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Research Paper

RP-HPLC Method Development and Validation of Upadacitinib (UDB) And Tofacitinib (TFC) In Bulk and Pharmaceutical Dosage Form

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ABSTRACT

UPADACITNIB(UDB) AND TOFACITNIB(TFC)In the present work, three simple, sensitive, and specific methods (First order Derivative Spectroscopy, Second order Derivative Spectroscopy, and RP-HPLC)have been developed for the quantitative estimation of Upadacitinib (UDB) and Tofacitinib (TFC) in bulk and pharmaceutical dosage forms.T A: DERIVATIVE SPECTROSCOPY METHOD A: FIRST ORDER DERIVATIVE SPECTROSPAR COPY A simple, specific, accurate, and precise First order Derivative Spectroscopy method was developed and validated for the estimation of UDB and TFC in pharmaceutical dosage forms. The stock solutions were prepared by weighing 100 mg of Standard UDB and TFC separately in 100 ml volumetric flasks with methanol. The final stock solutions were made to produce 200 µg/ml with distilled water. Further dilutions were prepared as per procedure and scanned at 267 nm for UDB and 260 nm for TFC. The linearity was found in the concentration range of 10-100 µg/ml for both drugs

INTRODUCTION

Analytical chemistry is inherently a quantitative Science. Whether determining the concentration of a species in a solution, evaluating equilibrium constant, measuring a reaction rate or drawing a correlation between a compounds structure and its reactivity. Analytical chemists make measurements and perform calculations.

SPECTROPHOTOMETRIC METHOD :-

This is most accurate method for determining the concentration of substance in solution, but the instruments are, of necessity, more expensive. A Spectrophotometer may be regarded as refined filter photoelectric photometer which permits the use of continuously variable and more nearly monochromatic bands of light.

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A DOUBLE BEAM UV - VISIBLE ABSORPTION SPECTROMETER

Most modern general purpose UV - Visible Spectrophotometers are double-beam instruments which cover the range between 200 - 800 nm by a continuous automatic scanning process producing the spectrum as a pen trace on calibr.

DRUGPROFILE

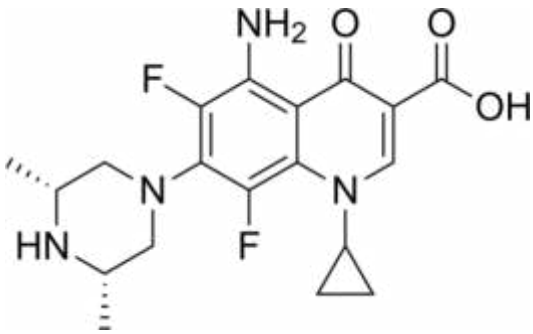

Fig:Structure of UDB

Molecular formula :C17H19F3N6O

Solubility :Rapidly soluble inWater and Ethanol.

TFC is an oral Janus kinase(JAK) inhibitor indicated for the treatment of autoimmune diseases such as rheumatoid arthritis, psoriaticarthritis, and ulcerative colitis.The molecular structure of TFC is shown in Fig

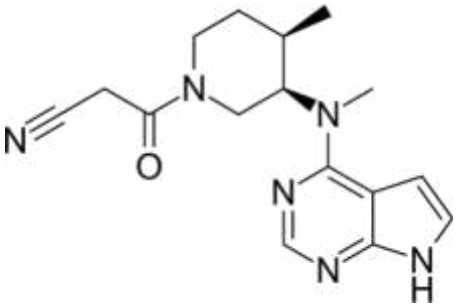

Fig: Structure of TCF

Molecular formula :C16H20N6O

Molecular weight : 312.37 gm / mole.

Characteristics: Bitter, crystalline and odourless.

Solubility: Rapidly soluble in Water and Ethanol.

METHODOLOGY

PART A: DERIVATIVE SPECTROSCOPY

METHOD A: FIRST ORDER DERIVATIVE SPECTROSCOPY

Preparation of stock solutions:

Standard UDB 100 mg and TFC 100 mg were each weighed and transferred to separate 100 ml volumetric flasks and dissolved in distilled water. The flasks were shaken and the volumes were made up to the mark with distilled water to give solutions containing 1000 µg/ml of UDB and 1000µg/ml of TFC, respectively. From each of these stock solutions,10ml was pipette out and placed into separate 100 ml volumetric flasks, and the volumes were made up to the mark with distilled water to give solutions containing 100 µg/ml of UDB and 100 µg/ml of TFC, respectively.

Selection of analytical concentration ranges:

From the standard stock solutions of UDB and TFC, appropriate aliquots were pipetted out into separate10 ml volumetric flasks ,and dilutions were made with distilled water to obtain working standard solutions of concentrations ranging from 10 to 150 µg/ml. The absorbance of the UDB solutions was measured at 267 nm, while the absorbance of the TFC solutions was measured at 287 nm. For both UDB and TFC, the analytical concentration range for the standard solutions was found to be 10-100 µg/ml. T

METHOD B: SECOND ORDER DERIVATIVE METHOD

Preparation of stock solutions:

Standard UDB 100 mg and TFC 100 mg were each weighed and transferred to separate 100 ml volumetric flasks and dissolved in distilled water. The flasks were shaken, and the volumes were made up to the mark with distilled water to give solutions containing 1000 µg/ml of UDB and 1000µg/ml of TFC, respectively. From each of these stock solutions,10ml was pipette out and placed into separate 50 ml volumetric flasks. The volumes were made up to the mark with

Distilled water to give solutions containing 200µg/ml of UDB and 200µg/ml of TFC, respectively.

Selection of analytical concentration ranges:

From the standard stock solutions of UDB and TFC, appropriate aliquots were pipetted out into separate10 ml volumetric flasks, and dilutions were made with distilled water to obtain working standard solutions of concentrations ranging from 1 to 100 µg/ml. The absorbance of these solutions was measured at 274 nm for UDB and at 287 nm for TFC. For the standard solutions, the analytical concentration range was found to be 20-80 µg/ml, and those values are given in Table 5.7.

Calibration curve for the UDB and TFC(20–80 µg/ml):

Appropriate volumes of aliquots from the standard UDB and TFC stock solutions were transferred to different volumetric flasks of 10 ml capacity. The volumes were adjusted to the mark with distilled water to obtain concentrations of 20, 30, 40, 50, 60, 70, and 80 µg/ml for both UDB and TFC. The absorbance spectra of each UDB solution against distilled water as a blankweremeasuredat274nm, and for each TFC solution, the absorbance was measured at 287 nm. The graphs of absorbance against concentration were plotted and are shown in Fig. 5.3. The regression equations and correlation coefficients were determined and reported in Table 5.8.

Sample preparation for determination of UDB and TFC from dosage form: Twenty tablets of two brands, each containing 100 mg of UDB and TFC, were weighed and finely powdered. The powder equivalent to 100 mg of each drug was accurately weighed and transferred to separate 100 ml volumetric flasks containing 25 ml of distilled water and sonicated for 5 minutes. The flasks were shaken and the volumes were made up to the mark with distilled water to give solutions of 1000 µg/ml for both UDB and TFC. These solutions were centrifuged at 2000 rpm for10minutesand carefully filtered through Whatman filter paper(No.41).From each solution, 10 ml was pipetted and diluted to 50 ml with distilled water to give solutions of 200 µg/ml, which were used for the estimation of UDB and TFC, respectively.

Validation of Spectrophotometric method:

All the parameters are same as Method A.

ANALYTICAL METHOD DEVELOPMENT

Various new analytical methods are required for controlling the quality of constantly growing new drugs. Alternate methods for existing (non-Pharmacopoeia) products are developed to reduce the cost and time for better precision and ruggedness. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit / demerits are made available.

VALIDATION[18] OBJECTIVE AND PARAMETERS OFANALYTICAL METHOD VALIDATION

ACCURACY

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

PRECISION

The precision of an analytical procedure expresses the closeness of agreement(degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Repeatability expresses the precision under the same operating conditions over a short interval of time.

REPRODUCIBILITY

The procedure is carried out by different analyst in different laboratories using different equipment, regents and laboratories setting.

SELECTIVITY

The selectivity of a method is a measure of how capable it is of measuring the analyte alone in the presence of other compounds contained in the sample.

SENSITIVITY

The sensitivity of method indicates how responsive it is to a small change in the concentration of an analyte. It

SPECIFICITY

An investigation of specificity should be conducted during the validation of identification tests and determination of impurities.

LINEARITY RANGE

The equation of a straight line takes the form.

Y= a+ b x

Where,,a" is the intercept of the straight with the y axis and,, b'is the slope of the line.The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

LIMITOFDEFECTION

. It is formally defined as follows.

X– Xb =3sb

Where X" is the signal from the sample. „Xb" is the signal from the analytical blank and Sb is the SD of the reading for the analytical blank. The detection limit is usually expressed as the concentration of analyte(percentage parts per million)in the sample. We can calculate LOD by using the following formula.

LOD=3*SD /slope of calibration curve SD =Standard deviation of intercepts.

LIMITOFQUANTITATION

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

ROBUSTNESS

This term refers to how resistant the precision and accuracy of an assay is to small variation in the method ,e.g.changes of instrumentation, slight variation in extraction procedure, sensitivity to minor impurities in reagents, etc.

RUGGEDNESS

Ruggedness is a measurement of reproducibility of test results under the variation in condition normally expected from laboratory to laboratory and from analyst to analyst.

VALIDATION OF ANALYTICAL METHOD:

1.Accuracy
2.Precision
3.Linearity
4.Limit of detection(LOD) 5.Limit of quantitation(LOQ)
6.Ruggedness:
7.Robustness:

RESULT AND DISCUSSION

TRAILS FOR METHOD DEVELOPMENT

Trail 1:

Mobile phase : Methanol: Water (80:20%v/v) **Column :** X-Bridge (4.6 ×150mm, 5µm particle size) Make; waters **Flow rate :** 2.0ml/min **Wavelength :** 252nm **Column temp :** 30ºC **Injection Volume :** 10µl **Run time :** 5 minutes

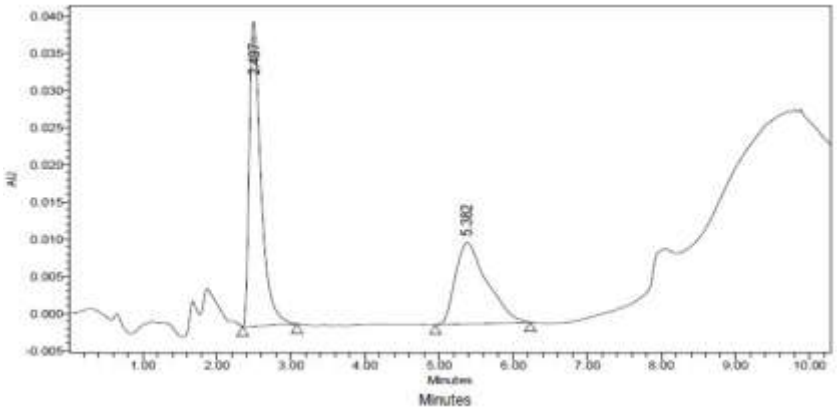

Fig. 10 (a) :- Chromatogram for Trail 1

Table 12 (a) :- Peak Results for Trail 1

S.No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP Plate count
1	Upadacitnib (UDB)	2.497	7585486	865987		1.21	5869
2	Tofacitnib (TFC)	5.382	653264	35628	4.65	1.19	8547

Observation: This trial shows improper separation of sample peaks and less plate count, improper baseline in the chromatogram. So more trials were required for obtaining good peaks.

Trail 2:

Mobile phase : Methanol: Acetonitrile (40:60 v/v)

Column : Hypersil C18 (4.6mm×250mm) 5µ Particle Size

Flow rate : 0.9 ml/min **Wavelength :** 246nm **Column temp :** Ambient **Injection Volume :** 5µl **Run time :** 10 minutes

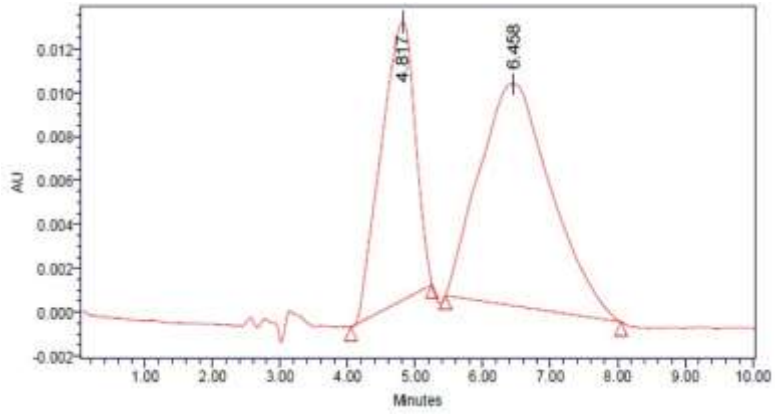

Fig. 10 (b) :- Chromatogram for Trail 2

Table 12 (b) :- Peak Results for Trail 2

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Upadacitnib (UDB)	4.817	8569885	685985		1.09	2163
2	Tofacitnib (TFC)	6.458	98569985	669584	1.23	1.06	3524

Observation: From the above chromatogram it was observed that the baseline is improper and sample peaks are not well separated. So it requires more trials to obtain well peaks.

Trail 3:

Mobile phas : Methanol: Water (60:40 % v/v)

Column : Symmetry C18 (4.6×250mm 5µm)

Flow rate : 0.9 ml/min **Wavelength** : 242 nm

Column temp : 33ºC **Injection Volume :** 10µl

Run time : 6 minutes

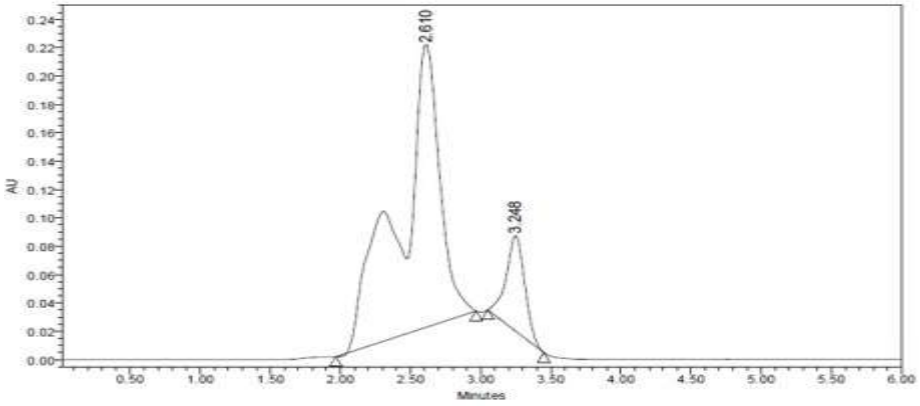

Fig. 10 (c) :- Chromatogram for Trail 3

Table 12 (c) :- Peak Results for Trail 3

S. No	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Upadacitnib (UDB)	2.610	2865985	365845		0.92	2368
2	Tofacitnib (TFC)	3.248	548556	4256	0.59	1.06	3525

Observation: This trial show very less plate count and sample peaks are not well separated, so more trials were required for obtaining good peaks.

PART A: DERIVATIVE SPECTROSCOPY

METHOD A: FIRST ORDER DERIVATIVE SPECTROSCOPY

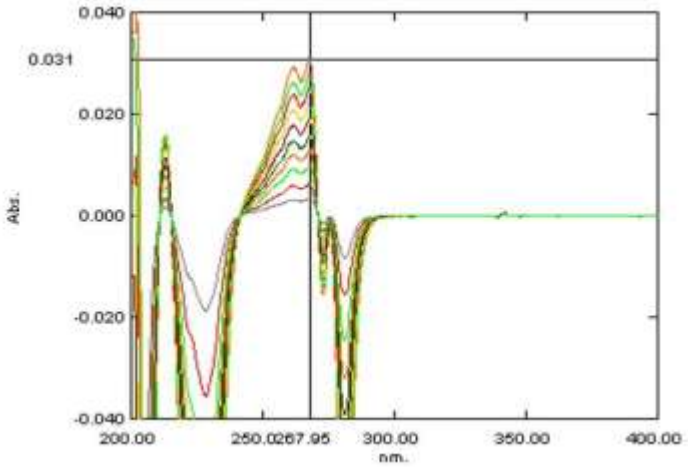

Table: 5.1. Results of Calibration curve at 267 nm for UDB and TFC by First order Derivative Spectroscopy

Sl. No.	Conc. (µg / ml)	Absorbance at 267 nm
1	10	0.003
2	20	0.007
3	30	0.010
4	40	0.013
5	50	0.016
6	60	0.019
7	70	0.022
8	80	0.025
9	90	0.028
10	100	0.031

Table: 5.2. Optimum conditions, Optical characteristics and Statistical data of the Regression equation in First order Derivative Spectroscopy

Parameter	First order Derivative Spectroscopy
λ_{max} (nm)	267
Beer's law limits (µg / ml)	10-100
Molar extinction coefficient (mol^{-1} cm^{-1})	0.03 $X10^4$
Sandell's sensitivity (µg / cm^2-0.001 absorbance units)	3.125
Regression equation (Y*)	Y = 0.0003 C + 0.0006
Slope (b)	0.0003
Intercept (a)	0.0006
Correlation coefficient (r^2)	0.9992

Table: 5.3. Determination of Accuracy results of UDB and TFC by First order Derivative Spectroscopy

Amount of sample (µg / ml)	Amount of drug added (µg / ml)	Amount Recovered** (µg / ml)	% Recovery ± SD**
Tablet 1			
10	9.0	19.08	100.89 ± 0.12
12	12	24.10	100.83 ± 0.30
Tablet 2			
10	9.0	18.95	99.45 ± 0.15
12	12	24.12	101.00 ± 0.24

****Average of six determinations Tablet 1: RINVOQ Tablet 2: REMATIB**

Table: 5.4. Determination of Precision results for UDB at 267 nm by First order Derivative Spectroscopy

Label claim (mg)	Amount found** (mg)	% Label claim	% RSD
100	99.67	99.67	0.06
100	99.50	99.50	0.02

**Average of six determinations

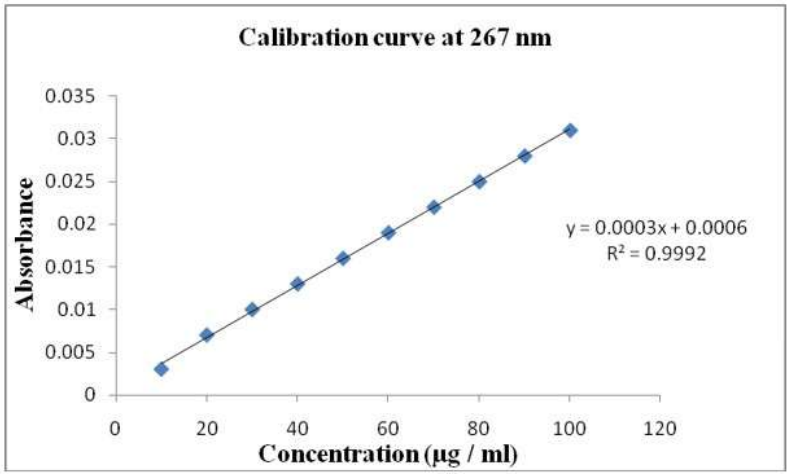

Fig: (5.2). Calibration curve for UDB and TFC at 267 nm by First order Derivative Spectroscopy

Table: 5.5. Repeatability data for UDB and TFC at 267 nm by First Order Derivative Spectroscopy

Conc. (µg / ml)	Absorbance			Mean	SD
10	0.003	0.004	0.003	0.003333	0.000577
20	0.007	0.008	0.007	0.007333	0.000577
30	0.010	0.011	0.010	0.010333	0.000577
40	0.013	0.013	0.014	0.013333	0.000577
50	0.016	0.016	0.017	0.016333	0.000577
60	0.019	0.019	0.020	0.019333	0.000577
70	0.022	0.021	0.022	0.0021666	0.000577
80	0.025	0.026	0.025	0.025333	0.000577
90	0.029	0.028	0.029	0.028667	0.000577
100	0.031	0.032	0.031	0.031333	0.000577

Table: 5.6. Ruggedness results for UDB and TFC at 267 nm by First order Derivative Spectroscopy

Sample	Label claim (mg)	Analyst I		Analyst II	
		Amount found** (mg)	% Recovery ± SD**	Amount found** (mg)	% Recovery ± SD**
Tab 1	100	99.73	99.73 ± 0.04	99.69	99.69 ± 0.01
Tab 2	100	99.84	99.84 ± 0.02	99.90	99.90 ± 0.03

** Average of six determinations. Tablet 1: RINVOQ Tablet 2: REMATIB

METHOD B : SECOND ORDER DERIVATIVE SPECTROSCOPY

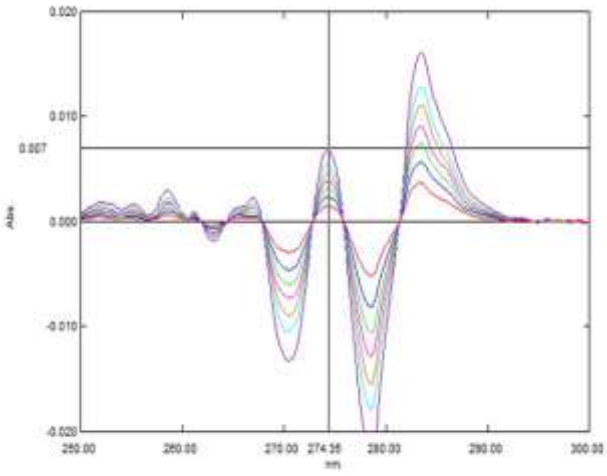

Fig: (5.3). Second order Spectra of UDB and TFC at 274 nm

Table: 5.7. Results of Calibration curve at 274 nm for UDB and TFC by Second order Derivative Spectroscopy

Sl. No.	Conc. (µg / ml)	Absorbance at 274 nm
1	20	0.001
2	30	0.002
3	40	0.003
4	50	0.004
5	60	0.005
6	70	0.006
7	80	0.007

Table: 5.8. Optimum conditions, Optical characteristics and Statistical data of the Regression equation in Second order Derivative Spectroscopy

Parameters	Second order Derivative Method
λ_{max} (nm)	274
Beer's law limits (µg / ml)	20 - 80
Molar extinction coefficient (mol^{-1} cm^{-1})	0.0019 X 10^4
Sandell's sensitivity (µg / cm^2-0.001 absorbance units)	15
Regression equation (Y*)	Y = 0.0001 C – 0.001
Slope (b)	0.0001
Intercept (a)	-0.001
Correlation coefficient(r^2)	1.000

***Y= b C + a where C is the concentration of UDB in µg / ml and Y is the absorbance at the respective lmax.**
****Average of six determinations.**

Table: 5.9. Determination of Accuracy results for UDB and TFC by Second order Derivative Spectroscopy
Table: 5.9. Determination of Accuracy results for UDB and TFC by Second order Derivative Spectroscopy

Amount of sample (µg / ml)	Amount of drug added (µg / ml)	Amount Recovered** (µg / ml)	% Recovery ± SD**
Tablet 1			
10	9.0	18.92	99.11 ± 0.13
10	10	19.90	99.00 ± 0.19
Tablet 2			
10	9.0	18.95	99.44 ± 0.15
10	10	20.02	100.25 ± 0.20

**Average of six determinations

Tablet 1: RINVOQ

Tablet 2: REMATIB

Table: 5.10. Determination of Precision results for UDB and TFC at 274 nm by Second order Derivative Spectroscopy

Label claim (mg)	Amount found ** (mg)	% Label claim	% RSD
100	99.67	99.67	0.09
100	99.76	99.76	0.10

**Average of six determinations

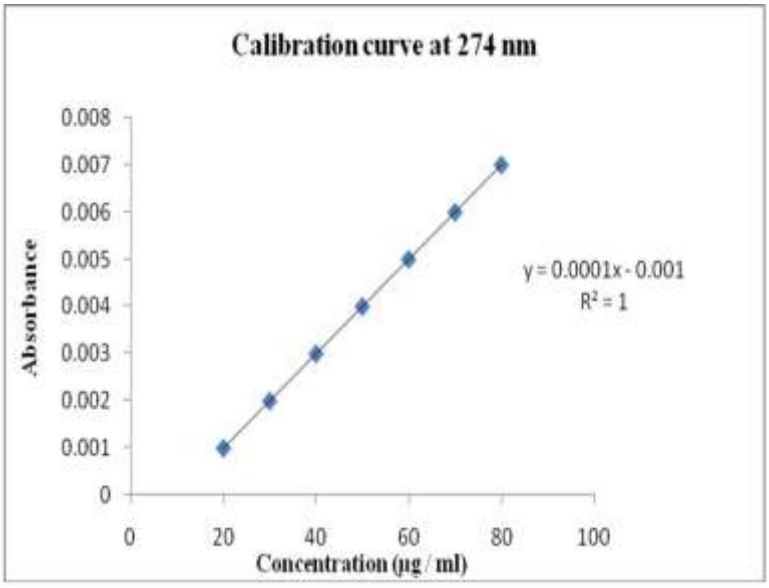

Fig: (5.4). Calibration curve of Linearity for UDB and TFC at 274 nm by Second order Derivative Spectroscopy

Table: 5.11. Repeatability data for UDB and TFC at 274 nm by Second order Derivative Spectroscopy

Conc. (µg / ml)	Absorbance**	SD
20	0.001333	0.000577
30	0.002333	0.000577
40	0.003333	0.000577
50	0.004333	0.000577
60	0.005333	0.000577
70	0.006333	0.000577
80	0.007333	0.000577

****Average of six determinations**

Table: 5.12. Ruggedness results for UDB and TFC at 274 nm by Second order Derivative Spectroscopy

Sample	Label claim (mg)	Analyst I		Analyst II	
		Amount found** (mg)	% Recovery ± SD**	Amount found** (mg)	% Recovery ± SD**
Tab 1	100	99.85	99.85 ± 0.09	99.90	99.9 ± 0.17
Tab 2	100	100.02	100.02 ± 0.43	99.95	99.95 ± 0.09

** Average of six determinations

**** Average of six determinations Tablet 1: RINVOQ Tablet 2: REMATIB**

PART B: HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

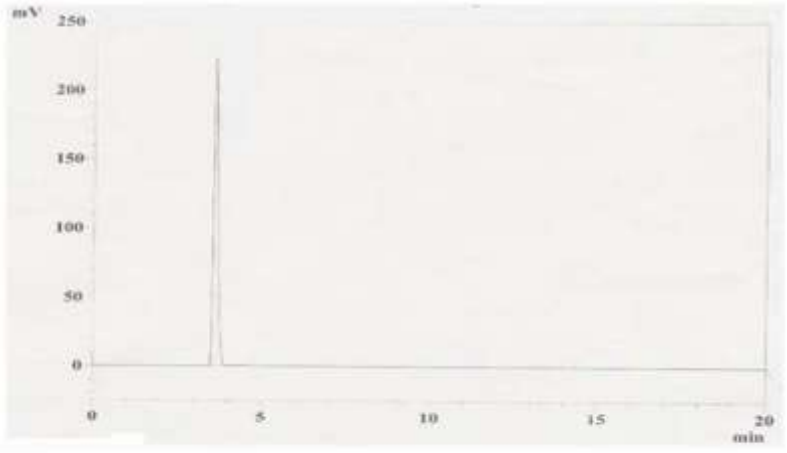

Fig: (5.3). Chromatogram of UDB and TFC at 270 nm

Table: 5.13. Parameters for HPLC

Instrument	High Performance Liquid Chromatography LC-20AT SHIMADZU – SPD 20A Detector
Injector	Rheodyne
Column	Phenomenex-Gemini RP C-18 (250 x 4.6 mm, 5 µm)
Wavelength	UV – Vis Detector SPD 20A (270 nm)
Flow rate	1.5 ml / min
Injection volume	20 µl
Mobile phase	81 : 19 v / v Triflouro acetic acid: Acetonitrile
pH	3.0 ± 0.5 Adjusted with Phosphoric acid
Temperature	30° C
Run time	20 minute

VALIDATION OF ANALYTICAL METHOD:

Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics are expressed in terms of analytical parameters.

ACCURACY:

The accuracy of the method will be inferred by establishing the precision and linearity of standard.

Table: 5.14. Accuracy results for UDB (for pure drug)

Area**	**0.250 (mg / ml)**	**0.375 (mg / ml)**	**0.500 (mg / ml)**	**0.625 (mg / ml)**	**0.750 (mg / ml)**
	1065470	1585971	2126500	2623413	3112651
% Assay	50.18	75.45	100.29	125.36	151.43
Theoretical %	50	75	100	125	150
% Accuracy	100.36	100.60	100.29	100.29	100.95

** Average of five determinations

Table: 5.15. Accuracy results for UDB and TFC (for dosage form)

Area**	0.250 (mg / ml)	0.375 (mg / ml)	0.500 (mg / ml)	0.625 (mg / ml)	0.750 (mg / ml)
	1065470	1585971	2126500	2623413	3112651
% Assay	50.34	75.56	100.33	125.41	151.49
Theoretical %	50	75	100	125	150
% Accuracy	100.68	100.75	100.33	100.33	100.32

** Average of five determinations

2. PRECISION:

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision expressed as standard deviation or relative standard deviation.

Table: 5.16. System precision results for UDB

Sl. NO.	R.T**	Peak area**
1	3.619	2046519
SD	0.005477	367.08
% RSD	0.151	0.020

** Average of five determinations

Method precision (for pure drug)

Table: 5.17. Method Precision results for UDB and TFC (for pure drug)

Sl. No.	Peak Area**	R.T**
1	2048790	3.619
% RSD	0.036	0.151

** Average of five determinations

Table: 5.18. Method Precision results for UDB and TFC (for dosage form)

Sl. No.	Peak Area**	R.T**	% Assay
1	2034506	3.62	99.42
% RSD	0.072	0.015	0.06

3. LINEARITY:

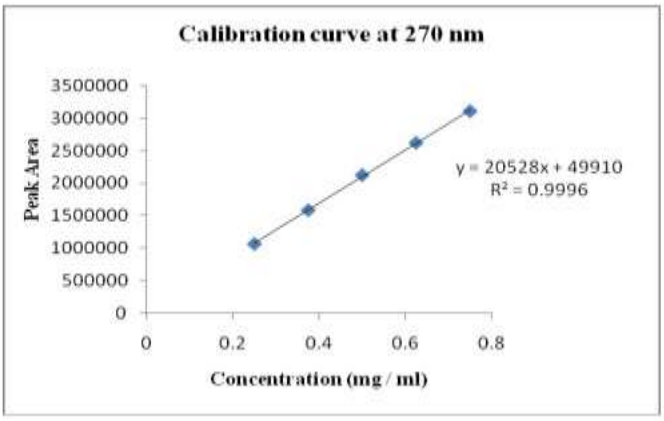

Fig: (5.4). Calibration curve of UDB at 270 nm

Table: 5.19. Linearity results for UDB and TFC

Conc. (mg / ml)	0.250	0.375	0.500	0.625	0.750
Peak area	1065405	1585725	2126607	2623390	3112623

Table: 5.20: Calibration parameters of UDB and TFC

Parameter	**Results**
Slope	20528
Intercept	49910
Correlation co-efficient	0.9996
Percentage Curve Fitting	99.96

2. LIMIT OF DETECTION (LOD):

Table: 5.21. LOD results of UDB and TFC

Injection No.	**Peak Area**	**R.T**
1	210	3.635
2	207	3.635
3	205	3.632
% RSD	1.21	0.04

LIMIT OF QUANTITATION (LOQ):

Table: 5.22: LOQ results of UDB and TFC

Injection No.	Peak Area	% Assay	R.T
1	640	99.95	3.639
2	638	99.41	3.632
3	642	99.72	3.635
% RSD	0.312	0.25	0.092

4.RUGGEDNESs

The ruggedness of an analytical method is determined by analysis of aliquots from homogenous lots by different analysts using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The assay of UDB was performed by different analyst and on different dates (days).

Table: 5.23: Ruggedness results of UDB and TFC (Day-1, Analyst-1)

Injection No.	Peak Area	% Assay	R.T
1	1799401	99.74	3.621
2	1800168	99.56	3.62
Avg.	1799785	99.65	3.62
%RSD	0.030	0.27	0.019

Table: 5.24. Ruggedness results of UDB and TFC (Day-2, Analyst- 2)

Injection No.	Peak Area	% Assay	R.T
1	2047564	99.85	3.625
2	2048694	99.8	3.615
Avg.	2048129	99.82	3.62
%RSD	0.039	0.03	0.195

4. ROBUSTNESS :-

The robustness of an analytical method is determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay.

Table: 5.25. (a) Chromatographic Condition: Change in flow rate

Sl. No	Change in flow rate	R.T
01	1.6ml / min	3.383
02	1.4ml / min	3.851

Table: 5.26. Robustness results of UDB and TFC (Flow rate -1.6)

Injection No.	Peak Area	% Assay	R.T
1	1683580	99.50	3.383
2	1683990	99.46	3.410
Avg.	16833785	99.48	3.396
%RSD	0.017	0.02	0.562

Table: 5.27. Robustness results of UDB (Flow rate -1.4)

Injection No.	Peak Area	% Assay	R.T
1	1921854	99.25	3.851
2	1921724	99.23	3.785
Avg.	1921789	99.24	3.818
%RSD	0.0047	0.01	1.22

Table: 5.28. (b) Chromatographic Condition: Change in mobile phase ratio

Sl. No	Composition of mobile phase	R.T
01	82 : 18	3.025
02	80 : 20	4.403

Table: 5.29. Robustness results of UDB and TFC (Triflouroacetic acid: Acetonitrile = 82 : 18, v / v)

Injection No.	Peak Area	% Assay	R.T
1	1497539	99.46	3.019
2	1497665	99.38	3.164
Avg.	1497602	99.42	3.025
%RSD	0.0059	0.05	0.28

Table: 5.30. Robustness results of UDB and TFC

(Triflouroacetic acid: Acetonitrile = 80 : 20, v / v)

Injection No.	Peak Area	% Assay	R.T
1	2237655	99.27	4.457
2	2237318	99.30	4.350
Avg.	2237486	99.28	4.403
% RSD	0.0106	0.02	1.71

Table: 5.30. Robusness results of UDB and

TFC (Triflouroacetic acid:Acetonitrile=80:20,v/ v

Injection No.	Peak Area	%Assay	R.T
1	**2237655**	**99.27**	**4.457**
2	**2237318**	**99.30**	**4.350**
Avg.	**2237486**	**99.28**	**4.403**
%RSD	**0.0106**	**0.02**	**1.71**

CONCLUSION

PARTA: DERIVATIVE SPECTROSCOPY

Method A:First Order Derivative Spectroscopy
Method B:Second Order Derivative Spectroscopy
The methods were validated interms of linearity, accuracy, precision, ruggedness, and robustness and used for the routine determination of UDB and TFC in bulk and in pharmaceutical dosage forms.
By comparing First and Second Derivative Spectroscopic methods, the results were found to be good and were expressed in Tables. The Second Derivative method was the best among the First order Derivative methods.

PART B:HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

In the present investigation, a simple ,sensitive, precise, and accurate RP-HPLC method was developed for the quantitative estimation of UDB and TFC (Tofacitinib) in bulk and pharmaceutical dosage forms.
The results expressed In Tables for HPLC are promising.The method is validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, ruggedness, and robustness. This method can be used for the routine determination of UDB and TFC in bulk and pharmaceutical dosage forms.

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