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Development & Validation of Stability Indicating RP-HPLC Method for Estimation of COPD Drug in Bulk & Pharmaceutical Dosage Form.

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ABSTRACT:

A simple, selective, accurate, precise, economic and stability-indicating RP-HPLC method for estimation of Pidotimod has been developed and validated in pharmaceutical dosage form. The drug was separated by using a mobile phase of methanol: water, (75:25 v/v) on a Cosmosil C18 (250mm×4.6ID) Particle size 5µ column at flow rate of 1.0 ml/min at surrounding temperature and detection was performed at 203 nm. The retention time was found to be 4.443 min for Pidotimod. The linearity was recognized in concentrations ranging from 10-50 µg/ml. The regression coefficient was 0.9983. Percentage assay of Pidotimod was found to be 100.009%. The Accuracy was calculated by percentage recovery studies at 50%, 100% and 150% at 30ppm, 40ppm and 50ppm respectively, which was found in between the limits of 98%-102% as per ICH Guidelines. The %RSD value of Pidotimod for Inter-Day Precision was found to be 0.15315% and for Intra-Day precision it was found to be 0.2780019%. Which is well within acceptance criteria (NMT 2%). In Robustness study the %RSD for Pidotimod at small change in flow rate is 0.27% and at small change in wavelength is 0.26%. The acceptance criteria are (NMT 2%). The limit of detection for Pidotimod was found to be 0.9189 and the limit of quantification was observed as 2.7847. For stability study, the drug was exposed to the stress conditions like acid (0.1 N HCl), base (0.1 N NaOH), oxidation (3% H2O2), Thermal and photolytic as per the recommendation of ICH guidelines and it was found that there was a considerable change in the peak area of Pidotimod, but not in the retention time. The results of the analysis were validated in terms of Linearity, Assay, Accuracy, Precision, Robustness, limit of detection, limit of quantification and stability study, as per ICH

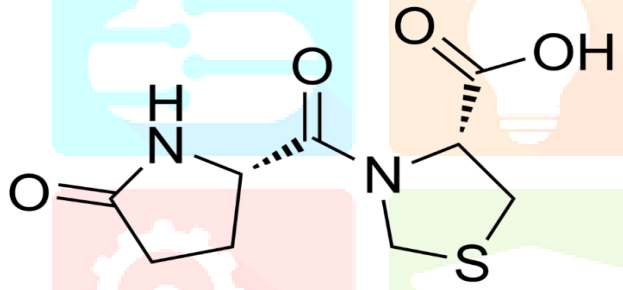
guidelines and were found to be satisfactory. The high recovery and low deviation confirm that the suitability of this method can be employed for the routine analysis of tablet containing Pidotimod.

KEYWORDS: Pidotimod, RP-HPLC, Validation, Stability study, ICH.

INTRODUCTION:

Pidotimod

Pidotimod (PDM), (R)-3-[(S)-(5-oxo-2-pyrrolidinyl) carbonyl]-thiazolidine- 4-carboxylic acid (Figure 1), is a biological response modifier with dipeptide structure, which can stimulate both primary and acquired immune response to virus and bacteria [1,2]. Studies demonstrated that PDM itself does not have antibacterial activity, but along with other antibacterial agents, can improve clinical symptoms of patients, promote recovery and limit hospital stay. It is often used in the treatment of repeated infections of the respiratory, urogenital, ear, nose, and throat systems. PDM therapy is a reliable, simple, and safe approach to treat children with recurrent respiratory infection. It has few adverse effects and demonstrates good safety and tolerability [3]. An extensive literature survey revealed that, few analytical methods have been reported for the quantification of PDM including HPLC-UV [4], HPLC–MS [5], HPLC-MS/MS [6], and HILIC-MS/MS [7]. There is one published report for the determination of residual organic solvents in PDM by GC [8]. Chiral separation of PDM by polysaccharide stationary phase has been reported by Zhang Lu Xing [9] so the aim of present study was to separate R and S enantiomers of Pidotimod on beta cyclodextrin based chiral stationary phase.



[Fig-1] – Structure of Pidotimod

Category	**Immunostimulant**
Chemical Name	(R)-3-((S)-5-Oxopyrolidine-2-carbonyl) Thiazolidine-4-carboxylic acid
Molecular Formula	$C_9H_{12}N_2O_4S$
Molecular Weight	244.27g/mol
Description	White powder.
Solubility	Soluble in Water and Methanol.
Storage	Store at 2° to 8°C
Melting point	192°c -198°c

Mechanism of Action [10]:

Pidotimod inhibits tumor necrosis factor α (TNF-α) induced increases in extracellular signal-related kinase (ERK) phosphorylation. It also increases nuclear factor κB (NFκB) expression and translocation to the nucleus. It is these to modulatory effects on ERK and NFκB signaling which are thought to produce the increase in toll-like receptor expression seen with pidotimod. Pidotimod increase maturation of dendritic cells responsible for presenting antigens to naive Th-cells. It also appears to result in a greater population these cells differentiating to Th1 cells which are believed to mediate the immune response to pathogens like bacteria and viruses. Lastly, pidotimod appears to increase antigen-specific antibody titer and cytotoxic response with antigen exposure. The precise mechanism and timeline of events leading to these effects is unknown.

EXPERIMENTAL SECTION

Materials and Methods:

A binary gradient system of 3000 series HPLC of analytical technologies ltd, was used for analysis & standard Pidotimod was procured from Wockhardt Ltd. Other chemicals like methanol, water, of HPLC grade were purchased from LobaChemie, Mumbai.

Method development & validation:

As per literature review and solubility test the standard Pidotimod was found soluble in methanol. As per the trials done on combination of methanol water system as mobile phase, it had shown better results at 203 nm wavelength and 75:25 v/v ratio of mobile phase as methanol: water.
The developed method was validated as per ICH Guidelines.

Mobile phase preparation: Take a mixture of Methanol: Water in the ratio of 75:25 v/v. Mix it well, sonicate it to degas.
Preparation of Diluent: Preparation of a mixture of Methanol: Water in the ratio of 75:25 v/v.
Preparation of Blank: Diluent is used as blank.
Preparation of Pidotimod Standard stock solution: weigh accurately10mg of pure drug dissolved in 10ml of solvent (solvent was used as our mobile phase only); this gives 1000ppm solution.
Preparation of Pidotimod Sample solution: Take 0.1, 0.2, 0.3, 0.4 and 0.5 ml of this solution and diluted individually in 10 ml of diluent solution, to make dilutions of 10, 20, 30, 40 and 50 ppm respectively.

RESULTS AND DISCUSSION

Optimization of chromatic condition:

After selection of mobile phase, the optimized chromatographic conditions for Pidotimod are as follows.

Table: Data of Pidotimod for optimization of chromatographic condition.

Sr. No.	Mobile phase	Flow rate	Wavelength	Injection volume	Observation	Conclusion
1	Methanol: water (70:30)	1.0ml/min	203	20µl	Unnecessary peak is observed	Method is rejected
2	Methanol: water (75:25)	1.0ml/min	203	20µl	Peak shape is proper	Method is selected for further validation.

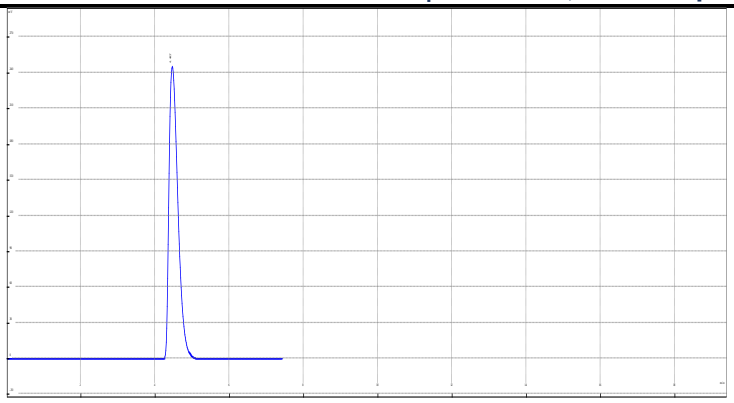


Figure: Chromatogram for Pidotimod trial 2

Table: The optimized chromatographic conditions for Pidotimod are as follows.

Sr. No.	Parameters	Optimized condition
1	Mobile phase	Methanol: Water
2	Flow rate	1.0 ml/min
3	Wavelength	203
4	Injection volume	20µl
5	Column	Cosmosil C18 (250mm×4.6ID) Particle size 5µ

Validation parameter:

Linearity: The linearity of the method was recognized by determining the absorbance of different concentrations of Pidotimod over a range of 10-50µg/ml.

Table: Linearity data of Pidotimod.

Conc.	Area
10	332433
20	622775
30	924162
40	1228909
50	1587562

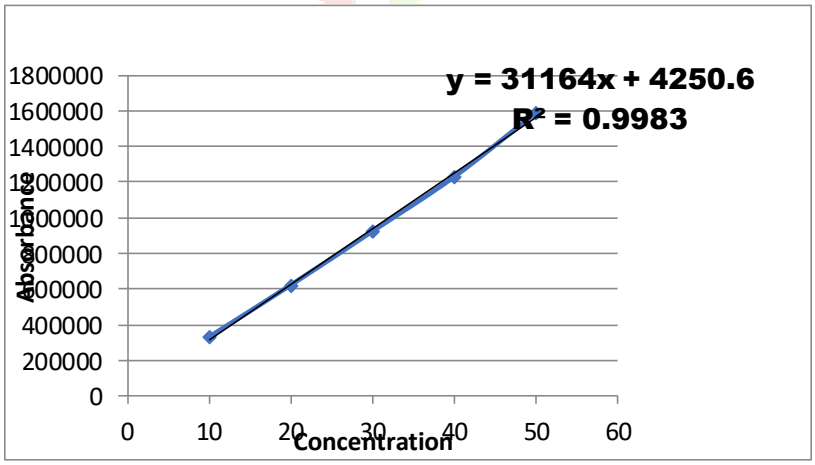


Fig: Calibration curve of drug sample

Assay: Assay of formulation available in the market was done by injecting sample corresponding to equivalent weight into HPLC system and recovery studies were carried out.

Table: Data of assay

Sr. NO.	Name of drug	Area of Standard	Area of Sample	% Assay
1	**Pidotimod**	924162	924243	100.009

Accuracy: To obtain accuracy of the proposed method, recovery studies were done by examining the samples, which were carried out by examining the measured concentration and the added concentration of the drug. Each sample was injected three times. The percent recoveries of drugs were estimated as follows.

Table: Accuracy data of Pidotimod

Sr. NO.	% composition	Sample Amount		Area of Sample	Amount Recovered in ppm	% Recovery	% Mean Recovery	% SD
		Sample Amount in ppm	Amount Added in ppm					
1	50% Recovery	20	10	922080	29.93	99.77	99.91	0.1070
		20	10	924126	29.99	99.99		
		20	10	924223	29.99	99.99		
2	100% Recovery	20	20	1224706	39.86	99.65	99.81	0.0048
		20	20	1227814	39.96	99.90		
		20	20	1227676	39.95	99.89		
3	150% Recovery	20	30	1582977	49.85	99.68	99.88	0.1170
		20	30	1587320	49.99	99.98		
		20	30	1586312	49.99	99.98		

Precision: Precision is one of the important factors which decides the reliability of an analytical method. The drugs Pidotimod was studied for the Interday and intraday precision studies, and the results are shown in below table.

Table: Interday and Intraday precision data of Pidotimod

Interday Precision		**Intra-day Precision**	
Day 1	**Area**	**Morning**	**Area**
	924162		924162
	925947		925947
	926278		926278
Day 2	922530	**Evening**	926348
	922865		931882
	923867		929599
Mean	924274.83	Mean	927369.33
SD	1415.3887	SD	2578.1041
% RSD	0.153135%	%RSD	0.2780019%

Robustness:

The robustness of planned method was obtained by analysis of sample from homogenous sets by differing physical parameters like change in flowrate, change in wavelength which may differ, but the responses were still within the limits of the assay.

Table7.8: Robustness data of Pidotimod

Level	**Pidotimod**		**Area**
	Retention time	**Tailing factor**	
Change in	**Flow rate (ml/min)**		
-0.2(0.8ml)	5.370	1.24	621636
0(1.0ml)	5.370	1.24	622775
+0.2(1.2ml)	3.613	1.29	625725
Change in	**Wavelength (nm)**		**%RSD 0.276403**
-2(201nm)	4.299	1.21	621032
0(203nm)	4.301	1.20	622775
+2(205nm)	4.343	1.21	625002
---	---	---	**%RSD 0.260822**

Limit of detection and limit of quantification:

According to ICH guidelines, the approach depends on the standard deviation of response and slope of calibration plots was used to determine detection and quantification limits.

LOD and LOQ values were estimated as [(standard deviation of repeatability)/ (Slope of the regression equation)] by multiplying with 3.3 and 10 respectively.

LOD= 3.3× (SD/Slope) (for LOD)
LOQ= 10× (SD/Slope) (for LOQ)
Where,

SD = Standard deviation of Y intercepts of the 5calibration curve.

Slope = Mean slope of 5 calibration curve.

Table7.9: Data of LOD and LOQ

Drug	**Pidotimod**
LOD (µg/ml)	0.918968
LOQ (µg/ml)	2.784753

Stability Study:

Stability indicating method of new drug undertaken in condition more severe than accelerated conditions. This study is helpful for elucidation of structure for degradation of products. It helps to analyzed chemical behavior of molecule that helps in development of formulation and packaging. The nature of stress testing will be depending upon individual drug substance and type of drug product involved.

Table7.10: Stability study of Pidotimod

Sr. NO.	Degradation	Area of Standard	Area of degraded Sample	Degraded upto %	Actual % degradation
1	Acid Degradation	1587562	1365562	86.016294167	13.98370583
2	Basic Degradation	1587562	1403076	88.37928849	11.62071151
3	H2O2 Degradation	1587562	1477649	93.07661685	6.92338315
4	Thermal Degradation	1587562	1543941	97.25232778	2.747672217
5	Photolytic Degradation	1587562	1553771	97.8715162	2.128483801

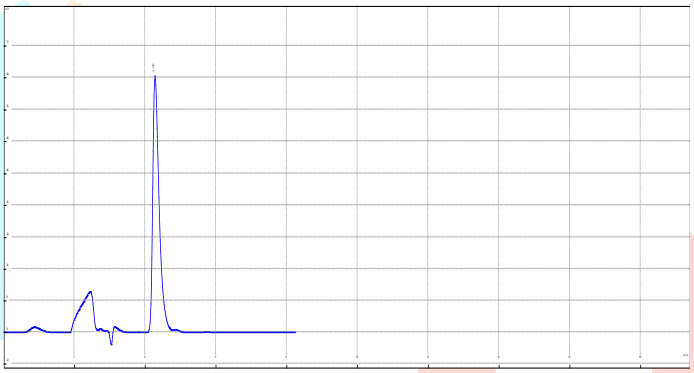


Fig7.27: Chromatogram for degradation study of Pidotimod after exposing to acid 0.1 N HCL

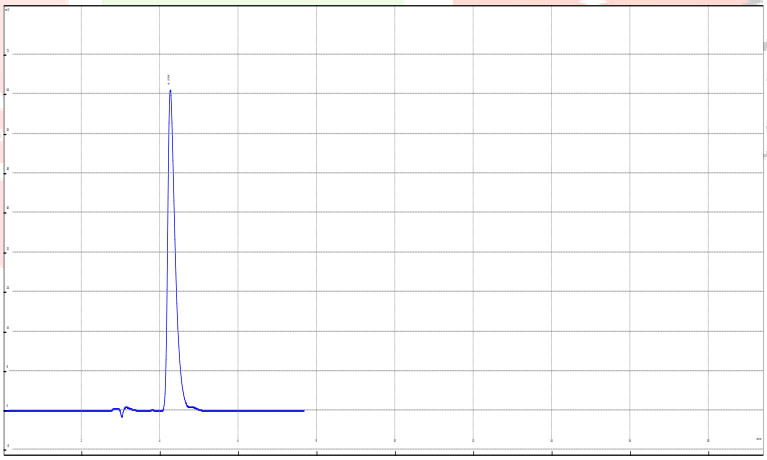


Fig7.28: Chromatogram for degradation study of Pidotimod after exposing to Base 0.1 N NAOH.

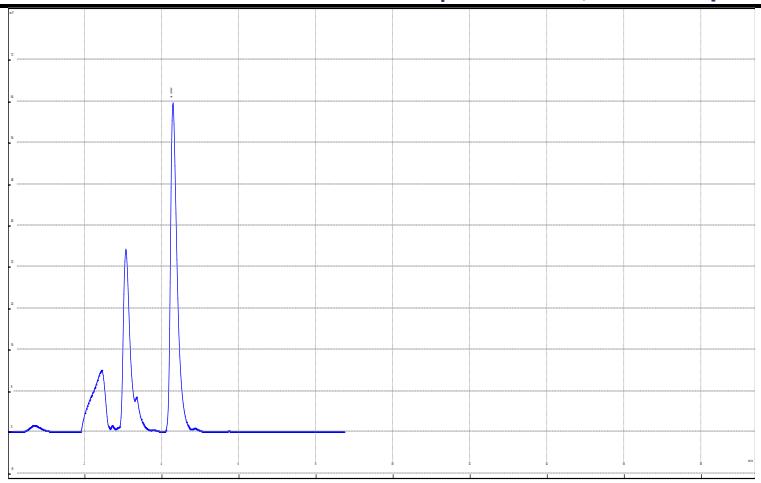


Fig7.29: Chromatogram for degradation study of Pidotimod after exposing to H2O2.

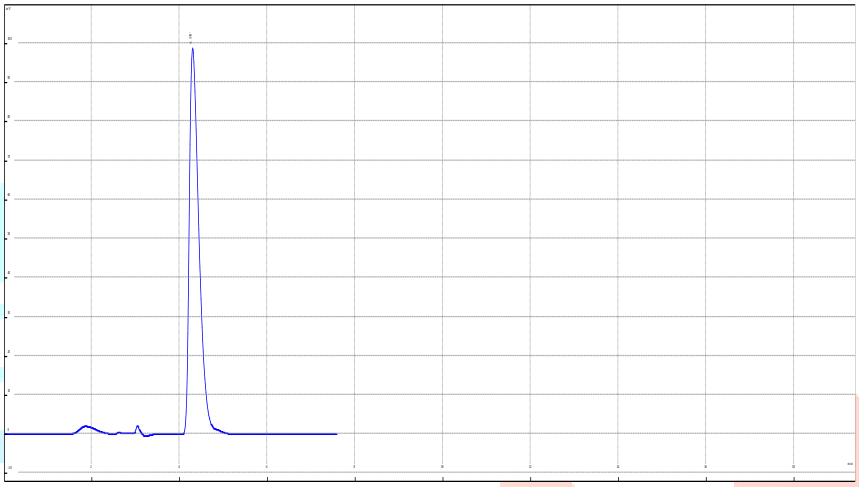


Fig7.30: Chromatogram for degradation study of Pidotimod after exposing to Thermal / dry heat.

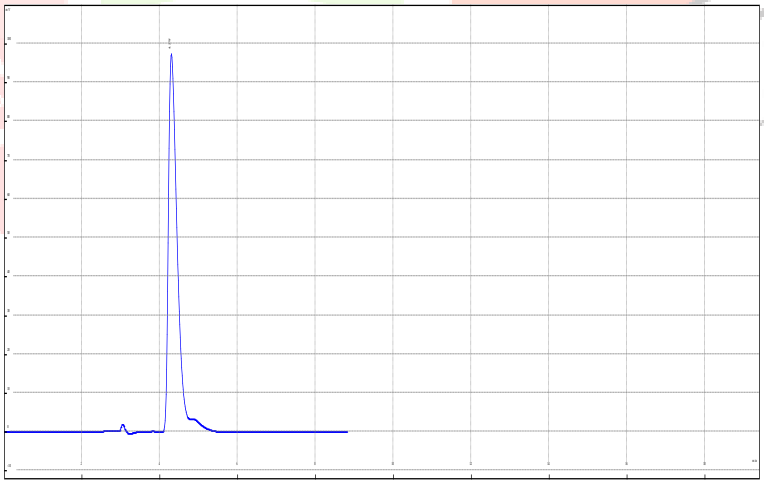


Fig7.31: Chromatogram for degradation study of Pidotimod after exposing to UV-Radiation

CONCLUSION

A simple, selective, accurate, precise, economic and stability-indicating RP-HPLC method for estimation of Pidotimod has been developed and validated in pharmaceutical dosage form. The drug was separated by using a mobile phase of methanol: water, (75:25 v/v) on a Cosmosil C18 (250mm×4.6ID) Particle size 5µ column at flow rate of 1.0 ml/min at surrounding temperature and detection was performed at 203 nm. The retention time was found to be 4.443 min for Pidotimod. The linearity was recognized in concentrations ranging from 10-50 µg/ml. The regression coefficient was 0.9983. Percentage assay of

Pidotimod was found to be 100.009%. The Accuracy was calculated by percentage recovery studies at 50%, 100% and 150% at 30ppm, 40ppm and 50ppm respectively, which was found in between the limits of 98%-102% as per ICH Guidelines. The %RSD value of Pidotimod for Inter-Day Precision was found to be 0.15315% and for Intra-Day precision it was found to be 0.2780019%. Which is well within acceptance criteria (NMT 2%). In Robustness study the %RSD for Pidotimod at small change in flow rate is 0.27% and at small change in wavelength is 0.26%. The acceptance criteria is (NMT 2%). The limit of detection for Pidotimod was found to be 0.9189 and the limit of quantification was observed as 2.7847. For stability study, the drug was exposed to the stress conditions like acid (0.1 N HCl), base (0.1 N NaOH), oxidation (3% H2O2), Thermal and photolytic as per the recommendation of ICH guidelines and it was found that there was a considerable change in the peak area of Pidotimod, but not in the retention time. The results of the analysis were validated in terms of Linearity, Assay, Accuracy, Precision, Robustness, limit of detection, limit of quantification and stability study, as per ICH guidelines and were found to be satisfactory. The high recovery and low deviation confirm that the suitability of this method can be employed for the routine analysis of tablet containing Pidotimod.

SUMMARY

Table: Summary of observed validated parameters for Pidotimod.

Sr. No.	Validation parameters	Acceptance criteria			Reported observation
	Assay	Assay data for Pidotimod			100.009%
1	**System suitability**	Retention time	Pidotimod		4.443 min
		Resolution should be Zero	Pidotimod		0.00
		Theoretical plates NLT 2000	Pidotimod		7629
		Tailing/Asymmetry factor NMT 2	Pidotimod		1.26
2	**Linearity**	Correlation coefficient	Pidotimod		0.9983
		Retention time	Pidotimod		4.443
3	**Accuracy and % recovery**	Acceptable limit is 98-102% Mean sample recovered for that three sample were prepared at different level RSD-NMT 2%			
			50%	**100%**	**150%**
		Pidotimod	99.91	99.81	99.88
4	**Precision**				
	Interday precision	RSD-NMT 2%	Pidotimod		0.15%
	Intraday precision	RSD-NMT 2%	Pidotimod		0.27%
5	**Robustness**	Critical Parameters			
					%RSD

		Change in flow rate (± 0.2 ml/min)	Pidotimod	0.27
		Change in wavelength (± 2 nm)	Pidotimod	0.26
6	**LOD**	Limit of detection	Pidotimod	0.918968
7	**LOQ**	Limit of quantitation	Pidotimod	2.784753
8	**Forced degradation**	**Degradation NMT 20%**		
		Acidic (0.1N HCL)	Pidotimod	13.98%
		Alkaline/Base (0.1N NaOH)	Pidotimod	11.62%
		Oxidative (3% H_2O_2)	Pidotimod	06.92%
		Photolytic	Pidotimod	2.12%
		Thermal	Pidotimod	2.74%

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