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Identification of urine samples obtained in forensic cases by fourier transform infrared spectroscopy

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Abstract

Forensic sciences are a multidisciplinary science that covers all branches of science for justice. Forensic genetics is one of the sub-branches of forensic sciences. It is a branch of science that contributes to establishing a connection between crime and criminal by using all kinds of biological evidence (blood, semen, saliva, urine, vaginal fluid, hair, hair, sweat, saliva, etc.) and reaching the perpetrator with the genetic information obtained. Serological and immunological tests are used in the identification of body fluids found as evidence at the crime scene. The number of serological tests used in the past and today is limited; not all body fluids can be analyzed routinely. Many of the existing tests are not directly specific to the body fluid but are intended to identify intermediate metabolites. One such example is the urine sample. In the identification of urine, the presence of creatinine and amines in the urine is often used. In addition, these tests require the expenditure of separate consumables to identify each body fluid. For this reason, with the development of technology, in recent years, the use of spectroscopic methods that provide cost-free and easy analysis in the identification of body fluids has increased. Fourier Transform Infrared (FTIR) spectroscopy is one of the spectroscopic methods used to identify body fluids from biological samples found at the crime scene without loss of quantity and without damaging the sample, quickly and without loss of consumables. This study investigated the identification of urine stains obtained from the crime scene with FTIR spectroscopy without using serological materials. Functional groups, which we can express as identification regions, were determined as 13 different distinguishing points in the relevant molecules and their usability in forensic serological analyses was determined.

Keywords: Forensic sciences, forensic genetics, identification of body fluids, FTIR spectroscopy, urine

Introduction

Forensic genetics is a branch of science that aims to reach the genetic information of the guilty person or persons based on biological samples (blood, semen, saliva, urine, feces, nails, teeth, bones, hair, etc.) found at the crime scene to reach the identity of the criminal in forensic cases such as sexual assault, murder, theft and to direct the investigation. Since all kinds of biological materials obtained at the crime scene are evidence that can lead us to the perpetrator, all kinds of biological materials encountered at the crime scene are evidence [1]. Biological stains on fabrics that can be obtained from any crime scene to be analyzed in the laboratory are not always visible. Therefore, they are very difficult to identify. Serological tests can be applied for unclear stains obtained from the crime scene [2-4]. In serological tests, more than one kit is used to detect a stain and this increases the cost. In addition, there is no specific test to determine what some biological samples are, while others are not sample-specific and determine metabolites. All components in body fluids are not different from each other. However, since the ratios of these common components in body fluids are different, body fluids can be identified. For example, although the urea component is common in sweat, urine, vaginal fluid, and semen, the amount of urea in urine is at a higher concentration than in sweat and semen. Therefore, urea is a distinguishing component of urine compared to other body fluids [5].

In routine, urine is not always detected and the number of direct urine-specific tests is limited. The identification of body fluids is very important for forensic science. Many body fluids are either

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invisible, present in very small quantities, or mixtures. Therefore, the identification of body fluids is not always easy. To identify a body fluid found at a crime scene, chemical or enzymatic tests, which are in the hypothetical test group and are limited in terms of sensitivity, are first used. Then, microscopic and immunological tests, which are in the group of confirmatory tests, are preferred [6]. FTIR (Fourier Transform Infrared) spectroscopy, a relatively new method for the identification of a stain, has been increasingly used in recent years. FTIR spectroscopy is a type of vibrational spectroscopy. This chemical analytical method, which measures the wave number against the infrared intensity of light, can be used to identify unidentified stains obtained at the crime scene. The advantage of this method is that the stains can be identified spectroscopically without loss of quantity and consumables in biological samples, leaving enough biological samples for DNA analysis, which is the next step [7,8]. When urine is evaluated specifically, the richness of biological sources such as urea, creatinine, uric acid, citrate, sulfate, phosphate, and nucleic acids in the urine structure stands out. These groups can be separated in urine by IR spectroscopy [3,9,10].

In order to eliminate such phenomena, FTIR spectroscopy, a new method for the identification of a stain, has been used in recent years. Thanks to FTIR spectroscopy, it is possible to determine what the stain is directly without cost and without damaging the sample. In the literature, there are no studies about forensic identification of urine samples by using FTIR spectroscopy. In this study, for the first time, urine samples on fabric were analyzed by using FTIR spectrophotometer and forensically identified to represent the samples from the crime scene.

In our study, the reference absorbance values were determined for urine in a non-forensic study conducted by Sarıgül et al. in 2021, in which spectroscopic analysis of urine was performed [10]. The overlaid IR spectrum of the distinguishing biomolecules in the urine sample of an adult individual is shown in Figure 1, and the functional groups of the urine components according to the spectrum and the wavelength numbers obtained in IR spectroscopy are shown in Table 1.

Table 1. Functional groups and wavelength numbers of urine [10]

Distinctive molecule*	Functional group	Wavelength number (cm^{-1})
Urea	N-H stretching	3420, 3334
	N-H deformation C-N-H vibrations	1609
***Urea*, creatinine**	N-H stretching	3196
***Creatinine,* NH_4^+**	Symmetric NH_4 stretching C-H (ring) stretching	3037
Urea, *uric acid*, creatinine, NH_4^+	N-H stretching $N-CH_3$ stretching C-H stretching	2815
Uric acid	N-H deformation	2673
***Urea*, uric acid, creatinine, proteins**	C=O stretching C=N stretching	1657
***Urea*, uric acid, creatinine**	C-H bending	1447
Uric acid, *creatinine*	C-N stretching	1345
***Creatinine*, uric acid, citrate**	C-N stretching CH2 rocking	1238
***Urea*, uric acid, citrate, sulfate**	C-NH2 stretching C-O stretching S-O stretching	1143
Creatinine	C-H stretching C-N-C stretching	1113
Urea, sulfate, *phosphate*, nucleic acids	P-O stretching NH2 stretching S=O stretching	1075
***Phosphate*, sulfate, nucleic acids**	S-O stretching P-OH stretching	929
Phosphate	P-OH bending	867
***Urea*, uric acid**	N-H wagging C-H (ring) bending	783

*The molecules shown in italic among the molecules that provide discrimination were found to have higher absorbance values at the wavelength number in question compared to the molecules with which they were written together

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When the spectra of urine and the components in urine were compared, it was observed that the same and/or different functional groups of the components gave similar peaks at similar wavelength numbers (Figure 1).

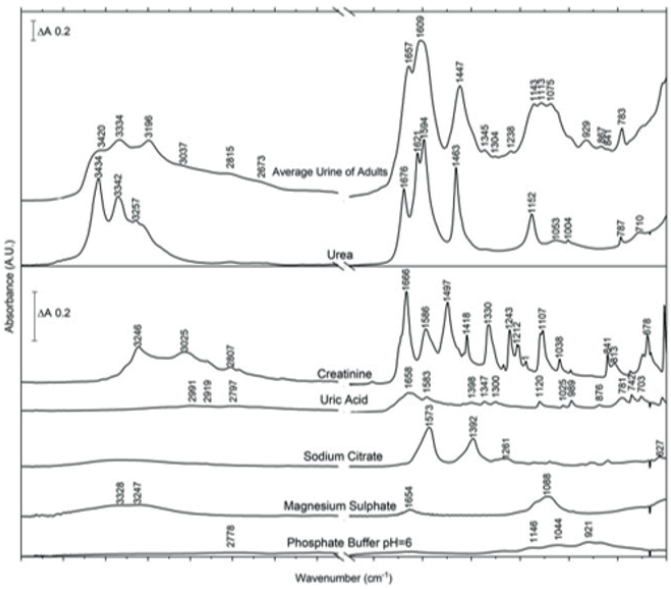

Figure 1. IR spectra of urine and its components [10]

This study aims to identify the urine stains that can be obtained from the crime scene with FTIR without any loss and to be used in the identification of urine samples in forensic cases so that it can be easily distinguished from other body fluids similar to urine such as seminal fluid.

Material and Methods

The sample group consisted of 40 healthy women and 40 men aged between 18-75 years. In the study, 80 individuals, all of whom volunteered and signed an informed consent form, were given sterile urine containers and asked to give urine samples. Before starting this study, Üsküdar University Non-Interventional Research Ethics Committee approval numbered 61351342/MAY 2022-34 was obtained.

Preparation of samples

To create a representative crime scene, two different fabric types, 100% cotton white fabric, and mixed patterned nylon fabric, were stained. The reason for using nylon fabrics is that stain detection on stained fabrics from the crime scene is easier on white- or solid-colored fabrics, whereas stain detection on mixed-patterned fabrics is not possible even under UV light. Considering such situations, it is also aimed to be able to analyze using FTIR spectroscopy. The fabrics preferred in the study were newly supplied and washed at 90°C to prevent any cross-contamination and then sterilized under UV light for 3 hours.

The two different fabric types were washed separately in an LG washing machine at 90°C and cotton program with detergent for 2 hours. The fabrics were allowed to dry in a closed environment. The scissors used to cut the fabrics were sterilized in an autoclave to prevent contamination. Each of the dried fabrics was cut as 10x7 cm2 and made ready. The cut fabrics were exposed to UV light for 3 hours and made ready for the staining process. The envelopes taken to pack the fabric pieces were also exposed to UV light for 3 hours and sterilized. Sterile fabric pieces were placed in sterile envelopes and packaged.

Creating the stains

The collected urine containers were stored at +4°C to prevent any bacterial contamination. During the staining process, one urine sample was stained with one piece of white cotton fabric and one piece of patterned nylon fabric. The fabric pieces were laid on a clean bench, and 3 mL of urine sample was dropped on them and allowed to dry at room temperature for 24 hours. The white cotton fabric pieces and patterned nylon fabric pieces were placed in sterile envelopes after the staining process and stored for examination in the FTIR spectrophotometer.

Collecting the spectral data

The study was carried out with an Agilent Cary 630 ATR-FTIR device. The dried stained fabrics were measured directly on the FTIR spectrophotometer device without any pretreatment in the range of 650-4000 cm^{-1} with 32 scans and 4 cm^{-1} resolution, and each sample was recorded separately. Before the analysis of each sample, the background was taken by scanning the stain-free area of the fabrics.

In our study, after 80 urine samples were analyzed for these functional groups, the mean value and standard deviation of the wavelengths obtained were calculated and the data belonging to the identification regions were determined.

After the data was collected, it was converted to .spc format. Then, the data in .spc format were converted to .csv format with the Spectragraphy program. PCA and Cluster analysis were performed with the Orange Data Mining program.

Results

In this study, the wavelengths of urinary urea, uric acid, creatinine, citrate, sulfate, and nucleic acid functional groups were successfully determined in all stained fabric types. As a result of the studies, peaks belonging to functional groups could not be detected in only 4 out of 80 fabric samples belonging to 40 women. In 80 fabric samples belonging to 40 men, peaks belonging to functional groups were detected completely. Spectrum images of male and female urine of cotton and nylon fabrics are shown in Figures 2-5.

In this study, the identification of urine using ATR-FTIR spectroscopy and the usability of ATR-FTIR spectroscopy in routine forensic analyses were discussed. According to the urine FTIR spectra of 80 individuals, it was determined that the molecules given in Table 2 and Table 3 contain functional groups belonging to urine, i.e. these functional groups are urine identification markers.

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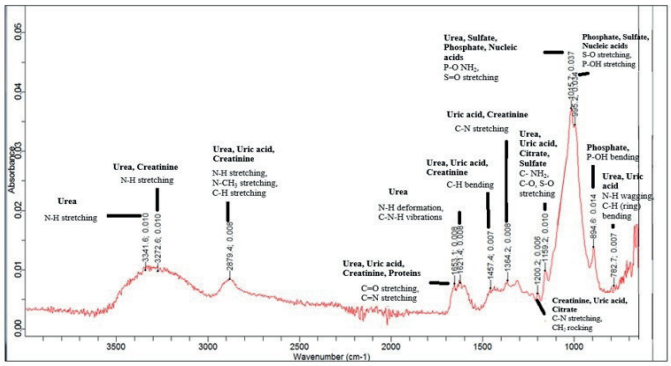

Figure 2. IR spectrum of female urine sample on nylon fabric

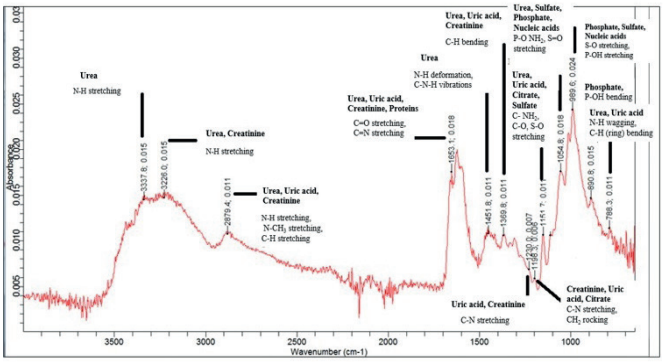

Figure 4. IR spectrum of male urine sample on nylon fabric

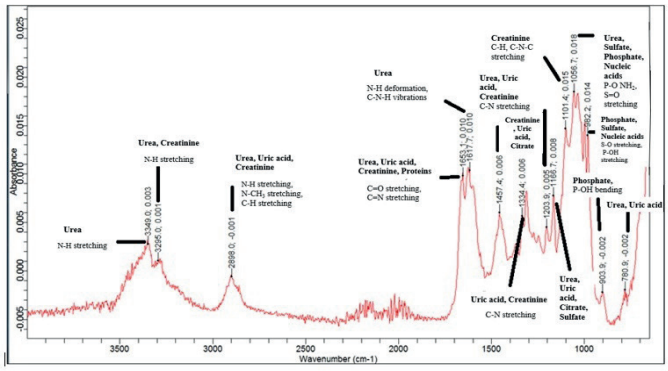

Figure 3. IR spectrum of female urine sample on cotton fabric

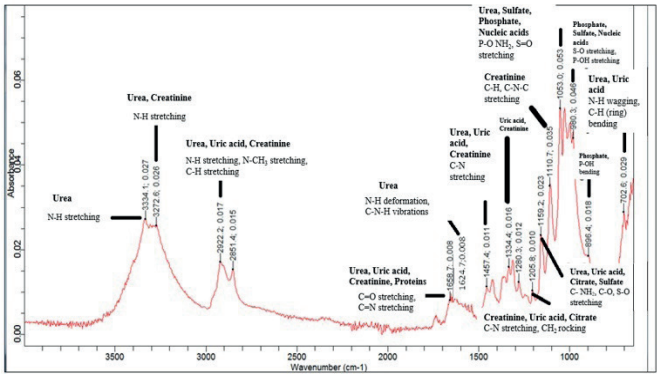

Figure 5. IR spectrum of male urine sample on cotton fabric

Table 2. Mean wavelengths of characteristic components of male and female urine samples

Distinctive molecule*	Functional group	Wavelength number (cm^{-1})	
		Female	Male
Urea	N-H stretching N-H deformation C-N-H vibrations	3341.5 1625.9	3342.1 1624.1
***Urea*, creatinine**	N-H stretching	3284.7	3285.6
***Urea*, uric acid, creatinine, proteins**	C=O stretching C=N stretching	1655.4	1655.5
***Urea*, uric acid, creatinine**	C-H bending	1455.1	1459.7
Uric acid, *creatinine*	C-N stretching	1352.8	1349.8
***Creatinine*, uric acid, citrate**	C-N stretching CH_2 rocking	1201.4	1200.2
***Urea*, uric acid, citrate, sulfate**	C-NH_2 stretching C-O stretching S-O stretching	1156.8	1155.5
Creatinine	C-H stretching C-N-C stretching	1108.2	1105.9
Urea, sulfate, *phosphate*, nucleic acids	P-O stretching NH_2 stretching S=O stretching	1040.2	1033.8
***Phosphate*, sulfate, nucleic acids**	S-O stretching P-OH stretching	990.8	986.6
Phosphate	P-OH bending	895.9	900.9
***Urea*, uric acid**	N-H wagging C-H (ring) bending	779.2	784.1

*The molecules shown in italic among the molecules that provide discrimination were found to have higher absorbance values at the wavelength number in question compared to the molecules with which they were written together

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Table 3. Mean values and standard deviation of wavelengths of characteristic components of urine samples by fabric type

Distinctive Molecule*	Functional group	Wavelength number (cm^{-1})	
		Cotton fabric	Nylon fabric
Urea	N-H stretching	3340.8	3343.4
	N-H deformation C-N-H vibrations	1625.5	1624.9
***Urea*, creatinine**	N-H stretching	3288.3	3282.1
***Urea*, uric acid, creatinine, proteins**	C=O stretching C=N stretching	1655.5	1655.5
***Urea*, uric acid, creatinine**	C-H bending	1456.7	1458.7
Uric acid, *creatinine*	C-N stretching	1340.3	1363.7
***Creatinine*, uric acid, citrate**	C-N stretching CH_2 rocking	1204.3	1197.0
***Urea*, uric acid, citrate, sulfate**	$C-NH_2$ stretching C-O stretching S-O stretching	1160.4	1152.2
Creatinine	C-H stretching C-N-C stretching	1106.9	-
Urea, sulfate, *phosphate*, nucleic acids	P-O stretching NH_2 stretching S=O stretching	1053.3	1013.1
***Phosphate*, sulfate, nucleic acids**	S-O stretching P-OH stretching	987.3	991.5
Phosphate	P-OH bending	905.1	892.3
***Urea*, uric acid**	N-H wagging C-H (ring) bending	781.6	781.9

*The molecules shown in italic among the molecules that provide discrimination were found to have higher absorbance values at the wavelength number in question compared to the molecules with which they were written together

When all the functional groups detected were evaluated separately, all wavelength number values at which the distinctive biomolecules peaked were given as averages. For example, N-H stretching due to urea was observed at 3341.5 cm^{-1} in women and 3342.1 cm^{-1} in men. The same groups were observed at 3340.8 cm^{-1} in cotton fabric and 3343.4 cm^{-1} in nylon fabric. N-H deformation and C-N-H vibrations arising from urea peaked at 1625.9 cm^{-1}, 1624.1 cm^{-1}, 1625.5 cm^{-1} and 1624.9 cm^{-1} in women, men, cotton fabric, and nylon fabric, respectively.

The N-H stretching arising from urea and creatinine peaked at 3284.7 cm^{-1} in women and 3285.6 cm^{-1} in men. The values of the same stretching in cotton and nylon fabrics were 3288.3 cm^{-1} and 3282.1 cm^{-1}, respectively. The C-H and C-N-C stretching arising from creatine peaked at 1108.2 cm^{-1}, 1105.9 cm^{-1}, and 1106.9 cm^{-1} in women, men, and cotton fabric, respectively, while the peaks thought to arise from creatine could not be detected in nylon fabric.

The C=O and C=N stretching of functional groups from urea, uric acid, creatinine, and proteins were observed at 1655.4 cm^{-1} and 1655.5 cm^{-1} in women and men, respectively, and peaked at 1655.5 cm^{-1} in both cotton and nylon fabric.

C-H bending detected in urea, uric acid, and creatinine molecules were at 1455 cm^{-1} in females, 1459.7 cm^{-1} in males, 1456.7 cm^{-1} in cotton fabric, and 1458.7 cm^{-1} in nylon fabric. C-N stretching from uric acid and creatinine molecules were observed at 1352.8 cm^{-1} in females and 1349.8 cm^{-1} in males. The peaks of these stretching in cotton and nylon fabrics were detected at 1340.3 cm^{-1} and 1363.7 cm^{-1}. N-H wagging and C-H bending of the ring structure, which is common in urea and uric acid molecules, were detected at 779.2 cm^{-1} in women, 784.1 cm^{-1} in men, 781.6 cm^{-1} in cotton fabric and 781.9 cm^{-1} in nylon fabric.

C-N stretching and CH_2 rocking from creatinine, uric acid, and citrate molecules were observed at 1201.4 cm^{-1} in women and 1200.2 cm^{-1} in men. The same functional groups were observed at 1204.3 cm^{-1} in cotton fabric and 1197.0 cm^{-1} in nylon fabric.

$C-NH_2$, C-O, and S-O stretching, which are thought to come from urea, uric acid, citrate, and sulfate molecules, were determined at 1156.8 cm^{-1} in females and 1155.5 cm^{-1} in males. The peaks of these groups in cotton and nylon fabrics were seen at 1160.4 cm^{-1} and 1152.2 cm^{-1}.

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P-O, NH_2, and S=O stretching arising from urea, sulfate, phosphate, and nucleic acids were detected at 1040.2 cm^{-1} in women and 1033.8 cm^{-1} in men. The peaks of these functional groups were observed at 1053.3 cm^{-1} in cotton fabrics and 1013.1 cm^{-1} in nylon fabrics. Phosphate, sulfate, and nucleic acid molecules S-O and P-OH stretching were observed to peak at 990.8 cm^{-1}, 986.6 cm^{-1}, 987.3 cm^{-1}, and 991.5 cm^{-1} in women, men, cotton fabric and nylon fabric, respectively. The peaks of P-OH bending belonging only to the phosphate molecule were determined at 895.9 cm^{-1}, 900.9 cm^{-1}, 905.1 cm^{-1}, and 892.3 cm^{-1} in women, men, cotton fabric, and nylon fabric, respectively.

Creatinine is one of the metabolites used to confirm the presence of urine during any measurement. According to the IR spectrum values, the peaks around 3500 cm^{-1} and 1650 cm^{-1} are characteristic of the N-H and C=O and C=N stretching in the creatinine molecule, respectively. Since the N-H and C=O peaks, which are also present in the structure of nylon, a polymer, are common with the creatinine molecule, we believe that they cannot directly confirm the presence of the relevant molecule. In addition, the fact that the C=N peaks around 1650 cm^{-1} and the C-H and C-N-C peaks around 1100 cm^{-1}, which are specific to the creatinine molecule, do not appear with the resonance structure arrangement that may occur on the nylon fabric suggesting that there may be an interaction between the nylon fabric and the urine sample on it, resulting in the attenuation of the peaks specific to urine and/or creatinine.

Another prominent feature was observed in the functional groups of urea, sulfate, phosphate, and nucleic acids. This group peaks at an average of 1053 cm^{-1} in cotton fabrics and 1013 cm^{-1} in nylon fabrics. The reason for this is thought to be due to the different bonding of similar molecular structures with the free atoms of the polymer structure in nylon fabric.

In addition, it is more difficult to take measurements in nylon fabric than in cotton fabric. Therefore, while it was observed that peaks were difficult to form in nylon fabric in functional groups in some samples, these problems were not experienced in cotton fabric.

No significant difference was found when we analyzed the wavelength numbers of the functional groups of female and male urine and the functional groups of different age groups (Figure 6). Cotton fabric samples were coded as P for women (example P1) and E after the fabric type and number for men (example P13E). Nylon fabrics were coded as N for women (example N1) and the same coding pattern as cotton for men (example N13E). PCA and Cluster analysis were performed with the Orange Data Mining program. From this point of view, it is concluded that there will be no difference between the wavelength numbers of the functional groups in women or men or people from different age groups when urine determination is performed by IR spectrum in stained fabrics from the crime scene.

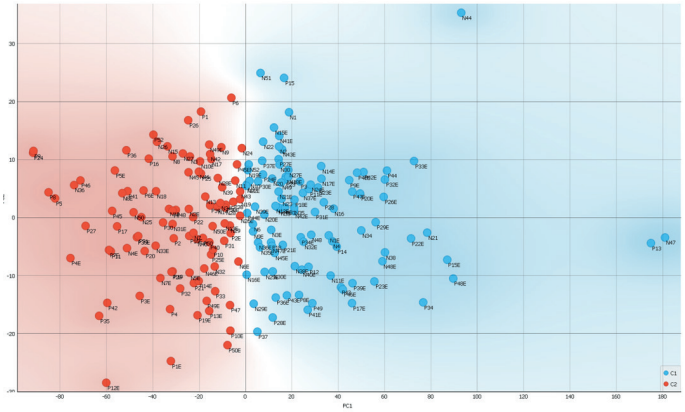

Figure 6. PCA and Cluster analyses results: cotton fabric samples were coded as P, nylon fabrics were coded as N. For men, the letter E has been added. *In the PCA and Cluster analysis, the samples are divided into two groups, c1 and c2, but these distinctions do not specify fabric type and sex

Discussion

In the urine analysis study conducted by Sarıgül et al. in 2021 using IR spectroscopy, the wavelength number values of urea, uric acid, creatinine, phosphate, sulfate, protein, nucleic acid, and citrate, which are the functional groups of urine, were determined and compared with our study [10]. In this study conducted by Sarıgül et al., a fabric sample was not used, direct samples were dripped directly into the device and it was not performed for forensic purposes. The wavelength numbers were determined according to the molecular structures of the functional groups by discriminating between men, women and different age groups.

Sarıgül et al. determined 17 functional groups originating from urea, creatinine, uric acid, various proteins, citrate, sulfate, phosphate, and nucleic acids. While the IR spectra of the related functional groups are shown in Figure 1, the functional groups common to our study are shared in Table 1. Thirteen of the 17 functional groups found in the aforementioned publication were detected at similar wavelength numbers in our present study and are given in Table 2 and Table 3, and parallel values were found with the literature.

In the study of Sarıgül et al., all functional groups were not analyzed individually when the difference between men and women was considered. In FTIR spectroscopy, interpretation was made based on absorbance values, and peaks in the 1390 cm^{-1} region were found to be slightly higher in women. Peaks after the 1200 cm^{-1} region were found to be lower. Apart from this, no significant difference was found in the wavelength numbers of the functional groups. Similarly, no significant difference was found between men and women in our study.

In the study conducted by Das et al. in 2021, ATR-FTIR spectroscopy and chemometric approach were used [11]. In the study, the functional groups of urine were determined by analyzing the ex-vivo transformation of semen, saliva, and urine as they dried, and urine samples were read directly into the

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device as liquid. No staining was performed on any fabric. In this study, only functional groups from urea, uric acid, creatinine, and proteins were analyzed. While the wavelength number for urea was determined as 3346 cm^{-1}, the wavelength number for urea, uric acid, creatinine, and proteins was found to be 1658 cm^{-1}.

The study conducted by Felix Zapata et al. in 2015 aimed to distinguish the external reflection of body fluid stains on fabrics using IR spectroscopy and chemometrics [9]. In the study, IR spectra were determined for the functional groups of urea in urine. In this study, urine stains were determined on 100% cotton t-shirts and jeans.

In our study, unlike the studies of Sarıgül, Das, and Zapata et al. two different fabrics, white cotton fabric and nylon patterned fabric, were used. Felix et al. analyzed only one metabolite of urine, urea. According to this study, the wavelength value determined for urea was 1665 cm^{-1} in cotton and 1634 cm^{-1} in denim fabric and compared to our study, urea peaked at 1625 cm^{-1} in cotton fabric. In nylon fabric, it peaked at 1624 cm^{-1}. Our results are in the direction that there will be a difference in wavelengths according to fabric type by Felix et al. [9].

Identification of body fluids found as evidence at a crime scene is an important detail for criminal investigations. Enzymatic and immunological tests are applied within the scope of serological tests for the identification of body fluids in routine. However, with the development of technology, new methods used on behalf of forensic sciences also come to the fore. One of these is FTIR spectroscopy. It enables the biological sample obtained from the crime scene to be read directly to the device without loss of sample and to obtain results.

In this study, the urine obtained from the crime scene was analyzed by IR spectroscopy on two different types of fabrics, white cotton, and nylon patterned fabric, and also as a result of staining from different sources, male or female. As a result of that, it was determined that urine samples that may be found on the fabric at the crime scene can be determined by ATR-FTIR spectroscopy method, and urine-specific identifier functional groups originate from urea, creatinine, uric acid, citrate, sulfate, phosphate, and nucleic acids in the urine structure. Considering the fabric types, it was determined that creatinine was not observed in nylon fabrics, and it can be said that this does not constitute an obstacle to urine detection and the presence of urine in the samples is provided by other biomolecules and functional groups.

Conclusion

In this study, urine samples stained on two different fabric types, white cotton and nylon patterned fabric, obtained from male and female individuals of different age groups to represent the evidence at the crime scene, were analyzed by FTIR spectroscopy.

In the study, it was determined that 14 different functional groups were involved in the identification of urine by FTIR spectrophotometer for forensic purpose. In addition, the wavelength number data of each functional group were evaluated as female - male and cotton - nylon fabric: It was determined that urine did not give different wavelength number in FTIR spectroscopy between different age groups in terms of women and men, and results were obtained at the same wavelength numbers. According to the PCA result, there is no clear distinction in terms of fabric types or sex.

As a result, it was determined that it is possible to identify urine in stained fabrics collected from the crime scene by using FTIR spectroscopy and this method can be used in forensic serological analyses in criminal laboratories.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical Approval
This study was carried out in Üsküdar University Institute of Addiction and Forensic Sciences Laboratory. Before starting this study, from Üsküdar University Non-Interventional Ethics Committee; 27.05. Ethics committee approval dated 2022 and numbered 61351342/MAY 2022-34 was obtained.

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