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Chemical Composition of Radiolucent Urinary Stones: A Fourier Transform Infrared Spectroscopy Study

Alireza Jafari
Guilan University of Medical Sciences
Siavash Falahatkar
Guilan University of Medical Sciences
Ehsan Aboutaleb
Guilan University of Medical Sciences
Haniyeh Ghafarzadeh
Guilan University of Medical Sciences
Mahan Azizzade Dobakhshari
mahanazizzademaz@gmail.com
Guilan University of Medical Sciences

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Abstract

Background

Radiolucent urinary stones are commonly presumed to represent uric acid calculi; however, the chemical composition of these stones may be heterogeneous. Accurate characterization of stone composition may provide clinically relevant information for understanding stone pathogenesis and optimizing individualized management. This study aimed to determine the FTIR-based chemical composition of radiolucent urinary stones and evaluate its association with demographic and clinical characteristics.

Methods

This cross-sectional study included 43 patients with radiolucent urinary stones who underwent percutaneous nephrolithotomy or endoscopic lithotripsy at Razi Hospital, Rasht, Iran. Retrieved stones were analyzed using Fourier transform infrared spectroscopy (FTIR). Stone composition was classified based on comparison of overall spectral patterns with reference spectra. Associations between stone composition and demographic characteristics, body mass index, stone size, medical history, dietary pattern, urine culture results, and history of urinary tract infection were assessed.

Results

Mixed stone compositions were identified in 60.5% of specimens, while pure uric acid stones accounted for 14.0%. The most frequent FTIR-based classification was uric acid plus calcium phosphate (53.5%), followed by pure calcium oxalate (25.6%). Overall, uric acid-containing compositions were identified in 74.4% of specimens. Age was significantly associated with stone composition ($p = 0.048$), with uric acid plus calcium phosphate becoming more frequent among older patients. No significant associations were observed between stone composition and sex, BMI, stone size, previous medical history, dietary pattern, urine culture results, or history of urinary tract infection.

Conclusion

Radiolucent urinary stones in this cohort demonstrated substantial compositional heterogeneity and should not be assumed to represent pure uric acid stones based solely on radiographic appearance. FTIR analysis identified frequent uric acid-containing mixed compositions, particularly uric acid plus calcium phosphate. These findings highlight the value of direct stone composition assessment in radiolucent calculi and support further investigation using larger cohorts with detailed metabolic evaluation to clarify the mechanisms underlying mixed stone formation.

1. Introduction

Urolithiasis represents a significant global health burden, with reported prevalence varying across populations and geographic regions (1–3). Urinary stones can cause acute pain, urinary obstruction, hematuria, recurrent urinary tract infection, and, in complicated or prolonged cases, impairment of renal

function (3, 4). Stone formation is multifactorial and is influenced by dietary, environmental, metabolic, and genetic factors (1, 2, 5, 6). Because urinary stones may contain different mineral and organic constituents, accurate characterization of stone composition is important for understanding their pathogenesis and for guiding appropriate treatment and strategies for preventing recurrence (7, 8).

Urinary stones are commonly characterized radiographically according to their visibility on plain abdominal radiography. Calcium-containing stones, including calcium oxalate and calcium phosphate stones, are generally more readily visualized on plain radiographs, whereas uric acid and matrix stones are classically associated with radiolucency (4, 5). Accordingly, the absence of radiographic visibility may lead to the clinical presumption that a stone is composed predominantly of uric acid. However, radiolucency is a radiographic characteristic rather than a definitive measure of chemical composition, and the composition of an individual radiolucent stone cannot be established solely from its appearance on plain radiography (4–6). This distinction is clinically relevant because radiolucent stones may require further characterization when their composition influences subsequent medical management and recurrence-prevention strategies (9, 10).

Accurate identification of stone composition has implications for both treatment and prevention of recurrent urolithiasis. Uric acid stones may be amenable to medical dissolution strategies based on urinary alkalinization, whereas calcium-containing stones generally require different preventive approaches directed toward their underlying metabolic and dietary risk factors (11). Mixed stones may present an additional clinical challenge because the presence of more than one constituent may complicate the selection of preventive strategies. Therefore, identification of the chemical constituents of a retrieved stone can provide clinically relevant information beyond that obtained from its radiographic appearance alone (7, 9, 10).

Several analytical methods are available for urinary stone characterization, including X-ray fluorescence, X-ray diffraction, thermal analysis, scanning electron microscopy, and Fourier transform infrared (FTIR) spectroscopy (7, 8, 12). FTIR spectroscopy identifies chemical constituents through their characteristic infrared absorption patterns and has been widely used for the analysis of both organic and inorganic components of urinary stones (7, 12). Its relatively rapid analysis and ability to detect multiple constituents from a small amount of powdered material make it particularly useful for routine stone analysis (12, 13). However, interpretation of spectra from mixed or chemically similar stones may be challenging because absorption bands can overlap; therefore, identification should be based on the overall spectral pattern and comparison with appropriate reference spectra rather than isolated absorption bands alone (12, 14).

Although urinary stone composition has been investigated in several populations, data specifically describing the chemical composition of radiolucent urinary stones in northern Iran remain limited. In particular, the distribution of chemical constituents among stones that are not visualized on plain radiography has not been well characterized in patients undergoing surgical stone treatment in Gilan Province. Therefore, this study aimed to determine the relative frequency of FTIR-based chemical

classifications among radiolucent urinary stones retrieved during percutaneous nephrolithotomy or endoscopic lithotripsy at Razi Hospital, Rasht, and to explore their associations with selected demographic and clinical characteristics.

2. Materials and Methods

2.1. Study Design and Setting

This analytical cross-sectional study was conducted at Razi Hospital, a tertiary referral center affiliated with Guilan University of Medical Sciences in Rasht, northern Iran, between April 2022 and March 2023. The study was designed to characterize the chemical composition of radiolucent urinary stones retrieved during percutaneous nephrolithotomy (PCNL) or endoscopic lithotripsy. The study protocol was approved by the Ethics Committee of Guilan University of Medical Sciences (IR.GUMS.REC.1402.233), and written informed consent was obtained from all participants before enrollment.

2.2. Study Population and Eligibility Criteria

Patients aged 18 years or older who underwent percutaneous nephrolithotomy (PCNL) or endoscopic lithotripsy for radiolucent urinary stones at Razi Hospital during the study period were eligible for inclusion. Written informed consent was obtained from all participants before enrollment. Patients with radio-opaque stones that were visible on plain radiography were excluded because the study specifically targeted radiolucent stones. Patients with severe underlying conditions, including renal failure, advanced diabetes mellitus, or malignancy, were also excluded because such conditions could potentially influence stone composition or the study findings. Patients who were unable to continue participation because of postoperative complications or lack of cooperation were excluded from the study.

2.3. Sample Size Calculation

The minimum sample size was calculated based on the estimated prevalence of radiolucent stones among patients undergoing surgical treatment at the study institution. Using a 95% confidence level and a 5% margin of error, the required minimum sample size was estimated to be 43 patients. Accordingly, 43 eligible patients were included in the study.

2.4. Assessment and Definition of Radiolucency

Radiolucency was defined as the absence of visible stone on preoperative plain abdominal radiography (kidney–ureter–bladder [KUB] radiography). KUB radiographs were routinely obtained on the morning of surgery; intravenous pyelography (IVP) was also used for stone localization when performed as part of the patient's preoperative evaluation. The radiographs were reviewed by a urology specialist, who classified stones as radiolucent when they were not visualized on plain radiography. The presence and location of the stone were confirmed using preoperative imaging, including ultrasonography or non-contrast computed tomography (NCCT), as documented in the clinical records.

2.5. Data Collection and Clinical Variables

Demographic, clinical, radiological, and stone-related data were collected using information from patients' medical records, preoperative imaging reports, and patient-reported medical history. The variables recorded included age, sex, body mass index (BMI), stone size, stone location, laterality, previous medical history, history of previous stone surgery, history of previous urinary stones, history of urinary tract infection (UTI), preoperative urine culture results, and predominant dietary pattern. Postoperative complications were also recorded. Stone size and location were obtained from the patients' clinical and imaging records. Stone location was categorized as the upper, middle, or lower calyx; renal pelvis; upper, middle, or lower ureter; or bladder. Predominant dietary pattern was recorded according to the patient's reported habitual diet.

2.6. Stone Collection, Preparation, and FTIR Analysis

Urinary stones were retrieved during percutaneous nephrolithotomy or endoscopic lithotripsy and transferred promptly to the laboratory for analysis. Following retrieval, the stones were thoroughly washed with sterile distilled water to remove residual blood and tissue material. Stone characteristics, including size, shape, and cross-sectional appearance, were documented using a stereomicroscope. The washed stones were subsequently dried and ground to obtain a powdered sample. According to the study protocol documented in the thesis, the stones were dried in an incubator at 40°C for 30 minutes before grinding. The powdered material was prepared from the entire stone sample to obtain a representative specimen for spectroscopic analysis. Approximately 10 mg of the resulting dry powder was used for FTIR analysis.

2.7. Fourier Transform Infrared Spectroscopy

2.7.1. Stone collection and preparation

Urinary stones were retrieved during percutaneous nephrolithotomy (PCNL) or endoscopic lithotripsy and transferred promptly to the laboratory for analysis. Following retrieval, the stones were thoroughly washed with sterile distilled water to remove residual blood and tissue material. Stone characteristics, including size, shape, and cross-sectional appearance, were recorded using a stereomicroscope. According to the study protocol documented in the thesis, the washed stones were dried in an incubator at 40°C for 30 minutes and subsequently ground to obtain a powdered sample. Approximately 10 mg of dry stone powder, obtained uniformly from the powdered whole-stone sample, was used for FTIR analysis.

2.7.2. FTIR spectroscopy

FTIR spectroscopy was performed using a PerkinElmer Spectrum Two spectrometer (PerkinElmer, USA). Approximately 10 mg of dry powdered stone was placed directly into the spectrometer for spectral acquisition. Infrared spectra were recorded over the range of 4000–400 cm^{-1}. The observed absorption patterns were compared with standard reference spectra available in the laboratory's chemical database and with previously published spectral data.

2.7.3. Spectral interpretation and chemical classification

The FTIR spectra were interpreted by comparison of their overall absorption patterns with reference spectra and published spectral assignments. Where multiple spectral features were present, the final classification was based on the concordance of the overall absorption pattern with reference spectra and published spectral assignments. The presence of individual constituents was assessed on the basis of concordant characteristic spectral features rather than a single absorption band. Based on the interpreted spectra, stones were classified as single-component or mixed-component specimens. The chemical categories identified in the study were pure uric acid, pure calcium oxalate, uric acid plus calcium phosphate, uric acid plus calcium oxalate, and uric acid plus calcium phosphate plus calcium oxalate.

2.8. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). Continuous variables, including age, body mass index (BMI), and stone size, were summarized as mean ± standard deviation. Comparisons of continuous variables across stone-composition groups were performed using analysis of variance (ANOVA) when the assumptions of parametric testing were met; otherwise, the Kruskal–Wallis test was used. Categorical variables were summarized as frequencies and percentages, and associations between categorical variables and stone-composition categories were evaluated using the chi-square test. A two-sided p-value < 0.05 was considered statistically significant.

2.9. Ethical Considerations

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and relevant national ethical guidelines. The study protocol was reviewed and approved by the Ethics Committee of Guilan University of Medical Sciences (IR.GUMS.REC.1402.233). Written informed consent was obtained from all participants before enrollment, including consent for the use of relevant clinical and medical data for research purposes. Patient information was kept confidential, and study data were coded to protect participants' identities. Access to identifiable medical information was restricted to the study investigators, and no personally identifying information was reported. Participants were informed of their right to withdraw from the study at any stage without any effect on their medical care.

3. Results

3.1. Study Population and Baseline Characteristics

A total of 43 patients with radiolucent urinary stones were included in the study. The mean age of the participants was 48.7 ± 15.2 years, with an age range of 23–78 years. Twenty-seven patients (62.8%) were male and 16 (37.2%) were female. The participants were distributed across three age groups: 6 patients (14.0%) were younger than 35 years, 21 (48.8%) were aged 36–55 years, and 16 (37.2%) were older than 56 years.

Regarding body mass index (BMI), 8 patients (18.6%) had a BMI of 18.5–24.9 kg/m², 25 (58.1%) were overweight (BMI 25.0–29.9 kg/m²), and 10 (23.3%) were obese (BMI ≥ 30 kg/m²). No participant had a

BMI < 18.5 kg/m².

The distribution of stone size showed that 10 stones (23.2%) measured < 10 mm, 26 (60.5%) measured 10–20 mm, and 7 (16.3%) measured > 20 mm. Stones were located on the right side in 24 patients (55.8%) and on the left side in 19 (44.2%). Anatomically, stones were located most frequently in the distal ureter (28.1%), followed by the proximal ureter (25.6%), renal pelvis (20.9%), and renal calyces (25.4%).

3.2. FTIR-Based Stone Composition

FTIR spectra from all 43 retrieved radiolucent urinary stones were evaluated by comparison with reference spectra and previously published spectral assignments. Based on the interpreted spectral patterns, five composition categories were identified: pure uric acid, pure calcium oxalate, uric acid plus calcium phosphate, uric acid plus calcium oxalate, and uric acid plus calcium phosphate plus calcium oxalate (Table 1).

Table 1
FTIR-based classification of retrieved radiolucent urinary stones

Stone Composition	Frequency (n)	Percentage (%)
Pure calcium oxalate	11	25.6
Pure uric acid	6	14.0
Mixed uric acid + CaP	23	53.5
Mixed uric acid + CaOx	2	4.7
Mixed Uric acid + CaP + CaOx	1	2.3
Total	**43**	**100**

Mixed-component classifications were identified in 26 of the 43 specimens (60.5%), whereas 17 specimens (39.5%) were classified as single-component stones. The most frequent FTIR-based classification was uric acid plus calcium phosphate, identified in 23 specimens (53.5%). Pure calcium oxalate was identified in 11 specimens (25.6%), while pure uric acid was identified in 6 specimens (14.0%). Uric acid plus calcium oxalate was identified in 2 specimens (4.7%), and uric acid plus calcium phosphate plus calcium oxalate was identified in 1 specimen (2.3%). Overall, spectral features consistent with uric acid were identified in 32 of 43 specimens (74.4%), either as a single-component classification or in combination with other constituents. The relative distribution of the FTIR-based classifications is shown in Fig. 1.

3.3. Association Between Stone Composition and Clinical/Demographic Variables

Associations between FTIR-based stone-composition classifications and selected demographic and clinical variables were evaluated (Table 2). Patient age was significantly associated with stone composition ($p = 0.048$). Among patients aged ≤ 35 years, pure uric acid and pure calcium oxalate were each identified in 33.3% of cases. In the 36–55-year age group, pure calcium oxalate and uric acid plus calcium phosphate were each identified in 38.1% of cases. Among patients aged ≥ 56 years, uric acid plus calcium phosphate was the predominant classification, accounting for 87.5% of cases, whereas pure calcium oxalate accounted for 6.3% (Table 2; Fig. 2).

Table 2
Association Between Stone Composition and Clinical Characteristics

Clinical characteristic	Category	Pure uric acid n (%)	Pure calcium oxalate n (%)	Uric acid + calcium phosphate n (%)	Uric acid + calcium oxalate n (%)	Uric acid + calcium phosphate + calcium oxalate n (%)	P-value
Age group	≤ 35 years	2 (33.3)	2 (33.3)	1 (16.7)	1 (16.7)	0 (0.0)	0.048
	36–55 years	3 (14.3)	8 (38.1)	8 (38.1)	1 (4.8)	1 (4.8)	
	≥ 56 years	1 (6.3)	1 (6.3)	14 (87.5)	0 (0.0)	0 (0.0)	
Sex	Male	3 (11.1)	10 (37.0)	12 (44.4)	1 (3.7)	1 (3.7)	0.200
	Female	3 (18.8)	1 (6.3)	11 (68.8)	1 (6.3)	0 (0.0)	
BMI category	18.5–24.9 kg/m²	1 (12.5)	3 (37.5)	4 (50.0)	0 (0.0)	0 (0.0)	0.530
	25.0–29.9 kg/m²	4 (16.0)	7 (28.0)	12 (48.0)	2 (8.0)	0 (0.0)	
	≥ 30 kg/m²	1 (10.0)	1 (10.0)	7 (70.0)	0 (0.0)	1 (10.0)	
Stone size	< 10 mm	0 (0.0)	3 (30.0)	6 (60.0)	0 (0.0)	1 (10.0)	0.533
	10–20 mm	6 (23.1)	5 (19.2)	14 (53.8)	1 (3.8)	0 (0.0)	
	> 20 mm	0 (0.0)	2 (28.6)	4 (57.1)	1 (14.3)	0 (0.0)	
Previous medical history	No	5 (19.2)	6 (23.1)	12 (46.2)	2 (7.7)	1 (3.8)	0.393
	Yes	1 (5.9)	5 (29.4)	11 (64.7)	0 (0.0)	0 (0.0)	
Previous stone surgery	No	2 (12.5)	5 (31.3)	7 (43.8)	2 (12.5)	0 (0.0)	0.709
	Yes	4 (14.8)	6 (22.2)	16 (59.3)	0 (0.0)	1 (3.7)	

Clinical characteristic	Category	Pure uric acid n (%)	Pure calcium oxalate n (%)	Uric acid + calcium phosphate n (%)	Uric acid + calcium oxalate n (%)	Uric acid + calcium phosphate + calcium oxalate n (%)	P-value
Preoperative urine culture	Negative	6 (15.0)	10 (25.0)	21 (52.5)	2 (5.0)	1 (2.5)	0.931
	Positive	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)	
History of UTI	No	6 (15.4)	11 (28.2)	19 (48.7)	2 (5.1)	1 (2.6)	0.429
	Yes	0 (0.0)	0 (0.0)	4 (100.0)	0 (0.0)	0 (0.0)	

No statistically significant association was observed between sex and stone composition (p = 0.200). Uric acid plus calcium phosphate was the most frequent classification in both males and females, accounting for 44.4% and 68.8% of cases, respectively (Table 2).

BMI category was not significantly associated with stone composition (p = 0.530). Uric acid plus calcium phosphate was the most frequent classification across the BMI categories evaluated (Table 2).

No statistically significant association was identified between stone size and stone-composition classification (p = 0.533) (Table 2). Uric acid plus calcium phosphate was the most frequent classification across the stone-size categories evaluated.

Among the 17 patients (39.5%) with a history of previous medical conditions, predominantly hypertension and diabetes mellitus, uric acid plus calcium phosphate was the most frequent classification, accounting for 64.7% of cases. No statistically significant association was observed between previous medical history and stone composition (p = 0.393) (Table 2).

A history of previous stone surgery was reported in 62.8% of patients. Among patients with a previous history of PCNL, uric acid plus calcium phosphate was the most frequent classification, accounting for 69.2% of cases; the corresponding proportion among patients with a previous history of endoscopic lithotripsy was 46.2%. The association between previous stone surgery and stone composition was not statistically significant (p = 0.709) (Table 2).

A protein-predominant dietary pattern was reported by 58.1% of patients. Among these patients, uric acid plus calcium phosphate was the most frequent classification, accounting for 52.0% of cases. No statistically significant association was observed between reported dietary pattern and stone composition (p = 0.724) (Table 2).

Preoperative urine cultures were positive in 3 patients (7.0%). Two of these patients (66.7%) had stones classified as uric acid plus calcium phosphate, and no statistically significant association was observed between preoperative urine culture results and stone composition (p = 0.931) (Table 2). A history of urinary tract infection was reported by 4 patients (9.3%), all of whom had stones classified as uric acid plus calcium phosphate. This association was not statistically significant (p = 0.429) (Table 2).

3.4. Representative FTIR Spectra

Representative FTIR spectra corresponding to selected stone specimens are presented in Figs. 3–5. According to the study's FTIR interpretation, specimen 9 was classified as pure uric acid, specimen 24 as uric acid plus calcium oxalate, and specimen 6 as pure calcium oxalate. The spectrum of specimen 9 showed prominent absorptions at approximately 3331, 1614, and 1315 cm^{-1}, together with additional features in the fingerprint region. The spectrum of specimen 24 demonstrated a prominent absorption near 1641 cm^{-1} and an additional sharp feature in the approximately 1300 cm^{-1} region. The spectrum of specimen 6 showed prominent absorptions at approximately 1614 and 1318 cm^{-1}, together with additional features in the lower-wavenumber region. In accordance with the analytical approach described in the Methods, interpretation of each spectrum was based on comparison of the overall absorption pattern with reference spectra and published spectral assignments rather than on isolated absorption bands.

4. Discussion

This study characterized the chemical composition of 43 radiolucent urinary stones retrieved during percutaneous nephrolithotomy or endoscopic lithotripsy at a tertiary referral center in Gilan Province, northern Iran, using FTIR spectroscopy. Mixed-component stones predominated (60.5%), and uric acid plus calcium phosphate was by far the most frequent classification (53.5%), followed by pure calcium oxalate (25.6%) and pure uric acid (14.0%). Spectral features consistent with uric acid were present, either alone or in combination, in nearly three-quarters of specimens (74.4%). Age was the only clinical or demographic variable significantly associated with composition (p = 0.048). These findings both confirm and refine the assumption that radiolucent stones are enriched for uric acid, while also indicating that a substantial calcium-bearing component frequently coexists within calculi that appear entirely invisible on plain radiography.

The high overall representation of uric acid in this study is unsurprising given that uric acid calculi are classically radiolucent, whereas calcium oxalate and calcium phosphate stones are more often, though not invariably, radiopaque (15). Nevertheless, the magnitude of uric acid positivity observed here exceeds the roughly 8–10% share that uric acid calculi typically occupy among unselected stone cohorts (15, 16), which is expected because our sample was deliberately restricted to radiolucent calculi rather than representing the full spectrum of stone formers. More notable is that, within this radiolucent subset, pure uric acid stones were in the minority; the dominant pattern was an admixture of uric acid with calcium phosphate. This pattern diverges from the pattern most commonly described in the general urolithiasis literature, where uric acid, when mixed, is most often reported in combination with calcium

oxalate rather than calcium phosphate (16, 17). In the present series, uric acid plus calcium oxalate accounted for only 4.7% of stones, considerably lower than the roughly one-third proportion reported for mixed uric acid–oxalate calculi in some Western cohorts (16).

The predominance of the uric acid–calcium phosphate combination in this study warrants mechanistic consideration. Uric acid crystallization is governed primarily by persistently acidic urine, with the undissociated, poorly soluble form predominating below the first dissociation constant of approximately pH 5.5 (15). Calcium phosphate precipitation is strongly influenced by urinary pH and other physicochemical factors. The coexistence of uric acid and calcium phosphate could therefore be consistent with changes in the urinary environment during stone growth, although this hypothesis cannot be tested directly in the present study. Insulin resistance has been proposed as one physiological driver of the chronically low urinary pH and impaired ammoniagenesis that predispose to uric acid stone formation (18), and metabolic comorbidities such as diabetes and hypertension have repeatedly been linked to uric acid urolithiasis in other cohorts (19, 20). Superimposed episodes of higher urinary pH, related to dietary shifts, transient reductions in animal protein intake, or intermittent alkaline tide, could plausibly permit intercalated calcium phosphate deposition around or within an initially uric acid–predominant nucleus. Importantly, it should not be assumed that this calcium phosphate component reflects urinary tract infection: non-infective calcium phosphate phases are recognized as a distinct etiological category from the infection-associated carbonate apatite and struvite that typically develop in persistently alkaline, infected urine (15). This distinction is consistent with our data, in which preoperative urine culture was positive in only 7.0% of patients and a history of urinary tract infection was reported in only 9.3%, arguing against infection as the principal explanation for the calcium phosphate component in most specimens. A direct test of the pH-oscillation hypothesis would require serial or 24-hour urinary pH measurements, which were not collected in this study and represent an important avenue for future work.

Age showed a strong and statistically significant association with stone composition, with uric acid plus calcium phosphate rising from 16.7% among patients 35 years or younger to 87.5% among those 56 years or older. This age-related shift toward uric acid–containing calculi is consistent with a broad body of evidence. Siener and colleagues reported that the proportion of uric acid calculi increases markedly after approximately age 50 in both sexes (16), and a large Chinese cohort similarly found that the prevalence of uric acid stones rose with advancing age while infection stones declined, with prevalence peaking in the sixth decade for men and the seventh for women (21). In a central Chinese series, uric acid stone formers tended to be older and to carry a higher burden of diabetes, hypertension, and elevated serum creatinine (20), findings that parallel the pattern observed in our own cohort, where uric acid plus calcium phosphate was the leading classification among patients with a prior history of hypertension or diabetes (64.7%). Age-related metabolic and urinary changes may contribute to the observed pattern; however, these mechanisms could not be evaluated directly in the present study because urinary pH, urinary ammonium excretion, and other metabolic parameters were not measured.

No statistically significant association was found between sex and stone composition in this study (p = 0.200), although uric acid plus calcium phosphate was numerically more common among women (68.8%) than men (44.4%). This directional pattern is broadly compatible with prior reports that carbonate apatite and calcium phosphate–type stones occur more frequently in women, whereas calcium oxalate and pure uric acid stones are more common in men (20, 22). A Taiwanese study similarly found male sex to be positively associated with uric acid stones and negatively associated with calcium phosphate stones (23). The absence of statistical significance may reflect the limited sample size, particularly the smaller number of female participants (n = 16), although a true absence of association cannot be excluded, and larger radiolucent-stone cohorts will be needed to confirm whether this pattern holds.

Body mass index and stone size were likewise not significantly associated with composition in this cohort. This null finding contrasts somewhat with mechanistic and epidemiological work linking obesity and insulin resistance to uric acid stone risk (18) and with reports that male uric acid stone formers carry a higher burden of obesity and hyperuricemia (21). However, in the present sample, 81.4% of patients fell into the overweight or obese categories, leaving comparatively little variance in BMI across which an association could be detected; a broader distribution of BMI, together with a larger sample, would likely be required to adequately test this relationship. Stone size similarly showed no statistically significant association with stone composition in this study.

Although only a minority of patients reported a history of urinary tract infection (9.3%) or had a positive preoperative urine culture (7.0%), all patients with a UTI history and two-thirds of those with a positive culture had stones classified as uric acid plus calcium phosphate. While this association did not reach statistical significance, it is worth noting in light of evidence from other cohorts that urinary tract infection is a recognized risk factor for calcium phosphate– and struvite-containing calculi (20) and that positive stone or urine culture, rather than metabolic abnormalities, has been identified as the strongest independent predictor of stone recurrence after percutaneous nephrolithotomy (24). Although these findings did not demonstrate a statistically significant association between infection-related variables and stone composition, the observed pattern warrants further investigation in larger cohorts incorporating stone culture, renal pelvis culture, and comprehensive metabolic evaluation.

A protein-predominant dietary pattern was reported by more than half of the cohort (58.1%) and, while not statistically associated with composition, was most frequently linked to uric acid plus calcium phosphate stones (52.0%). This is consistent with the well-established role of dietary purine and animal protein intake in promoting hyperuricosuria and lowering urinary pH, both of which favor uric acid crystallization (16). Notably, dietary intervention studies have shown that a balanced, reduced-protein diet can lower urinary uric acid supersaturation by close to half, largely through increases in urine volume and pH rather than through reduced uric acid excretion itself (16), reinforcing the potential value of dietary counseling as an adjunct to medical therapy in this population, even though our cross-sectional design cannot establish causality.

These findings carry several clinical implications. Because the majority of radiolucent stones in this study contained a calcium-bearing component in addition to uric acid, treatment decisions based solely on radiographic appearance may not accurately reflect stone composition or the suitability of medical dissolution (15).This reinforces the value of formal compositional analysis, rather than radiographic appearance alone, in guiding individualized recurrence-prevention strategies, a principle also emphasized in large-scale FTIR-based stone registries (17). FTIR spectroscopy is widely used for routine urinary stone analysis and can identify multiple chemical constituents through their characteristic infrared absorption patterns (10, 12, 14). In the present study, its application to a well-defined radiolucent subgroup provides regional data from northern Iran; however, the qualitative nature of the analysis and the absence of an independent confirmatory method should be considered when interpreting the reported classifications.

Taken together, these findings indicate that radiolucency on plain radiography, while a useful clinical clue toward uric acid involvement, should not be equated with a pure uric acid stone. In this northern Iranian cohort, most radiolucent calculi contained an admixed calcium phosphate component alongside uric acid, a pattern that increased sharply with age and that carries direct implications for individualized medical therapy and recurrence prevention. Larger, multicenter studies incorporating detailed urinary and serum biochemical profiling are needed to confirm these patterns and to clarify the pathophysiological basis of the uric acid–calcium phosphate combination observed here.

Limitations

This study has several limitations. The single-center design and modest sample size (n = 43) limited statistical power, particularly for subgroup comparisons such as BMI category, urine culture status, and UTI history, and may account for the number of biologically plausible associations that did not reach statistical significance. Unlike several comparator cohorts, this study did not incorporate direct biochemical measurements such as 24-hour urinary pH, uric acid, calcium, or oxalate excretion, or serum uric acid concentration, which would have allowed more direct testing of the proposed urinary pH-oscillation mechanism underlying the uric acid–calcium phosphate combination. In addition, FTIR analysis was performed on powder derived from the entire, homogenized stone, which, while representative of overall bulk composition, cannot distinguish core-from-shell layering; it therefore remains uncertain whether the coexisting uric acid and calcium phosphate phases represent an intimately mixed matrix or a layered structure reflecting sequential changes in urinary chemistry during stone growth. Dietary pattern was also captured as a self-reported categorical variable rather than through quantified nutrient intake, limiting precision. Finally, as a single-center cohort from Gilan Province, these findings may not generalize to other regions, given the well-documented geographic and ethnic variability in urinary stone composition reported elsewhere.

5. Conclusion

In this study of 43 radiolucent urinary stones from Gilan Province, FTIR spectroscopy showed that radiolucency on plain radiography does not reliably indicate pure uric acid composition. Most radiolucent stones were mixed, with uric acid combined with calcium phosphate being the most frequent classification, rather than pure uric acid or the uric acid–calcium oxalate combination reported in some other cohorts. Uric acid, either alone or in combination with other constituents, was identified in nearly three-quarters of specimens, confirming that radiolucent stones in this cohort were enriched for uric acid-containing compositions. The frequent co-occurrence of calcium phosphate could be consistent with changes in the urinary environment during stone growth; however, this hypothesis was not directly evaluated in the present study. Age was the only variable significantly associated with stone composition, with uric acid–calcium phosphate stones becoming markedly more common with advancing age, whereas sex, BMI, stone size, previous medical history, dietary pattern, urine culture, and history of UTI were not significantly associated with composition in this cohort.

These findings suggest that the chemical composition of radiolucent urinary stones should not be presumed from radiographic appearance alone. Formal stone analysis, including FTIR spectroscopy, may provide clinically useful information when stone composition is relevant to management and recurrence prevention. The strong age-related pattern observed in this cohort also warrants further investigation. Larger, multicenter studies incorporating direct urinary and serum biochemical measurements, including urinary pH and other metabolic parameters, are needed to confirm these findings and to clarify the mechanisms underlying the frequent coexistence of uric acid and calcium phosphate in radiolucent stones.

Declarations

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Guilan University of Medical Sciences, Rasht, Iran (Ethics Code: **IR.GUMS.REC.1402.233**). All participants provided written informed consent.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Clinical trial number

Not applicable

Competing Interests

The authors declare no competing interests.

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Declaration of AI use

AI (Claude, Anthropic) was used to assist in drafting portions of the Discussion and Conclusion; all content was verified and approved by the author(s).

Authors' Contributions

AJ: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Supervision.

SF: Methodology, Investigation, Resources, Writing – review & editing, Supervision.

EA: Methodology, Laboratory analysis, Validation, Writing – review & editing.

HG: Data collection, Investigation, Data curation, Project administration, Writing – original draft (thesis preparation).

MAD: Conceptualization, Methodology, Investigation, Writing – review & editing, Supervision.

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Figures

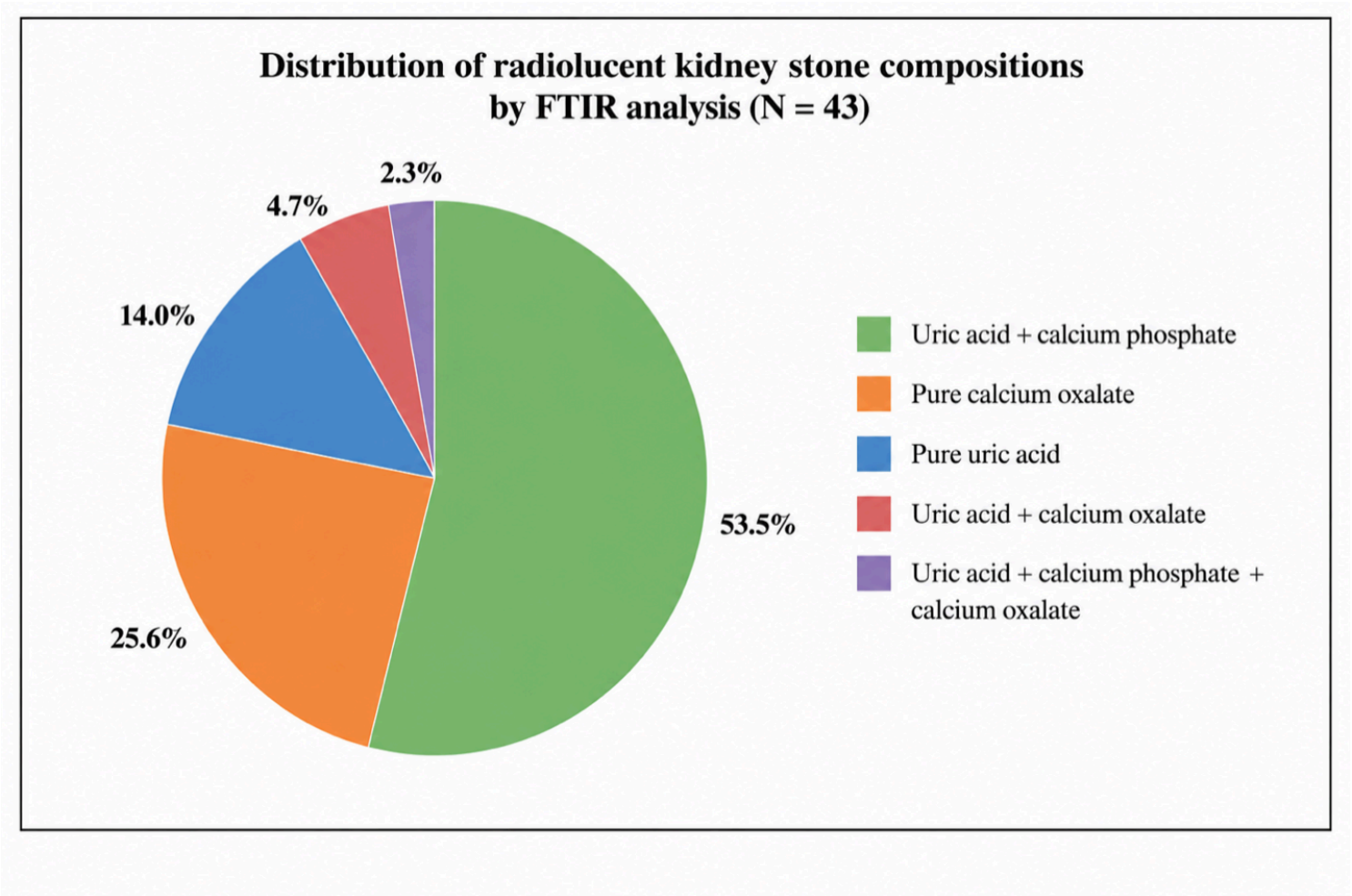


Figure 1

Distribution of FTIR-based classifications of radiolucent urinary stones (N=43). Uric acid plus calcium phosphate was the most frequent classification (53.5%), followed by pure calcium oxalate (25.6%) and pure uric acid (14.0%).

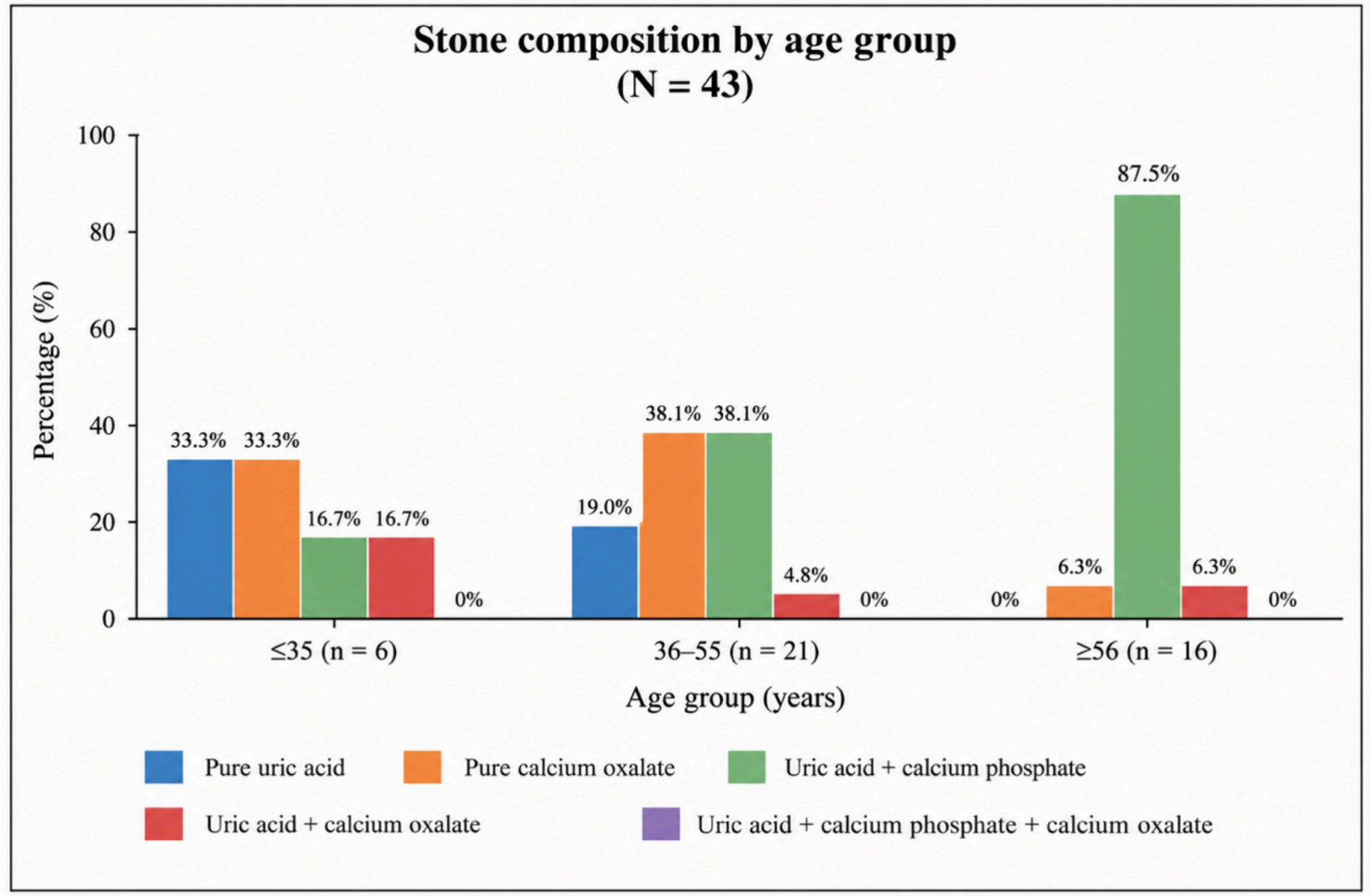


Figure 2

Stone-composition distribution across age groups based on FTIR classification (N = 43). Pure uric acid and pure calcium oxalate were each identified in 33.3% of patients aged ≤35 years. In the 36–55-year group, pure calcium oxalate and uric acid plus calcium phosphate each accounted for 38.1% of cases. Among patients aged ≥56 years, uric acid plus calcium phosphate was the predominant classification (87.5%). The association between age group and stone composition was statistically significant (p = 0.048).

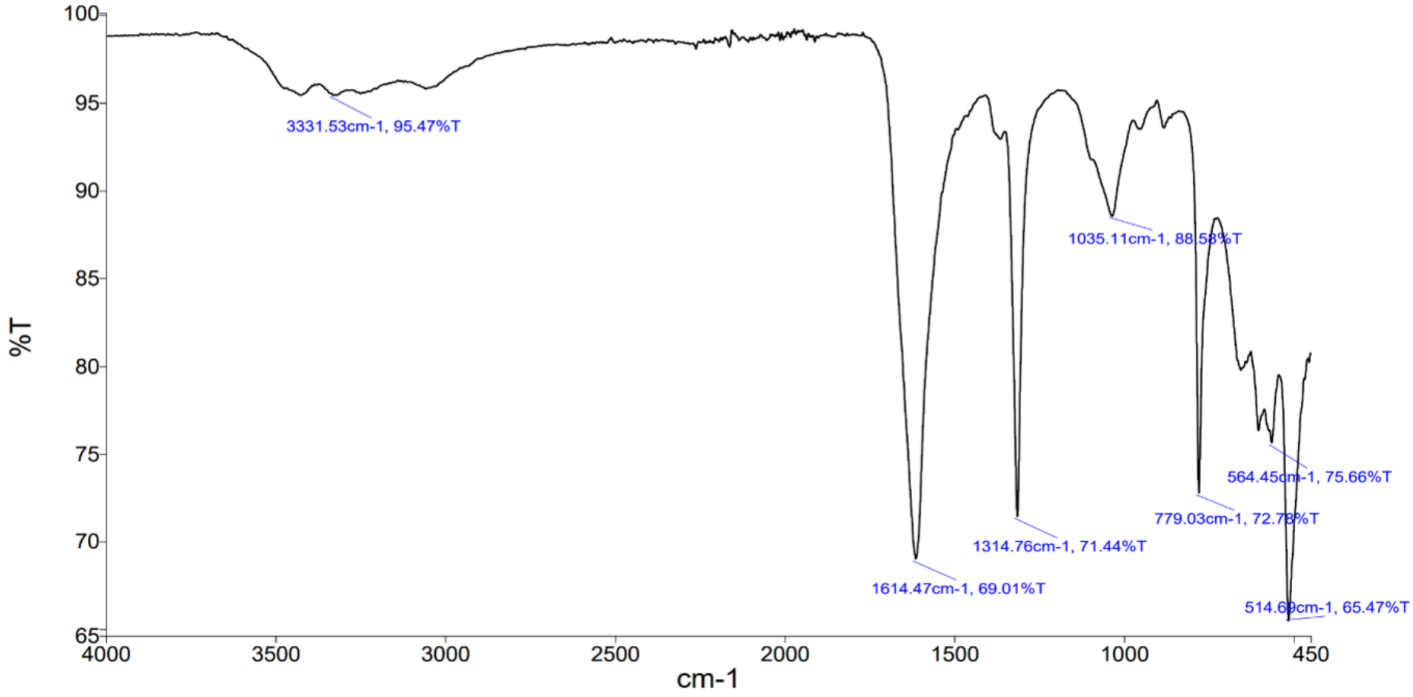


Figure 3

Representative FTIR spectrum of specimen 9, classified as pure uric acid according to the study's spectral interpretation. Prominent absorption features are observed at approximately 3331, 1614, and 1315 cm^{-1}, with additional absorptions in the fingerprint region.

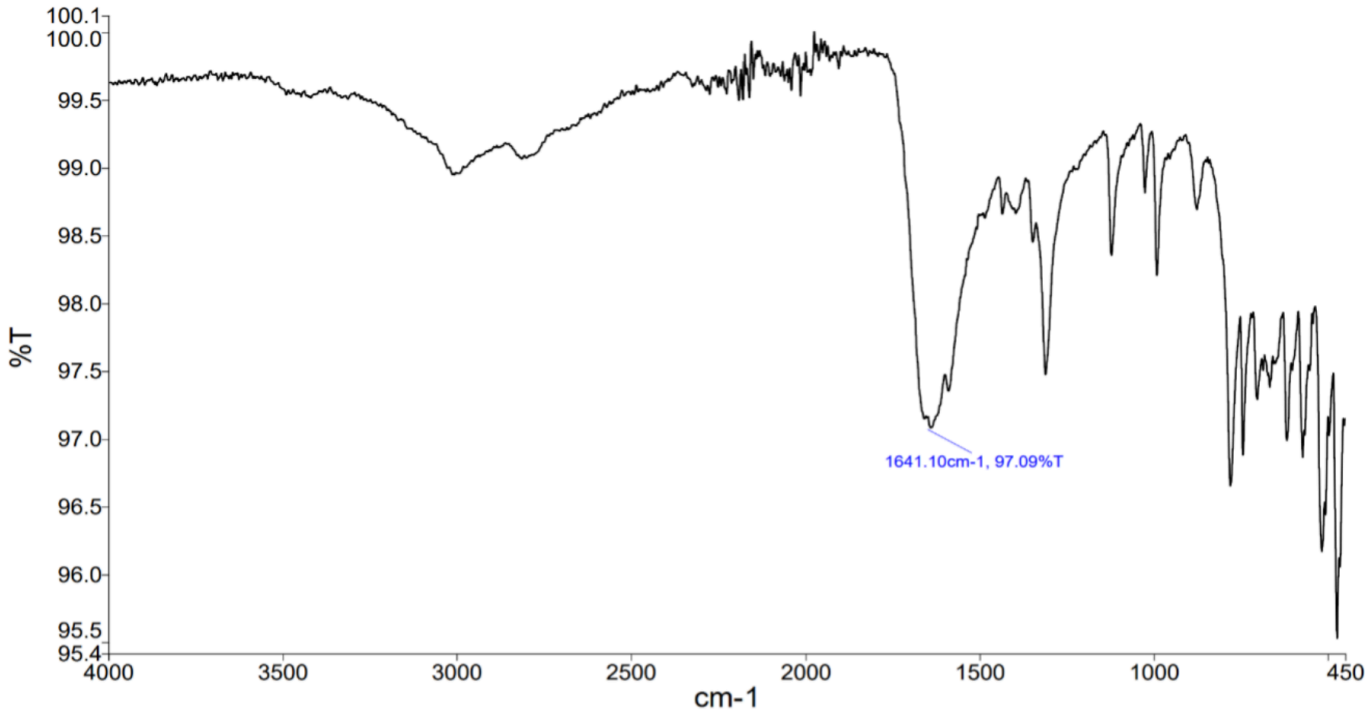


Figure 4

Representative FTIR spectrum of specimen 24, classified as uric acid plus calcium oxalate according to the study's spectral interpretation. The spectrum demonstrates a prominent absorption near 1641 cm^{-1} and an additional sharp feature in the approximately 1300 cm^{-1} region.

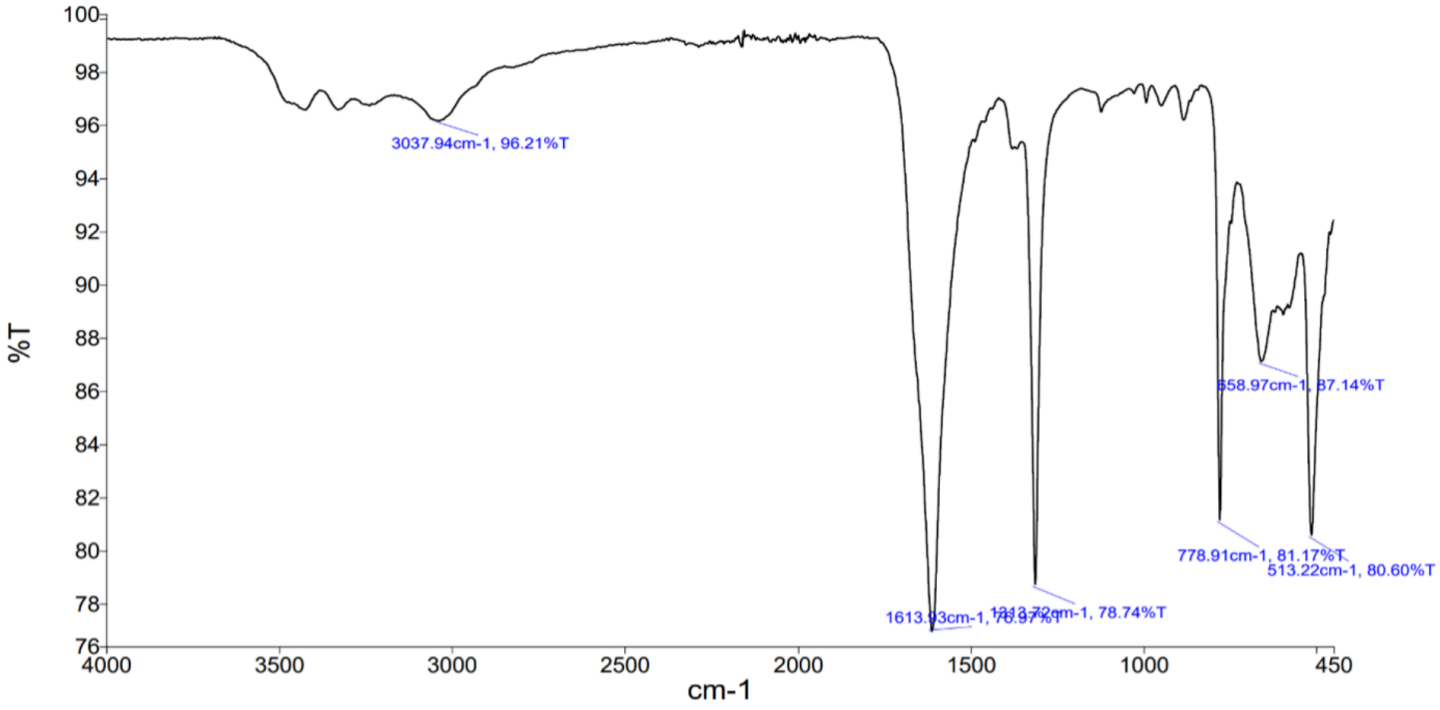


Figure 5

Representative FTIR spectrum of specimen 6, classified as pure calcium oxalate according to the study's spectral interpretation. Prominent absorption features are observed at approximately 1614 and 1318 cm^{-1}, together with additional absorptions in the lower-wavenumber fingerprint region.