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IN-VITRO INTERACTION STUDIES OF ANTIVIRAL DRUGS WITH NSAIDS BY LIQUID CHROMATOGRAPHY APPLICATION IN PHARMACEUTICAL FORMULATION AND HUMAN SERUM

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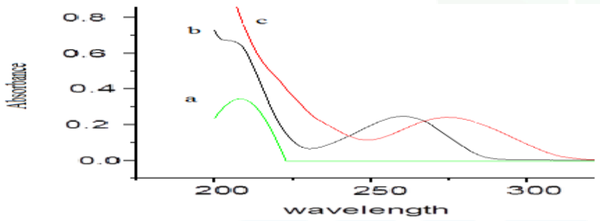
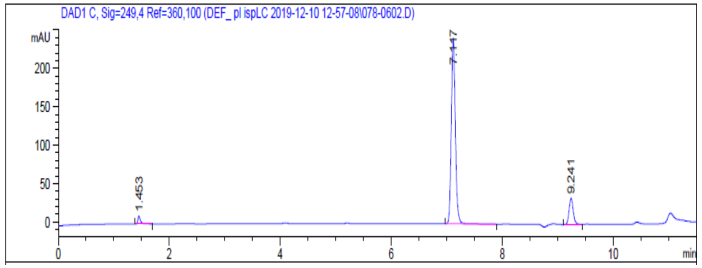
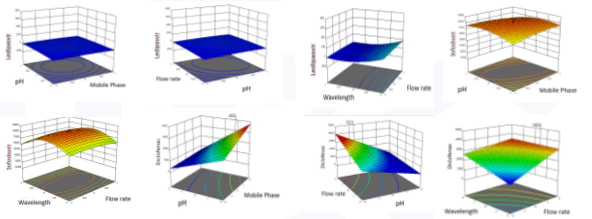


A simple, rapid and precise HPLC method was developed for the simultaneous method of Ledipasvir, Sofosbuvir and Diclofenac. The basic objectives for this research is to Create a cost effective and less time consuming method for the simultaneous method of three Drugs for this Aim Method Development was performed followed by Method Validation According to ICH guidelines. Ruggedness was performed by the help of Design of Experiment. And after that the method was applied on Pharmaceutical formulation and Analyzed on Human Serum.





Regression Characteristics					
Drugs	Intercept	Slope (S)	Correlation coefficient (R2)	LOQ (µg/mL	LOD (µg/mL
Diclofenac	15.369	13.909	0.997	0.00117	0.00039
Ledipasvir	2.0157	0.4942	0.999	0.00373	0.00123
Sofosbuvir	8.4944	178.48	0.999	0.01023	0.00338

System suitability test			
Drugs	N[a]	T[b]	Res[c]
Diclofenac	78643	0.0771	2.23
Ledipasvir	2482	0.0686	2.85
Sofosbuvir	49835	0.0743	44.38

N=theoretical plates, T=tailing factor, Res=resolution

Mobile phase: 0.1% Phosphoric Acid:Methanol (80:20)
Flow rate:1ml/min
Column: C_{18} Column (150 x 3.9mm and pore size of 5µm)
Wavelength: 249nm
Oven Temperature: 45°C

[illegible] ANOVA						
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	[illegible]	14	[illegible]	[illegible]	[illegible]	significant
A-Mobile Phase	[illegible]	1	[illegible]	[illegible]	[illegible]	
B-pH	[illegible]	1	[illegible]	[illegible]	[illegible]	
C-Flow Rate	[illegible]	1	[illegible]	[illegible]	[illegible]	
D-Wavelength	[illegible]	1	[illegible]	[illegible]	[illegible]	
AB	[illegible]	1	[illegible]	[illegible]	[illegible]	
AC	[illegible]	1	[illegible]	[illegible]	[illegible]	
AD	[illegible]	1	[illegible]	[illegible]	[illegible]	
BC	[illegible]	1	[illegible]	[illegible]	[illegible]	
BD	[illegible]	1	[illegible]	[illegible]	[illegible]	
CD	[illegible]	1	[illegible]	[illegible]	[illegible]	
A²	[illegible]	1	[illegible]	[illegible]	[illegible]	
B²	[illegible]	1	[illegible]	[illegible]	[illegible]	
C²	[illegible]	1	[illegible]	[illegible]	[illegible]	
D²	[illegible]	1	[illegible]	[illegible]	[illegible]	
Residual	[illegible]	[illegible]	[illegible]			
Lack of Fit	[illegible]	10	[illegible]	[illegible]	[illegible]	significant
Pure Error	[illegible]	5	[illegible]			
Cor Total	[illegible]	29				

[illegible] ANOVA						
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
A-Mobile Phase	[illegible]	1	[illegible]	[illegible]	[illegible]	
B-pH	[illegible]	1	[illegible]	[illegible]	[illegible]	
C-Flow Rate	[illegible]	1	[illegible]	[illegible]	[illegible]	
D-Wavelength	[illegible]	1	[illegible]	[illegible]	[illegible]	
AB	[illegible]	1	[illegible]	[illegible]	[illegible]	
AC	[illegible]	1	[illegible]	[illegible]	[illegible]	
AD	[illegible]	1	[illegible]	[illegible]	[illegible]	
BC	[illegible]	1	[illegible]	[illegible]	[illegible]	
BD	[illegible]	1	[illegible]	[illegible]	[illegible]	
CD	[illegible]	1	[illegible]	[illegible]	[illegible]	
A²	[illegible]	1	[illegible]	[illegible]	[illegible]	
B²	[illegible]	1	[illegible]	[illegible]	[illegible]	
C²	[illegible]	1	[illegible]	[illegible]	[illegible]	
D²	[illegible]	1	[illegible]	[illegible]	[illegible]	
Residual	[illegible]	15	[illegible]			
Lack of Fit	[illegible]	10	[illegible]	[illegible]	[illegible]	significant
Pure Error	[illegible]	5	[illegible]			
Cor Total	[illegible]	29				

[illegible] ANOVA						
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	significant
A-Mobile Phase	[illegible]	1	[illegible]	[illegible]	[illegible]	
B-pH	[illegible]	1	[illegible]	[illegible]	[illegible]	
C-Flow Rate	[illegible]	1	[illegible]	[illegible]	[illegible]	
D-Wavelength	[illegible]	1	[illegible]	[illegible]	[illegible]	
AB	[illegible]	1	[illegible]	[illegible]	[illegible]	
AC	[illegible]	1	[illegible]	[illegible]	[illegible]	
AD	[illegible]	1	[illegible]	[illegible]	[illegible]	
BC	[illegible]	1	[illegible]	[illegible]	[illegible]	
BD	[illegible]	1	[illegible]	[illegible]	[illegible]	
CD	[illegible]	1	[illegible]	[illegible]	[illegible]	
A²	[illegible]	1	[illegible]	[illegible]	[illegible]	
B²	[illegible]	1	[illegible]	[illegible]	[illegible]	
C²	[illegible]	1	[illegible]	[illegible]	[illegible]	
D²	[illegible]	1	[illegible]	[illegible]	[illegible]	
Residual	[illegible]	[illegible]	[illegible]			
Lack of Fit	[illegible]	10	[illegible]	[illegible]	[illegible]	significant
Pure Error	[illegible]	5	[illegible]			
Cor Total	[illegible]	29				

Tailing factor: 0.0686-0.0771

Regression Characteristics

Intercept: 2.0157-15.369

Correlation coefficient: **0.997-0.999**

%RSD for all three Drugs: 0.36-4.75

Precision of method

%Recovery: 96.44-104.58

Recovery in Serum
57.86-229.60
Interaction Studies by HPLC

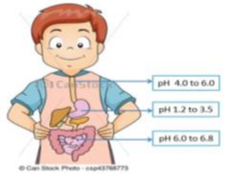
85% within 20 minutes

Simulating Human Stomach Environment

Buffer of pH 1.2 was prepared by dissolving 3.75g NaCl and 7 mL HCl (conc.) in 1 L distilled water followed by adjusting the pH with 0.1 N HCl.

Buffer of pH 4.5 was prepared by dissolving 2.99 g sodium acetate trihydrate and 1.4 mL acetic acid (conc.) in 1 L distilled water pH was set with 0.1 N acetic acid.

pH 6.8 buffer was prepared by dissolving 6.8 g sodium dihydrogen phosphate in 1 L distilled water and pH was adjusted with 0.1 N NaOH.



Current method is simultaneous method developed for the determination of antiviral drugs which were Sofosbuvir and Ledipasvir, with NSAID that was Diclofenac which is further optimized by Design of Experiment with Central Composite Model and Further Validated.
The proposed developed and validated HPLC method can effectively apply for the simultaneous estimation and quantification of Diclofenac with antiviral drug Sofosbuvir and Ledipasvir in reference standard, pharmaceutical formulations without interruption of tablet excipient with good separation and resolution of chromatographic peaks. All the parameters such as % recovery, accuracy, linearity and precision was set for the reliability of proposed method. A very short and quick HPLC Chromatographic method of run time of 12 min allow to perform analysis of analytes within limit of resolution .Linearity of the calibration curve was so good and correlation co-efficient greater than 0.997, not more than 4% RSD values were found all were not more than 4%. All samples results were in accordance with standards in the similar concentration. There were no peaks of excipients interruption found in chromatogram. The proposed method is applicable for the analysis in QC lab. The performance test i.e. vitro analysis (dissolution) was performed on dissolution USP type II apparatus using three buffers i.e. 1.2 buffer, 4.5 buffer and 6.8 buffer. The performance test results of formulated drug product for each tablet on all three buffers were found above 80%.

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