Ann Ist Super Sanità 2019 | Vol. 55, No. 3: 205-208
DOI: 10.4415/ANN_19_03_02

A combination of infrared spectroscopy and morphological analysis allows successfully identifying rare crystals and atypical urinary stones

Aniello Primiano[1,2], Silvia Persichilli[1,2], Pietro Manuel Ferraro[2,3], Angelo Minucci[2], Giovanni Gambaro[4], Andrea Urbani[1,2] and Jacopo Gervasoni[1,2]

[1]*Istituto di Biochimica e Biochimica Clinica, Università Cattolica del Sacro Cuore, Rome, Italy*
[2]*Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy*
[3]*Istituto di Medicina Interna e Geriatria, Università Cattolica del Sacro Cuore, Rome, Italy*
[4]*UOC di Nefrologia e Dialisi, Azienda Ospedaliera Universitaria Integrata di Verona, Verona, Italy*

Abstract

Background. The combination of infrared spectroscopy and morphological analysis significantly improves the urinary stone analysis. In addition to common urinary stones, it is not unusual to encounter spurious or factitious stones that, if not appropriately identified, can lead to errors in the diagnosis. In this study, we show the importance of Infrared Spectroscopy and the morphological analysis, for determining the presence of drugs crystals or atypical components in the calculi.

Methods. 1041 urinary stones were analyzed by morphocostitutional analysis, in addition the rare stones were analyzed by chemical spot test analysis.

Results. Among 1041 calculi analyzed, 1018 had a known composition, 23 samples were stones with rare composition or fake urinary stones.

Conclusions. Infrared spectroscopy (FT-IR), allows to identify, theoretically, any substance, including drug-containing calculi or calculi with unusual composition and identify false stones. This is mandatory to treat patients affected by urolithiasis with a personalized clinical approach.

Key words

- urolithiasis
- infrared spectroscopy
- morphocostitutional analysis
- rare stones

INTRODUCTION

Urolithiasis is caused by the deposition of crystals in the urinary tract; these formations start when the urine becomes supersaturated for a given salt. The presence in urine of promoters or inhibitors of crystallization may trigger the formation, growth, and aggregation of crystals. The crystals' aggregation can produce kidney stones of different composition. The most common kidney stones are composed of calcium oxalate monohydrate (COM, whewellite), calcium oxalate dihydrate (COD, weddellite), carbonate apatite (CA, dahllite), ammonium urate, magnesium ammonium phosphate (PAM, struvite), calcium hydrogen phosphate dihydrate (brushite), uric acid (AU0 anhydrous form and AU2 dihydrate form, uricite) and its salts and cystine. Spurious or factitious stones represent a very low percentage [1].

Nowadays different methodologies are available for the analysis of renal stones that include wet chemistry tests, X-ray crystallography, infrared spectroscopy (FT-IR) and stereomicroscopic study. Even if the guidelines on urolithiasis of the European Association of Urology [2] underline the obsolescence of wet chemistry and recommend the use of FT-IR, this technique is still widespread. FT-IR analysis allows more accurate results [3, 4] in the composition and discrimination of real from fake urinary stones. This technique, combined with morphological analysis (stereo microscope), is known as morphocostitutional analysis and allows a more accurate urinary stone stratification [5]. Furthermore, this technique can recognize both amorphous substances and drug-containing calculi, that represent a low percentage (2-3.5% as described in other articles) [1-6] but anyway deserve attention, because if not recognized they will never be appropriate treated.

In this study, we summarized the composition of urinary stones obtained by morphocostitutional analysis (stereomicroscopic combined with FT-IR analysis) and focused the attention on rare and fake stones in order

Address for correspondence: Jacopo Gervasoni, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Istituto di Biochimica e Biochimica Clinica, Università Cattolica del Sacro Cuore, Largo Agostino Gemelli 8, 00168 Rome, Italy. E-mail: jacopo.gervasoni@policlinicogemelli.it.

to demonstrate the clinical importance of the spurious stones identification. Furthermore, the rare stones were also analyzed by spot test analysis.

MATERIALS AND METHOD

Samples

We analyzed 1041 consecutive urinary stones from the Divisions of Nephrology and Urology of Agostino Gemelli University Hospital Foundation, collected by 705 males and 336 females (age range 19-75), after urological surgery or spontaneous expulsion. Shape, colour, size and weight were registered for each stone at the time of delivery. The stones submitted to analysis were washed with deionized water and dried at room temperature for 24 h. All the samples have been characterized by both stereo microscopic analysis and FT-IR method as described below.

Stereo microscopic analysis

Morphological analysis was conducted in accordance with our previous published method [7]. The sample analyzed by stereo-microscope was observed at different magnifications (from 7.5× up to 50×). The first step concerned the study of the surface of the stones; then they were sectioned and the components of the core, middle layer and outer layer were identified separately.

FT-IR analysis

The stones were powdered in a mortar and were mixed with an inert powdered support (dried potassium bromide) in a proportion of 0.5 to 2% in agate mortar. This mixture was transferred into an appropriate die and pressed at 10 t/cm^2 to form a transparent pellet 13 mm of diameter. The pellet, assembled in a holder, was placed in the IR beam of the spectrometer. The spectral region investigated was from 4000 to 400 cm^{-1}; 32 scans were averaged with a 4 cm^{-1} resolution for each spectrum. A background spectrum was collected before every analysis, for the sample blank. Once more a background spectrum was measured to provide a relative scale for the absorption intensity. Background spectra were performed at air or pure KBr pellet. Spectra were recorded by means of a Perkin Elmer Spectrum One [8]. Then spectra were computer-matched with the Euclidean search application, a tool of SPECTRA NICODOM IR Library (obtained from Nicodoms.r.o., Hlavni 2727 CZ-14100 Praha 4, Czech Republic, EU) that compares the unknown spectrum with reference spectra contained in the library between 4000 and 400 cm^{-1}. A report is then generated for the various stone components. The results of the automatic comparison for a spectrum identification were provided as a list of the best-fitting spectra with their score. The score value can range from 0.000 to 1.000. Score 1.000 indicates a perfect likeness between the unknown spectrum and the reference one. In each case, a visual inspection of the spectra was performed to check the results [9].

Chemical spot test

Spot test analysis for the qualitative tests of urinary calculi composition was performed according to kit instructions (Urinary Calculi Analysis kit, DiaSys, Diagnostic System GmbH, Holzheim, Germany). The assay consists of the addition of chemical reagents labeled R1 to R15 dropwise to the finely pulverized sample and placed into a vessel with 50 mL of distilled water. Then the appearance of certain colors, precipitates, or air bubbles would indicate positive results for one of the ions and cystine

This method allows detecting the presence of cystine and the following ions usually present in urinary calculi: carbonate, calcium, oxalate, ammonium, phosphate, magnesium, and urate.

RESULTS

Among 1041 calculi analyzed, 1018 (97.8%) had a common composition and in this group the most frequent substance was calcium oxalate as reported in literature [5].

The other remaining 23 (2.2%) samples were stones with rare composition or fake urinary stones.

As shown in *Table 1*, the spurious stones were composed principally of proteins (9/23; 39%). We have recorded also three drug stones (3/23; 13%) of atazanavir *(Figure 1)* in patients infected with human immunodeficiency virus (HIV) in treatment with this protease inhibitor [8] and one stone (1/23; 4%) of N-acetylsulfamethoxazole, the acetyl derivatives of sulphamethoxazole, that is associated with crystalluria and rarely responsible of urolithiasis [6].

Furthermore, we found two silicate urinary stones (2/23; 9%) and a peculiar case of false urolithiasis in a patient treated with synthetic absorbable suture that developed a polyglactine false stone (1/23; 4%).

We found a stone of aragonite (1/23; 4%) with not understood etiology.

Finally, we also detected six fake stones (6/23; 26%).

As reported in *Table 2*, we analyzed the rare stones also by chemical method. The results obtained showed the total disagreement of chemical analysis, as previously reported [9].

Table 1
Composition and frequency of 23 spurious stones

Spurious stone		
Composition	**Number**	**%**
N-acetylsulfamethoxazole	1	4.3
Atazanavir	3	13.0
Proteins	9	39.1
Alpha-quartz	2	8.7
Aragonite	1	4.3
Calcite	2	8.7
Bread	1	4.3
Tripalmitine	1	4.3
Wax-paraffin	1	4.3
Albite	1	4.3
Polyglactine	1	4.3

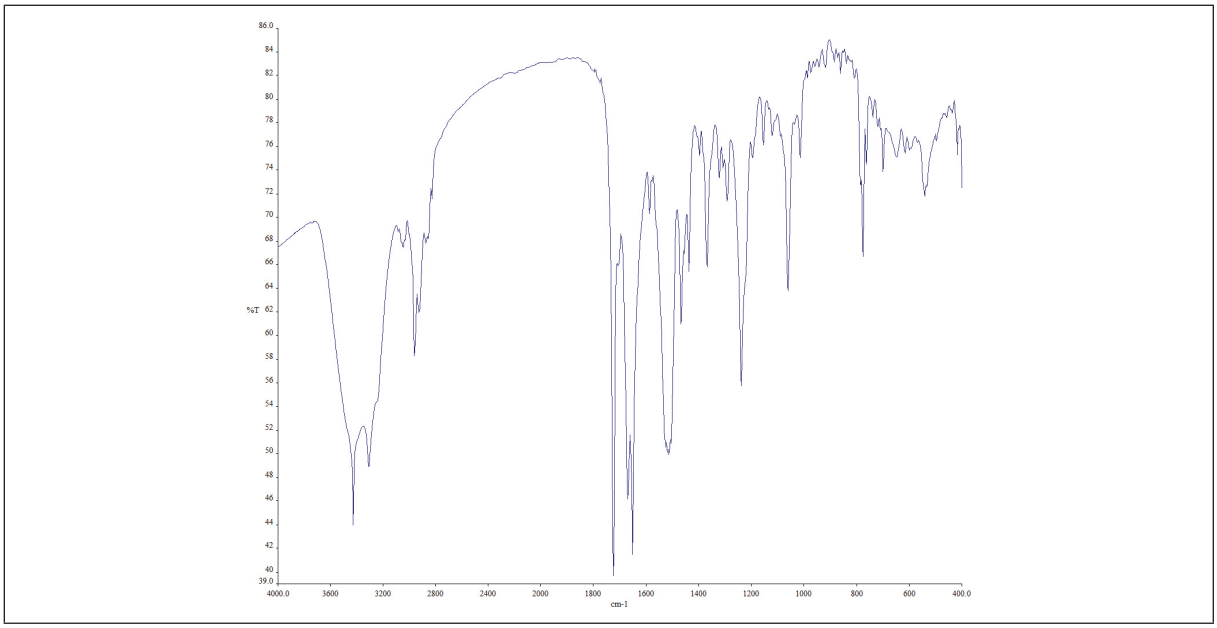

Figure 1
Infrared spectroscopy (FT-IR) spectrum of atazanivir.

DISCUSSION

Morphocostitutional analysis of urinary stone allows a more accurate urinary stone stratification in order to recognize both amorphous substances and drug-containing calculi.

In this study, we found 2.2% of stones with rare composition or samples resulted fake urinary stones. The results obtained by chemical analysis stress again the inaccuracy of this method, indeed the chemical analysis is not able to detect the rare stones and in addition it frequently gives wrong results that can induce the clinicians to incorrect diagnosis *(Table 2)*.

The identification of protein stones can be a marker of several pathologies, while the demonstration of a factitious stone may alert the clinicians to the possibility of a pseudo-medical complaint and influence patient management. The main clinical contexts for the growth of this kind of stone are pyelonephritis, glomerular kidney diseases, hematuria or crystal-induced bleeding, end-stage renal failure and long-term treatment with some antiseptic or antiviral drugs [5-11].

It is mandatory also to underline the possibility to identify drug stones, the knowledge of their composition can be very useful for clinician, because recurrences could be prevented by just discontinuing these drugs [12]. Moreover, the chemical analysis could not detect the drug-containing calculi and an incorrect biochemical composition could lead to a mistake in the medical treatment. Patients who habitually use magnesium trisilicate antacids can develop silicate urinary stone.

Table 2
FT-IR vs chemical spot test analysis

	Components detected	
FT-IR analysis (n)	**Chemical spot test**	**Comparison**
Atazanivir (3)	Oxalate	Disagreement
N-acetylsulfamethoxazole (1)	Ammonia / Magnesium	Disagreement
Proteins (9)	Oxalate / Calcium / Ammonia	Disagreement
Alpha-quartz (2)	ND	Disagreement
Calcite (2)	Carbonate	Agreement
Aragonite (1)	Ammonia	Disagreement
Polyglactine (1)	ND	Disagreement
Tripalmitine (1)	ND	Disagreement
Wax-paraffin (1)	ND	Disagreement
Albite (1)	Oxalate	Disagreement
Bread (1)	Oxalate	Disagreement
Total (23)		

A particular case of false urolithiasis was found in a patient treated with synthetic absorbable suture, in fact prolonged contact with salt solutions such as those found in the urinary tracts may result in a false calculus formation of polyglactine.

The 26% of spurious stones were caused by incorrect sample collection or Munchausen's syndrome psychopathology (albite, wax-paraffin, crumb of bread, tripalmitine) except for calcite stones that are not always factitious [13, 14].

With the chemical analysis, the identification of this substances was not possible and only thanks to the introduction of the new analytical approach that combines infrared spectroscopy and morphological study of kidney stones it is now possible to find substances considered rare, particular calculi, crystals of unexpected material and identify false stones.

CONCLUSION

The use of a reliable technique is of great importance in the management of urolithiasis expecially to identifying rare crystals and atypical urinary stones not identifiable by chemical analysis.

Authors' statements

No potential conflicts of interest relevant to this article were reported. The research did not receive specific funding, but was performed as part of the employment of the all authors. The data used to support the findings of this study have not been made available because of our Company policy.

Received on 23 February 2019.
Accepted 28 May 2019.

REFERENCES

1. Sies CW, Cadwallader J, Florkowski CM, Woods SH, George PM. Quartz renal calculi: were we being led up the garden path? Ann Clin Biochem. 2007;44(Pt 3):312-4. doi: 10.1258/000456307780480864
2. Türk C, Neisius A, Petrik A, Seitz C, Skolarikos A, Tepeler A, Thomas K. Urolithiasis. Available from: http://uroweb.org/guideline/urolithiasis/.
3. Baroni S, Garigali G, Primiano A, Gervasoni J, Fogazzi GB. Very unusual "needle- and pencil-like" uric acid crystals in the urine unmasked by infrared spectroscopy investigation. Clin Chim Acta. 2018;479:72-3. doi: 10.1016/j.cca.2018.01.018
4. Covarelli C, Primiano A, Garigali G, Caselli E, Gervasoni J, Baroni S, Fogazzi GB. Other very rare and poorly known uric acid crystals for the first time also shown by polarized light and definitively identified by infrared spectroscopy. Clin Chim Acta. 2018;484:148-9. doi: 10.1016/j.cca.2018.05.052
5. Daudon M, Dessombz A, Frochot V, Letavernier E, Haymann JP, Jungers P, et al. Comprehensive morphoconstitutional analysis of urinary stones improves etiological diagnosis and therapeutic strategy of nephrolithiasis. C. R. Chimie. 2016;19:1470-91. doi: 10.1016/j.cca.2018.05.052
6. Daudon M, Frochot V, Bazin D, Jungers P. Drug-induced kidney stones and crystalline nephropathy. Pathophysiology, prevention and treatment. Drugs. 2018;78(2):163-201. doi: 10.1007/s40265-017-0853-7
7. Gervasoni J, Primiano A, Ferraro PM, Urbani A, Gambaro G, Persichilli S. Improvement of urinary stones analysis combining morphological analysis and infrared spectroscopy. J Chem. 2018. Available fom: https://doi.org/10.1155/2018/4621256
8. Estepa LM, Levillain P, Lacour B, Daudon M. Infrared analysis of urinary stones: a trial of automated identification. Clin Chem Lab Med. 1999;37(11-12)1043-52. doi: https://doi.org/10.1515/CCLM.1999.153
9. Primiano A, Persichilli S, Gambaro G, Ferraro PM, D'Addessi A, Cocci A, et al. FT-IR analysis of urinary stones. A helpful tool for clinician comparison with the chemical spot test. Dis Mark. 2014;5 pages. Available from: http://dx.doi.org/10.1155/2014/176165
10. Hara M, Suganuma A, Yanagisawa N, Imamura A, Hishima T, Ando M. Atazanavir nephrotoxicity. Clin Kidney J. 2015;8(2):137-42. doi: 10.1093/ckj/sfv015
11. Cloutier J, Villa L, Traxer O, Daudon M. Kidney stone analysis: "Give me your stone, I will tell you who you are!". World J Urol. 2015;33:157-69. doi: 10.1007/s00345-014-1444-9
12. Matlaga BR, Shah OD, Assimos DG. Drug-induced urinary calculi. Rev Urol. 2003;5:227-31.
13. Christiansen FE, Andreassen KH, Sloth Osther PJ. Recurrent pure calcite urolithiasis confirmed by endoscopic removal and infrared spectroscopy in a malnourished anorectic female. J Endourol Case Rep. 2016;2(1):62-4. doi: 10.1089/cren.2016.0036
14. Alaya A, Nouri A, Belgith M, Saad H, Jouini R, Najjar MF. Changes in urinary stone composition in the Tunisian population: a retrospective study of 1301 cases. Ann Lab Med. 2012;32:177-83. doi: 10.3343/alm.2012.32.3.177