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Original Article

Stability Indicating Chromatographic Method Development and Validation for the Simultaneous Estimation of Nifedipine and Lidocaine in Cream Dosage Form by HPLC

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Abstract

A simple, novel, rapid, precise, accurate, specific and cost effective High performance thin layer chromatographic method has been developed and validated for Simultaneous Estimation of Nifedipine and Lidocaine in Cream Dosage Form. The Stationary Phase used was C_{18} Hypersil BDS column and the Mobile Phase used was Mixture of Buffer (pH 3.0): Methanol (50:50). The Developed Method was Validated As per International Conference on Harmonization (ICH) guidelines. Calibration Curve was found to be Linear and the Correlation Coefficient was found to be 0.998 and 0.999 for Nifedipine and Lidocaine respectively. The Limit of Detection for Nifedipine and Lidocaine was found to be 0.18µg/ml and 0.59µg/ml respectively. The Limit of Quantization for Nifedipine and Lidocaine was found to be 0.55µg/ml and 1.79µg/ml respectively. The Degradation Study Preformed are Oxidative, Acidic, Basic, Thermal and Photolytic.

Keywords: Validation; HPLC; Nifedipine; Lidocaine; Cream Dosage Form.

Introduction

Nifedipine (3,5 dimethy l 2,6 dimethyl 4(2nitrophenyl)1,4dihydropyridine3,5dicarboxylate) is a dihydropyridine calcium channel blocker that primarily blocks L-type calcium channels. Its main uses are as an antianginal and antihypertensive, although a large number of other indications have recently been found for this agent, such as Raynaud's phenomenon, premature labor, and painful spasms of the esophagus such as in cancer and tetanus patients. Lidocaine (2 diethylamino) N (2,6dimethylphenyl) acetamide) is a local anesthetic and cardiac depressant used as an antiarrhythmia agent.

Chronic anal fissure is the most common cause of anal pain associated with internal anal sphincter hypertonia. Reduction of hypertonocity is a special treatment for fissure healing. For this purpose chronic anal fissures were conventionally treated by anal dilatation or by lateral sphincterotomy. Hence the Combination of Nifedipine and Lidocaine in the form of Cream is used to Treat Anal Fissure. Various methods have been developed for the estimation of these two drugs separately, no method is developed for combination estimation.

Hence efforts are made to develop a HPLC method which used to find them in combination and to validate the HPLC method which is developed as per the ICH guideline, to get a better, reliable, accurate and easy method for the estimation of these two drugs.

Materials and Method

Instrumentation and Chromatographic Condition

Analysis was performed with RP-HPLC Instrument (Model: LC 10-AT) equipped with

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Software Spinchrom. The C_{18} (25 cm × 0.46 cm) Hypersil BDS column is used. It is having a fixed 20µL loop. Different combinations of mobile phases were tested so that to find a suitable mobile phase which can detect both the drugs at the same time. The appropriate mixture of mobile phase which can detect both the drug was found to be Buffer (pH 3.0): Methanol (50:50). The flow rate was adjusted at 1.0 ml/min with the run time of 10 min. The sample was injected with the injector into the fixed loop of 20µL and the system is run to find the specific wavelength. Standard solution of Lidocaine HCl (15µg/ml) and Standard solution of Nifedipine (3µg/ml) in Methanol were scanned between 200-400 nm using UV-visible spectrophotometer. Both solutions were scanned between 200 - 400 nm. Wavelength was selected from the overlay spectra of above solutions. The specific wavelength were both drug intersect each other was found to be 233nm. The identity of the compounds was established by comparing the retention times of compounds in the sample solution with those in standard solutions.

Materials and Reagents

Nifedipine was procured from RPG Life Science and Lidocaine from Oasis Laboratory. The other reagents used are Acetonitrile, water, Methanol, Acetic acid. All are HPLC grade reagents. The other materials used are UV Spectrophotometer (Shimadzu 1800), Analytical balance, PH meter, Ultrasonicator. The combined drug was found in Cream dosage form which is having name Anobliss which is manufacture by the Samarth Life science.

Preparation of Standard and Sample Solutions

Lidocaine HCl standard stock solution: (150µg/mL) A 15 mg of Lidocaine HCl was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with methanol.

Nifedipine Standard Stock Solution: (30µg/mL)

A 30 mg of Nifedipine was weighed and transferred to a 100 mL volumetric flask. and volume was made up to the mark with methanol and Take 1 ml from this solution and transfer to 10 ml volumetric flask and made up the to the mark.

Preparation of standard solution of binary mixtures of Lidocaine HCl (15 µg/mL) and Nifedipine (3 µg/mL)

Take 1 mL from the Lidocaine HCl stock solution and 1mL from Nifedipine stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Procedure for Analysis of Formulation

Take Cream equivalent to 15 mg Lidocaine HCl and 3mg of Nifedipine was transferred to a 100ml volumetric flask, shake for 15 minutes than put this solution on water bath for 15 minutes at 60 ^{o}C, Allow to cool the solution and made up volume up to the mark with mobile phase. The solution was filtered through Whatman filter paper no.42 and first few drops of filtrate were discarded. 10 ml of this solution was diluted to 100ml with mobile phase. The solution was injected 10 µl. The areas of resulting peak were measured at 233 nm.

Degradation Study

The Drug was employed for Oxidative, Thermal, Photolytic, Acidic and Basic Degradation condition. After the Degradation treatment were completed the solution were cooled at room temperature and were diluted to get the concentration of Lidocaine HCl (15 µg/mL) and Nifedipine (3 µg/mL). These solution is then run into the system and the chromatogram were recorded to assess the Stability of Sample. Specific Degradation conditions are as follows:

Oxidative Degradation

Oxidative decomposition studies were performed by refluxing One ml of stock solution was transferred in to 10 ml of volumetric flask. Two ml of 3% H_2O_2 solutions was added and mixed well and put for 3 hrs at 70 ^{o}C 250 ml Round bottom flask. After time period the content was cooled to RT. Then the volume was adjusted with diluent to get 15µg/ml for Lidocaine HCl and 3µg/ml for Nifedipine.

Thermal Degradation

Thermal Degradation studies were performed One ml of stock solution was transferred in to 10 ml of volumetric flask. The volumetric flask was stored in oven at 110°C for 4 hrs. Then the volume was adjusted with diluent to get 15µg/ml for Lidocaine HCl and 3µg/ml for Nifedipine.

Photo Degradation

Photo Degradation studies were performed One ml of stock solution was transferred in to 10 ml of

volumetric flask. The volumetric flask was keep in presence of Sunlight for 3 hrs. Then the volume was adjusted with diluent to get 15µg/ml for Lidocaine HCl and 3µg/ml for Nifedipine.

Acid Degradation

Acid decomposition studies were performed by Refluxing One ml of stock solution was transferred in to 10 ml of volumetric flask. Two ml of 0.1 N HCl solutions was added and mixed well and put for 4 hrs at 70 °C 250 ml Round bottom flask. After time period the content was cooled to RT. Then the volume was adjusted with diluent to get 15µg/ml for Lidocaine HCl and 3µg/ml for Nifedipine.

Base Degradation

Basic decomposition studies were performed by refluxing One ml of stock solution was transferred in to 10 ml of volumetric flask. Two ml of 0.1 N NaOH solutions was added and mixed well and put for 4 hrs at 70 °C 250 ml Round bottom flask. After time period the content was cooled to RT. Then the volume was adjusted with diluent to get 15µg/ml for Lidocaine HCl and 3µg/ml for Nifedipine

Method Development

Several Test were Performed in order to get a satisfactory separation of Nifedipine and Lidocaine with the different mobile phase and ratios. The appropriate mobile phase was found to be the mixture of Buffer (pH 3.0): Methanol (50:50). This mobile phase gives a proper separation and Resolution of Nifedipine and Lidocaine. The retention time of Nifedipine and Lidocaine on the analytical column was evaluated at a flow rate of 1 ml/min. The injection volume was 20µL. The retention time of standard and sample for Nifedipine and Lidocaine were satisfactory with good resolution. These mobile phase condition were the optimized to find whether is there any interference due to solvents.

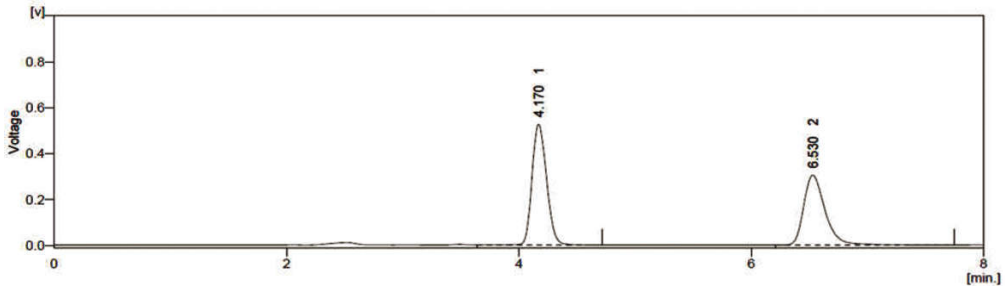

Fig. 1: Chromatogram for lidocaine (15µg/ml) and nifedipine (3µg/ml)

Method Validation

The method was validated for specificity, linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines.

Linearity

The linearity for Lidocaine HCl and Nifedipine were assessed by analysis of combined standard solution in range of 7.5-22.5 µg/ml and 1.5-4.5 µg/ml respectively, 5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Lidocaine HCl (150 µg/ml) and Nifedipine(30 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 7.5, 11.25, 15, 18.75 and 22.5 µg/ml and 1.5, 2.25, 3, 3.75 and 4.5 µg/ml for Lidocaine HCl and Nifedipine respectively

In term of Slope, Intercept and Correlation Co-efficient value. The graph of peak area obtained verses respective concentration was plotted.

Table 1: Linearity data for lidocaine

S. No.	Concentration (µg/ml)	Area
1	7.5	2319.102
2	11.25	3361.305
3	15	4592.486
4	18.75	5633.077
5	22.5	6823.593

Table 2: Linearity data for nifedipine

Sr. No.	Concentration (µg/ml)	Area
1	1.5	1957.077
2	2.25	2836.708
3	3	3748.038
4	3.75	4825.087
5	4.5	5790.415

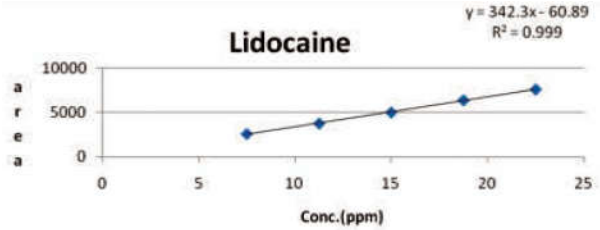

Fig. 2: Linearity curve for lidocaine

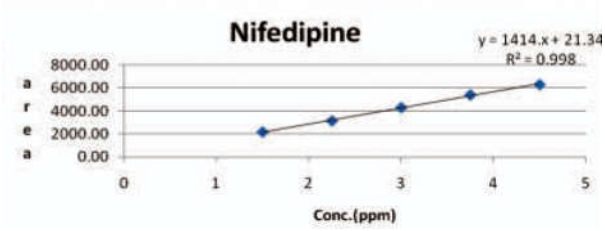

Fig. 3: Linearity curve for nifedipine

Precision

Results should be expressed as Relative standard deviation (RSD) or coefficient of variance.

A. Repeatability

Standard solution containing Lidocaine HCl (15µg/ml) and Nifedipine (3µg/ml) was injected six times and areas of peaks were measured and % R.S.D. was calculated.

B. Intra-Day Precision

Standard solution containing (7.5, 15, 22.5µg/ml) of Lidocaine HCl and (1.5, 3, 4.5µg/ml) of Nifedipine were analyzed three times on the same day and % R.S.D was calculated.

Inter-Day Precision

Standard solution containing (7.5, 15, 22.5µg/ml) of Lidocaine HCl (1.5, 3, 4.5µg/ml) of Nifedipine were analyzed three times on the different day and % R.S.D was calculated.

Table 3: Repeatability data for lidocaine

Sr. No.	Conc (µg/ml)	Lidocaine HCl Area	Mean ± S.D (n=6)	% R.S.D
1.	15	5090.69 5065.13 5070.01 5044.56 5040.07 5009.23	5053.28±28.32	0.56

Table 4: Repeatability data for nifedipine

Sr. No.	Conc (µg/ml)	Nifedipine Area	Mean ± S.D (n=6)	% R.S.D
1.	3	4293.54 4271.93 4240.94 4254.71 4155.87 4224.92	4240.32 ±47.77	1.13

Table 5: Intraday precision data for estimation of lidocaine HCl and nifedipine

S. No.	Conc. (µg/ml)	Lidocaine HCl Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Nifedipine Area Mean ± S.D. (n=3)	% R.S.D
1	7.5	2569.81 ± 3.87	0.151	1.5	2143.86± 24.94	1.16
2	15	5084.05± 15.91	0.31	3	4291.4.3± 19.20	0.45
3	22.5	7570.16± 15.22	0.20	4.5	6356.46±50.55	0.79

Table 6: Interday precision data for estimation of lidocaine HCl and nifedipine

S. No.	Conc. (µg/ml)	Lidocaine HCl Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Nifedipine Area Mean ± S.D. (n=3)	% R.S.D
1	7.5	2542.27±19.04	0.75	1.5	2141.09±21.41	0.99
2	15	5003.10±23.83	0.48	3	4205.55±40.79	0.97
3	22.5	7625.79±31.98	0.42	4.5	6431.53±26.92	0.42

Table 7: Recovery data for lidocaine HCl

S. No.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	7.5	6	6.00	99.99	99.55 ± 0.45
2		7.5	6	5.97	99.54	
3		7.5	6	5.95	99.10	
4	100 %	7.5	7.5	7.46	99.52	98.54± 0.86
5		7.5	7.5	7.37	98.26	
6		7.5	7.5	7.34	97.86	
7	120 %	7.5	9	8.97	99.66	98.99 ± 0.86
8		7.5	9	8.94	99.30	
9		7.5	9	8.82	98.03	

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for Lidocaine HCl was 99.72-100.02 %, while for Nifedipine, it was found to be in range of 99.87-100.17 %.

LOD and LOQ

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where,

SD = Standard deviation of intercepts Lidocaine HCl Nifedipine

LOD = 3.3 x (SD / Slope)= 3.3 x (61.36/342.36)
= 0.59 µg/ml

LOD = 3.3 x (SD / Slope) = 3.3 x (77.75/1414)
= 0.18 µg/ml

Lidocaine HCl Nifedipine

LOQ = 10 x (SD / Slope) = 10x(61.36/342.36)
= 1.79 µg/ml

LOQ = 10 x (SD / Slope) = 10 x (77.75/1414)
= 0.55 µg/ml

Table 8: Recovery data for nifedipine

Sr. No.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	1.5	1.2	1.20	99.92	98.69 ± 1.74
2		1.5	1.2	1.19	99.46	
3		1.5	1.2	1.16	96.70	
4	100 %	1.5	1.5	1.49	99.58	98.28 ± 1.13
5		1.5	1.5	1.46	97.48	
6		1.5	1.5	1.47	97.78	
7	120 %	1.5	1.8	1.79	99.62	97.92 ± 1.72
8		1.5	1.8	1.73	96.18	
9		1.5	1.8	1.76	97.97	

Table 9: LOD lidocaine and nifedipine

Lidocaine HCl	Nifedipine
LOD = 3.3 x (SD / Slope) = 3.3 x (61.36/342.36) = 0.59 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (77.75/1414) = 0.18 µg/ml

Table 10: LOQ lidocaine and nifedipine

Lidocaine HCl	Nifedipine
LOQ = 10 x (SD / Slope) = 10 x (61.36/342.36) = 1.79 µg/ml	LOQ = 10 x (SD / Slope) = 10 x (77.75/1414) = 0.55 µg/ml

Robustness

As defined by ICH, The robustness of an analytical procedure describes to its capability to remain unaffected by small and deliberate variations in method parameters. Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. pH of Mobile phase was changed (± 0.2) 3.2 and 2.8
3. Ratio of Mobile phase was changed(±2) Buffer: Methanol (48:22) and Buffer: Methanol (52:48)

Table 11: Robustness data for lidocaine

Sr. No.	Area at Flow rate (-0.2 ml/min)	Area at Flow rate (+0.2ml/min)	Area at pH (- 0.2)	Area at pH (+ 0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	5583.07	4563.41	4937.97	5125.37	5385.93	4805.39
2	5571.80	4554.17	4931.63	5120.19	5380.44	4776.65
3	5560.59	4531.30	4974.74	5111.43	5396.44	4786.02
% R.S.D	0.20	0.36	0.47	0.14	0.15	0.31

Table 12: Robustness data for nifedipine

Sr. No.	Area at Flow rate (-0.2 ml/min)	Area at Flow rate (+0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	4708.81	3848.82	4164.74	4322.82	4542.53	4052.92
2	4637.77	3841.16	4173.02	4318.46	4537.96	4028.60
3	4689.90	3807.21	4195.90	4287.20	4520.69	3987.59
% R.S.D	0.79	0.58	0.39	0.45	0.25	0.82

Table 13: Forced degradation studies of lidocaine

Parameter	Lidocaine HCl			
	Standard		Sample	
	Area	%Degradation	Area	%Degradation
Acid	3991.13	24.91	3946.00	25.76
Base	4304.57	19.01	4270.81	19.65
Thermal	4332.13	18.49	4455.23	16.18
Oxidation	3922.27	26.20	3936.41	25.94
Photo	4584.30	13.75	4686.55	11.82

Table 14: Forced degradation studies of nifedipine

Parameter	Nifedipine			
	Standard		Sample	
	Area	%Degradation	Area	%Degradation
Acid	3980.91	13.10	3884.50	15.20
Base	3702.77	19.17	3692.24	19.40
Thermal	3352.58	26.81	3328.94	27.33
Oxidation	3934.61	14.11	3801.56	17.01
Photo	3962.53	13.50	3979.14	13.13

Conclusion

The validated HPLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of Lidocaine and Nifedipine in combined Cream dosage form.

References

1. Dong WM. Modern HPLC for Practicing Scientists; A Wiley- Inter science publication, USA. 2006; pp 1-9.
2. Kazakevich Y and LoBrutto R. HPLC for pharmaceutical Scientists; A John Wiley and sons. 2007; pp 1-6.
3. Snyder LR., Kirkland JJ and Glajch LJ. Introduction to Modern Liquid Chromatography; 2nd Edn; A Wiley- Inter science publication, NY, USA. 1997; pp 5-42.
4. Snyder LR., Kirkland JJ and Glajch LJ. Practical HPLC Method Development; 2nd Edn; A Wiley-Inter science publication, NY, USA. 1997; pp 3-35.
5. Skoog DA., Holler FJ and Nieman TA. Introduction to UV Spectroscopy, Principle of instrumental analysis; 5th Edn; Thomson Brooks/Cole publication. 2005; pp 301.
6. Beckett AH and Stenlake JB. UV-visible Spectrophotometry: Practical Pharmaceutical Chemistry; 4th Edn; Part-II, C.B.S. Publishers, Delhi. 2001; pp 275-299.
7. FDA, "Guidance for Industry; Analytical Procedures and Methods Validation (Draft guidance), Food & Drug Administration," Rockville, US Department of Health and Human Services, 2000.
8. ICH, Validation of Analytical Procedures; Methodology, Q2 (R1), International Conference on Harmonization, IFPMA, Geneva 1996.
9. Golfam F, Golfam P, Khalaj A and Sayed M, "The effect of topical Nifedipine in treatment of chronic anal fissure" Acta Med Iran. 2010; 48(5): 295-299.
10. Drug profile for Nifedipine, http://www.drugbank.ca/drugs/DB01115 (Accessed on 25/09/2015)
11. Drug profile for Nifedipine, http://en.wikipedia.org/wiki/Nifedipine (Accessed on 12/09/2015)
12. Drug profile for Lidocaine, http://en.wikipedia.org/wiki/Lidocaine (Accessed on 13/09/2015)
13. Drug profile for Lidocaine, www.drugbank.ca/drugs/DB002181 (Accessed on 13/09/2015)
14. Indian Pharmacopoeia, THE INDIAN PHARMACOPOEIA COMMISSION GHAZIABAD Vol.II, 2010, pp 1779-1780.
15. USP30–NF25,Volume No. 27(3) pp 2569.
16. British Pharmacopoeia 2010, Vol-I &II, Medicinal and pharmaceutical Substances, Nifedipine
17. Uday YA, Patel SK, kumar D and Sandip AB, "Estimation of Nifedipine by reverse phase high performance liquid chromatography tablet dosage

form" Int. J. Pharm. and Life Sci. 2011; 2(3): 610-612.

18. Auda HS, Ghilzai NM, Najjar TA" HPLC Determination of Nifedipine In Human Plasma" King saud Univrsity, 1998
19. Sanka K, Gullapeli R, Patil N, Rao P and Divan PV, "Development and validation of RP-HPLC method for Nifedipine and its application for a novel proniosomal formulation analysis and dissolution study" Der Pharma Chemical. 2014; 6(1): 279-289.
20. Reddy S, Ahmad I, Nayak N, Thangam S, "Estimation of Nifedipine In Human Plasma By LC-MS/MS" Asian. J. Pharm. and Chem.. Res. 2013; 6(1): 83.
21. Rahman N, Azmi HNS, "New spectrophotometric methods for the determination of nifedipine in pharmaceutical formulations" Acta Biochemical Polonica. 2005; 52(4): 915-922.
22. Rahman N, Azmi HNS, "Validated Spectrophotometric Method for the Assay of Nifedipine in Bulk and Commercial Dosage Forms" Science Asia. 2006; 32: 429-435.
23. Abdallah I, Ibrahim A, Ibrahim N and Rizk N, "Simultaneous Determination of Atenolol and Nifedipine by Using Spectrophotometric Method with Multivariate Calibration and HPLC Method Implementing "Design of Experiment" Pharm. Anal. Acta. 2015; 6(6): 1-9.
24. British Pharmacopoeia 2010, Vol-I &II, Medicinal and pharmaceutical Substances, Lidocaine
25. Prathyusha PGS, Shanmungasundram P, Naidu PY, "Validated Stability indicating UPLC Method for the detection of Lidocaine and its degradation impurities in pharmaceutical dosage form, Int. J. Adv. in Pharm. Anal. 2013; 3(1): 1-10.
26. Putta M, Gurupandya BM, Sidha NS, "Spectrophotometric Determination Of Lidocaine In Bulk And Pharmaceutical Formulations" Inventi, 2013.
27. Patel PK, Patel DB, "Simultaneous Estimation of Nifedipine and Lidocaine in Cream by First Order Derivative Spectrophotometric Method" Inventi. 2013; 2013(3): 1-5.