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Electrospray and MALDI Mass Spectrometry in the Identification of Spermicides in Criminal Investigations

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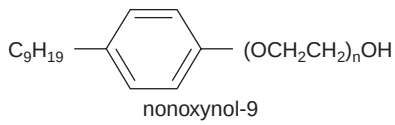
ABSTRACT: Electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry have been used to examine evidence in a sexual assault investigation. Because condoms are being used increasingly by sexual assailants and some condom brands include the spermicide nonoxynol-9 (nonylphenoxy polyethoxyethanol) in the lubricant formulation, the recovery, and identification of nonoxynol-9 from evidence items may assist in proving *corpus delicti.* A method was developed for the recovery of nonoxynol-9 from internal vaginal swabs and for its identification by reverse phase liquid chromatography/electrospray ionization mass spectrometry (LC ESI-MS), nanoelectrospray ionization (nanoESI) mass spectrometry, and high resolution MALDI Fourier transform mass spectrometry (MALDI-FTMS). The method was tested on extracts from precoitus, immediate postcoitus, and four-hours postcoitus vaginal swabs provided by a volunteer whose partner does not normally use condoms, but for this trial used a condom having a water-soluble gel-type lubricant that includes 5% nonoxynol-9 in its formulation. Subsequently, LC ESI-MS was used to identify traces of nonoxynol-9 from the internal vaginal swab of a victim of a sexual assault.

KEYWORDS: forensic science, nonoxynol-9, spermicide, condoms, sexual assault, LC, mass spectrometry, LC/electrospray MS, matrix-assisted laser desorption/ionization Fourier transform mass spectrometry (MALDI-FTMS), electrospray ionization mass spectrometry (ESI-MS), and nanoelectrospray ionization (nanoESI) mass spectrometry

Mass spectrometry has become an integral part of biological research primarily due to the establishment of matrix-assisted laser desorption/ionization (MALDI) (Fig. 1) and electrospray ionization (ESI) (Fig. 2). Both MALDI and ESI have greatly advanced our ability to analyze thermally labile molecules by providing an efficient means of generating intact, gas phase ions. Most significantly, MALDI and ESI have been used to gain molecular weight information on biological samples with unprecedented speed, accuracy, and sensitivity. Recent developments in instrumentation, along with new sampling methods have not only allowed for higher levels of sensitivity, increased mass range and better mass accuracy, but have also led to an increasing number of mass spectrometry-based applications (1). In these experiments we have applied these new mass spectrometry approaches to examine the spermicide, nonoxynol-9, to assist in a sexual assault investigation.



nonoxynol-9

Nonoxynol-9, a polyethoxylated nonylphenol, is a mixture of ethoximers with the molecular weight of each ethoximer being 44 amu [the weight of the ethoxy group, (—OCH_2CH_2—)] greater or lesser than its neighbors in the homologous series. Nonoxynol-9 is used in numerous products for vaginal insertion and is also a component of the lubricant formulation used in many brands of latex condoms. The recovery and identification of traces associated with condom use can provide important evidence in sexual assault cases (2–8).

Nonoxynol-9, whose most common trademark name is Igepal® CO-630, is prepared by the reaction of nonylphenol with ethylene oxide (9). The length of the alkyl chain is the same for all ethoximers, with the only difference being the length of the polyoxyethylene chain. The average number of ethylene oxide groups (the *n* value) is indicated by the number following nonoxynol. In various weight ranges (average *n* values), nonoxynols have many commercial uses as surfactants, detergents, emulsifiers, wetting agents, etc. (9). Neat nonoxynol-9 is a clear, colorless, viscous liquid which is a harsh detergent and would be very irritating to mucous membranes, so on latex condoms nonoxynol-9 is usually around 5 to 10% of the total lubricant formulation.

Octoxynol-9 (octylphenoxy polyethoxyethanol) is also used as a spermicide in some commercial products. Having just eight carbons on its alkyl chain, the various octoxynol ethoximers would be 14 amu (the weight of the methylene group, —CH_2—) less than the corresponding nonoxynol ethoximers.

A thin layer chromatography (TLC) method for nonoxynol-9 detection has been reported (10), but it is not reliable for forensic science purposes. Since nonoxynol-9 is a mixture of a wide range of ethoximers rather than a compound with a single molecular weight a broad streak, not a compact spot, is obtained from TLC analysis. Nonionic surfactants are found in many commercial laundry and other household products. By this TLC method, detergent residues extracted from evidence items such as clothing and bedding might be mistaken for nonoxynol-9.

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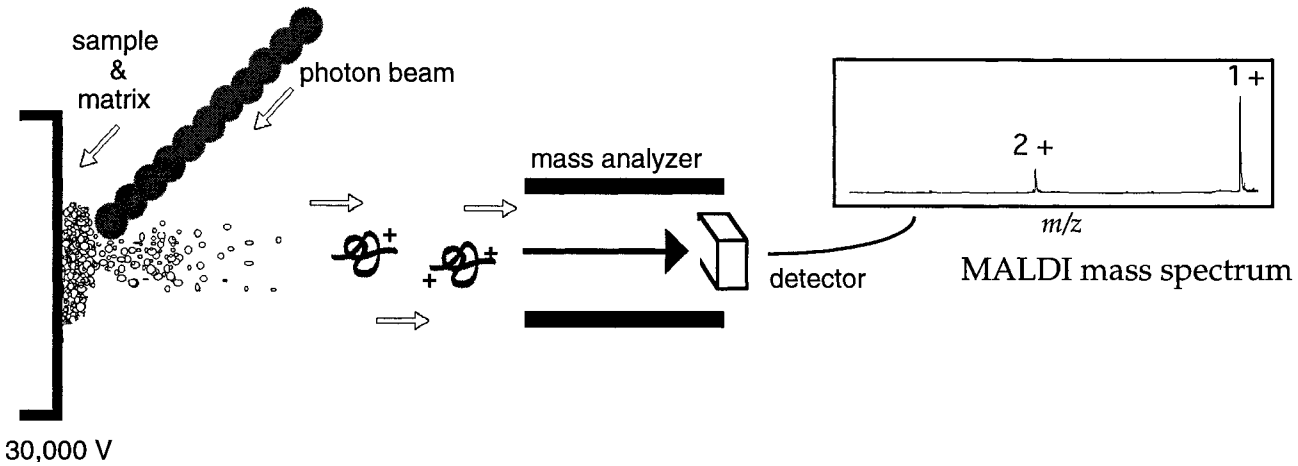

FIG. 1—*An illustration of matrix-assisted laser desorption ionization (MALDI). Gas phase ions are generated by the laser vaporization of a solid matrix/analyte mixture in which the matrix (usually a small crystalline organic compound) strongly absorbs the laser radiation and acts as a receptacle for energy deposition. This concentrated energy deposition results in the vaporization and ionization of both matrix and analyte ions.*

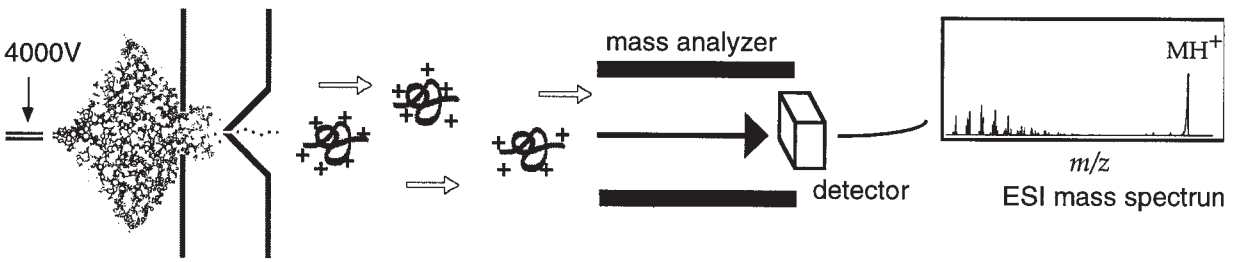

FIG. 2—*An illustration of electrospray ionization (ESI). ESI generates ions directly from solution (usually an aqueous or aqueous/organic solvent system) by creating a fine spray of highly charged droplets in the presence of a strong electric field. Subsequent vaporization of these charged droplets results in the production of gaseous ions.*

Methods for the extraction of nonoxynol-9 and its identification by Fourier transform infrared spectroscopy (FT-IR) and also by desorption chemical ionization mass spectrometry (DCI/MS) have been reported by Blackledge and Vincenti (3). However, the FT-IR method first involves the separation of nonoxynol-9 from a silicone oil-type condom lubricant; its separation from water-soluble, gel-type lubricants would be more difficult. Also, in actual evidence samples the quality of the FT-IR spectra might not be sufficient to unambiguously distinguish it from detergent residues having similar structures. DCI/MS was able to identify both the nonoxynol-9 and the silicone oil lubricant (polydimethylsiloxane), but very few forensic laboratories have access to the required instrumentation.

The silicone oil-type lubricant used in many condom brands is not water soluble. Its traces tend to remain in the vaginal vault and have been successfully recovered and identified as long as 24 h after intercourse (4). Also, it is a very strong absorber in the mid-range infrared spectral region and can be identified with FT-IR instrumentation found in most forensic laboratories (4).

Many condom brands employ a "natural" or water-soluble gel-type lubricant. Propylene glycol [$CH_3CHOHCH_2OH$ or (1,2-propandiol)], is the main ingredient in the gel used by one of the largest condom manufacturers. As part of the lubricant formulation, some brands also contain the spermicide nonoxynol-9 at 5 to 10% of the lubricant formulation. Propylene glycol can easily be identified using electron ionization (EI) with the gas chromatograph/mass spectrometer (GC/MS) instrumentation commonly found in forensic laboratories. However, propylene glycol is rapidly absorbed from the vaginal vault and within an hour or two after coitus its recovery and identification from internal vaginal swabs is unlikely. Nonoxynol-9 has a much higher molecular weight and both hydrophobic (the alkyl chain) and hydrophilic (the ethoxylate chain) groups. Its recovery and identification should be more promising, though it is not sufficiently volatile for analysis by GC/MS without derivitization. Soft ionization mass spectrometry methods, such as ESI and MALDI, which primarily produce molecular ions, generate spectra with polymers that are more readily interpreted than hard ionization methods (EI) that produce more fragmentation.

Since volatility is not required for liquid chromatography (LC), this instrumentation was considered for the analysis. However, use of ultraviolet detection alone to match retention times of the nonoxynol-9 standard to an evidence sample were considered to be potentially unspecific and insensitive for trace analysis. Therefore, the method of high-performance liquid chromatography/electrospray ionization (ESI) mass spectrometry (LC/MS), a comparatively soft ionization method with good sensitivity (<10 pmol/μL), was investigated. Normal phase LC would likely give a better separation of the various nonoxynol-9 ethoximers (11), but the solvents used in normal phase LC (i.e., hexane) are not routinely com-

patible with some types of ESI instruments (i.e., those utilizing a heated capillary). With normal phase LC of alkylphenol ethoxylates, both the alkyl groups and the polyethoxylate groups contribute to the possible separation of the various ethoximers. With reverse phase LC, where protic solvents are used in the mobile phases, only the alkyl groups contribute to the separation. The various nonylphenol ethoxylates all elute together regardless of the length of the polyethoxylate groups. For detection and identification by mass spectrometry, separation of the various ethoximers is not necessary.

In addition to LC/MS, nanoelectrospray ionization (nanoESI) mass spectrometry and matrix-assisted laser desorption/ionization with a high resolution Fourier transform mass spectrometer (MALDI-FTMS) were performed to demonstrate their usefulness in trace analysis of spermicidal agents. NanoESI is a relatively new technique, offering high sensitivity with low sample volume requirements. The technique is accomplished by loading μL volumes of sample solution into a glass capillary needle. Various types of capillaries are available, sealed or non-sealed, and in multiple sizes with different types of coatings for different types of analyses. In the instance of sealed capillaries, the needle is opened by gently touching the closed end against the orifice plate of the mass spectrometer to produce an opening with a diameter of approximately 10 μm. Once opened, the needle is positioned in front of the orifice at a distance of 1–2 mm. The sample is then directed into the mass spectrometer by a gas-tight syringe that allows a static backing pressure to be applied. The resulting flow rate is approximately 20–30 nL/min (12), allowing microliter sample volumes to be examined for extended periods of time with a minimal amount of sample consumed. One microliter of sample can last up to 30 min depending on the pressure applied to the syringe.

Materials and Method

A nonoxynol-9 standard (USP Reference) in the form of a 1 mL sealed glass ampule was donated by Carter-Wallace, Inc., New York, NY. A Trojan-Enz® brand latex condom (Carter-Wallace, Inc.) containing a water-soluble, gel-type lubricant with 5% nonoxynol-9 was used by the partner of the volunteer who provided the control internal vaginal swabs. Swabs were 6 in. (15.2 cm) long wood with cotton tips (Puritan®, Hardwood Products Co., Guilford, ME).

Sample Preparation

A stock solution of nonoxynol-9 standard was prepared by diluting 5 mg of the neat standard with 1 mL methanol. Serial dilutions of the stock solution were made to concentrations of 10 ng/μL, 1 ng/μL, 500 pg/μL, 50 pg/μL, and 5 pg/μL. For LC method development, a methanol extract of a new Trojan-Enz® condom was made, and this extract was run in sequence with the nonoxynol-9 standard to optimize run conditions.

Control samples were obtained from a volunteer whose partner does not normally use condoms, but for this trial used a condom having a water-soluble gel-type lubricant that includes 5% nonoxynol-9 in its formulation. Internal vaginal swabs were taken pre-coitus (control 1), immediately postcoitus (control 2), and four hours postcoitus (control 3) from the volunteer. Extraction of residues from cotton-tipped swabs was accomplished by rinsing each with 500 μL dichloromethane, which was evaporated under nitrogen. The dried residues were re-diluted with 100 μL methanol.

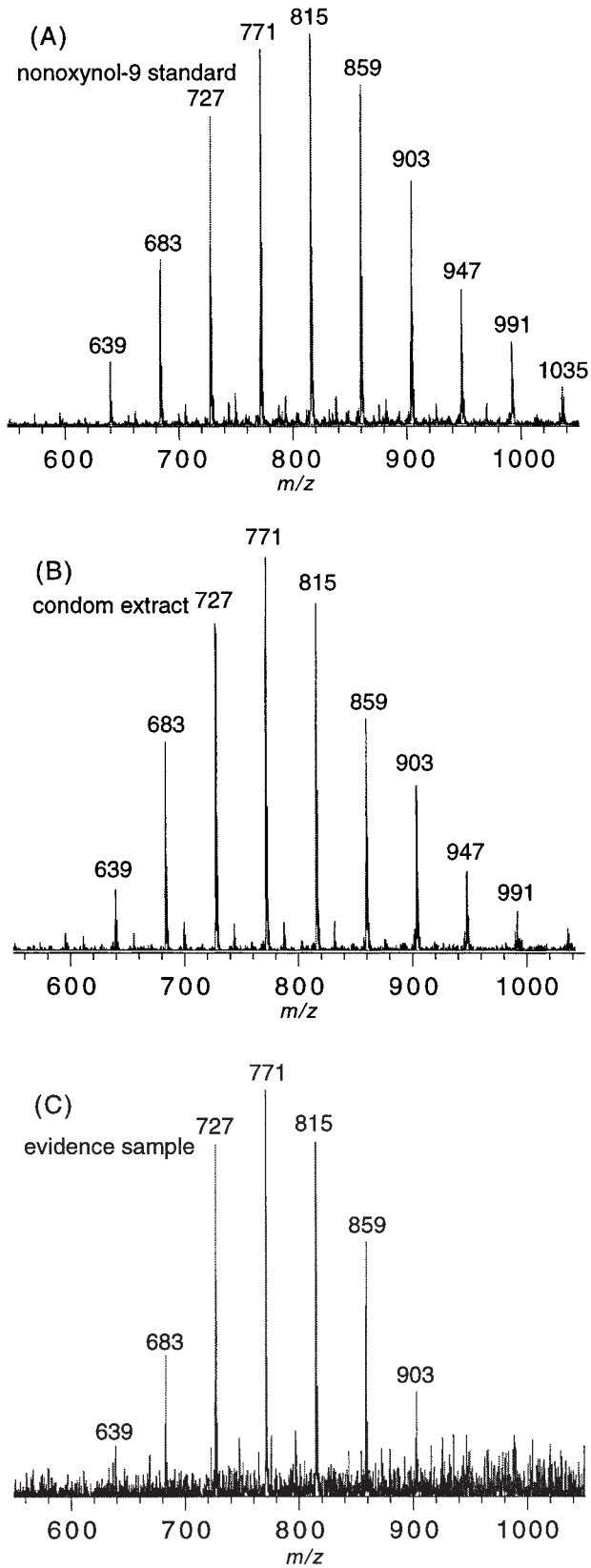

FIG. 3—*(A) Mass spectrum from LC/MS of nonoxynol-9 standard at 3 ng/μL. Average spectrum of 8–9 min. (B) Mass spectrum from LC/MS of nonoxynol-9 extracted from a Trojan-ENZ® latex condom. Average spectrum of 8–9 min. (C) Mass spectrum from LC/MS of evidence sample. Average spectrum of 9–10 min.*

In addition, an evidence swab taken between three and 4 h after a sexual assault was also prepared for analysis in the same manner as the controls.

LC/ESI-MS

Analysis of standard, control, and evidence samples was performed using a Hewlett-Packard 1090 Liquid Chromatograph (Avondale, PA) coupled with a Finnigan LCQ ion trap mass spectrometer (San Jose, CA). Positive ion-mode ESI was used with a capillary temperature of 220°C, capillary voltage of 13 kV, and spray voltage set at 4 kV. Nitrogen was used as the nebulizer and sheath gas. Ten microliter injections were made by autosampler onto a Zorbax SB-C_{18} rapid resolution 3.5 μm, 2.1 × 30 mm column. A gradient system of (A) 0.05% trifluroactic acid (TFA), and (B) methanol with 0.05% TFA was used with a flow rate of 150 μL/min. The gradient started with 75% B, and went to 85% B after 4 min, then to 100% at 9 min. UV was monitored at 214 nm.

NanoESI-MS

The control 2 sample and nonoxynol-9 standard were analyzed by a PE Sciex API III (Alberta, Canada) modified with a nanoESI source from Protana A/S (Denmark). Orifice setting was optimized at 100 V, with ESI voltage set at 650 V. Variation in orifice potential affected sensitivity but not ethoximer distribution. A curtain gas of ultrapure nitrogen was pumped into the interface at a rate of 0.6 L/min to aid evaporation of solvent droplets, and prevent particulate matter from entering the analyzer region. Desolvated ions entered the analyzer via the vacuum interface and were guided by the entrance optics. Normal-sized palladium coated, boro-silicate glass capillaries from Protana A/S were used for sample delivery.

MALDI-FTMS

The nonoxynol-9 standard was also analyzed using a HiResMALDI FTMS made by IonSpec Corp. (Irvine, CA). The instrument uses a 4.7-T superconducting magnet, nitrogen laser, and an RF-only quadrupole ion guide to transport ions from the external ion source to the FTMS analyzer (13). The data system, OMEGA, used to control instrument parameters and analyze Fourier transform signals, is also made by IonSpec Corp. Single microliters of samples were deposited on the instrument probe with 2,5-dihydroxybenzoic acid (150 mg/mL in MeOH) matrix.

Results and Discussion

Mass spectra taken by LC/MS of a 3 ng/μL nonoxynol-9 standard, an extract of the latex condom, and an extract of evidence

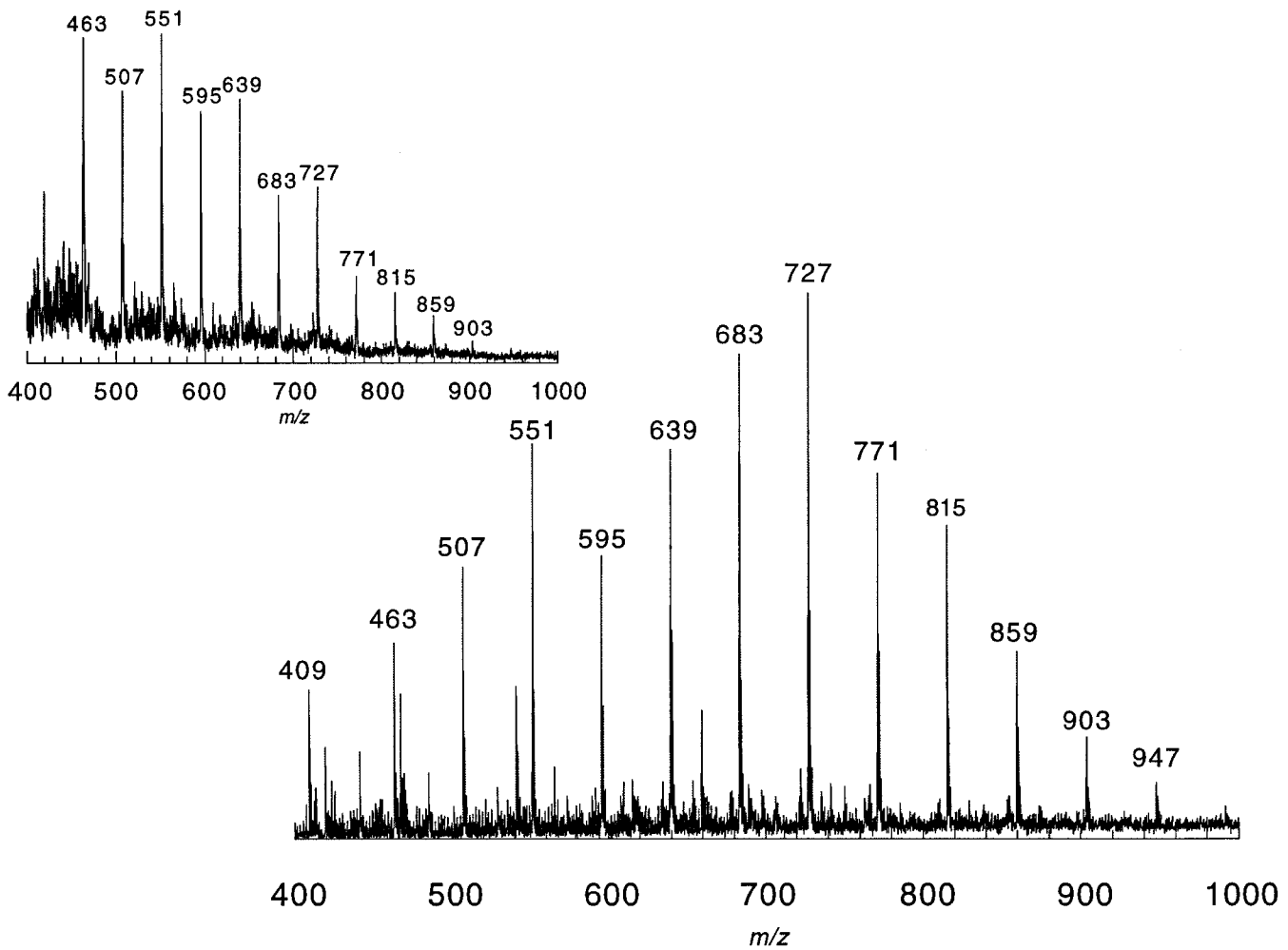

FIG. 4—*NanoESI mass spectrum of 1 ng/μL (1.6 pmol/μL) nonoxynol-9. Inset spectrum is the control 2 (postcoitus) sample by nanoESI.*

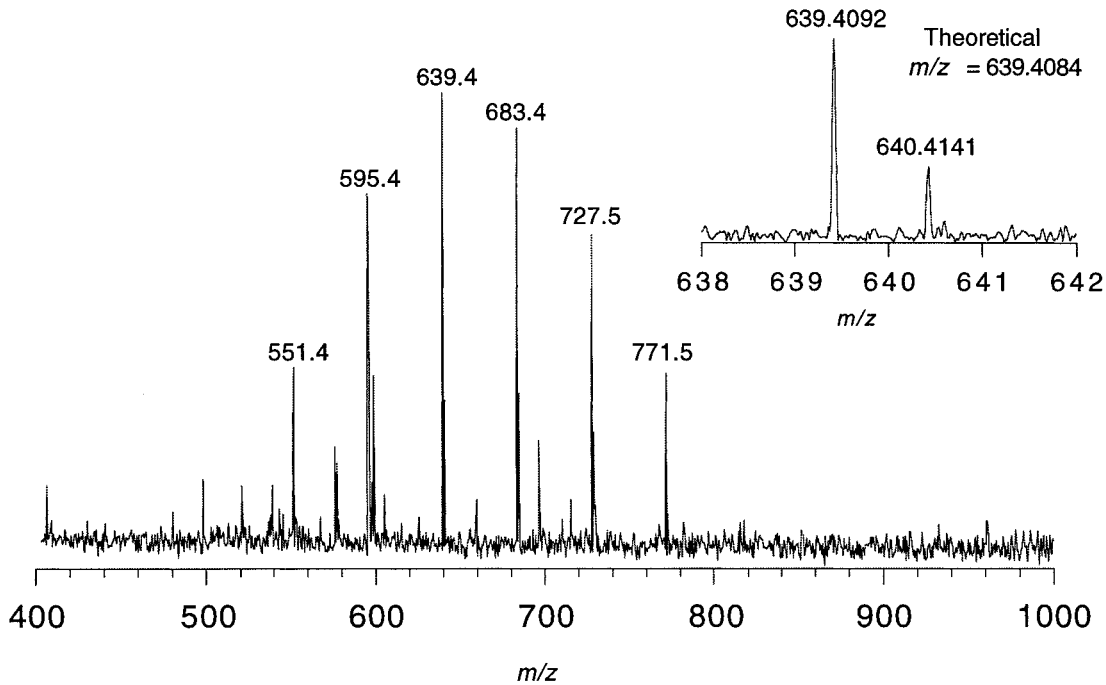

FIG. 5—*Mass spectrum from MALDI-FTMS of nonoxynol-9 standard at 4 ng/μL (8 pmol/μL). Inset spectrum shows the high resolution (14,500) of the spectrum, and exact mass determination with error of 1.2 ppm.*

swab are shown in Fig. 3a-c. Electrospray ionization MS primarily produces the sodium ion adducts of nonoxynol-9 (molecular weight of each ethoximer plus 23^+). This can be seen in the ion series: *m/z* 595 ($M_{n=8}$ + 23), *m/z* 639 ($M_{n=9}$ + 23), *m/z* 683 ($M_{n=10}$ + 23), etc. Previously, with DCI/MS, Blackledge and Vincenti (4) primarily obtained protonated molecular ions (molecular weight of each ethoximer plus 1^+). Not only does DCI/MS produce protonated molecular ions rather than Na^+ adducts, but the apparent ethoximer distribution is also lower. With DCI/MS the $n = 8$ ethoximer (573) is the most abundant, while with electrospray ionization MS it is the $n = 11$ (727) or $n = 12$ ethoximer (771). This could be due to a bias for more volatile (lower molecular weight) molecules in the DCI/MS method of ionization. However, nonylphenol ethoxylates with short polyethoxylate side chains have greater solubility in nonpolar solvents, while the solubility in polar solvents increases with increasing length of the polyethoxylate chain (11). Extraction of nonoxynol-9 residues with a relatively polar solvent such as methanol could produce a bias for ethoximers with longer polyethoxylate chains. Either method may be used for the detection of nonoxynol-9 traces just as long as the same method (and solvent) is used for questioned and known samples.

Under reverse phase LC conditions nonoxynol-9 appears to be easily retained in the system and traces can remain in the injection loop and/or column, which may be seen as background on subsequent runs. Therefore, although it is always good practice to run blanks between samples, it is especially important here. Perhaps a desirable protocol would be to first run blanks to assure the absence of low levels of nonoxynol-9 in the background; run a control that does not contain nonoxynol-9 but does contain a check substance that would give assurance that the LC/MS system is working correctly; then examine the evidence samples (blanks between each); and lastly run the nonoxynol-9 standard. Blanks are not needed for nanoESI, in which each sample is introduced to the mass spectrometer through a separate disposable glass capillary.

NanoESI is well-suited to trace analysis because of its high sensitivity, and low sample volume requirements. Additionally, the possibility of compromised results due to sample carryover from "sticky" compounds (such as nonoxynol-9) is eliminated because the samples do not all flow through the same source line, as with traditional ESI. Figure 4 shows the nanoESI spectra of the nonoxynol-9 standard and the control 2 sample (inset). The nonoxynol-9 standard is shown at 1.0 ng/μL (1.6 pmol/μL), although it was clearly detectable at half this concentration, 500 pg/μL (800 fmol/μL), though less well resolved. The control 3 sample (4 h postcoitus) was analyzed by nanoESI and MALDI-FTMS. Neither spectrum was well enough resolved to clearly show the presence of nonoxynol-9.

MALDI-FTMS is a technique which also offers good sensitivity with low requirements for sample volumes. Most importantly it can provide useful results through exact mass determination of compounds. In Fig. 5 the nonoxynol-9 standard is shown at 5 ng/μL (8 pmol/μL), with a single microliter of sample used in the analysis. The detection limit for the standard was 2.5 ng/μL (4 pmol/μL). The inset figure shows the high resolution of the spectrum (14,500) and exact mass determination with an error of 1.2 ppm. The control 2 sample was also analyzed and a few of the polyethoxylate peaks were detected. The spectrum is not included here.

Conclusion

LC/ESI-MS, nanoESI-MS, and MALDI-FTMS have been shown to be capable of identifying traces of the spermicide nonoxynol-9 from internal vaginal swabs taken postcoitus, and in an actual evidence sample. Although nonoxynol-9 traces could originate from other sources, various condom brands could at least

be included in the investigation. Investigator interviews with the victim and/or a survey of the victim's use of personal hygiene products may help to locate/eliminate other possible sources. Since their rapid absorption by the body cavity makes the recovery and identification of water-soluble lubricant components less likely, the recovery of nonoxynol-9 traces is especially important if a condom with a "natural" gel-type lubricant was used.

Acknowledgments

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