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Determination of Metals (Ca,Mg) and Non-metal(P) in Stones of a Anbar patients west of Iraq by Atomic Absorption Spectrometer(AAS) Technique

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ABSTRACT

Renal stone is a one common disease of urinary system and rarely studies about it. Therefore the aim of this research is to determine chemical compositions of stones using qualitative method and quantitative method to detect calcium, magnesium, and phosphorus ions. Forty three calculi (14 from female percentage 32.6% and 29 of male percentage 67.4%) were examined using visible spectroscopy method at different wave length for different elements while chemical qualitative tests were used for qualitative determine compositions of stone.

The analytical quantitative methods used for determination the concentration of calcium, magnesium, and phosphorus ions were accurate reliable and sensitive with standard deviation 0.004596, 0.00239, and 0.000756 for calcium, magnesium, and phosphorus ions respectively. Low detection limit (L.O.D.) were0.112, 0.0177, and 0.189 mg/dl for calcium, magnesium, and phosphorus ions respectively while the (RSD) were 1.4%, 2.36%, and 5.04% for calcium, magnesium, and phosphorus ions respectively.

Keywords: Renal stone, urinary system, qualitative methods, quantitative methods, and spectroscopy.

I. INTRODUCTION

Kidney stones are also called nephrolithiasis or urolithiasis, they are aggregation of materials or minerals and develop form a small stone or small crystals (crystalluria) in the kidney, ureter or bladder[1].Renal calculi also can be defined as the consequence of a variation in conditions of normal crystallization of urine in urinary system. In a healthy Peoples crystals do not form or are so small they are Extraction uneventfully (asymptomatic crystalluria) during urine stay in urinary tract. The rate of crystal nucleation is growth. Normal urine crystallization conditions change and may become a large crystals then cannot be easily eliminated according to the size. In some cases, change urinary conditions affecting crystallization are related to some diseases such as hyperparathyroidism and hypercalciuria [2]. crystalluriabecomes aggregation and then becomes stone when the urine becomes highly concentrated .In Normal conditions crystalluria pass through the urinary tract without problems. sometimes, if crystalluria become large enough, they may cause obstruction of the kidney drainage system which may result in severe pain, bleeding, infection or kidney failure. The obstruction sites of stone in the upper urinary system are located at the:

1 - Junction where the kidney meets the upper ureter.
2 - Mid portion of the ureter.
3 - Lower ureter at its entry into the bladder [3].

Renal calculi also can defined as a solid piece of material that forms in a kidney or other sites of urinary system when substances found in the urine become highly concentrated[4]. Risk factors responsible for contributing to stone formation have been identified, including environmental ,metabolic, dietary, racial, gender, obstructive uropathology and urinary tract infection.[5]bacteria of urinary tract infections (UTI) play an important role in the synthesis of renal stone[6].Renal stones are discover about 7000 years ago and maybe earlier. The earliest recorded example is bladder and kidney stones detected in Egyptian mummies dating to 4800 years B.C. (a urinary stone belonging to a 16-year-old boy). urinary stone was found in a boy from 3000 years ago in America[7].The existence of kidney stones has been recorded since the beginning of civilization, and lithotomic for the removal of stones is one of the earliest known surgical procedures[8].There are several types of kidney stones according to the type of crystals and compositions of which they consist. The majority are calcium oxalate stones, followed by calcium phosphate stones and uric acid stones. More rarely, struvite stones are produced by urea-splitting bacteria in people with urinary tract infections, and people with certain metabolic abnormalities may produce cystine stones[9]. Calcium salts, uric acid, cystine, and struvite ($MgNH4PO4$) are the basic constituents of most kidney stones in the western hemisphere. Calcium oxalate and calcium phosphate stones make up 85% of the total stones and may be Located in the same stone [10].

II. EXPERIMENTAL WORK AND RESULTS

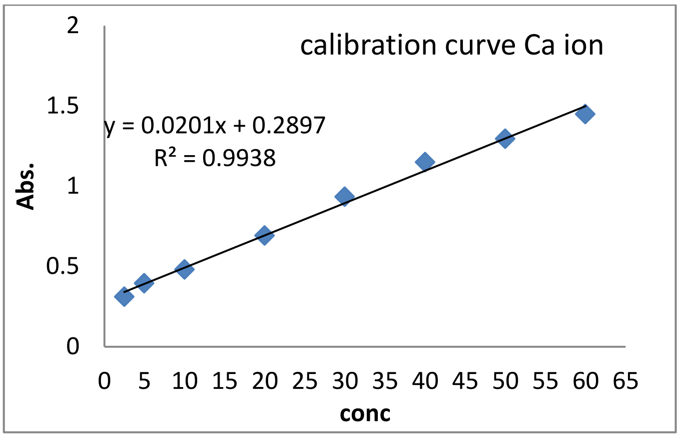
Forty three of stones (14 from female percentage 32.6% and 29 of male percentage 67.4%) were calculated from Anbar government patients. They were recruited from September 2013 until March 2014. Samples were collected from surgeries and laparoscopic operations in Urology Department in Ramadi Teaching Hospital and others of Anbar hospitals. After stone collected we wash it by Distilled Water and let it to dry then kept in plastic cup in room temperature and labeled to be used for qualitative and quantitative analysis .Information of patients and stones listed in Table (1).

1- Qualitative method :
   a) The stone grinding and take about 50 mg of the calculus powder was weighed and transferred into a test tube and 10 drops of reagent R1 were added. The mixture is called M1.
   b) Then mixture M2 was prepared by mixing 50 µl of M1 and 5 ml of distilled water. After M1 and M2 were prepared, according to the steps below, a drop of mixture M1 or M2 was transferred into each tube [11]. The result of qualitative chemical method showed in Table (2).

2- Quantitative method :
   a) Quantitative determination of calcium by visible spectrophotometer: 0.01 gm of powder stone was digested in 1 ml of 1N HNO_3, 16.5 N HNO_3, and deionizer water. The concentration of calcium was determined in different digest solution using Colorimetric method, without deproteinization, using o-cresolphthalei - ncomplexone. Interference due to Mg2+ ions is eliminated by 8-hydroxyquinoline (12)(13). Calibration curve for calcium was prepare using the same colorimetric method. The absorbance of samples read was fitted to the calibration curve Fig (1) to obtained the concentration of samples. the results of calcium are listed in Table (3). To obtained on the concentration of calcium in µg/gm we used the following relationship :

Concentration of calcium =

$$\frac{concentration\ in \frac{\mu g}{ml}\ x\ volume\ in\ ml}{weight\ in\ gm}$$

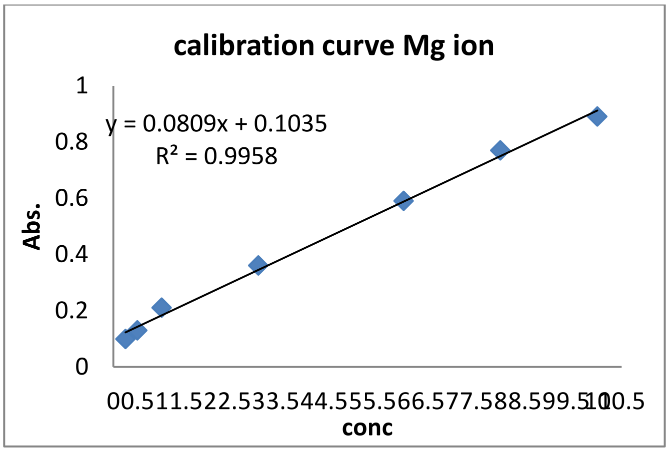


Fig(1) Calibration Curve of Ca ion

   b) Quantitative determination of magnesium by visible spectrophotometer : 0.01 gm of powder stone was digested in 1 ml of 1N HNO3, 16.5 N HNO3, and deionizer water. The concentration of magnesium was determined in different digest solution using Colorimetric method. The method based on the binding of calmagite, a metallochromic indicator and magnesium at alkaline PH with the resulting shifts in the absorption wavelength of the complex. The intensity of the cromophore formed is proportional to the concentration of magnesium. Calibration curve for magnesium was prepare using the same colorimetric method. The absorbance of samples read was fitted to the calibration curve Fig (2) to obtained the concentration of samples. the results of magnesium are listed in Table (4). To obtained on the concentration of magnesium in µg/gm we used the following relationship :

Concentration of magnesium =

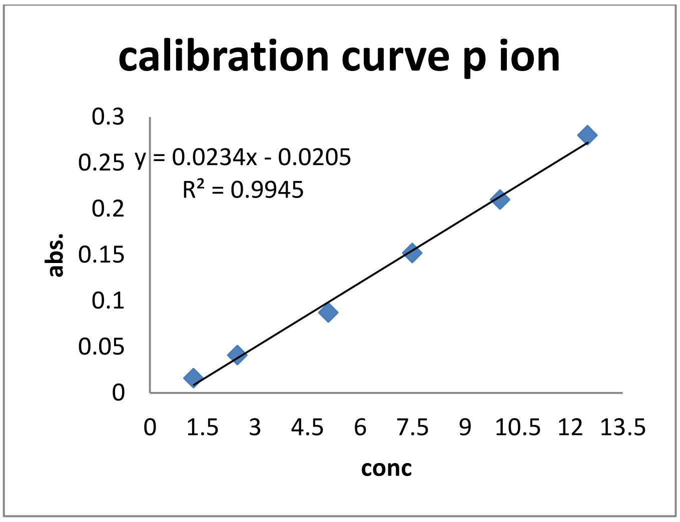
$$\frac{concentration\ in \frac{\mu g}{ml}\ x\ volume\ in\ ml}{weight\ in\ gm}$$



Fig(2) calibration curve of Mg ion

c) Quantitative determination of phosphorus by visible spectrophotometer: 0.01 gm of powder stone was digested in 1 ml of 1N HNO3, 16.5 N HNO3, and deionizer water. The concentration of magnesium was determined in different digest solution using Colorimetric method. Inorganic phosphate in sample reacts with molybdic acid to form a phosphomolybdic acid complex(14), which is reduced by ammonium iron (II) sulphate to molybdenum blue, which measured at 690 nm(15,16). Calibration curve for phosphorus was prepare using the same colorimetric method. The absorbance of samples read was fitted to the calibration curve Fig (3) to obtained the concentration of samples. the results of phosphorus are listed in Table (5). To obtained on the concentration of phosphorus in µg/gm we used the following relationship :

$$\text{Concentration of phosphorus} = \frac{concentration\ in \frac{\mu g}{ml}\ x\ volume\ in\ ml}{weight\ in\ gm}$$



Fig(3) calibration curve of P ion

Fig (4) picture of some stones

Table (1) information of patients and stones

Pat. No.	Age /year	sex	History of patient	Weight of Stone /gm	Color of Stone	rigidity
1.	65	Male	1 kidney only +stone former	21.2	Yellowish brown	Rigid
2.	35	Male	No History	3.3	Dark brown red	Rigid
3.	60	Male	No History	9.5	Brown orange	Rigid
4.	60	Male	D.M + stone former	20.03	Gray yellow	Rigid
5.	51	Male	stone former	0.32	Black	Soft
6.	73	Male	D.M+H.T+MI	7.4	White yellow	Soft
7.	41	Male	stone former	2.64	black yellow	Rigid
8.	25	Male	stone former	1.97	orange yellow	Soft
9.	30	Male	stone former	1.27	Orange yellow	Soft
10	40	Female	D.M+HT	0.95	yellow	Soft
11	40	Male	No History	0.59	Black brown	Soft
12	70	Male	No History	12.45	yellow	Rigid
13	60	Female	D.M+ Obesity	10.81	Black yellow	Rigid
14	40	Female	No History	0.86	Brown yellow	Soft
15	23	Male	No History	0.78	Brown yellow	Rigid
16	28	Male	No History	0.54	Brown yellow	Soft
17	70	Female	No History	2.15	Dark brown	Rigid
18	38	Male	No History	0.48	yellow	Soft
19	35	Male	No History	5.56	Black brown	Rigid
20	27	Female	D.M+HT	2.48	Black brown	Rigid
21	50	Female	No History	1.11	Gray yellow	Rigid
22	60	Male	D.M	14.08	Black brown	Rigid
23	43	Male	No History	6.76	Brown yellow	Soft
24	25	Female	No History	0.56	Brown	Rigid
25	23	Female	No History	0.8	Black brown	Rigid
26	33	Male	No History	0.9	Brown yellow	Rigid
27	28	Female	No History	1.5	Brown yellow	Soft
28	40	Female	D.M	0.1	Brown yellow	Soft
29	30	Male	No History	0.2	Brown	Soft
30	27	Male	stone former	1.54	Black	Soft
31	40	Male	D.M	2.7	Black	Rigid
32	35	Male	No History	0.14	White	Soft
33	28	Male	No History	0.10	Black	Soft
34	3	Female	No History	2.10	Brown	Rigid
35	33	Male	H.T	7.13	Dark brown	Rigid
36	3	Female	No History	7.34	Brown	Rigid
37	40	Female	D.M+HT	56.96	Yellowish white	Rigid
38	38	Male	stone former	36.86	Brownish yellow	Rigid
39	28	Male	No History	0.28	Gray	Soft
40	50	Male	D.M	1.69	Black	Soft
41	52	Female	No History	1.71	Yellow	Soft
42	49	Male	stone former	1.99	Gray	Soft
43	29	Male	No History	0.11	Brown	Soft

Table (2) concentration of Calcium in Stones

Stone No.	Visible technique					
	1N HNO_3 mg/dl	µg/gm	16.5N HON_3 mg/dl	µg/gm	D.I.W mg/dl	µg/gm
1.	7.2	72	15.0	150	4.96	49.6
.2	24	240	53.8	538	15.23	152.3
.3	22.2	222	28.3	283	10.89	108.9
.4	22.92	229.2	29.6	296	17.40	174
.5	30.12	301.2	58.4	584	19.30	193
.6	11.7	117	18.0	180	10.6	106
.7	28.98	289.8	36.6	366	17.7	177
.8	24.3	243	28.4	284	17.14	171.4
.9	17.1	171	22.0	220	10.5	105
.10	23.58	235.8	29.0	290	12.14	121.4
.11	29.1	291	40.1	401	17.1	171
.12	9.41	94.1	13.2	132	8.7	87
.13	28.2	282	42.6	426	17.3	173
.14	28.98	289.8	48.5	485	18.96	189.6
.15	29.1	29.1	38.2	382	15.6	156
.16	30.24	302.4	53.8	538	20.9	209
.17	29.04	290.4	52.4	524	21.3	213
.18	26.64	266.4	28.2	282	18.71	187.1
.19	35.59	355.9	38.8	388	15.66	156.6
.20	7.32	73.2	10.0	100	5.75	57.5
.21	48.5	485	53.4	534	27.5	275
.22	28.8	288	32.8	328	8.83	88.3
.23	48.7	487	50.2	502	20.2	202
.24	49.7	497	53.4	534	30.6	306
.25	48.7	487	51.4	514	10.4	104
.26	28.99	289.9	29.8	298	19.9	199
.27	34.5	345	40.2	402	18.0	180
.28	27.73	277.3	40.3	403	15.4	154
.29	11.88	118.8	12.84	128.4	5.3	53
.30	40.0	400	45.0	450	16.4	164
.31	42.3	423	44.22	442.2	16.995	169.95
.32	43.5	435	44.22	442.2	32.64	326.4
.33	40.3	403	42.11	421.1	12.12	121.2
.34	40.96	409.6	41.325	413.25	9.96	99.6
.35	32.46	324.6	39.15	391.5	17.34	173.4
.36	6.09	60.9	8.07	80.7	5.64	56.4
.37	40.1	401	42.1	421	36.94	369.4
.38	27.2	272	28.46	284.6	11.94	119.6
.39	24.87	248.7	27.47	274.7	14.4	144
.40	32.52	325.2	41.1	411	12.375	123.75
.41	19.1	191	20.59	205.9	10.2	102
.42	10.8	108	12.46	124.6	8.94	89.4
.43	42.39	423.9	44.82	448.2	20.75	207.5

Table (3) concentration of Magnesium in Stones

Stone No.	Visible technique					
	1N HNO_3 mg/dl	**µg/gm**	**16.5N HON_3 mg/dl**	**µg/gm**	**D.I.W mg/dl**	**µg/gm**
.1	1.253	12.53	1.795	17.95	0.876	8.76
.2	1.024	10.24	1.740	17.4	0.930	0.93
.3	1.296	12.96	2.702	27.02	0.932	9.32
.4	0.566	5.66	1.806	18.06	0.627	6.27
.5	1.923	19.23	3.597	35.97	1.001	10.01
.6	0.549	5.49	1.608	16.08	0.412	0.42
.7	1.204	12.04	2.696	26.96	0.798	7.98
.8	2.236	22.36	3.580	35.8	1.033	10.33
.9	1.696	16.96	2.570	25.7	0.924	9.24
.10	3.848	38.48	4.580	45.8	2.091	20.91
.11	4.213	42.13	6.558	65.58	3.667	36.67
.12	3.593	35.93	5.580	55.8	2.101	21.01
.13	0.474	4.74	1.610	16.1	0.420	0.42
.14	1.317	13.17	2.380	23.8	0.810	8.1
.15	3.543	35.43	4.114	41.14	2.350	23.5
.16	2.491	24.91	3.371	33.71	1.240	12.4
.17	2.375	23.75	3.580	35.8	0.932	9.32
.18	2.333	23.33	3.437	34.37	0.830	8.3
.19	4.992	49.92	5.410	54.1	2.246	22.46
.20	0.680	6.8	1.410	14.1	0.821	0.8
.21	3.092	30.92	4.443	44.43	2.111	21.11
.22	0.646	6.46	1.415	14.15	0.211	2.11
.23	5.952	59.52	6.580	65.8	3.180	31.8
.24	2.073	20.73	3.289	32.89	1.380	13.8
.25	3.700	37	4.349	43.49	2.360	23.6
.26	0.854	8.54	1.349	13.49	0.440	1.4
.27	0.798	7.98	1.415	15.15	0.864	1.64
.28	0.302	3.02	1.784	17.84	0.762	1.62
.29	0.562	5.62	1.410	14.1	0.370	1.7
.30	0.578	5.78	1.460	14.6	0.300	0.3
.31	1.962	19.62	2.844	28.44	1.296	12.96
.32	2.400	24	4.995	49.95	1.227	12.27
.33	0.938	9.38	1.964	19.64	0.616	6.16
.34	2.700	27	3.000	30	1.708	17.08
.35	0.560	5.6	2.012	20.12	0.426	4.26
.36	0.560	5.6	1.892	18.92	0.410	4.1
.37	4.41	44.1	5.982	59.82	3.576	35.76
.38	0.732	7.32	1.978	19.78	0.518	5.18
.39	0.726	7.26	1.994	19.94	0.514	5.14
.40	5.154	51.54	5.980	59.8	4.068	40.68
.41	0.678	6.78	2.000	20	0.354	3.54
.42	0.654	6.54	1.984	19.84	0.354	3.54
.43	4.614	46.14	5.286	52.86	3.570	35.7

Table (4) concentration of Phosphorus in Stones

Stone No.	Visible Spectroscopy					
	1N HNO_3 mg/dl	µg/gm	16.5N HON_3 mg/dl	µg/gm	D.I.W mg/dl	µg/gm
.1	0.221	2.21	0.336	3.36	0.140	1.4
.2	0.162	1.62	0.492	4.92	0.151	1.51
.3	0.882	8.82	1.408	14.08	0.173	1.73
.4	1.386	13.86	1.876	18.76	0.828	8.28
.5	6.840	68.4	8.780	87.8	2.050	20.5
.6	1.494	14.94	1.780	17.8	0.915	9.15
.7	2.430	24.3	2.516	25.16	2.020	20.2
.8	1.008	10.08	1.464	14.64	0.821	8.21
.9	0.666	6.66	1.465	14.65	0.180	1.8
.10	0.558	5.58	1.486	14.86	0.178	1.78
.11	1.548	15.48	1.884	18.84	0.784	7.84
.12	1.476	14.76	1.844	18.44	0.860	8.6
.13	1.548	15.48	1.868	18.68	1.001	10.01
.14	1.404	14.04	1.624	16.24	0.987	9.87
.15	0.912	9.12	1.624	16.24	0.323	3.23
.16	1.674	16.74	2.660	26.6	1.092	10.92
.17	1.404	14.04	1.480	14.8	0.892	8.92
.18	1.368	13.68	1.528	15.28	0.632	6.32
.19	0.264	2.64	1.344	13.44	0.024	0.24
.20	0.360	3.6	0.768	7.68	0.146	1.46
.21	0.258	2.58	0.300	3	0.021	0.21
.22	0.174	1.74	0.284	2.84	0.011	0.11
.23	5.802	58.02	8.600	86	3.546	35.46
.24	1.878	18.78	1.400	14	0.996	9.96
.25	0.378	3.78	0.585	5.85	0.121	1.21
.26	0.156	1.56	0.384	3.84	0.025	0.25
.27	0.992	9.92	1.824	18.24	0.667	6.67
.28	0.120	1.2	0.366	3.66	0.024	0.24
.29	0.126	1.26	0.180	1.8	0.024	0.24
.30	0.198	1.98	0.216	2.16	0.013	0.13
.31	0.348	3.48	0.394	3.94	0.120	1.2
.32	0.324	3.24	0.396	3.96	0.168	1.68
.33	0.150	1.5	0.192	1.92	0.130	1.3
.34	0.468	4.68	0.504	5.04	0.252	2.52
.35	0.276	2.76	0.312	3.12	0.204	2.04
.36	0.138	1.38	0.426	4.26	0.120	1.2
.37	5.250	52.5	5.490	54.9	3.318	33.18
.38	1.662	16.62	2.118	21.18	1.044	10.44
.39	0.438	4.38	0.462	4.62	0.402	4.02
.40	0.354	3.54	0.462	4.62	0.288	2.88
.41	0.234	2.34	0.306	3.06	0.168	1.68
.42	0.384	3.84	0.534	5.34	0.366	3.66
.43	0.270	2.7	0.318	3.18	0.234	2.34

III. DISCUSSION

The shape of stone are showing in Fig (4) are same mulberry stone, Jack stone, stag horn stone and other differential shapes. the stones are different in color, is dark in patient while pale in other patient. From information in Table (1) the range of age between (23-73) only two cases were 3 years .males were have higher Weight of stone always than Female. The first cause of higher Weight of stone in male it is the nature of diet and the environmental factor. Stone with calcium and magnesium was more rigid than other stones. From information's patient there are 24 patients didn’t have pathological history while 11 patients were under diabetic multiuse.. Result in Table (2) showed 40 stone 93.0% were content calcium while 3 stone 7.0% without calcium .magnesium was appear in 10 stone 23.3% and 33 stone without magnesium have percentage 76.7%, patients with Ammonium were 16.3% while 83.7% of patients didn’t have it. 37.2% of stones were positive carbonates while 62.8% were negative due to the most stones were having inorganic compositions. The results showed that most stones have uric acid 81.4% this percentage was high compare with other cities because the nature of diet, most patient intake sheep meat (high proteins) and derivatives of milk .for oxalate 88.4% were positive while 11.6% were negative. Results showed that only two patient have cystine. Tables (3,4,and 5) that 16.4 N of digest solution was the best solution to releasing the compositions of stones more than other digestion solutions, while deionizer water was slight to release the compositions. the quantitative method was more sensitive than qualitative method especially in samples (12,20,and 36). The analytical quantitative methods used for determination the concentration of calcium, magnesium, and phosphorus ions were accurate reliable and sensitive with standard deviation 0.004596, 0.00239, and 0.000756 for calcium, magnesium, and phosphorus ions respectively. Low detection limit (L.O.D.) were 0.112, 0.0177, and 0.189 mg/dl for calcium, magnesium, and phosphorus ions respectively while the (RSD) were 1.4%, 2.36%, and 5.04 % for calcium, magnesium, and phosphorus ions respectively.

IV. CONCLUSION

كان الهدف من هذا البحث هو لتقدير المكونات الكيميائية للحصىباستخدام الطرق النوعية والطرق الكمية لتقدير بعض الايونات مثل الكالسيوم والمغنسيوم والفوسفور اللاعضوي. تم فحص 43 حصى حيث كانت الحصى المجموعة من كلا الجنسين (14 حصى من النساء بنسبة 32.6% و29 حصى من الرجال بنسبة 67.4%). وقد تم فحصها وتحليلها كميا باستخدام مطيافية الضوء المرئي عند اطوال موجية مختلفة بينما تم استخدام الطرق النوعية لتحديد مكونات الحصى .

حيث كانت الطرق التحليلية الكمية التي تم استخدامها لتقدير تركيز ايونات الكالسيوم, المغنيسيوم, والفسفور اللاعضوي ذات دقة موثوقة وحساسية حيث كان نسبة الانحراف القياسي 0.004596 للكالسيوم و 0.00239 للمغنيسيوم و 0.000756 للفسفور. اما حد الكشف فقد كان 0.112, 0.0177, و 0.189 للكالسيوم والمغنيسيوم والفسفور على التوالي. اما الانحراف القياسي النسبي فقد كان 1.4%, 2.36%, و 5.04% للكالسيوم والمغنيسيوم والفسفور على التوالي.

الكلمات الافتيتاحيه : حصى الكلى, الجهاز البولي, الطرق النعية, الطرق الكمية, و قياس المطيافية .

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