Research Article

Stability Indicating HPLC Method for Simultaneous Estimation of Nicotinamide and Salicylic Acid

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ABSTRACT

The purpose of the present study was to develop stability indicating HPLC method for simultaneous estimation of Nicotinamide and Salicylic acid and to validate developed method in accordance with ICH guidelines. Stability indicating reverse phase high performance liquid chromatography method was developed for Nicotinamide and Salicylic acid. Various trials with aqueous phase and organic phase were performed and mobile phase was optimized. The separation was achieved using Chromatopak C-18 (250mm x 4.6 mm, 5 µm) column, mobile phase containing Methanol and Water (0.1% TEA and 0.15 gm Hexane sulphonic acid and pH 3.0 adjusted with Glacial acetic acid) in the ratio of (40:60 v/v), at a flow rate of 1.0 ml/min. The retention time for Nicotinamide and Salicylic acid was 4.343 min and 17.673 min respectively. Forced degradation study was performed by applying stress conditions like acid hydrolysis, alkali hydrolysis, and neutral hydrolysis, and oxidation, thermal and photolytic degradation. %degradation was calculated with respect to drugs and specificity.LC method was validated by ICH Q2 (R1) guideline. The linearity was observed in the concentration range of 225-315 µg/ml for Nicotinamide and 75-105 µg/ml for Salicylic acid. The correlation coefficient was found to be 0.9971 and 0.9985 for Nicotinamide and Salicylic acid respectively. Degradation in all conditions was found to be 10 -20%. The method specifically estimates both the drugs in the presence of all the degradant generated during forced degradation study. The developed method was specific and precise and can be used for simultaneous estimation of Nicotinamide and Salicylic acid.

Keywords : Nicotinamide, Salicylic acid, RP-HPLC method, forced degradation.

INTRODUCTION

Salicylic acid 2% and Nicotinamide 6% is used for the treatment of pimples and oily skin. It also suitable for acne and blemish prone skin [1, 2].Literature review reveals that there are several analytical methods available for estimation of Nicotinamide and Salicylic acid individually and in combination with other drugs. Assay method has been reported [3] for Nicotinamide and Salicylic acid, but no Stability indicating method has been reported.

Materials and methods

Nicotinamide and Salicylic acid are received as gift sample from pharma industries. Nylon 66 membrane filter (0.45µ), Hexane sulphonic acid, Sodium hydroxide, Acetonitrile, Methanol, Triethylamine, Glacial acetic acid, Hydrochloric acid, Hydrogen peroxide (30%) were also used.

HPLC method development [4, 5]

The conditions were optimized to obtain an adequate separation of the compounds and degradants. Various trials were taken during method development for optimization of method.

Preparation of standard stock solution

A standard stock solution of mixture of NIC and SAL was prepared by accurately weighing 30 mg NIC and 10 mg SAL drugs taking in a 10 ml of Volumetric flask and volume was made up with Methanol as diluents (Conc. Obtained was 3000 µg/mL NIC and 1000 µg/mL SAL).

Preparation of working standard solution

From the mixture of 3000 µg/mL NIC and 1000 µg/mL SAL, 1ml was taken and diluted with mobile phase to yield a solution with final concentration of 300 µg/mL NIC and 100 µg/mL SAL.

Procedure for forced degradation study [6]

Forced degradation with 0.1 N HCl up to 30 min, at RT; 0.1 N NaOH up to 90 min, at RT; 3%v/v H_2O_2 up to 60 min, at RT; photo degradation in UV light at 60 min and Dry Heat in Hot air oven for 30 min, at 70° C was performed. The % degradation was found to be 10 to 20% for Nicotinamide and Salicylic acid in the given condition using developed HPLC method.

The sample solutions (30 mg NIC and 10 mg SAL) were filtered through 0.22 μm Nylon membrane filter paper. Then sample solution was sonicated to degas the solution. This sample solution was injected in chromatographic system and area was obtained using validated HPLC method. Area obtained from the sample solution is compared to area of standard solution and % degradation was calculated for each condition.

Method Validation [7]

The method is validated as per the ICH Q2 (R1) Guideline for accuracy, precision, linearity-range, specificity and robustness. Validation parameters were determined statistically. System suitability was checked by repeatedly injecting (n = 6) standard solutions of 270 µg/mL NIC and 90 µg/mL for SAL

under the same chromatographic condition. The peak RT, theoretical plates and resolution was measured. % RSD of RT should not be more than 2%. theoretical plates and resolution must be more than 1500 and 2 respectively.The linearity was validated by preparing five concentrations in the range of 225-315 µg/mL for NIC and 75-105 µg/mL for SAL. Response was observed and correlation coefficient was found for the calibration curve for the drugs. The precision of the method was checked by repeatedly injecting (n = 6) standard solutions of 270 µg/mL NIC and 90 µg/mL for SAL under the same chromatographic condition. The peak area was measured. % RSD or CV of area should not be more than 2 %.Intraday precision and Interday precision was checked by statistical analysis of 3 responses repeating three times as per ICH guideline. Robustness was performed for different mobile phase ratio and must be within limit.

Table I: Data of Degradation study

Sr. No	Condition	% Degradation	
		NIC	SAL
1	Initial 1) Standard	-	-
	2) Test	-	-
2	Acid degradation 0.1 N HCl for 0 min. at RT	19.32	9.24
	0.1 N HCl for 30 min. at RT	19.95	10.25
	Formulation for 30 min at RT	17.10	12.56
3	Base degradation 0.1 N NaOH for 0 min. at RT	1.89	0.53
	0.1 N NaOH for 30 min. at RT	6.73	0.84
	0.1 N NaOH for 60 min. at RT	10.25	4.67
	0.1 N NaOH for 90 min. at RT	13.31	15.60
	Formulation for 90 min. at RT	11.30	14.40
4	Oxidative degradation 3% H_2O_2 for 0 min. at RT	6.15	2.84
	2) 3% H_2O_2 for 30 min. at RT	7.50	9.28
	3% H_2O_2 for 60 min. at RT	12.77	13.22
	Formulation for 60 min. at RT	10.90	11.52
5	Thermal degradation Hot air oven for 0min. at 70°C	17.82	11.63
	Hot air oven for 30min. at 70°C	22.06	11.88
	Formulation in hot air oven for 30min. at 70°C	19.75	12.97
6	Formulation in UV light for 60min. at 254 nm	10.47	10.09

Fig. I Chromatogram of 100µg/ml SAL &300µg/ml NIC

Assay

500 mg gel was accurately weighed (containing 10 mg SAL and 30 mg NIC) and was dissolved with Methanol in 10 ml volumetric flask. It was sonicated for 5-7 min to dissolve the drug completely as possible. This solution was filtered through Whatman filter paper (stock solution). The sample solution (270 µg/mL NIC and 90 µg/mL SAL) was injected in chromatographic system and area was obtained using validated HPLC method. The amount of NIC and SAL were estimated by applying obtained values to the regression equation of the calibration curve.

Result and discussion

Optimised HPLC Method

After different trials, Good peak separation with high resolution and less tailing for both drugs was obtained (Fig. I). Retention time of both drugs was satisfactory. Good separation with resolution more than 2 was achieved and theoretical plates were also within acceptance criteria.

The following chromatographic condition was finalized for further research. Nicotinamide and Salicylic acid were estimated on Chromatopak, peerless basic C_{18} (250 x 4.6 mm, 5 μm) column using Methanol and Water (0.1% TEA and 0.15 gm Hexane sulphonic acid and pH 3.0 adjusted with Glacial acetic acid) in the ratio of (40:60 v/v) as mobile phase with flow rate 1 ml/min and detection was carried out at 226 nm. The retention time of Nicotinamide and Salicylic acid were found to be 4.343 min and 17.673 min respectively.

Table II:Data for Linearity and Range

Sr. No	NIC		SAL	
	Concentration (µg/mL)	Area of NIC	Concentration (µg/mL)	Area of SAL
1	225	8776.507	75	3265.610
2	240	8918.125	80	3453.347
3	255	9172.119	85	3677.176
4	270	9331.118	90	3860.852
5	285	9547.354	95	4019.375
6	300	9710.242	100	4212.245
7	315	9892.350	105	4431.324

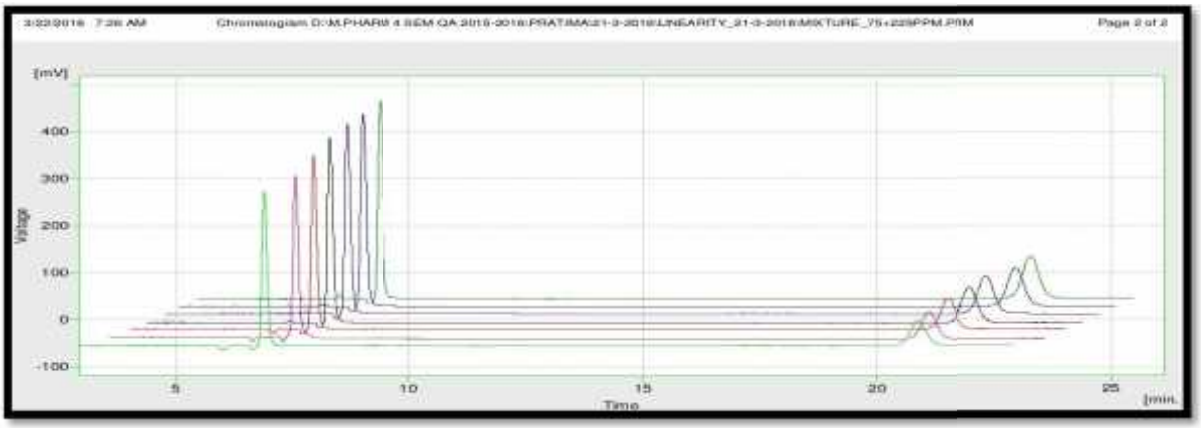

Fig. II Linearity of NIC and SAL in mixture.

Degradation Study

NIC and SAL both are degraded by all the forced degradation conditions. Sufficient degradation was observed in 0.1N HCl at 30min, in 0.1N NaOH at 90min, in 3% H_2O_2 at 60min, in hot air oven at 70°C at 30min and U.V light at 254 nm at 60 min respectively. Degradant peaks are well resolved from drug peaks.

Results of degradation study are presented in table I.

Method Validation

The linear response was observed over a range of 225-315 µg/mL for NIC and 75-105 µg/mL for SAL(Fig 2). The linearity and range data are presented in fig. II, III, IV and table II.

For NIC, regression equation was found to be **y = 12.6357x +5923.7606** and correlation co-efficient (R^2) was found to be **0.9971**.For SAL, regression equation was found to be **y = 38.2653x + 401.8304** and correlation co-efficient (R^2) was found to be **0.9985.**

Results of validation parameters are presented in table III. % recovery at different levels was found to be 98%-102%. Precision and robustness was less than 2%RSD. So, the developed method was precise, robust and accurate.

Analysis of Drugs in Marketed Formulation:

% Assay for Nicotinamide and Salicylic acid was found as 99.53 % - 99.97 % and 99.38 % - 100.09 % respectively(table IV).

Table III: Summary of Method Validation Parameters

Sr. No.	Validation Parameter	Result	
		Nicotinamide	Salicylic Acid
1	Linearity and range	225-315 µg/ml	75-105 µg/ml
2	co-relation coefficient	0.9971	0.9985
3	Precision: % RSD of repeatability, Intraday Precision and Interday precision	 0.887 0.573-1.015 0.621-0.836 (less than 2%)	 1.050 0.549-1.050 0.850-1.530 (less than 2%)
4	% RSD for Robustness parameters	0.374-0.855 (less than 2%)	1.077-1.607 (less than 2%)
5	LOD and LOQ	42.563 and 128.978	15.471 and 46.881
6	% Recovery at different levels (Accuracy)	99.22% - 100.15%	99.90% - 100.32%

Table IV: Analysis of marketed formulation for NIC and SAL by proposed method (n = 3)

Sample No.	Label Claim (mg/mixture)		Amount Found (mg/mixture)		% Label Claim (mg/mixture)	
	NIC	SAL	NIC	SAL	NIC	SAL
1	270	90	269.43	89.44	99.79	99.38
2	270	90	269.91	89.79	99.97	99.77
3	270	90	268.72	90.08	99.53	100.09
Mean			269.353	89.77	99.76	99.75
S. D.			0.599	0.320	0.221	0.356

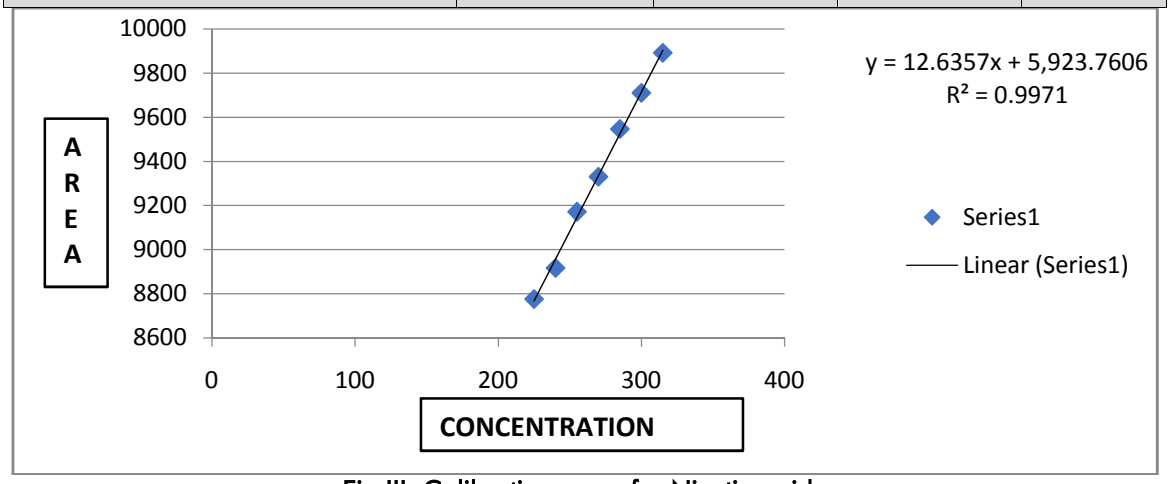

Fig.III: Calibration curve for Nicotinamide

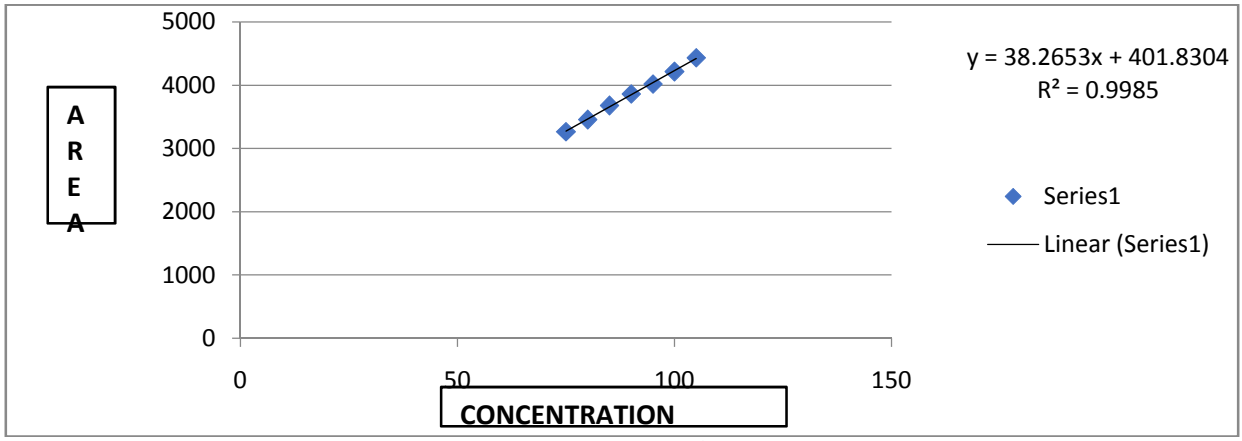

Fig. IV: Calibration curve for Salicylic acid

Conclusion

For determination of Nicotinamide and Salicylic acid, Stability indicating method was developed and validated as per the ICH Q2 (R1) Guideline and thus indicating general applicability of the method for analysis of marketed formulation. The proposed method is specific, rapid, accurate, precise, robust and has the ability to estimate Nicotinamide and Salicylic acid.

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