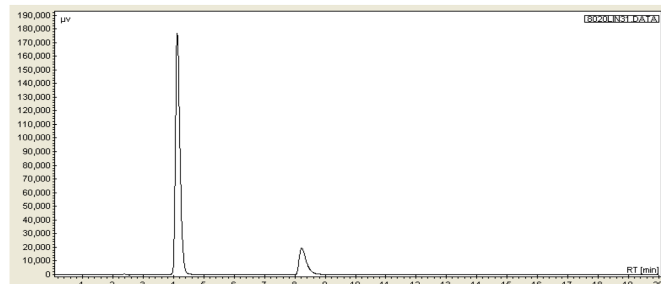

**Figure 3:** Chromatogram of standard solution

Methanol and HPLC water unable to elute the VIN, and in the same manner when we tried ACN, HPLC water in a different ratio (80:20). OPA was used in 0.2% concentration in the 80:20 ratio, and pH 5 was adjusted using NaOH. Using this mobile phase and flow rate 1 mL/min got the good peak and we have optimized this mobile phase and all the conditions and adjusted the retention time by reducing the organic solvent ACN in the mobile Phase (70:30).

### Specificity

After injection, the blank mobile phase into the HPLC system. The chromatogram has not shown in any type of interference at the retention time of ATS and VIN. The chromatogram is shown in Figure 2.

### Linearity and Range

A good correlation was found between the concentrations and peak area, which followed the Beers lamberts law. The concentration range for ATS 20-120 (µg/mL) and for VIN 5–30 (µg/mL) showed the regression coefficient of 0.997 for both drugs, respectively. The chromatogram of the standard solution is shown in Figure 3.

### Accuracy and Precision

The standard spiking method was used for the percent recovery experiment. The resulting spiked sample solutions (50, 100 and 150%) were analyzed in triplicate, and the results were compared with the standard concentration in the calibration curve and expressed as a percentage. The percent recoveries of ATS and VIN were found to be in the range of 98.888 to 101.403 and 99.30–100.99 respectively. The precision was satisfactory and confirmed by interday and intraday at three different concentration levels (50, 100, and 150%). The % RSD was not more than 2 for ATS as well as VIN.

### Robustness

The robustness of the method was evaluated by changing the operating analytical conditions. The % RSD was found in the range for the different varied conditions. There was not any change observed in the results and thus, it revealed that the developed method is robust. The results of robustness studies are depicted in Table 4.

### LoD and LoQ

The minimum concentration was assessed by the HPLC method for ATS 0.112 µg/mL and for VIN 0.0125 µg/mL. The HPLC method quantified the minimum concentration for ATS 0.356µg/ mL and for VIN 0.078 µg/mL.

### Assay results of Marketed Tablets

The marketed ATS and VIN tablets were analyzed by the proposed, validated SE and HPLC method. The sample solutions of respective concentrations for SE and HPLC method were analyzed. The percent recovery was calculated by comparing with label claim and the amount found—the percent recovery for ATS 98.9 and VIN 99.4 by SE method. By HPLC method 100.17 and 100.02 recoveries was found for ATS and VIN, respectively. The assay results of marketed dosage form by SE and HPLC method in Table 5. The chromatogram of the marketed sample is shown in Figure 4.

**Table 4.** Results of robustness studies

| *Chromatographic changes* | *Injected conc (µg/m)* | *Obtained conc (µg/mL)* | *% RSD* | *Chromatographic changes* | *Injected conc (µg/mL)* | *Obtained conc (µg/mL)* | *%RSD* |
|---|---|---|---|---|---|---|---|
| *Flow rate ( mL/min)* | | | | *Flow rate ( mL/min)* | | | |
| 0.9 | 80 | 79.90 ± 0.4 | 0.51 | 0.9 | 20 | 19.96 ± 0.1 | 0.622 |
| 1 | 80 | 80.02 ± 0.237 | 0.297 | 1 | 20 | 20.09 ± 0.141 | 0.703 |
| 1.1 | 80 | 80.11 ± 0.665 | 0.831 | 1.1 | 20 | 20.16 ± 0.161 | 0.799 |
| pH | | | | pH | | | |
| 4.8 | 80 | 79.58 ± 0.7 | 0.835 | 4.8 | 20 | 19.89 ± 0.15 | 0.753 |
| 5.0 | 80 | 80.27 ± 0.548 | 0.638 | 5.0 | 20 | 20.15 ± 0.168 | 0.828 |
| 5.2 | 80 | 80.63 ± 0.364 | 0.452 | 5.2 | 20 | 20.34 ± 0.19 | 0.945 |
| Composition of mobile phase (ACN: 0.2% OPA) | | | | Composition of mobile phase (ACN: 0.2% OPA) | | | |
| 65:35 | 80 | 80.18 ± 0.453 | 0.565 | 65:35 | 20 | 20.08 ± 0.17 | 0.840 |
| 70:30 | 80 | 80.64 ± 0.5 | 0.609 | 70:30 | 20 | 20.36 ± 0.1 | 0.516 |
| 75:25 | 80 | 80.82 ± 0.2 | 0.296 | 75:25 | 20 | 20.55 ± 0.15 | 0.728 |

Each value represents mean ± SD, n = 3

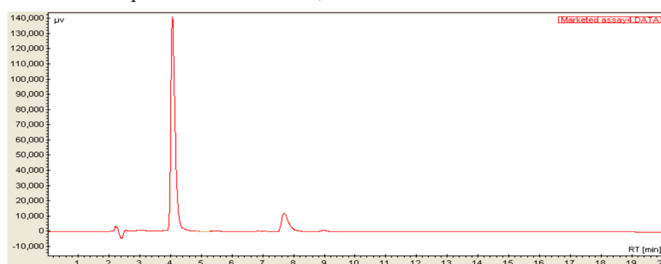


**Figure 4:** Chromatogram of marketed tablet formulation

**Table 5:** Assay results of combinatorial dosage form using proposed method

| *Method* | *UV Method* | | *HPLC Method* | |
|---|---|---|---|---|
| *Drug* | *ATS* | *VIN* | *ATS* | *VIN* |
| Label Claim (mg) | 10 | 5 | 10 | 5 |
| Amount found (mg) | 9.89 | 4.97 | 10.017 | 5.001 |
| % Recovery | 98.9 | 99.4 | 100.17 | 100.02 |

Each value represents mean, n = 3

## CONCLUSION

The proposed SE method and HPLC method for the combinational drug were validated successfully and revealed satisfactory results for all the parameters. Both methods showed all the results were quantified, % recovery and %RSD is also in the range that indicated that methods are very accurate, precise and specific UV spectroscopic methods and HPLC for the combination of ATS and VIN. Thus these methods can be used to estimate both drugs and combinational pharmaceutical dosage form of both drugs. Proposed methods are a time-saving method and economical. We have made a successful attempt to analyze the two drugs in two different tablet dosage form. We mixed it for analysis in a specific combination ratio and successfully analyzed and estimated ATS and VIN as per the label claim.

## REFERENCES


1. Kim MS, Jin SJ, Kim JS, Park HJ, Song HS, Neubert RHH, Wang SJP. Preparation, characterization and in vivo evaluation of amorphous atorvastatin calcium nanoparticles using supercritical antisolvent (SAS) process. European Journal Pharmaceutics and Biopharmaceutics. 2008; 69: 454–465.
2. Zhang Y, Li J, Yan C. An update on Vinpocetine: New discoveries and clinical implications. European Journal Pharmacology. 2018; 819: 30–34.
3. King DS, Wilburn AJ, Wofford MR, Harrell TK, Lindley BJ, Jones DW. Cognitive impairment associated with atorvastatin and simvastatin. Pharmacotherapy. 2003; 23(12): 1663–1667.
4. Serrano-Pozo A, Vega GL, Lutjohann D. Effects of simvastatin on cholesterol metabolism and Alzheimer disease biomarkers. Alzheimer Disease and Associated Disorders. 2010; 24(3): 220–226.
5. Klopfleisch S, Merkler D, Schmitz M. Negative impact of statins on oligodendrocytes and myelin formation in vitro and in vivo. Journal of Neuroscience. 2008; 28(50): 13609–13614.
6. Miron VE, Zehntner SP, Kuhlmann T. Statin therapy inhibits remyelination in the central nervous system. American Journal of Pathology. 2009; 174 (5): 1880–1890.
7. Elmowafy M, Ibrahim HM, Ahmed MA, Shalaby K, Salama A, Hefesha H. Atorvastatin-loaded nanostructured lipid carriers (NLCs): strategy to overcome oral delivery drawbacks. Drug Delivery. 2017; 24(1): 932-941.
8. Erturk S, Esram SA, Lale E, Samiye F. An HPLC method for the determination of Atorvastatin and its impurities in bulk drugs and tablets. Journal Pharmaceutical and Biomedical Analysis. 2003; 33 (5): 101–123.
9. Sangshetti JN, Mohammed A, Zahid Z, Rana ZA, Gonjari I, Dehghan MHG. Development and validation of RP-HPLC method for determination of Atorvastatin calcium and Nicotinic acid in combined tablet dosage form. Journal of Saudi Chemical Society. 2016; 20: S328-S333.
10. Yi FL, Da WC, Li XR, Xiu LZ, Jing Q. Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability. Journal of Controlled Release. 2006; 114: 53–59.
11. Sawant RL. Spectrophotometric methods for simultaneous estimation of rabeprazole sodium and aceclofenac from the combined capsule dosage form. Pharm Methods. 2011; 2(3): 193–197.
12. ICH Guidelines Q2 (R1). Validation of analytical procedures: text and methodology, international conference on harmonization. Geneva. 2005.