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A Review of the Forensic Applications for the Analysis of Biological Fluids Using Fluorescence Spectroscopy

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A review of the forensic applications for the analysis of

biological fluids using fluorescence spectroscopy

An honors thesis presented to the Department of Biological Sciences, University at Albany, State University of New York in partial fulfillment of the requirements for graduation with Honors in Biology and graduation from The Honors College

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Abstract

The use of science to compile information to assist in solving crimes is known as forensic science. Numerous bodily fluids, such as blood, semen, saliva, and vaginal fluid, are frequently discovered at crime scenes. This makes it crucial for forensic scientists to use analytical methods to examine these fluids to learn more about the crime, including the offender, victim, time since deposition, and other details. Although there are many techniques, the focus of this paper is fluorescence spectroscopy. Fluorescence spectroscopy is a rapid, inexpensive, and non-destructive technique used for forensic examination (Weber & Lednev, 2022). Fluorescence spectroscopy can be used to analyze the characteristics of the molecules and atoms in the samples, such as the presence of proteins and other biomolecules, which can help identify the type of fluid, and the time since deposition ("What Is Fluorescence Spectroscopy?" n.d.). For these reasons, it is important to analyze and compare fluorescence of all forensically relevant body fluids. In this paper, fluorescence spectroscopy techniques done on select body fluids (semen, sweat, urine, vaginal secretions, and saliva) will be discussed to determine effective techniques and possibilities for future research. Previous reviews have been done on peripheral and menstrual blood, which is why they will not be included in this review (Weber et al., 2021; Wójtowics et al., 2021).

Keywords: fluorescence spectroscopy, forensics, body fluids

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I would like to first thank Dr. Lednev for welcoming me into his lab and allowing me the opportunity to do research. My research mentor, Alexis Weber, has been a teacher to me through this process and has provided me with feedback so I can improve my skills. Dr. Lednev and Alexis have supported me through many obstacles, and I am grateful for the time and effort they put into me. I am also thankful to Dr. Thurman for agreeing to be my second reader and for sharing his thoughts on my work.

I am thankful to have many friends who supported me throughout my years at college and pushed me to do my best.

Last, thank you to my parents and my siblings because I would not be who I am without them, and I am grateful for the time we have had together. I would not be here without all my parents have done for me.

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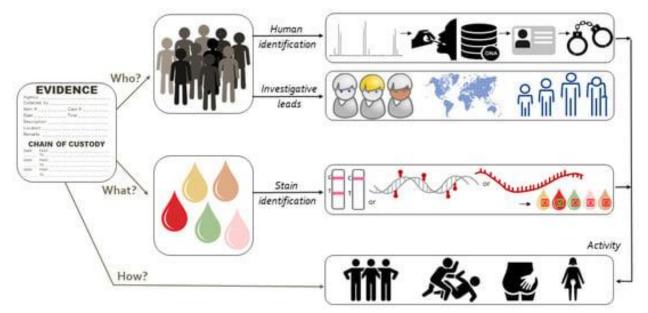
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Introduction

Forensic science is the application of science to the law to gather data about crimes. Many body fluids are commonly found at crime scenes, including blood, semen, saliva, and vaginal fluid. This makes it important to analyze these fluids using forensics to obtain information about the crime, including the perpetrator, the nature of the crime, victim, time since deposition, and more (Sijen & Harbison, 2021). Figure 1 shows the importance of body fluid analysis in context of the forensic process. There are a variety of tests that can be done on body fluids, including identification and species differentiation (Sijen & Harbison, 2021). Identifying the fluid is important because it is the first step before more analysis can be done and can influence the outcome of a case (Virkler & Lednev, 2009).

Figure 1



Importance of body fluid analysis.

Note. From Sijen & Harbison, 2021.

The different methods are split into presumptive and confirmatory tests. Presumptive tests are based on assumptions about the possible identity of the fluid and identify components that may be found in other fluids, which makes these methods prone to false positives. Presumptive tests can

also take a long time and destroy the sample. Examples include presumptive color tests. Presumptive color tests involve applying chemicals to the biological stain. Depending on investigator assumptions about the possible identity of the fluid, different tests will be used. For example, if a fluid is presumed to be blood, it can react with phenolphthalein, which will react with the heme in blood and turn pink (Bureau of Criminal Apprehension, n.d.). If the fluid is presumed to be saliva, it can be reacted with phadebas, which will detect amylase in saliva and release blue dye (Bureau of Criminal Apprehension, n.d.). Confirmatory testing is more specific and sensitive but also destroys the sample. For example, once a presumptive blood test shows a positive result, confirmatory tests can be used to confirm the identity of the fluid, including microscope tests (Virkler & Lednev, 2009). Microscope tests involve identifying blood cells by visualizing them in liquid blood (Virkler & Lednev, 2009). Seeing the red and white blood cells and fibrin confirms the presence of blood (Virkler & Lednev, 2009).

Testing can be based on each body fluid having a unique composition as shown in Table 1 (Zapata et al., 2015). Current and past methods of testing for each fluid are shown in Table 2 and more recent tests are shown in Table 3 (Virkler & Lednev, 2009). Table 2 and Table 3 show that there is a lack of confirmatory and non-destructive methods that can be applied to multiple fluids, which is why fluorescence spectroscopy can be useful (Virkler & Lednev, 2009).

Table 1

Blood	Semen	Saliva	Vaginal fluid	Urine	Sweat
Hemoglobin	Acid phosphatase	Amylase	Acid phosphatase	Urea	Chloride
Fibrinogen	Prostate-specific antigen	Lysozyme	Lactic acid	Creatinine	Sodium
Erythrocytes	Spermatozoa	Mucin	Citric acid	Uric acid	Urea
	Choline		Urea		
	Spermine		Vaginal peptidase		
	Semenogelin				
	Urea				

Components of body fluids.

Note. From Zapata et al., 2015.

Table 2

More recently developed tests.

Body fluid	Component	Classification	Method(s)
Blood	Whole fluid Hemoglobin Antibodies mRNA	ALS Spectroscopic Immunological	IR light XRF (iron) Lateral flow test strip – Antiglycophorin A SPTB/PBCD, HBA, ALAS2/SPTB (RT-PCR)
Semen	Whole fluid Elements mRNA Semenogelin	ALS Spectroscopic Immunological	Lumatec Superlight 400 XRF (zinc) PRM1, PRM1/PRM2, KLK (RT-PCR) RSID-Semen Test, Nanotrap Sg
Saliva	Whole fluid Amylase Antibodies mRNA	ALS Chemical Spectroscopic Immunological Immunological	Lumatec Superlight 400 SALIgAE [®] Fluorescence spectroscopy (swabbed from skin) RSID-Saliva Test Amylase ELISA, Lateral flow test strips HTN3, STATH/HTN3 (RT-PCR)
Vaginal fluid	Antibodies mRNA	Immunological Immunological	E2-17β ELISA MUC4, HBD-1/MUC4 (RT-PCR)
Sweat	Antibodies	Immunological	G-81 ELISA

Codes: grey: presumptive; dark grey: confirmatory; *italic*: non-destructive; and **bold**: applicable to multiple fluids.

Note. From Virkler & Lednev, 2009.

Table 3

Current and past tests.

Body fluid	Component	Classification	Method(s)
Blood	Whole fluid	ALS	Polilight [®]
	Hemoglobin	Chemiluminescent	Luminol, Fluorescein, Bluestar ²⁸
		Chemical	Benzidine, Kastle-Meyer, O-toludine, TMB/Hemastix [®] , LMG
		Crystal test	Teichman, Takayama
		Spectroscopic	UV-vis, Fluorescence (hematoporphyrin)
		Chromatographic	PC, TLC
	Elements	Spectroscopic	SEM-EDX
	Isozymes	Immunological	LDH
	Antibodies	Immunological	HemeSelect [™] , ABAcard [®] , HemaTrace [®] , Hexagon OBTI
		Immunological	ELISA
Semen	Whole fluid	ALS	Wood's Lamp, Bluemaxx BM500, Polilight [®]
	Enzymes	Chemical	SAP, LAP, GDA, CAP, y-GTP
	undymes	Immunological	GGT ELISA
	Choline	Crystal test	Florence test
	chonne	Chemical	Choline oxidase
		Chemiluminescent	Choline oxidase/Luminol solution
		Electrophoresis	Isotachophoresis
		Chromatographic	HPLC, PC, TLC
	Spermine	Electophoresis	Capillary tube electrophoresis
	Spermine		
	El	Crystal test	Barberio test, Puanen's test
	Elements	Chemical	Zinc paperstrip test
		Spectroscopic	SEM-EDX
	Spermatozoa	Microscopic	Christmas tree stain
	Antigens	Immunological	PSA, SVSA
	19-OH $F_{1\alpha}/F_{2\alpha}$		Radioimmunoassay
	Antibodies		1E5 ELISA, SAP ELISA
	Isozymes		LDH
Saliva	Whole fluid	ALS	UV light, High intensity quartz are tubes
	Amylase	Chemical	Starch-iodine, Phadebas [®] , Amylose Azure, Rapignost [®] -Amylas
	Antibodies	Immunological	ELISA, Immunodiffusion
	Elements	Spectroscopic	SEM-EDX
Vaginal fluid	Gly. epithelial cells	Chemical	PAS reagent
	Vaginal peptidase	Electrophoresis	Starch gel/L-valyl-L-leucine
	Antibodies	Immunological	Oestrogen receptors
	Lactate/citrate	Electrophoresis	Capillary isotachophoresis
Urine	Whole fluid	ALS	UV light
	Epithelial cells	Microscopic	Visualize cells (no stains)
	Urea	Chemical	Nessler's, DMAC, Urease/bromothymol blue
	Creatinine		Jaffe test, Salkowski test
	THP	Immunological	Sandwich ELISA, SP radioimmunoassay
	UA/UN	Chemical	TZ-UA One/Urea NB-test
	Elements	Spectroscopic	SEM-EDX
	17-Ketosteroid conj.	Chromatographic	HPLC, ESI-LC-MS, PC, TLC
Sweat	Elements	Microscopic	SEM-EDX

Codes: grey: presumptive; dark grey: confirmatory; italic: non-destructive; and **bold**: applicable to multiple fluids.

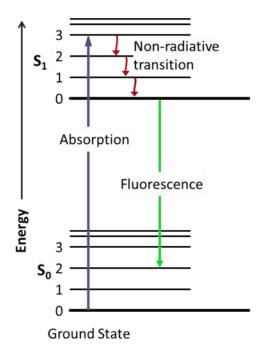
Note. From Virkler & Lednev, 2009.

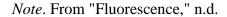
Fluorescence spectroscopy is a rapid, inexpensive, and non-destructive technique, which makes it ideal to use for forensic analysis. Fluorescence spectroscopy can be used to determine properties of the molecules and atoms in the samples, including the presence of specific proteins and other biomolecules, which can help determine time since deposition, and what body fluid it is ("What Is Fluorescence Spectroscopy?," n.d.). For these reasons, it would be beneficial to analyze and compare fluorescence of all forensically relevant body fluids. This paper will discuss the use of fluorescence spectroscopy to analyze body fluids with the exception of blood because there have already been reviews done on peripheral and menstrual blood (Weber et al., 2021; Wójtowics et al., 2021) within the Lednev laboratory. The goal is to determine effective techniques, avenues for future research, and gaps in current research.

When the molecules or atoms in the sample are excited by light, they emit light at a different wavelength, which is known as fluorescence (*An introduction to fluorescence spectroscopy*, 2000). Fluorescence spectroscopy analyzes the light emitted from a sample ("What Is Fluorescence Spectroscopy?," n.d.). Before the sample absorbs photons, it is in the singlet ground state, and it is promoted to a singlet excited state ("Fluorescence," n.d.). The excited state undergoes rapid thermal energy loss to the environment due to vibrations, which then causes a photon to be emitted from the lowest-lying singlet excited state ("What Is Fluorescence Spectroscopy?"). After absorption, there is also non-radiative relaxation with no photon emission ("Fluorescence," n.d.). Figure 2 shows a diagram of absorbance, non-radiative decay, and fluorescence.

Figure 2

Diagram of absorbance, non-radiative decay, and fluorescence.



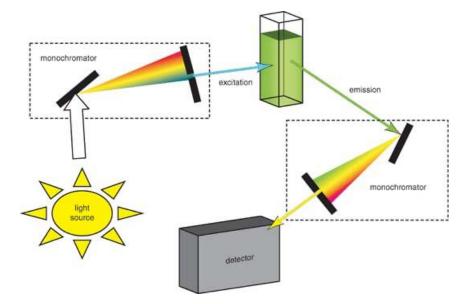


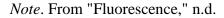
A fluorescence spectrometer works by shining a light of a specific wavelength on a sample that contains molecules or atoms that are capable of fluorescence (*An introduction to fluorescence spectroscopy*, 2000). The light is generated by the light source and is directed onto the sample using the optical system (*An introduction to fluorescence spectroscopy*, 2000). The sample is placed in the sample holder or cuvette, which allows the light to pass through it (*An introduction to fluorescence spectroscopy*, 2000). The monochromator is used to select the specific wavelength of light that will be used to excite the molecules or atoms in the sample (*An introduction to fluorescence spectroscopy*, 2000). The light that is emitted is directed towards a filter and detector for measurement and identification of the sample or changes in the sample ("What Is Fluorescence Spectroscopy?," n.d.). The data is sent to the data analysis system, which processes the data collected by the detector and displays the results in a usable format (*An introduction to*

fluorescence spectroscopy, 2000). Figure 3 shows a schematic representation of a fluorescence spectrometer.

Figure 3

Schematic representation of a fluorescence spectrometer.

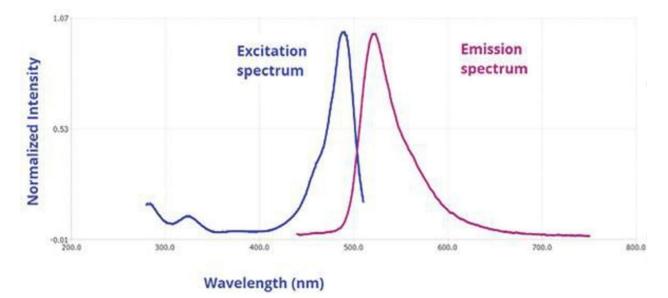




To measure steady state fluorescence spectra, a sample is placed in a fluorescence spectrometer and exposed to light of a specific wavelength. The amount of fluorescence that is emitted by a sample at a given time, as a function of the wavelength of the emitted light, is measured. A fluorescence emission spectrum is a graph that shows the intensity of fluorescence emitted by a sample as a function of the wavelength of the emitted light ("What Is Fluorescence spectroscopy?," n.d.). A fluorescence excitation spectrum is a graph that shows the intensity of fluorescence emitted by a sample as a function of the wavelength of the light used to excite the sample ("Fluorescence," n.d.). To measure a fluorescence excitation spectrum, a sample is placed in a fluorescence spectrometer and exposed to light of different wavelengths ("Fluorescence," n.d.). The emission and excitation spectra are mirror images of each other for a given fluorophore (or a sample that re-emits lights after light excitation and uses vibrational relaxation to return to its ground state), and the emission spectrum is usually at higher wavelengths ("What Is Fluorescence Spectroscopy?," n.d.; "Fluorescence," n.d.). Figure 4 demonstrates an excitation and emission spectra. Shifting toward a longer wavelength is a Stokes shift ("Fluorescence," n.d.).

Figure 4

Excitation and emission spectrum.



Note. From "What Is Fluorescence Spectroscopy?," n.d.

This paper will focus on analysis done on semen, vaginal secretions, saliva, sweat, and urine using fluorescence spectroscopy for forensic applications and will discuss implications of this work.

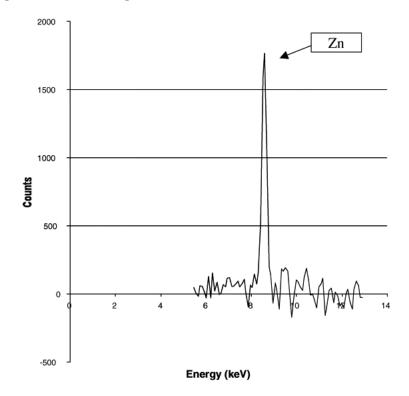
Semen

Fluorescence spectroscopy has been used to detect zinc levels as well as proteins and fluorescent oxidation products to identify semen.

Research using fluorescence spectroscopy was done by monitoring protein-lipid oxidation reactions, and aging models were used to determine the time since deposition of semen. They studied aging in dark conditions at room temperature. However, the paper explains that more research needs to be done in different environmental conditions and to determine the key components responsible for the fluorescent behavior of semen stains (Achetib, Wilk, et al., 2019). The study was able to successfully determine time since deposition for up to 16 days with a median absolute error of 1.7 days (Achetib, Wilk, et al., 2019).

A general-purpose x-ray fluorescence system was built to analyze trace and invisible materials at crime scenes; this instrument is non-destructive, uses elemental composition, is portable, and has been successful at detecting semen (Schweitzer et al., 2005; Trombka et al., 2002). Semen has high zinc concentrations, and the instrument was able to detect the significant zinc signal in around a minute (Trombka et al., 2002). Figure 5 shows the zinc peak from the semen sample spectrum. Figure 6 shows an image of the handheld instrument.

Figure 5

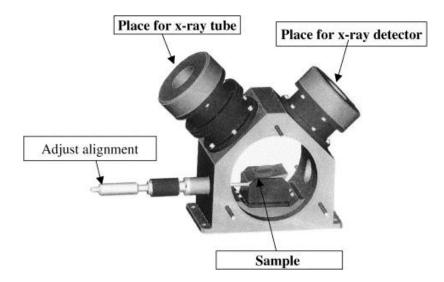


Zinc peak in semen sample.

Note. From Trombka et al., 2002

Figure 6

Schematic of the portable field XRF unit.



Note. From Trombka et al., 2002

Vaginal secretions

There are currently limited studies using fluorescence spectroscopy on vaginal secretions for forensic purposes. Techniques done on other fluids that might be applicable to vaginal secretions will be discussed in the conclusion.

Saliva

Fluorescence spectroscopy of saliva uses tryptophan, "an endogenous fluorophore in alphaamylase," as a prevalent probe in dried saliva stains on human skin (Upadhyay et al., 2023). Saliva was found to have an emission spectrum at 345–355 nm with excitation at 283 nm; however, the broad excitation peak at 283 nm and the 345–355 nm emission peak has been seen with semen and fingermarks as well (Achetib, Falkena, et al., 2023).

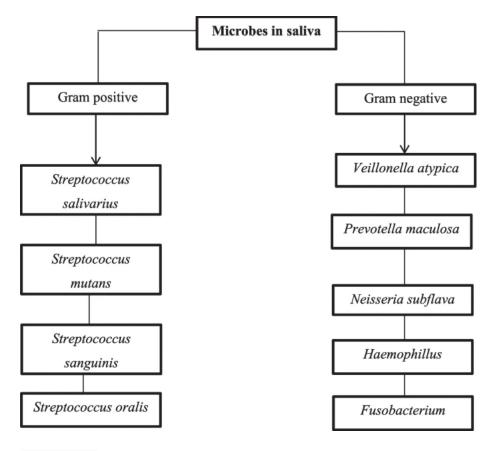
Soukos et al. (2000) demonstrated that fluorescence spectroscopy can be used to detect dried saliva rapidly and non-destructively on skin. Eighty-two volunteers were used, and water was the control. Swab samples were dissolved in 0.1M KCl solution, and emission spectra were obtained and "characterized by a principal maximum at 345-355nm with excitation at 282nm." The fluorescence emission intensity was more than the background readings from the control in 97.6%.

The fluorescence of saliva samples was similar to aqueous samples of pure amylase and tryptophan. The emission peak at 345-355nm with excitation at 282nm is a strong indicator of saliva.

Li et al. (2019) demonstrated that bacteria in saliva can be targeted with fluorescence spectroscopy to detect saliva. They used the nanoprobe, with fluorescence excitation/emission maxima at 320/410 nm, to detect *S. salivarius* levels. The detection limit was found to be 25 cfu·mL⁻¹, and the assay can be done in 40 min. The nanoprobe was used in samples with blood, urine, and semen and positive results were obtained for samples containing saliva while samples without saliva showed negative results. Figure 7 shows the different microbes that are in saliva.

Figure 7

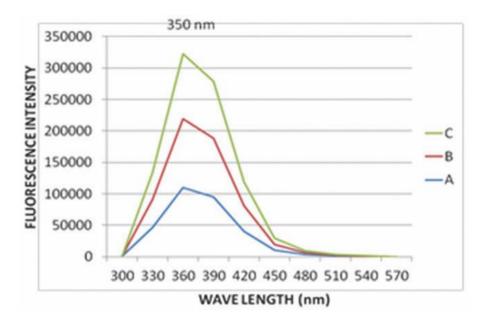
Microbes in saliva.



Note. From Upadhyay et al., 2023

Charan et al. (2018) confirmed that saliva can be detected using fluorescence spectroscopy due to saliva containing α -amylase, which has tryptophan (an aromatic amino acid constituent). When saliva is excited at 254 nm, there is a characteristic emission spectrum at 345-355 nm. A limitation is that this study had a small sample size. Figure 8 shows the emission spectrum for tryptophan.

Figure 8



Emission spectrum for tryptophan.

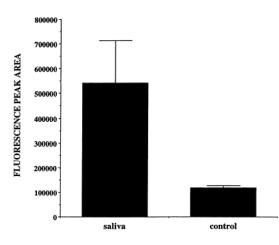
Denny et al. (2018) found that fluorescence spectroscopy can be used to detect saliva stains from the surface of a drinking glass. This study hypothesized that fluorescence spectroscopy can be used to detect saliva from other inanimate objects, including envelopes and cigars. The fluorescence emission spectra of dried saliva samples collected from drinking glasses were compared to the spectra from the undiluted liquid saliva from the same volunteers. The presence of saliva was predicted from the emission spectra of the enzyme amylase around 350 nm. The dried saliva stains and undiluted liquid saliva had an emission peak at 350 ± 5 nm and 345 ± 5 nm, respectively. The study also finds that fluorescence intensity and area under the curve can be used

Note. From Charan et al., 2018

to detect saliva. Figure 9 shows the fluorescence peak area for saliva vs the control. Figure 10 shows the average emission spectra when the saliva and control samples are excited at 282 nm.

Figure 9

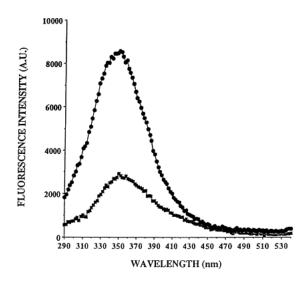
Fluorescence peak areas.



Note. From Denny et al., 2018

Figure 10

Average emission spectra.



Note. From Denny et al., 2018

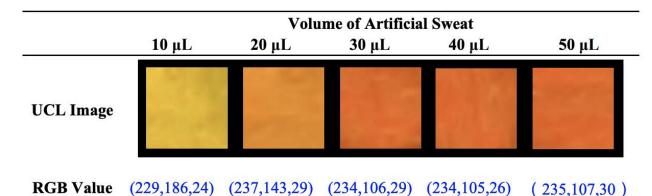
Nanda et al. (2011) also confirms that α amylase has a characteristic spectrum at 345-355 nm when excited at 282 nm using fluorescence spectroscopy. The sample size was 10, which could be a limitation.

Sweat

Studies on sweat are limited; however, Yu et al. (2023), van Dem et al., (2014), and Khosroshahi and Woll-Morison (2021) conducted research using fluorescence spectroscopy to detect fingerprints, and sweat is a component of fingerprints, which could mean these techniques can be used to design future studies for sweat detection using fluorescence spectroscopy. Khosroshahi and Woll-Morison (2021) found that sweat played a role in allowing fluorescence when analyzing fingerprints on glass using a 405 nm later and phase contrast microscope.

Hu et al. (2023) used visual wearable devices that detected changing urea levels using fluorescence spectroscopy. Urea is a major component of sweat. Figure 11 shows the change in the patch for different volumes of artificial sweat.

Figure 11



Patch based on volume of artificial sweat.

Note. From Hu et al., 2023

Urine

Studies with urine tend to focus on identifying drugs in urine because drug abuse is growing, and a rapid and consistent method is needed to determine the presence of these substances. Chung et al. (2001) demonstrated that non-aqueous capillary electrophoresis–fluorescence spectroscopy can be used to detect and identify 3,4-MDMA. They used a cryogenic

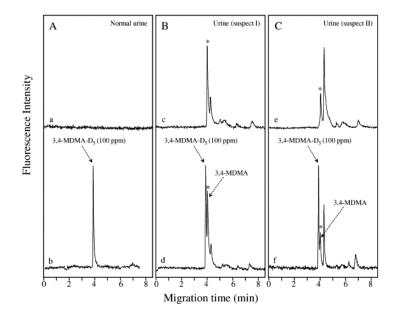
molecular fluorescence technique at 77 K. It took less than 12 min under optimized conditions to achieve baseline separation of the selected compounds.

Huang et al. (2003) used capillary electrophoresis/fluorescence spectroscopy to detect 3,4-MDMA isomers ingested through tablets in urine samples. They state that "several electrophoretic parameters, such as the concentration of β -cyclodextrin used in the electrophoretic separation and the amount of organic solvents required for the separation, were optimized" (Huang et al., 2003).

Fang et al. (2002) used nonaqueous capillary electrophoresis/fluorescence spectroscopy to detect 3,4-MDMA in urine. They found it is possible to detect 3,4-MDMA at the level 0.5 ppm without any pre-treatment in less than 5 min. After liquid–liquid extraction, the sample is condensed and "a detection limit of 50 ppb (S/N=3) can be achieved." As opposed to the conventional GC/MS method, it is not necessary to derivatize the 3,4-MDMA before injection. The GC migration time is more than 20 min. Figure 12 shows fluorescence intensity for different samples. Figure 13 shows changes to fluorescence area when analyzing a sample spiked with 3,4-MDMA and a spiked sample after liquid-liquid extraction.

Figure 12

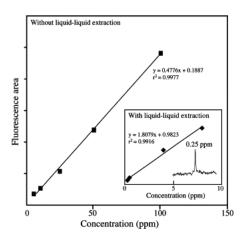
Fluorescence intensity per sample.



Note. From Fang et al., 2002

Figure 13

Fluorescence area for different samples.



Note. From Fang et al., 2002

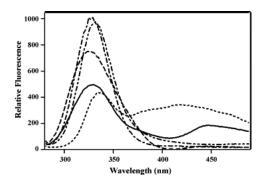
Multi-fluid studies

In addition to the studies presented for each body fluid, there are studies that included all or multiple of the fluids, which is a step towards developing a method that can be used on multiple fluids.

There was a study that used fluorescence spectroscopy to determine the emission spectra of semen, saliva, and urine from multiple excitation wavelengths over a wide range of wavelengths (Virkler & Lednev, 2009). This technique was rapid, usable for multiple fluids, non-destructive, has no contact with the sample, was able to cover a large region, and is highly sensitive (Virkler & Lednev, 2009). However, there might have been photodegradation from exposure to the 260-nm UV light (Virkler & Lednev, 2009). Figure 14 shows the emission spectra of the different fluids that were excited at 260 nm.

Figure 14

Fluorescence emission spectra.



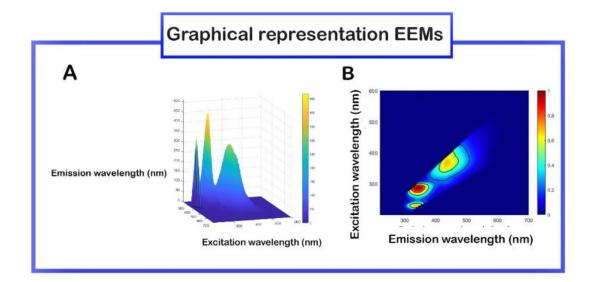
Note. Semen (—), saliva (----), and urine (-----). From Virkler & Lednev, 2009.

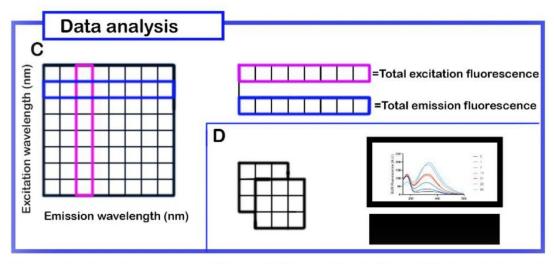
There was a study that used a handheld fluorescence device that could possibly be used at the scene of the crime to identify body fluids if combined with the right software (Virkler and Lednev, 2009). Research has not yet confirmed the device's usefulness at identifying fluids.

Achetib, Falkena, et al. (2023) analyzed semen, urine, and saliva as they aged at 81 excitation wavelengths from 200 to 600 nm and recorded emission from 220 to 700 nm. "The total emitted fluorescence intensities of specific fluorescent signatures in the UV–visible range were summed, and principal component analysis was performed to cluster the body fluids. Three combinations of four principal components allowed specific clustering of the body fluids" (Achetib, Falkena, et al., 2023). The study showed that 71.4% of unknown samples from a blind test could be correctly identified (Achetib, Falkena, et al., 2023). Figure 15 shows a simplified procedure for this study.

Figure 15

Simplified method for study.





Schematic overview of data analysis to produce the body fluid signatures. (A) 3D view of excitation and emission map (EEMs) of a 2 weeks old semen stain. (B) 2D view of corrected and normalized EEMs of two weeks old semen stain. (C) Compute the summed fluorescence of all excitation wavelengths for each emission wavelength (pink). Compute the summed fluorescence of all emission wavelengths for each excitation and emission wavelength (blue). (D) Repeat procedure for each excitation and emission wavelength for every donor on every time point, and plot the average fluorences of all donors for each time point showing the bodyfluid specific fluorescent signatures.

Note. From Achetib, Falkena, et al., 2023

Twitchett et al. (1978) combined high-performance liquid chromatography (HPLC), fluorescence spectroscopy, and radioimmunoassay to analyze urine samples with LSD and detect its metabolites. This technique was rapid and sensitive. This methodology has been used to analyze

LSD in body fluids for forensic and clinical purposes. Levels as little as 0.5 ng of LSD per ml can be detected using the minimum amount of sample.

Discussion

Due to the importance of analyzing body fluids to determine information about a crime, there is a need for body fluid analysis methods that can work on multiple body fluids and are nondestructive, rapid, and confirmatory. Since the fluorescence of body fluids was discovered, this characteristic has been used in forensics to analyze body fluids. Fluorescence spectroscopy is an upcoming method for body fluid analysis that could solve current problems. Because it is still relatively new, more studies are needed before fluorescence spectroscopy is accepted as an effective method.

A limitation is that x-ray fluorescence is based on elemental analysis of body fluids, which can make differentiation difficult for similar stains (Zapata et al., 2015). Since fluorophores emitting fluorescence in certain regions are common to all body fluids, further testing or other methods might be needed to allow differentiation of body fluids that have subtle differences in fluorescence in the same spectral region. Fluorescence spectroscopy has few and broad peaks, which could be a limitation when compared to other methods of spectroscopy (Virkler & Lednev).

Although some fluids (mainly vaginal secretions and sweat) have less research done, there are other studies that can be adapted and might be successful at identifying these fluids. For vaginal secretions, there was a study that used fluorescence spectroscopy for age determination of fingermarks; the authors state that the method—protein-lipid emulsions, which seems similar to the method done by Achetib, Wilk, et al. (2019) to estimate time since deposition of semen traces—might work for semen and vaginal fluid (van Dam et al., 2014).

While there are limitations and more research needs to be done, fluorescence spectroscopy seems promising for body fluid analysis. Studies mentioned earlier used portable instruments,

which indicates the possibility of having instruments that can identify fluids at the scene of the crime. This development will be helpful in sample collection. Studies mentioned earlier demonstrated that fluorescence spectroscopy is a method that can be used on multiple fluids, is confirmatory, nondestructive, rapid, portable, and inexpensive. As more research is done, fluorescence spectroscopy might be the solution to current problems with body fluid analysis methods.

References

- (2000). An introduction to fluorescence spectroscopy. PerkinElmer. https://www.chem.uci.edu/~dmitryf/manuals/Fundamentals/Fluorescence%20Spectroscop y.pdf
- Achetib, N., Falkena, K., Swayambhu, M., Aalders, M. C. G., & van Dam, A. (2023). Specific fluorescent signatures for body fluid identification using fluorescence spectroscopy. *Scientific Reports*, *13*. https://doi.org/10.1038/s41598-023-30241-7
- Achetib, N., Wilk, L. S., Schwarz, J. C. V., Lambrechts, S. A. G., van Leeuwen T. G., Aalders,
 M. C. G., & van Dam, A. (2019). Estimating the time of deposition of semen traces using
 fluorescence protein–lipid oxidation signatures. *Analytical Chemistry*, *91*(5), 3204-3208.
 https://doi.org/10.1021/acs.analchem.8b05625
- Bureau of Criminal Apprehension. (n.d.). *Serology*. Retrieved April 24, 2023, from https://dps.mn.gov/divisions/bca/bca-divisions/forensic-science/Pages/dna-serology.aspx
- Charan, K. R., Komali, G., Karunakar, P., Rajkumar, B., Pratima, S., & Reddy, L. (2018). Saliva as forensic evidence using fluorescent spectroscopy: A pilot study. *Journal of Clinical* and Diagnostic Research, 12(9), ZC27-ZC29. https://doi.org/10.7860/JCDR/2018/35796.12035
- Chung, Y.-L., Liu, J.-T., & Lin, C.-H. (2001). On-line identification of 3,4methylenedioxymethamphetamine in human urine by non-aqueous capillary electrophoresis–fluorescence spectroscopy at 77 K. *Journal of Chromatography B: Biomedical Sciences and Applications, 759*(2), 219-226. https://doi.org/10.1016/S0378-4347(01)00225-0

- Denny, S. E., Nazeer, S. S., T.T., S., Nair, B. J., & Jayasree, R. S. (2018). Forensic application of fluorescence spectroscopy: An efficient technique to predict the presence of human saliva. *Journal of Luminescence*, 203, 696-701. https://doi.org/10.1016/j.jlumin.2018.07.022
- Fang, C., Chung, Y.-L., Liu, J.-T., & Lin, C.-H. (2002). Rapid analysis of 3,4methylenedioxymethamphetamine: A comparison of nonaqueous capillary electrophoresis/fluorescence detection with GC/MS, *Forensic Science International*, *125*(2-3), 142-148, https://doi.org/10.1016/S0379-0738(01)00636-3
- Fluorescence. (n.d.). LibreTexts Chemistry. Retrieved April 24, 2023, from https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_ Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Spectroscopy/Elec tronic_Spectroscopy/Radiative_Decay/Fluorescence
- Hu, B., Kang, X., Xu, S., Zhu, J., Yang, L., & Jiang, C. (2023). Multiplex chroma response wearable hydrogel patch: Visual monitoring of urea in body fluids for health prognosis.
 Analytical Chemistry, 95(7), 3587–3595. https://doi.org/10.1021/acs.analchem.2c03806
- Huang, Y.-S., Liu, J.-T., Lin, L.-C., & Lin, C.-H. (2003). Chiral separation of 3,4methylenedioxymeth-amphetamine and related compounds in clandestine tablets and urine samples by capillary electrophoresis/fluorescence spectroscopy.
 ELECTROPHORESIS, 24(6), 1097-1104. https://doi.org/10.1002/elps.200390128
- Khosroshahi, M.E., & Woll-Morison, V. (2021). Visualization and fluorescence spectroscopy of fingerprints on glass slide using combined 405 nm laser and phase contrast microscope. *Journal of Visualization*, 24, 665–670. https://doi.org/10.1007/s12650-021-00745-3
- Li, X., Ding, Y., Ling, J., Yao, W., Zha, L., Li, N., Chang, Y., Wang, Y., & Cai, J. (2019). Bacteria-targeting BSA-stabilized SiC nanoparticles as a fluorescent nanoprobe for

forensic identification of saliva. *Microchimica Acta*, *186*. https://doi.org/10.1007/s00604-019-3890-y

- Schweitzer, J. S., Trombka, J. I., Floyd, S., Selavka, C., Zeosky, G., Gahn, N., McClanahan, T., & Burbine, T. (2005). Portable generator-based XRF instrument for non-destructive analysis at crime scenes. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, 241(1–4), 816-819. https://doi.org/10.1016/j.nimb.2005.07.137
- Sijen, T., & Harbison, S. (2021). On the identification of body fluids and tissues: A crucial link in the investigation and solution of crime. *Genes*, 12(11), 1728. https://doi.org/10.3390/genes12111728
- Soukos, N.S., Crowley, K., Bamberg, M. P., Gillies, R., Doukas, A. G., Evans, R., & Kollias, N. (2000). A rapid method to detect dried saliva stains swabbed from human skin using fluorescence spectroscopy. *Forensic Science International*, *114*(3), 133-138. https://doi.org/10.1016/s0379-0738(00)00292-9
- Trombka, J. I., Schweitzer, J., Selavka, C., Dale, M., Gahn, N., Floyd, S., Marie, J., Hobson, M., Zeosky, J., Martin, K., McClannahan, T., Solomon, P., & Gottschang, E. (2002). Crime scene investigations using portable, non-destructive space exploration technology. *Forensic Science International, 129*(1), 1-9. https://doi.org/10.1016/S0379-0738(02)00079-8
- Twitchett, P. J., Fletcher, S. M., Sullivan, A.T., & Moffat, A.C. (1978). Analysis of LSD in human body fluids by high-performance liquid chromatography, fluorescence spectroscopy and radioimmunoassay. *Journal of Chromatography A*, (150)1, 73-84. https://doi.org/10.1016/S0021-9673(01)92097-7

- Upadhyay, M., Shrivastava, P., Verma, K. & Joshi, B. (2023). Recent advancements in identification and detection of saliva as forensic evidence: A review. *Egyptian Journal of Forensic Sciences*, *13*. https://doi.org/10.1186/s41935-023-00336-3
- van Dam, A., Schwarz, J.C.V., de Vos, J., Siebes, M., Sijen, T., van Leeuwen, T.G., Aalders, M.C.G., & Lambrechts, S.A.G. (2014). Oxidation monitoring by fluorescence spectroscopy reveals the age of fingermarks. *Angewandte Chemie International Edition*, 53(24), 6272-6275. https://doi.org/10.1002/anie.201402740
- Virkler, K., & Lednev, I. K. (2009). Analysis of body fluids for forensic purposes: From laboratory testing to non-destructive rapid confirmatory identification at a crime scene. *Forensic Science International, 188*(1-3), 1-17. https://doi.org/10.1016/j.forsciint.2009.02.013
- Weber, A., & Lednev, I. K. (2022). Brightness of blood: Review of fluorescence spectroscopy analysis of bloodstains. *Frontiers in Analytical Science*, 2. https://doi.org/10.3389/frans.2022.906532
- Weber, A., Wójtowicz, A., & Lednev, I. K. (2021). Post deposition aging of bloodstains probed by steady-state fluorescence spectroscopy. *Journal of Photochemistry and Photobiology B: Biology*, 221, 112251. https://doi.org/10.1016/j.jphotobiol.2021.112251
- What Is Fluorescence Spectroscopy? (n.d.). HORIBA Scientific. Retrived April 24, 2023, from https://www.horiba.com/sgp/scientific/technologies/fluorescence-spectroscopy/what-is-fluorescence-spectroscopy/
- Wójtowics, A., Weber, A., Wietecha-Posłuszny, R., & Lednev, I. K. (2021). Probing menstrual bloodstain aging with fluorescence spectroscopy. *Science Direct*, 248, 119172. https://doi.org/10.1016/j.saa.2020.119172

Zapata, F., Fernández de la Ossa, M. Á., & García-Ruiz, C. (2015). Emerging spectrometric techniques for the forensic analysis of body fluids. *TrAC Trends in Analytical Chemistry*, 64, 53-63. https://doi.org/10.1016/j.trac.2014.08.011