

Trace Evidence Analysis in a Child Sexual Abuse Case - Silence Dares to Speak

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Abstract

There is a dire need of identification of analytical techniques suitable for the detection of trace evidence especially in sexual assault cases. We report an alleged case of sexual assault of a four year old girl child in which a jelly/cream could have been used as a lubricant and applied on her genital area. The samples taken from the child were deposited in an attempt to document the presence of traces of the jelly/cream involved. Fourier Transform Infra-Red Spectroscopy (FTIR) analysis allowed the identification of Petrolatum. Gas Chromatography-Mass Spectrophotometry (GC-MS) confirmed the presence of Methyl Stearate. Results of this investigation are relevant in the context and strongly suggest that a jelly composition containing petrolatum and methyl stearate as ingredients was in fact applied to the genital area of the child and the presence of same was also confirmed on the undergarment of the child.

Keywords: Child sexual abuse; Gas chromatography-mass spectrophotometry

Introduction

When the renowned poet of English literature P.B. Shelly wrote "If winter comes can spring be far behind", he was not possibly talking only about the change of seasons but also about the fact that change is the law of nature and it has to happen sooner or later and yes sometimes it comes with a heavy price. The brutal rape and murder of a girl student in the capital city of India on 16th December 2012 stirred the soul of every sensitive person to join the justified public protests against the senseless sexual violence to which females are subjected to in every part of the world even in the dawn of this twenty first century. The change has made way for itself now. Government has promised action and reforms in this domain and since then a lot of measures have been taken on the legal and administrative front and the incidence has awakened many Indians to the scale and prevalence of sexual violence in the country. Children and adolescents, irrespective of their race, culture, or economic status, appear to be at approximately equal risk for sexual victimization. Child sexual abuse is outlawed nearly everywhere in the world, generally with severe criminal penalties, including in some jurisdictions, life imprisonment or capital punishment [1,2]. The first published work dedicated specifically to child sexual abuse appeared in France in 1857: Medical-Legal Studies of Sexual Assault (Etude Médico-Légalesur les Attentats aux Moeurs), by Auguste Ambroise Tardieu, the noted French pathologist and pioneer of forensic medicine [3]. An adult's sexual intercourse with a child below the legal age of consent is defined as statutory rape [4] based on the principle that a child is not capable of consent and that any apparent consent by a child is not considered to be legal consent. Child Sexual abuse is engaging a child in any sexual activity that he/she does not understand or cannot give informed consent for or is not physically, mentally or emotionally prepared for. This includes using a child for pornography, sexual materials, prostitution and unlawful sexual practices [5]. The United Nations Convention on the Rights of the Child (CRC) is an international treaty that legally obliges states to

protect children's rights. Articles 34 and 35 of the CRC require states to protect children from all forms of sexual exploitation and sexual abuse. Accurate statistics on the prevalence of child and adolescent sexual abuse are difficult to collect because of problems of underreporting and the lack of one definition of what constitutes such abuse. Nineteen percent of the world's children live in India [6,7] which constitutes 42 percent of India's total population [8]. In 2007 the Ministry of Women and Child Development published the "Study on Child Abuse: India 2007". The study's main findings suggest that 53.22% of children reported having faced sexual abuse. Among them 52.94% were boys and 47.06% girls. The 'Protection of Children against Sexual Offences Bill, 2011' was passed by the Indian Parliament on May 22, 2012 [9].

Forensic experts typically identify sexual assault offenders by examining seminal fluid residues for sperm, proteins, blood grouping factors, and DNA profile. The body and the clothing worn at the time of the incident may contain trace evidence. Items commonly encountered include hair from any part of the body, clothing fibres, lubricants, petroleum jelly and lipstick. Lubricants have frequently been used in the past by sexual assailants because they facilitate the act [10,11]. The use of oil, gel, cream and condoms by the assailants in such assaults falls in the category of the non-biological valuable evidences [12]. As an alternative Forensic laboratories may be asked to look for traces of these products on evidence items in cases of alleged sexual assaults including child sexual abuse and therefore in such cases, the lubricant trace evidence may supplement biological evidence and be the primary physical evidence where biological evidences are not available.

Brief Sketch of the Case

We present here the detailed scientific analysis of the trace evidence of one case pertaining to child sexual abuse from the chemical examination point of view. As per information recorded by the mother of the girl child to the police officer on 07.09.2013, it was alleged that their driver had coerced the four year-old girl child while dropping her to their residence from her school and fondled with her private parts

by putting his fingers therein. The case was registered under section 376 IPC and 5/6 POCSO ACT on 07.09.2013 at 13.00 hrs. without any delay and the girl child was taken for medical examination immediately. The mother also stated that after taking her child into confidence, the child was able to narrate the unfortunate incidence and told that the same driver had done the heinous act with her many times earlier also but she remained silent as she did not understand the brutality of the act she was subjected to and thus did not speak up. It was also alleged that the driver had rubbed some jelly or cream on her genital area. The exhibits were deposited in the Chemistry Division of Forensic Science Laboratory (FSL) Delhi on 13.09.2013, and were asked by the investigating officer to look for traces of the unidentified chemical in the oral swabs, vaginal swabs, and the undergarment worn by the child at the time of the heinous incidence. The medical report submitted along with the forwarding letter for examination clearly mentioned "Redness at the Vulvar Region, Vaginal Hymen not intact, ruptured (not fresh)". The case was undertaken for examination on priority basis on 19.09.2013.

Experimental Set-Up

Description of exhibits and sample protocol

Three exhibits consisting of oral swab, vaginal swab and undergarment, belonging to all girl children were forwarded to the laboratory, kept in a duly sealed cardboard box labelled "Sexual Assault Evidence Collection Kit" (Figure 1).

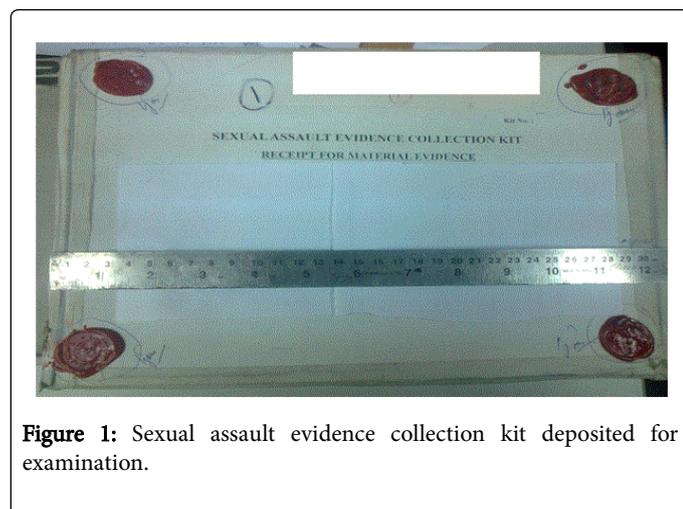


Figure 1: Sexual assault evidence collection kit deposited for examination.

Biological sample protocol

Biological samples originating from evidence samples were contained in victim sexual assault evidence kits. They were stored at 4°C in a refrigerator located in a secured area. The evidence samples which were sent to us and examined were from Victim Kit (Figure 2): A dry cotton viscose swab (Figure 3) contained in a plastic wrapper labelled 'oral swab'(OS) and a dry cotton viscose swab with pale yellow coloured dirty stains sticking on the bud which was contained in a plastic wrapper labelled 'vaginal swab' (VS). Upon original examination of the victim the vaginal swab and oral swab had been used to prepare microscope slides which were forwarded to Biology Division for detection of spermatozoa. The cotton swabs were placed in disposable glass test tubes, covered with a minimum amount of

diethyl ether and vortexed for a few seconds and later subjected to chemical and instrumental analysis after requisite sample preparation.

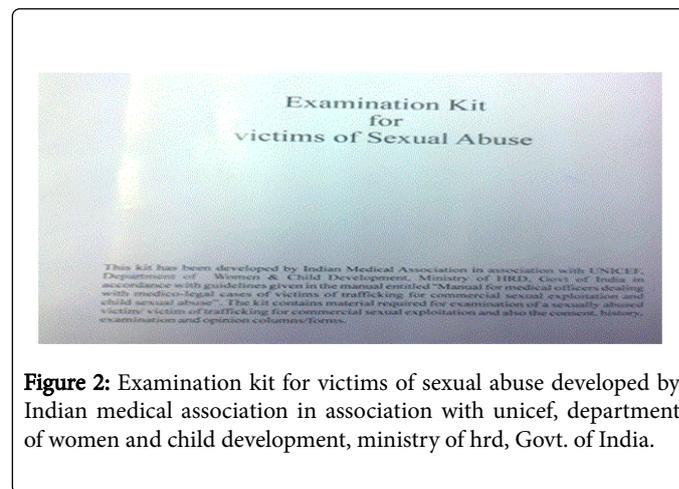


Figure 2: Examination kit for victims of sexual abuse developed by Indian medical association in association with unicef, department of women and child development, ministry of hrd, Govt. of India.



Figure 3: Exhibits-oral swab and vaginal swab.

Non-biological sample protocol

The clothing worn at the time of the incident may contain trace evidence and thus the undergarment (UG) a blue coloured panty having pale yellow coloured dirty stains on it was also submitted for examination (Figure 4). A portion of the UG was also sent to Biology Division for examination. The sampling and examination was carried out as per guidelines of the manual of DFS (Directorate of Forensic Science Services, Ministry of Home Affairs, Govt. of India).



Figure 4: Exhibit- under garment.

Materials and Methods

Chemicals

The solvents, Diethyl ether, Chloroform, Hexane and Anhydrous Sodium Sulphate (Merck, India) used were of analytical grade. Potassium Bromide (Merck) was of IR grade.

Extraction method

All the three exhibits (OS, VS and UG) were separately subjected to steam distillation. Clear oily droplets were observed in the distillate and the distillate was further extracted using chloroform and diethyl ether. The two extracts were combined and divided into three different phases (n-hexane, ethyl acetate and water) using liquid extraction. The organic phases of the extracts were further passed through anhydrous sodium sulphate and the solvent was removed by evaporation under stream of nitrogen. The dried extracts were further reconstituted with hexane and subjected to different chromatography systems including column, and thin layer-chromatography on various stationary and mobile phases in order to get an idea to detect the presence of a non-polar hydrocarbon constituent of the unknown compound.

Instrumental methods

The target compounds after extraction were characterised by FTIR and further by GC-MS which aids in interpretation of data by way of mass fragmentation pattern and comparison with the literature/library matching.

Fourier transform infra-red spectroscopy (FT-IR): FT-IR has wide applicability in characterisation of compounds and structure elucidation. It provides a great deal of information and the beauty and utility of this technique lies in the identification of the compounds. The IR spectra are like a fingerprint for identification of compounds as no two spectra is identical. It is a complementary technique for UV-VIS, NMR & GC-MS. A Perkin Elmer Spectrum GX FT-IR Spectrometer was used to analyse the exhibits. The operating parameters of the spectrometer were as follows: resolution: 4 cm, number of scans 12, scan range 400-4000 cm^{-1} , Interval-1.0, Units-%, Detector TGS (Triglycene sulphate). Canadian Forensic spectral library software was accessed as an aid to work out the identification of the compound. Halide disk method (KBr pellet) was used for the sample preparation.

Gas chromatography-mass spectrometry: Gas chromatography (GC) was used for peak separation, coupled with mass spectrometry (MS) for peak identification of the analytes. Interpretation on mass of GC-MS was done using the database. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the National Institute of Standards and Technology (NIST) library. The name, molecular weight and structure of the components were ascertained.

An Agilent 6890 GC with MS was used to analyse the entire sample. The instrument had Agilent 19091 J-333 HP-5 Column packing with 5% Phenyl Methyl Siloxane operating in electron impact mode at 70 eV; with MSD Detector. The operating parameters setting of the instrument were: Oven Temperature 300 °C, Injection Temperature – 280 °C, Injection volume – 1 μL , Mode – Split, Carrier Gas – Helium, Flow Rate – 70.9 ML/min., Detector Temperature 290°C. The instrument accesses international library software (NIST).

Results

FTIR was used in this case to identify the compound present in the solvent extracts of the three exhibits. The spectral analysis of the extracts of exhibits VS and UG shows high intensity bands between 3000 cm^{-1} and 2800 cm^{-1} , medium intensity bands between 1500 cm^{-1} and 1300 cm^{-1} , and they show low intensity bands between 750 cm^{-1} to 700 cm^{-1} . For the detection of compound in the exhibits their spectra's were matched with the compounds present in the library by accessing the Canadian Forensic Spectral Database and Vaseline was found to be the closest hit to our suspected compound. Further the spectra of the extracts of VS and UG matched with that of the standard spectra of petrolatum (Figure 5). These results not only confirm the GC-MS findings, they illustrate very low-tech and rapid 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.

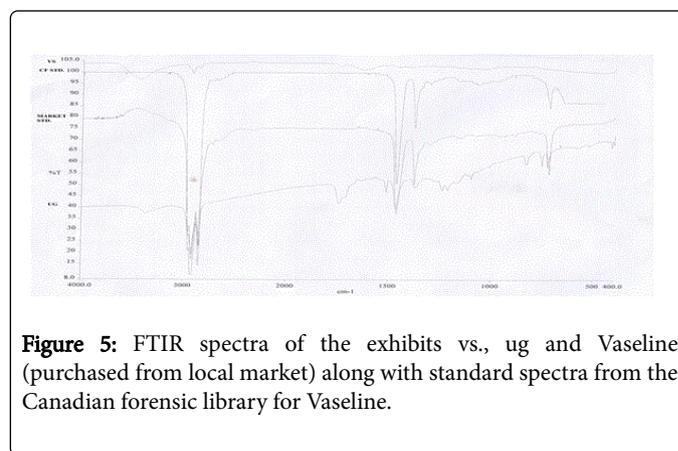


Figure 5: FTIR spectra of the exhibits vs., ug and Vaseline (purchased from local market) along with standard spectra from the Canadian forensic library for Vaseline.

On perusal of the ingredients compiled in the glossary for Vaseline, we found that it has around forty five official entries as per the information provided on the website of the company [13]. We critically examining all the 45 ingredients through exhausting literature survey in order to establish the most probable compound which would assist in the confirmation of the result obtained by FTIR for the VS and UG samples. Petrolatum and Methyl Stearate were identified as the most probable markers to confirm the identity of the chemical in question. The hydrocarbons and the target compounds were determined by GC/MS with the assistance of NIST library database (Figure 6). On study of petrolatum in detail by way of various publications and patents, it was found that the compound had been initially patented in 1872 by Robert A. Cheeseborough [14] and is one of the basic raw materials used for cosmetic and relieving ordinary xerosis [15]. Since petrolatum is produced from crude oil, it consists of hydrocarbons of paraffin group and may also contain small amount of unsaturated hydrocarbons. It is pertinent to mention here that on freezing and thawing the extract a very evident waxy layer was visually observed and this is in line with the fact that petrolatum is considered to be not simply a mixture of liquid paraffin and solid paraffin but to exist as a colloid in which a solid wax forms the external phase and a liquid oil forms the internal phase.

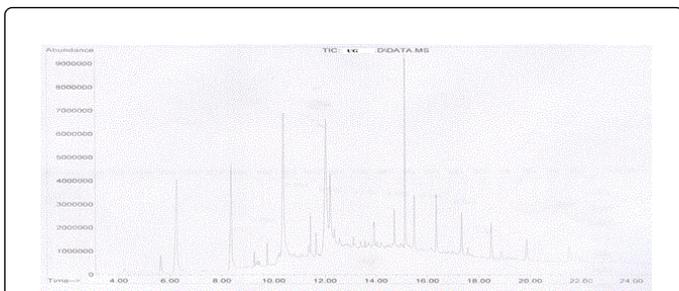


Figure 6: Chromatogram of the exhibit obtained by gas chromatography.

Literature reveals that one of the principle constituents of petrolatum is tetracosane [16] and this constituent was also identified in the extracts of UG. On further detailed examination of patent available in the literature regarding petrolatum, a reference was found that charge producing agents consisting of negatively charged hydrophilic molecules including oleic acid (9-Octadecenoic acid) and palmitic acid (n-Hexadecanoic acid) along with some other constituents are also the part of the composition [17]. Both these fatty acids were confirmed by GC/MS. The GC/MS analysis of the extract resulted in few compounds of interest, comprising the constituents of

the extracted material, out of which we identified four most definite ones which corroborated with our experimental, analytical and literature search which includes, methyl stearate (Figure 7), n-hexadecanoic acid (Figure 8), 9-octadecenoic acid (Figure 9), and tetracosane (Figure 10). Further one more important component (Figure 11) phenol,2,4-bis-(1,1-dimethylethyl) of the possible composition was identified which may have been used as a chemical intermediate for the synthesis of UV stabilizers or antioxidants [18] and it has been reported to have antibacterial properties as well [19]. In the mass spectra of normal alkanes, m/z 57 is the base peak which is present in tetracosane. The most abundant ion in normal saturated acid ester mass spectra is m/z 74. The molecular ion peaks of methyl stearate observed at 298 is as expected. It is interesting to note that methyl stearate show $\text{CH}_3\text{OC} (= \text{OH}^+) \text{CH}_2$ fragment and appears at $m/z=74$ as the base peak (100%) which is the result of McLafferty rearrangement. The molecular ion peaks of other constituents present in the composition as identified were also in accordance with the m/z values of their respective fragments as expected (Table 1). Extracts of OS were negative for any detectable traces of the compounds which are attributed to the reason that in addition to the limitations of the analytical techniques, the detection of trace lubricant is also dependent on the length of time it persists on skin and mucosal surfaces. This is in turn affected by environmental factors such as temperature and moisture [20,21]

Sl.No	RT min	Name of the compound	Molecular Formula	Area %	Molecular weight	Principle ions at m/z
1.	5.63	Phenol,2,4-bis(1,1-dimethylethyl)	$\text{C}_{14}\text{H}_{22}\text{O}$	1.24	206	206, 191 91, 74, 57
2.	10.40	n-Hexadecanoic acid	$\text{C}_{16}\text{H}_{32}\text{O}_2$	17.63	256	256, 73 213, 129
3.	11.65	Methyl stearate	$\text{C}_{19}\text{H}_{38}\text{O}_2$	0.99	298	298, 74, 143,55
4.	12.05	9-Octadecenoic acid	$\text{C}_{18}\text{H}_{34}\text{O}_2$	16.54	282	282, 264, 83, 55, 111
5.	13.93	Tetracosane	$\text{C}_{24}\text{H}_{50}$	0.83	338	338, 57 85, 141

Table 1: Details of major components obtained and identified by Gc-MS in the exhibit

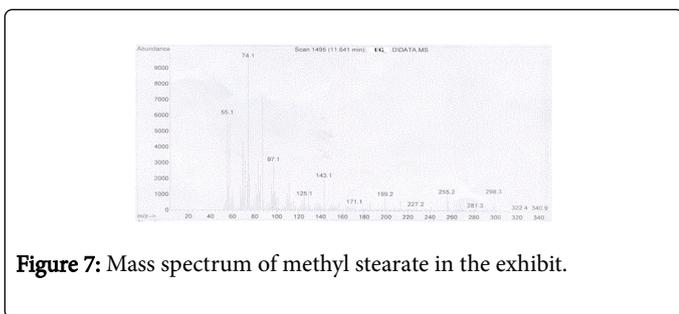


Figure 7: Mass spectrum of methyl stearate in the exhibit.

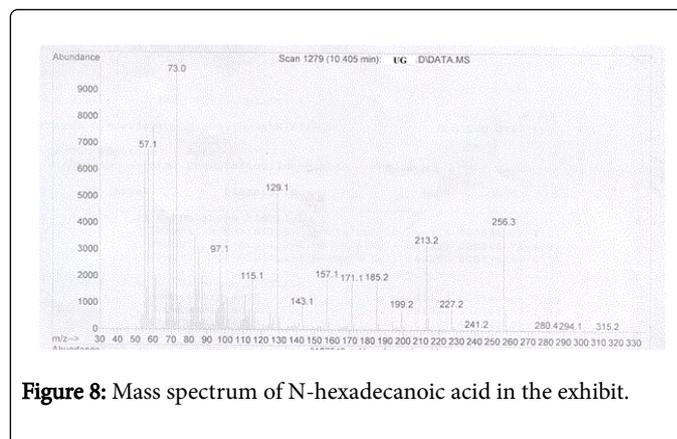


Figure 8: Mass spectrum of N-hexadecanoic acid in the exhibit.

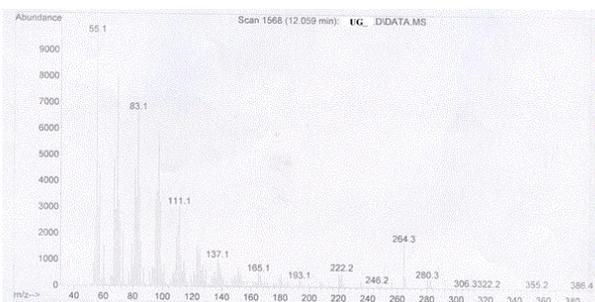


Figure 9: Mass spectra of oleic acid in the exhibit.

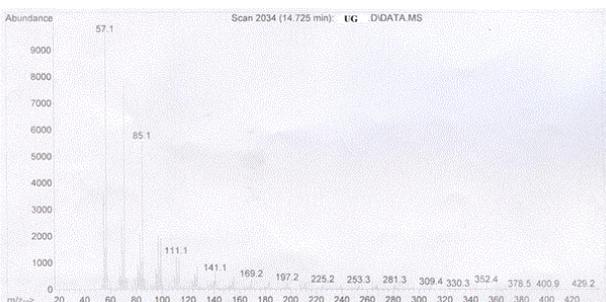


Figure 10: Mass spectra of tetracosane in the exhibit.

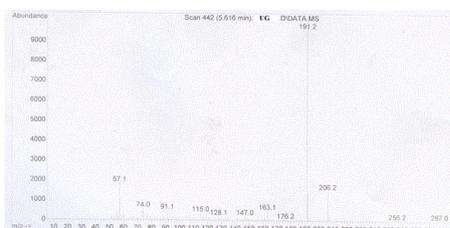


Figure 11: Mass spectra of Phenol,2,4-Bis (1,1-Dimethylethyl) in the exhibit.

The purpose of this study is to appreciate and acknowledge the well-established analytical methods that are available for detection of an unknown compound in traces. The intent is not to provide an exhaustive list of analytical methods, the intention is to identify and use well-established methods which are indispensable aid to forensic scientists in rapid and accurate analysis with confidence. GC-MS has been shown to be a very sensitive and effective method for identifying traces of those molecular species that cannot be separated and identified by FTIR but are in a mass range of a few hundred to a few thousand Daltons. In the case discussed here MS could clearly distinguish the main constituents. In the dirty samples commonly encountered in forensic science casework, it is unlikely that FTIR methods could make such distinctions [22-24]. Child sexual abuse is indeed a very serious but to put it very honestly a hidden problem in India and it has been proved by intensive research that children are

often sexually abused by people known to them. The fear of social stigma or the lack of faith in institutions prevents many people from even reporting cases related to child sexual abuse and as such there is a need for longitudinal data that provides prospectively measured indicators of children pre-assault functioning levels [25]. By enacting the Protection of Children from Sexual Offences Act in 2012, the Government of India has taken a significant step in acknowledging the rampant sexual abuse of the country's children [26]. The forensic fraternity is undoubtedly an integral part of the Justice delivery system and the society looks forward to us to be sensitive towards cases which are categorised in this domain. The forensic scientists are the one who can literally be an instrument in dissecting the unfortunate profound silence towards such cases.

We have documented our scientific and technical findings with all humility in a salubrious spirit without any prejudices and bias in an effort to melt the frozen turbulence in the mind of many like us.

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