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RESEARCH ARTICLE

Development and validation of a stability indicating RP-HPLC method for estimation of daclatasvir in pharmaceutical dosage form

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ABSTRACT

Introduction: The aim of the present investigation is to develop and validate a novel, accurate, precise, and sensitive and stability indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantitative determination of daclatasvir in pure and pharmaceutical formulation. **Materials and Methods:** The method development was found to be having suitable applications for routine quality control analysis. Pharmaceutical analysis plays a key role in the Quality Assurance and Quality Control of bulk drugs. Analytical chemistry involves separating, identifying, and determining the relative amounts of components in a sample matrix. Pharmaceutical analysis is a specialized branch of analytical chemistry. Pharmaceutical analysis derives its principles from various branches of sciences such as physics, microbiology, nuclear science, and electronics. Qualitative analysis reveals the chemical identity of the sample. **Results:** To establish an optimized method, two trails were conducted with different compositions. Finally, the mobile phase selected for the analysis was composed of acetonitrile and 0.1% formic acid buffer adjusted to pH 4.5 in the ratio of 40: 60 v/v at flow rate of 1 mL/min. The maximum response was observed at 305 nm and was optimized for measuring the absorbance. The sharp peak of daclatasvir was retained at 4.34 min. **Conclusion:** By the obtained results and reports, the developed RP-HPLC method for the estimation of daclatasvir was found to be more efficient and accurate.

Keywords: Reverse-phase high-performance liquid chromatography, Daclatasvir, Quality assurance and quality control

INTRODUTION

Pharmaceutical analysis plays a key role in the Quality Assurance and Quality Control of bulk drugs. Analytical chemistry involves separating, identifying, and determining the relative amounts of components in a sample matrix. Pharmaceutical analysis is a specialized branch of analytical chemistry. Pharmaceutical analysis derives its principles from various branches of sciences such as physics, microbiology, nuclear science, and electronics. Qualitative analysis reveals the chemical identity of the sample. Quantitative analysis establishes the relative amount of more of these species or analytes in numerical terms. Qualitative analysis is required before a quantitative analysis can be under taken. A separation step is usually a necessary part of both a qualitative and quantitative analysis. The results of typical quantitative analysis can computed from two measurements. One is the mass or volume of sample to be analyzed and second is the measurement of some quantity that is proportional to the amount of analyte in that sample and normally completes the analysis.[1-10]

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Typical instrumental techniques

The methods of estimation of drugs are divided into physical, chemical, physic chemical, and biological ones. Of them, physical and physic chemical methods are used mostly. Physical methods of analysis involve the studying of the physical properties of a substance. They include determination of the solubility, transparency or degree of turbidity, color density or specific gravity (for liquids), moisture content, melting, freezing, and boiling points. Physicochemical methods are used to study the physical phenomenons that occur as a result of chemical reactions. Among the physicochemical methods are optical refractometry, polarimetry, emission and fluorescent methods of analysis, photometry including photo Colorimetry, spectrophotometry, Nephelometry, and Turbidimetry, electrochemical (Potentiometry, Amperometry, Coulometry, Voltametry, and polarography), and chromatography (column, paper, thin layer, gas-liquid, and high-performance liquid chromatography [HPLC]) methods are generally preferable. Methods involving nuclear reactions such as nuclear magnetic resonance (NMR) and paramagnetic resonance are becoming more and more popular. The chemical methods include the gravimetric and volumetric procedures, which are based on complex formation, acid-base, precipitation, and redox reactions. Titrations in non-aqueous media and complexometry have been widely used in pharmaceutical analysis, whenever the existing amounts are in milligram level and the interferences are negligible. The methods (HPLC, GLC, NMR, and Mass Spectroscopy) of choice for assay in volve sophisticated equipment that is very costly and poses problems of maintenance. Hence, they are not in the reach of most laboratories and small-scale industries, which produce bulk drugs and pharmaceutical formulations. However, this sophisticated equipment usage eliminates the difficulties encountered in the determination of minute amounts of degradation products or the analysis of the metabolites of drugs in body fluids.[2,11-14]

Advances in both chemistry and technology are making new techniques available and expanding the use of existing ones. Photo acoustic spectroscopy is an example of an emerging analytical technique. A number of existing techniques have been combined to expand the utility of the component methods. Gas Chromatography–Mass Spectrometry, Inductively Coupled Plasma-Mass Spectrometry, and Gas Chromatography-Infrared Spectroscopy are examples of successful hyphenated methods.

HPLC

HPLC is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. To obtain satisfactory flow rate, liquid must be pressurized to a few thousands of pounds per square inch.

The rate of distribution of drugs between stationary and mobile phase is controlled by diffusion process, if diffusion is minimized, a faster and effective separation can be achieved. The technique of HPLC is so called due to its improved performance when compared to classical column chromatography. Advances in column technology, high-pressure pumping system, and sensitive detect or shave transformed liquid column chromatography into high-speed, efficient, accurate, and highly resolved method of separation [Figure 1].

HPLC basic instrumentation

Drug profile

Daclatasvir is a direct-acting antiviral agent against Hepatitis C Virus (HCV) used for the treatment of chronic HCV genotype 1 and 3 infections. It is marketed under the name DAKLINZA [Figure 2].

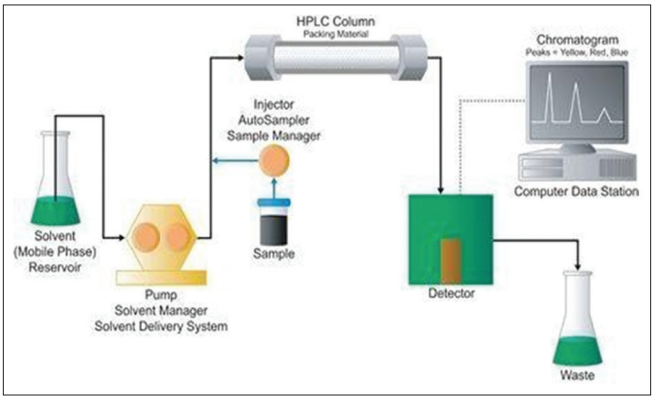

Figure 1: Instrumentation of high-performance liquid chromatography

IUPAC name
Dimethyl N,N'-([1,1'-biphenyl]-4,4'-diylbis{1H-imidazole-5,2-diyl-[(2S)-pyrrolidine-2,1-diyl][(2S)-3-methyl-1-oxobutane-1,2-diyl]}) dicarbamate.

Molecular formula
$C_{40}H_{50}N_8O_6$.

Molecular weight
738.88 g/mol.

Chemical structure

Description
White powder.

Solubility
Poor solubility in water and ethanol at neutral pH. Highly soluble at lower pH ranges.

Storage
It should be stored at room temperature (25°C) in an air tight container.

MATERIALS AND METHODS [TABLES 1-3]

Preparation of reagents and solutions

0.1% Formic acid buffer solution
0.1 mL of formic acid was transferred to 100 mL of volumetric flask and diluted with water and then adjust the pH to 4.5 using triethylamine.

Mobile phase
Sixty volumes of acetonitrile were to be mixed with 40 volumes of 0.1% formic acid buffer and then filtered through 0.22 µm nylon membrane vacuum filtration. The mobile phase was sonicated for removal of unwanted gases.

Preparation of diluent
Acetonitrile and formic acid buffer in the ratio of 30:70 v/v was used as diluent.

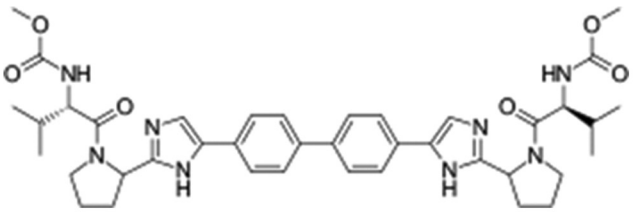

Figure 2: Structure of daclatasvir physicochemical properties

Table 1: Instruments used

HPLC	Agilent 1260 infinity binary pump
HPLC software	Open lab solutions
UV-Visible Spectrophotometer	Shimadzu UV 1800
UV-Visible Spec. software	UV probe
Ultrasonicator	Remi
pH meter	Systronics 335
Electronic balance	Shimadzu
Syringe	Agilent manual syringe
HPLC column	Zobrax C_{18} [250×4.6 mm] 5µ

HPLC: High-performance liquid chromatography

Table 2: Reagents used

Water	Triple distilled water
Acetonitrile	HPLC Grade
Triethylamine	AR Grade
Formic acid	AR Grade

HPLC: High-performance liquid chromatography

Table 3: Drugs used

Daclatasvir	Yarrow chemicals
Daclatasvir 60 mg	Obtained from local pharmacy

Preparation of standard stock solutions
Daclatasvir standard stock solution was made by dissolving 5 mg of pure form of drug in 10 mL diluent and sonicated for 10 min to get the primary standard stock solution containing 500 µg/mL. Working standard solution was prepared by further dilution with mobile phase.

Preparation of sample solution
Five tablets of Daclatasvir were weighed and crushed them to a fine powder and transfer the powder weight equivalent to 5 mg to 10 mL volumetric flask and dissolved in mobile phase. The volume was made up to mark with diluent. The mixture was allowed to stand for 30 min with intermittent sonication to

ensure complete dissolution. Finally, the prepared solution was filtered through a 0.22 μm membrane filter. The filtrate was diluted further with mobile phase to get the working sample solution.

Detection of wavelength

The diluted solutions contain 10 μg/mL of daclatasvir in diluent which is prepared and record the spectrum on UV spectrophotometer; the solutions were scanned between 200 and 400 nm using diluents as blank. The peaks of maximum absorbance wavelengths were observed. The wavelength was found to be 305 nm for daclatasvir.

RESULTS AND DISCUSSION

Method development

An attempt has been made to develop a novel, simple, and rapid method for quantification of daclatasvir in pure and tablet dosage form. To establish an optimized method, two trails were conducted with different compositions Finally, the mobile phase selected for the analysis was composed of acetonitrile and 0.1% formic acid buffer adjusted to pH 4.5 in the ratio of 40: 60 v/v at flow rate of 1 mL/min. The maximum response was observed at 305 nm and was optimized for measuring the absorbance. The sharp peak of daclatasvir was retained at 4.34 min [Table 4].

Trial-1

Preparation of calibration curve

A series of dilutions were made from the working standard solution in the range of 5–25 μg/mL for assessment of linearity. The calibration curve was plotted by taking the concentration on X-axis and peak area on Y-axis. The results are shown in table and linearity graph is depicted in Table 5.

System suitability

System suitability is a parameter which is used to assess the performance of HPLC instrument. To evaluate the system performance, the parameters such as theoretical plates, resolution, and asymmetric factors should be determined. The results are shown in given Table 6.

Table 4: Trail 1 Chromatographic conditions

Mobile phase	Acetonitrile: 0.1% Formic acid
Ratio	40:60 v/v
Column	ODS C_{18} (250 × 4.6 mm × 5μ)
Wavelength	305 nm
Flow rate	1.0 mL/min
Run time	5 min
Diluents	50:50 v/v
Observation	Peak was observed after 5 min

Table 5: Preparation of standard solutions of Daclatasvir

Stock solution (mL)	Amount of solvent (mL)	Conc. (μg/mL)
0	10	0
0.1	9	5
0.2	8	10
0.3	7	15
0.4	6	20
0.5	5	25

Table 6: Results for system suitability

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	4.21	1109103	5063	1.44
2	4.23	1106495	5168	1.43
3	4.23	1109041	5046	1.47
4	4.22	1109678	5146	1.46
5	4.23	1127349	5142	1.44
6	4.22	1115986	5023	1.43
7	4.24	1102356	5120	1.44
8	4.23	1125463	5129	1.42
9	4.23	1110322	5107	1.46
10	4.22	1106094	5098	1.4
Mean	4.226	1112188	-	-
%RSD	0.1943	0.7056	-	-

Table 7: Results for linearity

S. No.	Conc. (μg/mL)	Peak area
1	0	0
2	5	645364
3	10	1166094
4	15	1661904
5	20	2182712
6	25	2692583
Slope		106119
Intercept		64959
Regression equation		Y=106119x+64959
Correlation coefficient		R^2 = 0.998

Table 8: Results for accuracy

Spike level [%]	Amount of Daclatasvir added (ppm)	Peak Area	Conc. Found (µg/mL)	% Recovery	% Mean recovery
50	15	2332413	9.88	98.81	98.81
50	15	2333309	9.88	98.81	
50	15	2332478	9.88	98.82	
100	20	2830614	9.75	97.52	98.44
100	20	2824810	9.71	97.16	
100	20	2884230	10.06	100.64	
150	25	3368446	9.99	99.96	99.88
150	25	3381563	10.07	100.72	
150	25	3351764	9.89	98.98	

Table 9: Results for robustness

S. No.	Parameter	Optimized	Used	Rt (min)	Peak area	%RSD
1	Flow rate	1mL/min	0.8 mL/min	5.27	1910399	0.76
			1.0 mL/min	4.18	1009103	0.25
			1.2 mL/min	3.52	1006495	0.21
2	Wavelength	305 nm	303	4.24	1089041	0.53
			305	4.18	1009103	0.23
			308	4.26	1195084	0.54
3	Mobile phase	ACN: Buffer (60:40)	55:45	4.54	1097919	0.36
			60:40	4.18	1009103	0.45
			65:35	4	1147349	0.58

Table 10: Degradation data

Type of degradation	Peak area	% Assay	% Amount degraded
Acid	1123354	92.3	7.7
Alkali	1148267	95.92	4.08
Oxidative	1048652	90.37	9.63
Neutral	1137594	93.05	6.95

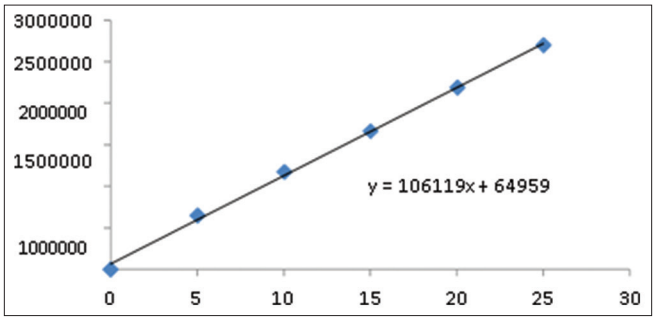

Figure 3: Calibration curve of linearity of daclatasvir

Specificity

Specificity is the ability of a method to resolve the peaks of analyte in presence of other components. It was assessed by recording the chromatograms of blank, placebo, standards, and sample.

Linearity

Linearity was performed by preparing different concentrations, that is, 5–25 µg/mL from standard stock solution of daclatasvir. The absorbance was measured at 305 nm. Each measurement was carried out in triplicate. Plot the graph between the concentration and peak area. In this calibration curve, the straight line obeyed linearity in the concentration ranges of daclatasvir. The correlation coefficient and linearity results are represented in Tables 7-10 and the chromatograms are shown in Figure 3.[8,15-18]

CONCLUSION

A novel, simple, linear, accurate, and rapid stability indicating reverse phase (RP)-HPLC method was established for the quantitative determination of daclatasvir in pure form and pharmaceutical dosage form. The developed method was validated for parameters such as linearity, accuracy, precision, LOD, LOQ, and robustness and obtained satisfactory results.

By the obtained results and reports, the developed RP-HPLC method for the estimation of daclatasvir was found to be more efficient and accurate.

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