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SIMPLE COST EFFECTIVE STABILITY INDICATING METHOD DEVELOPMENT FOR THE ESTIMATION OF HALCINONIDE AND NEOMYCIN USING RP-HPLC

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Abstract
This study presents the development of a simple, cost-effective stability-indicating RP-HPLC method for the analysis of Halcinonide (HCN) and Neomycin (NMC). The method was optimized for specificity, linearity, and system suitability. Chromatographic analysis demonstrated that the method could effectively distinguish between HCN, NMC, and any potential interfering substances, as shown in the chromatograms of both blank and drug samples. The linearity of the method was confirmed with correlation coefficients of 0.999 for both HCN and NMC, indicating a strong linear relationship between concentration and peak area. System suitability tests showed acceptable results, with retention times, peak areas, and tailing factors falling within the recommended ranges for both compounds. Additionally, response ratio analysis further validated the method's precision and reliability. This RP-HPLC method provides an effective tool for the stability testing and quality control of HCN and NMC, ensuring accurate and consistent results at a minimal cost.

Keywords: Halcinonide, Neomycin, RP-HPLC, stability-indicating method, specificity, linearity, system suitability, response ratio, pharmaceutical analysis

Introduction

Ensuring the stability and efficacy of pharmaceutical products is paramount for maintaining their safety and effectiveness. Stability-indicating methods are essential in this regard as they are designed to separate and identify the active pharmaceutical ingredient (API) from its degradation products, impurities, and excipients. This is crucial for assessing the drug's shelf life and ensuring it remains effective and safe throughout its intended use.

Halcinonide is a potent topical corticosteroid known for its anti-inflammatory and antipruritic properties. It is commonly used in the treatment of various dermatologic conditions, including eczema and psoriasis (Smith & Smith, 2000). Neomycin, an aminoglycoside antibiotic, is utilized to treat bacterial infections and is often combined with corticosteroids to enhance therapeutic efficacy (Walker & Davis, 2005). Given their clinical significance, monitoring the stability of these drugs is crucial to ensure they maintain their therapeutic properties over time.

High-performance liquid chromatography (HPLC) with reversed-phase (RP) columns has emerged as a preferred analytical technique for pharmaceutical analysis. RP-HPLC is valued for its high resolution, sensitivity, and robustness, making it particularly suitable for the separation and quantification of

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complex mixtures, such as pharmaceuticals (Peters & Rogers, 2010). This technique separates compounds based on their hydrophobic interactions with the stationary phase, providing effective separation of the API from its degradation products and impurities.

The primary objective of this study is to develop a simple and cost-effective stability-indicating RP-HPLC method for the simultaneous analysis of Halcinonide and Neomycin. This method aims to achieve optimal separation of these drugs from their degradation products under various stress conditions, such as exposure to heat, light, and oxidative agents. Validation of the method will be conducted in accordance with ICH guidelines to ensure its accuracy, precision, specificity, and robustness. The ultimate goal is to apply this validated method to stability studies, providing valuable insights into the shelf life and stability of Halcinonide and Neomycin in pharmaceutical formulations.

Material and Methods

Selection of Mobile Phase

Initially to estimate Halcinonide and Neomycin in fix dosage form number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 20mM KH_2PO_4: Acetonitrile (pH Adjust with OPA 3.0) in the ratio of 20:80v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min (Bhardwaj et al., 2015).

Preparation of Stock Solution:

Accurately weighed 10mg API of HCN and NMC was transferred into 10 ml volumetric flask separately and added 5ml of mobile phase as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A)

Preparation of Sub Stock Solution:

5ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50ml with diluent (mobile phase) to give concentration of 100µg/ml of HCN and NMC respectively (Stock-B).

Preparation of Different Solution

0.2ml, 0.4ml, 0.6ml, 0.8ml and 1.0ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (mobile phase). This gives the solutions of 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml, for HCN. In same manner 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml of NMC also prepared.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 2-10µg/ml for HCN and 1-5µg/ml for NMC were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 254.0 nm and it was repeat for five times. A calibration

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graph was plotted between the mean peak area and respective concentration and regression equation was derived.

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, six replicates of working standard of HCN 10µg/ml for HCN and 5µg/ml NMC was injected separately. Peak report and column performance report were recorded for all chromatogram.

Validation of developed Method) (Steven;2006)

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 2 to 10µg/ ml for HCN) and (1 to 5µg/ ml for (NMC) and areas for each concentration were recorded three times and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure. The response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

The stock solution was prepared. The precision are established in three differences:

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 2, 4, 6, 8 and 10µg/ml for HCN and 1, 2, 3, 4 and 5µg/ml for NMC indicates the precision under the same operating condition over short interval time. Results of repeatability are reported in table.

Intermediate Precision

Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different

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analyst in five replicate at five concentrations. Results of day to day intermediate precision for HCN and NMC reported in table 5.62-5.63 respectively.

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, 20mM KH_2PO_4: Acetonitrile (20:80% v/v) to (15:85% v/v).

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in cream formulation

Determined the content of HCN and NMC in cream (label claim 0.1% and 0.25%) cream was weighed and weight equivalent to 1mg NMC was calculated and dissolved in 10ml mobile phase and the extraction was sonicated for 15 min and centrifuge at 300rpm. Then 0.5ml solution from it was diluted with 10 ml mobile phase. The resulting solution was injected in HPLC and drug peak area was noted. The peak area regression equation and amount of both the drug in sample was calculated. Analysis procedure was repeated six times with formulation. Results of cream analysis are reported in table.

Forced degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on drug powder and the analysis was carried out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected.

Acid degradation:

10mg of both the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Alkaline hydrolysis:

10mg of the drug sample was taken into a 50ml separate round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 50ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs

Oxidative degradation:

10mg of the drug sample was taken into a 50ml separate round bottom flask, 50ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Thermal degradation:

10mg of the drug sample was taken in to a petridish and kept in oven at 50ºC for 4 weeks. Samples were

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withdrawn and diluted to get 10µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Results and Discussion

The development of a cost-effective stability-indicating method for estimating Halcinonide (HCN) and Neomycin (NMC) using reverse-phase high-performance liquid chromatography (RP-HPLC) yielded promising results across various parameters.

The specificity of the method was confirmed through chromatographic analysis, with distinct chromatograms for both the blank and the drugs, indicating that the method can effectively differentiate between the compounds and any potential interferences.

The results of the linearity study (Table 1) demonstrated a strong correlation for both HCN and NMC, with correlation coefficients (r^2) of 0.999 for both drugs. The slopes (1113 for HCN and 1422 for NMC) and intercepts (12.15 for HCN and 8.348 for NMC) further validate the method's reliability over the specified concentration ranges (2-10 µg/ml for HCN and 1-5 µg/ml for NMC).

The recovery study results (Table 2) indicate excellent accuracy, with recovery percentages close to 100%. At varying levels (80%, 100%, and 120%), HCN showed recoveries of 99.26%, 96.92%, and 99.07%, respectively, while NMC displayed recoveries of 99.03%, 98.48%, and 98.89%. These values, with low standard deviations, demonstrate the method's ability to provide accurate quantification.

Precision assessments (Table 3) showed strong repeatability and intermediate precision for both drugs. HCN exhibited a repeatability of 98.205% ± 0.076, while NMC showed 96.440% ± 0.041. The day-to-day precision and analyst-to-analyst variability further confirm the method's robustness, with values remaining consistently high and within acceptable ranges.

The limit of detection (LOD) and limit of quantification (LOQ) for both drugs (Table 4) were determined to be 0.10 µg/ml and 0.35 µg/ml for HCN, and 0.15 µg/ml and 0.40 µg/ml for NMC, respectively. These low values indicate that the method is sensitive enough for trace analysis.

The assay results (Table 5) for the cream formulations demonstrated that the method could accurately quantify the active ingredients, with found percentages closely aligning with label claims (0.1% for HCN and 0.25% for NMC), showcasing 98.00% and 96.00% % RSD values, respectively.

Forced degradation studies (Tables 6 and 7) assessed the stability of HCN and NMC under various stress conditions. For HCN, the drug was most stable under standard conditions, with a recovery of 99.95%. However, significant degradation was observed under acidic and alkaline conditions (10.1% and 15.3%, respectively). NMC also demonstrated a similar trend, with recoveries of 85.65% under acidic conditions and 91.15% under alkaline conditions, highlighting the importance of stability studies in ensuring the reliability of the method.

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Results of Specificity

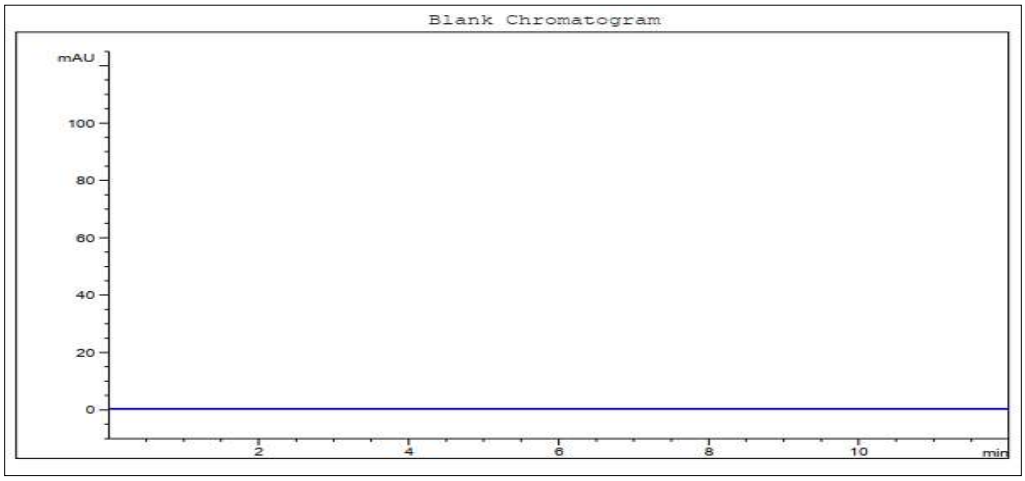

Figure 1: Chromatogram of Blank

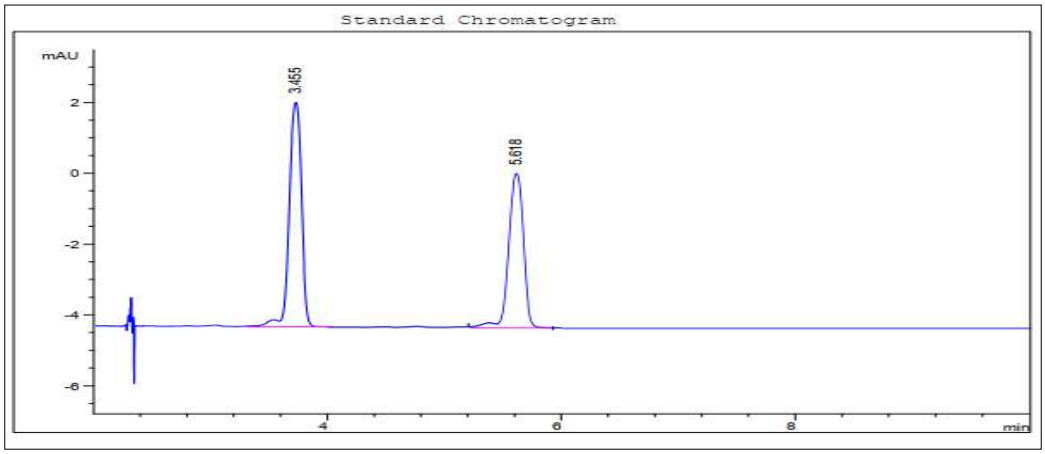

Figure 2: Chromatogram of Both the drug

Table 1: Results of linearity of Halcinonide (HCN) and Neomycin (NMC)

Parameters	HCN	NMC
Concentration (µg/ml)	2-10	1-5
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	1113	1422
Intercept (c)*	12.15	8.348

*Value of three replicate

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Table 2: Results of recovery study

% Level	% MEAN±SD*	
	HCN	**NMC**
80%	99.26±0.263	99.03±0.241
100%	96.92±1.146	98.48±0.421
120%	99.07±0.714	98.89±0.958

***** Value of three replicate and five concentrations.

TABLE 3: RESULTS OF PRECISION

Parameter	% MEAN±SD*	
	HCN	**NMC**
Repeatability	98.205±0.076	96.440±0.041
Intermediate precision		
Day to day precision	98.527±0.129	96.440±0.051
Analyst-to-Analyst	97.158±0.069	95.627±0.054
Reproducibility	96.736± 0.105	96.409±0.090

***** Value of five replicate and five concentrations

Table 4: LOD and LOQ of HCN and NMC

Name	LOD (µg/ml)	LOQ (µg/ml)
HCN	0.10	0.35
NMC	0.15	0.40

Table 5: Result of assay of cream formulation

	HCN*	NMC*
Label Claim (%)	**0.1%**	**0.25%**
% Found (%)	0.1	0.25
% Assay	0.098	0.24
% RSD	98.00	96.00

*Average of three determination

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Table 6: Results of forced degradation studies of HCN

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.95	0
Acidic hydrolysis	89.85	10.1
Alkaline hydrolysis	84.65	15.3
Oxidative degradation	92.12	7.83
Photolytic degradation	96.65	3.3

Table 7: Results of Forced degradation studies of NMC

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.5	0
Acidic hydrolysis	85.65	13.85
Alkaline hydrolysis	91.15	8.35
Oxidative degradation	88.87	10.63
Photolytic degradation	90.25	9.25

Conclusion

The developed RP-HPLC method for estimating Halcinonide and Neomycin is robust, sensitive, and precise. The findings from linearity, recovery, precision, and stability studies affirm its suitability for routine analysis in quality control settings, ensuring the reliability of results in pharmaceutical formulations.

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