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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF NICOTINAMIDE AND CLINDAMYCINE IN BULK AND PHARMACEUTICAL FORMULATIONS

Anil Kumar S.M.*, A. Satishkumar Shetty, N. D. Satyanarayan

Department of Pharmaceutical Analysis, National College of Pharmacy, Shimoga 577201, Karnataka, India.

ABSTRACT

The objective of the current study was to develop a simple, accurate, precise and rapid RP-HPLC method and subsequently validate as per ICH guidelines for the determination of Nicotinamide (NIC) and Clindamycine (CLI) using mobile phase [A mixture of 0.02M disodium hydrogen phosphate buffer and acetonitrile (pH-2.9), in the ratio of 71:29 v/v was considered to be the optimal composition] as the solvent. The proposed method involves the measurement of retention time at selected analytical wavelength. 195.0 nm was selected as the analytical wavelength. The retention time of NIC and CLI was found to be 1.864 and 3.642 respectively. The linearity of the proposed method was investigated in the range of 2-10 µg/ml (r = 0.9999) for NIC and 10-50 µg/ml (r = 0.999) for CLI respectively. The method was statistically validated for its linearity, accuracy and precision. Both inter-day and intra-day variation was found to be showing less % RSD (Relative Standard Deviation) value indicating high grade of precision of the method.

Keywords: RP-HPLC method, Nicotinamide, Clindamycine, Validation.

INTRODUCTION

Nicotinamide (NIC) [pyridine-3-carboxamide] is also known as niacinamide or nicotinic acid amide, is the amide of nicotinic acid. NIC is a water-soluble vitamin and is part of the vitamin B group. NIC has demonstrated anti-inflammatory actions that may be of benefit to patients with inflammatory skin conditions. These conditions include acne vulgaris, and the compound can suppress antigen-induced, lymphocytic transformation and inhibit 3'-5' cyclic AMP phosphodiesterase. NIC has demonstrated the ability to block the inflammatory actions of iodides known to precipitate or exacerbate inflammatory acne [1, 2].

Clindamycine (CLI) [2S,4R)-N-{2-chloro-1-[(2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6- (methyl sulfanyl) oxan-2-yl] propyl}-1-methyl-4-propylpyrrolidine-2-carboxamide] is used primarily to treat infections caused by susceptible anaerobic bacteria, including infections of therespiratory tract, skin and soft tissue infections,and peritonitis.

Inpatients with hypersensitivity to penicillins, CLI may be used to treat infections caused by susceptible aerobic bacteria as well. It is also used to treat bone and joint infections, particularly those caused by Staphylococcus aureus. Topical application of clindamycin phosphate can be used to treat mild to moderate acne [3, 4]. Combination of CLI and NIC is available as marketed formulation and prescribed by physician and dermatologist for the treatment of mild to moderate acne vulgaris and to reduce inflammatory acne lesions [5].

On the literature survey it was found that Clindamycin and Nicotinamide can be estimated independently and in combination with other drugs by several HPLC and Spectrophotometric methods [6-9]. But no method found for simultaneous estimation of CLI and NIC. Hence an attempt has been made to develop a simple, accurate,precise and reproducible RP-HPLC method for

Corresponding Author:-Anil Kumar S.MEmail:- anil_manchi@rediffmail.com

simultaneous estimation of NIC and CLI in combined dosage forms with validation as per recommendation of ICH guidelines [10].

EXPERIMENTAL

Chemicals and reagents

The working standards of NIC and CLI were gifted from Strides Arco labs Ltd, Bangalore. The ointment formulation of NIC and CLI (Label claim: Nicotinamide 4% and Clindamycine 1 %), Acnestar (Mankind Ltd,) were purchased from the local market. Acetonitrile and water (HPLC grade) were obtained from Merck Ltd Mumbai, India. Disodium Hydrogen Phosphate buffer was obtained from Sd Fine chemicals Pvt Ltd Mumbai, India. pH-2.9 was adjusted with O-Phosphoric acid obtained from Sd Fine chemicals Pvt Ltd Mumbai, India.

Instrument used

A Shimadzu class HPLC unit accomplished with SPD-20AD UV-Visible detector;Enable C18 (250*4.6*5) Column (Shimadzu); LC-20 AD Pump; Quantitative HPLC was performed on a isocratic mode with 20 µl injection of sample loop (manual). The output signal was monitored and integrated using software LAB SOLUTIONS (Shimadzu).

Preparation of Mobile phase

The HPLC grade acetonitrile was filtered through 0.4µm membrane filter and 0.02M of disodium hydrogen phosphate in HPLC water (7.163gm in 1000ml of water) was filtered through 0.4µm membrane filter. Mobile phase was prepared by mixing 710 ml of buffer with 290 ml of acetonitrile and pH-2.9 was adjusted by ortho-phosphoric acid and sonicated for 15 min.

Preparation of standard stock solution

50 mg each of standard NIC and CLI was weighed accurately and transferred to two separate 50 ml volumetric flasks. Both the drugs were dissolved in 40 ml of mobile phase and sonication for 15 min and then volume was made upto the mark with mobile phase (solution–A). Further the stock solutions were diluted to get 20 µg/ml final concentration of NIC and 100 µg/ml final concentration of CLI standard stock solution of each drug (solution–B). These stock solutions were filtered through 0.4µm membrane filter.

Preparation of calibration curves

Appropriate dilutions were prepared separately and 20 µl of each was injected into the HPLC system and their chromatograms were recorded under the same chromatographic conditions as described below. Peak areas were recorded for all the peaks and a standard calibration curve of AUC against concentration was plotted.

Chromatographic condition

The mobile phase containing both buffer and acetonitrile in the ratio of 71:29 was selected as the optimum composition of mobile phase, because it was found that this solvent system resolved both the components ideally. The flow rate was set to 1.5 ml/min and UV detection was carried out at 195.0nm. The mobile phase and samples were degassed by sonication for 15 min and filtered through 0.4 µm membrane filter paper. All determinations were performed at constant column temperature (25^0C).

Selection of analytical concentration range

Appropriate aliquots were pipetted out from the standard stock solution (solution B) into a series of 10 ml volumetric flasks. The volume was made upto the mark with the mobile phase to get a set of solutions having the concentration range, ranging from 2-10 µg/ml and 10-50 µg/ml of NIC and CLI respectively. Triplicate dilutions of each of the above mentioned concentrations were prepared separately and from these triplicate solutions, 20 µl of each concentration of the drug was injected into the HPLC system two times separately and their chromatograms were recorded under the same chromatographic conditions as described above. Peak areas were recorded for all the peaks and a standard calibration curve of AUC against concentration was plotted.

Analysis of ointment formulation

From the volume ofointment formulation, a quantity containing 2 mg of Nicotinamide was measured accurately and transferred to a 10 ml volumetric flask, volume was made upto mark with mobile phase to get 200 µg/ml (stock A). The contents were sonicated for 15 minutes and the final volume was made upto the mark with mobile phase.

The above prepared solution was filtered through 0.4 µm membrane filter paper and was used as standard stock solution. Appropriate aliquot was pipetted out from the standard stock A and was further diluted with the mobile phase to obtain a mixture containing 4 µg/ml of NIC and 10 µg/ml of CLI (By standard addition method). A replicates mixture containing 4 µg/ml of NIC and 10 µg/ml of CLI were prepared as above from the standard stock solution. A 20 µl volume of each sample mixture was injected into the sample injector of HPLC system and their chromatograms were recorded under the same chromatographic conditions as described above. The area of each peak was determined at 195.0 nm and the amount of drug present in the sample mixture was determined.

Method validation

The developed analytical method was subjected to various validation parameters such as linearity, limit of quantification (LOQ), limit of detection (LOD), accuracy, precision, recovery studies and reproducibility as per the ICH guidelines.

RESULT AND DICUSSION

The present manuscript deals with simultaneous estimation of NIC and CLI in combined ointment dosage form by RP- HPLC method using mobile phase as the solvent. The developed method is based upon estimation of both the drugs by determining the area under curve of the chromatogram at selected analytical wavelength.

The linearity of the proposed method was established by least square regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 2-10 µg/ml for NIC (r^2 = 0.9999) and 10-50 µg/ml CLI (r^2 = 0.999) respectively, along with the summary of validation and System suitability parameters as shown in the Table 1.

Recovery studies were also performed to determine the accuracy and precision of the proposed method. Recovery experiments were performed at three levels, 80%, 100% and 120% of the labeled amount of both the drugs as shown in Table 2.

Three replicate samples of each concentration levels were prepared and the percentage recovery at each level (n = 3), and mean % recovery (n = 3) were determined and Statistical validation data for accuracy determination summarized in Table 3.

Intra-day precision as estimated by assaying samples containing 6 µg/ml of NIC and 30 µg/ml of CLI, six times and the results were averaged for statistical evaluation. The assay results and statistical validation data for intra-day precision are summarized in Table 4 and 5.

Inter-day precision was evaluated by analyzing a set of quality control samples containing 6 µg/ml of NIC and 30 µg/ml of CLI, replicates were analyzed on three consecutive days. The determination of inter-day precision and statistical validation data for inter-day precision is summarized in Table 6 and 7.

Both intra-day and inter-day variation showed less % RSD value indicating high grade of precision of the method.

The robustness was evaluated by analysing the samples by varying few parameters like wavelength and flow rate. The determination of robustness and statistical validation data is summarized in Table 8 and 9.

The validation results obtained confirm the suitability of the proposed RP-HPLC method for simple, accurate and precise analysis of NIC and CLI in pharmaceutical preparations. The proposed method does not need prior separation of NIC and CLI before analysis. In addition it is suitable for application without interference of excipients and can be applied directly to the commercial preparations without previous treatment.

Table 1. Summary of validation and System suitability parameters of NIC and CLI

Parameters	NIC	CLI
Linear range (µg/ml)	2-10	10-50
Slope	19352	6788.1
Intercept	480.67	2380.8
Regression coefficient (r^2)	0.9999	0.999
Limit of Detection (µg/ml)	0.086	0.1524
Limit of Quantification (µg/ml)	0.262	0.462
Retention time (min)	1.864	3.642
Tailing factor	1.300	1.163
Resolution factor	12.077	
Theoretical plate	4859	6416

Table 2. Recovery of NIC and CLI in spiked standard drug solution

Level of % recovery	Amount present (µg/ml)		Amount of standard drug added (µg)		Total amount recovered (µg)		% Recovery	
	NIC	CLI	NIC	CLI	NIC	CLI	NIC	CLI
80%	2	10	1.6	8	3.63	17.91	101	99.5
	2	10	1.6	8	3.58	18.19	99.5	101.1
	2	10	1.6	8	3.57	17.67	99.2	98.2
100%	2	10	2	10	3.99	20.05	99.76	100.26
	2	10	2	10	3.99	20.28	99.99	101.4
	2	10	2	10	3.97	20.12	99.4	100.6
120%	2	10	2.4	12	4.36	22.11	99.2	100.5
	2	10	2.4	12	4.39	21.83	99.81	99.27
	2	10	2.4	12	4.40	21.84	100.15	99.28

Where n *= 3

Table 3. Statistical Validation Data for Accuracy determination

Components	Mean*(%)	Standard Deviation*	% Relative standard deviation*	Standard Error*
NIC	99.77889	0.581032	0.582086	0.237253
CLI	100.0122	0.91504	0.916275	0.373638

Where n *= 3

Table 4.Determination of intra-day precision of NIC and CLI respectively

Sr. no	Amount present(µg)		Amount found(µg)		% Recovery	
	NIC	CLI	NIC	CLI	NIC	CLI
1	6	30	6.02	29.94	100.4	99.80
2	6	30	5.99	29.86	99.9	99.53
3	6	30	6.09	30.12	100.16	100.4
4	6	30	5.98	29.92	99.85	99.73
5	6	30	5.99	30.22	99.89	100.73
6	6	30	6.13	30.04	101.09	100.13

Table 5. Statistical validation data for determination of intra-day precision

Components	Mean*	Standard deviation*	% Coefficient of Variation*	Standard Error*
NIC	100.15	0.4724	0.4716	0.1928
CLI	100.05	0.4531	0.4528	0.1850

*n=6

Table 6. Determination of inter-day precision of NIC and CLI respectively

Sr. no	Amount present(µg)		Amount found(µg)		% Recovery	
	NIC	CLI	NIC	CLI	NIC	CLI
DAY-1						
1	6	30	5.99	29.94	99.89	99.80
2	6	30	5.98	29.89	99.78	99.63
3	6	30	5.99	30.12	99.84	100.40
4	6	30	6.10	29.76	100.21	99.20
5	6	30	6.09	30.16	100.16	100.50
6	6	30	5.99	29.74	99.92	99.13
DAY- 2						
1	6	30	5.98	29.76	99.78	99.20
2	6	30	5.99	29.94	99.84	99.80
3	6	30	5.99	29.74	99.92	99.13
4	6	30	5.99	30.12	99.89	100.40
5	6	30	6.10	30.16	100.21	100.50
6	6	30	6.09	29.89	100.16	99.63
DAY- 3						
1	6	30	6.08	29.73	100.12	99.10
2	6	30	5.99	29.75	99.90	99.16
3	6	30	5.99	29.93	99.89	99.78
4	6	30	6.09	30.15	100.16	100.44
5	6	30	5.98	29.90	99.80	99.66
6	6	30	5.99	30.12	99.86	100.32

Table 7. Statistical validation data for determination of inter-day precision

Components	Mean*	Standard deviation*	% Coefficient ofVariation*	Standard Error*
NIC	99.60	0.5125	0.5146	0.2093
CLI	99.57	0.5793	0.5817	0.2365

Where n*= 3

Table 8. Determination of Robustness of NIC and CLI respectively

Levels	Retentiontime		Tailingfactor	
	NIC	CLI	NIC	CLI
FlowRate				
-0.3	1.884	3.663	1.322	1.179
0	1.864	3.642	1.300	1.163
+0.3	1.847	3.622	1.275	1.134
Wavelength				
+2	1.894	3.709	1.286	1.152
0	1.864	3.642	1.300	1.163
-2	1.829	3.568	1.321	1.189

Table9. StatisticalvalidationdataofdeterminationofRobustness for Changeinmethodparameters

Parameters					(%)Coefficientofvariance	
	NIC	CLI	NIC	CLI	NIC	CLI
FlowRate						
Retentiontime	1.865	3.642	0.01852	0.02050	0.993	0.5629
Tailingfactor	1.299	1.158	0.02351	0.02281	1.80	1.969
	1.8623	3.345	0.03253	0.06158	1.746	1.689
	1.302	1.168	0.1761	0.019	1.352	1.626

The Chromatogram of Nicotinamide, Clindamycine and formulation by RP-HPLC Method are reported (Fig.3,4 and 5) and calibration curve was plotted (Fig.6, and 7).

Fig 1. Chemical Structure of Nicotinamide

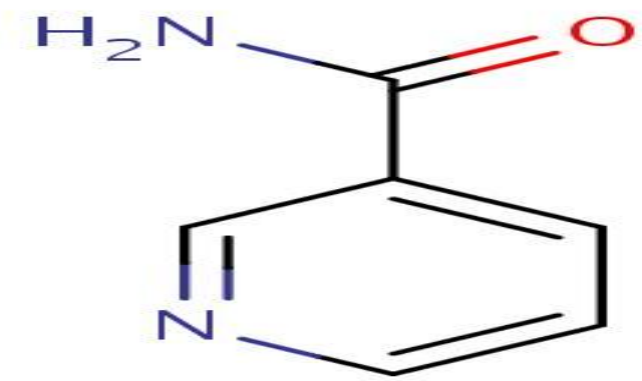


Fig 2. Chemical Structure of Clindamycin

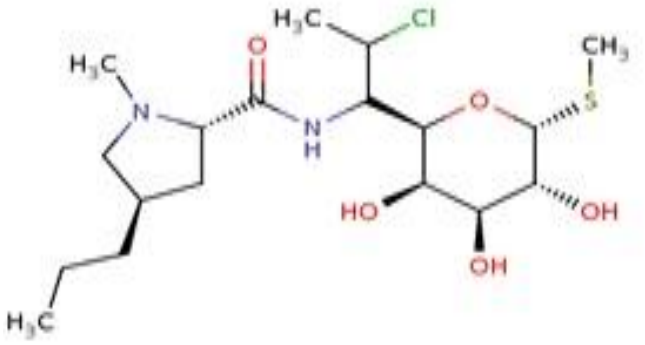


Fig 3. Chromatogram showing retention time of Nicotinamide

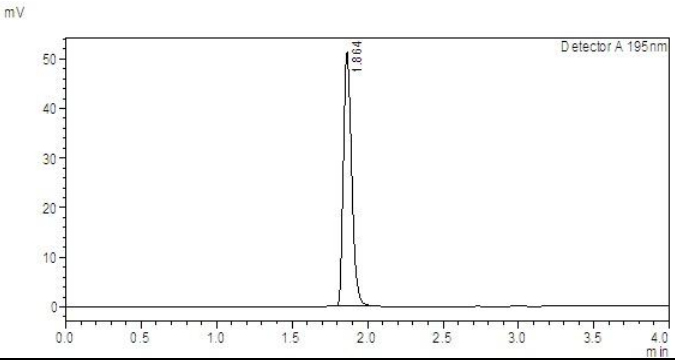


Fig 4. Chromatogram showing retention time of Clindamycine

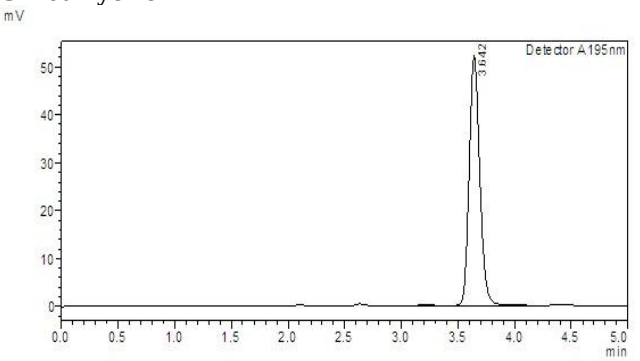


Fig 5. Chromatogram showing retention time of Nicotinamide and Clindamycin in formulation

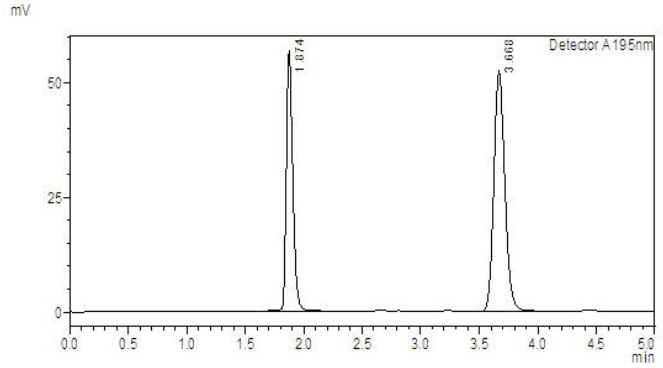


Fig 6. Calibration curve of Nicotinamide at 195.0 nmby RP-HPLC Method

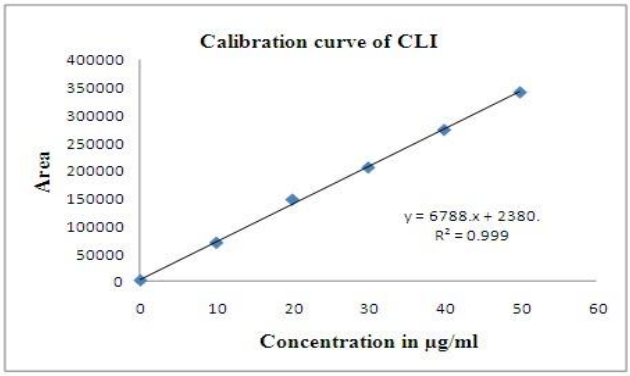
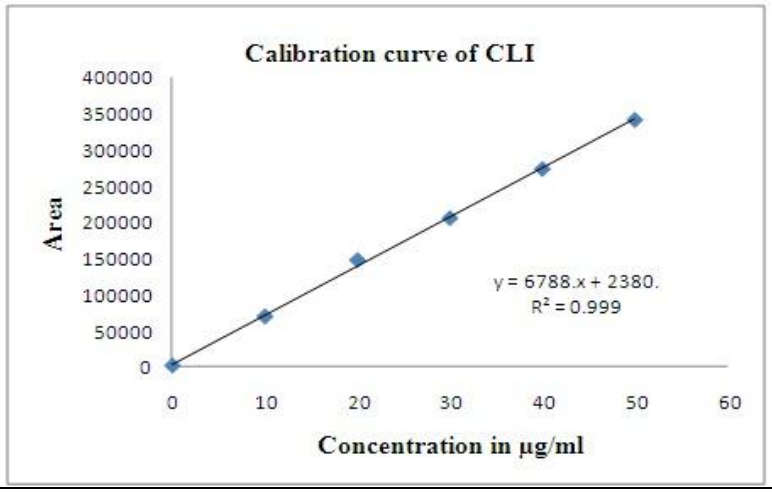


Fig 7. Calibration curve of Clindamycine at 195.0 nm by RP-HPLC Method



CONCLUSION

Proposed study describes a new RP-HPLC method for the estimation of NIC and CLI in combination using simple mobile phase. The method gives good resolution between the compounds with a short analysis time. The method was validated and found to be simple, sensitive, accurate and precise. So the developed method can be used conveniently for analysis of NIC and CLI in combined pharmaceutical dosage forms.

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CONFICT OF INTEREST

There is no conflict of interest.

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