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ORIGINAL PAPER

The establishment of a standard and real patient kidney stone library utilizing Fourier transform-infrared spectroscopy with a diamond ATR accessory

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Abstract This investigation highlights the establishment of a real patient kidney stone library utilizing Fourier transform-infrared spectroscopy with a diamond attenuated total reflection accessory (FT-IR ATR) and the construction of a standard FT-IR ATR (sFTIRATR) library using OMNIC spectral math arithmetic operations for kidney stone analysis. This is necessary because reference spectra in commercial libraries provided with specialized software are usually complied using synthesized crystalline compounds which can exhibit changes in intensity, position and/or characteristic profile of reflectance bands when compared with authentic biological stone compositions. Currently, there is no published literature for the Republic of Ireland (RoI) on stone type and prevalence. The results obtained from the establishment of the real patient kidney stone library were a representative selection of kidney stones found in the population, and thereby provided an accurate picture of the present epidemiology of kidney stones in the RoI. The results of 188 patients were compared with those from our newly constructed sFTIRATR library and existing methods, namely wet chemical analysis, and FT-IR ATR utilizing an ATR algorithm and potassium bromide search libraries. We found that for the optimum quantitative analysis of kidney stone mixtures, FT-IR ATR spectroscopy utilizing a standard FT-IR ATR library, supported by a real patient kidney stone library, applying library searching accurately provides the molecular and crystalline species of stone constituents present in an unknown kidney stone sample, providing some predicative value in diagnosing medical conditions. Our data suggest that the epidemiology for nephrolithiasis in the RoI is similar to other Western nations.

Keywords Kidney stones · FT-IR ATR · Library searching · Real patient kidney stone library · Crystalline species

Introduction

Nephrolithiasis (kidney stone disease) is a common and recurrent chronic urological multiethnic disease with multifactorial etiopathogenesis [1]. It is a worldwide problem afflicting 5–15% of the population. No geographical, cultural or racial group is spared this painful medical condition and its significant morbidity with occasional mortality. Nephrolithiasis is in itself not a true diagnosis, but a clinical manifestation from a diverse array of underlying pathologies [2]. It has now become apparent that establishing the correct molecular and crystalline identification of kidney stone components provides correlations with pathophysiological conditions, and can help prevent stone recurrence, which should be the hallmark for stone management [3]. Indeed, knowing the precise molecular and crystalline composition of a kidney stone can guide physicians towards the correct stone aetiopathogeny and the implementation of the optimum treatment strategy for the patient [3, 4]. The variability in kidney stone fragility to shock waves is considerable, even within crystalline groups defined by the same molecular composition. Today, extracorporeal shock wave

This study has been approved by the Mater Misericordiae University Hospital ethics committee and has been performed in accordance with ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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lithotripsy (ESWL) is the primary surgical modality for nephrolithiasis, producing small kidney stone fragments for stone analysis. This necessitates a laboratory technique, which can accurately quantify minute amounts of stone material [5, 6].

Numerous approaches have been implemented for the analysis of kidney stones. These include sophisticated techniques such as thermogravimetry, polarized microscopy, scanning electron microscopy with energy dispersion, X-ray crystallography and infrared spectroscopy, as well as simpler methodologies such as qualitative dry 'spot tests' and quantitative wet chemistries [6–9]. Traditionally, wet chemical analysis has been the most common method used in routine practice in hospital laboratories due to its ease of use and low cost [10]. However, wet chemical analysis has many disadvantages when compared with Fourier transform-infrared (FT-IR) spectroscopy [11]. FT-IR spectroscopy utilizes a diversity of sample holders and is gaining widespread popularity [6].

The current method for analysis of kidney stone composition in our laboratory is the traditional "gold standard" wet chemical analysis. This method determines the constituents of stones on a combined qualitative and quantitative basis. It is reliant on automated chemistry analysers and the application of in-house established calculations with derived stoichiometric ion-association graphs. The most likely stone combinations are, respectively, derived. Wet chemical analysis is very time consuming and necessitates larger amounts of sample [10]. Wet chemical methods can miss rare and unidentified material, and with the exception of pure kidney stones can only indicate the presence of certain individual ions rather than a specific compound [9, 11]. Significantly, wet chemical analysis methods cannot distinguish crystalline species, which are necessary to provide the correct clinical diagnosis [3, 12].

Our laboratory purchased a Thermo Nicolet 380 FT-IR spectrometer with a smart orbit diamond crystal attenuated total reflection (ATR) accessory to overcome these numerous drawbacks. The ATR accessory does not necessitate sample pre-treatment allowing the use of neat stone material. Currently, there are no commercially available reference ATR libraries for quantitative stone analysis with FT-IR spectrometers. Therefore, a major drawback with the current search libraries provided with the FT-IR ATR instrument is the necessary application of a patented ATR conversion algorithm to convert the neat sample reflectance spectra into a compatible absorbance spectra for library searching, utilizing the provided potassium bromide-derived OMNIC coded (KBrOC) library. A KBr tablet transmission sample holder would appear to solve this problem. However, analysis using an FT-IR KBr method requires sample pre-treatment. The preparation of KBr pellets is time consuming and pellet breakage warrants the repeat of the technique [6]. This is not suitable if the initial sample amount is small, which can occur following ESWL therapy. To overcome these drawbacks, we developed a new comprehensive and easy to use standard FT-IR ATR (sFTIRATR) library using OMNIC spectral math arithmetic operations.

For the optimum quantitative analysis of kidney stone mixtures, library searching or other comparative algorithms can be used. If this is to work, a spectral library of real patient kidney stones must be established. The reference spectra in commercial libraries provided with specialized software are normally compiled using synthesized crystalline compounds which can show changes in intensity, position and/or characteristic profile of reflectance bands when compared with actual biological stone compositions. To combat these discrepancies, we propose the development of a real patient kidney stone library and utilize it as a reference in conjunction with the newly constructed sFTIRATR library for library searching [4]. To establish the real patient library, the most accurate quantitative composition for each sample was assigned after initial interpretation of the results from each library search method, the wet chemistry method and confirmation of the stone composition by visual inspection of the spectra. Subsequently, each specific designated real kidney stone composition was added to a new library resulting in the establishment of the real patient kidney stone library in ATR reflectance spectral format. An unknown sample spectrum can then be compared to a number of different library spectra using a search programme for automatic comparison and the optimum-fitting spectrum obtained. The precise quantitative stone signature produced will ultimately provide correlations with aetiologies and ensure implementation of the optimum therapeutic regimen to prevent kidney stone recurrence for the stone former.

Our laboratory is the reference centre for kidney stone analysis for the Republic of Ireland (RoI). There is currently no published data on the prevalence and kidney stone type in the RoI. It is proposed from the results following the establishment of the real patient kidney stone library which is a representative selection of kidney stones found in the country that this study will also provide an accurate epidemiological picture of kidney stones for the RoI.

Materials and methods

FT-IR ATR method

A ThermoNicolet 380 FT-IR Spectrometer with a smart orbit diamond crystal ATR accessory was used for analysis. Standardization involved measurement of a polystyrene transmission standard comprising wave number positions and absorbances of known IR bands. The samples were stored in the dark and measured at room temperature.

Approximately, 2 mg of pulverized kidney stone material was applied to the flat surface of the durable and chemically inert type IIA diamond crystal and placed into position by the pressure tower containing a torque screw producing a spectrum within 140 s. The reproducibility of this pressure and uniform optical contact between the sample and crystal was assured by the swivel pressure tower containing a torque screw that ensures the amount of pressure applied on the sample is kept constant. The analyser was equipped with Thermo Scientific OMNIC™ software. The KBr-derived kidney stone libraries provided with the OMNIC software consists of the following parts: a basic kidney stone library containing close to 800 spectra of the most common kidney stone mixtures (was not used for method comparison) and the advanced KBrOC library containing approximately 18,000 spectra including potential interferences. Similar to our constructed ATR library, these provided libraries were constructed from single components and mixed to theoretically build all possible binary and ternary mixtures. A special algorithm, the NICODOM kidney stone analysis kit, was used for any major discrepancies in the results of the method. Based on the instructions supplied from the manufacturer, and the capability of the instrument, a scan resolution of 4 cm^{-1} was selected, together with a scan number of 128 and the reference standards were scanned in the mid-IR region from 4,000 to 400 cm^{-1}. The analysis range was subsequently adjusted from 4,000 to 400 cm^{-1} to the fingerprint area 2,000–400 cm^{-1} for all analysis by FT-IR ATR.

A background spectrum was collected before every analysis. A background spectrum must be measured to provide a relative scale for the absorption intensity and blank subtraction. This was a measurement with no sample in the crystal. This could then be compared to the measurement of the sample spectrum resulting in a spectrum, which has all of the instrumental characteristics removed. This ensures that all spectral characteristics are strictly due to the kidney stone sample. After each measurement, the smart orbit diamond crystal was wiped with a dry clean tissue followed by cleaning with 70% isopropanol.

Construction of the standard FT-IR ATR library

Volmer et al. [6] made binary and ternary mixtures by weighing and mixing pure commercial components to construct their search library. We constructed our FT-IR ATR library from measured IR reflectance spectra from 12, mostly commercially available, fine homogenous powder components and mixed in various proportions using instrument-specific OMNIC software (see Table 1). Usually no more than three organic/inorganic components constitute any stone of which seven commonly occur in all kidney stones (components with 'a' in Table 1). These components are as follows: calcium hydrogen phosphate (CaHP), calcium oxalate monohydrate (CaOxMH), calcium oxalate dihydrate (CaOxDH), carbapatite (CA), hydroxyapatite (HA), magnesium ammonium phosphate (MAP) and uric acid (UA).

The number of components is limited and the mixtures build a closed semi-stoichiometric set, 171 binary and 348 ternary mixtures of the seven most commonly occurring components, and some additional likely binary and ternary combinations of rarer and common components were constructed. The spectral contribution of each component is additive, so all binary and ternary combinations of components were constructed from the pure standards using OMNIC spectral math arithmetic operations in linear ranges of 0–100%, in step sizes of 10%. Performance of these arithmetic operations on the spectra required experience in visual spectral interpretation and offered extensive

Table 1 Primary compounds used for the construction of the sFTIRATR library

Component names	Alternate component names	Abbreviations	Component sources
Calcium oxalate monohydrate[a]	Whewellite	CaOxMH	Alfa Aesar
Calcium oxalate dihydrate[a]	Weddellite	CaOxDH	Patient
Calcium hydrogen phosphate[a]	Brushite	CaHP	Sigma-Aldrich
Hydroxyapatite[a]	Hydroxylapatite	HA	Sigma-Aldrich
Carbapatite[a]	Carbonate apatite	CA	Patient
Magnesium ammonium phosphate[a]	Struvite	MAP	Sigma-Aldrich
Uric acid[a]		UA	MP Biomedicals
Cystine		CYST	Sigma-Aldrich
2,8-Dihydroxyadenine		2,8-DHA	Sigma-Aldrich
Xanthine		XAN	Sigma-Aldrich
Ammonium urate		AmU	MP Biomedicals
Calcite		CTE	Alfa Aesar

[a] Seven commonly occurring components in all kidney stones

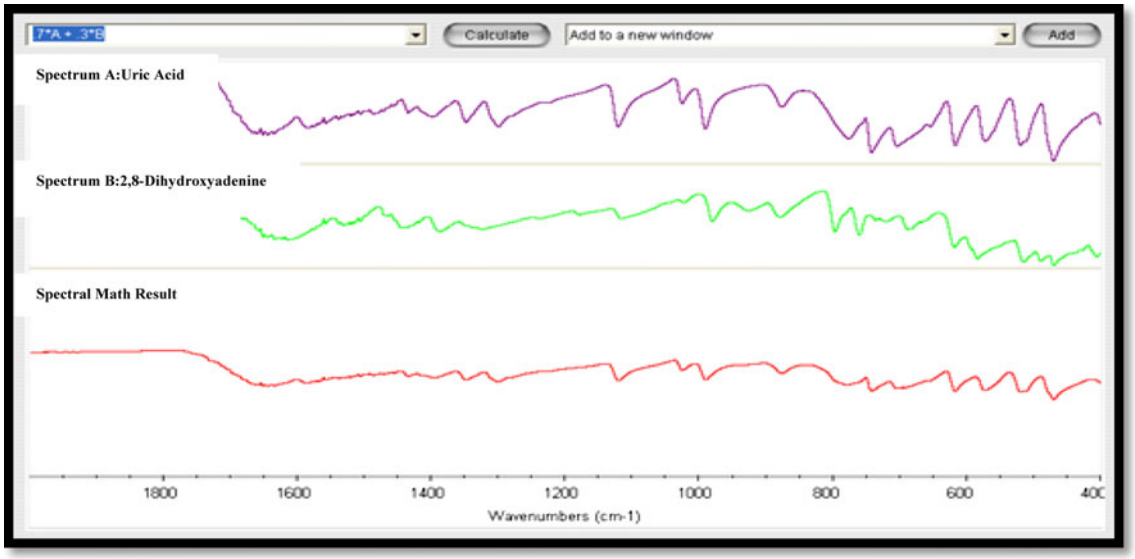

Fig. 1 A 70 + 30% binary construction from two primary pure standards using OMNIC spectral math arithmetic operations

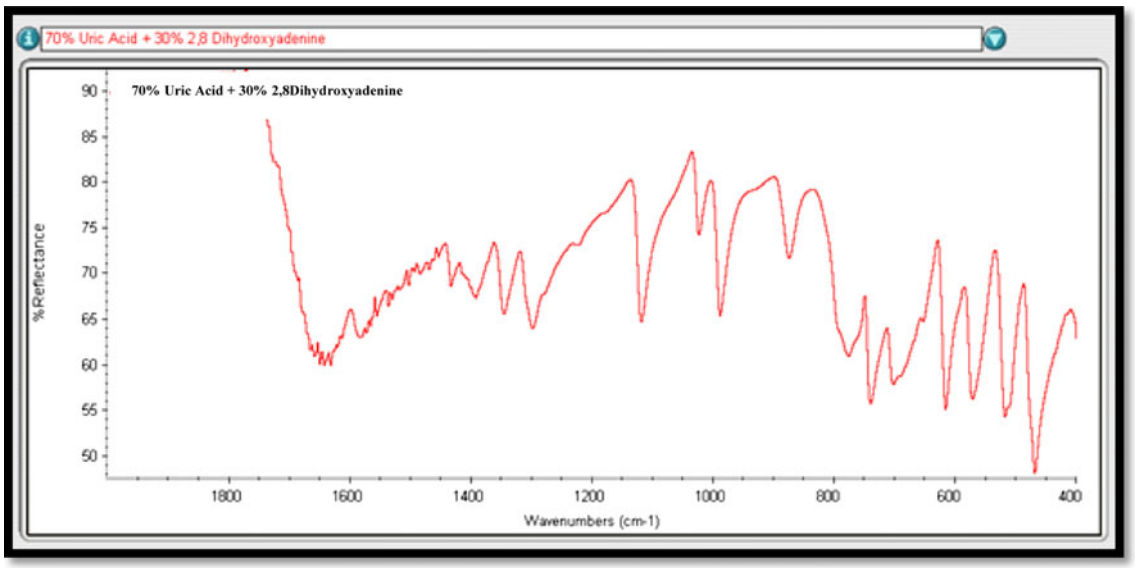

Fig. 2 A resultant 70% uric acid + 30% 2,8-dihydroxyadenine binary constructed standard

application possibilities. Figure 1 depicts the construction of a binary mixture indicating the principles on how OMNIC spectral math arithmetic operations work. Here, 70% UA + 30% 2,8-dihydroxyadenine (2,8-DHA) is constructed as a reference compound by adding 0.7 spectrum A (100% pure UA) to 0.3 spectrum B (100% pure 2,8-DHA). The spectral math result of 70% UA + 30% 2,8-DHA is then added to the sFTIRATR library for library searching (Fig. 2). In total, an easy to use comprehensive and flexible sFTIRATR library incorporating the most frequent mixture types was created containing 531 spectra. This library is not exhaustive and additional custom spectra utilizing the OMNIC software to produce binary and ternary admixtures can be added if deemed clinically relevant.

Although CA and CaOxDH are two of the most common components in kidney stones, they could not be obtained from commercial companies. Instead, they were obtained by a selection based on sample purity. Purity was established by comparison of IR spectra with (1) the reflectance spectra of 100% CA and 100% CaOxDH used by another user for construction of a kidney stone library (2) by confirming the presence of calcium phosphate (CaP) or calcium oxalate (CaOx) with wet chemical analysis and (3) further verified by a NICODOM atlas (the NICODOM Kidney Stones Analysis software package has been created by spectroscopists and medical doctors for quantitative analysis of kidney stone compositions using Thermo Nicolet FT-IR spectrometers with OMNIC software).

Establishment of the real patient kidney stone library

A total of 191 real human kidney stones were collected consecutively from hospitals and some general practitioner surgeries throughout the RoI. Stones retrieved were from non-invasive ESWL treatments, spontaneous passage from lithiasic patients and surgical removal. Each whole sample was previously homogenously crushed and analysed by the in-house wet chemical method, and both core and surface were microscopically and physically examined, with enough stone concrement left for FT-IR ATR analysis. Prior to FT-IR ATR analysis, each sample was again carefully mixed and pulverized using a pestle and mortar. This global powder was then analysed at room temperature to quantify the relative proportions of the various components. FT-IR ATR does not require mixing the sample with an infrared inactive material such as KBr prior to analysis. Grinding and pressing the sample numerous times is not required and sample pre-treatment is minimal. This is primarily because ATR measurements are independent of sample thickness, provided the sample is thicker than the sampling depth. Owing to the measurements being independent of sample thickness, FT-IR ATR can yield accurate spectra allowing for quantitative stone analysis [13]. The quantitative composition obtained from the whole stone powder for each sample was used for the study material. It was derived using the first hit on library searching, firstly from (1) a reflectance library search using the new sFTIRATR library, secondly by (2) application of a patented ATR conversion algorithm to convert the reflectance spectra into absorbance spectra, followed by smoothing and baseline correction for library searching utilizing the KBrOC library provided with the analyser software. The true quantitative composition for each patient sample was assigned following interpretation of the results from each library search method and the visual inspection by two trained scientific operators. In addition, comparison of these results to those previously analysed by the in-house wet chemical method contributed to the elucidation of the true quantitative composition. Subsequently, each specific designated real kidney stone composition was added to a new library resulting in the establishment of the real patient kidney stone library. All spectra for the real patient kidney stone library were added and stored in the neat ATR reflectance form. This is identical to the spectra produced from stone analysis using an FT-IR ATR spectrometer and of the spectral format of the newly formed sFTIRATR library. It is very important to understand that if a sample is ATR corrected, it is impossible to revert back from the resulting absorbance spectrum to the original reflectance spectrum.

The NICODOM kidney stone analysis kit was used only as a backup for any discrepancies in the library search results. Similar to the KBrOC library, this advanced analysis software algorithm utilizes a KBr transmission-derived library with the useful addition of a "reliability factor" as an indication of the libraries' ability to describe the composition of the stones. It also calculates the "matrix content" of the sample, which is very beneficial for low search matched stones, requiring further investigation [14].

Republic of Ireland kidney stone epidemiology

Following the initial interpretation of the results from each search library with careful visual inspection of the spectra by two trained scientists and the wet chemistry method results, the most accurate stone composition was applied to each sample for formation of the real patient kidney stone library. Subsequently, each specific designated real kidney stone composition was used to present an accurate picture of the epidemiology of kidney stones in the RoI.

The calcareous (calcium-containing) stones were defined as follows: (1) CaOx stones were defined as any kidney stone containing >70% calcium oxalate (either CaOxMH or CaOxDH alone, or a combination). (2) Calcium phosphate stones were defined as those with >30% apatite (CA or HA alone, or a combination) or with >30% CaHP. (3) Stones were classified as calcium phosphate when the calcium phosphate (CA, HA or CaHP) content was $\geq$30% with $\leq$70% calcium oxalate. (4) Mixed calcium oxalate–calcium phosphate stones were categorized if the calcium oxalate content was $\leq$70 plus $\geq$10 to $\leq$30% calcium phosphate content. All other stone types were non-calcareous. MAP stones were defined as any kidney stone containing $\geq$10% MAP. If MAP was present with CA, it was considered an infectious MAP stone [10, 15, 16].

Wet chemical detection of ammonia for MAP presence

Add 1 ml of 10% NaOH to a few milligrams of crushed stone followed by gentle shaking to dissolve it. The presence of a dull orange precipitate after the addition of two drops of Nessler's reagent is confirmation of ammonia and the presence of MAP. A lighter yellow colouration is sometimes seen when the ammonium ion is present in small concentrations. Ammonium sulphate was used as a positive control.

Data analysis

GraphPad Prism 5 was used to calculate Spearman's correlations between the combined qualitative and quantitative wet chemistry method, the sFTIRATR library and the KBrOC library. GraphPad Prism 5 was also used for the non-parametric Wilcoxon signed-rank test for comparison of the quantitative sFTIRATR library method and the KBrOC library. After the application of a Bonferroni

correction, a P value <0.005 was considered statistically significant. The first hit of highest percentage match was used for both libraries for standardization. Bland–Altman agreement plots were used to describe the agreement between these two methods for the seven main stone components using Microsoft Office Excel 2007. This programme was also used for the bar charts comparing the average percentage distribution of the main stone components in females and males according to age group. SPSS version 11.5 was used to show the age and sex distribution of 191 patients with kidney stones in the RoI.

Results

Initially for the construction of the sFTIRATR library, the 100% standard compounds were scanned in the mid-IR region from 4,000 to 400 cm^{-1} wave numbers. However, we felt that the region from 4,000 to 2,000 cm^{-1} was susceptible to noise and possible water vapour interference, which could reduce the percentage match on library searching and cause misleading results. The analysis wave number range was adjusted from 4,000–400 cm^{-1} to 2,000–400 cm^{-1} for both development of the sFTIRATR library and analysing patient samples to establish the real kidney stone library.

From the reference standards, the diagnostic bands identified for the seven main components were as follows: for CaOxMH the bands around 1,314, 778, 511 cm^{-1}; CaOxDH 1,321, 776, 513 cm^{-1}; CaHP region around 1,000, 576, 520 cm^{-1}; HA 1,024, 600, 561 cm^{-1}; CA 1,416, 1,023, 600, 560 cm^{-1}; MAP 1,431, 992 cm^{-1} and for UA 1640 and characteristic regions at 1,583–1,299 cm^{-1} and 775–468 cm^{-1}, respectively.

Correlations between the three kidney stone analysis methods

Spearman's correlation coefficient was performed to assess the degree of relationship between the three methods (see Table 2). For CaOx, all methods showed a near perfect positive correlation with each other. The sFTIRATR library correlated best with each method, with $r_s = 0.971$ with the KBrOC library and $r_s = 0.945$ with the wet chemistry method. For CaP, a high positive correlation was also observed. Similar to CaOx, the sFTIRATR library correlated best with the KBrOC method, with $r_s = 0.935$. The sFTIRATR and KBrOC library methods had close to identical correlation coefficients when compared with the wet chemistry method for CaP agreement. Statistically weaker correlations were observed with MAP among the three methods. The sFTIRATR library produced positive correlations with both the KBrOC and wet methods, $r_s = 0.666$ and 0.699, respectively. In contrast, for CaOx and CaP molecular complexes, the sFTIRATR library showed a closer agreement with the wet chemistry method for MAP. The KBrOC and the wet chemistry method correlated weakly positively for MAP, $r_s = 0.566$.

Table 2 Spearman's correlation coefficients between the three methods

Molecular component	sFTIRATR (r_s)	KBrOC (r_s)	Wet (r_s)
CaOx			
sFTIRATR	1	0.971	0.945
KBrOC		1	0.930
Wet			1
CaP			
sFTIRATR	1	0.935	0.906
KBrOC		1	0.907
Wet			1
MAP			
sFTIRATR	1	0.666	0.699
KBrOC		1	0.566
Wet			1

The Wilcoxon signed-rank test was used as a non-parametric equivalent to the paired t test. A Bonferroni correction was applied to an initial cutoff value of 0.05 to give a cutoff of 0.005. This was to take into account that eight comparisons (7 main components +1 for all) would be performed, i.e., a cutoff of 0.05 divided by 8 gives a cutoff of 0.00625, which was rounded off to the 0.005 cutoff. Using this P value cutoff of 0.005, significant differences were found to exist between the KBrOC and sFTIRATRlibrary methods for CaOxMH, CaOxDH, CaHP, HA and MAP, respectively (see Table 3).

Comparison of the KBrOC library and sFTIRATR library methods

Three of the 191 samples were excluded from the data set because of very poor library percentage matches for both methods (<70%). The agreement between the KBrOC and sFTIRATR library methods, as obtained from the Bland–Alt-

Table 3 Wilcoxon signed-rank test for the sFTIRATR and KBrOC quantitative methods

Crystalline/molecular species	P value (cutoff 0.005)
CaOxMH	<0.0001
CaOxDH	<0.0001
CA	0.504
HA	0.001
CaHP	0.003
UA	0.065
MAP	<0.0001
All	0.813

man plots for the results of the seven most common components for 188 patient samples, is shown in Fig. 3. From this comparison, 12 samples showed a 30% difference and 2 showed a 40% difference between both methods. For example, a sample constituting a binary combination of CaOxMH + HA was composed of 90% CaOxMH and 10% HA with the sFTIRATR library, whereas it comprised 60% CaOxMH + 30% CaOxDH + 10% CA with the KBrOC library method. Therefore, the resulting data point [x (mean of both methods), y (KBrOC–sFTIRATR)] for the stone crystalline component CaOxMH was (75, −30). A sample containing CaOx was composed of 100% CaOxMH with the sFTIRATR library compared to 70% CaOxMH + 30% CaOxDH with the KBrOC library method. Five of the samples which produced a 30% difference contained 100% UA. Each of these was composed of 100% UA with the sFTIRATR library, whereas each consisted of 70% UA + 30% 2,8-DHA with the KBrOC library method. The resulting data points (x, y) for UA in these cases overlapped were (85, −30). The two samples which showed a 40% difference were composed of 90% CA and 10% MAP with the sFTIRATR library compared to 50% CA + 30% MAP + 20% whitlockite with the KBrOC library method. Therefore, the resulting data point for CA was (70, −40). From the Bland–Altman method agreement plot for both methods, the KBrOC method has a tendency to overestimate CaOxDH and CA. In contrast, the sFTIRATR library displays a positive bias for CaOxMH and HA when compared with the KBrOC library.

Composition identification of poorly matched samples using the NICODOM kidney stone analysis kit

Application of the NICODOM kidney stone analysis kit and visual inspection of the spectra produced accurate stone compositions for two poorly matched library search results. The first sample consisted of 30% CA + 40% AmU + 30% CaOxMH with a reliability index of 81 and matrix content of 8%. The second sample consisted 60% UA + 30% AmU + 10% CaOxMH with a reliability index of 86 and matrix content of 0%. These real stone ternary compositions were subsequently added to the real patient library.

Establishment of the real patient kidney stone ATR library

One patient sample of the 191 analysed produced a very poor and unreliable percentage match between methods. Consequently, this was not added to the real patient library. Application of the NICODOM atlas showed that it constituted the uncommon CaP crystalline species amorphous carbonated calcium phosphate. When a stone sample is analysed by FT-IR ATR, the spectrum produced for the sample is in percentage reflectance format. All spectra in the real patient kidney stone library were added and stored in this neat ATR reflectance form. The real patient library contained 42 pure biological stones, 73 binary and 75 ternary mixtures. Numerous binary and ternary combinations were very similar comprising the same components in different proportions (see for examples Table 4).

Republic of Ireland kidney stone epidemiology

For 191 patient kidney stone demographics, gender-wise comparisons disclosed that the majority of kidney stones were derived from male patients (69%) with an overall male: female sex ratio of 2.2:1. Age-wise comparison of the data revealed that the majority of stones were recovered from patients in the age groups 40–49 years (Fig. 4).

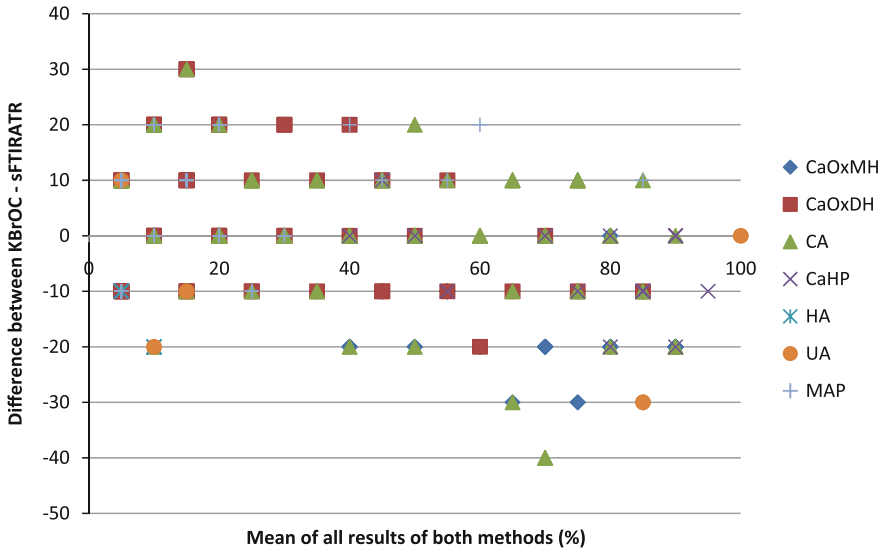

Fig. 3 Bland–Altman agreement plot of the results for the seven most common components in 188 kidney stones as analysed by the KBrOC and sFTIRATR library methods. 95% confidence interval lines of the differences between both methods were omitted for clarity (14 in all)

❖ 95% confidence interval lines of the differences between both methods were omitted for clarity (14 in all).

Table 4 Examples of pure, binary and ternary mixtures of kidney stones added in establishing the real patient library

Pure crystalline/molecular
100% CaOxMH
100% CA
100% Cystine
100% UA
Binary combinations
50% CaOxMH + 50% CA
60% CA + 40% CaOxMH
50% CA + 50% MAP
80% CaHP + 20% CaOxDH
90% CaOxMH + 10% HA
Ternary combinations
50% CaOxDH + 20% CaOxMH + 30% CA
50% CaOxDH + 20% CaOxMH + 30% HA
50% CA + 40% MAP + 10% CaOxDH
70% CaOxMH + 20% CaOxDH + 10% CA
50% CA + 30% CaOxMH + 20% CaOxDH

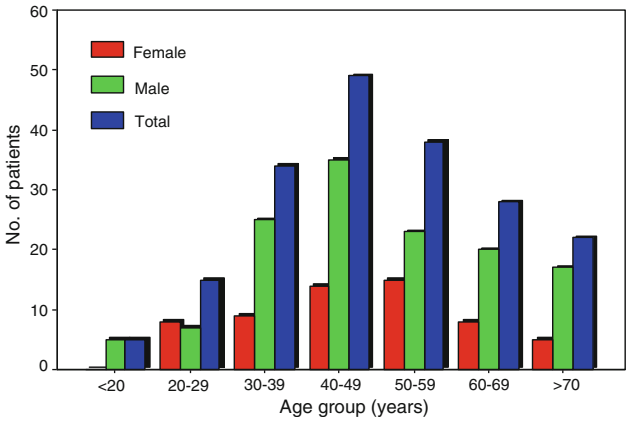

Fig. 4 Age and sex distribution of 191 patients with kidney stones in the RoI

All 191 stones were genuine pathological kidney stones, with no stones of dubious origin. The stone classification systems in the literature vary considerably and suffer from a lack of standardization. We implemented our classification definitions as per Pak et al. [16]. The results of the stone composition analysis revealed that 63 patients (33%) were afflicted with pure stones. CaOx stones were the most common pure molecular stone accounting for 62% of all pure stones. Interestingly, the crystalline form CaOxMH, the thermodynamically stable form of calcium oxalate, was present in 66% of all kidney stones and accounted for 41% of the pure CaOx stones; the remainder was a mixture of the two hydrated CaOx forms. CaOxDH did not occur in a pure form in either male or female within any age group. It must be stressed that calcium oxalate monohydrate was not further divided into the two groups of papillary and non-papillary CaOxMH.

CaOx and CaP were found as the major or at least a minor component in 74 and 70% of all kidney stones, respectively. When stones were classified based upon the classification criteria used to define calcareous stones, CaOx was the most prominent stone type in the RoI accounting for 40% of total kidney stones followed by CaP constituting 34%. CA was the commonest crystalline form of CaP and was the second most common crystalline species after CaOxMH for our data series. Interestingly, CaHP was typically present as a pure form or as the major component combined with 10% CaOxDH. CaHP accounted for 6% of all stones. HA was present only in negligible amounts in 6% of total stones. Various binary and ternary mixtures of different crystalline forms of CaOx mixed with CaP were present in 55% of all stone types. However, based on the classification criteria used to define calcareous stones in our series, it was disclosed that mixed CaOx–CaP stones (CaOx $\leq$ 70% and CaP > 10% but $\leq$30%) were the most common mixed stone group. They accounted only for 6% of all kidney stone types. MAP was the third most abundant stone type accounting for 9%, with 88% of MAP stones found in binary combination with CA. UA and cystine were found solely as pure forms and accounted for 4 and 3% of the total stone types in the RoI and 1% of all stone types were attributable to AmU stones. Rarer stones such as xanthine and 2,8-DHA were absent in our series.

Stone composition in females

Our data series revealed no one specific molecular component abundant throughout all age groups in females. No stones were present for the <20 years age group (Fig. 5). CaOx was the most abundant molecular component for the age classes 20–29 and from 50–59 years through to old age. CaOxMH was the most abundant crystalline CaOx phase and the ratio of CaOxMH to CaOxDH increased dramatically among the age groups 50–59 and 60–69 years, reaching 8:1. However, CaOxDH was close to a ratio of 1:1 at the more extreme age groups of 20–29 and >70 years old, respectively. In the age classes 30–39 and 40–49 years, CaP was the most prevalent molecular stone component: the average percentage proportion of stone components attributed to these classes was 61 and 54%, respectively. For the CaP crystalline species in these age classes, CA was the predominant phase. Overall in the age class 20–29 years, CA was also the most prevalent crystalline species (28%). HA was identified in some age groups but only in minute amounts, never exceeding 2% of the average percentage proportion of stone components in the respective age groups. CaHP was present in all age groups between 20 and 69 years old, its average percentage ranging from 5% to a

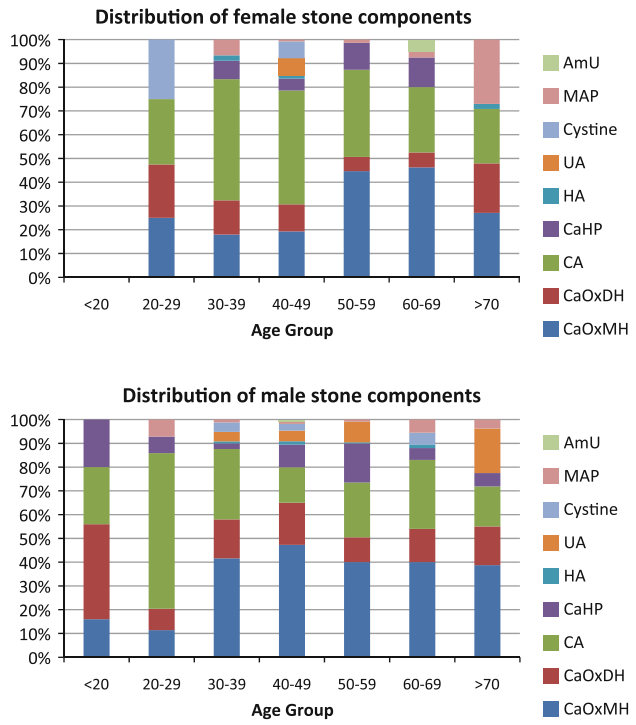

Fig. 5 Average percentage distribution of the main stone components in females and males according to age group

peak of 13%. CaHP was absent at the extremes of life in females. Throughout our series, UA was found in only one female patient and was in the age group 40–49 years old. AmU had a very low prevalence with one female observed in the 60–69 years age group. MAP was most prevalent at old age, accounting for 26% of the average percentage of stone components in patients older than 70 years of age. Between the ages 40–59 years, the average percentage of MAP was especially low at 1%, but was still clinically significant. Cystine had a frequency peak for females in the age group 20–29 years of 25% of the average percentage of stone components.

Stone composition in males

With the exception of the 20–29 years age group, CaOx was the most abundant molecular component of stones observed in males. With regard to the CaOx crystalline species, our data series revealed that CaOxDH was more abundant in the early years up to 20–29 years age group (Fig. 5). CaOxDH was 2.5 times more prevalent than CaOxMH in young males under 20 years. From 30 years to old age, CaOxMH supersedes CaOxDH as the predominant crystalline hydration phase. In contrast to females, among the age classes 30–39 and 40–49 years, CaOx was the most prevalent molecular stone component, their average percentage of stone components accounting for 58 and 66%, respectively. Similarly to females, CaP was the main stone component in the 20–29 years group with CA the preponderant crystalline phase (66%). However, this was the only age class where CaP was the most abundant molecular component. The same pattern was observed in this data series for males as in females for HA. HA never exceeded 2% of the average percentage proportion of stone components in any age group, with CA presenting as the markedly predominant apatite crystalline species. CaHP was present in all age groups in males and accounted as high as 20 and 17% of the total average percentage proportion of stone components in young males <20 and 50–59 years, respectively. In general, the prevalence of UA increased with age, accounting for 19% of the average percentage in elderly men over 70 years old. However, in our series, UA was not found in the age group 60–69 years, which accounted for 15% of all kidney stones in males. Similar to females, AmU had a very low prevalence with one male presenting with an AmU nephrolith in the 40–49 years age group. MAP was more prevalent at age extremes, but its average percentage proportion never exceeded 7%. Interestingly, cystine had a frequency peak for males attributable to 5% of the total average percentage proportion of stone components in the age group 60–69 years.

Discussion

We describe an optimum method for the quantitative analysis of kidney stone composition using an FT-IR ATR spectrometer with library searching utilizing a newly constructed sFTIRATR library and a real patient kidney stone library. This method makes use of an FT-IR spectrometer with a diamond crystal ATR accessory and will replace the existing wet chemical method because of its numerous drawbacks. Kasidas reported that 80% of participants involved in a urinary kidney stone external quality assurance scheme used wet chemical analysis, with the remainder of laboratories using more sophisticated physical techniques like IR spectroscopy (14%). Wet chemical analysis has been traditionally used for its ease and low cost; however, IR spectroscopy was far superior overall in analytical performance. Conventional wet chemical analysis is laborious, its limit of detection is poor for its qualitative tests and it requires at least 10–15 mg of sample. This is in stark contrast to FT-IR ATR spectroscopy, which is highly sensitive and selective with a sample requisite less than 1 mg [15]. All wet chemical methods cannot detect rare and unidentified material and, with the exception of pure kidney stone types, wet chemical analysis can only indicate the presence of individual ions and radicals rather than specific stone compositions. Wet chemical analysis does not easily detect rare drug-induced or metabolic compounds such as

xanthine and 2,8-DHA [9–11]. Even in the absence of any detectable metabolic abnormality, the precise crystalline stone composition may allow the institution of prophylactic therapy [10]. It is now becoming more apparent that the most appropriate therapeutic regimen to prevent kidney stone recurrence also relies on quantitative assessment of the actual crystalline composition of the kidney stone [1, 4]. Significantly, wet chemical analysis methods cannot distinguish crystalline species. This differentiation of molecular components into its respective crystalline phases provides predicative value in diagnosing medical conditions and also helps predict treatment response with ESWL [16]. For example, CaHP stone formers are more difficult to treat, requiring more ESWL treatment cycles for complete comminution of stones when compared with apatite stone formers [17].

The new sFTIRATR library was validated compared with the KBrOC library provided with the analyser. The current in-house wet chemistry method measures the constituents of stones on a combined qualitative and quantitative basis and was correlated for total molecular components obtained compared to the two IR search library method results. The precise quantitative composition for each patient stone sample was assigned after computer-aided interpretation of the results from the automatic search comparison for each spectral data library and the wet chemistry method correlations plus confirmatory visual spectral inspection by two trained scientific operators. Subsequently, each assigned real kidney stone composition was added to a new library, resulting in the establishment of the real patient kidney stone library. Both the sFTIRATR library and real patient kidney stone library are in the same spectral reflectance format and accordingly sample spectra can be library searched instantaneously against these libraries for a precise quantitative result, comparing like with like and requiring no spectral manipulations.

Molecular composition and crystalline forms influence the absorbance (and hence reflectance spectrum) of IR radiation and the resulting spectrum is characteristic of each specific compound or mixture corresponding to the rotational and vibrational frequency of the bonds of atoms comprising the stone [4]. The principle diagnostic IR bands observed from the newly constructed sFTIRATR library were in agreement with other studies [1, 4, 18–20]. It was found that the region from 4,000 to 2,000 cm^{-1} was susceptible to noise and possible water vapour interference, which could reduce the percentage match on library searching and generate misleading results. Also, all kidney stones consist of a complex amalgam of an inorganic crystalline mineral phase and organic material [21]. This organic material is a non-crystalline organic matrix phase, which may be composed of all types of organic biomolecules accounting for 2–10% of total dry weight, which can produce strong reflectance bands around 3,000 cm^{-1} [17, 22]. For these reasons, the analysis range was adjusted from 4,000–400 to 2,000–400 cm^{-1} for construction of both the sFTIRATR library and analysis of all patient samples for the establishment of the real kidney stone library. This fingerprint area (2,000–400 cm^{-1}) contains peaks, which have more precise spectral definition and are characteristic of a specific compound or mixture containing bands that are significant to distinguish molecular and crystalline species [6].

Library searching can be implemented for quantitative analysis of kidney stones [14]. Universally, the commercial libraries from the manufacturers derive the reference spectra using synthesized crystalline material, which can demonstrate changes in peak intensity, positional changes at different wave numbers and/or characteristic profile of reflectance bands when compared with real biological stone compositions (see Fig. 6) [4]. Stones which constitute binary or ternary mixtures can have overlapping reflectance bands, resulting in the best-fitting spectrum produced on library searching against synthesized standard libraries misidentifying stone components. As crystalline species distinction best correlates with clinical pathology and is imperative for optimum stone management, these misidentifications could prevent elucidating the true nephrolithiasis mechanism(s) and indicate the incorrect underlying disorder(s) [15]. For these reasons and to eradicate these discrepancies, it is essential to establish a real patient kidney stone library. This ultimately can then be used as a reference in conjunction with the sFTIRATR library for library searching to properly identify the type of stone disease and clues to aetiopathogeny [3, 14]. Also an added advantage with this real patient library is, because it has been constructed using consecutive patient samples which are representative of the population, it contains many spectra similar to new and unknown patient samples for easier and confident identification.

Method comparison

Spearman's correlation was used to assess the relationship between the three methods principally because in contrast to FT-IR methods, the wet chemistry method cannot distinguish crystalline species and measures the constituents of stones on a combined qualitative and quantitative basis producing a total molecular component quantitative result. Therefore, the total molecular components were compared. The high positive correlation between the main chemical/molecular complexes, CaOx and CaP, was offset by a weaker positive correlation for MAP. However, this can be explained. With library searching, an unknown sample spectrum is compared to a number of different library spectra using a search programme for automatic comparison and the best-fitting spectrum is acquired. For standardiza-

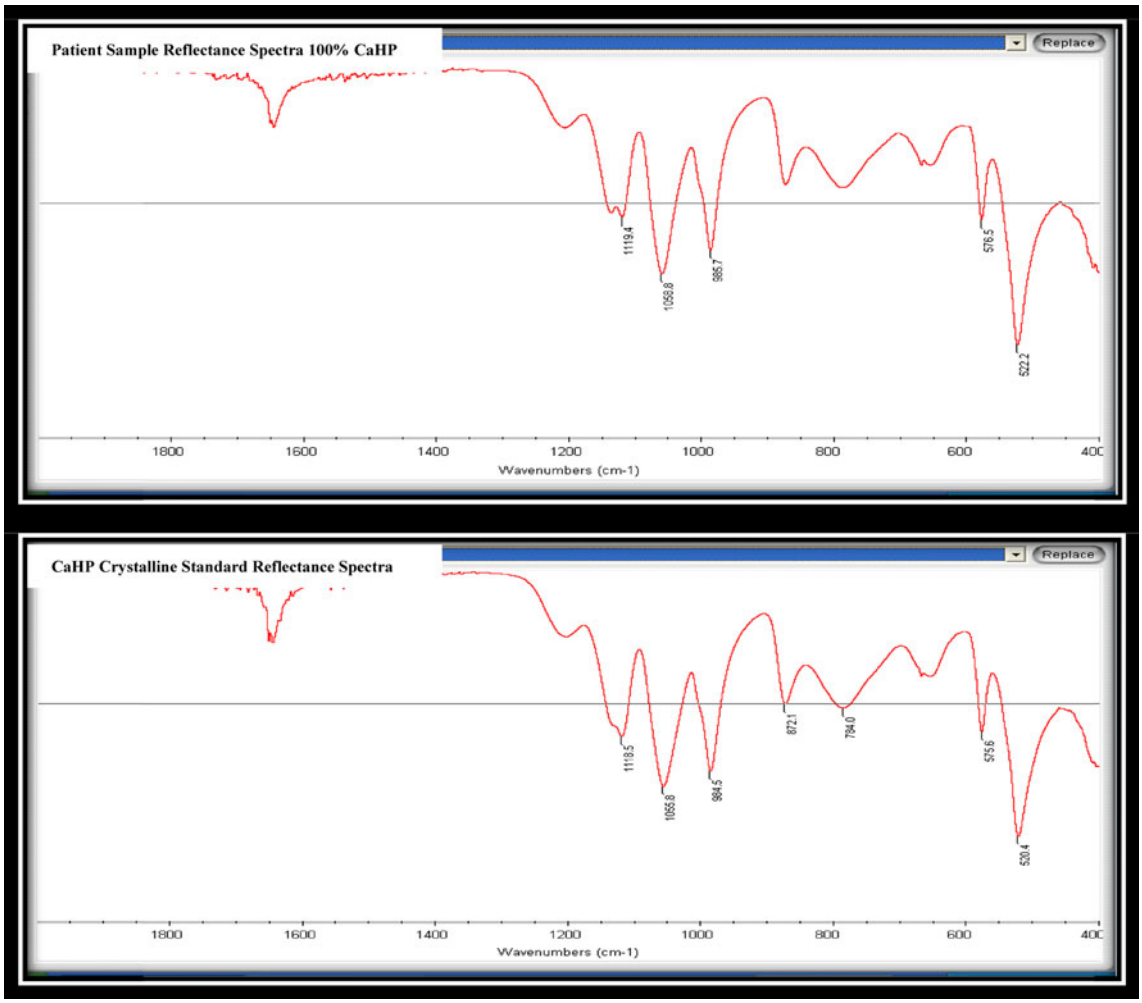

Fig. 6 Example of how a real patient sample (*top*) can exhibit changes in peak intensity, positional changes at different wave numbers and/or characteristic profile of reflectance bands when compared with library reference spectra (*bottom*) and vice versa

tion for comparison of methods, we accepted the first hit which was the highest percentage match obtained. However, no library prediction is perfect and small inaccuracies may occur for some components that are absent in a sample. The total outcome for every sample analysis is 100% and small impurities or variable matrix content may distort spectra, whereby the main component(s) are readily identified but 10% of a minor component may be misidentified. This minor component could be of crucial clinical significance in some cases (e.g. for infectious MAP or AmU stones). It is imperative to acknowledge that the first hit using library searching is not always the correct kidney stone composition. Library spectra can be very similar and the match values can also be very similar. There may be a difference in percentage match between the first and second hit, etc. of as little as 0.01%. Initial interpretation of additional library spectra matches followed by visual inspection is therefore imperative before the final reportable true kidney stone composition can be obtained.

A striking observation using both search libraries was the difference in the percentage match with library standards. A matched value close to 100 indicates that both samples consisted of the same components in about the same ratio. Sample analysis utilizing the sFTIRATR library had consistently best-fitting sample spectrum matches >94%. In contrast, analysis of the same samples after applying the ATR correction and searching with the KBrOC library produced best-fitting sample spectrum matches between 80 and 92%.

Our series data disclosed that on seven occasions where MAP was absent, the newly constructed sFTIRATR library incorrectly detected 10% MAP in combinations with the other main stone components on the first hit. This has treatment implications because MAP is correlated to urea-splitting urinary tract infections (UTI's) and requires antibiotic intervention [3, 23]. This 10% MAP misidentification had a tendency to occur with high percentage CA patient samples. The standard CA has a wide absorption band at

1,023 cm^{-1} corresponding to the stretching vibration of phosphate and carbonate groups. For some real patient samples, this band appeared to shift slightly with a decrease in wave number for this diagnostic band. The synthesized standard MAP has a wide diagnostic band at 992 cm^{-1} corresponding to the stretching vibration of the phosphate group [4, 20]. Ultimately, this patient sample band shift utilizing the computerized reference library match of the closest spectrum measured as a slight band overlap, resulting in a small proportion of MAP misidentification. This patient sample band displacement at this position highlights the necessity for a real patient library. Encouragingly on every occasion, the second or third highest match had the correct stone composition and was negative for MAP using the sFTIRATR library.

Significantly, the wet chemistry method was correct for MAP identification for all samples in our data series. To solve these small systematic inaccuracies in the sFTIRATR library prediction of patient samples for 10% MAP, the simple wet chemical Nessler's test for detecting the presence of ammonia could be used as a testing adjunct. The authentic patient samples in the real kidney stone library will also be very helpful in such circumstances. The KBrOC library misidentified MAP on numerous more occasions and is supported by the weak positive correlation $r_s = 0.566$ with the wet chemistry method. A striking example is seen with a 100% CA patient sample. Following the necessary procedure of applying the patented ATR conversion algorithm to convert the 100% CA sample reflectance spectra from the ATR analysis into a compatible absorbance spectra for library searching utilizing the KBrOC library, 80% CA + 20% MAP was detected. This result pattern for the KBrOC library was common for patients with a high CA content. CA alone is more frequently associated with UTIs caused by non-urea splitting microorganisms, e.g. *E. coli* [3, 24]. In contrast, if CA is in combination with MAP in a kidney stone, then UTIs by urea-splitting microorganisms, e.g. Pseudomonas is considered the main cause [3, 23]. In these circumstances, analysis with the KBrOC library only is highly suggestive of a different aetiology, thereby preventing the most appropriate pharmacological intervention. This erroneous stone composition pattern is also evident when the 100% CA standard used for constructing our sFTIRATR library and a pure CA sample from another user is ATR corrected. An inaccurate 80% CA + 20% MAP stone composition is obtained using the KBrOC library.

Using the Wilcoxon signed-rank test, significant differences were found to exist between the KBrOC and sFTIRATR library search methods for CaOxMH, CaOxDH, CaHP, HA and MAP. Bland–Altman analysis complements these findings showing moderate biases to exist between the two methods for the respective crystalline forms. Predictably, when comparing the seven components in unison with the same statistical analysis, no significant difference was found to exist between the two groups. This is likely due to the fact that the sFTIRATR library and in particular the KBrOC library method are prone to either over- or underestimating certain crystalline components. As the composition is reported as a 10% step size, the positive bias for one component would result in a negative bias for another, effectively causing the two to cancel one another out when making comparisons of multiple components. This concept is highlighted with CaOx stones. The KBrOC library generally underestimates CaOxMH and overestimates CaOxDH when compared with the sFTIRATR library. We are highly confident that the sFTIRATR library is the most accurate and sensitive method for CaOx identification and distinguishing CaOxMH from CaOxDH in the correct proportions. In the case of an early pure CaOxMH patient stone, the presence of CaOxDH is detected by an increase in percentage reflectance (decrease absorbance) for the sharp band at 1,314 cm^{-1} and an increase in the 778/511 cm^{-1} reflectance ratio relative to that found for pure CaOxMH. In contrast, CaOxMH may be detected in a nearly pure CaOxDH patient stone by a sharpening decrease in percentage reflectance of the 776 cm^{-1} band [25].

When the 100% CaOxMH synthesized reference standard utilized for construction of the sFTIRATR library was ATR corrected using the ATR conversion algorithm and measured using the KBrOC library, a 80% CaOxMH + 20% CaOxDH concentration was identified. This pattern was also evident for 100% pure CaOxMH patient samples, and with samples of lower concentrations of CaOxMH the difference was less. This discrepancy between the ratio of percentages for the two hydrated forms of CaOx is highlighted by the statistical significant difference ($p < 0.0001$) obtained. However, importantly on a molecular level they still constitute the same overall CaOx percentage. For example, a 90% CaOxMH + 10% CaOxDH measured by the sFTIRATR library compared to a 70% CaOxMH + 30% CaOxDH measured with the KBrOC library are both 100% molecular CaOx. Clinically, there is no significance with these differences, as a predominance of CaOxMH is mainly associated with hyperoxaluric conditions, whereas predominant CaOxDH is mainly linked to hypercalciuria conditions and an increased risk of stone recurrence [26, 27]. Nephrolithiasis should never be mistaken as a true diagnosis as such, but is essentially a clinical manifestation of an underlying defect. This is particularly evident with calcareous stones, because calcareous kidney stone formation can evolve from an extensive range of underlying pathological disorders. Therefore, the establishment of a kidney stone should not signify an end point for any diagnostic or therapeutic efforts, but should be the start of a more rigorous clinical and metabolic investigation [2].

The statistical significant difference between the two methods for CaHP had also clinical significance because of the minor components co-identified. Our data revealed that CaHP stones tend to occur as pure forms or as the predominant chemical species in binary combination with a CaOxDH. A strikingly common pattern observed with pure CaHP stones was (1) the sFTIRATR library recognized the neat spectra as 100% CaHP; (2) after ATR conversion on occasions the KBrOC library produced a match of predominantly CaHP with a small percentage hit for the presence of MAP. It would appear that after the ATR conversion of the complex CaHP spectrum, there is misinterpretation from the resulting absorbance spectra around the distinctive around region at 1,000 cm^{-1} corresponding to the stretching vibration of the monohydrogen phosphate anion: splitting in three to four bands, which overlap with the diagnostic band at 992 cm^{-1} for MAP [4, 18]. Based on this KBrOC library distinction, the presence of MAP could not be overlooked, indicating a possible UTI, acute or chronic, caused by urea-splitting bacteria.

The statistically significant difference between the two methods for HA was primarily due to the sFTIRATR library, correctly identifying it as opposed to the KBrOC library not recognizing it, always labelling it as CA. The maximum percentage of HA in stones when present was only 20% of the total components, and it typically presented as a minor crystalline component of the total molecular CaP in stones with predominantly CA. The diagnostic value of identifying HA in such circumstances is less apparent. Both the HA and CA spectra are very similar and it seems after ATR conversion of the neat reflectance spectrum, it alters the spectrum enough to misidentify HA as CA. The principle IR diagnostic band for the carbonate vibration for CA at 1,416 cm^{-1} helps distinguish it from HA. Some authors report that all apatite stones contain some degree of carbonation, and particularly in mixtures this carbonate can make it difficult to differentiate CA and HA [17, 24].

Five of the 12 patient samples which showed a 30% difference between both methods constituted 100% UA. They were correctly identified as 100% UA with the new sFTIRATR library. In contrast after ATR conversion, they each consisted of 70% UA + 30% 2,8-DHA with the KBrOC method. This misreading again highlights how the ATR correction can give erroneous results, especially as both UA and 2,8 DHA have specific spectral features that allow differentiating them with good sensitivity. This misinterpretation has crucial clinical significance. 2,8-DHA nephrolithiasis is a rare entity. This autosomal recessive metabolic disorder arises from a deficiency of the salvage enzyme adenine phosphoribosyl transferase. Adenine is consequently metabolized by xanthine oxidase resulting in 2,8-DHA crystalluria and a nephrolith presentation [28]. It is clear that analysis using the sFTIRATR library would correctly direct treatment towards correcting acidic urine with urinary alkalization, the cornerstone of medical management for UA stones. Medical evaluation directed at underlying associated medical conditions found with UA stones such as primary gout, and myeloproliferative and lymphoproliferative disorders may also be warranted in some circumstances to unveil the underlying pathophysiology for the UA stone formation [29]. In contrast, the KBrOC library search results could erroneously orient physician guidance towards the singular pathophysiology of the rare metabolic disease 2,8-dihydroxyadeninuria and indicate allopurinol treatment [28].

Only 2 of the 188 patient samples exhibited maximum differences of 40%. The two samples which showed a 40% difference were composed of 90% CA and 10% MAP with the sFTIRATR library, whereas they consisted of 50% CA + 30% MAP + 20% whitlockite with the KBrOC library method. Although our newly developed sFTIRATR library did not contain whitlockite, we were very confident it was not present in both samples. If a compound is not present in a search library, the percentage match will be low. On both occasions, the best-fitting spectrum obtained was a 96% match. The quantity and quality of the kidney stone components of the most similar library spectrum is known and, because the match value was close to 100, this was indicative that both samples consisted of the same components in very similar proportions, i.e. 90% CA and 10% MAP. In both cases, the application of the ATR conversion algorithm to the neat reflectance spectra acquired from the FT-IR ATR analysis for library searching with the KBrOC library appears to be altered, resulting in the whitlockite misidentification. The presence of whitlockite is often encountered in patients with chronic UTI induced by non-urea splitting microorganisms. However, if either whitlockite or CA is present with MAP in a kidney stone, then UTI by urease-positive microorganisms is considered the primary aetiology [3, 30]. Therefore, in these two specific cases, a stone comprising 90% CA and 10% MAP or 50% CA + 30% MAP + 20% whitlockite would suggest the same aetiopathogeny and treatment strategy. However, this misidentification of whitlockite cannot be overlooked and could have clinical implications in other kidney stone compositions.

The two kidney stone compositions which exhibited poor library matches for both methods were quantified using a special algorithm, the NICODOM kidney stone analysis kit and supported by visual inspection of the spectra. The samples consisted of 30% CA + 40% AmU + 30% CaOxMH and 60% UA + 30% AmU + 10% CaOxMH and both contained normal matrix content. Both libraries produced poor results primarily because they did not contain these rare ternary combinations in their respective library inventory. The library search can only give a high percentage match and precise stone component composition only if

the components are available in the library. To combat this, these patient sample compositions were added to the real patient library and various ratios of these components could be mixed using OMNIC spectral math arithmetic operations and added to our newly constructed sFTIRATR library for future library searching.

Republic of Ireland kidney stone epidemiology

Our laboratory is the reference centre for kidney stone analysis for the RoI. The results obtained from the establishment of the real patient kidney stone library, which were a representative selection of the kidney stones found in the country over a 2-year period, provided an accurate picture of the present epidemiology of kidney stones in the RoI. Over time, the kidney stone distribution and composition will be further supported by a larger number of kidney stones analysed.

In the RoI, nephrolithiasis mainly afflicted adults aged 20–70 years old. This is consistent with reports from other industrialized countries [23, 31–33]. Paediatric nephrolithiasis is an uncommon occurrence in developed nations and no kidney stones were received from females under 20 years old in our data series of 191 stone formers [34]. A preponderance of male urinary stone disease was observed, with over twice as many males afflicted as females. This is in conformity with the findings from other studies [23, 32, 33]. Calcareous stones constituted 74% of all kidney stones, the primary chemical complexes being CaOx and CaP. These findings are also consistent with reports from other industrialized countries [15, 35]. Based on the classification criteria used to define calcareous stones in our series, CaOx was the predominant stone type overall in the RoI population. This predominance was observed in males with CaOx stones constituting 47% of all stones. However, interestingly the frequency of CaOx in females only accounted for 25% of all stone types, with CaP predominant, attributable for 49%. The marked prevalence of CaOx in males was almost twice that of the second most abundant kidney chemical stone component, namely CaP. With regard to crystalline species, CaOxDH was 2.5 times more frequent than CaOxMH in young males under 20 years old, but its prevalence markedly decreased thereafter stabilizing at approximately 16% of the average percentage of all stone components. Conversely in females, the proportion of CaOxDH present in all stones was highest at 20–29 years, then steadily decreased until at >70 years old close to peak levels were observed again. CaOxDH is linked to a high calcium/oxalate ratio, which is suggestive of disorders linked to high urinary calcium content, frequently observed in hypercalciuric patients [4]. Some studies have shown that increased calcium intake from diet or supplements does not increase the prevalence of kidney stones and an inverse relationship between calcium intake and stone risk has been observed [36]. However, the high CaOxDH observed in mature women may be related to provision of calcium and vitamin D supplements to treat and prophylactically prevent osteoporosis post-menopause. This supplementation may increase urinary calcium excretion, which could result in the main pathophysiological factor for CaOxDH stones, hypercalciuria [3, 23]. One may speculate that the overall stone frequency may not be increased, but the proportion of CaOxDH relative to other crystalline components is raised in older females.

Our series data highlight a striking find: females as a gender group have not only a marked increased prevalence of CaP kidney stones when compared with males in all age classes as reported by various authors [23, 37], but also a preponderance of CaP over CaOx kidney stones. CA was the predominant crystalline species and is frequently associated with hypocitraturia, medullary sponge kidney, UTIs caused by non-urea splitting bacteria, hypercalciuria linked to complete or incomplete renal distal tubular acidosis and primary hyperparathyroidism [3, 4]. Many of these clinical pathologies are more prevalent in females and is a plausible explantation why CaP stones are more common in women when compared with men [38].

Interestingly, although CaP stones were preponderant in females, the actual type of crystalline phase distribution was dependent on gender. For example, CaHP was more prevalent in males compared to females. CaHP accounted for 32% of total CaP stones in males and 9% of all male kidney stones. In contrast, CaHP accounted for 7% of all female stones, but comprised only 15% of total CaP female kidney stones. This is a marked difference between the two genders, especially considering CaOx is the predominant stone type in males as opposed to CaP in females. This suggests the role of different pathogenic mechanisms compared with other CaP stones [23]. CaHP frequency of 6% in the RoI population is significantly higher than in other industrialized countries [4, 17, 23]. CaHP is found in patients with hypercalciuria associated with proximal tubular phosphate leak, hypophosphatemia and hyperphosphaturia. It is also frequently associated with cases of primary hyperparathyroidism or sarcoidosis [3, 4].

MAP stones were more common in females than males and were most prevalent at old age. In addition, the majority of infectious MAP stones comprised various MAP proportions mixed in binary combination with various CA proportions. This is indicative of either current or past UTIs and usually necessitates pharmacological intervention [2, 16]. It also points to infectious factors in the pathogenesis of CA stones [23].

UA stones were three times more frequent in males than females and increased in prevalence with age. This was

very apparent in men beyond 70 years of age where a peak frequency for UA nephrolithiasis was observed. Possible explanations have been reported for this increased incidence of UA nephrolithiasis in the elderly. These include defective ammoniagenesis and an increased presentation of the metabolic syndrome with age, both decreasing urine pH and resulting in an increased propensity for UA nephrolithiasis [39]. The incidence of AmU stones was only 1%. In each case, AmU was combined in a ternary mixture with CaOxMH and either CA or UA in adults. Pure AmU stones are endemic in children in developing countries, but are extremely rare in Western developed countries and we would not expect to find them in our series [11]. In industrialized nations, AmU stones are strongly associated with hyperoxaluria, ammonia produced from urease-positive bacteria in UTIs or chronic diarrhoeal syndromes particularly from laxative abuse [11, 16, 40].

Cystine stones constituted 3% of the kidney stones in the RoI, which is in conformity with the other studies [4, 16]. Cystinuria, the result of an autosomal recessive disorder in renal tubular and intestinal transport of dibasic acids, predisposes to the formation of cystine stones. Owing to the inherited nature of this disease, the majority of cystine stones would be expected to be found in the early decades of life. Yet interestingly with our series, its presentation was found throughout various age groups and was even observed in the 60- to 69-year group. This may be in part due to the major challenge for cystine stone formers. Stone-free durations are generally short with concurrent high recurrence rates. Although best managed by a multidisciplinary approach, hydration is the mainstay of treatment. Patients are advised to wake during the night to drink water to prevent stone recurrence. This social inconvenience alone results in poor patient compliance and is a plausible explanation for the consistent prevalence of cystine nephrolithiasis across age groups in the RoI [41].

Conclusion

In conclusion, an FT-IR spectrometer with an ATR accessory utilizing the newly constructed sFTIRATR library method is superior to the KBrOC library FT-IR spectrometer ATR accessory method and the wet chemistry analysis. It compares the neat reflectance ATR spectra measured to the newly constructed sFTIRATR search library negating the use of spectral manipulations, comparing like with like, producing highly sensitive, selective and rapid measurements with unambiguous results for kidney stone composition. Currently there are no ATR libraries available in the reflectance spectral format for library searching with FT-IR ATR instruments. Instead, they must use KBr transmission-derived libraries, which necessitate the application of patented ATR algorithms for spectral compatibility to allow library searching. Our method of comparison discloses that this ATR correction adds another variable, which can distort the patient spectra and is prone to error resulting in misleading component identification. This ultimately can indicate erroneous stone aetiopathogeny.

Reference spectra in commercial libraries provided with specialized software are customarily complied using synthesized crystalline compounds. When compared with authentic biological stone samples, manufactured standards can exhibit changes in peak intensity and size, which can correlate directly with the quantity of the specific component and exhibit changes in wave number position and/or characteristic profile of reflectance bands. To confront such significant discrepancies, a real patient kidney stone library must be established and used as a reference in conjunction with the newly constructed sFTIRATR library for library searching. We established a real patient library by assigning a true quantitative composition to each patient kidney stone following library searching, wet chemistry analysis and visual inspection of the measured spectra. This established library is not exhaustive and additional real patient spectra will continue to be added over the ensuing years.

Our data suggest that the epidemiology for nephrolithiasis in the RoI is similar to other Western societies. The continuation of building the real patient kidney stone library will enable us to further explore the mineral constituents for the RoI population using a multivariate epidemiological approach.

We predict that for the optimum quantitative analysis of kidney stone mixtures, FT-IR ATR spectroscopy utilizing a reference FT-IR ATR library, supported by a real patient kidney stone library, applying library searching will accurately provide the molecular and crystalline forms of stone constituents present in an unknown kidney stone sample. We recommend visual inspection by experienced scientific staff as essential using this diagnostic modality. This will ultimately benefit the stone former, providing invaluable guidance for physicians towards predicting correlations with specific pathophysiological conditions, orientating metabolic evaluations and the implementation of the most appropriate treatment strategy which is essential for prevention of kidney stone recurrence.

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