**Research Article** **CODEN: AJPAD7** **ISSN: 2321 - 0923**

**Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry**

**Journal home page: www.ajpamc.com**

# DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR DETERMINATION OF LOPINAVIR IN BULK AND TABLET DOSAGE FORM

**Sonali Narsing Koli*[1], Rekha Pritishkumar Mudke[1], Adavirao Venkatrao Belvotagi[2]**

[1*]Department of Quality Assurance, D.S.T.S. Mandal's College of Pharmacy, Solapur, Maharashtra, India.
[2]Department of Pharmaceutical Chemistry, D.S.T.S. Mandal's College of Pharmacy, Solapur, Maharashtra, India.

**ABSTRACT**

A simple, economic, sensitive, precise and accurate UV spectroscopic method was developed and validated for determination of Lopinavir in bulk and tablet dosage form. Adequate drug solubility and maximum assay sensitivity was found in Acetonitrile at 220nm. Calibration graph constructed at 220 nm was linear in concentration range of 10-30 µg/ml with correlation coefficient of 0.999. The method was validated as per ICH guidelines in terms of linearity (within 10-30 µg/ml), accuracy (% recovery), precision (inter-day and intraday), specificity and robustness. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.130µg/ml and 0.394µg/ml respectively. Therefore, the proposed method is suitable and can be adopted for the determination of Lopinavir from pharmaceutical dosage form in routine quality control analysis.

**KEYWORDS**

Lopinavir, UV Spectrophotometer, Acetonitrile and Method validation.

**Author for Correspondence:**

Sonali Narsing Koli,
Department of Quality Assurance,
D.S.T.S. Mandal's College of Pharmacy,
Solapur, Maharashtra, India.

**Email:** kolisonali55@gmail.com

## INTRODUCTION

Lopinavir is an anti-HIV, anti-infective and antiretroviral agent. Lopinavir is a Protease Inhibitor. The mechanism of action of lopinavir is as a HIV Protease Inhibitor, and P-Glycoprotein Inhibitor, and Cytochrome P450 3A Inhibitor, and Organic Anion Transporting Polypeptide 1B1 Inhibitor. Lopinavir is chemically designated as (2S)-N-[(2S, 4S, 5S)-5-[[2-(2, 6-dimethylphenoxy) acetyl] amino]-4-hydroxy-1, 6-diphenylhexan-2-yl]-3-methyl-2-(2-oxo-1, 3-diazinan-1-yl) butanamide.

Its molecular formula is $C_{37}H_{48}N_4O_5$ and molecular formula is 628.81gm/mol[1].

Lopinavir is white fine powder soluble in methanol, ethanol, acetonitrile, chloroform, Dimethyl form amide, dimethyl sulfoxide. Literature survey reveals that the Lopinavir has been estimated: in rat plasma and pharmacokinetics study by LC[3], by UV Spectroscopic method[4-5], by HPLC[6-8], *in vitro* study in soft gel capsules[9], by HPTLC method[10]. The present work is a simple, sensitive, accurate and precise Spectrophometric Method for the estimation of Lopinavir in API and its Pharmaceutical Dosage Forms with the help of Acetonitrile solvent.

## MATERIAL AND METHODS

### Instruments

For weighing, a calibrated weighing balance (Shimadzu) of 1mg sensitivity was used. A Systronic UV-visible double beam spectrophotometer- 2201 was used. All the glass wares and were made of borosilicate and were calibrated.

### Chemicals

Lopinavir pure drug was gifted by Aurobindo Pharma Ltd., India and Tablets of 200 mg strength were purchased from the local pharmacy in Solapur under commercially available brand name Lopimune (Cipla), Acetonitrile LR was used in this study.

### UV Spectroscopic Method

### Solvent Selection

Lopinavir is soluble in Acetonitrile (ACN) so, ACN is used as the solvent.

### Preparation of Standard Stock Solution

The standard stock solution Lopinavir (LPR) was prepared by transferring, accurately weighed 10 mg of Lopinavir into 10mL volumetric flask containing 5mL Acetonitrile, dissolved properly. Then volume was made up to the mark by using Acetonitrile to give a concentration of 1000µg/mL. From this, 2.5mL of the solution was transferred to a 25mL volumetric flask and make up the volume with Acetonitrile to give a concentration of 100µg/mL which is a standard stock solution and it is further diluted with ACN to get concentration range of 10-30µg/mL.

### Determination of Absorption Maxima

The standard stock solution of 100µg/mL was scanned in the range of 400-200nm to determine the wavelength of Maximum Absorption. The drug showed Absorption maxima at 220nm.

### Preparation of Calibration Curve

For the preparation of calibration curve, the concentration of 10-30µg/mL were prepared by pipetting out 1, 1.5, 2, 2.5 and 3mL of the 100µg/mL solution into 10mL volumetric flasks and made up the volume with Acetonitrile.

The absorbance of each solution was measured at 220nm against Acetonitrile as blank. Calibration curve of the Lopinavir was plotted by taking the absorbance obtained on the y-axis and concentration of the solution on the x-axis. The curve showed linearity in the range of 10-30µg/mL with correlation coefficient 0.999. (Showen in Figure No.3)

### Quantitative Analysis of Tablet Dosage Form

Twenty tablets were weighed accurately and powdered. Powder equivalent to 10mg Lopinavir (LPR) was weighed and transferred to a 10 mL volumetric flask. It was dissolved in 10mL Acetontrile and sonicated for 15 minutes to get a homogeneous solution. Then it was filtered through a 0.45µ Whatman filter paper. A final concentration of 100µg/mL of LPR was prepared. This solution was filtered through filter paper to remove some un-dissolved excipients. After filtration, from this 2.5mL was taken and diluted to 10mL with ACN which gives 25µg/mL solution and the absorbance of the solution was measured at 220 nm.

### Method Validation

The developed method was validated as per ICH guidelines for the following parameters:

### Linearity

1, 1.5, 2, 2.5, 3mL of standard LPR solution was transferred into a series of 10mL volumetric flasks. The volume was made up to the mark with ACN to obtain the concentration of 10, 15, 20, 25, 30µg/mL. Then absorption of these solutions was recorded and the graph was plotted of absorption against concentration. The correlation coefficient ($r^2$) of least square linear regression of LPR was calculated.

**Range**

The Range of the analytical method was decided from the interval between upper and lower level of calibration curve by plotting curve.

**Accuracy**

Recovery study was carried out by the standard addition method by adding a known amount of LPR to the pre-analyzed sample at three different concentration levels that is 80%, 100%, 120% of assay concentration and percent recovery were calculated. 1.5mL of tablet solution was transferred to 4 different 10mL volumetric flasks (labelled as blank, 80%, 100%, 120%) separately and 0, 0.8 , 1, 1.2mL of 100µg/mL standard solution was added respectively and the volume was made up to the mark with ACN. Absorbances were noted for these samples. Standard deviation and % RSD was calculated. Accuracy is reported as % recovery, which was calculated from the expression as equation given below,

% Recovery = Observed value / True value ×100

**Precision**

The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same sample under the prescribed conditions. The precision of the method was determined in terms of repeatability and intra-day and inter-day precisions. Intra-day and inter-day precision (Intermediate Precision)

Intraday precision was determined by analyzing the drugs at concentrations (20µg/mL) and each concentration for three times, on the same day. Inter-day precision was determined similarly, but the analysis being carried out daily, for two consecutive days.

**Repeatability**

Repeatability of the method was determined by analyzing six samples of same concentrations of the drug (20µg/mL). Absorbance of each was measured.

**Robustness**

The robustness of the developed method is its capacity to remain unaffected by small changes in altered conditions. To determine the robustness of the method, the wavelength of analysis was deliberate and the assay was evaluated. The effect of detection wavelength was studied at ±5 nm.

**Ruggedness**

Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

**Limit of Detection**

Detection limit was determined based on the standard deviation of absorbance of same concentration that is a standard solution of LPR (20µg/mL) and LOD calculated by LOD = 3.3 (SD/S) Where, SD- standard deviation; S= slope of the curve

**Limit of Quantification**

Quantification limit was determined based on the standard deviation of peak area of same concentration that is standard solution LPR (20µg/mL) prepared six times and LOQ calculated by LOD = 10(SD/S) Where, SD= standard deviation; S= slope of Curve.

## RESULTS

Determination of wavelength of maximum absorption the wavelength of maximum absorption was found to be 220 nm.

**Linearity**

The linearity of this method was determined at ranges from 10-30 µg/mL for Lopinavir. The regression equation was found to be *Y=0.0191x +0.031*, $R^2$=0.999.

The linearity for Lopinavir was found to be linear in the range of 10-30 µg/mL with $R^2$= 0.999 and the straight line equation as *y= 0.00191x+0.031*

**Accuracy**

The accuracy of the analytical method for Lopinavir was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 220nm and results were expressed in terms of % recoveries.

**Precision**

The precision (measurement of intra-day, inter-day, repeatability) results showed good reproducibility with the relative standard deviation (% RSD) below 2.0 %. This indicated that method was highly precise.

**Ruggedness (20µg)**

Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

## DISCUSSION

**Preliminary Analysis of Lopinavir**

Preliminary analysis of Lopinavir such as description, solubility was performed.

**UV-spctrophotometry for Lopinavir**

Lopinavir being UV absorbing has been successfully employed for its quantitative determination by UV Spectrophotometric method. Being soluble in Acetonitrile, stock solutions and working standards were prepared in Acetonitrile .The maximum wavelength of absorption of drug was determined by taking scan of the drug solution in the UV region (200-400 nm). The correlation of the standard curve for the drug was 0.999. The accuracy was from 99.56-102% at 220nm. The proposed method showed absorption maxima at 220nm and obeyed Beer's law in the concentration range of 10-30µg/mL. The limit of detection (LOD) was found to be 0.130µg/mL and limit of quantification (LOQ) to be 0.394µg/mL respectively. All statistical data prove validity of the proposed method, which can be applied for routine analysis of Lopinavir.

**Assay of tablet formulation**

Amount of drug present in tablet formulation was calculated using equation at 220nm, and *y=0.0191 xs+0.031* and amount of Lopinavir were found to be 99 % of label claim respectively. This method can be employed for routine analysis of Lopinavir.

**Table No.1: Results obtained in the determination of LPR in tablet dosage form**

| S.No | Tablet formulation | Label claim | Amount taken | Amount found | Assay % |
|---|---|---|---|---|---|
| 1 | Lopimune | 200 mg | 25 µg/mL | 24.9µg/mL | 99.60% |

**Table No.2: Linearity table**

| S.No | Conc. | Absorbance |
|---|---|---|
| 1 | 10 | 0.221 |
| 2 | 15 | 0.313 |
| 3 | 20 | 0.420 |
| 4 | 25 | 0.509 |
| 5 | 30 | 0.600 |

**Table No.3: Table for accuracy**

| S.No | Level of % Recovery | Amount of tablet sample (mL) | Amount of standard drug added (µg/mL) | Amount added (µg) | Amount found (µg/mL) | % Recovery |
|---|---|---|---|---|---|---|
| 1 | 0 | 1.5 | 0 | 0 | 0 | 0 |
| 2 | 80 | 1.5 | 0.8 | 23 | 22.9 | 99.56% |
| 3 | 100 | 1.5 | 1 | 25 | 25.2 | 100.8% |
| 4 | 120 | 1.5 | 1.2 | 27 | 27.8 | 102% |

**Intra-day Precision**

**Table No.4: Intra-day morning precision**

| S.No | Concentration (µg/mL) | Absorbance | SD | % RSD |
|---|---|---|---|---|
| 1 | 20 | 0.446 | | |
| 2 | 20 | 0.446 | | |
| 3 | 20 | 0.445 | 0.000753 | 0.169 |
| 4 | 20 | 0.446 | | |
| 5 | 20 | 0.445 | | |
| 6 | 20 | 0.447 | | |
| | | $\bar{y}=0.445$ | | |

**Table No.5: Intra-day afternoon precision**

| S.No | Concentration (µg/mL) | Absorbance | SD | % RSD |
|---|---|---|---|---|
| 1 | 20 | 0.455 | | |
| 2 | 20 | 0.454 | | |
| 3 | 20 | 0.450 | 0.0001751 | 0.0386 |
| 4 | 20 | 0.454 | | |
| 5 | 20 | 0.454 | | |
| 6 | 20 | 0.453 | | |
| | | $\bar{y} = 0.453$ | | |

**Table No.6: Intra-day evening precision**

| S.No | Concentration (µg/mL) | Absorbance | SD | % RSD |
|---|---|---|---|---|
| 1 | 20 | 0.434 | | |
| 2 | 20 | 0.436 | | |
| 3 | 20 | 0.435 | | |
| 4 | 20 | 0.435 | 0.000753 | 0.1731 |
| 5 | 20 | 0.435 | | |
| 6 | 20 | 0.436 | | |
| | | $\bar{y} = 0.435$ | | |

**Inter-day Precision**

**Table No.7: Inter-day morning precision study**

| S.No | Concentration (µg/mL) | Absorbance | SD | % RSD |
|---|---|---|---|---|
| 1 | 20 | 0.465 | | |
| 2 | 20 | 0.463 | | |
| 3 | 20 | 0.464 | | |
| 4 | 20 | 0.464 | 0.000753 | 0.1622 |
| 5 | 20 | 0.465 | | |
| 6 | 20 | 0.464 | | |
| | | $\bar{y}$ = 0.464 | | |

**Table No.8: Inter-day afternoon precision study**

| S.No | Concentration (µg/mL) | Absorbance | SD | % RSD |
|---|---|---|---|---|
| 1 | 20 | 0.470 | | |
| 2 | 20 | 0.471 | | |
| 3 | 20 | 0.470 | | |
| 4 | 20 | 0.470 | 0.000516 | 0.109 |
| 5 | 20 | 0.471 | | |
| 6 | 20 | 0.470 | | |
| | | $\bar{y}$ = 0.470 | | |

**Table No.9: Interday evening precision study**

| S.No | Concentration (µg/mL) | Absorbance | SD | % RSD |
|---|---|---|---|---|
| 1 | 20 | 0.488 | | |
| 2 | 20 | 0.489 | | |
| 3 | 20 | 0.488 | | |
| 4 | 20 | 0.488 | 0.000516 | 0.105 |
| 5 | 20 | 0.489 | | |
| 6 | 20 | 0.488 | | |
| | | $\bar{y}$ = 0.488 | | |

**Repeatability**

**Table No.10: Repeatability study**

| S.No | Concentration (µg/mL) | Absorbance | SD | % RSD |
|---|---|---|---|---|
| 1 | 20 | 0.455 | | |
| 2 | 20 | 0.455 | | |
| 3 | 20 | 0.454 | | |
| 4 | 20 | 0.455 | 0.00408 | 0.89 |
| 5 | 20 | 0.455 | | |
| 6 | 20 | 0.455 | | |
| | | $\bar{y}$ = 0.454 | | |

**Limit of Detection**

**Table No.11: For Limit of Detection**

| | |
|---|---|
| LOD (µg/mL) | 0.130 µg/mL |

**Limit of Quantification**

**Table No.12: For Limit of Quantification**

| | |
|---|---|
| LOQ (µg/mL) | 0.394 µg/mL |

**Robustness (30µg)**

**Table No.13: Robustness study**

| S.No | Wavelength (nm) | Absorbance | SD | % RSD |
|---|---|---|---|---|
| 1 | 220 | 0.487 | | |
| 2 | 221 | 0.489 | | |
| 3 | 222 | 0.494 | | |
| 4 | 223 | 0.497 | 0.08122 | 1.8 |
| 5 | 224 | 0.460 | | |
| 6 | 225 | 0.422 | | |
| 7 | 226 | 0.405 | | |
| 8 | 227 | 0.389 | | |
| 9 | 228 | 0.245 | | |
| | | $\bar{y}$ = 0.432 | | |

**Table No.14: For Ruggedness**

| **Analyst-1** | | | |
|---|---|---|---|
| **S.No** | **Concentration (µg/mL)** | **Absorbance** | **Statistical analysis** |
| 1 | 20 | 0.445 | |
| 2 | 20 | 0.445 | Mean = 0.446 |
| 3 | 20 | 0.445 | SD = 0.004021 |
| 4 | 20 | 0.446 | % RSD = 0.90 |
| 5 | 20 | 0.445 | |
| 6 | 20 | 0.445 | |
| **Analyst-2** | | | |
| 7 | 20 | 0.441 | |
| 8 | 20 | 0.440 | Mean = 0.440 |
| 9 | 20 | 0.440 | SD=0.000516 |
| 10 | 20 | 0.441 | %RSD=0.11 |
| 11 | 20 | 0.440 | |
| 12 | 20 | 0.440 | |

**Table No.15: For Summary**

| S.No | Parameters | Values |
|---|---|---|
| 1 | Beer's Law limit (µg/mL) | 10-30 |
| 2 | Absorption maxima (nm) | 220 |
| 3 | Standard regression equation | 0.0191x+0.031 |
| 4 | Correlation coefficient ($R^2$) | 0.999 |
| 5 | Accuracy | 96.56-102% |
| 6 | Precision (% RSD)<br>Repeatability | 0.16<br>0.89 |
| 7 | LOD (µg/mL) | 0.130 |
| 8 | LOQ (µg/mL) | 0.394 |
| 9 | Robustness (%RSD) | 1.8 |
| 10 | Ruggedness | 0.90 and 0.11 |
| 11 | Assay (%) | 99.60 % |

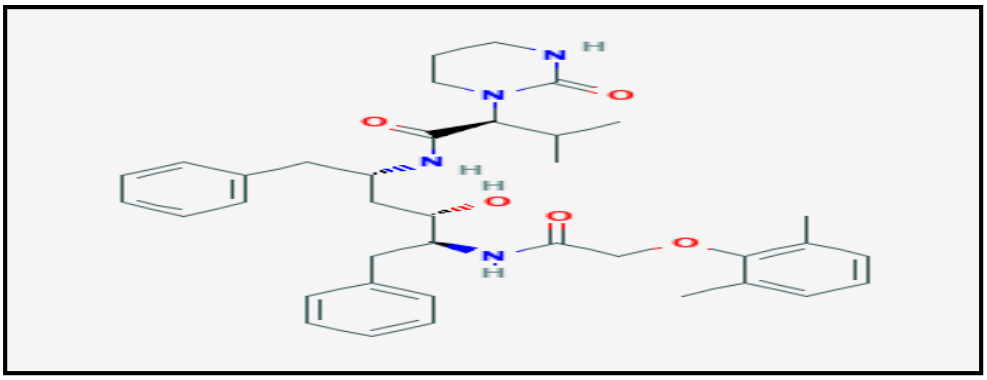

**Figure No.1: Chemical structure of Liponavir**

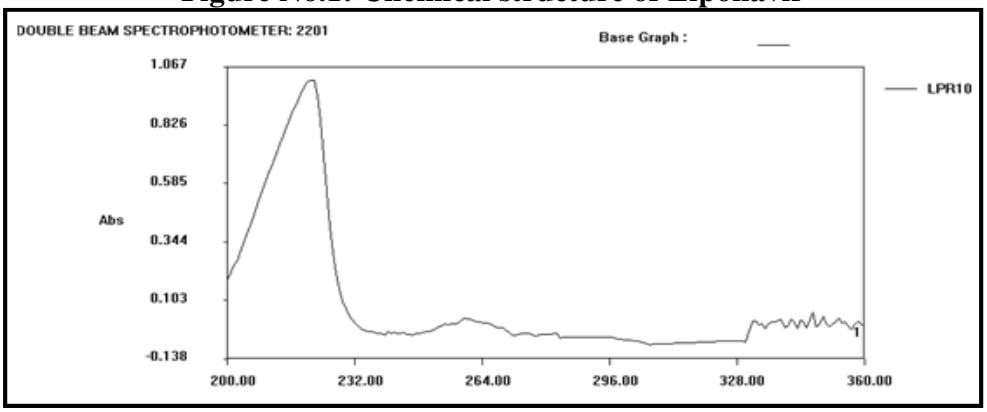


**Figure No.2: Wavelength of maximum absorption of Lopinavir**

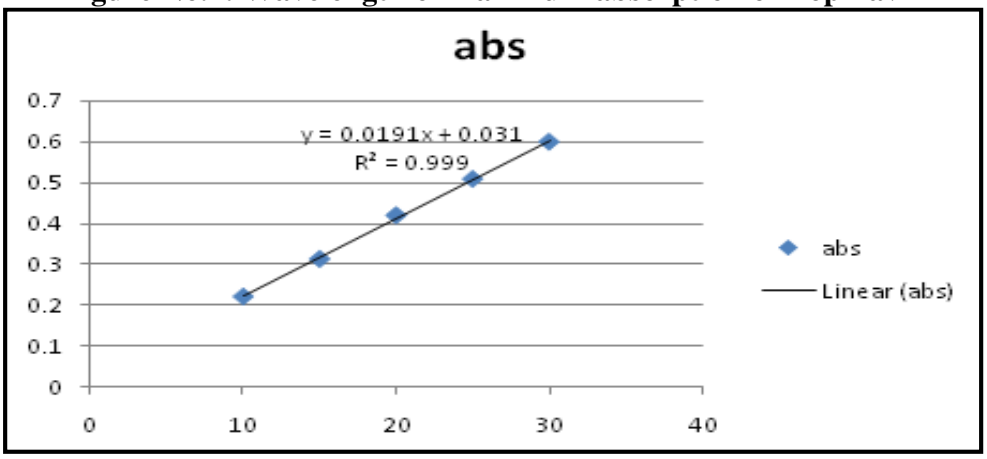


**Figure No.3: Linearity graph of Lopinavir**

## SUMMARY AND CONCLUSION

Summary of UV Spectrophotometeric Method of Lopinavir.

## CONCLUSION

The UV-Spectrophotometric method was developed and it is found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination of Lopinavir in API and its tablet dosage form without any interference from the excipients. This method can be effectively applied for the routine analysis of Lopinavir in API. Its advantages are the low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

## ABBREVIATIONS

UV-Ultra Violet
API- Active Pharmaceutical Ingredient

## ACKNOWLEDGEMENT

The authors are very thankful to the Principal of D.S.T.S. Mandal's College of Pharmacy, Solapur, Maharashtra, India and cooperative staff for providing the required facilities and guidance to carry out this research work.

## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

## BIBLIOGRAPHY

1. National Centre for Biotechnology Information, *PubChem Compound Database,* CID11243969. Available from:https://pubchem.ncbi.nlm.nih.gov/compound/Lopinavir#section=Top [accessed 10 march 2019].
2. International conference on Hormonization. ICH Q2 (R1), Validation of analytical procedures: text and methodology, 2005.
3. Rahul V, Aditya M, Ravi P. Simple, rapid and validated LC determination of Lopinavir in rat plasma and its pharmacokinetics studies, *Scientia Pharmaceutica*, 79(4), 2011, 849-863.
4. Namratha S, Vijayalaxmi A. UV spectrophotometeric method development and validation of Lopinavir in bulk and in pharmaceutical dosage form, *CIBTech Journal of Pharmaceutical Sciences*, 6(2), 2017, 1-4.
5. Hetal T, Ketul P. A first derivative spectrophotometric method for the estimation of Lopinavir in tablets, *Chronicles of Young Scientists*, 1(3), 2010, 22-25.
6. Madhukarb A, Jagadeeshwar K, Naresh K, Vinod A, *et al.* Simple and sensitive analytical method development and validation of Lopinavir in bulk drug by RP-HPLC, *Der Pharma Chemica*, 3(6), 2011, 494-499.
7. Mohan S, Vijayalakshmi R, Dhanaraju M D. Development and validation of RP-HPLC method for determination of Lopinavir in bulk and pharmaceutical dosage form, *International journal of Research in Pharmacy and Chemistry*, 2(2), 2012,413-417.
8. Vijayalaxmi A, Namratha S. Method development and validation of Lopinavir in tablet dosage form using reversed phase high performance liquid chromatography, *Asian Journal of Pharmaceutical and Clinical Research*, 11(4), 2018, 1-4.
9. Deepthi K, Jane M, Hemanth K. Estimation of Lopinavir by RP-HPLC, *Research Journal of Pharmacy and Technology*, 12(1), 2019, 251-253.
10. Eliane D, Laura M, Pedro F, Maria B. Development and validation of dissolution test for Lopinavir, a poorly water-soluble drug in soft gel capsules based on in-vivo data, *Journal of Pharmaceutical and Biomedical Analysis*, 47(3), 2008, 547-552.
11. Monali B, Sanjay W. HPTLC method development and validation for the estimation of Lopinavir and Ritonavir in capsule dosage form, *Der Pharma Sinica*, 5(5), 2014, 56-60.

**Please cite this article in press as:** Sonali Narsing Koli *et al.* Development and validation of UV spectroscopic method for determination of lopinavir in bulk and tablet dosage form, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry,* 6(4), 2018, 155-163.