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Original article

DETERMINATION OF THE SPERMICIDES NONOXYNOL-9 IN A GEL PHARMACEUTICAL FORMULATION BY RPLC

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Abstract

The purpose of the research described herein was to develop and validate a reversed-phase liquid chromatographic (RPLC) method for the commercially available spermicides nonoxynol-9 (1.90-2.10%, w/w) in a gel formulation. The chromatographic separation was achieved with methanol-water (83:17, v/v) as mobile phase through a Nucleosil Cyano column by UV detection at 289 nm. The calibration curve was linear over the concentration range of 0.05-0.35 mg/mL (R^2 = 0.9997). The recovery ranges were from 99.87-100.04% from a gel formulation. The mean percent relative standard deviation values for intra- and inter-day precision studies were less than 1.0%. The method was adequate sensitive with detection limit of 0.0065 µg/mL. The assay method remains unaffected by small but deliberate variations, thus it was robust.

Key words: *Spermicides, Nonoxynol-9, Gel formulation, RPLC, Method development, Method validation*

Spermisit Nonoxynol-9'un Farmasötik Jel Formülasyonlarinda RPLC ile Miktar Tayini

Bu çalışmanın amacı, spermisit içeren jel formundaki piyasa preparatlarında nonoxynol-9 (%1.90-2.10) miktar tayini için ters faz sıvı kromatografisi (RPLC) yöntemi geliştirmek ve valide etmektir. Kromatografik ayırım, "nucleosil cyano" kolon ve mobil faz olarak methanol-su (83:17, h/h) kullanılarak 289 nm'de UV detektörü yarmıyla gerçekleştirildi. Kalibrasyon doğrusu (R^2=0.9997) 0.05-0.35 mg/ml konsantrasyon aralığında elde edilmiştir. Jel formülasyonundan etkin maddenini geri kazanım değerleri %99.87-%100.04 aralığında yer almıştır.Gün içi ve günler arası kesinlik çalışmalarıyla elde edilen rölatif standart sapma yüzdelerinin ortalaması %1.01'den küçük bulunmuştur. Yöntem, 0.0065 µg/mL tayin sınırı ile yeterince hassas bulunmuştur. Miktar tayini küçük ama göze çarpan varyasyonlardan etkilenmemiş ve böylelikle yöntemin kesinliği doğrulanmıştır.

Anahtar kelimeler: *Spermisit, Nonoxynol-9, Jel formülasyonu, RPLC, Yöntem geliştirme, Yöntem validasyonu*

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INTRODUCTION

Nonoxynol-9 (nonylphenoxy-polyethylleneoxy-ethanol, $C_{33}H_{60}O_{10}$, (Figure 1) is a non-ionic surfactant and one of the most common active spermicidal ingredients in commercially available vaginal contraceptives. Women have used vaginal products in attempts to prevent pregnancy for centuries. Nonoxynol-9 is the only vaginal spermicides currently available in the United States (although other products are marketed in other countries). Products containing nonoxynol-9 have been available over the counter for nearly fifty years. Nonoxynol-9 is comprised of multiple oligomers due to its synthesis from nonyl phenol and ethylene oxide that vary in ethyleneoxide chain length and consequently in their molecular weights (1).

Nonoxynol-9 is used as multiple oligomers as the active component in various formulations ranging from gels to creams which are administered into the vaginal cavity. The basis of this compound activity is associated with the structural affinity of nonoxynol-9 for membrane lipids, which serves to cause rapid immobilization and cell death by disruption of sperm membrane integrity (2, 3). Consequently, frequent high dose usage of these contraceptive agents have been reported to have the same effect within the female tract, causing local lesions of the cervicovaginal epithelium and a burning sensation of the genitals of either one or both partners (4). Furthermore, a recent survey has shown that the existence of such lesions can increase the rate of the Human Immunodeficiency Virus (HIV) infection (5-8).

In the literature only one analytical method for the determination of nonoxynol-9 in vaginal lavage fluid using normal-phase LC with bonded phase aminosilica column has been reported (9). The purpose of this study was therefore to develop a simple, sensitive, precise and robust reversed-phase liquid chromatography (RPLC) method for the determination of nonoxynol-9 in gel formulation.

Analytical method development and validation is an important part of analytical chemistry and plays a major role in the discovery, development, and manufacture of pharmaceuticals. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency and performance of drug product 'quality' essential for drug safety and efficacy. RPLC is the analytical method of choice in pharmaceutical analysis because of its specificity and sensitivity. Finally, the developed analytical method was validated to assess the validity of research data means determining whether the method used during the study can be reliable to provide a genuine, account of the intervention being evaluated. As a best practice (10-14), in the subsequent investigation, the new RPLC method was fully validated on the basis of International Conferences on Harmonization (ICH) Q2 (R1) (15) guideline.

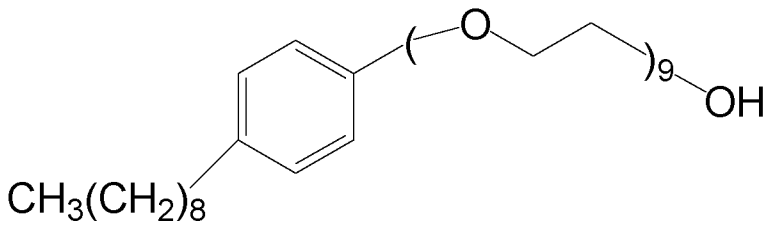

Figure 1. Chemical structure of nonoxynol-9.

EXPERIMENTAL

Chemical and reagents

Methanol (HPLC-grade) and nonoxynol-9 were obtained from Sigma-Aldrich (Gillingham, UK). Distilled water was de-ionised by using a Milli-Q system (Millipore, Bedford, MA).

LC instrumentation and conditions

The Knauer LC system (Berlin, Germany) equipped with a model 1000 LC pump, model 3950 autosampler, model 2600 photodiode-array (PDA) detector and a vacuum degasser was used. The data were acquired via ClarityChrom Workstation data acquisition software. RPLC analysis was performed isocratically at 30°C using a Nucleosil CN (150 x 4.6 mm, 10 μm) column (Jones Chromatography, Hengoed, UK). The mobile phase consisted of a mixture of methanol/water (83:17, v/v) was used. The flow rate was 1.0 mL/min and injection volume was 10 μL. The eluent was monitored with a UV detector at 289 nm. The mobile phase was filtered through 0.45 μm membrane filter and continuously degassed with on-line degasser.

Standard preparation

Nonoxynol-9 (0.2 g) was accurately weighed and added to a 100 mL volumetric flask and dissolved in a HPLC grade methanol (stock solution 1). A 10 mL aliquot of stock solution 1 was diluted to 100 mL in the mobile phase, yielding a final concentration of 0.2 mg/mL.

Sample preparation

An accurately weighed amount (1.0 g) of nonoxynol-9 sample gel was dissolved in 100 mL mobile phase. The sample was filtered through a sample filtration unit (0.45 μm) and injected into the LC system.

RESULTS AND DISCUSION

Method development

Initially, two analytical columns were studied in order to reach an acceptable separation, thus specificity and selectivity. Initially, Lichrosorb C_8 (150 x 4.6 mm, 5 μm) column was exploited. The Lichrosorb column gave a poor peak shape with high tailing value (3.44) and longer retention time as 5.35 min (Figure 2a) for the analyte peak. Nucleosil CN column (150 x 4.6 mm, 10 μm) produced a peak with superior band shape (tailing value 1.05) and column efficiency with much shorter retention time (2.03 min) (Figure 2b) under the same analytical conditions given in Section LC instrumentation and conditions

Tests were performed at different temperature between 25°C and 35°C at 5°C steps, to study the influence of this parameter. The results showed that the variation of the temperature between 25°C and 35°C did not significantly affect any of the chromatographic parameters and only decreased the retention time of the analyte so 30°C was selected as the working temperature.

The choice of wavelength is essential to accomplish a sensitive chromatographic assay. The optimal wavelength for nonoxynol-9 detection was established using the scan range of 200 to 400 nm. It was established that 289 nm was the optimal wavelength to maximize the signal.

Robustness testing was performed during the method development phase to optimize final RPLC conditions. A RPLC method must prove to be able to remain unaffected by small deliberate variations in method parameters, thus showing its own reliability during normal usage. In this study, robustness of the method was evaluated by the analysis of nonoxynol-9

under different experimental conditions such as deliberated changes in the composition of the mobile phase, column temperature and flow rate. The percentage of methanol in the mobile phase was varied ±5%, the column temperature was varied ±5°C and, the flow rate was varied ±0.2 mL/min. Besides, the method was applied using different lots of Nucleosil CN columns. Their effects on the retention time (t_R), tailing factor (T), and theoretical plate numbers (N) were studied as shown in Table 1.

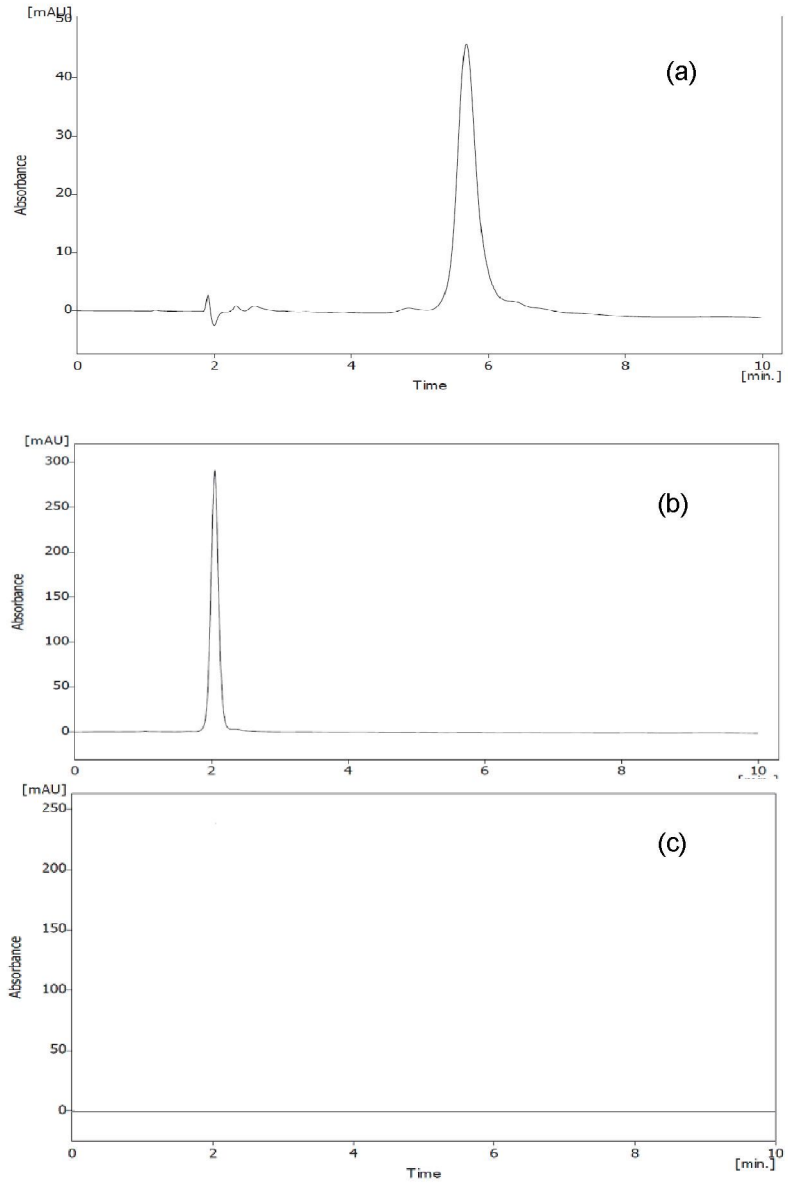

Figure 2. RPLC chromatogram obtained from injections of (a) using Lichrosorb C_8 (150 x 4.6 mm, 5 µm) column; (b) Sample using Nucleosil CN (150 x 4.6 mm, 10 µm) column; and (c) placebo sample using Nucleosil CN (150 x 4.6 mm, 10 µm) column.

Table 1. Robustness data of the developed RPLC method.

Method parameter	Modification	t_R (min)	T	N
	78	2.09	1.25	6786
Methanol (%)	83	2.03	1.00	6745
	88	2.00	1.05	6648
	0.8	2.08	1.13	6835
Flow rate (mL/min)	1.0	2.00	1.16	6743
	1.2	1.98	1.12	6794
	25	2.01	1.12	6638
Temperature (°C)	30	2.03	1.05	6746
	35	2.00	1.25	6642

Method validation

The method was fully validated (15) with respect to linearity, range, accuracy, precision, specificity, limits of detection, limit of quantitation and stability of analytical solutions.

Linearity and range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample. Linearity graph was established in the range of 0.05-0.35 mg/mL, at the concentrations of 25, 35, 50, 75, 100, 125, 150, and 175%, of the theoretical concentration in the test preparation, n = 3). Standard solutions of nonoxynol-9 were prepared by serial dilution in mobile phase to yield concentrations of 0.05, 0.07, 0.10, 0.15, 0.20, 0.25, 0.30, and 0.35 mg/mL. For calibration, weighed linear regression analysis was applied. The calibration graph established was linear (r^2 = 0.9997) within the tested range of 0.05-0.35 mg/mL (Table 2).

Table 2. Linearity assessment of the RPLC method for the assay of nonoxynol-9.

Concentration (mg/mL)	Concentration as percent of 0.20 mg/mL of nonoxynol-9	Peak area (mAU.s)	RSD (%)	n
0.050	25	897	0.26	3
0.070	35	1302	0.47	3
0.100	50	1838	0.49	3
0.150	75	2769	0.65	3
0.200	100	3702	0.28	3
0.250	125	4655	0.32	3
0.300	150	5638	0.25	3
0.350	175	6669	0.62	3
Determination coefficient (r^2): 0.9997				
Equation for regression line: y = 18973x – 46.663				

Stability of analytical solutions

The stability of nonoxynol-9 samples and standard solutions containing 0.20 mg/mL of nonoxynol-9 were chromatographed immediately after preparation and then re-assayed after storage at room temperature for 48 h. Solutions stored in a capped volumetric flasks on a

laboratory bench under day light for 48 h, and were stable with no significant change (<1% response factor) in nonoxynol-9 concentration over this period (Table 3).

Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day variation). The repeatability (intra-day precision) of the method was evaluated by assaying six replicate injections of the nonoxynol-9 at 100% of test concentration (0.2 mg/mL). The %RSD of the retention times (min) and areas of nonoxynol-9 peak were found to be less than 0.14% (Table 3).

Intermediate precision (inter-day variation) was demonstrated by two analysts using two LC systems and evaluating the relative peak area percent data across the two LC systems at three concentration levels (75%, 100%, and 125%) that cover the assay method range (0.05-0.35 mg/mL). The mean and %RSD across the systems and analysts were calculated from the individual relative percent peak area mean values at the 75%, 100%, and 125% of the test concentration. The %RSD values for both instruments and analysts were less than 0.26% (Table 3) and illustrated the good precision for the analytical method.

Table 3. Method validation results of nonoxynol-9.

Validation step	Parameters	Acceptance criteria*	Results
Standard stability	% change in response factor	< 2%	0.09
Sample stability	% change in response factor	< 2%	0.11
Repeatability	t_R (min) %RSD	< 2%	0.09
(n = 6)	Peak area %RSD		0.13
Intermediate precision	Instrument %RSD	< 2%	0.22
(n = 6)	Analyst %RSD	< 2%	0.25
LOD	Signal-to-noise ratio	S/N = 3:1	(s/n = 3.2), 0.0065 µg/mL
LOQ	Signal-to-noise ratio	S/N = 10:1	(s/n = 10.2), 9.40 µg/mL
System suitability	Peak area %RSD	< 2%	0.12
(n = 10)	Retention times (min) %RSD	< 2%	0.09
	Tailing factor	≤ 2	1.11
	Theoretical plates	> 2000	6758

*References 11, 16

Accuracy/recovery studies

The accuracy of the method was evaluated by means of recovery assay, adding known amounts of nonoxynol-9 reference standard to a known amount of gel formulation in order to obtain three different levels (75%, 100%, and 125%) of addition. The samples were analysed and the mean recovery was calculated. The data presented in Table 4 shows the recovery of nonoxynol-9 in spiked samples met the evaluation criteria for accuracy (100 +/-2.0% over the range of 80 to 120% of target concentration).

Table 4. Recovery studies of nonoxynol-9 from samples with known concentration.

Sample #	Percent of nominal	Amount of nonoxynol-9 (μg/mL)		Recovery (%, n = 5)	Bias%	RSD (%, n = 3)
		Added	Obtained			
1	75	150	149.8	99.87	0.13	0.18
2	100	200	199.8	99.90	0.10	0.22
3	125	250	250.2	100.08	-0.08	0.29
Mean				99.95		

Specificity

The RPLC-PDA/UV purity isoplot chromatogram was produced and results demonstrated a good separation of the nonoxynol-9. The isoplot chromatogram data consist of PDA UV/Vis absorption spectra from 200 to 400 nm for each point along the chromatogram. Injections of the extracted placebo were also performed to demonstrate the absence of interference with the elution of the nonoxynol-9. These results demonstrate (Figure 2c) that there was no interference from the other materials in the gel formulation and, therefore confirm the specificity of the RPLC method.

The forced degradation studies were performed to evaluate the specificity of nonoxynol-9 under four stress conditions (heat, UV light, acid, base). Solutions of nonoxynol-9 were exposed to 60°C for 1h, UV light using a UVL-56 lamp for 24h, acid (1M hydrochloric acid) for 24h and base (1M sodium hydroxide) for 4h. A summary of the stress results is shown in Table 5. No significant degradation was observed under any stress conditions studied and therefore confirm the specificity of the RPLC method.

Table 5. Summary of forced degradation studies.

Stress conditions	Sample treatment	Concentration (mg/mL)	t_R (min)	Assay (%)	Area (mAU s)
Reference	Fresh solution	0.2	2.03	99.6	2414.18
Acid degradation	1M HCl for 24 h	0.2	2.05	100.0	2417.23
Base degradation	1M NaOH for 4 h	0.2	2.05	98.9	2346.14
Heat degradation	60°C for 1 h	0.2	1.97	98.8	2376.22
Light degradation	UV Light for 24 h	0.2	2.00	99.9	2402.38

Limits of detection and quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) of nonoxynol-9 was determined based on standard deviation (σ) of response and slope (s). Nonoxynol-9 solutions were prepared in the range 0.004-150 μg/mL and injected in triplicate. Average peak area of analyte was plotted against concentration. LOD and LOQ were calculated by using the following equations:

$LOD = (3.3\ \sigma)/s$
$LOQ = (10\ \sigma)/s$

The LOD was (S/N ratio 3.2) 0.0065 μg/mL and LOQ was (S/N ratio 10.2) 9.40 μg/mL for nonoxynol-9 with %RSD less than 0.24% for six replicate injections (Table 3).

System suitability test

A system suitability testing was performed by injecting six replicate injections of a solution containing 0.2 mg nonoxynol-9/mL. The RSD of the peak area responses and retention time (min) were measured, giving an average of 0.12% for areas and 0.09 for retention times (n = 10). The tailing factor (T) for each nonoxynol-9 peak was 1.11, the theoretical plate number (N) was 6758, and the retention time (t_R) variation was less than 0.09% for six replicate injections as shown in Table 3. The RPLC method met these requirements within the accepted limits (11, 16).

CONCLUSION

This is the first RPLC method developed and validated for the determination of nonoxynol-9 in gel formulation using UV detection. A simple isocratic mobile phase was used without adding buffer. Sample preparation and analytical procedure run times are short (<3 min). The proposed method for its validity was validated and results showed excellent linearity (R^2 = 0.9997), good precision (RSD < 1.0%) and excellent recovery (>99.8%). This proposed validated robust method can be reliably used in routine analysis in quality control for release of raw materials, bulk gel samples and finished products congaing nonoxynol-9 compound.

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