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A Validated Rp-Hplc Method for the Estimation of Daclatasvir Dihydrochloride in Bulk and Pharmaceutical Formulation

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ABSTRACT: A new sensitive, specific, linear, precise and accurate RP-HPLC method was developed and validated for estimation of Daclatasvirdihydrochloridein Bulk and Pharmaceutical Tablet Formulations. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, Shimadzu shim pack C18 (250mm x 4.5µm x 5µ) column. Shimadzu Prominence-i LC-2030C Plus equipped with Auto sampler as the instrument model. Methanol (100% v/v) was used as a solvent in mobile phase at a flow rate of 1.0 ml /min with injection volume of 10 µg/ml UV detection was performed at 316 nm. The Linearity was established forDaclatasvirdihydrochloride in the range of 2-20 µg/ml with correlation coefficient of 0.9993. LOD and LOQ were found to be 1.63 µg/ml and 4.95 µg/ml respectively. Retention time of Daclatasvirdihydrochloridewere found to be 2.8 min. % Recovery was found to be 99.76 to 100.49 and %RSD was found with in 2. The method has been validated according to ICH guidelines for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed validated method was successfully applied for reliable quantification of Daclatasvirdihydrochloridein bulk and pharmaceutical dosage form.

KEYWORDS: Daclatasvirdihydrochloride, RP-HPLC, Validation, Pharmaceutical formulations.

I. INTRODUCTION:

Daclatasvir is an inhibitor of hepatitis C virus (HCV) NS5A protein. It is an orally direct-acting antiviral with potent pangenotypic activity. It is a first in class direct acting antiviral agent which binds to and inhibits the function of the HCV protein NS5A. It is marketed beneath the name DAKLINZA and is contained in day by day oral tablets as hydrochloride salt form.

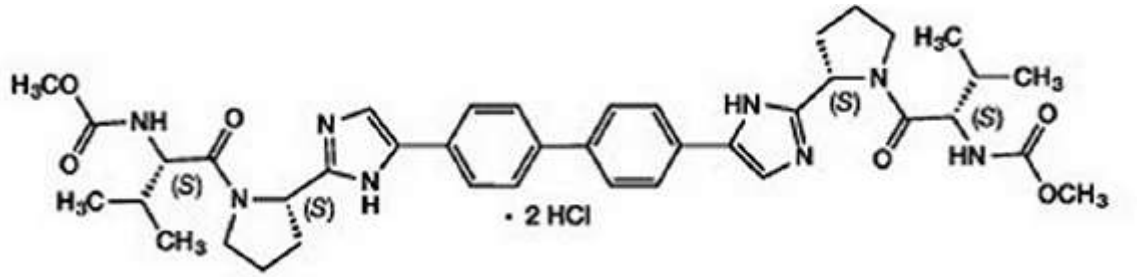

Fig.1: Chemical structure of Daclatasvirdihydrochloride.

Daclatasvirdihydrochloride is chemically known as 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)carbamate dihydrochloride with a molecular formula of $C_{40}H_{50}N_8O_6$ 2HCl and a molecular weight of 738.8 g/mol. Daclatasvir drug substance is white to yellow powder and it is freely soluble in water (>700 mg/ml), soluble in DMSO.

Literature survey revealed that there were few analytical methods have been reported for the determination ofDaclatasvirdihydrochloride in pure drug and pharmaceutical dosage forms by using RP- HPLC[1-13] so far.

The aim of present work is to develop and validate a novel, rapid, simple, precise and specific Area under curve Spectrophotometric method for

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estimation of Daclatasvirdihydrochloridein bulk and tablet dosage form.

II. MATERIALS AND METHODS:

Instrumentation:

Chromatographic separation was performed on a Shimadzu Prominence-i LC-2030C plus equipped with Auto sampler comprising a variable wavelength programmable UV detector. Shimadzu shim pack C18 (250mm x 4.5µm, 5µ) column is used.

Materials and Reagents:

Daclatassvirdihydrochloride pure drug was obtained as a gift sample from MSN laboratories Hyderabad and its pharmaceutical dosage form Daclatassvirdihydrochloride 20 tablet labelled claim 60mg from local pharmacy manufactured by ZydusHeptiza Pharma India Ltd.

Methanol was obtained from Bharathi College of pharmacy, Bharathinagara, KM Doddi, Maddur Taluk, Mandya District, India. Distilled water was used throughout the experiment.

Chromatographic conditions:

Table 1: HPLC method development parameters.

HPLC method development parameters	
Column	C18, 250mm X 4.5 µ m X 5µ
Column Temperature	30°C
Wavelength	316nm
Run time	10min
Injection Volume	10 µL
Flow rate	1.0 mL / min
Diluents	Mobile phase
Elution	Isocratic

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Preparation of solutions Mobile phase :
Methanol (100% v/v) was used as solvent.

Preparation of sample Standard Solution:
The formulation tablets of Daclatasvirdihydrochloride(Daclahep - 60mg) were crushed to give finely powdered material. From the Powder prepared a 100mg of Daclatasvirdihydrochloridewas accurately weighed, transferred in a 100 ml volumetric flask, add 30 ml of diluents and sonicate to dissolve and dilute to volume with diluent. Transfer 10 mL of standard stock solution into 100 ml volumetric flask and dilute to volume with diluent, and an appropriate concentration of sample was prepared at the time of analysis. 10µl of these solutions were injected in triplicate into HPLC system and the peak areas were recorded.

Preparation of Standard Solution:
10 mg of Daclatasvirdihydrochloride was dissolved in 10ml of Methanol in 10 ml volumetric flask (1000µg/ml). Further dilution was made from above in such a way that the final concentrations consist of 2, 4, 8, 10, 12, 20 µg/ml

System suitability requirements from stock and standard solutions:
Tailing factor: NMT 1.131
Theoretical Plates: NLT 4678

III. RESULTS AND DISCUSSION:

Validation of the proposed method:
The proposed method was validated as per ICH guidelines.[14] The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

Specificity:
From the chromatograms of blank, standard (Prepared from Formulation). It was found that there is no interference due to excipients in the tablet formulation and also found good retention time. The specificity results are shown in Table 2.

Table 2: Specificity of Daclatasvirdihydrochloride:

Name of the solution	Retention time in min
Blank	0
Daclatasvirdihydrochloride(STD)	2.8

BLANK

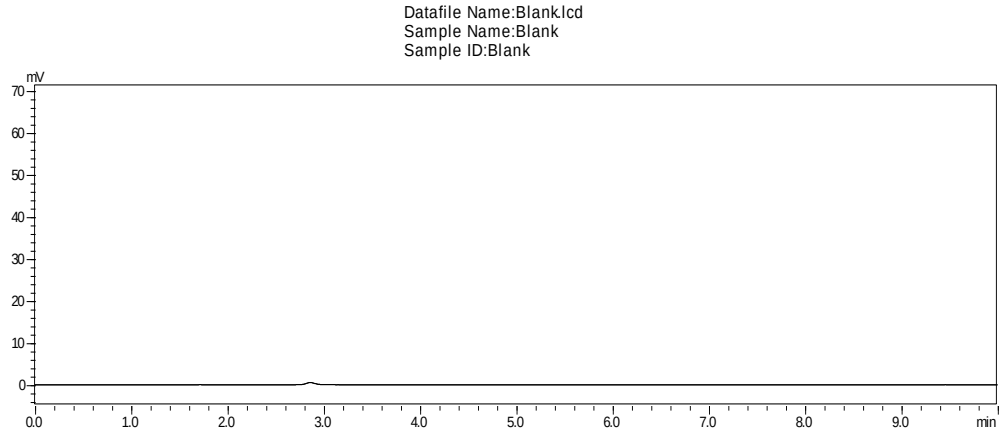

Figure 2: Chromatogram of Blank solution of Daclatasvirdihydrochloride.

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DACLATASVIR DIHYDROCHLORIDE STANDARD 100mcg/ml

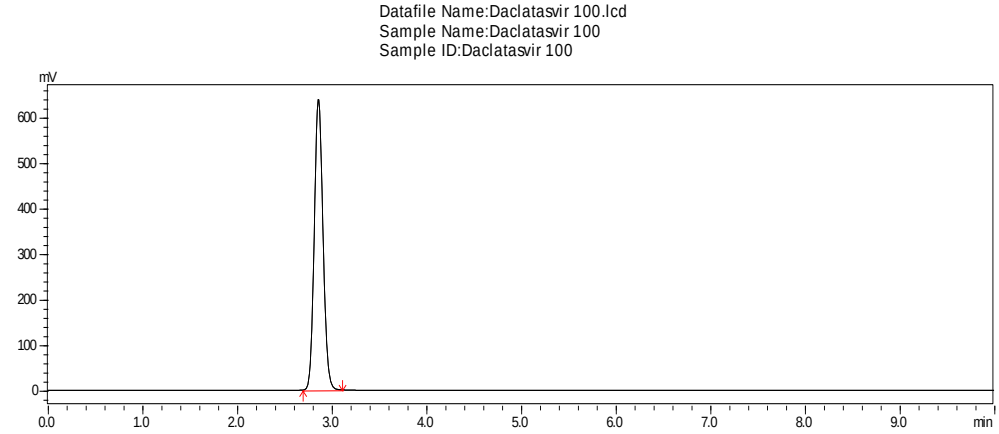

Figure 3: Chromatogram of standard solution of Daclatasvirdihydrochloride.

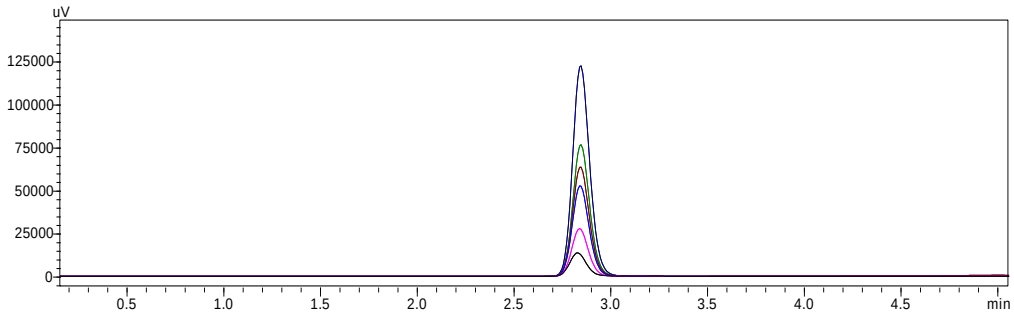

Figure 4: Overlain Chromatogram of Daclatasvirdihydrochloride.

Linearity:

The linearity of the response of the drug was verified at six concentration levels, ranging from 2-20mcg/ml of Daclatasvirdihydrochloridein each linearity level were prepared. 10µl of each concentration was injected into the HPLC system. The response was read at 316nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were presented in Table 3.

Table 3: Linearity of Daclatasvirdihydrochloride.

Concentration (µg/ml)	Peak area*(mv)
2	86320
4	170578
8	326799
10	394431
12	473467
20	760549

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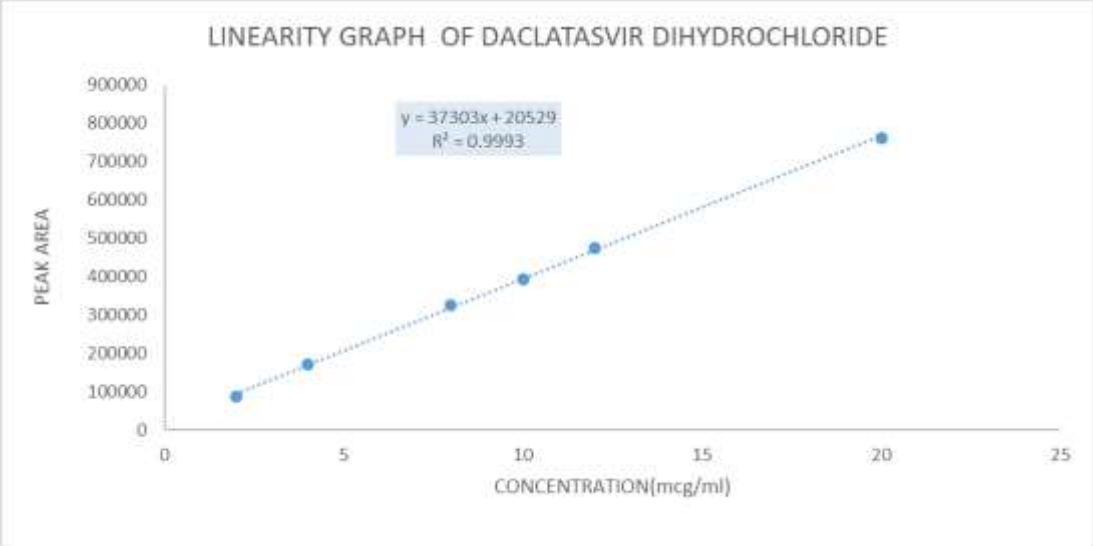
*Average of six determinations



Precision:

Precision of the method was performed as intraday precision, Inter day precision. To study the intraday precision, six replicate standard solutions of Daclatasvirdihydrochloridewere injected. % RSD was calculated and it was found to be 1.267 and interday precision done same as intraday, six replicate standard solutions of Daclatasvirdihydrochloridewere injected. % RSD was calculated and it was found to be 1.389 which are well within the acceptance criteria of not more than 2.0. Results of system precision are shown in Table 4.

Table 4: Results of Precision ofDaclatasvirdihydrochloride.

SI. NO	Intra Day Name	Studies Peak area	Inter Day Name	Studies Peak area
1	Injection-1	330131	Injection-1	320456
2	Injection-2	332382	Injection-2	331303
3	Injection-3	333720	Injection-3	331389
4	Injection-4	335149	Injection-4	333484
5	Injection-5	342471	Injection-5	330054
6	Injection-6	333184	Injection-6	329354
	AVG	334506.17	**AVG**	329340
	STDEV	4239.626	**STDEV**	4575.149
	%RSD	1.2674	**%RSD**	1.3891

Accuracy:

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 50, 100 and 150% of the label claim of the tablet (60 mg ofDaclatasvirdihydrochloride). The average recoveries of three levels of Daclatasvirdihydrochloridewere found to be 99.76 – 100.24 %. The results are shown in Table 5.

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Table 5: Results of recovery ofDaclatasvirdihydrochloride.

Recovery level	Amount taken (mcg/ml)	Peak Area	Average	% Recovery ± SD*	% RSD
50%	4	342912 340373 341424	341569.66	100.2425 ± 0.74	0.74519
100%	8	657383 656718 645733	653278	99.902 ±1.42	1.42639
150%	12	949808 944528 943180	945838.7	99.7686 ±0.73	0.74071

*Average of three determinations

Limit of Detection and Limit of Quantification:

The limit of detection is an analytical method in which the smallest amount of analyte in a sample which can be reliable detected by the analytical method. The limit of quantitation is an individual analytical procedure in which the smallest amount of the analyte in sample which can be quantitatively determined LOD and LOQ were calculated using formula LOD = 3.3(SD)/S and LOQ = 10(SD)/S. Results were shown in Table 6.

Table 6: System suitability parameters.

Parameters	Daclatasvirdihydrochloride
Linearity	2-20mcg/ml
Regression equation	y = 37303X+ 20229
Correlation coefficient	R^2 = 0.9993
Retention time	2.8 min
Run time	10 min
Limit of Detection (LOD)	1.635µg/ml
Limit of Quantitation (LOQ)	4.954µg/ml
Tailing factor	1.138
Theoretical Plate	4242

Ruggedness:

The ruggedness of test method by carrying out precision study in six preparation of sample on a single batch sample by different analysts and by different instrument, the results of the precision study are tabulated as below Table 7. The % RSD values are less than 2.

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Table 7: Results of Ruggedness ofDaclatasvirdihydrochloride:

By changing the Analysts:

Concentration(mcg/ml)	T1	T2	Mean	SD	%RSD
2	86514	85220	85867	914.996	1.06559
4	170678	171578	171128	636.396	0.37188
8	316899	317716	317307.5	577.706	0.18206
10	395431	399331	397981	2757.716	0.69397
12	483567	475519	479543	5690.795	1.18671
20	761841	759433	760637	1702.713	0.22385

By changing the Instrument:

Concentration(mcg/ml)	T1	T2	Mean	SD	%RSD
2	88124	87891	88007.5	164.7558	0.18720
4	181332	182273	181802.5	665.3874	0.36599
8	320457	325177	322817	3337.544	1.03388
10	400176	401042	400609	612.3544	0.15285
12	498751	495338	497044.5	2413.355	0.48554
20	771355	769158	770256.5	1553.513	0.20168

Robustness:

Robustness is the measure of the capacity of the analytical method to remain unaffected by small but deliberate variation in the procedure. The robustness of the method was evaluated by analyzing the system suitability standard and evaluating system suitability parameter data after varying, individually, the HPLC pump flow rate (±0.2 ml/min), column temperature (±5°C) and detection wavelength (±2 nm) shown in Table 8.

Acceptance criteria: System suitability should pass as per test method at variable conditions.

Table 8: Robustness results for Daclatasvirdihydrochloride.

Robustness	Condition	Tailing Factor	% RSD
Flow Rate	Decreased(-0.2ml/min)	1.13	1.242
	Increased (+0.2ml/min)	1.129	
Column Temperature	Decreased (-5^{0}C)	1.136	0.284
	Increased (+5^{0}C)	1.134	
Wavelength	Decreased (-2nm)	1.131	0.768
	Increased (+2nm)	1.132	

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ASSAY:

Brand name	Available form	Label claim	Amount found	Assay
DACLAHEP	Tablets	60mg	59.35mg	99.66

IV. CONCLUSION:

The present analytical method was validated as per ICH guidelines[14] and met the acceptance criteria. It was concluded that the developed analytical method was simple, accurate, economical and sensitive, and can be used for routine analysis of Daclatasvirdihydrochloridein bulk drug and pharmaceutical dosage forms.

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