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Analytical Method Development And Validation Of Niclosamide By RP-HPLC.

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ABSTRACT

This research covers the development of an RP-HPLC method for estimating niclosamide. The developed method was verified in terms of Specificity, Accuracy, Linearity, LOD, LOQ, and Robustness according to ICH requirements. The precision findings for inter-day and intra-day were excellent enough to demonstrate that the proposed Method was exact and reproducible. The Assay experiment revealed that the niclosamide content measured in the tablet dose was free of excipient interference, indicating that the devised approach was specific. The recovery of standard drugs added was 99-100%.for niclosamide, indicating that the suggested approach was accurate. In the concentration ranges of 10-60 µg/ml, a good linear connection was seen for Standard Drug of niclosamide and Tablet Sample of nicosamide. Nicosamide correlation coefficient was determined to be 0.9999. Following analysis by many analysts, it was discovered that the RP-HPLC technique for the identification of niclosamide was robust. The % RSD for Robustness was well within the bounds, ensuring the suggested Method's robustness. The LOD for niclosamide Standard Drug was 0.2085 µg/ml. The LOQ of niclosamide for Standard Drug was determined to be 0.6321 µg/ml. This demonstrated that the developed RP-HPLC technique was simple, linear, precise, accurate, robust, and rugged, and that it could be easily used for routine quality control analysis of niclosamide from its pharmaceutical dosage form and bulk medication.

KEYWORDS

Niclosamide, RP-HPLC, Recovery, Analysis.

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INTRODUCTION

Niclosamide (chemical name 2,5-dichloro-4-nitrosalicylanilide) is an anthelmintic medication used to treat worm infestations in humans and animals. [1] It prevents the reproduction of a variety of acute respiratory disorders. [2] Niclosamide (NCL) is available in tablet and suspension forms. Niclocide pill is a BCS Class II medication. Niclosamide is one of the most important orally delivered medications with extensive evidence. [3] In the tapeworm, niclosamide decouples oxidative phosphorylation. The authors created a liquid chromatographic technique and UV spectroscopy to serve as quick and accurate methods for determining Niclosamide in bulk and pharmaceutical dosage forms. The validation study is carried out in accordance with the ICH guidelines. [4]

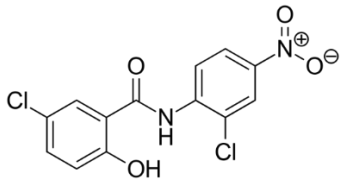

Figure 1. Chemical structure of Niclosamide

MATERIALS AND METHOD

Niclosamide were obtained as a memento sample from GSK pharma Ltd., Navi Mumbai. Acetonitrile, methanol HPLC grade Rankem New Delhi, Methanol Hong yang chemical corp. china, Milli-Q water it was purified by milli Pore Corporation. The analysis was carried out on Agilent HPLC using C18 Column (250mm x 4.6mm), 5µm particle size. The analysis was carried out on UV Lab India UV visible spectrometer (Jasco, 630).

Standard Stock solution of Niclosamide

Accurately weigh and transfer 10 mg Niclosamide working standard into 10 ml volumetric flask as about dilute Methanol prepared in completely and make volume up to the mark with the same solvent to get 1000g/ml standard (stock solution) and 15 min sonicate to dissolve it and 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with mobile phase methanol: Water solvent (0.05%TEA pH adjusted with OPA). The resulting 10g/ml solution was chromatographically analysed using mobile phases of varying strengths and the chromatographic conditions listed below. [5]

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Determination of λ max of niclosamide

Niclosamide standard stock solution diluted separately with diluents to a final concentration of 10g/ml. The solution was scanned with a UV-Visible Spectrometer in spectrum mode between 400nm and 200nm. [6]

Selection of Analytical wavelength

Standard solutions were scanned in the 200-400nm range against a reference of 10 ml methanol and volume made using water solvent system. Niclosamide was discovered to have a chosen wavelength of 254 nm in water.

Selection of Mobile phase:

Niclosamide was put into the HPLC apparatus and ran in various solvent systems. A variety of solvent mixtures were tested in order to establish the best chromatographic conditions for successful separation. Following various mutations and combinations, it was discovered that a mixture of Acetonitrile: water with a PH of 3.0 produces satisfactory results when compared to other mobile phases.

Preparation of sample solution

Accurately weigh and transfer 10 mg Niclosamide working standard into 10 ml volumetric flask as about dilute Methanol prepared in completely and make volume up to the mark with the same solvent to get 1000g/ml standard (stock solution) and 15 min sonicate to dissolve it and 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with mobile phase Acetonitrile: water with adjusted PH 3.0. The resulting 10g/ml solution was chromatographically analysed using mobile phases of varying strengths and the chromatographic conditions listed below.

Chromatographic condition[7-9]

Table 1. Chromatographic conditions for Method development

Analytical column	Agilent C18 Column (250mm x 4.6mm), 5µm particle size.
Mobile Phase	Acetonitrile : water (80:20% V/V) adjusted PH 3.0 with OPA
Column temperature	Ambient
Injection volume	20µl
Flow rate	1.0 ml/min
Detection	254 nm
Run time	10 min

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Assay of Niclosamide[5,10]

Weigh 20 Niclosamide tablets, calculating the average weight of 4.076 mg, then correctly weigh and transfer the sample equivalent to 42.5 mg Niclosamide into a 10 ml volumetric flask. Add around 10ml of diluent and sonicate to completely dissolve it and bring the volume up to the mark with diluents. Filter through a 0.45 m filter after thoroughly mixing. Pipette 0.2ml of the aforesaid stock solution into a 10 ml volumetric flask and dilute with diluents to the mark. (20µg/ml). The basic test chromatogram for Niclosamide The quantities of Niclosamide per tablet shown in were estimated by extrapolating the area value from the calibration curve. With tablet formulation, the analysis technique was done five times. Calculated tablet assay for %label claim for %RSD.

Analysis of Tablet formulation of Niclosamide[11-13]

Weigh 20 Niclosamide tablets, calculating the average weight of 4.076 mg, then correctly weigh and transfer the sample equivalent to 42.5 mg Niclosamide into a 10 ml volumetric flask. Add around 10ml of diluent and sonicate to completely dissolve it and bring the volume up to the mark with diluents. Filter through a 0.45 m filter after thoroughly mixing. Pipette 0.2ml of the aforesaid stock solution into a 10 ml volumetric flask and dilute with diluents to the mark. (20µg/ml). To obtain the final concentrations, suitable dilutions of niclosamide were prepared from this solution, and the solutions were then filtered through a 0.45 m filter. A sample is injected, and a peak at 254 nm is noticed.

RESULTS AND DISCUSSION

The Results of Method Development and Validation of Niclosamide all the Analytical Data with Chromatograms were given as follows.

Determination of λ max of Niclosamide:

UV absorption of 10 µg/mL solution of Niclosamide in methanol was generated and absorbance was taken in the range of 200-400 nm. λ max. Concentration of 10 µg/ml. solution was scanned using UV-Visible Spectrophotometer.

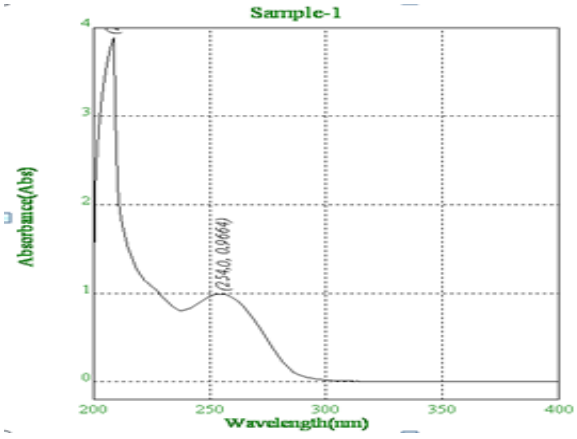

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Figure 2. UV Spectrum for Standard Drug Niclosamide

HPLC METHOD DEVELOPMENT

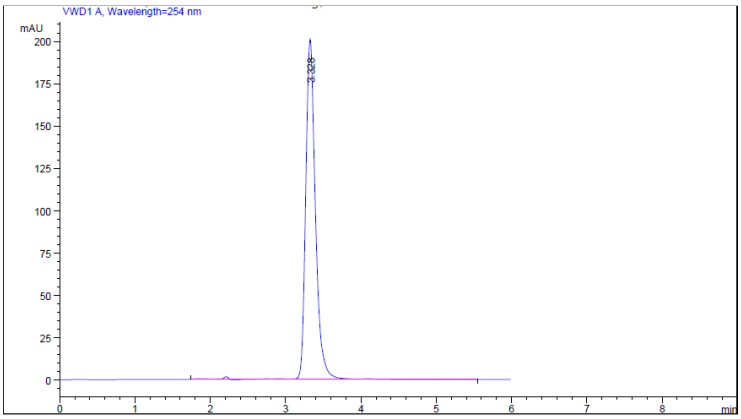

Figure 3. Chromatogram of the niclosamide drug

Table 2. Chromatograph for Tablet Sample of Niclosamide.

No.	RT[min]	Area[mV*s]	Symmetry	TP	Resolution
1	3.30	1828.97	0.75	3185	0.00

VALIDATION

Accuracy

Accuracy of method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 99-100%.

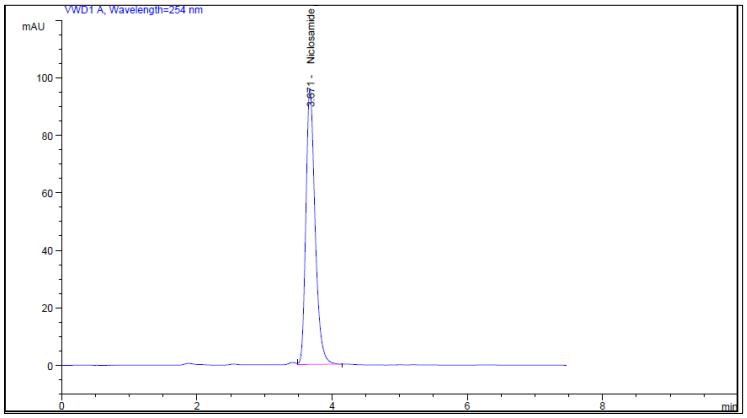

Figure 4. Accuracy data for propose HPLC method.

Table 3. Statistical Validation of Recovery Studies Niclosamide

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Drug	Sr. No	Level (%)	Amt. taken (µg/ml	Amt. Added (µg/ml	Amt. found ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
Niclosamide	1	80%	10	8	18±0.08	8.0±0.08	100.06±0.98
	2	100%	10	10	19.97±0.01	20.58±0.01	99.73±0.15
	3	120%	10	12	21.94±0.03	11.94±0.03	99.97±0.27

Table 4. Statistical Validation of Recovery Studies Niclosamide

Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation*	% RSD
80%	Niclosamide	100.06	0.98	0.98
100%	Niclosamide	99.73	0.15	0.15
120%	Niclosamide	99.97	0.27	0.27

Precision

The method was established by analyzing various replicates standards of Niclosamide All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded. The result obtained for intraday is shown in below table 5 respectively.

Table 5. Intra-Day Inter day variability for Niclosamide

Drug	Conc. (µg/ml)	Intraday Precision			Inter day Precision		
		Mean± SD	%Amt. Found	%RSD	Mean± SD	%Amt. Found	%RSD
Niclosamide	20	1778.58± 0.96	100.27	0.16	1767.22±0.96	99.45	0.05

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	30	2482.61± 11.40	99.33	0.44	2485±4.49	99.63	0.17
	40	3189.66± 2.10	99.43	0.06	3185.67±4.54	99.55	0.13

Linearity

The calibration curves exhibited linear relationship of peak area to concentration in the range 10-60 µg/mL for Standard Drug of Niclosamide The regression coefficients (r^2) for Standard Sample of Niclosamide 0.9999, maintaining good correlation close to unity. The graph of concentration Vs. Average area was plotted which is showing straight line passing through all points. So as per ICH guidelines, the proposed HPLC method for the determination of Standard Drug of Niclosamide found to be linearity.

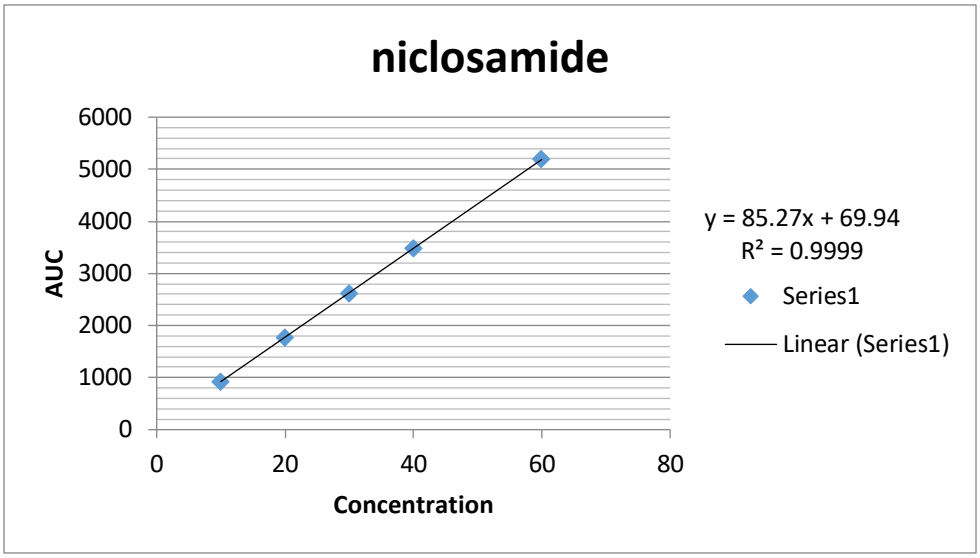

Figure 5. Linearity Graph for Standard Drug of Niclosamide

Limit Detection

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope the limit of detection (LOD) may be expressed as:

LOD = 3.3 (SD)/S

= 3.3 X 5.39/85.27

= 0.2085

Limit Quantification

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope. The quantitation limit (LOQ) may be expressed as:

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LOQ = 10 (SD)/ S
=10 X 5.39/85.27
= 0.6321

Robustness

Results of the robustness study showed that the elution order and resolution for both components were not significantly affected. RSD of both components were examined and found to be well Within the limit of 2.0%. The plate count and asymmetry factor were well within the acceptable USP limits, ensuring that the proposed method was robust and was capable of providing data of acceptable quality.

Table 6. Result of Robustness Study of Niclosamide

Parameters	Conc.(µg/ml)	Area (mean ±SD)	%RSD
MP composition(81 ml+ 19ml) ACN + 0.1%water with OPA	10	853.69± 2.02	0.24
MP composition(79 ml+ 21ml)ACN + 0.1% (OPA)water	10	695.25 ±1.58	0.23
Wavelength change 253 nm	10	918.9± 2.02	0.22
Wavelength Change 255 nm	10	920.35± 1.75	0.19
Flow rate change(1.1ml)	10	840.1± 113.23	13.48
Flow rate change(0.9ml)	10	796.60± 2.02	0.25

CONCLUSION

Niclosamide method development and validation by RP-HPLC is accurate, precise, robust, and specific. The approach was shown to be superior to previously described methods due to its shorter retention period, use of an inexpensive and easily accessible mobile phase, UV detection, and improved peak resolution. The comparatively short run time will allow for the quick measurement of multiple samples in routine and quality-control examination of diverse formulations containing Niclosamide. The suggested approximated technique was found to be simple, precise, accurate, and fast for determining Niclosamide from Tablets forms, and the mobile phase is easy to manufacture and affordable. An attempt was made to develop an RP-HPLC technique for estimating niclosamide. The RP-HPLC technique was designed and validated in accordance with ICH recommendations, employing a mobile phase consisting of an 80:20 combinations of acetonitrile and water with a PH of 3.0, at a flow rate of 1.0 ml/min. The stationary phase used an

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Agilent C18 Column (250mm x 4.6mm), with a particle size of 5m. Niclosamide has a retention time of 3.30 minutes. At 254 nm, the eluent was identified. It was discovered to be simple, accurate, exact, inexpensive, and reproducible. As a result, the proposed approach may be utilized for routine quality control analysis of Niclosamide in bulk and dosage form.

CONFLICT OF INTEREST

None declared by authors.

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