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Development, Validation and Stability Study of UV Spectrophotometric Method for Determination of Daclatasvirin Bulk and Pharmaceutical Dosage Forms

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Abstract : A simple, specific and economic UV spectrophotometric method has been developed using as a solvent methanol:water (8:2) to determine the daclatasvir content in bulk and pharmaceutical dosage formulations. The quantitative determination of the drug has been carried out at a predetermined $\lambda_{max}$of 317nm, it was proved linier in the range 2-12 µg/mL and exhibited good correlation coefficient (R^2=0.998) and excellent mean recovery (98-100.09%). The method was validated statically and by recovery studies for linearity, precision, repeatability and reproducibility as per ICH guideline. The obtained results proved that the method can be employed for the routine analysis of daclatasvirin bulk as well as in the commercial formulations.
Key Words : Daclatasvir, UV Spectroscopy, Validation, Stress Studies.

1. Introduction

Daclatasvir is an inhibitor of hepatitis C virus (HCV) NS5A protein. It is a new, oral, direct-acting antiviral with potent pangenotypic activity [1]. It is a first in class direct acting antiviral agent which binds to and inhibits the function of the HCV protein NS5A. NS5A is involved in both viral RNA replication and virus particle assembly. A putative inhibitor-binding region spanning amino acids 21-30 of NS5A was identified [2]. In vitro studies suggested that DCV could interfere with protein–protein interactions at the stage of membranous web biogenesis, which can explain potent inhibition of replication-complex formation, resistance, effects on lipid droplet distribution and virion release [3].Chemically Daclatasvir Dihydrochloride is methyl(1S)-1- (2S)-2-(5-(4'- (2-(2S)-1- (2S)-2-(methoxycarbonyl) amino)-3-methylbutanoyl)-2-pyrrolidinyl)-1H-imidazol-5-yl)-4-biphenylyl)-1H-imidazol-2-yl)-1-pyrrolidinyl)carbonyl)-2-methylpropyl) carbamatedidi hydrochloride (Fig.1).The synthesis involves an alkylation and formation of the imidazole ring, a coupling reaction and the formation of the dihydrochloride salt. Daclatasvir is a white to yellow crystalline non-hygroscopic powder [4].

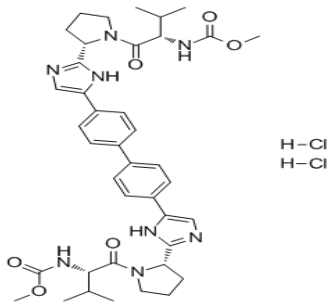

Figure 1- Chemical Structure of Daclatasvir Dihydrochloride

Literature survey reveals that only an LC-MS study has been reported for the quantification of Daclatasvir in human plasma. No UV spectrophotometric method has been reported for the estimation of Daclatasvir.In this study, efforts were made to develop a simple, easy and economic UV spectrophotometric method using a diluents composed of methanol:water (8:2) for the determination of daclatasvir in raw material as well as in the marketed dosage formulations. The developed methoid was optimized and validated as per the guidelines of International Council on Hormonisation (ICH) and demonstrated excellent specificity, linearity, precision and accuracy for daclatasvir.

2. Materials and Methods

2.1 Instruments

A Shimadzu UV–visible spectrophotometer (UV1800, Shimadzu Corporation, Kyoto, Japan)was used for all absorbance measurements with matched quartz cells.

2.2 Materials

All chemicals and reagents were of analytical grade. Daclatasvir in the form of Daclatasvir dihydrochloride powder withcertificate of analysis was provided by Cipla Research Centre, Mumbai. Pharmaceuticalgrade excipients were obtained from Pharmaceutical Technology Lab. of Maharashtra.

2.3 Determination of wavelength of maximum absorption

A standard stock solution of daclatasvir(100 µg/mL) was prepared using diluents to further obtain 10 µg/mL.an UV spectroscopic scanning (190-400 nm) was carried out with final diluted solution to determine λ_{max} for the detection of daclatasvir using diluents as a blank.

2.4 Linearity and Range

For linearity study, six solutions at different concentrations (2, 4, 6, 8, 10 and 12 mg/mL) were prepared using six different aliquots of stock solution, and the obtained data were used for the linearity calibration plot. Limit of detection (LOD) and limit ofquantification (LOQ) for the assay were also calculated

2.5 Intra-day precision (repeatability) and inter-day precision study (intermediate precision)

Daclatasvir tablets were finely powdered and the sample stock solution of 10mg/mL was prepared followingthe same dilution pattern of stock solution. Three different aliquots of stock solution were then diluted to 10 mL to obtain the concentrations of 4, 6 and 8 mg/mL. This procedure was repeated in the following days.

2.6 Stability study

Samples prepared for repeatability study were preserved for 24 h at room temperature and analyzed on the following day to test for short-term stability.

2.7 Accuracy/recovery study

This study was carried out using pre-formulated granules containing pure daclatasvir dihydrochloride and common excipients. Calculation was done from the label claim and the average weight of the final product. Previously used dilution pattern was followed for the granules to obtain three concentrations—80%, 100% and 120% of reference solution.

2.8 Specificity in the presence of excipients

The test for the specificity was carried out using only excipients. Spectra for placebo granules, blank, and sample were compared. Secondly the specificity was determined by subjecting the sample solution to accelerated degradation by heat (60 °C) for 48 h in order to verify that none of the degradation products interfered with the quantification of the drug.

2.9 Assay of content of Daclatasvir in selected marketed brands

Market brands of daclatasvir tablet from different manufacturers were randomly selected and analyzed using the newly developed and validated method. Sample solutions of each brand (10 mg/mL) were also prepared and assayed for content of daclatasvir against the standard. The content of daclatasvir in the marketed brands was determined using standard calculations.

2.10 Stress degradation studies

i. Photolytic Degradation

Specific amount of drug daclatasvir was weighed accurately & putted into the UVchamber for three days. After three days 10mg drug was weighed and madestock solution (100µg/mL) with diluents. Then an appropriate concentration(10 mg/mL) wasprepared & absorbance was measured in UV spectrophotometer.

ii. Thermal Degradation

Drug was taken in a Petri dish which was previously cleaned & dried then was put it into the oven for 48 hrs then it was taken out & weighed 10mg drug was weighed and made stock solution (100µg/mL)with diluents. Then an appropriate concentration(10µg/mL) wasprepared & absorbance was measured in UV spectrophotometer.

iii. Acid Degradation

0.01N HCl was taken in a 10 ml volumetric flask then accurately weighed 10mg drug daclatasvir was dissolved in it. Then the solution was refluxed for 4 hrs then from this solution an appropriate concentration(10µg/mL) was prepared using diluents & absorbance was measured in UV spectrophotometer.

iv. Alkali Degradation

0.01N NaOH was taken in a 10 ml volumetric flask then accurately weighed 10mg drug daclatasvir was dissolved in it. Then the solution was refluxed for 4 hrs then from this solution an appropriate concentration(10µg/mL) was prepared using diluents & absorbance was measured in UV spectrophotometer.

v. Oxidation with H_2O_2

3% H_2O_2solution was taken in a 10 ml volumetric flask then accurately weighed 10mg drug daclatasvir was dissolved in it. Then the solution was kept in dark for 4 hrs then from this solution an appropriate concentration(10µg/mL) was prepared using diluents & absorbance was measured in UV spectrophotometer.

3. Results and discussion

3.1 Method development and optimization

Daclatasvir is almost insoluble in aqueous medium and freely soluble in organic solvents like methanol. During the development phase, the use of a few milliliters of methanol with water as the diluent resulted in preferable outcome in UV analysis. The solvent composition was optimized to water (2):methanol (8). The pre-determined wavelength of maximum absorption (λ_{max}) was 317 nm. (Fig. 2)

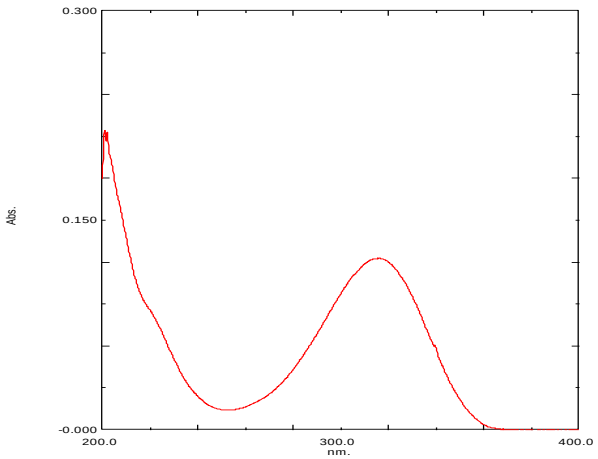

Figure 2- UV Spectrum of Daclatasvir Dihydrochloride

3.2 Method validation

3.2.1 Linearity and range

The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of the samples in the range of 2.0–12.0 mg/mL was linear with a correlation coefficient (R^2) greater than 0.998 (Table 1). The LOD and LOQ were calculated as 0.858 mg/mL and 2.60 mg/mL respectively.

Table1 :Linearity data

Concentration µg/ml	Absorbance
2	0.064
4	0.127
6	0.178
8	0.238
10	0.305
12	0.367

3.2.2 Intra-day and inter-day precision

The intra-day and inter-day precision study (Table 2) of the developed method confirmed adequate sample stability and method reliability where all the RSDs were below 2%.

Table 2:Intra-day and inter-day precision determined for three different concentrations of daclatasvir (n=3).

Concentration µg/mL	Intra-day precision			Inter-day precision		
	Absorbance measured	RSD (%)	Average potency (%)	Absorbance measured	RSD (%)	Average potency (%)
4	0.128	0.142	98.62	0.130	0.132	98.12
8	0.249	0.112	97.98	0.246	0.098	98.10
12	0.386	0.099	99.27	0.105	0.133	98.34

3.2.3 Stability

Stability study's results were within the acceptance range (Table 3) and indicated the samples stability over 24 h(short-term).

Table 3: Short term stability determined by the proposed method (n=3).

Concentration declared µg/mL	Concentration found µg/mL	RSD (%)	Average potency (%)
4	0.126	0.172	98.36
8	0.246	0.132	98.22
12	0.381	0.129	98.00

3.2.4 Accuracy/Recovery

Results within the range of 98.00–100.97% ensure an accurate method (Table 4) as well as indicate non-interference with the excipients of formulation.

Table 4:Accuracy/Recovery for three different concentrations of daclatasvir by the proposed method.

Dosage form	Label Claim	Amount added	Recovery (%)
Pre-formulated granules	300 mg	80	99.01
		100	99.25
		120	99.69

3.2.5 Specificity in the presence of excipients

The specificity of the analytical method was proved by comparing the spectra of placebo and degradation productof sample solution with that of accuracy sample (Fig. 3).

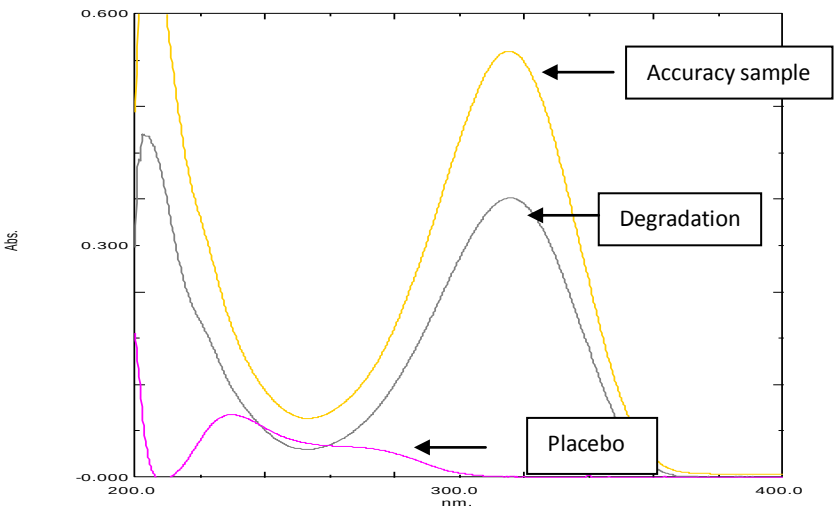

Figure 3: Specificity of the method determined by comparing the spectra of accuracy sample, placebo and degradation products

3.2.6 Stress degradation studies

The study conducted (Table5)shows that there is degradation of drug under the stress conditions like photolytic, alkali & oxidation.

Table 5: Summary of stress degradation results

Stress condition	Degradation%	Remark
Photolytic	38	Unstable
Thermal	09	Stable
0.01N HCl	15	Stable
0.01N NaOH	89	Unstable
H_2O_2	69	Unstable

3.2.7 Content of daclatasvir in marketed brands

Daclatasvir content of three marketed products determined by the proposed method (Table 6) was in good agreement with the label claims and was in the range of 98.45–100.50% with the RSD values of 0.107–0.140% respectively.

Table 6: Content of daclatasvir in marketed products

Brand	Label claim (mg)	Amount found	Potency	RSD (%)
Brand A	300	296.8	98.93	0.125
Brand B	300	298.3	99.43	0.140
Brand C	300	297.01	99.00	0.107

4. Conclusion

The results and the statistical parameters demonstrate that the proposed UV spectrophotometric method is simple, rapid, specific, accurate and precise. Therefore, this method can be used for the determination of daclatasvir either in bulk or in the dosage formulations without interference with commonly used excipients and related substances.

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References

1. McCormack PL., Daclatasvir: A review of its use in adult patients with chronic hepatitis c virus infection, Drugs, 2015; 75(5): 515-524.
2. Assessment Report of Daclatasvir from European Medicines Agency (EMA). Available from:http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Public_assessment_report/human/003768/ WC500172849.pdf.
3. Bunchorntavakul C, Reddy KR, Review article: the efficacy and safety of daclatasvir in the treatment of chronic hepatitis C virus infection, Alimentary Pharmacology and Therapeutics, 2015; 42(3): 258-272.
4. Chakravarthy AV, Sailaja B.B.V, Method development and validation of assay and dissolution methods for the estimation of daclatasvir in tablet dosage forms by reverse phase HPLC, European Journal of Pharmaceutical And Medical Research, 2016; 3(7): 356-364.
5. Mohammed A. Othman et al, Stability Indicating HPLC Method Development and Validation for Determination of Daclatasvir in Pure and Tablets Dosage Forms., Indo Am. J. P. Sci, 2016; 3(12): 1565-1572.
6. SreekanthNadig and Jane T Jacob, Quantitative Estimation of DaclatasvirIn Drug Substances and Formulated Drug Product By UPLC, Der Pharmacia Lettre, 2016; 8 (13): 280-284.
7. ICH harmonized tripartite guideline, Q2(R1), Validation of analytical procedures: text and methodology," in International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2005.
8. Snyder LR, Kirkland JJ, Glajch JL. Handbook of Practical HPLC Method Development. 2 nd ed., Hoboken, NJ, USA; Wiley-Blackwell, 1997.
9. Center for Drug Evaluation and Research (CDER), Reviewer Guidance: Validation of Chromatographic Methods. CMC 3, 1994.
10. Blessy.M., Development of forced degradation and stability indicating studies of drugs-A review, Journal of Pharmaceutical Analysis, 2014; 4(3):159–165.
11. Guidance for industry, analytical procedures and methods validation for drugs and biologics. Draft Guidance, U.S. Department of Health and Human Services, Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), CMC, 2014.
12. Sachin B. Gholve, Rani R. Ajgunde, Omprakash G. Bhusnure and Sanjay S. Thonte, Analytical method development and validation by QbD approach – A review, Der Pharmacia Sinica, 2015; 6(8):18-24.
13. Shabir GA, John LW, Arain SA, Bradshaw TK. Evaluation and application of best practice in analytical method validation. J of Liquid Chroma and Related Tech, 2007; 30(3): 311–333.
14. HanaaSaleh, Gamal H. Ragab, Mohammed A. Othman, Stability indicating hplc method development and validation for determination of daclatasvir in pure and tablets dosage forms, Indo American Journal of Pharmaceutical Sciences, 2016; 3 (12): 1565-1572.
15. Mark Green J. A practical guide to analytical method validation. Analytical Chemistry, 1996; 68(9): 305A–309A.
16. USP 37, NF 32. Validation of compendial methods, chapter 1225. The United States Pharmacopeial Convention, Rockville, Md, USA, 2014.

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