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RATIO DERIVATIVE-ZERO CROSSING AND SUCCESSIVE DERIVATIVE OF RATIO SPECTRA FOR SIMULTANEOUS DETERMINATION OF UREA, CREATININE, AND URIC ACID IN HUMAN URINE SAMPLES

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ABSTRACT. Two simple precise and accurate spectrophotometric techniques are developed for simultaneous determination of ternary mixture of urea, creatinine, and uric acid in humane urine sample. The first technique is a ratio derivative zero - crossing where measured urea at 216.61 nm, creatinine at 260.56 nm, and uric acid at 283 nm. The second technique is a successive derivative of ratio spectra where urea, creatinine, and uric acid measured at 203 nm, 261 nm, and 283 nm, 287 nm, respectively. These procedures do not need separation. The proposed methods showed excellent linear range over the concentration ranges of 1.0–15.0 µg/mL, 1.0–16.0 µg/mL, and 2.0–15.0 µg/mL for urea, creatinine, and uric acid, respectively. The recoveries ranged from 97.10% to 101.9% for urea, 97.22% to102.70 % for creatinine, and 97.45% to102.55 % for uric acid with relative standard deviations less than 1.56% for urea, 3.87% for creatinine, and 3.71% for uric acid. The analytical eco-scale and green analytical procedure index tools were used to evaluate how the proposed procedures will affect the environment. The simultaneous quantification of urea, creatinine, and uric acid in human urine samples can be accomplished with remarkable effectiveness by using the suggested approaches.

KEY WORDS: Urea, Creatinine, Uric acid, Derivative spectrophotometry

INTRODUCTION

Urea is a simple compound of low molecular weight with chemical formula $(CO(NH_2)_2$, Figure 1(a) [1]. Urea is a nitrogenous product of protein metabolism, produced mainly in the live [2]. Eighty to ninety percent of the nitrogen excreted by humans is made up of urea, which is then primarily transported by the circulation to the kidneys and removed in the urine [3]. Urea may be up to 50-fold more concentrated in urine samples [4]. Serious issues with the human body could result from the high percentage of urea in the blood [5].

Creatinine (2-amino-1-methyl-2-imidazoline-4-one), with chemical formula $C_4H_7N_3O$ as shown in Figure 1(b), is a substance found in both human blood and urine [6, 7]. The kidney filters CRT in blood without any reabsorption [8, 9]. Creatine is predominantly synthesized in the liver, and the balance between its production and kidney excretion regulates its levels in a blood [10]. The CRT concentration in a serum is maintained by the balance between its generation and excretion by the kidneys [11]. CRT is a vital biomarker for renal role identification and for normalising variations in concentrations of urinary drugs/metabolites [12].

Uric acid (UA) is the metabolic product of purine nucleosides in human body. It is a heterocyclic organic compound with the chemical formula ($C_5H_4N_4O_3$), Figure 1(c), which is primarily generated in the liver [13], intestines, muscles, kidneys, vascular endothelium, and various other tissues, [14]. Between 60% and 70% of the body's total UA is eliminated by the kidneys[13] and excreted by urine [15].

In recent years, different analytical methods such as spectrophotometric method [5, 15-17] ion chromatography [9, 18], high performance liquid chromatography [3, 19-23], gas chromatography mass spectrometry [24, 25] reversed-phase liquid chromatography with tandem

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mass spectrometry [26], high-performance liquid chromatography/isotope dilution mass spectrometry [27], liquid chromatography–tandem mass spectrometry [2, 28] and chemiluminescence [29], liquid chromatography/mass spectrometry [30] for estimation of urea, creatinine and UA levels in human serum or plasma samples, as well as urine have been developed.

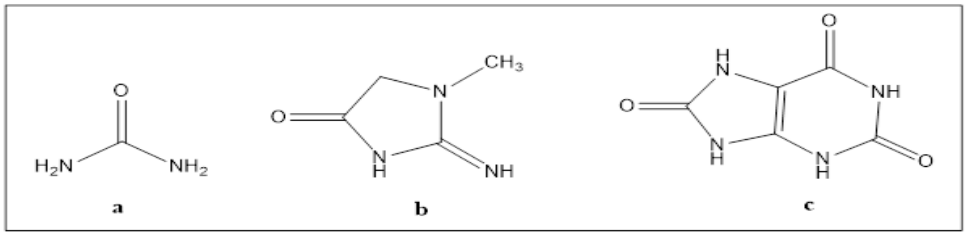


Figure 1. Chemical structures of (a) urea, (b) creatinine, and (c) uric acid.

This study aims to develop new and very simple methods to solve overlapping spectra in their ternary mixtures without preliminary separation steps. Two different spectrophotometric methods for the simultaneous quantification of urea, CRT and UA in human urine samples were utilised. The results of these techniques are compared to those of the cobas 6000 fully automated analyzer's analysis. The analytical eco-scale and analytical greenness are two of several green metrics in use, and it has been found to be an excellent quantitative tool to verify greenness of an analytical method. It compares various parameters and steps for the entire analytical process.

EXPERIMENTAL

Apparatus

A Shimadzu UV-Visible double beam spectrophotometer (model UV1800, Japan) with a fixed 1 nm bandwidth and 1 cm quartz cell was utilized for spectrophotometric measurement, and computer was connected to a double beam spectrophotometer in order to recording zero order spectra and the computer loaded with software UV Probe program was used for recording the different orders derivative spectra for each one of urea, CRT and U.A solutions in the derivative spectrophotometric determination ternary systems. All computations were performed with Matlab 6.5 and Microsoft Excel.

Chemical and reagents

All of the reagent and chemical compounds used in this investigation were analytical grade. Sodium hydroxide (Scharlau – Spain) reagent grade 99.5%, urea (Sigma Aldrich-USA) powder ≥98%, creatinine (Sigma Aldrich-USA) anhydrous, ≥98%, uric acid (Sigma Aldrich-USA) ≥99%, crystalline.

Standard stock solution

Standard solutions of urea, creatinine and uric acid (100 µg/mL) were prepared by dissolving 0.0100 g each of urea, CRT in distilled water (DW) [17, 31] and UA in small amount of 0.1 M NaOH [15] and diluting to 100 mL in a volumetric flask with distilled water then stored at room temperature. Working solutions were prepared daily diluting the stock solutions.

Sample preparation

Urine samples were collected from fifty human twenty five male and twenty five female. All of the samples were collected in Hawler hospital and Khanzad laboratory Erbil city.

2.0 mL of collected human urine in plastic bottle were centrifuged at 2000 rotations per min for 10.0 min and the supertant filtered transported to anather tube then the pH of all samples were adjusted between (8.0 - 9.0) with 0.1 M NaOH to avoid uric acid preseptation. A urine specimen was diluted with distilled water (DW) until its signal fell within the linear range of the calibration graph [32, 33]. Validity of the methods was assessed by spiking the urine samples by known amounts of standard solution.

Analytical eco-scale analysis

The result of analytical eco-scale analysis is the score that is calculated by subtracting penalty points from the basis of 100 points. The penalty points are assigned for high amounts and high hazards connected with utilization of chemicals, high energy consumption, occupational hazards and generation of wastes [34, 35]

RESULTS AND DISCUSTION

Urea, CRT, and UA are three compounds, and their normal UV absorption spectra are completely overlapped in the wavelength range between 190 and 400 nm Figure 2. As a result, determination of three compounds in ternary mixture simultaneously is impossible by classical spectrophotometry to resolve a mixture. Therefore, two different techniques of derivative spectrophotometry have been used to reduce interference and resolve the overlapped spectra. Figure 2 shows normal absorption spectra of 15.0 µg/mL urea, 10.0 µg/mL CRT, and 10.0 µg/mL UA against distilled water as a blank solution.

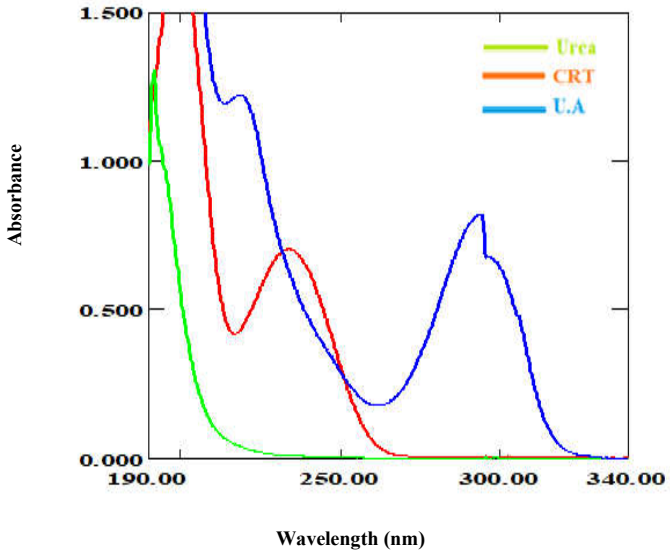


Figure 2. Normal absorption spectra of 15.0 µg/mL urea, 10.0 µg/mL CRT, and 10.0 µg/mL UA against distilled water as a blank solution.

Derivative ratio spectrum zero - crossing method (DRSZC)

In this method, the absorption spectrum of the ternary mixture solution of urea, CRT, and UA in DW were divided by the standard spectrum of 15.0 µg/mL urea in the same solvent and the ratio spectrum of CRT-UA were obtained, which could be considered as that of a binary mixture (CRT and UA) where the ternary mixture (urea, CRT, and UA) is divided by urea. Figure 3(a) shows the first derivative of the ratio spectra which was plotted with intervals of $\Delta\lambda$ = 8 nm. The concentrations of CRT and UA. can be determined by measuring the signals at 260.56 nm for CRT (zero-crossing point of UA) and 283 nm for UA (zero-crossing point of CRT). Similar to how the ratio spectrum of urea-CRT was created, the stored spectra of the ternary mixture solution of CRT, UA, and urea in DW were divided by the standard spectrum of 3.0 µg/mL UA in the same solvent. Figure 3(b) displays the first derivative of the ratio spectrum, which was plotted with intervals of $\Delta\lambda$ = 8 nm and concentration of urea in the ternary combination was determined by measuring the signals at 216.61 nm (the zero-crossing point for CRT). In a ternary mixture, several urea, CRT, and UA mixture compositions were created and tested between 1.0-15.0 µg/mL urea and 1.0-16.0 µg/mL CRT.

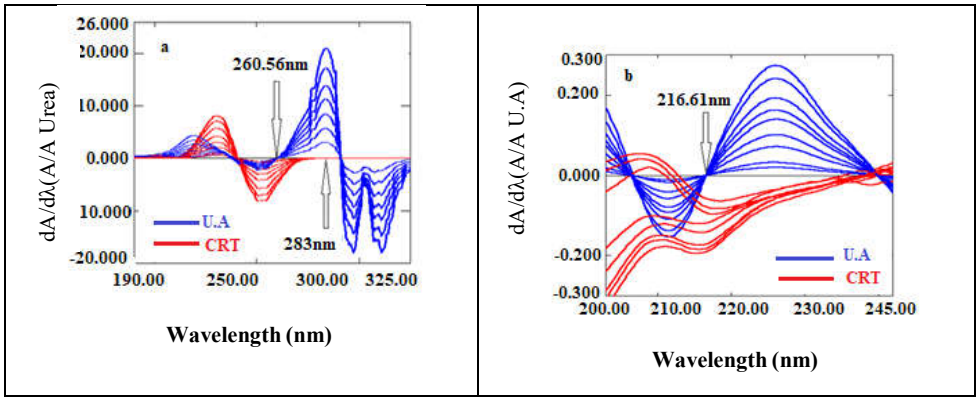


Figure 3. First derivative ratio spectra of (a) CRT (1.0-16.0) µg/mL and of UA (2.0-15.0) µg/mL, using (15.0 µg/mL Urea as divisor) in DW ($\Delta\lambda$ = 8 nm) and (b) Urea (1.0-15.0) µg/mL and of CRT (1.0-16.0) µg/mL, using (3.0 µg/mL UA as divisor) in DW ($\Delta\lambda$ = 8 nm).

Optimization of the method

There are two essential factors that need to be tested to optimize derivative ratio spectrum-zero crossing method for determining ternary mixture; devisor concentration and value of $\Delta\lambda$.

Effect of divisor concentrations

The most important parameters that need to be optimized are the effect of divisor concentrations on the calibration curves [36] various divisor concentrations were tested for each of urea, CRT and UA (3.0, 5.0, 8.0, 10, 12, 15) µg/mL. It was noticed that the standard solution 15.0 µg/mL of urea for determining CRT and UA and 3.0 µg/mL of UA for determining urea in their pure and ternary mixture were found suitable.

Effect of the value of wavelength (Δλ)

The first derivative of the ratio spectra was affected by Δλ. As the Δλ values increase, the noise level decline slightly. Therefore, testing at different Δλ and Δλ = 8 nm regarded to be suitable for determination urea, CRT and UA in ternary mixture.

Successive derivative ratio spectra method (SDRS)

To determine urea, the stored spectra of the ternary mixture with different concentrations of urea in the range between 190-400 nm divided by a standard spectrum of 5.0 µg/mL of CRT, the ratio spectra was obtained. Then the first derivative of these ratio spectra attained by using Δλ = 4 nm. Next the second ratio spectra were obtained through dividing these vectors (first derivative of the ratio spectra) to the first derivative of the ratio spectra (UA/CRT) 5.0 µg/mL for each of UA and CRT. Then the first derivative of the second ratio spectra was attained at Δλ = 4 nm and scale factor 10 as shown in Figure 4(a). The urea concentration was estimated through measuring the signal at 203 nm of urea in the presence of CRT and UA.

Similar to this, the ratio spectra were obtained by dividing the stored spectra of the ternary mixture with various concentrations of CRT in the range of 190-400 nm by a standard spectrum of 5.0 g/mL of urea. Then, using Δλ = 4 nm, the 1D of these ratio spectra was obtained. By dividing these vectors (1D of the ratio spectra) by the 1D of the ratio spectra (UA/urea) 5.0 µg/mL for each of UA and urea, the second ratio spectra were later obtained. Then, using a scaling factor of 10, the 1D of the second ratio spectra was obtained at Δλ = 4 nm (Figure 4 (b)). By detecting the CRT signal at 261 nm while it was in the presence of urea and UA., the concentration of CRT was calculated.

The ratio spectra were generated by dividing the stored spectra of the ternary combination with various concentrations of UA in the range between 190 and 400 nm by the reference spectrum of 5.0 µg/mL of urea. The 1D of the ratio spectra was then obtained at Δλ = 4 nm. The second ratio spectra was then created by dividing these vectors (1D of the ratio spectra) by the 1D of the ratio spectra (CRT/urea) 5.0 µg/mL for each CRT and urea. Then, using a scaling factor of 10, the 1D of the second ratio spectra was obtained at Δλ = 4 nm (Figure 4 (c)). By detecting the signal of UA at 282 nm and 287 nm, the concentration of UA was calculated in the presence of urea and CRT.

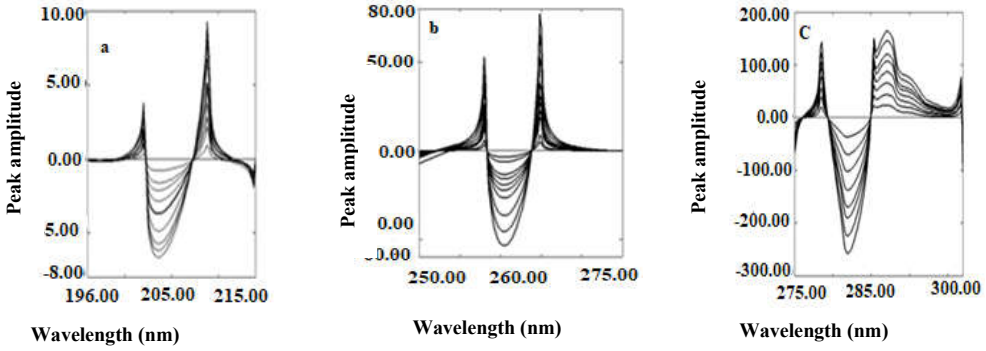


Figure 4. Successive derivative ratio spectra for determination of (a) urea, (b) CRT, and (c) U.A in DW at Δλ = 4 nm with scaling factor 10.

Optimization of the method

There are two essential factors that need to be tested to optimize SDRS method for determining ternary mixture.

Effect of divisor concentration

Divisor concentration's impact on the calibration equation's correlation coefficient, slop, intercept, and detection limit was investigated, as well as the method's selectivity. For this, several divisor concentrations (2.0, 5.0, 8.0, 10, 12, and 15) µg/mL have been investigated for each of the following: CRT and UA for urea determination, urea and UA for CRT determination, and urea and CRT for UA determination. Markedly altering the divisors concentration had a considerable impact on the method selectivity. Hence 5.0 µg/mL of each of urea, CRT and UA was used as divisors.

Effect of the working wavelength (Δλ)

The calibration graphs were built using the lowest or maximum of the SDRS with regard to wavelengths. The amount of Δλ and its effect on the creation of ratio spectra are another parameter that needs to be tuned. Since different Δλ were being tested for this purpose, Δλ = 4 nm with a scaling factor of 10 was employed.

Calibration curve and statistical data

DRSZC and SDRS were used to get various calibration curves. The ternary combination was evaluated by evaluating various mixtures of urea, CRT, and UA. The linearity range of the two suggested techniques over the concentration ranges were 1.0-15.0 µg/mL for urea, 1.0-16.0 µg/mL for CRT, and 2.0-15.0 µg/mL for UA. The high value of the regression coefficients, which is greater than 0.999, however, shows that the calibration graphs had good linearity. The main results are shown in Table 1. The accuracy and precision of the suggested techniques were calculated at three dissimilar concentrations of each compound in the ternary combination (5 replicate measurements). The results of error studies not less than than ±5.0 % which designates that the proposed methods have acceptable accuracy. Also the RSD values for determination of compounds are betwene 0.29 -3.87%.

Table 1. The statistical parameters for quantification of ternary mixture Urea, CRT and U.A using the proposed techniques.

Methods	Compounds	λ_{max} (nm)	Linearity µg/mL	Regression equation	R^2	LOD µg/mL	LOQ µg/mL
DRSZC	Urea	216.61	1.0 – 15.0	Y = 0.009x + 0.0537	0.9995	0.215	0.651
	CRT	260.56	1.0 – 16.0	Y = 0.2679x -0.0207	0.9996	0.285	0.864
	UA	283	2.0 – 15.0	Y = 1.3713x + 0.2453	0.9997	0.450	1.363
SDRS	Urea	203	1.0 – 15.0	Y = 0.4017x + 0.283	0.9994	0.240	0.727
	CRT	261	1.0 – 16.0	Y = 3.2084x -0.0508	0.9997	0.130	0.393
	UA	282	2.0 – 16.0	Y = 17.037x + 0.4946	0.9998	0.200	0.607
	UA	287	2.0 – 15.0	Y = 11.095x -0.773	0.9997	0.445	1.348

Study of interferences

The effect of different compounds, 10000 µg/mL of (glucose, Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, CO_3^{2-}, SO_4^{2-}, and PO_4^{3+}), on the simultaneous determination of ternary mixture of urea, CRT with UA

using two different spectrophotometric techniques were studied. Results indicated that the compounds under study with the mentioned concentration did not interfere in the proposed method for urea, CRT, and UA.

Application of methods

The suggested techniques were applied successfully for simultaneous quantification of ternary combination of urea, CRT with UA in human urine samples using a single standard addition method and cobas 6000 fully automated analyzer technology for analysis of human urine samples collected in Erbil city. The results of the application and recovery study are summarized in tables 2 and 3.

Table 2. Simultaneous quantification of urea, UA, and CRT in human urine samples using propose technique.

No. of sample	Found (µg/mL) (DRSZC method)			Found (µg/mL) (SDRS method)			Found (µg/mL) by Cobas 6000		
	Urea	CRT	UA	Urea	CRT	UA	Urea	CRT	UA
1	5106	857	522	5102	865	524	5100	853	530
2	6012	917	634	5997	902	634	6005	918	640
3	5012	472	732	5021	482	733	5025	486	740
4	11209	450	1210	11210	450	1214	11207	460	1220
5	4108	651	1113	4115	653	1120	4106	659	1118
6	5105	713	1251	5098	704	1245	5110	715	1255
7	5412	1848	603	5415	1849	605	5400	1851	615
8	6013	1923	1357	6017	1905	1350	6002	1922	1362
9	8691	1040	326	8698	1046	322	8700	1052	330
10	6910	1108	812	6919	1115	814	6900	1120	820
11	9259	1240	757	9264	1240	760	9250	1245	762
12	7114	1510	661	7124	1515	664	7103	1521	668
13	4140	1213	457	4142	1220	459	4150	1226	465
14	4312	1808	926	4315	1810	928	4300	1819	934
15	8508	909	505	8518	907	517	8502	917	520
-16	6388	698	845	6398	710	862	6400	700	860
17	4720	1862	612	4715	1872	614	4700	1878	620
18	5462	1635	433	5457	1645	435	5450	1650	432
19	4033	581	365	4037	595	364	4020	600	371
20	8658	1586	616	8665	1585	625	8650	1590	625
21	7810	1658	815	7820	1645	810	7800	1651	819
22	3190	647	742	3195	655	755	3200	662	750
23	8911	589	911	8917	580	910	8901	575	918
24	4240	1072	814	4245	1074	812	4250	1076	815
25	6110	1126	712	6112	1120	713	6120	1129	721
26	2542	307	413	2538	305	439	2550	311	432
27	7288	1254	569	7297	1242	568	7300	1252	562
28	6415	1248	558	6421	1265	561	6402	1260	569
29	2510	419	457	2520	420	460	2520	430	472
30	2615	208	351	2617	208	365	2622	215	355
31	1972	157	546	1978	156	565	1983	155	570
32	3140	316	610	3145	312	625	3150	318	621
33	1770	236	657	1772	235	655	1778	240	672
34	4145	352	386	4148	338	380	4155	345	391
35	1212	142	425	1218	145	420	1227	150	432

36	6135	252	576	6152	263	570	6137	265	581
37	2215	276	768	2224	270	765	2215	275	771
38	1812	225	802	1817	220	814	1824	220	810
39	1610	150	613	1625	165	625	1628	170	630
40	1090	126	472	1093	136	478	1099	140	480
41	1204	147	522	1212	145	535	1218	149	150
42	1312	152	593	1307	148	615	1316	160	620
43	1070	448	926	1081	440	922	1090	452	928
44	1008	171	844	1025	163	854	1030	165	861
45	1108	152	487	1128	158	480	1134	155	488
46	1078	137	928	1084	135	925	1096	146	935
47	2086	276	857	2085	274	854	2100	272	858
48	5113	486	605	5121	490	605	5127	495	613
49	4812	495	656	4814	492	656	4824	500	660
50	8109	492	707	8123	510	707	8131	512	715

Table 3. Recoveries % for simultaneous quantification of urea, CRT and UA in human urine samples using propose methods.

No. of sample	Recovery, % (DRSZC method)			Recovery, % (SDRS method)		
	Urea	CRT	UA	Urea	CRT	UA
1	98.14	99.15	98.84	98.32	99.08	99.15
2	98.67	98.55	100.15	98.65	98.85	98.78
3	99.12	100.60	99.08	99.15	99.00	98.55
4	100.76	99.20	99.30	100.40	98.65	97.70
5	98.89	98.45	98.56	98.15	97.86	101.30
6	98.65	99.12	100.35	97.80	100.65	98.50
7	99.21	98.23	101.25	98.85	99.08	97.45
8	99.08	100.30	99.40	97.65	101.40	98.65
9	99.35	99.10	98.70	99.10	100.65	101.24
10	100.46	98.35	99.20	98.45	99.20	98.56
11	101.03	98.17	100.78	97.50	98.85	99.10
12	98.19	99.12	98.85	98.15	99.02	98.94
13	98.78	99.06	99.20	101.40	99.30	97.65
14	98.65	97.45	98.65	99.03	100.88	99.00
15	97.58	98.65	101.35	98.65	97.55	101.34
16	99.25	101.23	102.45	99.00	100.45	97.78
17	101.15	100.55	99.15	98.55	99.15	96.14
18	98.45	99.15	97.10	100.86	98.30	98.56
19	96.31	98.70	98.48	98.55	97.95	98.45
20	98.82	98.25	100.65	101.20	101.35	100.36
21	98.45	99.20	99.30	96.25	98.45	97.60
22	97.85	100.35	99.10	98.30	100.88	98.55
23	99.02	98.65	98.70	98.75	101.22	99.15
24	98.51	99.25	97.28	100.78	99.03	101.40
25	100.65	98.34	97.80	98.44	97.65	97.20
26	101.24	98.30	99.20	101.65	98.05	98.55
27	98.17	97.55	101.30	99.08	99.40	102.15
28	97.76	98.11	100.60	97.65	100.55	99.18
29	99.20	98.80	99.25	98.40	97.40	98.84
30	98.60	101.35	99.12	96.20	101.35	97.60
31	98.44	100.78	101.20	101.20	100.66	98.46
32	99.31	98.39	98.55	99.06	98.45	99.05

33	99.30	99.25	99.25	97.86	98.24	97.90
34	100.75	97.26	100.78	101.26	99.15	98.40
35	9815	98.32	101.34	98.40	101.45	101.60
36	97.45	99.10	98.32	100.75	100.72	98.82
37	97.81	98.57	98.60	98.66	99.10	97.68
38	98.50	98.50	99.11	101.15	98.24	97.45
39	98.68	97.22	97.50	97.10	98.86	98.68
40	99.05	99.06	101.35	98.35	100.45	97.90
41	97.44	98.67	101.20	97.45	98.20	98.15
42	98.35	98.35	99.14	98.85	99.10	101.55
43	97.11	97.80	98.25	101.20	97.82	100.60
44	98.40	98.40	98.57	98.68	101.45	98.52
45	98.65	98.65	97.66	97.45	102.15	98.20
46	101.85	101.85	99.20	100.63	99.15	97.70
47	100.62	100.60	98.63	99.78	98.35	99.35
48	101.55	101.45	100.55	101.35	102.70	102.25
49	98.45	98.45	98.75	98.84	98.45	97.45
50	98.15	98.16	99.02	98.60	98.70	98.62

Comparison with other methods

A comparison has been done between some of analytical variables obtained from the proposed methods for determination of urea, CRT, and UA with literature method [30] and the results summarized in Table. 6.

Table 6. Comparison of the proposed methods with literature methods.

Urea			
Analytical parameter	DRZCR	DRZCR	Literature method [30]
Linearity range (µg/mL)	1.0 - 15.0	1.0 -15.0	2.0 - 40.0
LOD (µg/ml)	0.215	0.240	-
Recovery (%)	96.31- 101.85	96.20 -101.65	96.4 – 100.5
RSD (%)	0.53	0.35	1.8
R^2	0.9995	0.9994	9.9999
CRT			
Analytical parameter	DRZCR	DRZCR	Literature method [30]
Linearity range (µg/mL)	1.0-16.0	1.0-16.0	0.13 – 2.0
LOD (µg/ml)	0.285	0.130	-
Recovery (%)	97.26 – 101.35	97.40 – 102.70	95.4-101.6
RSD (%)	0.23	0.48	0.30
R^2	0.9996	0.9997	0.9979
UA			
Analytical parameter	DRZCR	DRZCR	Literature method [30]
Linearity range (µg/mL)	2.0 – 15.0	2.0 – 15.0	2.0 – 15.0
LOD (µg/ml)	0.450	0.200	____
Recovery (%)	97.10- 102.45	96.14 – 102.25	97.3 – 104.6
RSD (%)	0.27	0.12	0.10
R^2	0.9997	0.9998	0.9986
Application	Urine	Urine	Urine

Greenness assessment

The analyst's first aim is to develop an environmentally friendly method that can be easily employed in routine analysis and has high efficiency and low cost. The goal of green analytical

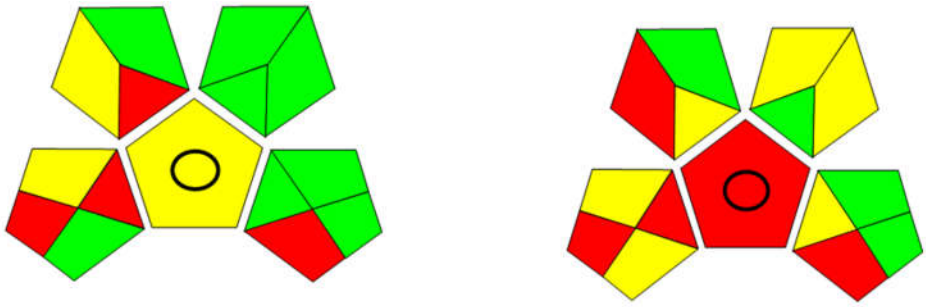
chemistry (GAC) is to develop an ecological approach that doesn't harm the environment or deal with dangerous compounds. Utilizing two analytical tools, the proposed methods' "greenness" was evaluated.

Analytical eco-scale (AES)

Analytical eco-scales are one of the greenness assessment tools that are capable of assessing the efficacy of the technique and extracting quantitative information about the method's environmental compatibility, taking into account the employed chemicals, instruments, and created waste. This tool relies on computing the AES value, which is found by assigning penalty points to all elements that have a negative impact on the environment, adding up all of these points, and then subtracting from a base (100 points). The approach has a good green profile the greater the eco-scale value (> 85) that is obtained. Scores greater than 50 indicate competent green analysis, while scores < 50 indicate insufficient green analysis [36, 37]. The result of eco-scale values for proposed method and literature method [30] are shown in Table 5.

Table 5. Analytical comparison between eco-scale penalty points of the proposed technique and other literature method ones for assessment of greenness.

Methods [30]	Proposed methods	Literature method
Reagents		
Sodium hydroxide	1	-
Potassium hydroxide	-	1
Acetonitrile	-	4
Ammonium acetate	-	2
Water	0	-
Instrument		
Energy	0	2
Centrifuge	1	1
Occupational hazards	-	-
Oven	0	2
Wastes	3	3
Total penalty points	5	15
Analytical eco-scale (Total score)	95	85



(a) Proposed method (b) Literature method [30]

Figure 5. Analytical comparison between pictograms of (a) the proposed methods and (b) other literature method ones for assessment of greenness.

Green analytical procedure index (GAPI)

GAPI is a cutting-edge instrument that assesses the analytical process's overall methodology's greenness based on 15 factors. According on the strength of the environmental influence, five pentagrams were created and colored, each divided into three or four sections. Whereas yellow indicated a medium environmental impact, red indicated a strong environmental effect, green indicated a low environmental impact [37]. The literature technique displayed four green shaded areas, compared to eight green shaded portions for the proposed methods as shown in Figure 5.

CONCLUSION

In this work, two sensitive and reliable spectrophotometric methods (DRSZC and SDRS) were used for the determination of (urea, CRT, and UA) simultaneously in a ternary mixture of humane urine samples without preliminary separation process. In addition, it is rapid and low-cost method when compared with other methods such as chromatographic methods. The methods developed showed satisfactory validation parameters in terms of linearity, LOD which is lower than 0.215, 0.130, and 0.200 µg/mL for urea, CRT, and UA, respectively. The efficiency of the proposed methods can be illustrated by the mean percentage recovery values which were between 97.10 % and 102.7 with relative standard devotion less than 3.87%. The impact of the proposed methods on the environment was assessed by the eco scale and green analytical procedure index (GAPI) tools, where the results take that proposed methods were the greenest than literature methods. As a result, all the two proposed methods have a good accuracy, precision, and sensitivity and are typically suited for the estimation of urea, CRT, and U.A in urine samples.

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