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RP-HPLCMETHOD DEVLOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LIDOCAINE, NIFEDIPINE AND IMIDAZOLIDINYL UREA IN THE TOPICAL DOSAGE FORM

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ABSTRACT
A simple, accurate, precise, specific Reverse phase High performance liquid chromatography method was developed and validated for simultaneous estimation of lidocaine, nifedipine, and imidazolidinyl urea in pharmaceutical formulation. The approach was validated for lidocaine, nifedipine, and imidazolidinyl urea using ICH recommendation over a range of 15-75 µl/mL and 3-15 µl/mL and 2-10 µl/mL for lidocaine, nifedipine, imidazolidinyl urea respectively. An analytical column HYPERSIL ODS C_{18}250 nm * 4.6 mm was utilized. At a flow rate of 1.0 ml/min. the mobile phase was Acetonitrile:methanol:0.05m potassium dihydrogen phosphate buffer(40:35:25v/v) ratio. The elution was examined using UV detector with detection wavelength of 225 nm. The retention time for lidocaine, nifedipine, and imidazolidinyl urea are 4.091min, 8.291min and 5.521min respectively. Accuracy, precision, repeatability, robustness and specificity in accordance with ICH guidelines. The method can be successfully employed for the simultaneous determination of lidocaine, nifedipine and imidazolidinyl urea in pharmaceutical formulation.

KEYWORDS: Lidocaine,Nifedipine, Imidazolidinyl urea, RP-HPLC method

INTRODUCTION

Lidocaine at the 1.5% w/w,0.3% w/w for Nifedipine and 0.2% w/w Imidazolidinyl urea is use for treatment of fissures when it is combined with a topical medication and applied directly to the perianal area.
When detailed review of literature was carried out, following points were concluded.

- There are two stability indicating methods reported for estimation of both the drugs from cream but neither of the methods is able to check the presence of preservatives present in the formulation (Which itself is the active ingredient "IMIDUREA").
- (Meshram DB,Mehta K, Mishra P. Stability Indicating Analytical Method for the Simultaneous Estimation of Lidocaine and Nifedipine in the Combined Dosage Form. *Der Pharma Chemica.* **2018**.10(1): 60-66
- Modi T, Patel B, Patel J. Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Lignocaine HCl and Nifedipine in Cream. Research & Reviews: Journal of Pharmaceutical Analysis. **2016**. 5(1): 1-37.
- Furthermore, in both the reported methods the retention time for lidocaine is about 2.5 minutes at which, column dead volume is expected. There is no meaning of carrying out stability study with Rt nearing to column dead volume hence there is a need for a new method which has a better handling capacity for lidocaine.

By considering all the above mentioned points, it was decided to develop and validate a new RP-HPLC method for estimation of Lidocaine, nifedipine along with added preservatives from their combined pharmaceutical topical preparation.

Materials and Methods

Instrument specification

Table 1 Instrument specification for RP HPLC

Make	Shimadzu
Model	LC 2010
Type	Binary Gradient
Detector	UV detector
Software	LC solution
Column	Hypersil ODS C_{18} (250*4.6 mm, 5 Micro-meter)
Pump	High Pressure Gradient (Reciprocating pump)

Table 1 Instrument specification for weighing balance

Make	Mettler Toledo
Sensitivity	0.1 milligram
Minimum weighing Capacity	1 milligram

Table 2 Instrument specification for melting point apparatus

Make	Gallenkamp
Design No.	889339

Table 3 Instrument Specification for UV double beam Spectrophotometer

Make	: Shimadzu
Model	: UV 1800
Type	: Double beam spectrophotometer
Detector	: Photodiode
Scanning Range	: 190 – 1100
Output	: %T & Absorbance
Software	: U.V. Probe 2.42

HPLCMETHODDEVELOPMENT

SELECTIONOF ANALYTICAL/DETECTIONWAVELENGTH

A Working standard of LID (15 µg.ml^{-1}) and NIF(3 µg.ml^{-1}) and IMID (2µg.ml^{-1}) using methanol as a solvent, were scanned in UV 200-400 nm region and overlapped

Sample preparation

	Preparation of solution
Master Stock Solution:	Accurately weighed LID+NIF+IMID (150 mg+30 mg+20 mg) dissolved in 100 ml methyl alcohol (1500+300+200 µg.ml^{-1})
Standard Solution	Withdraw 100µlfrom Master StockSolutionandmakeupto10mlwith methyl alcohol LID+NIF+IMID (15+3+2µg.ml^{-1})

RESULTS AND DISCUSSION

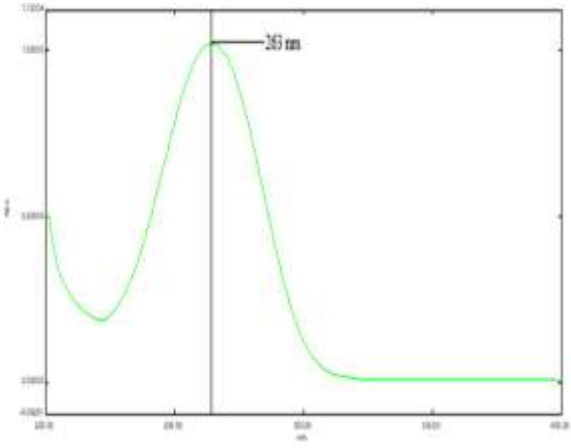
Identification of Lidocaine

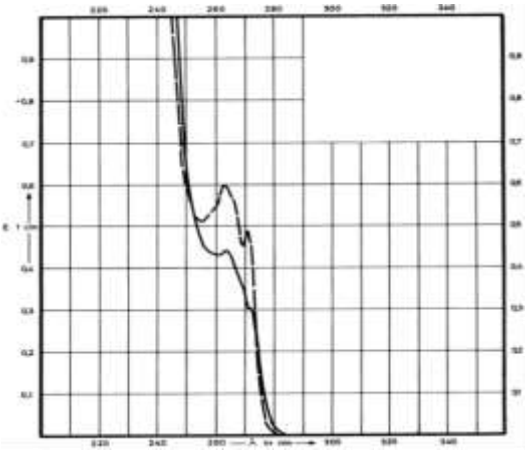
Identification by melting point

Table 2 Melting point data of Lidocaine

Drug	**Reported Melting Point**[17]	**Observed Melting Point**
Lidocaine	68.5 °C	68-70 °C

Identification by UV spectrophotometry



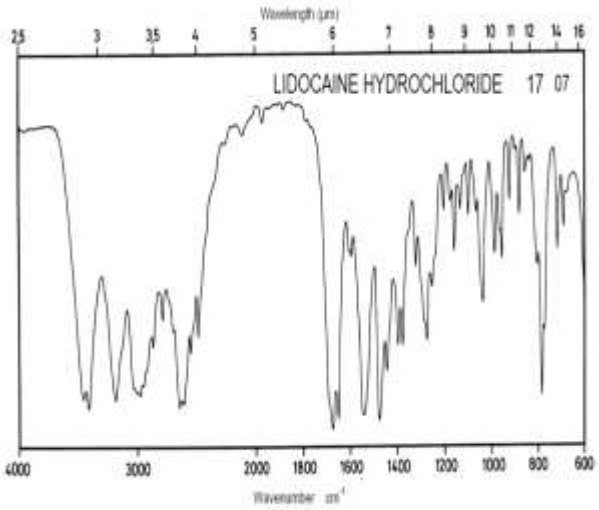


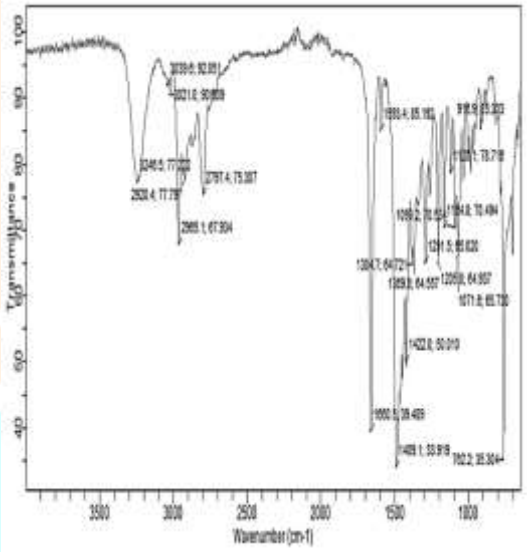
Recorded UV Spectra of Lidocaine (5 µg/mL) Reference UV Spectra of Lidocaine[46]

Table 3Comparison of reported and observed λ max

Drug	Solvent	Reported λ max	Observed λ max
Lidocaine	Methanol	263 nm	263 nm

Identification by IR Spectra





Reference IR Spectrum of Lidocaine[47] Recorded IR Spectrum of Lidocaine

Interpretation of IR Spectrum of Lidocaine

Type of Functional group/ Bond present	Reported frequency (cm^{-1})	Observed frequency (cm^{-1})
N-H stretch	3240	3246
=C-H stretch	3013	3039
-C-H Stretch	2950-2800	2925, 2960
C=O stretch	1680 (Amide)	1660
C-H bending	1450-1470	1489
C-N stretching	1350-1200	1369
N-H bending	1650-1580	1593
C-H Bending (Methyl)	760	762

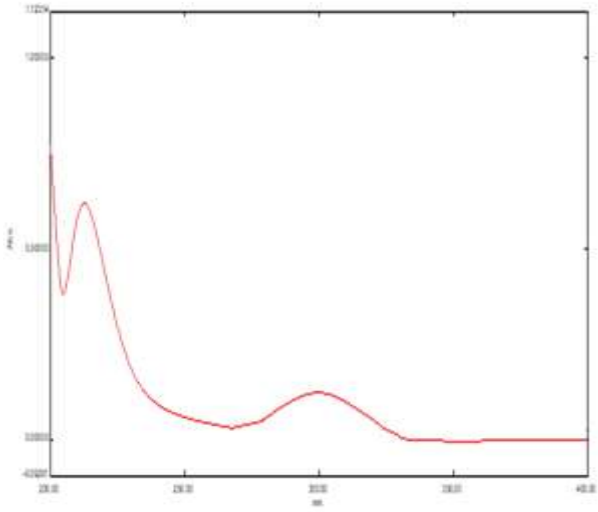
Identification of Nifedipine

Identification by melting point

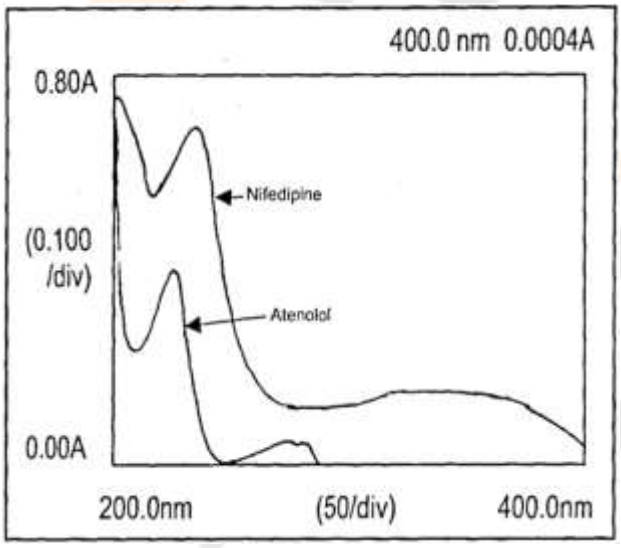
Table 4Melting point data of Nifedipine

Drug	Reported Melting Point[22]	Observed Melting Point
Nifedipine	172-174°C	170-173°C

Identification by UV spectrophotometry



Recorded UV Spectra of Nifedipine (5 µg/ml)

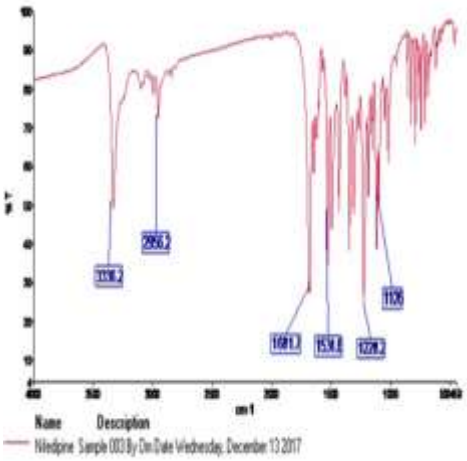


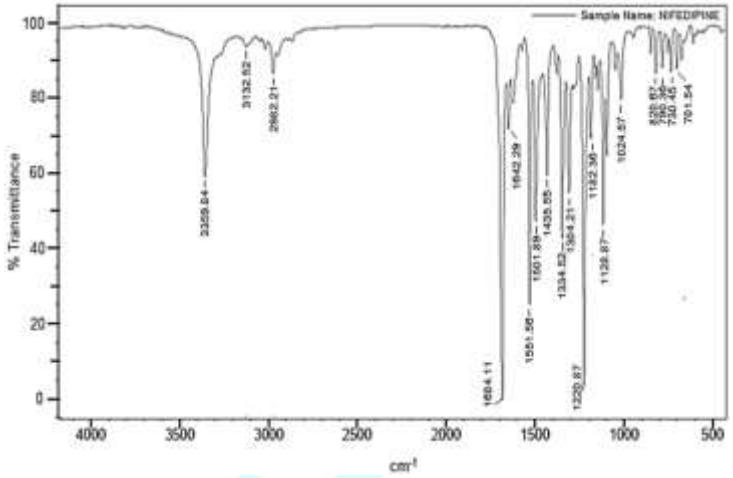
Reference UV Spectra of Nifedipine[48]

Comparison of reported and observed λmax

Drug	Solvent	Reported λmax	Observed λmax
Nifedipine	Methanol	235 nm	236 nm

Identification by IR Spectra



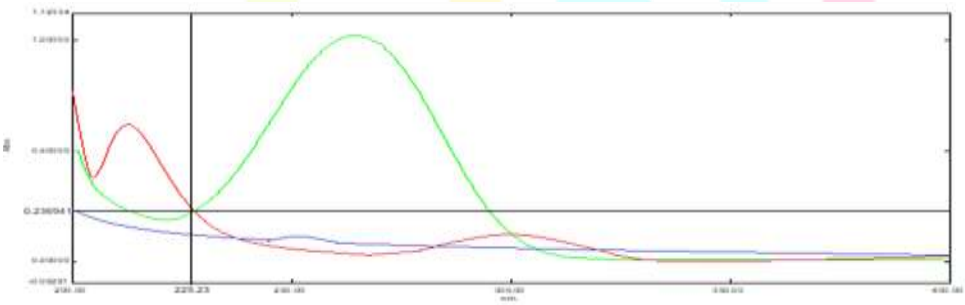


Reference IR Spectrum of Nifedipine[49] Recorded IR Spectrum of Nifedipine

Interpretation of IR Spectrum of Nifedipine

Type of Functional group/ Bond present	Reported frequency (cm^{-1})	Observed frequency (cm^{-1})
C=O stretch	1680	1684.11
C-O stretch	1251	1304.21
N-H Stretch	3400-3200	3359.84
=C-H stretch	3013	2982.21
C-H Bending	1470-1450	1435.55
87N=O Stretch	1479	1501.89
C-N Stretch	1259	1220.87

Reverse Phase High Performance Liquid Chromatography
Selection of Analytical/Detection wavelength

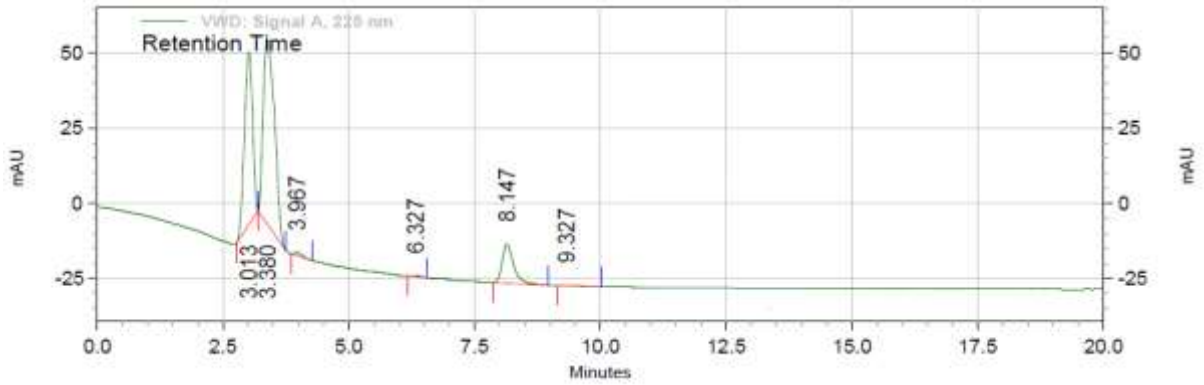


Mandatory requirements for selection of analytical wavelength in HPLC with UV detection is that both the drugs should give adequate response at selected wavelength.
When, individual solution, having concentration of 15 µg/ml of LID and 3 µg/ml of NIF and 2 µg/ml of IMID was scanned between 200-400 nm (Methanol as solvent) and overlapped, the primary observation was that, at 225 nm both NIF and LID showed adequate response and it was only wavelength at which IMI shows appropriate response and hence 225 nm was selected as analytical wavelength.

Optimization of Chromatographic Conditions

Trial 1

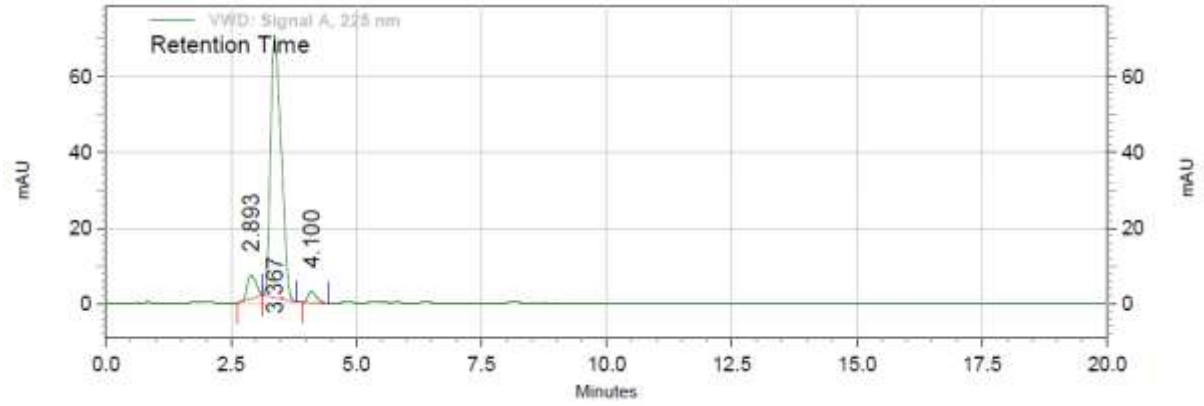
Column: Hypersil C18 (250*4.6 mm, 5µm)
Mobile Phase: Acetonitrile: Water (70:30 v/v),Detection: 225 nm
Flow rate: 1 ml/minRun Time: 20 minutes
Observation: Separation with interference observed.



Trial 1: Chromatogram of LID+NIF+IMID (30+6+4 µg.ml^{-1})

Trial 2

Column: Hypersil C18 (250*4.6 mm, 5µm)
Mobile Phase: Acetonitrile: 0.05M Potassium dihydrogen phosphate buffer (50:50 v/v),
Detection: 225 nm
Flow rate: 1 ml/min
Run Time: 20 minutes
Observation: Separation with inadequate resolution observed.

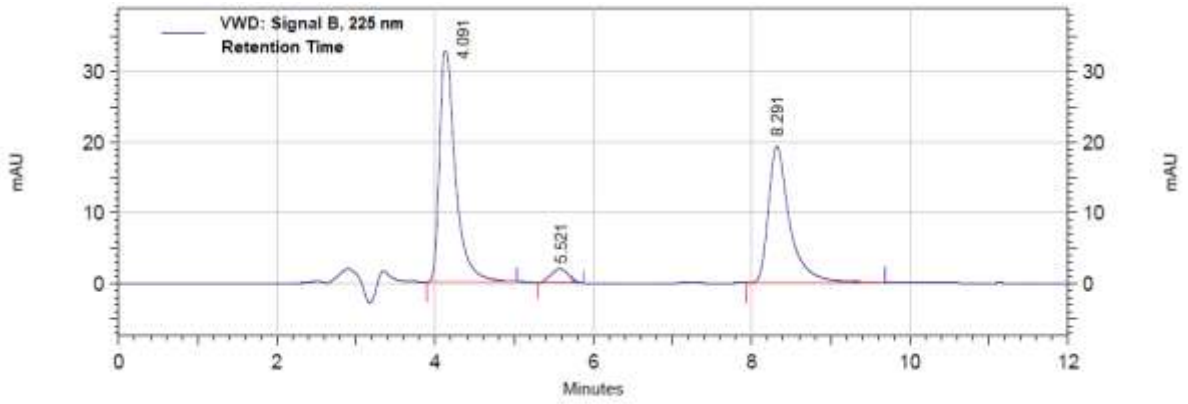


Trial 2: Chromatogram of LID+NIF+IMID (30+6+4 µg.ml^{-1})

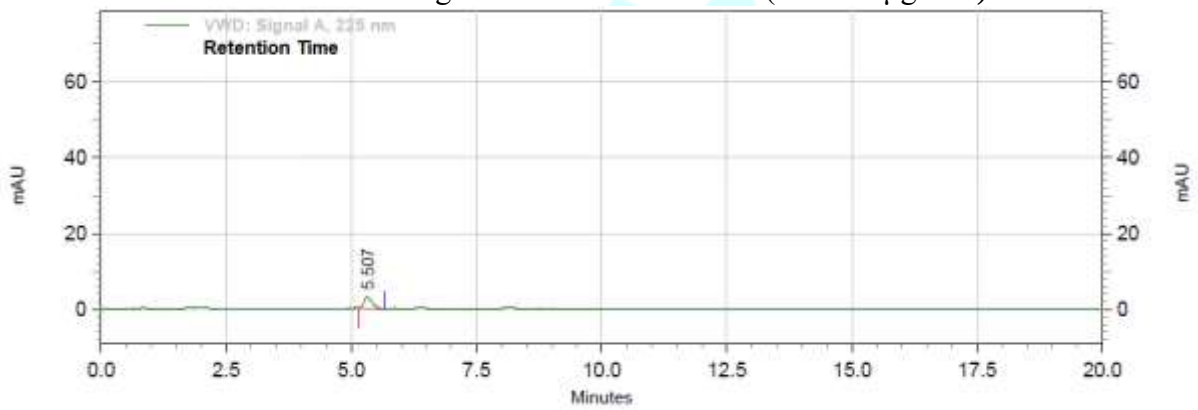
Trial 3

Column: Hypersil C18 (250*4.6 mm, 5µm)
Mobile Phase: Acetonitrile: Methanol: 0.05M Potassium dihydrogen phosphate buffer (40:35:25 v/v)
Detection: 225 nm
Flow rate: 1 ml/min
Run Time: 20 minutes

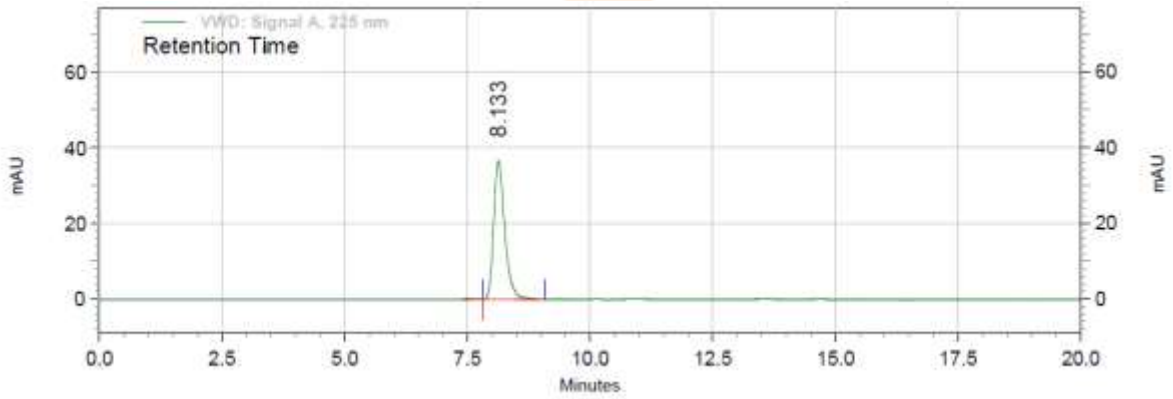
Observation: Separation with adequate resolution observed.



Trial 3: Chromatogram of LID+NIF+IMID (30+6+4 µg.ml^{-1})



Chromatogram of IMID (6 µg.ml^{-1}) for peak identification



Chromatogram of NIF (10 µg.ml^{-1}) for peak identification

Optimized Chromatographic Condition

Table 4 Optimized Chromatographic Condition

Stationary Phase	HYPERSIL ODS C18, 250 mm*4.6 mm
Mobile Phase	Acetonitrile: Methanol: 0.05M Potassium dihydrogen phosphate buffer (40:35:25 v/v)
Detection wavelength	225 nm
Flow rate	1 ml/minute
Run Time	20 minutes
Retention Time	LID: 4.091 min, IMID: 5.521 min, NIF: 8.291

System Suitability Parameters

Table 6 System suitability parameter for LID+NIF+IMID (15+3+2 $\mu g.ml^{-1}$)

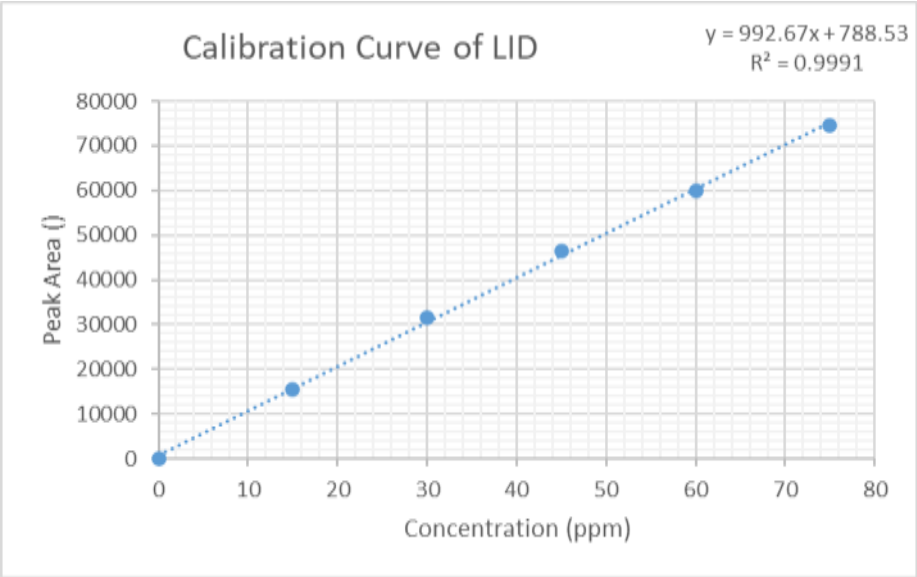
Parameter	**LID**			**IMID**			**NIF**		
	Mean	**± SD (n=3)**	**RSD**	**Mean**	**± SD (n=3)**	**RSD**	**Mean**	**± SD (n=3)**	**RSD**
Retention time (R_t)	4.06	0.04	0.87	5.53	0.04	0.74	8.26	0.01	0.12
Tailing Factor	1.43	0.02	1.54	1.04	0.01	1.35	1.63	0.02	1.11
Number of theoretical plates	57593.00	324.82	0.56	4779.67	59.53	1.25	27445.33	138.02	0.50
Resolution (R_s)	3.60	0.07	1.85				7.17	0.04	0.56
	Resolution with IMID						Resolution with IMID		

Validation of developed RP-HPLC method for estimation of LID+ NIF+ IMID

Linearity and Range

Table 7 Linearity data of LID

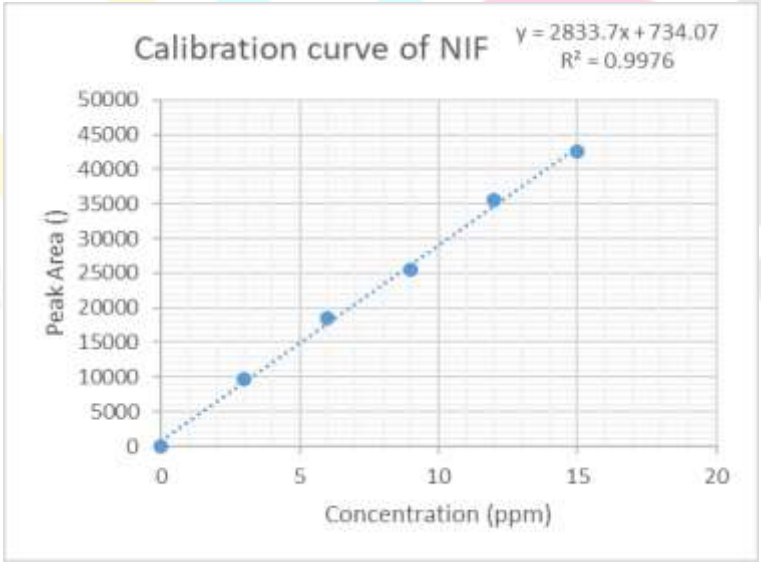
Sr. No.	**Concentration (µg/ml)**	**Mean area (µV. s)**	**± SD (n=5)**	**RSD**
1	15	15507.2	215.91	1.39
2	30	31547.8	349.76	1.11
3	45	46544.6	497.30	1.07
4	60	59869	558.76	0.93
5	75	74614.4	307.07	0.41



Calibration curve of LID

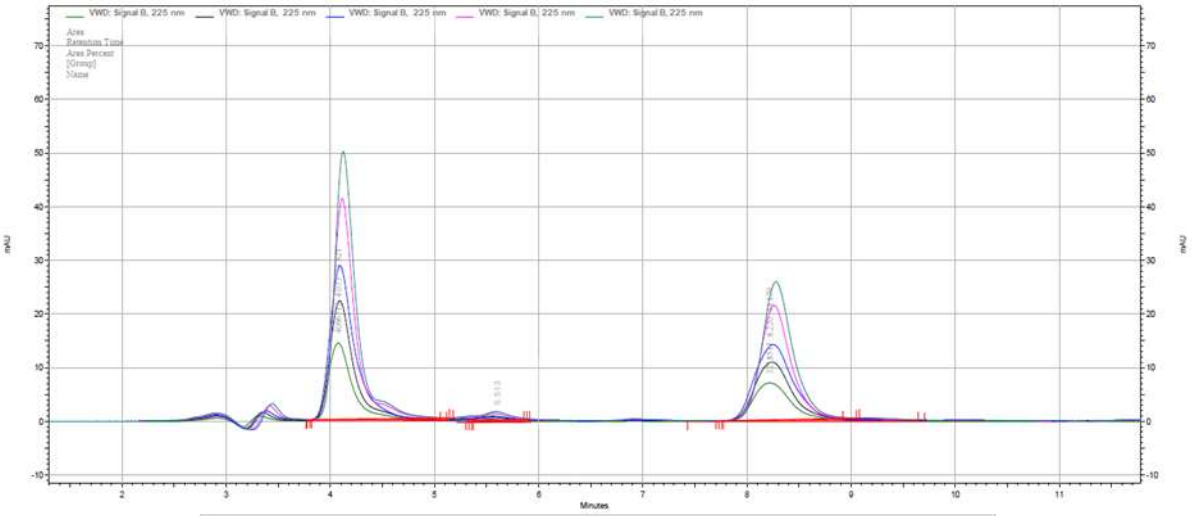
Linearity data of NIF

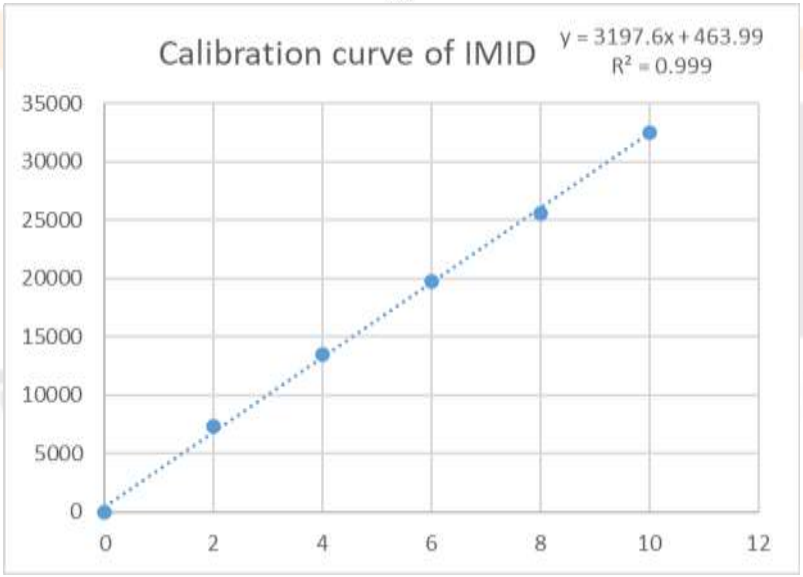
Sr. No.	Concentration (µg/ml)	Mean area (µV. s)	± SD (n=5)	RSD
1	3	9722.8	158.56	1.63
2	6	18517.4	292.13	1.58
3	9	25504.4	325.61	1.28
4	12	35579.6	383.53	1.08
5	15	42595.8	269.49	0.63

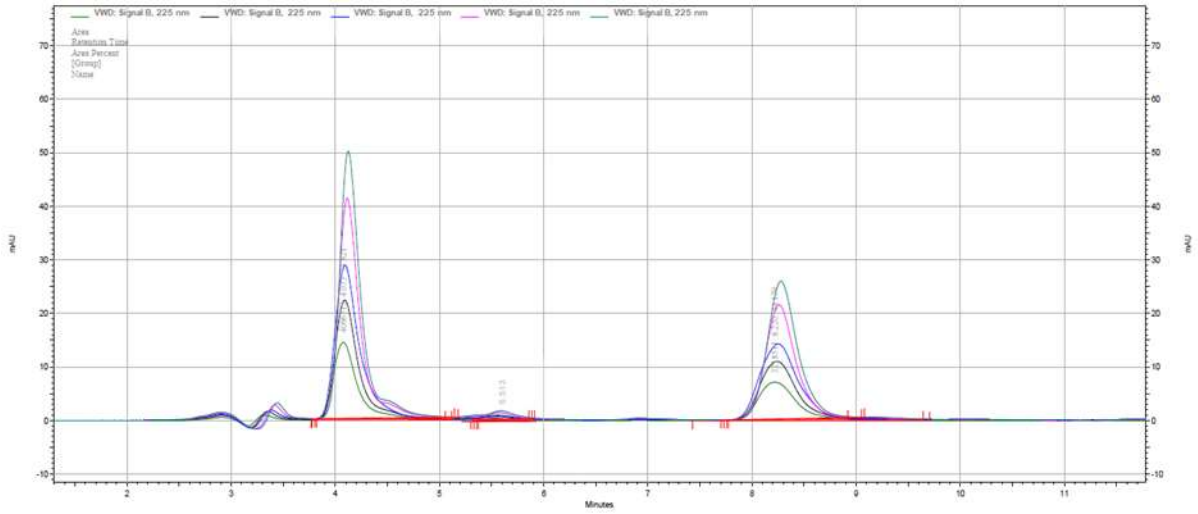


Linearity data of IMID

Sr. No.	Concentration (µg/ml)	Mean area (µV. s)	± SD (n=5)	RSD
1	2	7297.2	131.89	1.81
2	4	13524.4	168.16	1.24
3	6	19761.8	239.40	1.21
4	8	25580.2	288.46	1.13
5	10	32549.8	227.46	0.70







Overlain Chromatogram for linearity

Conclusion:

As per ICH guidelines the value of R^2 should be greater than 0.995, and observed R^2 for given concentration range for LID,NIF, and IMID is 0.9991, 0.9976, and 0.999, respectively.

Hence, we can say that developed method is linear over the range of 15-75 µg/mL, 3-15 µg/mL and 2-10 µg/mL for LID,NIF, and IMID, respectively.

Repeatability

Table 8 Repeatability data of LID

Sr. No.	Concentration (µg/mL)				
	15	30	45	60	75
1.	15426	31475	46124	59897	74982
2.	15715	31521	46824	60214	74863
3.	15221	31945	47245	58978	74521
4.	15732	31774	46056	59811	74485
5.	15442	31024	46474	60445	74221
MEAN	15507.2	31547.8	46544.6	59869	74614.4
± SD (n=5)	215.91	349.76	497.30	558.76	307.07
RSD	1.39	1.11	1.07	0.93	0.41

Table 9 Repeatability data of NIF

Sr. No.	Concentration (µg/mL)				
	3	6	9	12	15
1.	9825	18745	25414	35772	42774
2.	9454	18156	25863	35812	42712
3.	9744	18247	25472	35974	42789
4.	9854	18674	25027	35114	42563
5.	9737	18765	25746	35226	42141
MEAN	9722.8	18517.4	25504.4	35579.6	42595.8
± SD (n=5)	158.56	292.13	325.61	383.53	269.49
RSD	1.63	1.58	1.28	1.08	0.63

Table 10 Repeatability data of IMID

Sr. No.	**Concentration (µg/mL)**				
	2	4	6	8	10
1.	7112	13563	19887	25895	32441
2.	7332	13227	19719	25316	32534
3.	7215	13624	19423	25742	32889
4.	7425	13587	19711	25714	32611
5.	7402	13621	20069	25234	32274
MEAN	7297.2	13524.4	19761.8	25580.2	32549.8
± SD (n=5)	131.89	168.16	239.40	288.46	227.46
RSD	1.81	1.24	1.21	1.13	0.70

Conclusion:

As per ICH guidelines the value of RSD should be less than 2, and observed RSD is less than 2 for all concentrations of for LID,NIF, and IMID.

Hence, we can say that developed method is repeatable over the range of 15-75 µg/mL, 3-15 µg/mL and 2-10 µg/mL for LID,NIF, and IMID, respectively

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of Detection (LOD):

LID	NIF	IMID
LOD = 3.3 x (σ/S) = 3.3 x (128.19 / 992.676) = 0.426 µg/mL	LOD = 3.3 x (σ/S) = 3.3 x (151.19 / 2833.68) = 0.176 µg/mL	LOD = 3.3 x (σ/S) = 3.3 x (105.18 / 3197.64) = 0.108 µg/mL

Limit of Quantification (LOQ):

LID	NIF	IMID
LOQ = 10 x (σ/S) = 10 x (128.19 / 992.676) = 1.29 µg/mL	LOQ = 10 x (σ/S) = 10 x (151.19 / 2833.68) = 0.53 µg/mL	LOQ = 10 x (σ/S) = 10 x (105.18 / 3197.64) = 0.32 µg/mL

Accuracy

Quantity in mcg/ml

	LID					
	50%		100%		150%	
	Amount of drug recovered	%Recovery	Amount of drug recovered	Recovery	Amount of drug recovered	%Recovery
	14.81	98.73	29.85	99.50	44.84	99.64
	14.73	98.20	29.68	98.93	44.52	98.93
	14.86	99.07	29.71	99.03	44.62	99.16
MEAN	14.80	98.67	29.75	99.16	44.66	99.24
SD	0.07	0.44	0.09	0.30	0.16	0.36

	NIF					
	50%		100%		150%	
	Amount of drug recovered	%Recovery	Amount of drug recovered	Recovery	Amount of drug recovered	%Recovery
	2.97	99.00	5.94	99.00	8.86	98.44
	2.95	98.33	5.89	98.17	8.83	98.11
	3.01	100.33	5.96	99.33	8.94	99.33
MEAN	2.98	99.22	5.93	98.83	8.88	98.63
SD	0.03	1.02	0.04	0.60	0.06	0.63

	IMID					
	50%		100%		150%	
	Amount of drug recovered	%Recovery	Amount of drug recovered	Recovery	Amount of drug recovered	%Recovery
	1.98	99.00	3.96	99.00	5.93	98.83
	1.97	98.50	3.93	98.25	5.91	98.50
	2.01	100.50	3.99	99.75	5.96	99.33
MEAN	1.99	99.33	3.96	99.00	5.93	98.89
SD	0.02	1.04	0.03	0.75	0.03	0.42

Intra-day and Inter-day Precision

Intra-day Precision									
	LID(mcg/ml)			NIF(mcg/ml)			IMID(mcg/ml)		
	15	45	75	3	9	15	2	6	10
	15408	46109	74258	9539	25381	42308	7119	19691	32381
	15683	4632	73836	9714	25129	42169	7237	19431	32762
	15411	45514	74728	9812	25493	42711	7124	19513	32542
MEAN	15500.67	45951.67	74274.00	9688.33	25334.33	42396.00	7160.00	19545.00	32561.67
SD	157.91	383.99	446.22	138.30	186.43	281.51	66.73	132.92	191.26
RSD	1.02	0.84	0.60	1.43	0.74	0.66	0.93	0.68	0.59

Inter-day Precision									
	LID(mcg/ml)			NIF(mcg/ml)			IMID(mcg/ml)		
	15	45	75	3	9	15	2	6	10
	15311	46218	74285	9489	25288	42217	7266	19389	32428
	15420	46682	73826	9722	25634	42638	7412	19624	32644
	15721	47121	74818	9786	25873	42921	7389	19766	32908
MEAN	15484.00	46673.67	74309.67	9665.67	235598.33	42592.00	7355.67	19593.00	32660.00
SD	212.36	451.56	496.46	156.31	294.13	354.25	78.50	19.40	240.40
RSD	1.37	0.97	0.67	1.62	1.15	0.83	1.07	0.97	0.74

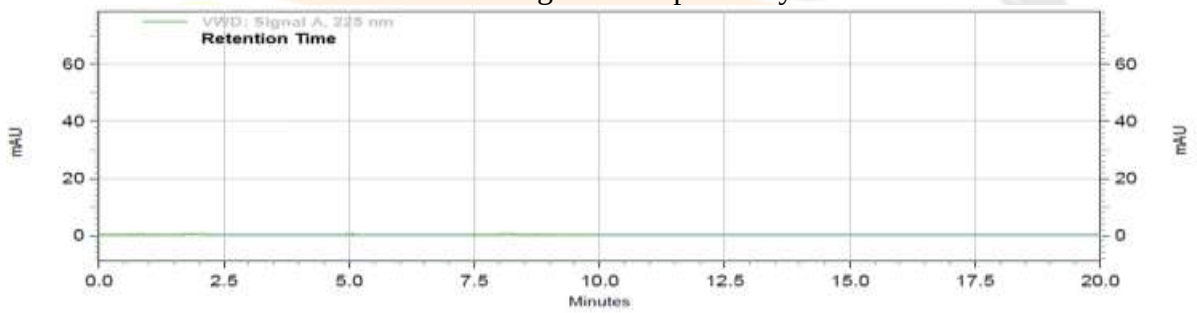
Robustness

Parameter	Level of Change	Effect on assay volume					
		LID		NIF		IMID	
		Assay ± SD	RSD	Assay ± SD	RSD	Assay ± SD	RSD
Flowrate	0.9 mL/min	99.23 ± 0.43	0.44	99.19 ± 0.86	0.87	98.46 ± 0.22	0.22
	1.1 mL/min	98.49 ± 0.27	0.28	99.04 ± 0.41	0.42	98.53 ± 0.28	0.29
Mobile Phase Composition	42:33:25	99.75 ± 0.45	0.45	98.35 ± 0.21	0.22	98.41 ± 0.41	0.42
	40:37:23	98.94 ± 0.77	0.78	98.99 ± 0.95	0.96	98.56 ± 0.45	0.46
	38:35:27	100.32 ± 1.21	1.21	98.62 ± 0.44	0.45	98.34 ± 0.34	0.35

(n = 3 determinations)

Specificity

Chromatogram for Specificity



Specificity of the method was adjudged by injecting the mobile phase in optimized chromatographic condition, it was observed that no interference observed from mobile phase.

Assay

Drug	Amount taken (µg/mL)	Amount found (µg/mL)	% Assay
LID	30	29.63 ± 0.48	98.77 ± 0.49
NIF	6	5.93 ± 0.33	98.83 ± 0.34
IMID	4	3.96 ± 0.52	99.08 ± 0.53

(n = 3 determinations)

Summary and conclusion

Table 11 Optimized Chromatographic Condition	
Stationary Phase	HYPERSIL ODS C18, 250 mm*4.6 mm
Mobile Phase	Acetonitrile: Methanol: 0.05M Potassium dihydrogen phosphate buffer (40:35:25 v/v)
Detection wavelength	225 nm
Flow rate	1 ml/minute
Run Time	20 minutes
Retention Time	LID: 4.091 min, IMID: 5.521 min, NIF: 8.291

Table 5 Validation parameters

Parameter	Limit	Result			Conclusion
		LID	NIF	IMID	
Linearity and Range	R^2> 0.995	0.9991 (15-75µg/mL)	0.9976 (3-15 µg/mL)	0.999 (2-10 µg/mL)	Method was linear
Repeatability	RSD < 2	0.41-0.39	0.63-1.63	0.70-1.81	Method was repeatable
LOD	-	0.426µg/mL	0.176 µg/mL	0.108 µg/mL	-
LOQ	-	0.129 µg/mL	0.533 µg/mL	0.328 µg/mL	-
Intraday Precision	RSD < 2	0.60-1.02	0.66-1.43	0.59-0.93	Method was precise
Inter-Day Precision	RSD < 2	0.67-1.37	0.83-1.62	0.74-1.07	Method was precise
% Recovery	98 - 102 %	98.67-99.24 %	98.63-99.22 %	98.89-99.33 %	Method was accurate
Robustness	RSD < 2	0.28-1.21	0.22-0.96	0.22-0.46	Method was robust
Assay	-	98.77 ± 0.49	98.83 ± 0.34	99.08 ± 0.53	-

Thus, we found that method was comply with all the validation parameters according to ICH Q2R1 guideline

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