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Method development and validation of nifedipine and lignocaine by RP-HPLC

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Abstract

For the validation of the nifedipine and lignocaine assay by reverse phase high performance liquid chromatography, in both pure form and tablet dosage form, a straightforward, quick, and exact approach has been established. ACN, Methanol, and perchloric acid were used as the mobile phase in chromatography on a Kromasil 100-5-C18 (4.6 x 150 mm, 5 m) column at a flow rate of 1.0 ml/min. 210 nm was used for detection. Nifedipine and lignocaine had retention times of 3.30 and 5.820.02 min, respectively. In the concentration range of 10–50 mg/ml of nifedipine and 20–100 mg/ml of lignocaine, the approach yields linear responses. The method precision for the assay result was less than 2.0%RSD, and the Nifedipine and lignocaine individual assays should be between 98% and 102.0%, respectively. The technique is helpful for pharmaceutical and bulk formulation quality control.

Keywords: Nifedipine; Lignocaine; Assay; RP-HPLC; Validation

1. Introduction

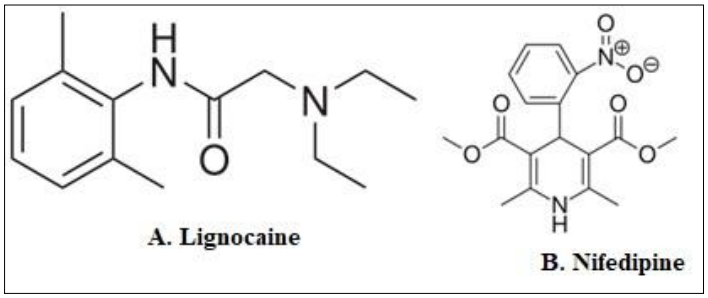

Figure 1 Chemical structures of A) Lignocaine B) Nifedipine

Chemically, Lignocaine is 2-(diethylamino)-N-(2,6-dimethylphenyl) acetamide, whereas Nifedipine is 3,5-dimethyl 2,6-dimethyl. -4-(2-nitrophenyl) -1,4-dihydropyridine (See Figures 1 and 2, respectively) 3,5-dicarboxylate (Figures 1 and 2 respectively). While lignocaine is an anaesthetic, nifedipine is an antihypertensive. The combination is widely used to treat persistent anal fissures. Lignocaine and nifedipine are recognised standards (USP) by the Indian Pharmacopeia (IP), British Pharmacopeia (BP), and United States of Pharmacopeia [1-3].A review of the literature revealed that different techniques, including stability indicating methods, HPLC-Tandem Mass (MS/MS) spectrometry, Reverse Phase High Performance Liquid Chromatography (RP-HPLC), and UV spectroscopy, were available for the estimation of lignocaine, whereas Ultra Performance Liquid Chromatography (UPLC), MS/MS, HPLC, and UV spectroscopic techniques were reported for the estimation of nifedipine in single or combined dosage forms [4–18]. A UV spectroscopic method for determining lignocaine and nifedipine concurrently was also published [19]. In this study, a stability indicating

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approach that was created and validated was used to estimate lignocaine and nifedipine simultaneously in bulk and their topical dose form.

2. Material and methods

2.1. Instrumentation

The Enable C18 G RP column (250 4.6 mm, 5 m) and Shimadzu Prominence UFLC LC-20AD with UV-detector were utilised. LC solution software was used for data collecting and integration.

2.2. Materials

Authentic drug samples of lignocaine and nifedipine were obtained from hetero labs, hyderabad, India.

2.3. Reagents

The following items were purchased from Merck Specialties Private Limited, Mumbai: HPLC grade methanol and water, GR grade ammonium acetate, hydrochloric acid, and sodium hydroxide. Hydrogen peroxide and glacial acetic acid of the AR and GR grades were purchased from Sigma Aldrich.

2.4. Sample preparation

The necessary amounts of lignocaine and nifedipine were dissolved in methanol to create the standard stock solutions. These solutions were sufficiently diluted to yield lignocaine and nifedipine concentrations of 150 g/ml and 30 g/ml, respectively. Using an accurate scale, 5 g of cream containing 0.3% w/w nifedipine and 1.5% w/w lignocaine, or the weight equivalent of 15 mg nifedipine and 75 mg of lignocaine, was transferred to a 100 ml volumetric flask and around 70 ml of methanol was added. It was sonicated for 15 minutes after 30 minutes of swirling. Methanol was then added to get the level up to 100 ml. The mixture was well blended, then a 0.45 nylon syringe filter was used to filter it.

3. Results and discussion

3.1. Optimization of chromatographic conditions

The mobile phase was composed of various ratios of acetonitrile, water, methanol, and various buffer solutions, and it was used to chromatograph the mixed standard stock solution, which contained 150 mg/ml of lignocaine and 30 mg/ml of nifedipine. Lignocaine and nifedipine have suggested pKa values of 7.9 and 5.3, respectively. The choice of buffers with pH values close to their pKa values causes the analytical method's robustness to be inconsistent. This led to the discovery that the 20 mM ammonium acetate buffer, pH 4.8 adjusted with glacial acetic acid, was appropriate. The combined spectra of both medications in Figure 3 recommended that the detection wavelength should be 231 nm. Since it produced symmetrical peaks for both lignocaine and nifedipine in bulk and had a flow rate of 1 ml/min, the ratio of 65:35% v/v of buffer with methanol was found to be the most effective.

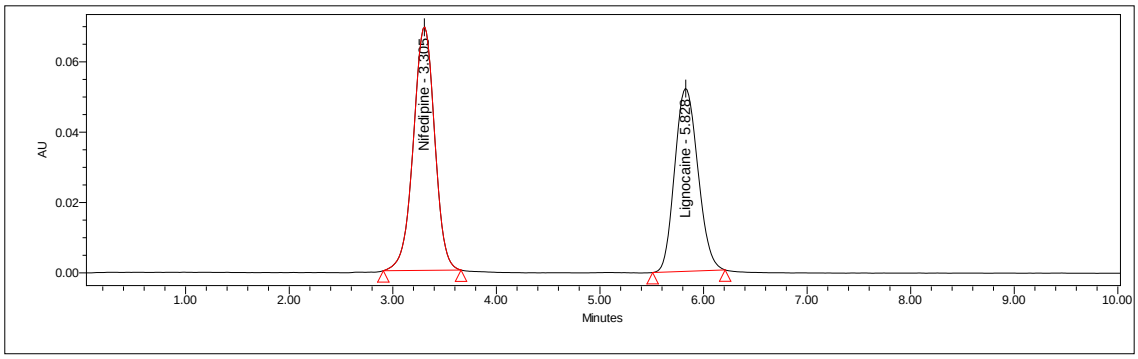

Figure 2 Overlain spectra of nifedipine and lidocaine

3.2. Validation of proposed method

After method development and optimization, validation of the proposed method was carried out as per Q2 (R1) guidelines.

3.3. System suitability testing

In order to verify that the system suitability parameters were satisfied, six duplicates of a solution mixture containing lignocaine (150 g/ml) and nifedipine (30 g/ml) were injected. Chromatograms were then recorded. It was possible to achieve a resolution of more than 2, a tailing factor of less than 1, and a % RSD of repeatability of less than 2. (Figure 3).

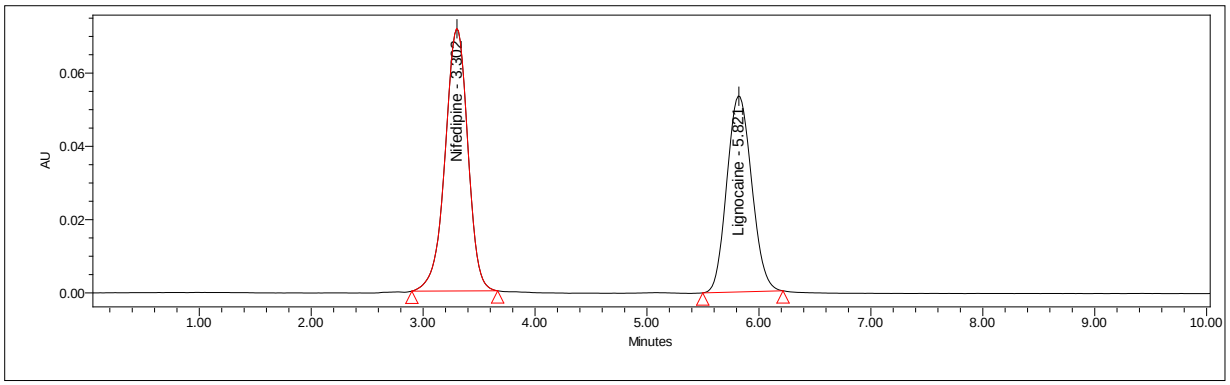

Figure 3 System suitability Standard chromatogram

3.4. Linearity

For lignocaine and nifedipine, a calibration curve was plotted for the concentration ranges of 24-36 g/ml and 120-180 g/ml, respectively. Regression line equation and correlation coefficient were used to describe linearity. The result is shown in Table 1.

Table 1 Results of Linearity

Lignocaine		Nifedipine	
Concentration (µg/ml)	**Mean area**	**Concentration (µg/ml)**	**Mean area**
75.0	3748407	15.0	2858017
112.5	5625287	22.0	4288483
150.0	7505762	30.2	5729400
187.6	9379421	37.5	7159610
225.1	11307691	45.0	8617062

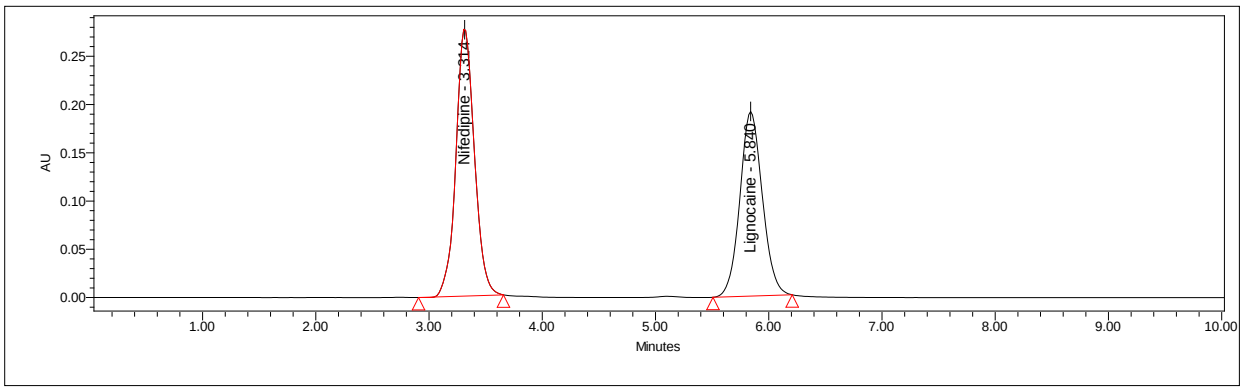

Figure 4 Chromatogram of lignocaine and nifedipine

3.5. Precision

The precision of the instrument was checked by repeatedly injecting (n=6) solution of nifedipine (30 µg/ml) and lignocaine (150 µg/ml). The results of precision studies are summarized in Table 2. The % RSD was found within the acceptable limit, i.e. < 2.

3.6. Recovery studies

The approach's accuracy was guaranteed by the use of the conventional addition procedure. The pre-analyzed sample solution of the marketed product had known concentrations of NIF and LID (80, 100, and 120%) standard solutions added to it. The mixes were tested, and the outcomes for both medications were compared to what was anticipated. (Table 2).

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

LOD and LOQ were calculated from the linearity studies. The standard deviation of the response and slope was calculated and applied to the following Equations:

$$LOD=3.3\sigma/S \quad LOQ=10\sigma/S$$

Where, σ=Standard deviation of the response, S=Slope of calibration curve, obtained LOD and LOQ are summarized in Table 2.

3.7. Robustness

By varying the parameters of flow rate (0.2 ml/min), mobile phase composition (0.2), and detecting wavelength (2 nm), robustness of the approach was investigated. The purposeful modifications to the flow rate, mobile phase composition, and wavelength did not significantly affect the assay result. The are shown in Table 2.

Table 2 Results of robustness

Parameters		**Nifedipine**	**Lignocaine**
Specificity		**No interference from excipients present in the formulation and fro degradants product indicatespecific nature of method**	
Linearity range		15-45 µg/ml	75-225.1 µg/ml
Slope		191764.309	50598.473
Intercept		64602.704	100744.569
Correlation coefficient (r^2)		1.000	1.000
Precision (%RSD)	Repeatability (n=6)	0.7	0.9
	Interday (n=3)	0.02-0.25	0.02-0.17
	Intraday (n=3)	0.02-0.62	0.01-0.23
Accuracy (% Recovery) (n=3)		100.7-100.3	100.4-100.1
LOD		0.43 µg/ml	2.31 µg/ml
LOQ		1.31 µg/ml	7.10 µg/ml
Robustness		No significant change	No significant change

4. Conclusion

The development of the analytical method involved researching several parameters. First off, it was discovered that the maximal absorbance of nifedipine was at 210 nm while that of lignocaine was at 278 nm. The peaks purity was outstanding, and the typical wavelength will be 210 nm. The 20 l injection volume chosen provided a suitable peak area. The study column, Kromasil 100-5-C18, 250X4.6mm, 5.0m, was chosen for its acceptable peak form. Case days. Because of the good peak area, satisfactory retention time, and good resolution, the flow rate was set at 1.0 ml/min. As a result

of the well-symmetrical peaks and good resolution, the mobile phase with the ratio of Methanol: ACN: Perchloric acid (50:50:0.1)v/v/v was fixed. Thus, the suggested study made use of this mobile phase. The current recovery was determined to be linear and exact over the same range, 98.0-101.50. The precision of the system and the procedure were both confirmed to be precise and within bounds. The detection limit for nifedipine was determined to be 3.305 and for lignocaine to be 5.828. Curve fitting, correlation coefficient, and a linearity investigation were all successful. For both medicines, it was discovered that the analytical method was linear over the range of 20-80 ppm of the target concentration. The analysis passed the tests for ruggedness and robustness. The relative standard deviation in both circumstances was very acceptable.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest.

References

[1] Indian Pharmacopoeia, Government of Indian, Ministry of Health and Family welfare, Published by Indian Pharmacopoeia Commission,2007, 2, 1442-1445 and 3, 1299.

[2] British Pharmacopoeia, London Her Majesty's, Stationary office 5th Edi., 2007, 2, 1468 and 3, 1243-1244.

[3] United State Pharmacopoeia-NF, Washington: By authority of U.S. Pharmacopeia Convention, 2008, 3, 2807 and 2, 2526.

[4] P. Prathyusha, P. Shanmugasundaram, P. Naidu, Int. J. Adv. Pharm. Anal., 2013, 3, 18.

[5] K. Kumar, R. Thiruvengada, V. Begum, N. Tanveer, Int. J. Pharm. Pharm. Sci., 2012, 4, 610.

[6] L. Dal, P. Mazzucchelli, J. Chrom. A., 1999, 854, 3-11.

[7] C.B. Swezey, J.L. Ponzo, Clin. Biochem., 1984, 230-232.

[8] A. Suhail, S. Safeena, S. Patni, Int. J. Res. Pharm. Sci., 2012, 2, 78-88.

[9] S. Nada, W.A. Nouruddin, M. Hamed, W.M. Osman, Chrom. Sep. Tech., 2013, 4, 1-6.

[10] A. Suhail, S. Safeena, S. Patni, Int. J. Sci. Res. Pub., 2012, 2, 1-8.

[11] J. Abrahum, R. Prasanna, T. Sivakumar, Int. J. Inn. Pharm. Sci. Res., 2014, 2, 1337-1338.

[12] E. Weijden, M.P. Broek, FF. Ververs, J. Chromatogr. B. Anal. Technol. Biomed. Life. Sci., 2012, 881-882.

[13] W. Dan, J. Kun, Y. Shuyan, Q. Feng, L. Xiumei, L. Famei, J. Chrom. B., 2011, 879, 1827-1832.

[14] A. Bijay, R. Sangha, S. Mamata, Int. J. Pharm. Biol. Sci. Arch., 2014, 5, 89-94.

[15] R. Revathi, T. Ethiraj, V. Saravanan, V. Ganeshan, V. Saranya, T. Sengottuvel, Int. J. Pharm. Pharm. Sci., 2010, 2, 74-76.

[16] Z. Dragica, S. Suzana, S. Olgica, K. Elena, M. Kalina, J. Krume, J. Emilija, K. Igor, T. Jasmina, Z. Dime, J. Chrom. B., 2006, 839, 85-88.

[17] R. Nafisur, N. Syed, Acta. Biochem. Pol., 2005, 52, 915-922.

[18] S. Shelke, S. Sable, S. Neharkar, V. Mathdevru, Int. Res. J. Pharm., 2012, 3, 360-364.

[19] P. Patel, D. Patel, Inventi J., 2013, 1-5.