

CASE REPORT

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A Case Study on Forensic Polymer Analysis by DIOS-MS: The Suspect Who Gave Us the SLIP[®]*

ABSTRACT: New technology was used to identify traces of a commercial barrier/spermicide in evidence from a case of a man accused of rape of a minor. Examination of vaginal swabs performed by another laboratory had been negative for seminal fluid or other sources of DNA from the suspect and we were asked to examine the remaining swabs for any traces that might have originated from the commercial product. Encare[®] consists of vaginal inserts having a suppository-like shape. They contain the spermicide, nonoxynol-9, in a matrix consisting of approximately two parts polyethylene glycol (PEG) 1000 to one part PEG 1450, plus minor inorganic components added to produce foaming. Portions of the cotton from vaginal swabs from the victim and penile swabs from the suspect were extracted with methanol and subsequently examined by desorption ionization on silicon time-of-flight mass spectrometry (DIOS TOF MS). Low levels of PEG in the same mass range as Encare[®] were found on two separate vaginal swabs from the victim and one penile swab from the suspect. Subsequent to these findings, the suspect (through his attorneys) provided us with a sample of SLIP[®] Plus, a commercial sexual lubricant that also contains nonoxynol-9. Traces of PEG in the same mass range as Encare[®] were found in this sample, while no PEG was found in a sealed sample of SLIP[®] Plus provided by the manufacturer. At trial the suspect's attorneys stipulated that their client had added some Encare[®] to the SLIP[®] Plus sample he had provided.

KEYWORDS: forensic science, sexual assault, desorption ionization on silicon time of flight mass spectrometry, polyethylene glycol, nonoxynol-9

Because they facilitate the act, lubricants (1,2) have frequently been used in the past by sexual assailants. But with advances in DNA technology lubricant use without condoms has diminished due to general public knowledge one may be positively identified by the DNA present in their seminal fluid. However, as an alternative to condoms or other methods of birth control, there are available several commercial products that after insertion deep into the vagina followed by a wait of about 10 to 15 min provide a measure of contraceptive protection through the combination of a physical barrier and spermicidal action. Forensic laboratories may be asked to look for traces of these products on evidence items in cases of alleged child sexual abuse, or when the accused acknowledges that sex occurred but claims it was consensual.

Desorption ionization on silicon (DIOS) (3) is a new sample ionization technique for laser desorption-ionization mass spectrometry. Upon the advent of matrix-assisted laser desorption ionization time of flight [MALDI-TOF] mass spectrometry, high molecular weight molecules could be analyzed directly from small amounts of sample (4,5). MALDI-MS, however, relies upon the addition of

low molecular weight matrix compounds that often interfere with the analysis of small and moderate molecular weight analytes. For samples in the range of just a few thousand Daltons or less the matrix ions produce an interfering background that raises the minimum level of detection. With DIOS the analyte is deposited on a prepared silicon surface. The silicon acts as a well for the energy pulses from the laser, that in turn cause the desorption/ionization of the analyte without pyrolysis, fragmentation, or a background of matrix ions. Figure 1 illustrates typical sensitivity and mass ranges allowed by different ionization techniques. DIOS TOF MS has an added advantage in that it is tolerant of the contaminants typically found in forensic casework samples. The following case illustrates the method's impressive minimum levels of detection. The specificity of DIOS-MS for members of a homologous series is also compared with that of Fourier transform infrared spectroscopy (FTIR).

Case Summary

It was alleged a man had coerced his 14-year-old stepdaughter to have sex with him. It was also alleged that prior to sex he had provided her with a contraceptive commercial product, Encare[®]. This product (Fig. 2) is a vaginal insert that provides both a physical barrier and spermicidal action. Shaped much like a suppository and having a paraffin-like texture, prior to sex an individual insert is placed deep into the vagina. Product literature recommends a wait of 10 min before engaging in sex.

After examinations of some vaginal swabs from the victim by another laboratory had proved negative for seminal fluid or any other sources of the suspect's DNA, we were asked to look for traces of the Encare[®] product on the remaining swabs from the

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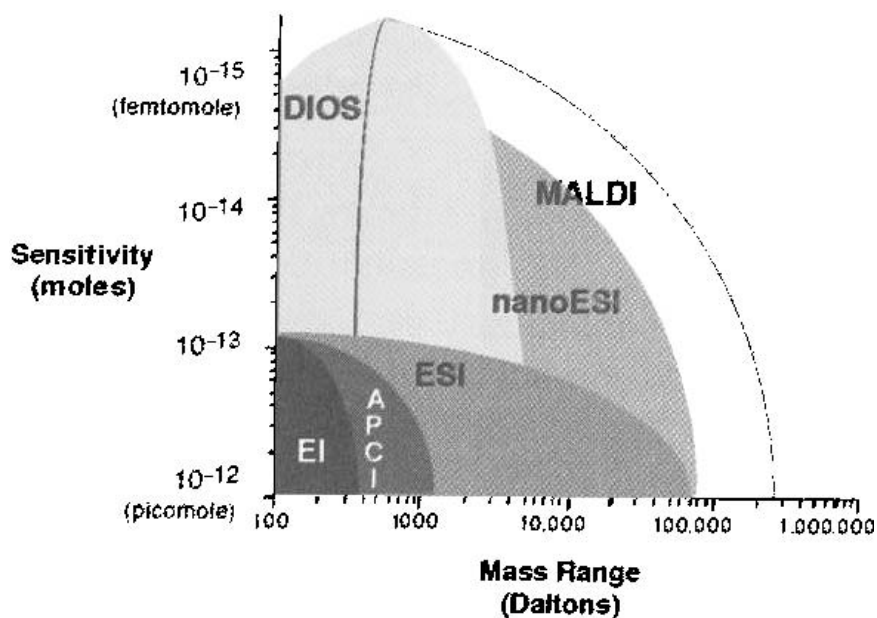


FIG. 1—A glance at the typical sensitivity and mass ranges allowed by different ionization techniques provides a clear answer to the question of which are the most useful; electron ionization (EI), atmospheric pressure chemical ionization (APCI) and desorption ionization on silicon (DIOS) are somewhat limiting in terms of upper mass range, while electrospray ionization (ESI), nanospray ionization (nanoESI), and matrix assisted laser desorption ionization (MALDI) have a high practical mass range.

*Used with permission from Fig. 1.5, p 14, *The Expanding Role of Mass Spectrometry in Biotechnology*, Gary Siuzdak, *The Scripps Research Institute*, MCC Press, San Diego, CA, 2003.



FIG. 2—Encare[®] vaginal contraceptive inserts box and below a strip containing six individual inserts.

victim as well as two penile swabs obtained from the suspect (the victim claimed that the accused had not worn a condom).

Materials and Methods

Chemicals

A nonoxynol-9 standard (IGEPAL[®] CO-630) in the form of a 1 mL sealed glass ampoule was donated by Carter-Wallace, Inc.,

New York, NY. PEG standards of 300, 1000, and 1500 average molecular weights and octoxynol-9 (IGEPAL[®] CA-630) were purchased from Sigma-Aldrich (Milwaukee, WI), and used without further purification. Encare[®] vaginal contraceptive inserts with nonoxynol-9 (Blair Labs; Columbus, IN) and Ramses[®] Extra Spermicidally Lubricated brand latex condoms (Schmid Laboratories; Sarasota, FL) were extracted into methanol/water solutions as described in the results. An Encare[®] insert was used by the female partner of a volunteer couple who provided the control vaginal

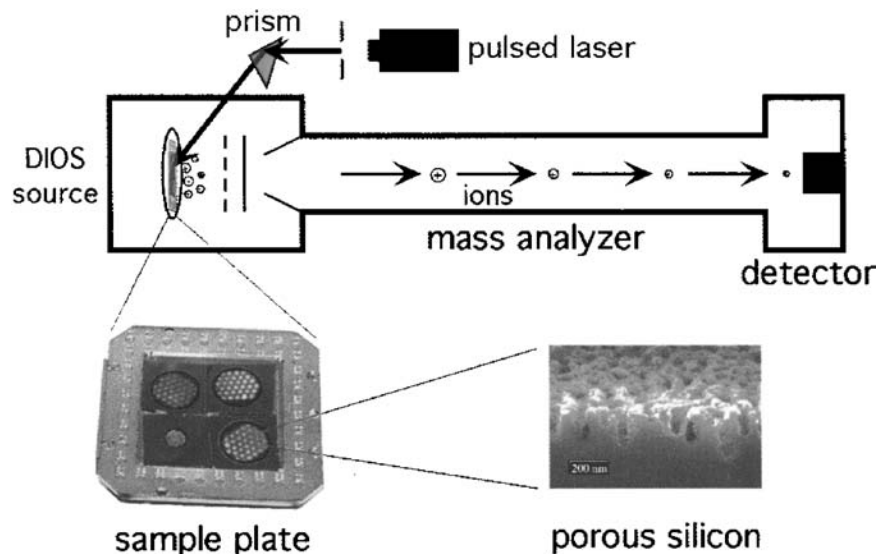


FIG. 3—DIOS surfaces are attached to the modified commercial MALDI sample plates. In the source, analyte ions are directly desorbed from the DIOS surface, and subsequently accelerated into the mass analyzer. The scanning electron microscopy figure (lower right) shows the typical morphology of the DIOS surfaces used in this analysis.

*Used with permission from Fig. 1, Thomas JJ, et al., *Analytica Chimica Acta* 442 2001;442:183–90, Elsevier Science.

swabs. Swabs were 6 in. (15.2 cm) long wood with cotton tips (Puritan®, Hardwood Products Co., Guilford, ME). Two sealed bottles of SLIP® Plus, Trimensa Pharmaceuticals, Newbury Park, CA, were donated by the manufacturer.

Biological Sample Protocol

Biological samples originated from evidence samples contained in victim and suspect sexual assault evidence kits. These are provided to hospitals and law enforcement agencies. They were stored at 4°C in a refrigerator located in a secure area. The only evidence samples examined were: Victim Kit: (a) a cotton swab contained in a cardboard box labeled “vaginal”; (b) a cotton swab contained in a glass test tube with red rubber stopper. Upon original examination of the victim this vaginal swab (b) had been used to prepare a microscope slide. Suspect Kit: (c) a cotton swab contained in a cardboard box labeled “urethral meatus”; (d) a cotton swab in a cardboard box labeled “glans/shaft.” As a control, a volunteer couple used an Encare® insert and vaginal swabs were obtained immediate post-coitus and 8 h post-coitus. The cotton was removed from the swabs with different disposable scalpels (Feather® sterile single-use disposable scalpels, Fisher Scientific, Pittsburgh, PA). The cotton was then placed in disposable glass test tubes, covered with a minimum amount of methanol and vortexed for a few seconds.

Preparation of Samples for Mass Spectrometry

For DIOS TOF MS, samples are pipetted onto silicon wafers contained in a modified sample plate for use in the TOF MS instrument. Samples are allowed to dry prior to their insertion in the instrument. The surface of the silicon is first prepared by etching with a 25% HF/ethanol solution under white light illumination for 1 min with a current density of 5 mA/cm². This was followed by rapid oxidation with ozone followed by chemical etching in 5% HF/ethanol for 1 min. After washing with ethanol the surfaces were ready for use. Details of DIOS chip preparation procedure with illustrations are available elsewhere (6), and prepared chips may now be obtained

commercially (7). More uniform sample distribution with minimal sample spreading occurs if the sample is dissolved in distilled water rather than organic solvents, so all methanol extracts were diluted with distilled water. Additionally, DIOS TOF MS is actually more amenable to low sample concentrations than high (8). Higher sample concentrations (i.e., millimolar concentrations) require higher laser intensities that lead to greater background noise and poorer resolution leading to poor sensitivity and lowered accuracy. Therefore, polymer standard samples were diluted approximately 1:2000 with deionized water, and methanol extracts of the cotton from the swabs from the volunteer couple were diluted (4x) with deionized water.

Mass Spectrometry

Figure 3 shows a schematic of the DIOS TOF MS setup. All DIOS TOF MS experiments were performed on a Voyager DE-STR, time-of-flight mass spectrometer (PerSeptive Biosystems, Framingham, MA) equipped with a pulsed nitrogen laser operated at 337 nm. More detailed instrument conditions are described in Ref 6.

Results and Discussion

One Encare® insert weighs 3.76 g. Of this, 1.82 g is PEG 1000 and 0.98 g is PEG 1450. Also present are 100 mg nonoxynol-9, and tartaric acid (these last three are added to produce foaming). It was fortuitous that the Encare® matrix consisted of PEG. It is a simple straight chain polymer with a repeat unit of 44 Da. (the mass of the ethoxylate group, $-\text{CH}_2\text{CH}_2\text{O}-$). Therefore under soft ionization conditions (promote the formation of molecular ions with minimal fragmentation) mass analysis of a mixture of PEG molecules will produce a series of peaks separated by 44 Da. PEG is often used in mass spectrometry as a calibration standard for mass accuracy and for determining minimum levels of detection, and is therefore very familiar to experienced practitioners.

The spermicide, nonoxynol-9, is an alkyl phenol ethoxylate. That is, attached to a benzene ring it has an alkyl chain of nine

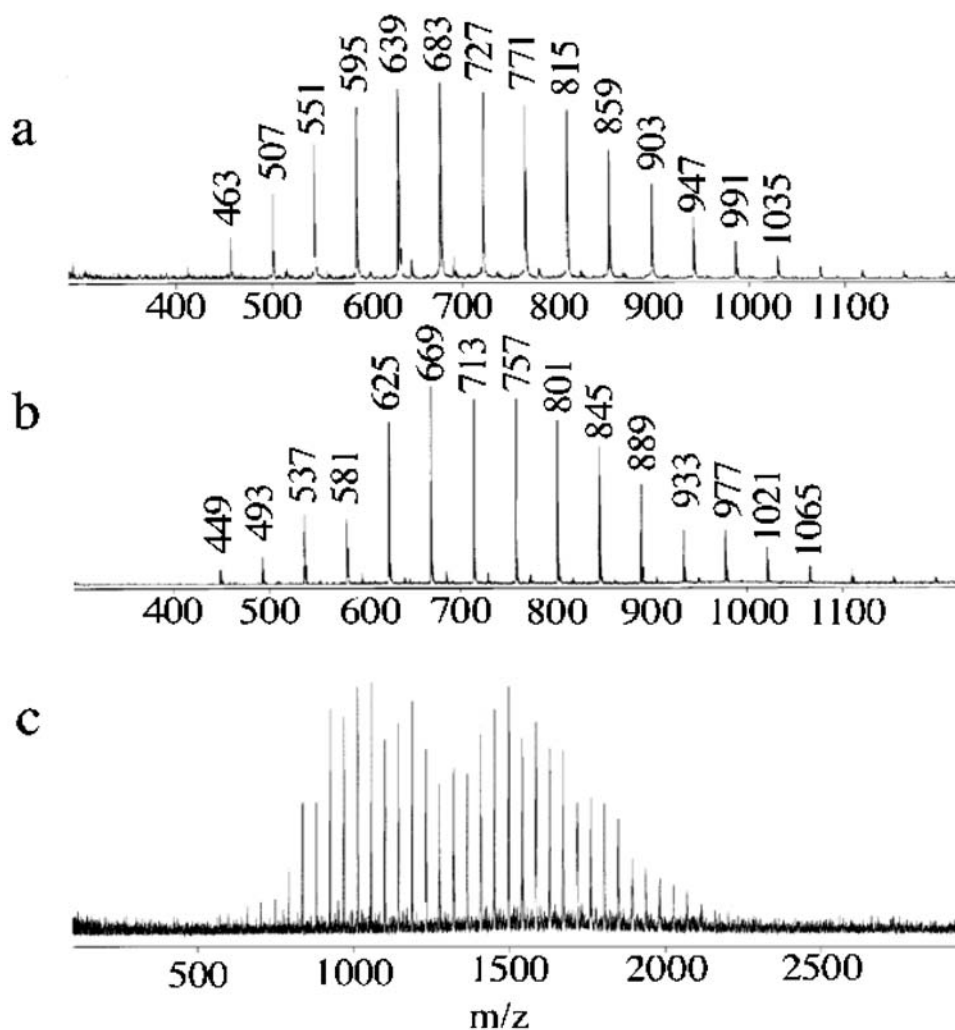


FIG. 4—DIOS-MS spectra of: (a) nonoxynol-9 (nonylphenylpolyethylene glycol); (b) octoxynol-9 (octylphenylpolyethylene glycol); and (c) a mixture of PEG 1000 and PEG 1500.

*Used with permission from Fig. 3, Thomas JJ, et al., *Analytica Chimica Acta* 2001;442:183–90, Elsevier Science.

carbons, and para to this is an ethoxylate chain. Commercial samples of nonoxynol-9 are mixtures having an average or most abundant ethoxylate chain length of nine ethoxy groups. So like PEG the mass spectrum of nonoxynol-9 will consist of a series of peaks each separated by 44 Da. However, due to the mass of the benzene ring with attached 9-carbon alkyl group, the molecular weights of the nonoxynol-9 series are easily distinguished from PEG. Although much less frequently encountered in commercial products, octoxynol-9 also finds use as a spermicide. Octoxynol-9 is just like nonoxynol-9 except that it has one less methylene in its alkyl side chain. Therefore, the molecular weights of the octoxynol-9 series will be 14 Da (the weight of a $-\text{CH}_2-$ group) less than the corresponding members of the nonoxynol-9 series.

Figures 4a and 4b show that DIOS TOF MS easily distinguished the nonoxynol-9 and octoxynol-9 series. Figure 4c shows the spectrum for a mixture of PEG 1000 and PEG 1500. Is PEG present in any other products for vaginal insertion? Vaginal contraceptive films consist of nonoxynol-9 in a matrix of a 2-in. by 2-in. sheet of plastic film. However, the film is made of polyvinyl alcohol, not PEG. Some condom brands utilize PEG as a liquid lubricant, but it is in a much lower mass range. Figure 5 shows the spectrum for the lubricant from a Ramses[®] Extra condom (contains PEG and nonoxynol-9). Clearly, the PEG present could not be confused with

that in the Encare[®] product. Figure 6 shows the results obtained from an immediate post-coitus swab when a volunteer couple used Encare[®]. Notice that any traces of nonoxynol-9 are buried among the peaks on the low mass side of the PEG matrix. Under the ionization conditions present in the DIOS TOF MS experiment all peaks (PEG and spermicides) are present as Na^+ adducts (molecular weight plus 23). Sodium adduct species are easily differentiated from potassium adducts ($\Delta m/z = 16$ Daltons) and polymers differing by one methylene group ($\Delta m/z = 14$ Daltons).

Figure 7a shows the DIOS TOF MS results from an extract from sample a (a deep vaginal swab from the victim). A series of peaks was obtained each separated by 44 Da and with molecular weights corresponding to PEG in the same mass range as Encare[®]. The second deep vaginal swab from the victim, sample b, was also positive for PEG in the same mass range but at lower concentration levels (spectrum not shown). Figure 7b shows the results from an extract from sample d (penile swab of suspect, glans and shaft). Albeit at lower concentration levels, it too is positive for PEG in the same mass range as Encare[®]. Extracts of a second penile swab (urethral meatus) from the suspect, sample c, were negative for any detectable PEG traces.

The suspect initially denied having ever used Encare[®] when he engaged in sex. However, subsequent to our findings, he claimed

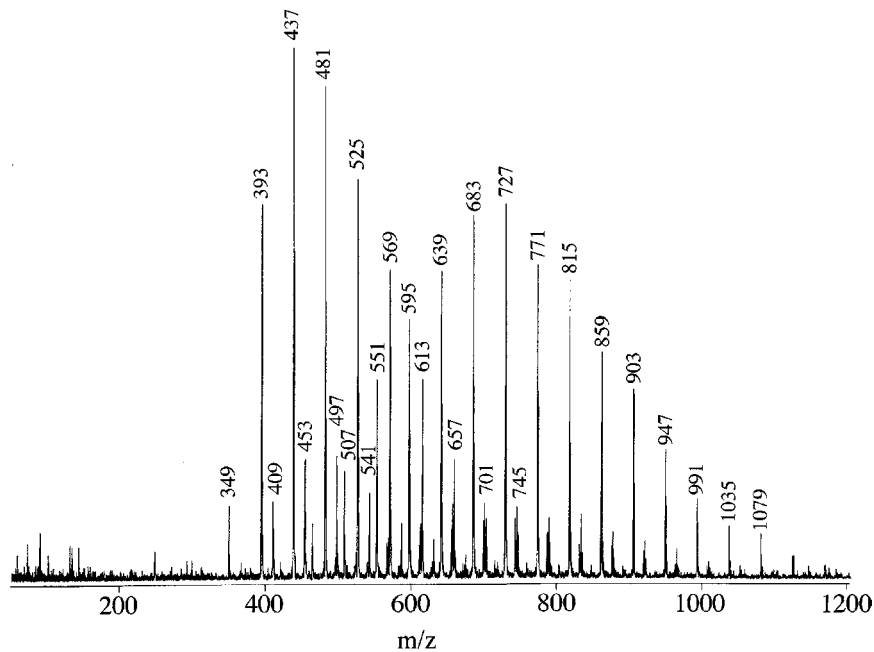


FIG. 5—DIOS mass spectrum of PEG lubricant [peak grouping with 437 Da ($n = 9$) maximum and 44 Da between adjacent peaks) and nonoxynol-9 spermicide [peak grouping with 727 maximum ($n = 11$) and 44 Da between adjacent peaks] from a Ramses® Extra spermicidally lubricated condom.
*Used with permission from Fig. 4, Thomas JJ, et al., *Analytica Chimica Acta* 2001;442:183–90, Elsevier Science.

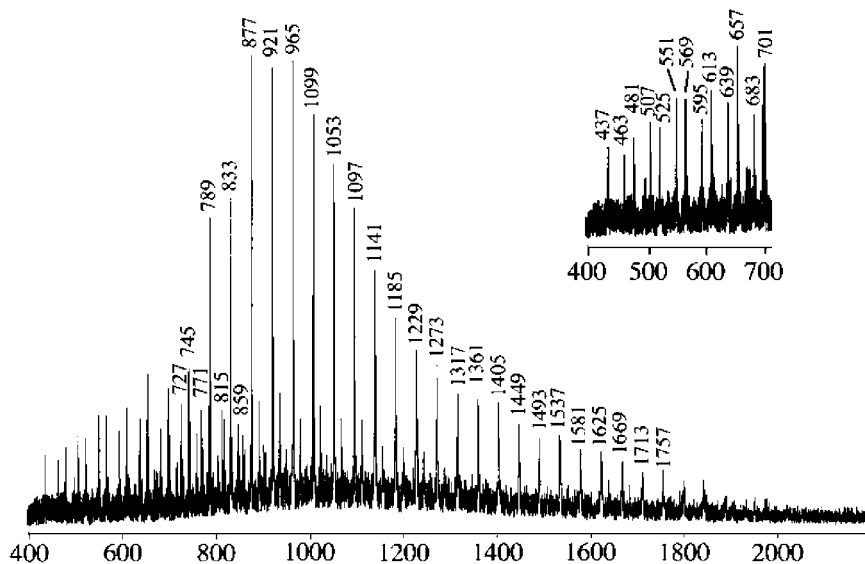


FIG. 6—DIOS mass spectrum from a swab extract from a volunteer immediately following the use of an Encare® vaginal contraceptive insert. Nonoxynol-9 peaks are buried within the low mass side of the PEG spectrum (peaks at 463, 507, 551, 639, and 683 Da in the insert on the upper right).
*Used with permission from Fig. 5, Thomas JJ, et al., *Analytica Chimica Acta* 2001;442:183–90, Elsevier Science.

that he sometimes used a commercial lubricant product and that this might account for our findings. The court told his attorneys to either provide a sample of this product for testing, or they would not be allowed to refer to it at trial. Eventually, the suspect (through his attorneys) provided us with a previously opened bottle of SLIP® Plus lubricant. Figure 8a shows the front of the bottle as received, and Fig. 8b shows the back. Notice that someone had scratched out the list of ingredients, and also the address information of the manufacturer. However, the manufacturer of SLIP® Plus was easily determined to be TRIMENSA Pharmaceuticals, Newbury Park, CA, and they agreed to send us two sealed bottles of SLIP® Plus as well as a list of its ingredients. The list included purified water,

glycerin, and nonoxynol-9 as the first, second, and fourth most abundant ingredients, respectively. Not only was PEG absent, but a chemist for TRIMENSA Pharmaceuticals stated that PEG had never been used in any of their products.

Figure 9a shows the DIOS TOF MS spectrum obtained from one of the two bottles of SLIP® Plus provided by the manufacturer, and Fig. 9b is the spectrum obtained from the bottle the defendant had provided to us through his attorneys. Because the mass of glycerin (mol. wt. = 92 Da) is below the mass range of the DIOS experiment, nonoxynol-9 dominates the spectrum, but low levels of PEG are clearly seen extending out to beyond 1500 Daltons in Fig. 9b while any evidence of PEG is completely absent in Fig. 9a.

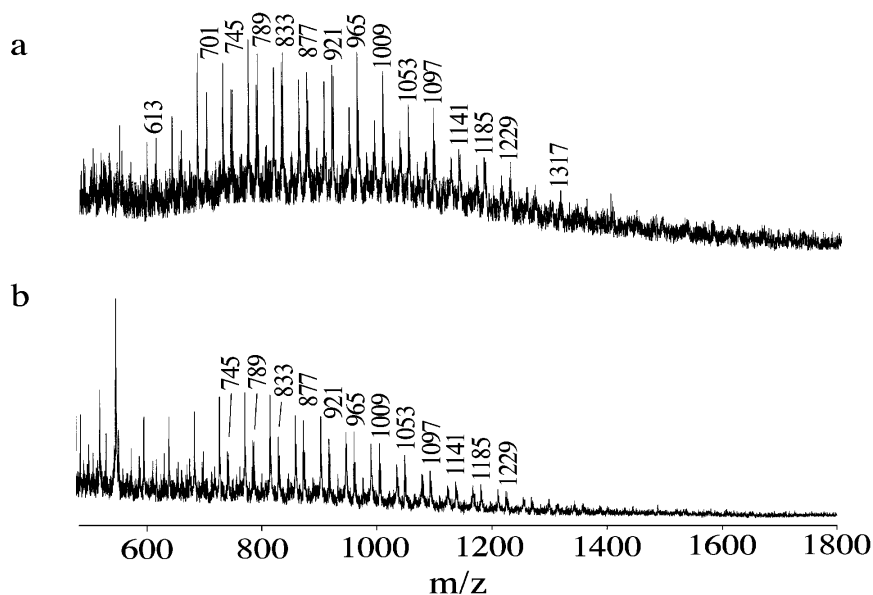


FIG. 7—DIOS mass spectrum showing a pattern of PEG in the same range as the Encare® product. Samples taken from: (a) deep vaginal swab from alleged victim, and (b) penile (glans and shaft) swab from suspect.

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FIG. 8—SLIP® PLUS bottle provided by the suspect (through his attorneys). Notice scratch marks (right) on the back of the bottle obliterating the ingredients list and the distributor's address.

Fourier transform infrared spectroscopy (FTIR) was also used to compare the liquid contained in the SLIP® Plus bottle from the defendant with the liquid in one of the bottles from the manufacturer. For FTIR, the samples were prepared as thin smears of the neat liquids on 3M IR Cards (3M Company, St. Paul, MN). In the sampling area, these cards have a thin film of porous polyethylene. Spectra were ratioed against a background spectrum of a blank card. Figure 10a shows the spectrum obtained when the card was first prepared. Below it is a spectrum for glycerin, the closest hit produced by a library search. This card was allowed to sit out in the open air on top of the FTIR bench for just over a month, and then the FTIR spectrum of any chemicals still remaining on the card was obtained. Figure 10b shows that most of the glycerin as well as most of the nonoxonyl-9 have evaporated from the card, while a library search of the spectrum now gives PEG as the closest hit. These results not only confirm the DIOS TOF MS findings, they illustrate a very low-tech method that may have application for identifying a

higher molecular weight component in a mixture when it is present together with more volatile (lower molecular weight) components.

Conclusions

DIOS TOF MS has been shown to be a very sensitive and effective method of identifying traces of those molecular species that cannot be separated and identified by gas chromatography/mass spectrometry (GC/MS), but are in a mass range of a few hundred to a few thousand Daltons. In the case discussed, DIOS TOF MS could clearly distinguish the spermicides nonoxonyl-9 and octoxonyl-9, as well as easily distinguish different molecular weight ranges of PEG. In the dirty samples commonly encountered in forensic science casework, it is unlikely that FTIR methods could make such distinctions.

The first trial resulted in a hung jury despite the fact that the defendant's attorneys stipulated that their client had added some

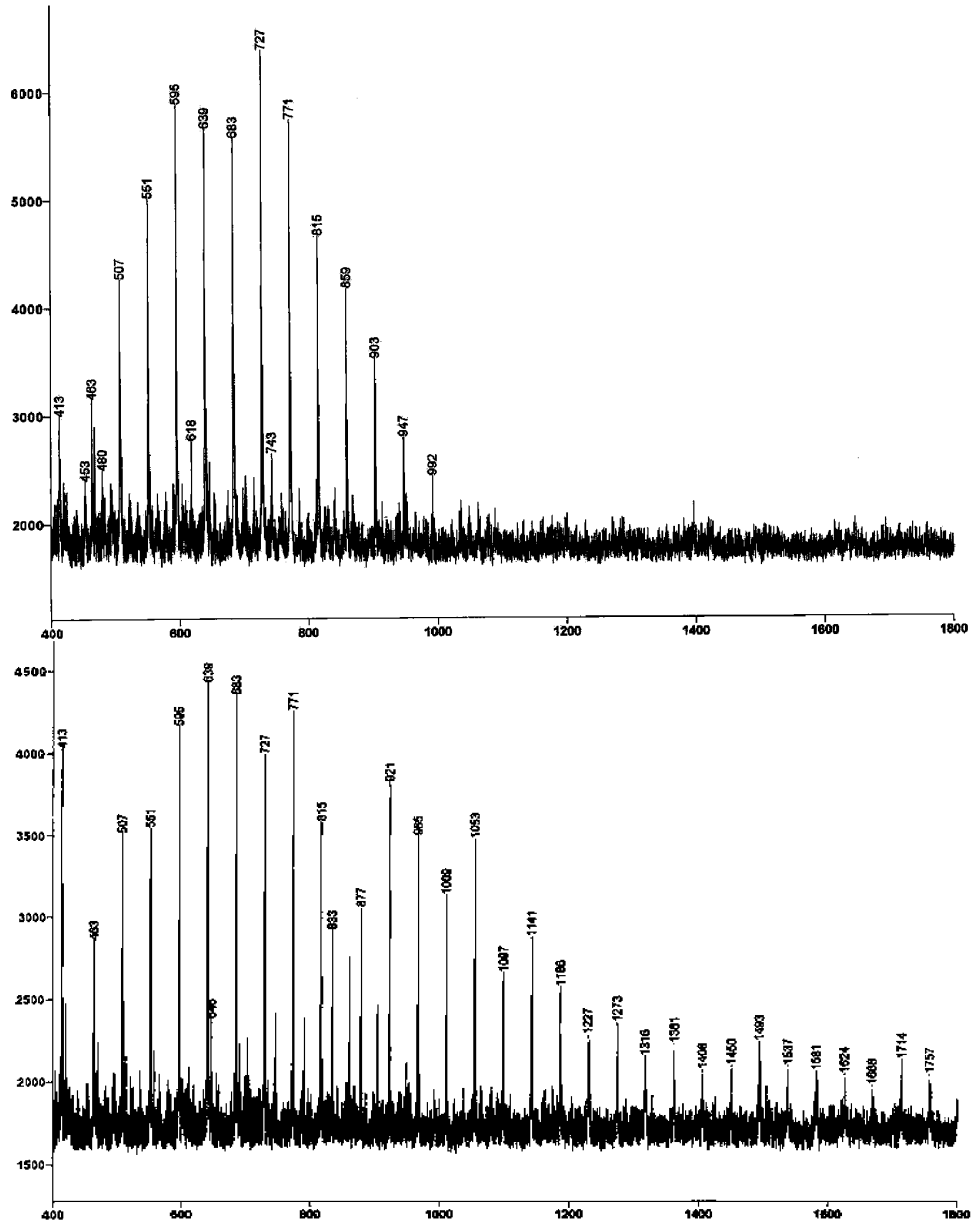


FIG. 9—DIOS mass spectrum from liquid contained in a SLIP® PLUS bottle provided by the manufacturer (top), and the liquid from the SLIP® PLUS bottle provide by the suspect. At trial his attorneys stipulated their client had added some of the Encare® product to the bottle.

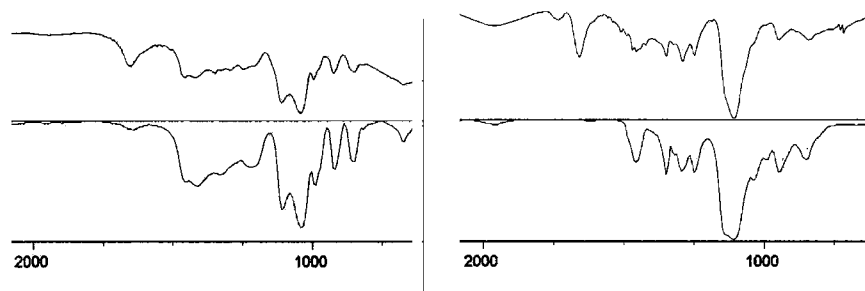


FIG. 10—FTIR spectrum as a thin film on a 3M IR card of the liquid from the bottle of SLIP® PLUS provided by the suspect (top left), with (below left) the spectrum for glycerin, the closest hit on library search, and (upper right) the spectrum just over one month later after the card had been open to the air, and (below right) the spectrum for PEG which is now the closest hit. In the intervening time period the lower molecular weight components (primarily glycerin and nonoxynol-9) have evaporated from the card leaving the higher molecular weight PEG.

Encare[®] to the sample of SLIP[®] Plus he had provided. It appeared that the lack of any DNA evidence associating the defendant with the victim or the crime scene was the major concern for those jurors voting for acquittal. A second trial lasted almost five weeks and again resulted in a hung jury (9 for conviction to 3 for acquittal), and the state decided not to try the case again.

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