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N. Gould · P. C. Hallson · G. P. Kasidas · C. T. Samuell
T. B. Weir

Rapid computer-assisted infrared analysis of urinary calculi using photoacoustic detection

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Abstract The application of commercial spectrum-analysing software to quantitative analysis of urinary stones by Fourier transform infrared spectrophotometry is described. The infrared technique is straightforward in comparison with other stone analysis procedures of similar scope and affords significant time savings. The use of partial least squares regression in the analysis program enables better quantitation of stone components than has been hitherto possible using infrared methods. All the principal and many less common stone constituents can be detected and measured. Photoacoustic detection was employed, thus enabling non-destructive analysis with minimal sample preparation. A comparison is made between the infrared procedure and the hybrid thermogravimetric plus "wet" chemistry technique, which it has superseded for routine urinary stone analysis in the authors' department.

Key words Infrared analysis · Urinary calculi
Photoacoustic detection

Accurate knowledge of stone composition is essential to the study of the aetiology of urinary stone formation and for deciding medical regimens. Techniques proposed for stone analysis have been the subject of numerous reports. Thus wet chemistry [13, 23], X-ray diffraction [16, 19], optical microscopy [14], thermogravimetry [18] and infrared transmission spectroscopy [2, 15] have all been tried in the quest for an accurate and straightforward method. None of these is, however, fully quantitative and often such methods require skill both in operation and to interpret results. In addition the instrumentation needed is often expensive.

Beischer [2] in 1955 was among the first to recognise the utility of infrared (IR) spectroscopy in identifying urinary stone materials. Early attempts to apply this technique were, however, attended by problems in spectral interpretation and the infrared analysis of calculus materials remained at best semi-quantitative. In recent years with the interfacing of microcomputers to infrared spectrometers these difficulties have lessened and the arrival of spectrum-analysing computer programs has enabled significant improvements to be made [22].

The authors describe here the application of commercial general purpose software, using partial least squares (PLS) regression, to quantitative stone analyses.

Materials and methods

Infrared analysis

A Nicolet 510 Fourier transform infrared spectrophotometer coupled to a Nicolet computer (Nicolet Instruments, Madison, USA) and fitted with an MTEC photoacoustic detector (MTEC Photoacoustic, Ames, USA) was used to obtain the spectra. Spectra analysis was carried out using the "PLS Quant" quantitative analysis program (Nicolet Instruments, Madison, USA) after appropriate calibration.

To obtain spectra for calibration purposes the following materials were used: calcium oxalate monohydrate and cystine (BDH, Poole, UK); calcium hydrogen phosphate ("brushite") and anhydrous uric acid (Aldrich, Gillingham, UK); and xanthine and 2:8-dihydroxyadenine (Sigma, Poole, UK). Hydrated apatite, calcium oxalate dihydrate, magnesium ammonium phosphate hexahydrate (MAP $6H_2O$), ammonium urate and uric acid dihydrate were also used and were prepared in the authors' laboratory [1, 11].

Calibration protocols

Mixtures used to obtain calibration spectra were made by grinding stone component materials in their pure chemical form in known combinations and proportions by weight. The IR spectra of these

N. Gould · G. P. Kasidas · C. T. Samuell · T. B. Weir
Department of Chemical Pathology, University College London
Medical School, Windeyer Building, Cleveland Street,
London W1P 6DB, UK, Fax: +44 (71) 3 80 94 96

P. C. Hallson (✉)
Institute of Urology and Nephrology, University College London
Medical School, Windeyer Building, Cleveland Street,
London W1P 6DB, UK

Table 1 Constituents of the mixtures used for calibration in the four PLS Quant spectrum-analysing programs (*MAP* $6H_2O$ magnesium ammonium phosphate hexahydrate)

	Program			
	1	2	3	4
Calibrant materials	Calcium oxalate monohydrate	Cystine	Anhydrous uric acid	Xanthine
	Calcium oxalate dihydrate	MAP $6H_2O$	Uric acid dihydrate	Ammonium urate
	MAP $6H_2O$	Apatite	Calcium oxalate monohydrate	Dihydroxyadenine
	Anhydrous uric acid	Brushite		
	Apatite		Calcium oxalate dihydrate	
	Brushite			

Table 2 Regions of the infrared spectrum scanned during operation of each of the four PLS Quant programs. These bands were selected to optimise the discrimination of the stone components in analysis (*MAP* $6H_2O$ magnesium ammonium phosphate hexahydrate)

Program	Use	Number of calibration samples	Spectral regions scanned (cm^{-1})
1	Common stone components	72	1800–500
2	Cystine with MAP $6H_2O$ and calcium phosphates	30	1750–500
3	Hydrates of urid acid and of calcium oxalate	36	3750–2600 2150–400
4	Uncommon components	72	3750–2327 1800–500

mixtures were entered onto the PLS Quant calibration program together with their composition as a percentage by weight. The calibration operation yielded the PLS Quant spectrum-analysing programs required.

The components of the calibrant mixtures for the four PLS Quant analysing program which were developed are shown in Table 1. The proportions (percentage weight) of each material in the calibrant mixtures were widely spaced to cover the concentrations of the analytes from 1 to 100%. At the same time these proportions were set out in such a way as to minimise correlation between component concentrations, this being essential for PLS to operate properly. The absence of such correlations was confirmed by the software program after the concentration data had been entered onto the worksheet, since it was then possible to generate instantly a plot of each component concentration against each other for visual inspection. The calibration software automatically placed the more extreme reference sample concentration values into the calibration set. It then divided the remaining ones between the calibration and validation set. During the calibration operation the number of mathematical factors which are optimal for the performance of each model were automatically computed. The calibration program calculated the errors associated with the validation standards as the number of factors increased in a stepwise manner. When the errors became minimal the correct number of factors was selected and the computation stopped. During the development of each program a series of trial calibrations was carried out during which the spectral regions scanned and the resolution (data point spacing) was optimised. This was enabled by observing changes in the predicted concentrations of the reference samples which were automatically computed by the current calibration model. In general it was found that increases in the spectral areas scanned led to better results (apart from those regions associated with atmospheric CO_2 and bound water). Table 2 shows the optimal spectral regions and the number of calibrant mixtures used in each program. Program 1 is a general program which was designed to analyse the most frequently occurring stone constituents. Programs 2 and 3 were intended for the analysis of cystine and uric acid stones, respectively, together with the other stone materials with which they frequently occur in combination. Certain uncommon calculus components were assigned to a rarity program, number 4.

Calculus analysis

Typically a 30-mg quantity (minimum about 1 mg) of the stone sample, previously dried at 37 °C, was used. Quantities <1 mg can be used with some deterioration in quantitation; nevertheless the qualitative results remain satisfactory. The material was crushed to powder in an agate mortar. After grinding, the material was scraped from the surface of the mortar and reground. The powder was transferred to an aluminium sample dish (7×1 mm) which was inserted into the sample holder of the photoacoustic detector. The apparatus was sealed and flushed with helium (10 ml/s) for 30 s and the sample scanned 128 times during 3.5 min. The resulting spectrum was rationed against a background spectrum (of a standard black material) to yield a difference spectrum in photoacoustic spectroscopy units. This was labelled and saved on hard disk. The operator time required from grinding to saving of the spectrum was approximately 7 min.

Spectrum analysis

Small and systematic errors were generated during spectral analysis of calculus material when using PLS Quant analysing programs 1, 2 and 3, and this matter is discussed later. Simple sets of rules were therefore written to limit these errors and these procedures were conveniently saved as separate computer correction programs.

The spectra of the urinary stones for analysis were recovered from disk and entered into the PLS Quant spectral-analysing program chosen. The analysis operation, which took several seconds per spectrum, displayed and printed out the uncorrected assay results. These were keyed into the appropriate correction program, giving the final adjusted analysis.

Thermogravimetric analysis

The IR plus PLS Quant analyses were compared with a quantitative hybrid procedure employing thermogravimetry and "wet" chemistry (TGA/WC) which has been employed for many years in the authors' department [18, 23].

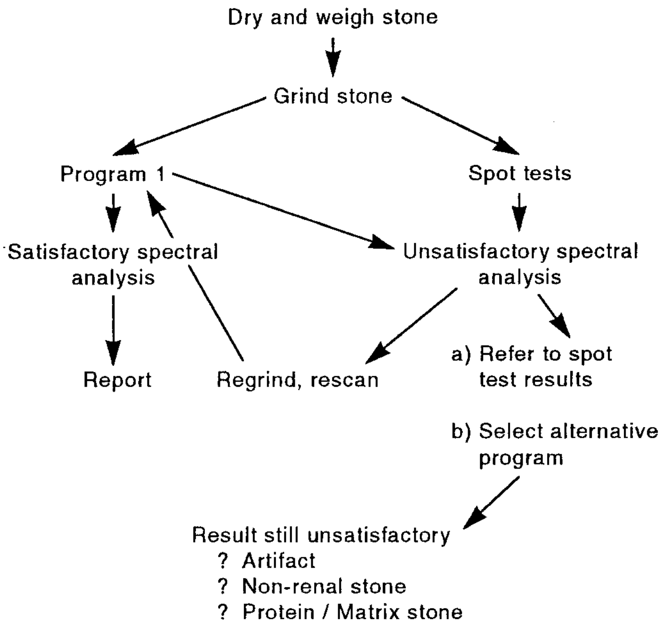

Fig. 1 Flow diagram showing sequence of operations during urinary stone analysis by IR/PLS

Qualitative chemical tests

The presence of cystine and the ammonium ion were detected by spot tests using the nitroprusside reaction [21, 23] and Nessler's [6, 23], respectively. Oxalate and urate were detected enzymatically using oxalate oxidase and uricase, respectively, the H_2O_2 formed being coupled, via peroxidase, to colour-forming reagents. The presence of carbonate in a sample was indicated by effervescence after addition of concentrated HCl. These tests aid in the selection of PLS Quant programs best suited to the analysis of individual stone samples especially when the stone composition is complex.

Sample for method evaluation

One hundred and fifty-seven stones submitted to the laboratory were analysed by TGA/WC and by the IR/PLS scheme outlined in Fig. 1. To evaluate the performance of program 1 when limited amounts of stone substance were available 26 stone samples, weighing between 0.9 and 7.1 mg (mean 3.7 mg), were analysed. In order to assess the accuracy of this program in predicting the extent of calcium oxalate hydration, eight mixtures of calcium oxalate monohydrate and calcium oxalate dihydrate (as pure chemicals) were weighed out and assayed by IR/PLS.

Results

The stone samples used for IR/PLS evaluation covered the range of expected stone composition. Thirty-one percent of the reference stones were single compounds whilst 61% and 8% contained two and three components, respectively. For these purposes hydrates of calcium oxalate and uric acid and both forms of calcium phosphate (apatite and brushite) were classed as single components. Figure 2a and 2b shows the correlations found between the combined calcium oxalate hydrates and the combined calcium phosphates (apatite + brushite) respectively, as estimated by the two methods. Infrared plus PLS Quant estimates of calcium phosphates slightly exceed those determined by thermogravimetry with wet chemistry. Predictions of total calcium oxalates by IR/PLS are, on the other hand, slightly below the TGA/WC values.

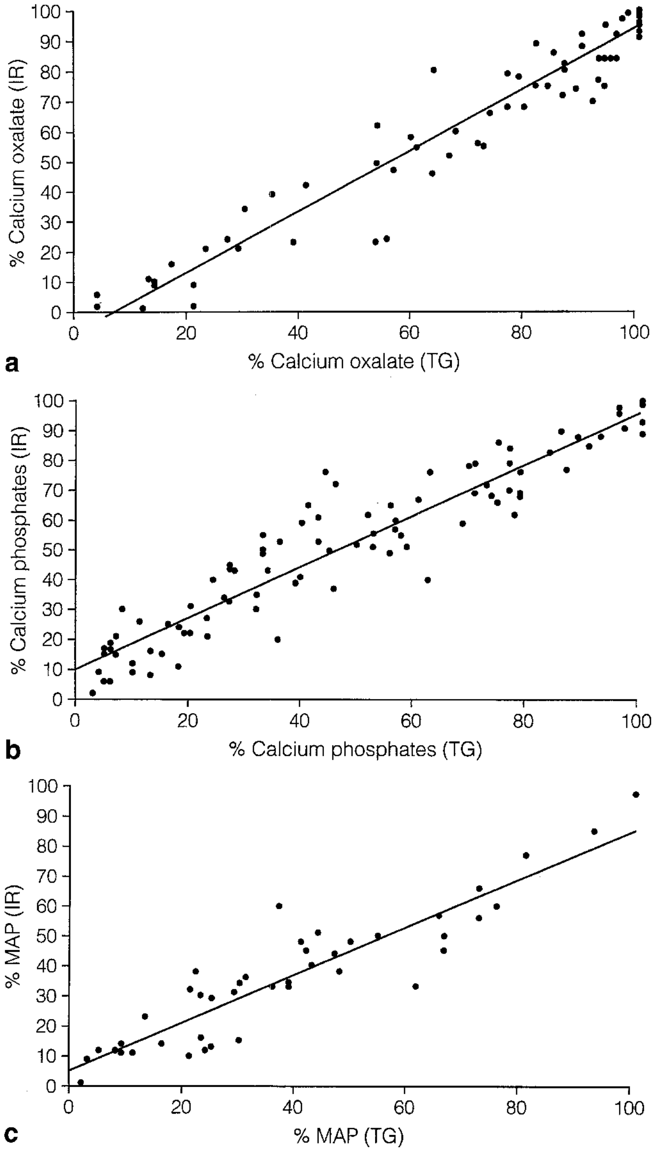

Fig. 2a–c Correlations between the analysis of urinary stone constituents by infrared spectroscopy using program 1 (*IR*) and thermogravimetric analysis/wet chemistry (*TG*) for: **a** Calcium oxalate monohydrate plus dihydrate, $n = 79$, $r = 0.97$, $y = 1.02x - 6.8$. **b** Total calcium phosphates (apatite + brushite), $n = 95$, $r = 0.95$, $y = 0.86x + 10.33$. **c** Magnesium ammonium phosphate $6H_2O$, $n = 44$, $r = 0.92$, $y = 0.8x + 5.4$

The hydration of MAP when present in renal stones is evidently slightly variable [5], which may, in part, account for the somewhat poorer correlation with TGA/WC for MAP compared with those of calcium oxalate and phosphate (Figure 2c). Cystine, on the other hand, with numerous strong and distinct absorption peaks in the IR

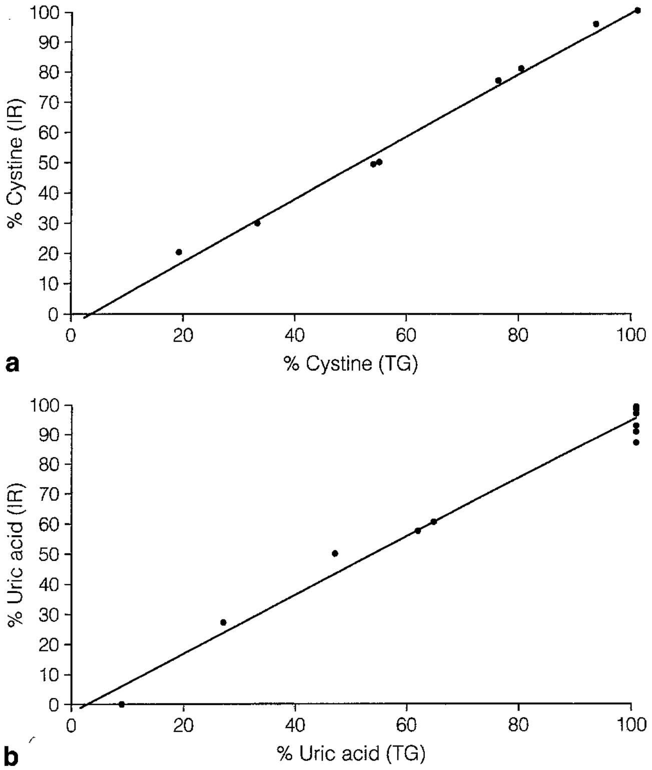

Fig. 3a, b Correlations between infrared spectroscopic assay (*IR*) and thermogravimetric plus wet chemical analysis (*TG*) for two organic stone constituents: **a** Cystine analysed using program 2, $n=9$, $r=0.99$, $y=1.04x-3.6$. **b** Total uric acid (anhydrous+hydrated forms) analysed using program 1, $n=13$, $r=0.99$, $y=0.98x-2.7$

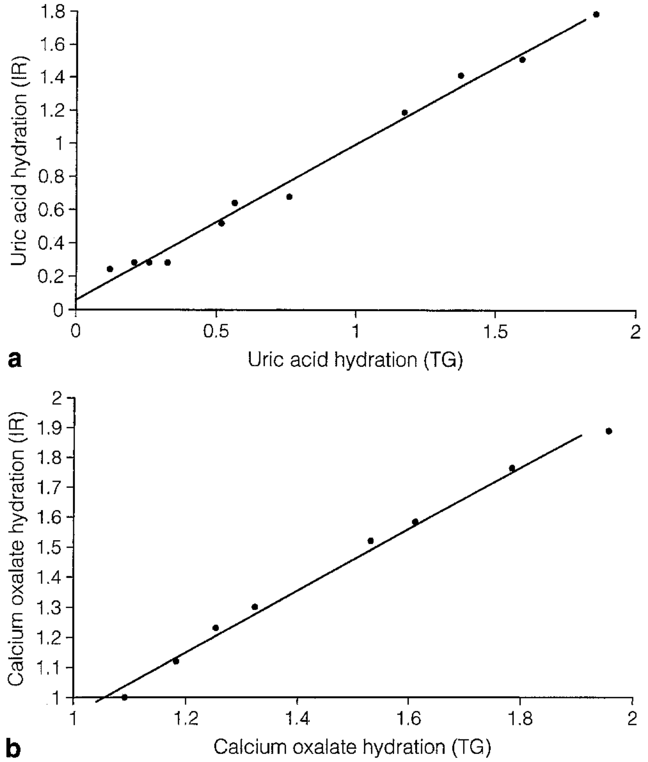

Fig. 4a, b Correspondence between estimates of hydration of two stone constituents, uric acid and calcium oxalate, obtained by the infrared technique described (*IR*) and by thermogravimetry and wet chemistry (*TG*). (The six samples richest in uric acid dihydrate were prepared from uric acid stone material to which known amounts of pure uric acid dihydrate had been added). **a** Uric acid analysed using program 3, $n=11$, $r=0.99$, $y=0.95x+0.06$. **b** Calcium oxalate analysed using program 1, $n=8$, $r=0.99$, $y=1.033x-0.09$

“fingerprint region” of $1400-650\,\mathrm{cm}^{-1}$, gives a good correlation between the two methods (Fig. 3a). Probably for the same reason, the assay of anhydrous uric acid in program 1 also correlates well with thermogravimetry (Fig. 3b). However, the occasional presence in stones of the dihydrate of uric acid in significant amounts upsets the correct operation of program 1. To overcome this a separate urate program (No. 3) was provided to enable the analysis of the two forms uric acid together with the two hydrates of calcium oxalate which often occur in association in urate stones. The correlation between estimates of uric acid hydration is good (Fig. 4a). The relationship between the true hydration of calcium oxalate in the test samples and the IR/PLS estimates appears in Fig. 4b. Pearson’s correlation coefficient was 0.996.

The rarity program (No. 4) has been shown to be effective in identifying the uncommon stone materials either as pure chemicals or as calculus material. A full infrared analysis was possible for the 26 small stones and the correlation with the TGA did not differ significantly from the analysis of the 157 larger stones referred to earlier. The actual and PLS Quant predicted compositions of some urinary stone and artificial mixtures prepared to evaluate the PLS Quant programs are shown in Table 3.

Discussion

In 1955 Beischer [2] pioneered the application of infrared spectroscopy to the analysis of urinary calculi. Beischer was able to visually identify spectral features which corresponded to the presence of specific stone constituents with the aid of specially prepared spectral templates and his method was adopted by subsequent workers [20]. The subjective element in such estimation was lessened when computer search facilities became available for the automatic comparison of spectra [5, 15], but analyses remained semi-quantitative at best. Rule-based systems also evolved which relied upon the interpretation of salient spectral features in a systematic way [7, 17]. Rule-based methods were sometimes combined with computer spectral search facilities [12]. More recently, infrared analysis has been attempted by Volmer et al. using a mathematical technique for spectral analysis [22].

Like Volmer, the present method employs spectral analysis based upon partial least squares regression, thus eliminating any need for visual or computer comparison of absorption peaks. PLS is a multivariate statistical procedure which has been successfully applied to both infrared and ultraviolet quantitative spectral analysis. The

Table 3 Comparison of the percentage composition of prepared mixtures of pure chemicals (*Mix*) and urinary stone materials (*Stone*) as determined by thermogravimetric (*T*) and Fourier transform infrared analysis (*F*) (*MAP* magnesium ammonium phosphate hexahydrate)

Program 1	No.	Sample	Calcium oxalate				MAP		Uric acid		Apatite	Brushite	Apatite	Brushite
			H_2O	$2H_2O$	H_2O	$2H_2O$								
			T	T	F	F	T	F	T	F	T	T	F	F
	1	Mix	75	0	68	0	0	0	25	32	0	0	0	0
	2	Mix	10	31	6	25	0	0	60	68	0	0	0	0
	3	Mix	70	0	65	0	0	0	0	0	30	0	34	1
	4	Stone	30		0	34	2	1	62	58	5		3	3
	5	Stone	56		40	5	0	0	30	36	13		12	6
	6	Stone	64	16	46	22	0	0	0	1	20		27	4
	7	Stone	0	100	10	90	0	0	0	0	0	0	0	0
	8	Stone	0	0	0	0	55	50	0	0	45		50	0
	9	Stone	0	0	0	0	44	51	0	0	56		49	0
	10	Stone	8	77	6	80	0	0	0	0	15		13	2
	11	Stone	0	0	0	0	93	85	0	0	7		15	0
	12	Stone	32	47	15	63	0	0	0	0	20		18	4
	13	Stone	41		15	27	0	0	27	27	32		30	0
	14	Stone	0	0	0	0	0	0	0	0	0	100	0	100
	15	Stone	0	0	0	1	41	48	0	0	59		51	0
	16	Stone	13		0	11	24	12	0	0	63		67	9
	17	Stone	0	0	3	0	100	97	0	0	0	0	0	0
	18	Stone	20	80	13	80	0	0	0	6	0	0	1	0

Program 2	No.	Sample	Cystine		MAP		Apatite	Brushite	Apatite	Brushite
			T	F	T	F	T	T	F	F
	1	Mix	76	77	21	18	3	0	3	2
	2	Mix	55	50	9	13	36	0	37	0
	3	Stone	80	81	0	8	0	20	0	11
	4	Stone	100	100	0	0	0	0	0	0
	5	Stone	100	100	0	0	0	0	0	0
	6	Stone	0	2	31	31	69		65	2

Program 3	No.	Sample	Calcium oxalate				Uric acid			
			H_2O	$2H_2O$	H_2O	$2H_2O$	Anhydrous		$2H_2O$	
			T	T	F	F	T	F	T	F
	1	Mix	58	0	53	0	42	47	0	0
	2	Mix	25	0	24	0	75	76	0	0
	3	Stone	0	0	0	0	74	74	26	26
	4	Stone	0	0	0	0	90	85	10	14
	5	Stone	0	0	0	0	94	88	6	12
	6	Stone	4		0	4	80	82	16	14

method is useful when there is little information available about which spectral frequencies are important but no shortage of data for use for calibration purposes. The method depends upon estimating in an iterative manner a linear combination of spectral features in such a way that the concentrations of the materials giving rise to them are optimally predicted. PLS Quant is Nicolet software (utilising PLS algorithm 2) which fully incorporates calibration and prediction computation facilities and thus requires no special mathematical knowledge on the part of the user. Details of the PLS approach are described by Haaland and Thomas [10]. Volmer [22] pointed out that qualitative stone compositions which are unlike the calibration set result in deviations by PLS regression from the actual composition. Whilst this is our experience also, we have found it possible to arrange the four PSL Quant programs in such a way that calculus component which usually occur in combination can be assayed using a single program and without difficulty. PLS spectral analysis functions optimally when the target concentrations fall in a limited range. Component concentrations of urinary stones vary from zero to 100% and therefore it was necessary to limit the number of components in each calibration set to ensure satisfactory analytical performance. Hence four separate sets were developed in order to analyse a wide range of stone constituents. Relatively smaller deviations from the true composition arise as the result of the presence of other materials which are also unrepresented in the calibration set. Thus the presence of proteinaceous stone matrix substances and the small variations in chemical composition due, for example, to carbonate in apatite and to water in both apatite and MAP [5] often give rise to small but false-positive reports of substances present in the calibration set. Apatitic stone materials are particularly prone to cause errors of this kind evidently because this family of compounds possess different basic molecular formulae [8, 9] in addition to their variable hydration and carbonation. Hence they cannot be effectively mimicked in any calibration procedure. This difficulty is reflected in the correlation of calcium phosphate assays with thermogravimetry. The errors are often directly related to the proportions of actual stone components present in the sample. Thus such deviations are to some extent predictable and amenable to straightforward arithmetic correction. These corrections can be quickly and conveniently applied using a second computer correction program. Choice of the wrong PLS Quant analysis program in the first place will frequently result in an assay total which either significantly exceeds or is markedly below 100%. This prompts the operator to select an alternative program.

As stated, the four PLS Quant analysis programs have been designed to enable those components which are commonly found in association in stones to be present in the same calibration set. Hence a wrong choice of analysis program is unlikely and, with the support of spot test results, easily corrected if it happens.

Some atypical calculus materials have been placed in the rarity program (No. 4), whilst the IR spectra of others are saved on hard disk in the computer. Inevitably only a small number of such stones were available to us and this, so far, has prevented a thorough evaluation of the rarity program. Such components almost always appear as the sole constituent of a stone and the program is effective in determining these as the major component. Other rare compounds, such as silica or calcium carbonate, may be identified by checking their characteristic absorption peaks and confirming any correspondences by overlaying the unknown spectrum upon that of the authentic material on the computer screen.

The specificity of the PLS Quant analysis programs requires calibrant compounds with near identical spectral features to those found in the sample to be analysed. It was therefore necessary to prepare certain of these in the author's laboratory since they were commercially unobtainable. These preparations, all straightforward, have been published [1, 11]. Following a satisfactory calibration this operation does not have to be repeated.

One innovation in the IR/PLS scheme described was the use of photoacoustic detection (PAD) in the spectrophotometer. When PAD is used in infrared spectroscopy the surface of the sample under study is excited with modulated infrared radiation from the source. Absorption of the radiation by the sample results in heating and thermal expansion. This, in turn, gives rise to sound waves in the helium atmosphere of the detector. The sound waves vary in sympathy with the absorbed radiation and a microphone is used to convert them into corresponding electrical signals. This form of detection is important when precious samples must be preserved or when they are very opaque or both.

The use of PAD avoids the inconvenience of sample preparation which was necessary for ealier IR analysis, such as mulling the sample with nujol or subjecting it to high pressures, which risks altering its chemical composition [3]. Furthermore PAD enables non-destructive analysis and precious small samples are preserved. Milling of the stone is all that is necessary for sample preparation; however, consistency in grinding technique is important to ensure good results [4, 24]. Consistency in packing the material when loading the sample dish also pays dividends in this respect. Most stones in the present study were dried at 37 °C before grinding and only in a few instance was more intensive drying helpful.

During development the present method was compared with the accurate and well-tried TGA/WC technique for stone assay, which has been successfully employed at the Middlesex and St. Peter's Group Hospitals for many years. The IR/PLS method showed acceptable correlation with TG/WC but is simpler and more straightforward to apply and requires only a fraction of the time and effort.

Further improvement is doubtless possible and the extension of this analytical tool to IR microcopy of urinary crystals, post-lithotripsy fragment and stone section scanning may provide further applications.

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References

1. Babić-Ivančić V, Füredi-Milhofer H, Milat O, Brown WE, Gregory TM (1985) The conditions for precipitation of uric acid and sodium acid urate. In: Schwille PO, Smith LH, Robertson WG, Vahlensieck W (eds) Urolithiasis and Related Clinical Research. Plenum, New York, p 785
2. Beischer DE (1955) Analysis of renal calculi by infrared spectroscopy. J Urol 73:653
3. Bellanato J (1990) Infrared spectroscopy of urinary calculi. In: Buck AC, Wickham JEA (eds) Renal Tract Stone. Churchill-Livingstone, Edinburgh p 45
4. Belton PS, Wilson RH, Saffa AM (1987) Effects of particle size on quantitative photoacoustic spectroscopy using a gas-microphone cell. Anal Chem 59:2378
5. Berthelot M, Cornu G, Daudon M, Helbert M, Laurence C (1987) Computer-aided infrared analysis of urinary calculi. Clin Chem 33:2070
6. Dawson RMC, Elliot DC, Elliot WH, Jones KM (1969) Data for Biochemical Research, 2nd edn. Oxford University Press, London p 619
7. Gault MH, Ahmed M, Kalra J, Senciall I, Cohen W, Churchill D (1980) Comparison of infrared and wet chemical analysis of urinary tract calculi. Clin Chim Acta 104:349

8. González-Díaz PF, Hildago A (1976) Infrared spectra of calcium apatites. Spectrochimica Acta 32A: 631
9. González-Díaz PF, García-Ramos JV, Santos M (1979) Composition of apatites in human urinary calculi. Calcif Tissue Int 28: 215
10. Haaland DM, Thomas EV (1988) Partial least squares methods for spectral analysis. 1. Relation to other quantitative calibration methods and the extraction of qualitative information. Analytical Chemistry 60: 1193
11. Hallson PC, Kasidas GP, Samuell CT (1993) Preparation of calibrant materials for renal calculi analysis – some problems solved. Communications in Laboratory Medicine 2: 5
12. Hesse A, Gergeleit M, Schüller P, Möller K (1989) Analysis of urinary stones by computerized infrared spectroscopy. J Clin Chem Clin Biochem 27: 639
13. Hodgkinson A (1971) A combined qualitative and quantitative procedure for the chemical analysis of urinary calculi. J Clin Pathol 24: 147
14. Laskowski D (1965) Chemical microscopy of urinary calculi. Anal Chem 37: 1399
15. Lehmann CA, McClure GL, Smolens I (1988) Identification of renal calculi by computerized infrared spectroscopy. Clin Chim Acta 173: 107
16. Morriss R, Beeler M (1967) X-ray diffraction analysis of 464 urinary calculi. Am J Clin Pathol 18: 413
17. Oliver LK, Sweet RV (1976) A system of interpretation of infrared spectra of calculi for routine use in the clinical laboratory. Clin Chim Acta 72: 17
18. Rose GA, Woodfine C (1976) The thermogravimetric analysis of renal stones (in clinical practice). Br J Urol 48: 403
19. Sutor DJ, Wooley SE (1970) The composition of urinary calculi by X-ray diffraction. Br J Urol 42: 302
20. Tsay Y-C (1961) Application of infrared spectroscopy to the analysis of urinary calculi. J Urol 86: 838
21. Varley H, Gowenlock AH, Bell M (1980) Practical Clinical Biochemistry, Vol. 1, 5th edn. Heinemann, London p 1175
22. Volmer M, Bolck A, Wolthers BG, Ruiter AJ de, Doornbos DA, Slik W van de (1993) Partial least-squares regression for the routine analysis of urinary calculus composition with Fourier transform infrared analysis. Clin Chem 39: 948
23. Westbury EJ, Omenogor P (1970) A quantitative approach to the analysis of renal calculi. J Medical Laboratory Technology 27: 462
24. Yang CQ, Fateley WG (1986) The effect of particle size on Fourier-transform infrared photoacoustic spectra. J Molecular Structure 146: 25