ANALYSIS OF HUMAN URINARY STONES IN REPUBLIC OF MOLDOVA IN 2015-2023 BY INFRARED SPECTROSCOPY

ANALIZA CALCULILOR URINARI UMANI DIN REPUBLICA MOLDOVA ÎN 2015-2023 PRIN SPECTROSCOPIE INFRAROŞIE

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The method of Fourier transform infrared attenuated total reflectance (ATR FT-IR) spectroscopy is perspective to use for studying the chemical composition of urinary stones resulting from urolithiasis. 270 urinary stones of patients living in the territory of the Republic of Moldova were investigated. The results of stone composition can provide valuable information for the diagnosis, treatment and recurrence prevention of urolithiasis. The most common are oxalate stones (70%), uric acid (27%), phosphates (23%), struvite (4.9%). Carbonates (0.4%), cystine (1.5%), brushite (1.1%) were only rarely diagnosed. A spectral database of urinary stones of mixed type and in pure form was compiled, the spectra of which were obtained by ATR FT-IR spectroscopy method. The frequencies of absorption of functional groups and anions of chemical components of urinary stones were identified, according to which stones were identified. It has been shown that the IR spectra recorded using the ATR FT-IR accessory can be successfully used to study the composition of urinary stones along with the IR transmission spectra in KBr pellets.

Metoda de spectroscopie în infraroşu cu transformată Fourier cu un accesoriu ATR este promiţătoare pentru studiul compoziţiei chimice a pietrelor urinare formate ca urmare a urolitiazelor. Au fost examinate 270 de calculi urinari. Rezultatele acestor studii pot fi utilizate în viitor pentru a diagnostica cauzele formării de calculi urinari şi urolitiaza. Pietrele urinare apar la bărbaţii şi femeile care locuiesc pe teritoriul Moldovei în vârstă de 18-75 de ani în raport de 1,4: 1. Cele mai frecvente calculi sunt calculii de oxalat (70%), acidul uric (27%), fosfaţii (23%), struvitul (4,9%). Carbonaţii (0,4%), cistina (1,5%) şi brushite (1,1%) sunt rare. A fost compilată o bază de date spectrală de calculi urinari de tip mixt şi în formă pură, ale căror spectre au fost înregistrate folosind accesoriul ATR (reflecţie totală internă atenuată). Au fost identificate

frecvenţele de absorbţie ale grupărilor hidroxil funcţionale şi ale anionilor componentelor chimice ale calculilor urinari, care au fost folosite pentru identificarea acestora. S-a demonstrat că spectrele IR înregistrate folosind accesoriul FT-IR ATR pot fi utilizate cu succes pentru a studia compoziţia pietrelor urinare împreună cu spectrele de transmisie IR ale tabletelor în KBr.

Keywords: *ATR FT-IR spectroscopy, Infrared spectroscopy, Urolithiasis, Urinary stones.*

INTRODUCTION

Urinary lithiasis occupies an important place in the structure of urological diseases with a percentage of 10-40% [1]. Nephrolithiasis has an estimated frequency between 1.0% and 12.0% of the general population causing high disability (approximately 11% of patients with kidney stones) [2]. The high rate of invalidation places nephrolithiasis in third place among urological pathologies after neoplasms and urinary infections [2]. Currently, in the Republic of Moldova, there is an increase in the incidence of urolithiasis, which from 2005 to the present has occupied the first place in the structure of diseases in urological clinics, leaving behind inflammatory pathologies and prostate adenoma. The prevalence of urolithiasis is about 10% in the country's population [2].

Infrared spectroscopy is a modern analytical method for studying the chemical composition of urinary stones. Together with researchers and doctors from medical institutions of the Republic of Moldova, the qualitative chemical compositions of kidney stones from patients suffering from urolithiasis were studied using Fourier transform infrared attenuated total reflectance (ATR FT-IR) spectroscopy method. The composition of kidney stones may indicate a possible etiopathogenic mechanism responsible for their formation.

MATERIALS AND METHOD

The spectra were recorded on IR Fourier spectrometer SPECTRUM 100 (PERKIN ELMER, USA) using an ATR (Attenuated Total Reflectance) accessory. The use of the ATR accessory makes it possible to significantly simplify sample preparation for sample analysis and reduce the time of its analysis. The studied urinary stones were preliminarily grinded in an agate mortar. The powder of the substance was placed on the ZnSe-ATR crystal embedded in the accessory, pressed with a clamping utility and started the measurement. The spectra were recorded in the range 4000 – 650 cm^{-1}. 270 urinary stones obtained from patients of the Department of Urology and Surgical Nephrology (Nicolae Testemitanu State University of Medicine and Pharmacy), the Section of Urology (Timofei Mosneaga Republican Clinical Hospital), Novamed Polyvalent Hospital, IMSP SCM "Holy Trinity" of Chisinau of the Republic of Moldova were investigated. 270 patients (157 men and 113 women, aged 18-75 years) were from different cities and regions of the Republic of Moldova and Transnistria. For interpretation, electronic databases of IR spectra of individual substances, atlases of IR spectra, and other reference and scientific literature on IR spectroscopy were used [3-14].

RESULTS AND DISCUSSIONS

Our research objectives were studying the IR spectra of urinary stones, determination of what type of stones is most common among the population of the Republic of Moldova. The results of the studies were subsequently used to make a diagnosis and prescribe the necessary treatment. Another objective of our research was comparative studying the IR spectra of urinary stones in pure form and as part of complex mixtures using the ATR accessory, identifying which absorption bands correspond to anions and functional groups of urinary stones. Table 1 presents the results of the analysis of the composition of the studied urinary stones. The right column shows the percentage of urinary stones of a certain type. Urinary stones occur in 57.5% of men and 42.5% of women aged 18-75 years, the ratio is 1.4: 1. Oxalate stones are the most common, they account for 70% of stones, both in pure form and in the composition of impurities and constituents of mixed stones. In second place is uric acid and its modifications in the form of uric acid dehydrate and urate in pure form, as well as in the composition of impurities and constituent components of mixed-type stones, they account for 27%. In third place are phosphates 23%, which are found only with impurities or in the composition of stones of a mixed type, in fourth place - struvite - 4.9%, rare carbonates - 0.4%, cystine - 1.5%, brushite - 1.1%. The total % of the above content was not 100%, as the calculations took into account the number of stones with impurities and mixed type (Table 1).

Table 1

The chemical composition of the studied urinary stones of 270 patients of the Republic of Moldova (2015 – 2023).

Chemical composition	%
$CaC_2O_4 \cdot H_2O$ (whewellite)	21.5
$CaC_2O_4 \cdot H_2O$ with impurities $Ca_3(PO_4)_2$	9.4
$CaC_2O_4 \cdot H_2O$ in the composition of mixed stones	14.6
$CaC_2O_4 \cdot 2H_2O$ (weddellite)	2.6
$CaC_2O_4 \cdot 2H_2O$ with impurities of other substances	3.8
$CaC_2O_4 \cdot 2H_2O$ in the composition of mixed stones	18.4
$CaC_2O_4 \cdot H_2O + CaC_2O_4 \cdot 2H_2O$	24.0
$CaC_2O_4 \cdot H_2O + CaC_2O_4 \cdot 2H_2O$ with impurities and as part of mixed stones	46.1
Uric acid	16.1
Uric acid in the composition of mixed stones	6.4
Uric acid dehydrate in pure form and as part of mixed stones	2.6
Ammonium urate in pure form and as part of mixed stones	1.9
$Ca_3(PO_4)_2$ with impurities of other substances	5.6
$Ca_3(PO_4)_2$ in the composition of mixed stones	17.6
$CaCO_3$ with impurities of other substances	0.4

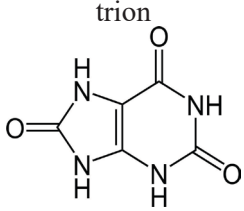
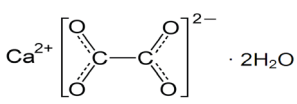
$MgNH_4PO_4 \cdot 6H_2O$ (struvite) in pure form and with impurities of other substances	1.1
$MgNH_4PO_4 \cdot 6H_2O$ (struvite) in the composition of mixed stones	3.8
Cystine in pure form and with impurities of phosphates	1.5
$CaHPO_4 \cdot 2H_2O$ (brushite) in pure form and with impurities of phosphates	1.1

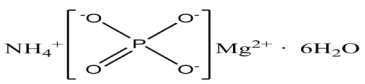
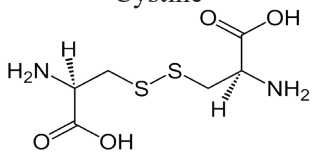
Data in the literature are sometimes given on urinary stones containing phosphate ions in the form of dahlite, apatite, hydroxyapatite, whitlockite [3-5, 7, 8, 10, 13, 14]. At the same time the authors do not give the IR spectra of these substances and give the IR spectra of calcium phosphate mixed with struvite or carbonates, mistakenly believing that they belong to phosphate minerals. In fact, dahlite, apatite, hydroxyapatite and other phosphate minerals have a specific IR spectrum that differs from the usual IR spectrum of calcium phosphate [8, 10, 14].

The conditions for the formation of minerals in nature, obtained synthetically, differ from the conditions for the formation of urinary stones in the kidneys. Therefore, we have no right to confirm that dahlite, apatite, hydroxyapatite are present in the kidneys, since these are geological minerals, and not biological minerals. But according to the results of IR spectroscopy, we can confirm with 100% probability that there were phosphate ions in urinary stones (Table 2). Table 2 shows the correlation of absorption frequencies of urinary stones of different chemical structures by functional groups and anions.

Table 2

Correlation of absorption frequencies of urinary stones of different chemical structures by functional groups and anions

Name and structure of substance	Frequency of absorption bands, cm^{-1}	Functional groups or anions
Uric acid 7,9-dihidro-1H-purin-2,6,8 (3H) – trion 	2989, 2821 1641 1588 1436, 1400, 1348, 1305 1122 778, 742, 704	ν (N-H) ν (N-H), ν (C=O) δ (N-H) δ (N-H) δ (N-H), ν (C-N) ν (C-N) δ (N-H)
Weddellite $CaC_2O_4 \cdot 2H_2O$ 	3440, 3331 1611 1320 774 (medium and wide for $CaC_2O_4 \cdot$ **$2H_2O$**)	ν (O-H) ν_{as} (C=O), ν_{as} (C-O) ν_s (C=O), ν_s (C-O) δ (O-C=O)

Whewellite $CaC_2O_4 \cdot H_2O$	3431, 3333, 3260, 3064 1611 1314 779 (very strong and narrow for $CaC_2O_4 \cdot$ **H_2O**)	ν (O-H) ν_{as} (C=O), ν_{as} (C-O) ν_s (C=O), ν_s (C-O) δ (O-C=O)
Struvite $MgNH_4PO_4 \cdot 6H_2O$ 	2899 1647 1432, 1389 996	ν (O-H), NH_4^+ ν (O-H) ν (NH_4^+) ν (PO_4^{3-})
Calcium phosphate $Ca_3(PO_4)_2$	995-1015 (very strong and wide) 671 (very strong and narrow)	ν (PO_4^{3-}) ν (PO_4^{3-})
Calcium carbonate $CaCO_3$	1391, 871, 711	ν (CO_3^{2-})
Brushite $CaHPO_4 \cdot 2H_2O$	3536, 3480, 3260, 3268, 3163, 1324 1646 1203, 782 1134, 1118, 1057, 985 871	ν (O-H) ν (H-O-H) δ (P-O-H) ν (P-O) ν (P-O-H)
Cystine 	2988, 2915, 2741 2656, 2583 1620, 1578, 845 1620, 1578, 1482,872, 674 1578, 1482, 1403, 1296, 775 1380, 1192, 1124, 872 1336 1296 1088 1267, 1255, 1041 962	ν (NH_2), ν (CH_2) ν_{as} (S-CH_2) ν_{as} (NH_2), δ (N-H) ν_{as} (COO^-) ν (C-C) δ (CH_3) δ (C-H) ν (C-C-N) ν (C-N) ν (N-CH)
Note: ν - stretching vibrations, δ - deformational vibrations		

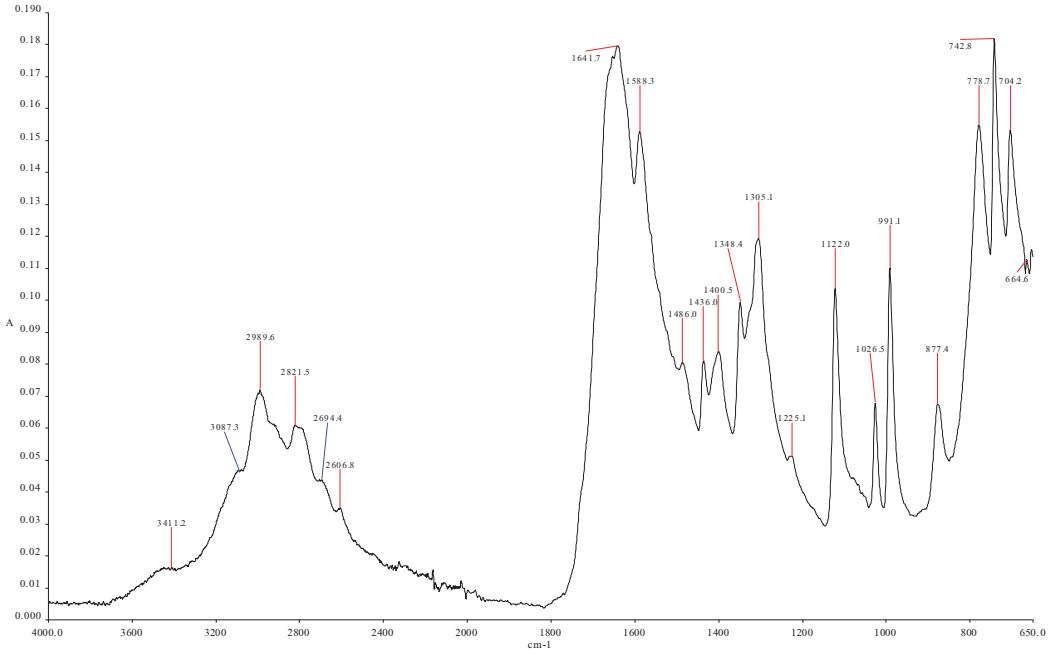


Fig. 1. The IR spectrum of uric acid.

In the IR spectrum of uric acid absorption bands are observed at 2989, 2821, 1641, 1588, 1436, 1400, 1348, 1305, 778, 742, 704 cm^{-1}, corresponding to the stretching and bending vibrations of the N-H imino group, 1641 cm^{-1} - stretching vibrations of the carbonyl group C=O, 1122 cm^{-1} - stretching vibrations of the C-N bond of uric acid (Fig 1., Table 2).

In the IR spectrum of weddellite in the range of 3500 - 3000 cm^{-1} two fitting absorption bands of crystallization water 3440, 3331 cm^{-1} are observed, which form a wide band related to symmetric and asymmetric stretching vibrations of the O-H hydroxyl group. Bands in the region of 1611 and 1314 cm^{-1} correspond to symmetric and asymmetric stretching vibrations of C-O of the oxalate group. Also, the bands in the region of 1611, 1314 cm^{-1} belong to asymmetric and symmetric vibrations of the C=O carbonyl group, the band 779 cm^{-1} belong to the bending vibrations of O-C=O of the oxalate part (Fig 2., Table 2).

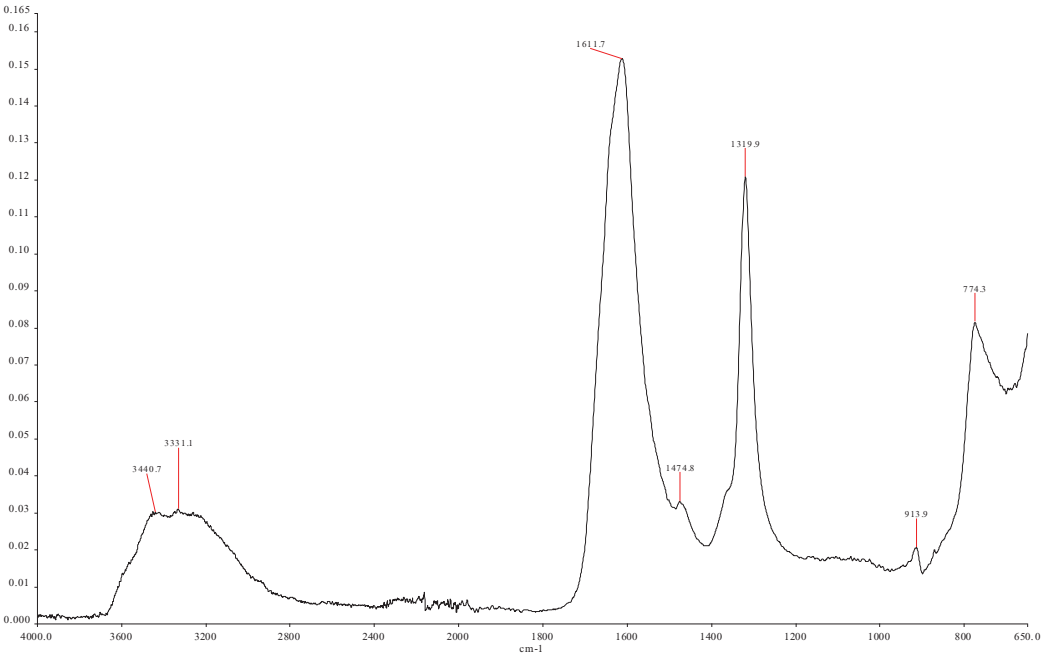


Fig. 2. IR spectrum of weddellite (calcium oxalate dehydrate) $CaC_2O_4 \cdot 2H_2O$

In the IR spectrum of whewellite in the range of 3500 - 3000 cm^{-1} 5 absorption bands of crystallization water 3476, 3434, 3329, 3250, 3053 cm^{-1} are observed, they are related to symmetric and asymmetric stretching vibrations of the O-H hydroxyl group. Bands of 1611 and 1314 cm^{-1} are also observed which correspond to symmetric and asymmetric stretching vibrations of C-O of the oxalate group. Also, the bands in the region of 1611, 1314 cm^{-1} belong to asymmetric and symmetric vibrations of the C=O carbonyl group, and 779 cm^{-1} belong to the bending vibrations of O-C=O of the oxalate part (Fig 3., Table 2).

Despite the fact that whewellite and weddellite differ from each other by only one water molecule, their spectra can be distinguished by the bands of stretching vibrations of crystallization water in the region of 3500 – 3000 cm^{-1}. Whewellite has 5 bands, the intensity of which is less than 2 bands of weddellite 3440, 3331 cm^{-1} (Fig. 2, 3). Also these two oxalates differ in the intensity of the 779 cm^{-1} O-C=O band of the oxalate part, this peak is a strong narrow band for whewellite, and it is a medium and wide band for weddellite (Fig. 2, 3, Table 2).

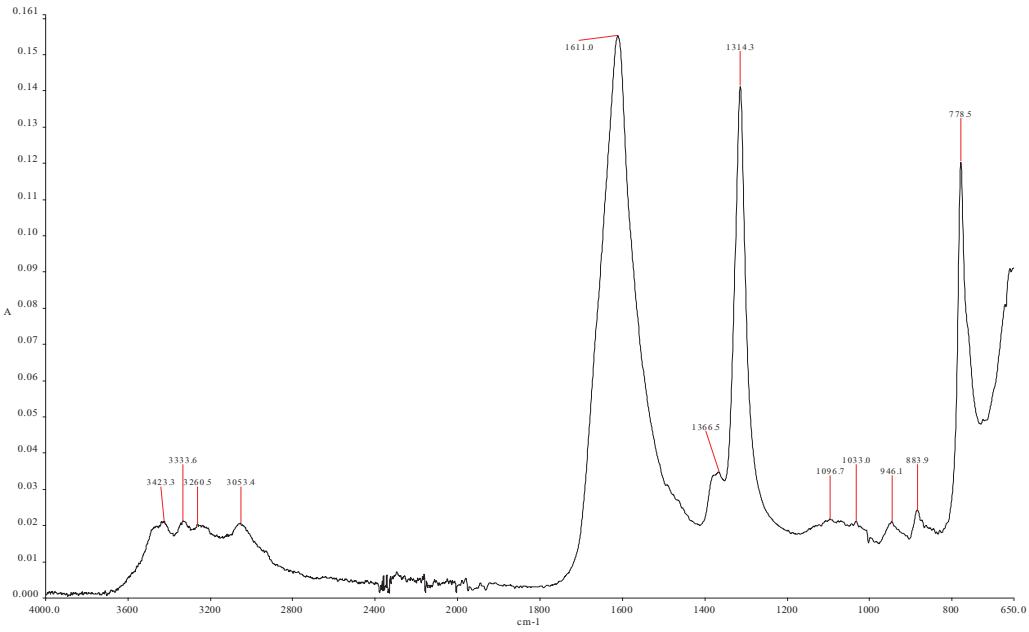


Fig. 3. IR spectrum of whewellite (oxalat de calciu monohidrat) $CaC_2O_4 \cdot H_2O$.

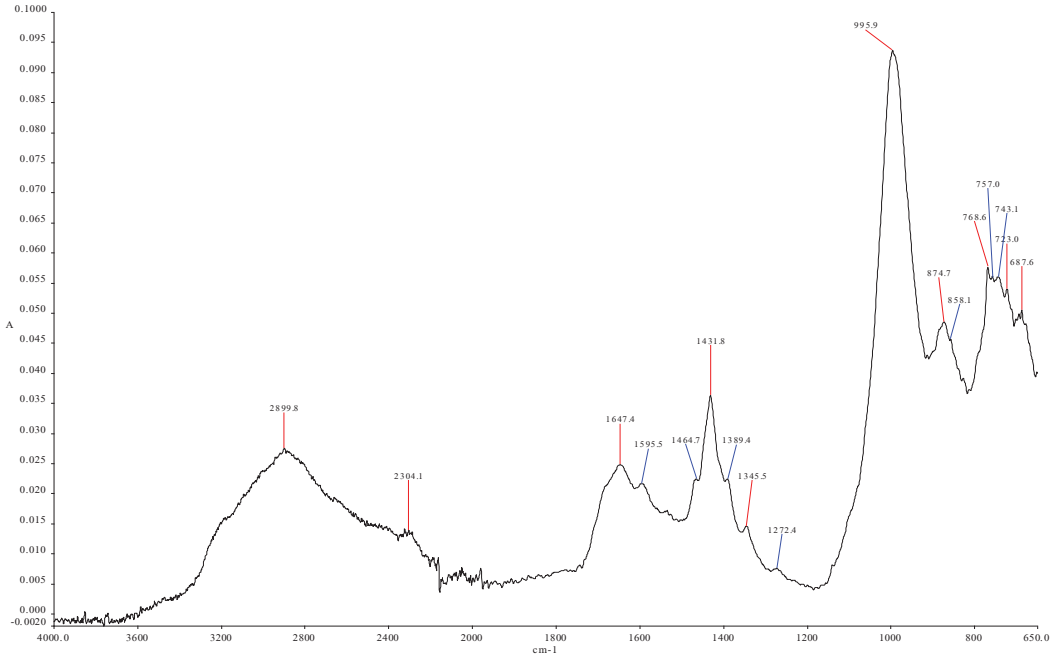


Fig. 4. IR spectrum of struvite (magnesium ammonium phosphate hexahydrate) $MgNH_4PO_4 \cdot 6H_2O$

In the IR spectrum of struvite the band at 2899 cm^{-1} corresponds to the stretching vibrations of the hydroxyl OH group and the NH^{4+} anion. The band at 1647 cm^{-1} is the stretching vibrations of the OH group of water, the bands at 1432, 1389 cm^{-1} are the vibrations of the NH^{4+} ion, the band at 996 cm^{-1} is the vibrations of the PO_4^{3-} ion. (Fig 4, Table 2).

CONCLUSIONS

The method of Fourier Transform Infrared Attenuated Total Reflectance Spectroscopy is perspective to use for studying the chemical composition of urinary stones resulting from urolithiasis. 270 urinary stones (157 men (57.5%) and 113 women (42.5%), aged 18-75 years, the ratio is 1.4 : 1) of patients living in the territory of the Republic of Moldova were examined. The results of stone composition can provide valuable information for the diagnosis, treatment and recurrence prevention of urolithiasis. The most common are oxalate stones (70%), uric acid (27%), phosphates (23%), struvite (4.9%). Carbonates (0.4%), cystine (1.5%), brushite (1.1%) were only rarely diagnosed. A spectral database of urinary stones of mixed type and in pure form was compiled, the spectra of which were obtained by ATR FT-IR spectroscopy method. The frequencies of absorption of functional groups and anions of chemical components of urinary stones were identified, according to which stones were identified. It has been shown that the IR spectra recorded using the ATR FT-IR accessory can be successfully used to study the composition of urinary stones along with the IR transmission spectra in KBr pellets.

References:

1. TURK, C., KNOLL, T., PETRIK, A. *Guidelines on urolithiasis. European Association of urology*. 2015. ISBN 978-94-92671-19-6. Available: https://d56bochluxqnz.cloudfront.net/documents/full-guideline/EAU-Guidelines-on-Urolithiasis-2023.pdf / [Accessed: 27.09.2023]
2. Ceban, E., et al. Litiaza urinară – o provocare a medicinei contemporane, sub semnul realizărilor ştiinţifice. În: *Revista de Ştiinţă, Inovare, Cultură şi Artă „Akademos”*. 2021. nr.4, vol.63, p.87-100. https://doi.org/10.52673/18570461.21.4-63.11
3. PRIMIANO, A, et al. FT-IR analysis of urinary stones: a helpful tool for clinician comparison with the chemical spot test. In: *Disease Markers*, 2014. vol.2014, p.1-5. https://doi.org/10.1155/2014/176165
4. CLOUTIER, J., et al. Kidney stone analysis: “Give me your stone, I will tell you who you are!”. In: *World journal of urology.* 2015. nr.2, vol.33, p.157-169. https://doi.org/10.1007/s00345-014-1444-9
5. GERVASONI, J., et al. Improvement of Urinary Stones Analysis Combining Morphological Analysis and Infrared Spectroscopy. In: *Journal of Chemistry*. 2018. vol.2018, p.1-7. https://doi.org/10.1155/2018/4621256

6. BARANNYK, K., et al. Complex determination of the identification of urinary stones in patients residents of the industrial region. In: *Grail of Science.* 2023. nr.24, p.669-676. https://doi.org/10.36074/grail-of-science.17.02.2023.123
7. HERMIDA, F. J. Analysis of human urinary stones and gallstones by Fourier transform infrared attenuated total reflectance spectroscopy. In: *Journal of Applied Spectroscopy*. 2021. nr.1, vol.88, p.215-224. https://doi.org/10.1007/s10812-021-01160-0
8. THEOPHANIDES, Th. (Ed.). *Infrared Spectroscopy - Materials Science, Engineering and Technology*. InTech, 2012. 526 p. ISBN: 978-953-51-0537-4. Available: https://www.intechopen.com/books/1591 / [Accessed: 27.09.2023]
9. STUART, B. *Infrared Spectroscopy: Fundamentals and Applications.* United Kingdom: John Wiley & Sons, 2004. 224p. ISBN: 9780470854273. https://doi.org/10.1002/0470011149 . Available: https://www.pharmaresearchlibrary.com/wp-content/uploads/2013/04/Infrared-Spectroscopy-Fundamentals-and-Applications-Barbara-Stuart.pdf / [Accessed: 27.09.2023]
10. MILLER, F., WILKINS, C. Infrared Spectra and Characteristic Frequencies of Inorganic Ions. In: *Analytical Chemistry*. 1952. nr.8, vol.24, p.1253–1294. https://doi.org/10.1021/ac60068a007
11. Spectral Database for Organic Compounds SDBS. Available: https://sdbs.db.aist.go.jp/sdbs/cgi-bin/cre_index.cgi / [Accessed: 27.09.2023]
12. Kidney Stones IR spectra. FT IR Infrared Library. Available: http://www.ftir.cz/KID.html / [Accessed: 27.09.2023]
13. KOCADEMIR, M., et al. Structural characterization and vibrational studies of human urinary stones from Istanbul, Turkey. In: *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2016. Vol. 160, p.1–7. https://doi.org/10.1016/j.saa.2016.01.055
14. GRASES, F., SÖHNEL, O., GOMILA, I. Structure and Composition of Non-Infectious Phosphate Calculi Formed in Patients with Low and High Urinary Phosphate Concentrations. In: *Open Journal of Urology*. 2013. nr. 3, p.12-20. http://dx.doi.org/10.4236/oju.2013.31003

Acknowledgement. *This work is a part of the Moldovan State Program (2020-2023) "Study and management of pollution sources to develop recommendations for implementing measures to mitigate the negative impact on environment and human health", Project number: 20.80009.7007.20.*