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DEVELOPMENT AND VALIDATION OF SIMPLE SPECTROSCOPIC METHODS FOR THE ESTIMATION OF HEPATITIS-C DRUG DACLATASVIR DI HYDROCHLORIDE IN PURE FORM

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ABSTRACT

Three simple, sensitive, rapid and accurate spectrophotometric methods have been proposed for the estimation of daclatsvir di hydrochloride (Dimethyl *N*,*N*'-([1,1'-biphenyl]-4,4'-diylbis{1*H*-imidazole-5,2-diyl-[(2*S*)-pyrrolidine-2,1-diyl][(2*S*)-3-methyl-1-oxobutane-1,2-diyl]})dicarbamate) in pure form. These methods were based on the measurement of absorbance of FC reagent [METHOD-A], Methyl orange [METHOD-B] and Cresol red [METHOD-C] at 553nm, 425nm and 432nm respectively. Reaction conditions have been optimized to obtain coloured complexes of higher sensitivity and longer stability. The absorbance increased linearly with increase in concentrations of daclatasvir.The complexes obeyed Beer's law over the concentration range of 25 - 125 µg/mL, 30 - 70 µg/mL and 30 - 70 µg/mL in method A, method B and method C respectively.The common excipients and additives did not interfere in their determination. The developed methods have been successfully applied for the estimation of daclatasvir in bulk samples.

Keywords
Daclatasvir (DAC), Spectrophotometry, FC reagent, Methyl orange, Cresol red.

Introduction

Hepatitis C is a liver disease produced by the hepatitis C virus (HCV) and can cause liver cirrhosis, liver failure, and liver cancer. The standard treatment for HCV is pegylated-interferon (Peg-IFN) and ribavirin (RBV), however these agents caused side effects such as bacterial infections, anemia, haematological toxicity, neutropenia and anorectal symptoms [1, 2]. Telaprevir and boceprevir were the first generation direct-acting protease inhibitors that were developed and approved for the treatment of genotype I chronic hepatitis C. However, they have to be co-administered with interferon and ribavirin therefore they were associated with their common side effects so theireffectiveness were limited [3, 4]. Second-generation direct-acting antiviral drugs were developed and aimed to have a high pangenotypic activity with fewer undesirable side effects. These drugs include daclatasvir that have effective antiviral activity and genotypic coverage [5, 6].

Daclatasvir is a potent nucleotide analogue which causes a structural modification of NS5A, leading to a loss of action and resulting in an inhibition of virion formation [7,8]. Only few techniques were reported for thequantitative determination of DAC including different spectroscopic and chromatographic method [9,10,11,12,13,14].The present study was aimed to develop simple, sensitive, rapid and accurate spectrophotometric methods for the estimation of daclatasvir di hydrochloride (DAC) in pure form by using fc reagent, methyl orange and cresol red indicator. The developed method was validated as per ICH guidelines[15].

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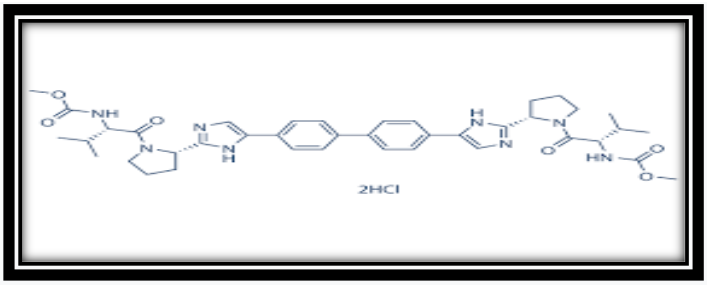

Figure no-1 Structure of daclatasvir di hydrochloride

Materials and Methods

Instruments required

Systronics double beam spectrophotometer and shimadzu digital weighing balance.

Chemicals required

a) **0.05N Naoh solution:**Weigh accurately about 2grams ofNaOHand transfer it into a 1000ml volumetric flask dissolve it with 100ml of distilled water and make up the volume up to the mark with distilled water.
b) **FC reagent 5%:**pipette out 5ml of fc reagent into a beaker and add 10ml of distilled water and mix well.
c) Methyl orange indicator.
d) Cresol red indicator.
e) Acetic acid.
f) Chloroform.

Sample preparation (1000 µg/ml):

Weigh accurately about 100mg of daclatasvir sample and transfer it into a clean dry 100ml volumetric flask. Dissolve it in a 10ml of methanol and then make up the volume up to the mark with distilled water.

Experimental procedure:

Method A: (Using fc reagent)

From the standard stock solution aliquots of 0.25-1.2ml (25-125 µg/ml) were placed into a series of calibrated 10ml volumetric flasks. To these flasks add 0.5ml of 0.05n naoh solution followed by the addition of 0.5ml of fc reagent mix well and kept the flasks aside for 5minutes and make up the final volume up to the 10ml mark with 0.05n naoh solution. Measure the absorbance at 553nm against reagent blank.

A standard graph was plotted by taking concentration on x-axis and absorbance on y-axis.

Method B: (Using methyl orange indicator)

From the standard stock solution aliquots of 0.3-0.7ml (30-70 µg/ml) were placed into a series of separating funnels and add 1ml of methyl orange indicator and followed by the addition of 1ml of acetic acid. Make up the volume 10ml with chloroform and shake the separating funnels for 10min and kept a side for 15minutes. After 15minutes the chloroform layer was collected into a series of test tubes and measure the absorbance at 425nm against reagent blank.

A standard graph was plotted by taking concentration on x-axis and absorbance on y-axis.

Method C: (Using cresol red indicator)

From the standard stock solution aliquots of 0.3-0.7ml (30-70 µg/ml) were placed into a series of separating funnels and add 1ml of cresol red indicator and followed by the addition of 1ml of acetic acid. Make up the volume 10ml with chloroform and shake the separating funnels for 10min and kept a side for 15minutes. After 15minutes the chloroform layers was collected into a series of test tubes and measure the absorbance at 425nm against reagent blank.

A standard graph was plotted by taking concentration on x-axis and absorbance on y-axis.

Results

Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample. The linearity range for method-A is 25-125 µg/ml, for method-B is 30-70µ and for method-C is 30-70µg/ml. Linearity results for method-A, B and C are shown in table-1,2,3.

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Figure 1. Method –A linearity graph

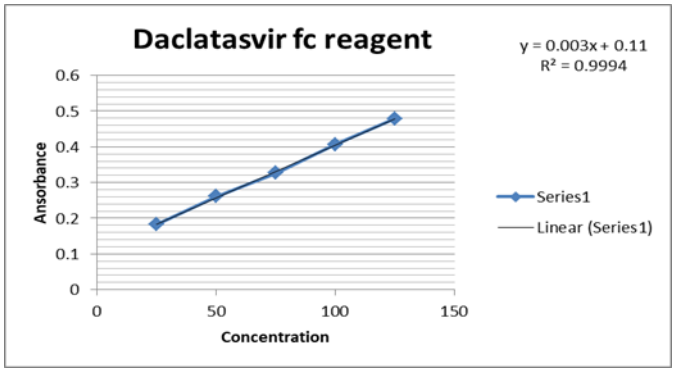


Figure 2. Method –B linearity graph

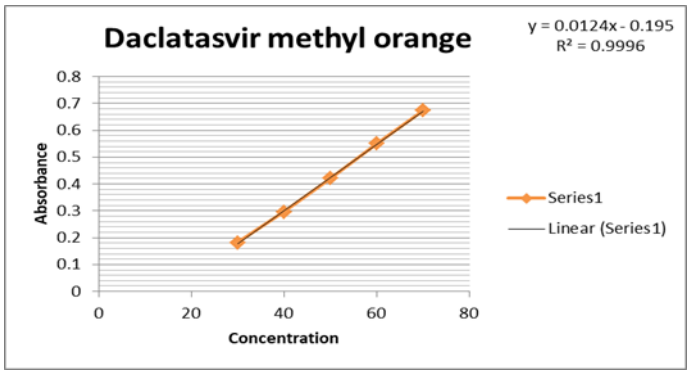
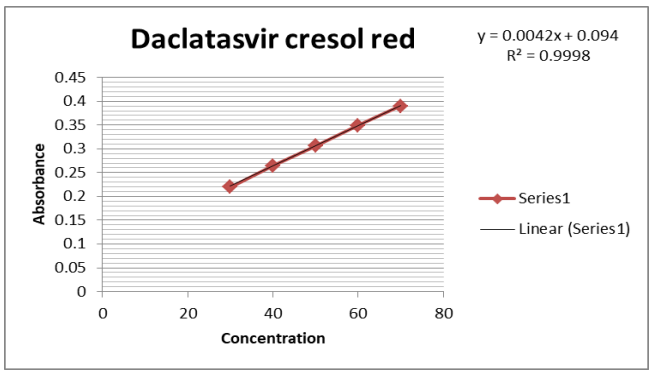


Figure 3. Method –C linearity graph



Linearity tables

Table-1 method-A linearity data

Concentration (µg/ml)	Absorbance	Statistical Analysis	
25	0.183	Slope	0.003
50	0.261	y-Intercept	0.11
75	0.327		
100	0.407	Correlation Coefficient	0.9994
125	0.479		

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Table-2 method-B linearity data

Concentration (µg/ml)	Absorbance	Statistical Analysis	
30	0.181	Slope	0.0124
40	0.296	y-Intercept	0.195
50	0.420		
60	0.550	Correlation Coefficient	0.9996
70	0.673		

Table-3 method-C linearity data

Concentration (µg/ml)	Absorbance	Statistical Analysis	
30	0.221	Slope	0.0042
40	0.265	y-Intercept	0.095
50	0.306		
60	0.349	Correlation Coefficient	0.9999
70	0.390		

Precision

Precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogeneous sample. Precision is classified in to two types. Inter day precision and Intraday precision. Results for Inter day precision and Intraday precision are in table no- 4 to 9.

Inter day precision

Table-4 method-A Inter day precision data

Trail	Concentration (µg/ ml)	Absorbance
1	75	0.384
2	75	0.378
3	75	0.376
4	75	0.379
5	75	0.383
6	75	0.373
Mean	0.378	
Standard deviation	0.001907	
%RSD	0.50	

Table-5 method-B Inter day precision data

Trail	Concentration (µg/ ml)	Absorbance
1	50	0.456
2	50	0.449
3	50	0.457
4	50	0.459
5	50	0.452
6	50	0.447
Mean	0.453	
Standard deviation	0.00213	
%RSD	0.47	

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Table-6 method-C Inter day precision data

Trail	Concentration (µg/ ml)	Absorbance
1	50	0.312
2	50	0.308
3	50	0.308
4	50	0.306
5	50	0.309
6	50	0.311
Mean	0.309	
Standard deviation	0.000979	
%RSD	0.31	

Intraday precision

Table-7 method-AIntraday precisiondata

Trail	Concentration (µg/ ml)	Absorbance
1	75	0.339
2	75	0.339
3	75	0.340
4	75	.0341
5	75	0.342
6	75	0.342
Mean	0.340	
Standard deviation	0.000616	
%RSD	0.18	

Table-8 method-B Intraday precision

Trail	Concentration (µg/ ml)	Absorbance
1	50	0.423
2	50	0.423
3	50	0.421
4	50	0.420
5	50	0.420
6	50	0.421
Mean	0.421	
Standard deviation	0.00063	
%RSD	0.14	

Table-9 method-C Intraday precision

Trail	Concentration (µg/ ml)	Absorbance
1	50	0.308
2	50	0.312
3	50	0.309
4	50	0.311
5	50	0.312
6	50	0.310
Mean	0.310	
Standard deviation	0.00074	
%RSD	0.23	

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Limit of detection [LOD]

Table-10 LOD data

Method-A	Method-B	Method-C
3.3σ/slope	3.3σ/slope	3.3σ/slope
3.3*0.0006164/0.003	3.3*0.00063/0.0124	3.3 * 0.00074/ 0.0042
0.67	0.16	0.58

Limit of quantification [LOQ]

Table-11LOQ data

Method-A	Method-B	Method-C
10σ/slope	10σ/slope	10σ/slope
10*0.0006164/0.003	10*0.00063/0.0124	10*0.00074/0.0042
2.05	0.50	1.7

Robustness

It is performed by changing the wavelength to determine the method robustness. Results for the robustness are shown in table no-10.

Table-10 Robustness data

Method-A		Method-B		Method-C	
Wavelength (nm)	**Absorbance**	**Wavelength (nm)**	**Absorbance**	**Wavelength (nm)**	**Absorbance**
553	0.477	425	0.487	432	0.314
548 (-)	0.465	420 (-)	0.478	427 (-)	0.303
558 (+)	0.470	430 (+)	0.480	437 (+)	0.306

Sandell's sensitivity

Table-11 Sandell's sensitivity data

Method-A	Method-B	Method-C
0.001×1/slope	0.001×1/slope	0.001×1/slope
0.001×1/0.003	0.001×1/0.0124	0.001×1/0.0042
0.33	0.08	0.23

Molar absorptivity

Tabel-12 Molar absorptivity data

Method-A	Method-B	Method-C
A=ƐCL	A=ƐCL	A=ƐCL
Ɛ=A/CL	Ɛ=A/CL	Ɛ=A/CL
0.327/75×1	0.420/50×1	0.306/50×1
0.0043	0.0084	0.0061

Discussion and Conclusion

Three simple, sensitive, rapid and accurate spectrophotometric methods have been developed And validated for the estimation of daclatsvir di hydrochloride in API form.

The proposed spectrophotometric methods for the daclatasvir are simple and accurate. The statistical parameters and validation parameters data clearly indicate the reproducibility and repeatability of the proposed methods. The methods are found to be free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control is well established by performing the analysis by using this method for determination of daclatasvir in pure form. Hence, the proposed methods can be utilized in the pharmaceutical laboratories and industries.

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