International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (4): 357-363
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved

Analytical Method Development and Validation for Quantitative Estimation of Nonoxynol-9 In Dosage Form by RP-HPLC

Hemanth Mandava*, Naga Malleswari. G, Rama Rao. N.
Department of Pharmaceutical Analysis, Chalapathi institute of Pharmaceutical Sciences, Lam, Guntur, AP, India.

Received: 06 Jul 2019 / Accepted: 08 Aug 2019 / Published online: 1 Oct 2019
***Corresponding Author Email:** hemanthmandava@gmail.com

Abstract

The aim of the present work was to develop and validate a simple, efficient, economical method for the estimation of Nonoxynol-9.by reverse phase high pressure liquid chromatography. For Nonoxynol-9 Chromatography was performed on Waters Nova-Pak, C18,3.9mm with mobile phase containing Methanol: water (80:20) at a flow rate of 1 mL/min and eluents were monitored at 280 nm The retention times of Nonoxynol-9 7.307 min and showed a good linearity in the concentration range of 0.50 to 1.50 mg/mL for Nonoxynol-9, with a correlation coefficient of 0.999. The validation characteristics included Specificity, Linearity, Accuracy, Precision, Robustness and Stability. Validation acceptance criteria were met in all cases. The percent recoveries ranged between 97 – 103 %, RSD < 2%. The method could be successfully used for the analysis of Nonoxynol-9 in Drug product.

*****

INTRODUCTION:

Nonoxynol-9, (2-[2-[2-[2-[2-[2-[2-[2-[2-(4-Nonylphenoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethanol. Sometimes abbreviated as N-9, is an organic compound that is used as a surfactant. It is a member of the nonoxynol family of non-ionic surfactants. N-9 and related compounds are ingredients in various cleaning and cosmetic products. It is widely used in contraceptives for its spermicidal properties. As a spermicide, it attacks the acrosomal membranes of the sperm, causing the sperm to be immobilized. Nonoxynol-9 is the active ingredient in most spermicidal creams, jellies, foams, gel, film, and suppositories

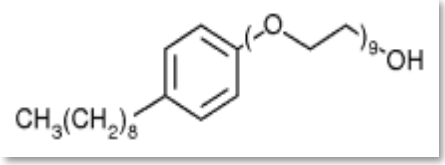

Figure 1: Structure of Nonoxynol-9

MATERIALS AND METHODS:

Chemicals used: water, methanol, Nonoxynol-9.

3.1 Nonoxynol-9

3.1.1 Selection of Wavelength for Detection by Scanning in Uv

The working standard solution of Nonoxynol – 9 was scanned in the UV region and spectrum was recorded. Distilled water was used as the blank. Solutions were scanned on spectrophotometer in the UV range of 200-400nm. It was seen that at 278 nm maximum absorbance was found. In HPLC, proper peak response was observed using 280nm. Hence, 280 nm was selected as the wavelength for estimation in HPLC.

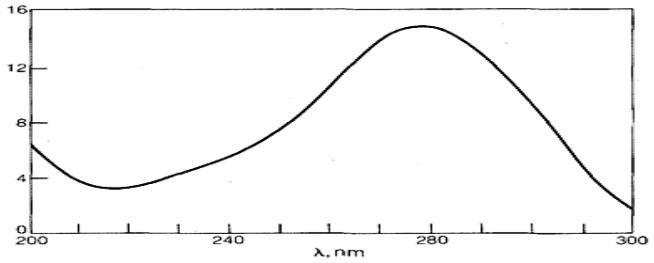

Figure 2: Spectra of Nonoxynol – 9 showing λ max of 280nm

3.1.2 Preparation of Solutions for Estimation of Nonoxynol–9

Standard Preparation of Nonoxynol: Accurately Weigh 100 mg Nonoxynol – 9 in to 100 mL Volumetric flask, dissolve and dilute to volume with Mobile Phase. Mix Well. Filter a portion of the Standard through 0.45 µ Nylon filter discarding the first 3 mL of Filtrate.

Sample Solution preparation:

- Weigh the sponges and determine the average weight of sponge.
- Cut the sponges in to small pieces approximately 10-20 mm across.
- Weigh an amount of sponge equivalent to one sponge, about 7grams.

Extraction procedure:

- Cut the sponges in to small pieces approximately 10-20 mm across.
- Extract the material with mobile phase by compressing and releasing the sponge for approximately 2 min.
- Repeat the process by using 20 ml of mobile phase three times.
- Transfer above extract in to 100ml volumetric flask and make up volume by using Mobile phase (methanol: water) (80:20).

3.2 Calculations:

Formula for Calculation for Content of Nonoxynol – 9 / SA & BA in Sample:

$$\textbf{Nonoxynol – 9/ SA \& BA} = \frac{(Area\ Sample)\times(STD\ Wt)\times(P)}{(Area\ STD)\times(100mL)} \times 11.11 \times ASW$$

ASW = Average Sponge Weight in grams
P = Decimal Purity for the standard

3.3 RESULTS:

Table No: 1 Assay Data of Nonoxynol – 9 / SA & BA in Sample

S.NO	Name of Sample	Area		Result	Limits	Assay
		Standard	Sample			
1	Nonoxynol - 9	2449364	2130719	0.984	1.00 g	98.4%
2	Sorbic acid	309816	283216	0.095	0.1 g	95.4%
3	Benzoic acid	118833	98123	0.077	0.08 g	96.2%

3.3.1 Optimized method:

Chromatographic conditions:

Mobile phase: Methanol: water (80:20)
Maximum wavelength: 280nm
Injection volume: 20µl
Run time: 15min

Flow rate: 1.0ml/min
Column: Waters Nova-Pak, C18, 3.9mm
Elution: Isocratic.

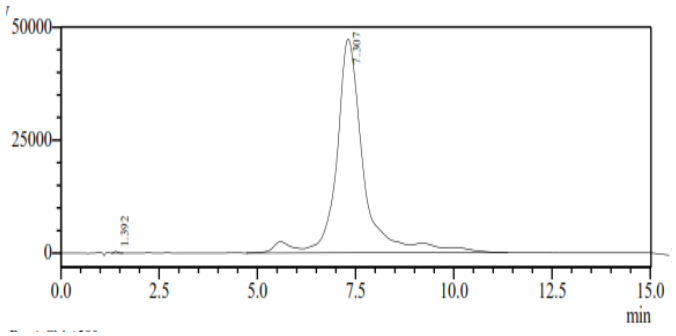

Figure 3: Optimized method Chromatogram

4.1 METHOD VALIDATION:

Validation of an Analytical Procedure is to demonstrate that it is Suitable for its Intended Purpose. The following parameters were evaluated.

4.2 SYSTEM SUITABILITY:

System suitability testing is an integral part of any analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analysed constitute an integral system factor are parameters that are normally used in assessing the column performance. These parameters include column efficiency, resolution, tailing factor, related standard deviation, number of theoretical plates, relative retention time and capacity factor. System Suitability was performed by injecting Blank Followed by six injections of working standards.

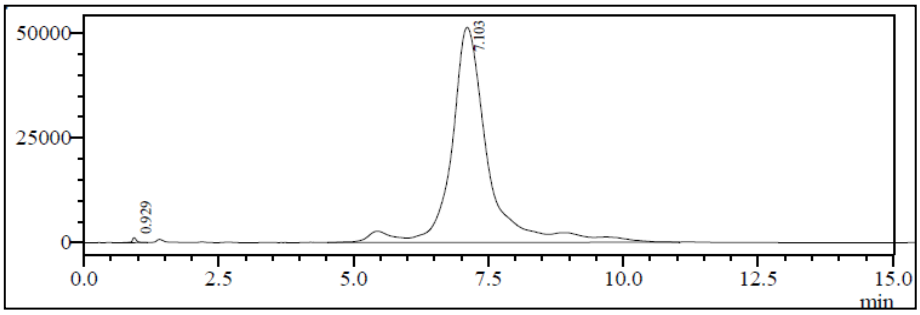

Figure 4: System suitability Chromatogram

Table 2: System suitability Data

S.No.	System Suitability Parameters	Results	Acceptance criteria
1	Tailing factor (T_f)	1.355	NMT 2
3	Theoretical plates(N)	3836	NLT 2000
5	%RSD	0.04	≤ 2

4.1.2 SPECIFICITY:

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present such as impurities, degradation products, and excipients. Specificity was performed by injecting the Blank followed by placebo and there should be no chromatogram at the retention time of API. Placebo is defined as the Today Sponge without API (Nonoxynol-9)

4.1.3 LINEARITY:

Linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. The linearity of the Nonoxynol-9 peak area responses were determined from 50 % to 150 % of the nominal method concentration, 1mg/mL, by preparing 5 concentrations covering the expected range of the method.

Table 3: Linearity Data

S.NO	Concentration(mg/ml)	Peak Area	Acceptance criteria
1	0.50	1225658	
2	0.80	1915559	
3	1.00	2448844	R^2 Should not Less than 0.99
4	1.20	2995708	
5	1.50	3708474	
Correlation coefficient(r^2)		0.999	
Slope (m)		2522824	

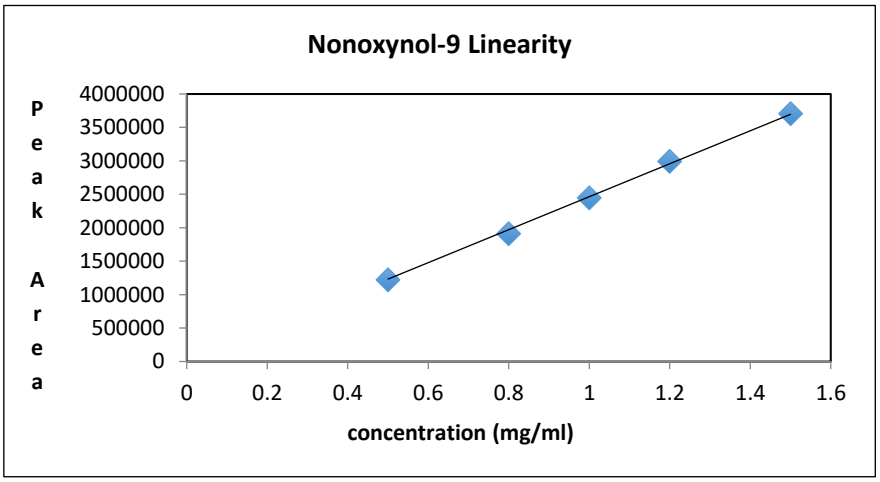

Figure 5: Linearity plot of Nonoxynol–9

4.1.4 ACCURACY:

The accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or as an accepted reference value and the value found. Accuracy is calculated as the percentage of recovery by the assay of the known added amount of the analyte in the sample.

Recovery studies were performed to assess the accuracy of the method, where known amounts of analyte were spiked into Solution and recovered. Three concentrations were prepared covering the range of the method.

Acceptance criteria: The % Recovery for each level should be NLT 96 % and NMT 104 %

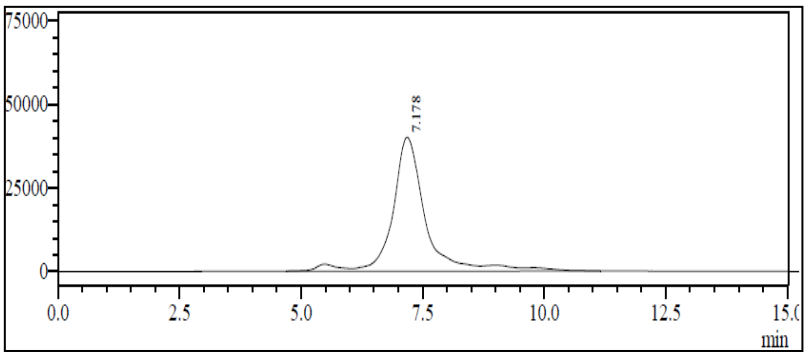

Figure 6: Chromatogram of sample- 80 %

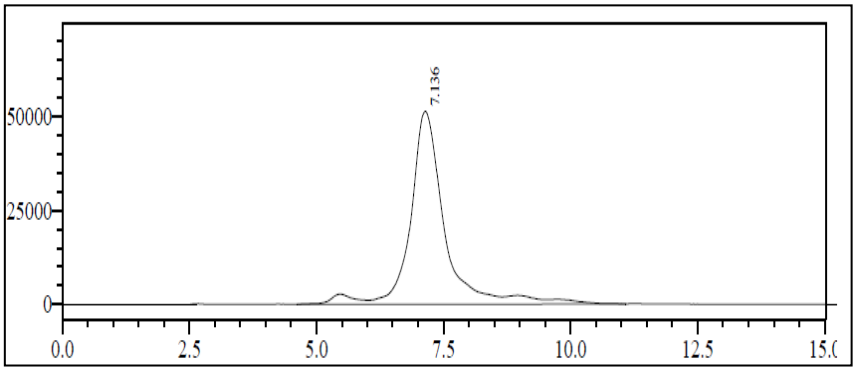

Figure 7: Chromatogram of sample- 100 %

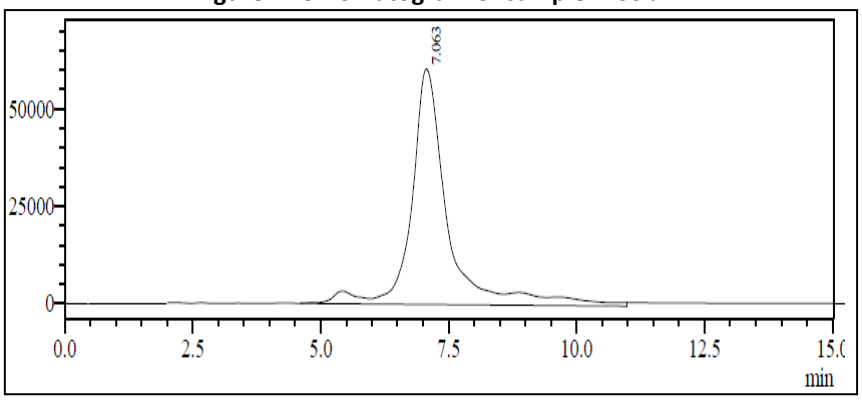

Figure 7: Chromatogram of sample- 120

Table 4: Accuracy Data

S.NO	Sample	Area of Spiked	% Recovery	Mean% Recovery	Acceptance criteria
1	80 %	1912992	102.9		
2	80 %	1914201	103.0	102.9	
3	80 %	1911750	102.8		
4	100 %	2443561	100.4		% Recovery Should be In between 96 – 104 %
5	100 %	2445200	100.5	100.5	
6	100 %	2445397	100.5		
7	120 %	3010992	98.8		
8	120 %	2999496	98.4	98.5	
9	120 %	3000276	98.4		

4.1.5 Repeatability:

Standard Repeatability will be determined by Six multiple injection at 100% of nominal concentration. Repeatability was calculated by % RSD and it should be less than

Table No: 5 Repeatability Data

S.NO	Sample	Peak Area	Acceptance criteria
1	1	2449003	
2	2	2447227	
3	3	2448289	
4	4	2447038	
5	5	2448545	% RSD ≤ 2
6	6	2444636	
	STDEV	1578.13	
	AVG	2447456.6	
	%RSD	0.06	

4.1.6: Robustness:

It is a measure of its capacity to remain unaffected by small but deliberate variations in procedural parameters listed in the procedure documentation and indication of its suitability during normal usage.

Examples of typical variations in assay, impurities and dissolution method validation by HPLC are

1. Effect of variation in mobile phase composition.
2. Effect of variation in pH of mobile phase.
3. Effect of mobile phase flow rate.
4. Effect of variation in Temperature.

Table 6: **Robustness Data**

S.No	Parameter	Nonoxynol – 9		
		RT (min)	Asymmetry	Theoretical plate count
1	Standard	7.169	1.373	2732.315
2	Change in organic phase ratio-78%v/v	9.106	1.292	2511.394
3	Change in organic phase ratio-82%v/v	6.656	1.364	2837.121
4	Change in flow rate- 0.8 ml/ min	7.156	1.372	2729.596
5	Change in flow rate- 1.2 ml/ min	6.879	1.361	2736.705

4.1.7 ASSAY:

The following formula used for the calculation of percentage content

$$\text{Assay} = \frac{\text{Cs}}{\text{Ct}} * \frac{\text{Pt}}{\text{Ps}} * 100$$

Where,

Cs = Concentration of Standard solution

Ct = Concentration of Test solution

Pt = Peak Area of Standard solution

Ps = Peak Area of Test solution

Table 7: **Assay Data**

S.no	Solution	Conc.	Peak area	Peak area	Assay
1	Standard	1 mg/ml	2433419	2445200	99.58
2	Sample		2443561	2445397	99.86

ACKNOWLEDGEMENT:

The authors acknowledge Chalapathi institute of Pharmaceutical Sciences, Lam, Guntur, Andhra Pradesh for providing all the necessary facilities and infrastructure to carry out the research.

REFERENCES:

1) Douglas A Skoog, James Holler F. Principles of Instrumental Analysis, 5th ed.,
2) Frank A Settle. Handbook of Instrumental Techniques for Analytical Chemistry, Pearson ed., 2004.
3) Willard, Merritt, Dean and Settle. Instrumental Methods of Analysis, 7th ed., New Delhi: C.B.S publications.
4) Sethi P D. High Performance Liquid Chromatography Qualitative analysis of pharmaceutical formulations, 2nd ed., New Delhi: C.B.S publications; 1995.
5) Lunn G and Schmuff N R. HPLC Methods for Pharmaceutical Analysis, 4th ed., New York: Wiley; 1997.
6) Ahuja S, Michael W Dong. Hand Book of Pharmaceutical Analysis by HPLC, 6th ed., New Delhi: Elsevier; 2009.
7) Jeffery G H et al. Vogel's Textbook of Quantitative Chemical Analysis, 5th ed., England: Longman House; 1989.
8) McMaster C M. HPLC- A Practical User's Guide, 2nd ed., New Jersey: John Wiley & Sons; 2007.
9) Lloyd R Snyder, Joseph J Kirkland, Joseph L Glajesh. Practical HPLC Method Development, 2nd ed., 1997.
10) ICH–Guidelines Q2A. Validation of Analytical Procedures: Definition and terminology, (CPMP III/5626/94); Geneva, Switzerland; March 1995.
11) ICH–Guidelines Q2B. Validation of Analytical Procedures: Methodology, (CPMP/ICH/281/95); Geneva, Switzerland; November 1996.
12) ICH-Guidelines Q3B. Validation of Analytical Procedures: Methodology, Geneva, Switzerland; November 1996.
13) Validation of Analytical Methods and Procedures, www.labcompliance.com
14) Govind J Kher. Studies on Development of Analytical Method Validation and Applications for some Drugs, 2011.
15) ICH–Guidelines Q1A(R2). Stability Testing of New Drug Substances and Products, (CPMP/ICH/2736/99); Geneva, Switzerland; March 2003.

16) Shabir, Ghulam A.; Bradshaw, Tony K Determination of the Spermicides Nonoxynol - 9 in a Gel Pharmaceutical Formulation by RPLC. Turkish Journal of Pharmaceutical Sciences. 2011, Vol. 8 Issue 1, p43-51.
17) 3.1.2 Chhonker YS, Chand asana H, Kumar L, Sharma VL, Gupta G, Bhatta RS Quantitative determination of microbicide spermicide 'nonoxynol-9' in rabbit plasma and vaginal fluid using LC-ESI-MS/MS J Chromatogr B Analyt Technol Biomed Life Sci. 2014 Aug 15;965:127-32.
18) Bhimireddy Venkata Rami Reddy et al. Normal phase HPLC (NRP-HPLC) coupled with a photodiode array detector for the quantitative determination of (S)-enantiomer in API substance and as well as drug product. Journal of Pharmacy Research 2013; Vol. 7(6): 546-550.
19) Gary J. Beck , Dulcie Kossak simple, sensitive assay for the spermicide nonoxynol-9 in biological fluids by high-performance liquid chromatography J Pharm Sci. 1990 Nov;79(11):1029-31.
20) Walter BA1, Digenis GA 5. chromatographic (HPLC) analysis of oligomeric components of the spermicidal nonoxynol-9. Pharm Res. 1991 Mar;8(3):409-11.
21) Arambasić MB1, Zlatković V, Dimitrijević R, Ilić N Biological and chemical quality control of spermicidal raw material nonoxynol-9 6. Boll Chim Farm. 2002 Sep-Oct;141(5):343-7.
22) TIAN Wen-li,YANG Jiang-Shan, SONG Qing-yu Development of HPLC Method for Detection of Cleavage Reagent Nonoxynol-9 Chinese Journal of Biologicals, Year 2011 , Issue 12 , Page 1503-1506
23) Jun - Xian Zhang1,2, Ming - Ming Miao1, Xue - Mei Niu2, Su - Juan Wang1, Yi Han1, Ke - Qin Zhang2 and Cheng - Ming Zhang1 Simultaneous Quantitative Determination of Nonoxynol - 9, Acrylamide, Sorbic Acid and Sodium Cyclamate in Toothpaste by HPLC-MS/MS 8. Asian Journal of Chemistry 01/2015; 27(4):1194-1198.
24) Nawaporn Anantasinkul, Supawan Chongthamawat Determination of Nonoxynol-9 in Lubricant Extracted from Contraceptive by HPLC 9. Contraception. 1997 Apr;55(4):261-3.
25) Saad B1, Bari MF, Saleh MI, Ahmad K Simultaneous determination of preservatives (benzoic acid, sorbic acid, methylparaben and propylparaben) in food stuffs using high-performance liquid chromatography. Journal of Chromatography a Volume 1073, Issues 1–2, 6 May 2005, Pages 393–397
26) Jankana Burana-osot, Leelawan Arunsingkharat, Matsarin Naksuk, Supapan Naungnamjain and Thanapon Saetun Validation of a HPLC Method for the Determination of Benzoic Acid and Sorbic Acid in Noodles 2. Chiang Mai J. Sci. 2014; 41(2): 370-382
27) Gomaa, A.M., Amer, M.E, Attallah, E.R. and Abo Elhassan, A.F. Validation of analytical method for determination of sorbic acid and benzoic acid in juices and carbonated beverages 3. Journal of Applied Sciences Research; Mar2013, Vol. 9 Issue 3, p1472.
28) krishna prasad rai, sovita shrestha Benzoic Acid Residue in Nepalese Fruits and Vegetable Products 4. J. Food Sci. Technol. Nepal, Vol. 6 (110-113), 2010.
29) M. Madhu1, V. Sreeram, A. V. D. Nagendra kumar Validated RP-HPLC method for the determination of Benzoic acid in bulk and pharmaceutical formulation Pelagia Research Library 2014, 5(5):52-60.
30) Bahruddin Saada, Md. Fazlul Baria, Muhammad Idiris Saleha Simultaneous determination of preservatives (benzoic acid, sorbic acid, methyl paraben and propyl paraben) in foodstuffs Journal of Food Composition and Analysis Volume 20, Issues 3–4, May 2007, Pages 220–225.
31) Marsili NR1, Lista A, Fernandez Band BS, Goicoechea HC, Olivieri AC New method for the determination of benzoic and sorbic acids in commercial orange juices J Agric Food Chem. 2004 May 5;52(9):2479-84.
32) Ittipon Techakriengkrai, Ranee Surakarnkul Analysis of benzoic acid and sorbic acid in Thai rice wines Journal of Food Composition and Analysis 20 (2007) 220–225
33) http://www.drugs.com/mmx/nonoxynol-9.html
34) http://www.chemspider.com/Chemical-Structure.7843323.html
35) http://link.springer.com/article/10.2165/00003495-199652010-00008
36) http://www.ordainhealth.com/Marketing/ProductDetails.aspx?div=Intenz&ProdId=I22