Forensic Science International 280 (2017) 87–94

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Comparative analysis of condom lubricants on pre & post-coital vaginal swabs using AccuTOF-DART

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ARTICLE INFO

Article history:
Received 16 May 2017
Received in revised form 26 August 2017
Accepted 4 September 2017
Available online xxx

Keywords:
Forensic science
Nonoxynol
Sexual assault
Pre- and post-coitus samples
Direct Analysis in Real Time Mass Spectrometry
Time of flight

ABSTRACT

In this study we demonstrate the use of Direct Analysis in Real Time Mass Spectrometry (DART) as a powerful tool for detection of nonoxynol in vaginal fluid post contact with a condom, enabling rapid tracing and added evidences in sexual assault crimes. Vaginal fluid was sampled using cotton swabs and glass rods and measured directly with DART. Sample preparation using water, hexane, methanol, and dichloromethane extraction, was explored for comparison and optimization of signals. Nonoxynol was detected up to eight hours after sampling. Optimal sampling conditions and mass spectrometry parameters are reported and discussed.

1. Introduction

In the past decade, there has been a significant increase in sexual assault cases in which the perpetrators use condoms. Since the advent of sophisticated DNA analysis, sexual offenders often use condoms to avoid leaving DNA samples at the crime scene. In sexual assault cases, lubricant trace evidence may supplement biological evidence, or may be the primary physical evidence when biological evidence is unavailable. Lubricants are used in condoms to preserve and extend shelf life. The lubricants often contain additives that aid in minimizing the chances of pregnancy through their spermicidal activity. Research done in the past, using various instrumentation, focused on studying the traces of additives and condom lubricants in condoms in order to be able to discriminate between condom brands [1]. While most of the methods and instrumentation employed were indeed sensitive, only occasionally were they discriminative [1].

Nonoxynol is the most common spermicidal ingredient. It may appear as a mixture of compounds that possess the constant nonylphenol moiety with varying lengths of para-oriented ethoxy chains that are all equally effective [2]. This mixture is a non-ionic surfactant, that is usually found in concentrations between 5% and 10% in spermicidal lubricant formulas. The spermicidal effect comes from disrupting the production of enzymes required for the sperm to enter the egg [3]. Nonoxynol is a good target compound for identification of the presence of lubricant due to its persistent nature as it lingers in the vaginal vault. While there could be several compounds present on condoms, it is more reliable to resort to the recognizable pattern of nonoxynol such that it appears amongst the mixture of other ion peaks. Since nonoxynol has varying lengths of the repeating ether unit $-CH_2CH_2O-$ unit with a mass of 44, it distinctly stands out amongst other peaks. Furthermore, the presence of the multiple oxygen atoms renders for a facile ionization process, which out competes the ionization of other possible analytes present from the vaginal swab. This results in an enhancement in the detection limit. Nonoxynol is also stable enough and does not react with other compounds possibly present in vaginal fluids, thereby serving as an unambiguous marker for the use of condoms carrying that mixture of spermicide. Several types of analyses have already been reported for the forensic analysis of vaginal fluid including Electrospray Ionization and Matrix Assisted Laser Desorption Ionization [4], High Pressure Liquid Chromatography [5], Gas Chromatography Mass Spectrometry [6], and Capillary Electrophoresis [1a]. Many of these methods require extensive sample preparation or run time.

The overall goal of this investigation was to find both a sensitive and discriminatory methodology to analyze condoms' residues and volunteers' vaginal swabs.

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http://dx.doi.org/10.1016/j.forsciint.2017.09.005
0379-0738/

Direct Analysis in Real Time Accurate Time of Flight Mass Spectrometry (DART-AccuTOF-MS or DART) [7] has been used in the past as a very powerful technique for detecting and quantifying organic molecules in biological matrices [8]. DART was also used in the forensic analysis of condom lubricants and even fingerprints on condom wrappers [9]. In this study, DART AccuTOF was used to investigate both condoms' residues and the vaginal fluid samples. In addition, several sampling strategies, sample treatments, and instrument parameters were explored for nonoxynol detection. The inherent advantages of DART ionization will be discussed, as well as direct sampling and sampling after extraction.

2. Materials and method

2.1. General

Due to the heterogeneous nature of DART-AccuTOF-MS, optimization of both sample preparation and tuning of the instrument took place before comparing condom types. Special attention was paid to the collection of the samples, the sample treatments before DART analysis, and ionization to detection parameters and spectral interpretation. Igepal® CO-630, 9% (v/v) was used as a positive control for nonoxynol in sampling and extraction experiments in addition to pre-coital samples from both vaginal fluid and condoms. A total of 208 samples were taken and analyzed for this study. Trojan®-ENZ, Ultra-Ribbed, Trojan®-ENZ Her Pleasure Sensations, and Beyond Seven Plus® condoms were used for the analysis. This study was submitted and approved by the John Jay College Institutional Review Board (IRB).

2.1.1. Sampling

All samples were collected from the vaginal vault of a volunteer with the aid of a speculum. The couple did not use additional lubricants of any kind and allowed at least seven days between collection events. No health implications were observed, such as a urinary tract infection, throughout the sampling times. Collection events took place immediately and up to eight hours after coitus to investigate the longevity of measurement ability. Controls were necessary including: a positive control of the lubricant without any vaginal fluid, a negative control of vaginal fluid without lubricant and vaginal fluid with lubricant. Cotton swabs were used instead of Nylon swabs to avoid anticipated sensitivity to ionization out-competing the detection of the nonoxynol mixture. Sterile glass rods and spoons were also used to avoid contamination from cotton residues and to achieve a direct exposure to the ionization region.

2.1.1.1. Cotton sampling. Sterile cotton swabs were used for sample collection with control groups including: a blank, pre-coital condom and vaginal swabs, as well as post-coital condom and vaginal swabs. The blank was treated without any swabbing activity. The condoms were swabbed with cotton swabs with a slight pressure in the streak to allow the contact between the absorbing material and the surface of the condom before and after coitus. Vaginal swabs were collected before and after intercourse, via repeated circular motions in the vaginal vault.

2.1.1.2. Glass sampling. Glass rods or spoons (Chemglass Life Sciences) approximately six inches in length were used to collect samples from the vaginal vault including: a blank, pre-coital condom and vaginal swabs, and post-coital condom and vaginal swabs. The resulting samples were freeze-dried and analyzed with DART-AccuTOF-MS.

2.1.2. Sample treatments

The collected samples were either directly sampled by DART, or treated with solvents to isolate nonoxynol hoping to enhance the detection by DART. Solvent extractions were carried out with a series of solvents such as 10 mL of methanol, hexane, double distilled water (ddH_2O), or dichloromethane (CH_2Cl_2) for both cotton swabs and glass rods.

We have observed from previous experimentation with DART techniques that direct measurement is best with smaller molecules. However, with larger and stickier molecules, e.g. nonoxynol, dilution often enhanced the volatilization process and it was critical to not over dilute samples. Water in general has shown to enhance ionization of ethers and esters. We chose a diverse range of solvents from the nonpolar hexane across to the polar water. Depending on the content of the mixture of lubricant

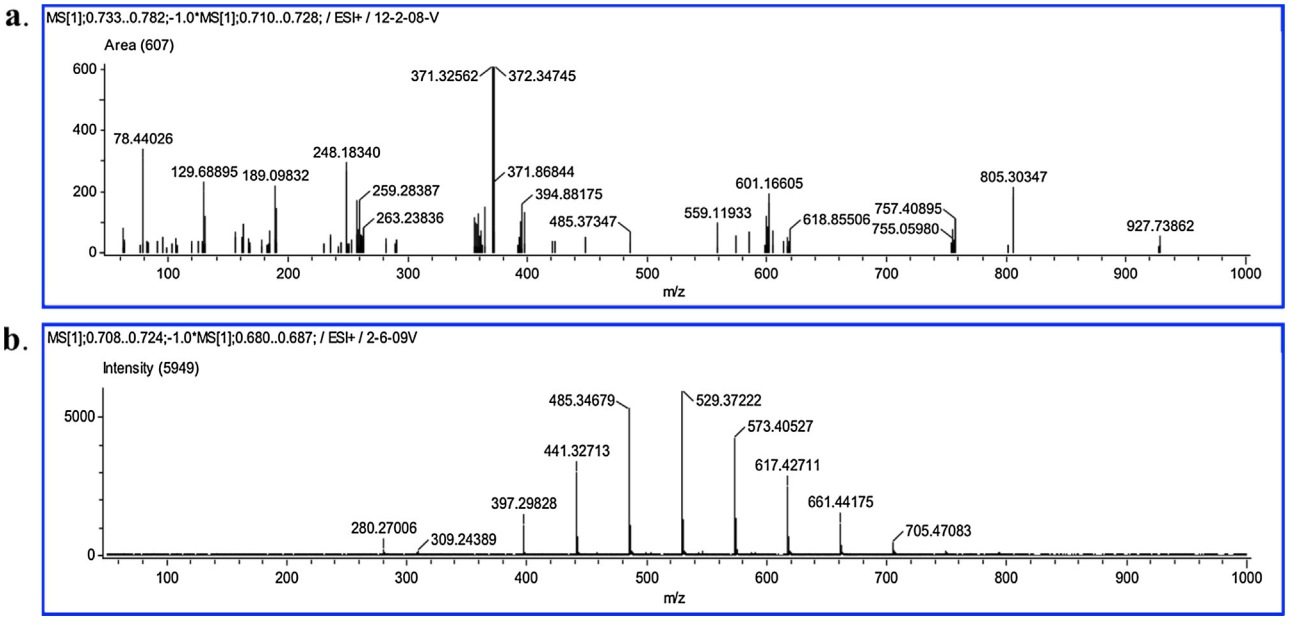

Fig. 1. (a) Trojan Enz® — Pre-coital condom cotton swab; methanol wash. (b) Trojan Enz® — Pre-coital condom sample collected with glass rod; methanol wash.