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DEVELOPMENT OF NEW SOLVENT SYSTEM FOR SEPARATION OF SEMINAL PROTEIN

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ABSTRACT

The identification of semen is often an important issue in the investigation of rape and other crimes involving sexual assault. The most commonly used procedures for semen identification focus on the visualization of sperm or of prostatic acid phosphatase activity. Methods involving the visualization of spermine, choline, or semen antigens are used less often. In this research paper developed 9 solvent systems were used for the separation of seminal protein such as Chloroform : Acetic acid: Acetone, Acetic acid : Acetone, Acetic acid: ethanol: Acetone, Acetic acid: Normal Saline, Chloroform: Methanol, Methanol: Ammonia, Acetic acid: Methanol, Acetic acid: n- butanol: Water, Nitric acid: Ethanol. Out of these solvent systems it was found that Acetic acid: Acetone gave better result as compare with conventional solvent system (1NHCl).

INTRODUCTION

Semen, a viscous mucilaginous liquid, is a human body fluid present in human males. It is faint yellow in color and has a characteristic odour called seminal odour. A total volume of 3ml is ejaculated per ejaculation. Spermatozoa (10%) Seminal Plasma (90%) Epithelial Cells (<1%) Spermatozoa contain lipids, proteins (Protamine and histone etc.) and enzymes (dehydrogenases and transferases) and are produced in the testis by spermatogenesis. A spermatozoon is about 50 microns in length, consisting of a flat and oval-shaped head and a tail. The nucleus occupies the major portion of the head whereas the tail of spermatozoa is responsible for its motility. Sexual assault is a crime of violence against a person's body and will. Sex offender use physical and psychological aggression to victimize, in the process often threatening a victim's sense of privacy, safety, and well-being. Sexual assault can result in physical trauma and significant mental anguish and suffering for victims. A great number of cases received in a forensic science laboratory involve sexual offences, making it necessary to examine exhibits for the presence of seminal stain. There is a need of separation of seminal protein, visualization of sperm and semen even if present in small quantity; no matter how old stains are.

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The stains are usually found on clothing of the victims and the suspects especially on their undergarments. They may found on bed- sheets, on pillow cover, on carpet, on flour etc. They may also be found on the person of victim and the suspect. Vaginal and anal swab and smears of the victims of sexual offences and also the urethral swabs and smears of the suspects are often required to be examined in the laboratory for the visualization and characterization of semen. The identification of seminal stains is frequently of great value in medico legal practice, particularly in cases of alleged rape, sexual assault, sexual homicide or even adultery. One of the primary aims of the forensic laboratory sexual offence investigations is to sample and examine smears or other biological material taken from the assailant or the Victim or stains found on cloths or linen or any other evidence concerning the assault for the presence of semen, with the potential to link them. In cases of sexual assault, the forensic examination of evidence for seminal stains can actually be considered a two-step process. Firstly, the stain must be located. Secondly, the stain will be examined to prove its identity; possibly it may even be tested for the blood type of the individual from whom it originated. Furthermore, forensic scientists can successfully link seminal material to an individual suspect by DNA typing. Oshio, (1988), explained that sexual crimes may involve males who have an abnormally low sperm count, a condition known as Oligospermia, or they may involves individuals who have no spermatozoa at all in their seminal fluid (Aspermia).

Table 1. Developed color in various solvent systems over T.L.C. plate by spraying reagent

S.N.	Used Solvent system for separation of seminal protein	Developed colour with Dragondroff reagent	Developed colour with Potassium idoplatinate reagent
1	Chloroform: Acetic acid: acetone	Light orange	Purple
2	Acetic acid: Acetone	Fine orange	Dark brown
3	Acetic acid: ethanol: acetone	Light orange	Purple
4	Acetic acid: Normal saline	No colour	No colour
5	Chloroform: methanol	No colour	No colour
6	Methanol: Ammonia	No colour	No colour
7	Acetic acid: Methanol	Light orange	Purple
8	Acetic acid: methanol: water	No colour	No colour
9	Nitric acid: ethanol	Yellow/ orange	No colour

Table 2 Comparison of result between conventional solvent system and developed solvent system

Sr. no.	Solvent system	Developed colour with dragondroff reagent	Developed colour with potassium idoplatinate reagent
1	Conventional solvent system (1N HCl)	orange	Brown
2	Developedsolvent system (acetic acid : acetone)	Fine orange	Dark brown

The finding of seminal constituents in a rape victim is important evidence substantiating the fact that sexual intercourse has taken place, but their absence does not necessarily mean that a rape did not occur.

MATERIALS AND METHODS

For the separation of seminal protein through different solvent system in comparison with conventional solvent system (1N HCl) First of all collection of semen sample wants more attention in preservation process, Fresh Semen sample up to 5ml were collected in sterile container containing preservative from the different Medical Pathology Lab of Allahabad. Collected semen samples were brought to the Laboratory of Department of Forensic Science for analysis was kept at room temperature. After this a one drop of semen taken with the help of micropipette and put on the precoated silica gel plates **(**Silica Gel 60F-254E, Merck, Darmstadt, Germany) at the middle part of lower side of TLC plate and left for a min. for dryness. After dryness of spot TLC was kept under developing chamber and treated with different types of developed solvent system for the separation of seminal protein.

After that TLC plate was taken out from the developing chamber and dry with the help of air and also use spraying reagent for the separation of seminal protein like Dragondroff for separation of choline and Potassium idoplatinate for spermine protein. According to Levonen, (1960), employed ascending paper chromatography in isopropano1: acetic acid: water:: 50:10:40. 10µl of semen in a stain was required for a positive result. Using Dragendoff"s reagent, spermine appeared as a pink spot, while choline appeared as a deep purple spot. Hessel *et al.* (1967), reported the visualization of spermine and choline from as little as 1 µl semen by thin layer chromatography. 1N HCl was used as an extraction medium. Choline was detected with Dragendoff"s reagent and spermine was detected by over spraying with potassium idoplatinate reagent. After drying T.L.C. plate in lower 2/3 portion of T.L.C. plate was sprayed with Dragondroff reagent for the visualization of Choline spot and the upper 1/3 portion of the T.L.C. plate was sprayed with potassium iodoplatinate reagent for the visualization of Spermine spot, because the density of Spermine is less than density of Choline, Dodd (1984), after few minutes developed colour was observed and recorded. Different types of developed solvent system are Chloroform: Acetic acid: Acetone, Acetic acid: Acetone, Acetic acid: ethanol: Acetone, Acetic acid: Normal Saline, Chloroform: Methanol, Methanol: Ammonia, Acetic acid: Methanol, Acetic acid: methanol: Water and Nitric acid: Ethanol etc. used for the separation of seminal protein with the help of spraying reagent dragondroff reagent for choline and idoplatinate reagent for spermine.

RESULTS

It is concluded that developed solvent system Acetic acid: Acetone fairly matches with the conventional solvent system 1NHCl and it can be used as substitute in place of conventional solvent system because result of both solvent system gives same result in comparison of developed colour of choline and spermine protein on TLC plate.

DISCUSSION

It was observed that the result of present research work was found very much similar to the result obtain by Yano, (1970); method used 0.01N HCl as solvent system for the separation of seminal protein through TLC plate. It was observed that the visualized intensity of separated spot was fairly better as compare to visualization by dragondroff reagent and potassium idoplatinate reagent and separated spot through conventional solvent system. This developed solvent system can be utilized as substitute solvent system in place of conventional solvent system. In other way this developed solvent system that is Acetic acid: Acetone was less harmful and less corrosive in nature as compare to conventional solvent system 0.01N HCl because sometimes highly acidic solution degrades the nature of protein. So we can replace and also used this developed solvent system for the separation of seminal protein. The nature of new solvent system is less acidic and safe to use. From the present research work it is confirmed that developed new solvent system Acetic acid: Acetone has shown remarkable result and very much similar to conventional solvent system 1N HCl.

Conclusion

After applying these developed solvent systems at the place of conventional solvent system for the separation of seminal protein, the combination of acetic acid: acetone was given better result in the comparison of other tried solvent system.

Therefore it is concluded that this developed solvent system can be used as substituted solvent system for separation of seminal protein that is Choline and Spermine. Developed solvent system acetic acid: acetone is better than conventional solvent system 1N HCl. One more advantage of developed solvent system is that it is less harmful as compare to conventional solvent system because of its less acidic characteristic, so it can be used as a safer and as a best solvent system.

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