Original Article

Electrochemical oxidation behavior of hydrochlorothiazide on a glassy carbon electrode and its voltammetric determination in pharmaceutical formulations and biological fluids

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Ali F. Alghamdi*

Department of Chemistry, Faculty of Science, Taibah University, Madinah, Saudi Arabia

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ABSTRACT

The electrochemical oxidation behavior of hydrochlorothiazide (HCT) on a glassy carbon as a working electrode was investigated in Britton–Robinson (B–R) buffer pH 3, by using anodic stripping voltammetry (ASV) and cyclic voltammetry (CV). This drug gave a well-defined voltammetric oxidation peak at + 1200 mV versus an Ag/AgCl reference electrode. The electrochemical oxidation process was shown to be irreversible and diffusion controlled, with adsorption characterized over the entire pH range. The optimized conditions, such as accumulation time and potential, scan rate, frequency, pulse amplitude, varying of working electrodes, and instrumental parameters were studied. The calibration graph for HCT was obtained from 4×10^{-6} to 4×10^{-5} M (correlation coefficient = 0.997) using the developed electroanalytical method (ASV). The detection limit of this drug was 4.3×10^{-9} M. ASV and CV techniques with adequate precision and accuracy have been developed and applied for direct determination of HCT in commercial tablets without separation or extraction procedures and biological fluids such as urine and plasma.

1. Introduction

Hydrochlorothiazide (HCT) is a diuretic drug of the thiazide class, which acts by inhibiting the ability of the kidneys to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance [1,2]. HCT is a calcium-sparing diuretic; it can help rid the body of excess water, but retain calcium. HCT is frequently used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, and the prevention of kidney stones [3]. It is also sometimes used for hypercalciuria, Dent's disease, and Ménière's disease.

Hydrochlorothiazide is named 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide, as recorded in the IUPAC system. The chemical formula of HCT is $C_7H_8ClN_3O_4S_2$.

Anodic stripping voltammetry (ASV) is the most widely used form of stripping analysis. It is considered a voltammetric method for quantitative determination of trace concentrations for heavy metals, dyes, drugs, and so on. The analyte of interest is electroplated on the working electrode surface during a deposition step, and oxidized from the electrode during the stripping step, so the current is measured during the stripping step. The oxidation of species is recorded as a peak in the current signal at the potential at which the species begins to be oxidized. Thereafter, the stripping step can be either linear, staircase, square-wave, or pulse. Many reviews have been published which illustrate the wide spectrum and scope of ASV applications in the analysis of toxic metals [4], pharmaceutical compounds [5–12], and in forensic science [13].

Cyclic voltammetry (CV) is the most widely used technique for acquiring qualitative information about electrochemical reactions. The power of CV results from its ability to rapidly provide considerable information on the thermodynamics of redox processes and the kinetics of heterogeneous electron transfer reactions and on coupled chemical reactions or absorption processes. CV is often the first experiment performed in an electrochemical study. In particular, it offers a rapid location of redox potentials of the electroactive species, and convenient evaluation of the effect of media on the redox process [14].

ASV is used as an analytical quantitative method capable of monitoring the signal of HCT at low concentration levels. Hence, a wide variety of analytical methods were found to determine trace concentrations of many drugs such as HCT in pharmaceutical formulations and biological fluids [5–12]. Instrumental techniques of chemical analysis were successively used to determine HCT that included spectrophotometry [15–17], chromatography [17,18], liquid chromatography-UV [19], HPLC [20–23], and other electrochemical methods [17,24,25].

The glassy carbon electrode has been used to determine some chemical compounds in different literatures, as modified electrode to enhance electrochemical study [26–30]. An electrochemical detection used to analysis most compounds in the previous studies [31–33].

ASV is considered a powerful electrochemical procedure that enhances the sensitivity of HCT pharmaceutical content in some pharmaceutical formulations. No published article for analysis of HCT using ASV has been reported in the literature. Therefore, this research was carried out to study the ASV behavior of HCT in order to develop an effective and sensitive electrochemical method for determination it in pharmaceutical formulations and biological fluids.

2. Methods

2.1. Apparatus

ASV and CV measurements were carried out using 797 VA Computrace (Metrohm, Herisau, Switzerland) controlled by (VA Computrace 2.0) control software. Anodic stripping and cyclic voltammograms were printed via a HP color laserjet CP1215 printer (China). A three electrode system was used, including a glassy carbon electrode as the working electrode. pH values were studied using a Hanna instrument pH211 (Romania). An Oxford adjustable micropipette (Huawei, Ireland) was used to measure microliter volumes of the drug standard solutions. The Labofuge 200 instrument, Heraeus Sepatech (Germany) was used to centrifuge the urine and plasma samples, which were then suitable for voltammetric analysis.

2.2. Reagents

The chemicals used were of analytical reagent grade and used without further purification. HCT stock solution of 1×10^{-2} M was prepared by dissolving the appropriate amount of HCT in distilled water in a 25 mL volumetric flask. The drug stock solution was stored in a dark place. The standard solutions of HCT with lower concentrations were prepared by diluting the stock solution with distilled water daily. Britton–Robinson (B–R), carbonate, phosphate, and acetate supporting buffers were prepared for the voltammetric analysis of HCT [34].

2.3. Procedure

The general procedure which applied for getting all voltammograms was as follows: 10 mL of B–R buffer pH 3 was injected in to a dry and clean voltammetric cell and then a required standard solution of HCT was added. All buffer solutions were purged with a nitrogen gas for nearly 3 minutes initially, while these solutions were stirred. The deposition potential of 0.0 V versus an Ag/AgCl reference electrode was applied to a glassy carbon electrode while the solution was stirred for 20 seconds. Anodic scans were carried out over the range 800–1700 mV. The voltammetric measurements were performed at room temperature (25°C).
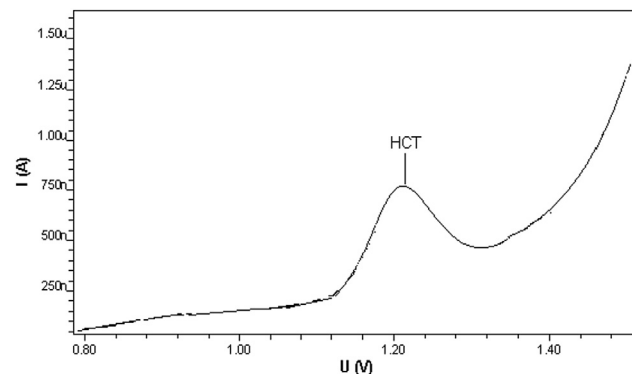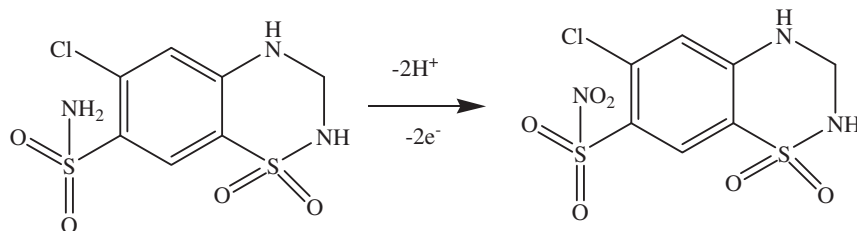

Fig. 1 – **Anodic stripping voltammogram of hydrochlorothiazide (HCT) in Britton–Robinson buffer at pH 3, experimental conditions: t_{acc} = 20 seconds, E_{acc} = 0.0 V, scan rate = 150 mV s^{-1}, drug concentration = 6 × 10^{-6} M.**

Scheme 1 – Structure and suggested oxidation mechanism of hydrochlorothiazide drug.

3. Results and discussion

3.1. Anodic voltammetric behavior of HCT

Initially, investigation of HCT indicated that it was deposited effectively onto the solid electrode (glassy carbon) and it could be monitored by ASV after scanning the applied potential in the positive direction. For instance, the electrochemical accumulating of 6×10^{-6} M HCT solution for 20 seconds in B–R supporting buffer pH 3, yielded a single well-defined anodic voltammetric peak at 1200 mV versus the Ag/AgCl reference electrode, as shown in Fig. 1, which shows the anodic stripping voltammogram for HCT. The anodic voltammetric signal is thought to have resulted from the oxidation process through the common active site of the primary amine group (NH_2) outside the aromatic ring, where the primary amine function (NH_2) is oxidized to the nitro group (NO_2). The proposed electrochemical oxidation mechanism is shown in Scheme 1.

By contrast, the resulting oxidized compound was formed as an irreversible reaction, so no cathodic peak was recorded. This irreversible characteristic nature of HCT electrochemical process was confirmed by the CV technique for measuring HCT solution (8×10^{-5} M) at a 50 mV/second scan rate in B–R buffer pH 3, as shown in Fig. 2. The multicyclic voltammograms of 8×10^{-5} M of HCT in B–R buffer at pH 3 are shown in Fig. 3, which illustrates that the surface of the glassy carbon electrode was saturated by the analyzed drug after the first sweep, so the values of the current were gradually decreased.

The electrochemical oxidation process was shown to be diffusion controlled with adsorption characterized at different scan rates over the r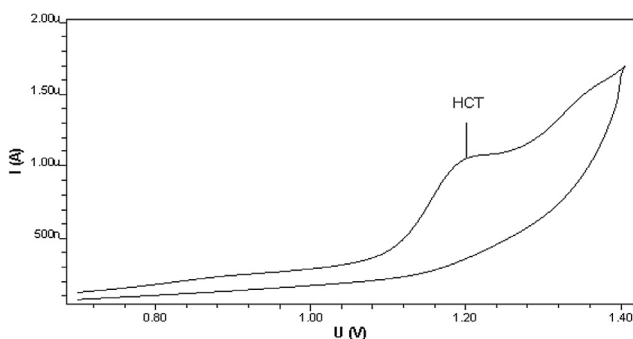ange 10–80 mV/second, as shown in Fig. 4. Through this analysis, a linear relationship between the oxidation peak current and scan rate was observed.

3.2. Optimization of experimental parameters

3.2.1. Effect of buffer solution and pH
The study of a suitable medium is a very important parameter for the ASV determination of HCT. Herein, 5×10^{-6} M of HCT was analyzed by ASV in B–R, acetate, phosphate, and carbonate buffers at different pH values (3, 7, and 10) after 30 seconds preconcentration time at 0.0 V deposit potential. The best anodic stripping response in terms of current value, the peak shape, and the smoothness of baseline was observed when the B–R buffer was used. Therefore, B–R buffer was selected as the optimum condition for future experiments. It was generally noticed that the current height of the obtained ASV signal reached its maximum value in acidic media (B–R pH 3). By contrast, the influence of pH variation over the range 2–7 on the anodic current of 5×10^{-6} M HCT was also investigated, and the results are shown in Fig. 5. The highest current was recorded at pH 3, which was selected as the optimum value for further studies.

3.2.2. Effect of accumulation operators
An accumulation process of the studied drug on the glassy carbon electrode surface is another important operator for the sensitive determination of HCT. On the working electrode surface, the accumulated drug depends on the length of the time over which the deposit method is allowed to proceed, in addition to the intensity of anodic stirring and applied deposition (accumulation) potential. The anodic oxidation current of 5×10^{-6} M for HCT solution was measured as a function of

Fig. 2 – Cyclic voltammogram of 8×10^{-5} M hydrochlorothiazide (HCT) at Britton–Robinson buffer pH 3.0, scan rate 100 mV/second, [I(nA) = 780].
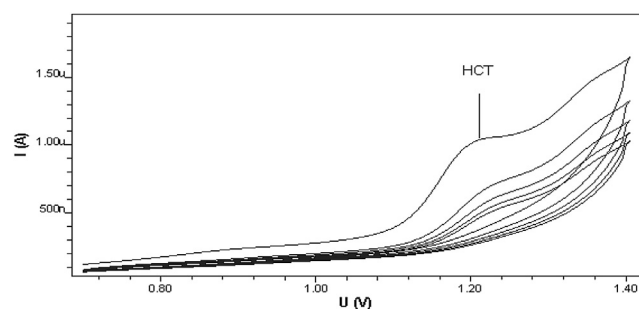

Fig. 3 – Multicyclic voltammograms of 8×10^{-5} M hydrochlorothiazide (HCT) at Britton–Robinson buffer pH 3.0, scan rate 100 mV/second [I (nA)$_{scans}$ = 750, 400, 300, 180 and 100].
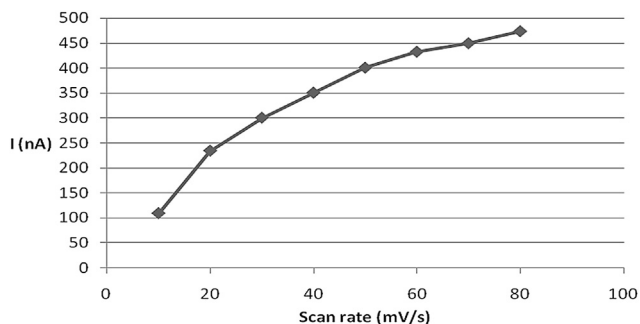
Fig. 4 – Relationship between oxidation peak current and scan rate for 8×10^{-5} M of hydrochlorothiazide in Britton–Robinson buffer pH 3.0.

Fig. 6 – Effect of accumulation time on 5×10^{-6} M hydrochlorothiazide in Britton–Robinson buffer, pH3, and $E_{acc} = 0.0$ V.

accumulation time, as seen in Fig. 6. Through this study, an almost linear relationship between the ASV peak current and accumulation time was recorded from 0.0 seconds to 20 seconds; thereafter, the anodic current decreased. Therefore, 20 seconds was adopted as the optimum for the following ASV studies.

In a different study, the accumulation potential effect on the peak current of this drug was recorded by variation of accumulation potential from +0.8 V to −0.6 V. The maximum anodic peak current value was obtained with an accumulation potential of 0.0 V, which was selected as the optimum value in future experimental studies.

3.2.3. Effect of scan rate, amplitude, and frequency

In general, an ASV response depends on various parameters related to the applied potential which was scanned. The anodic current of HCT was found to be proportional to the scan rate, particularly at high scan rate values. The studied scan rate over the range 50–250 mV/second caused the ASV current to increase linearly, but 150 mV/second produced the best shape of peak and it was selected for further works. Furthermore, the influence of the pulse amplitude on the voltammetric signal was also evaluated over the range 10–100 mV. The anodic current of HCT increased almost linearly with the pulse amplitude over this range, but a 30 mV value produced the best shape of peak, so it was selected for further works. By contrast, the influence of frequency on the ASV peak current was studied over the range 10–80 Hz, as can
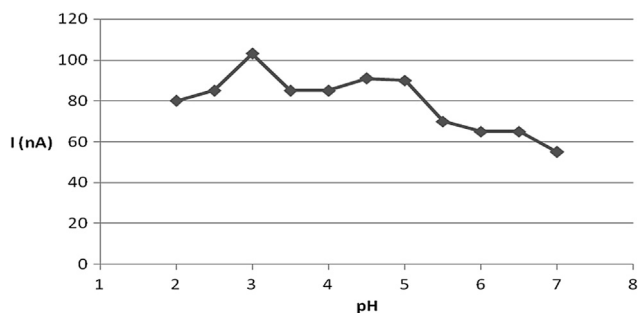
be seen in Fig. 7. A linear relationship was observed over 10–50 Hz; thereafter, the anodic current decreased. A frequency of 50 Hz was adopted as the optimum value.

3.2.4. Effect of instrumental factor and working electrodes

The influence of convection rate on the monitored anodic current was evaluated over the range 0–3000 rpm, as shown in Fig. 8. There was a high anodic current at 1000 rpm, so this value was chosen as the optimum value in further experiments.

By contrast, the effect of the working electrode for analyzed HCT was investigated. Gold, platinum, ultra trace graphite, and glassy carbon were used as working electrodes; the resulting platinum and ultra trace graphite did not record any anodic signal, however gold recorded a high coupling current with an unsuitable peak shape. A high and good anodic peak current was observed by use of a glassy carbon electrode, which was selected over the voltammetric analysis for this drug.

3.3. Analytical performance

3.3.1. Calibration graph

According to optimum experimental conditions, a good linear relationship was obtained between HCT electrochemical anodic signal and its concentration through the range 4×10^{-6} to 4×10^{-5} M (Fig. 9). The parameters of the HCT concentration-anodic current straight line were calculated by

Fig. 5 – Effect of pH on the anodic current of 5×10^{-6} M hydrochlorothiazide in Britton–Robinson buffer, $t_{acc} = 30$ seconds, and $E_{acc} = 0.0$ V.
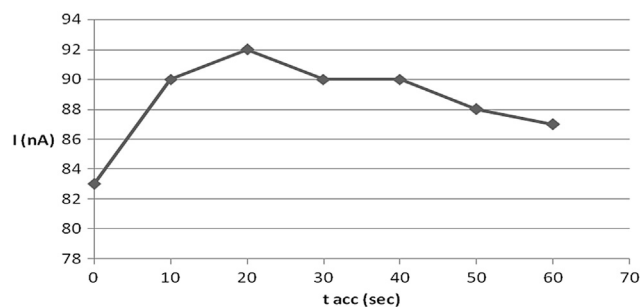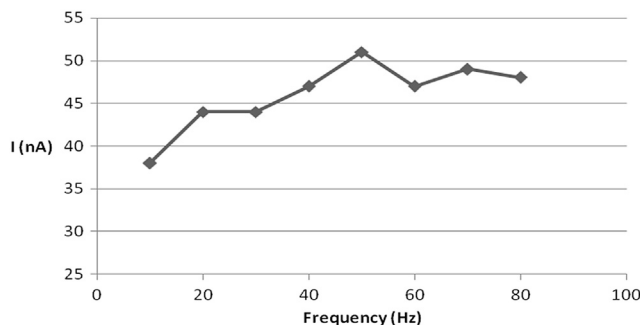

Fig. 7 – Effect of frequency on 5×10^{-6} M hydrochlorothiazide in Britton–Robinson buffer, pH = 3, $t_{acc} = 20$ seconds, $E_{acc} = 0.0$ V, 150 mV/second, and 30 mV amplitude.

Fig. 8 – Effect of convection rate on 5×10^{-6} M hydrochlorothiazide in Britton–Robinson buffer, pH = 3, t_{acc} = 20 seconds, E_{acc} = 0.0 V, 150 mV/second, 30 mV amplitude, and 50 Hz.
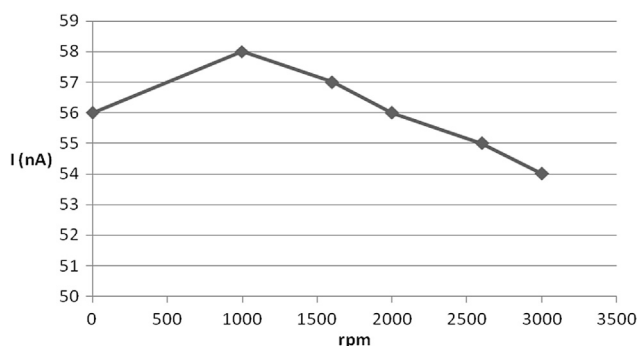
Table 1 – Voltammetric determination of hydrochlorothiazide (HCT) in its commercial tablets by the anodic stripping voltammetry method.		
Labeled content	(mg)	Recovery %
Monozide tablets	24.00	96
25 mg HCT	23.75	95
	23.75	95
	23.75	95
	23.50	94
Mean	95%	
SD (standard deviation)	±0.71	

using the least-squares method. The regression equation of the calibration curve is:

$$i_p \text{ (nA)} = 1.4 \times 10^7 \text{ C (M)} + 27.45, r = 0.997, n = 9$$

where i_p is the ASV (nano ampere), C is the HCT molar concentration and r is the correlation coefficient.

3.3.2. Limit of detection
The limit of detection defined as three times the signal-to-noise ratio (S/N = 3) reached in the optimum experimental conditions for monitored HCT drug was 4.3×10^{-9} M (1.3 ppb). This remarkable sensitivity shows the preference of the ASV technique over the other analytical techniques, such as the electrochemical technique, which only achieved a 5 ppb detection limit [25] and the conventional HPLC method with a 1 ppm detection limit [22]. The applied ASV approach obviously enhanced the sensitivity compared to the cited analytical methods.
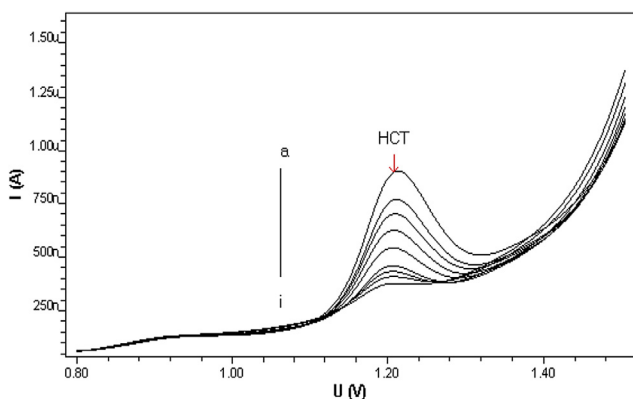

Fig. 9 – Calibration graph voltammograms of hydrochlorothiazide (HCT) in Britton–Robinson buffer, pH = 3.0, t_{acc} = 20 seconds, E_{acc} = 0.0 V, and scan rate = 150 mV/second. Drug concentration (M): a = 4×10^{-5}; b = 3×10^{-5}; c = 2.5×10^{-5}; d = 2×10^{-5}; e = 1.5×10^{-5}; f = 1×10^{-5}; g = 8×10^{-6}; h = 6×10^{-6}; i = 4×10^{-6} M.

3.3.3. Reproducibility
The electroanalytical precision of this developed method was evaluated from reproducibility of eight determinations of 5×10^{-6} M HCT in B–R buffer at pH 3.0. A relative standard deviation of 0.02% was calculated, which indicated the reproducible deposit and monitoring of the studied HCT drug.

3.3.4. Recovery
The recovery of the developed method, which gives the accuracy of the used ASV technique, was studied by electroanalyzing spiked B–R buffer solution containing 5×10^{-6} M HCT via the optimized ASV procedure. The mean recovery of five measurements was found to be $98 \pm 1.0\%$.

3.3.5. Stability
The ASV signal of 5×10^{-6} M HCT was investigated by monitoring the ASV current every 10 minutes. The measured electrochemical response seemed to be fixed over the studied time range (0–90 minutes). This study demonstrates the stability of the analyzed drug under the optimum experimental conditions.

3.4. Interferences study

The influence of some possible interfering substances usually present as ingredients in pharmaceutical formulations (such as starch, sucrose, and so forth) on the anodic current for HCT was also evaluated. These interferences were studied by adding appropriate amounts at different concentrations (1-, 5-, and 20-fold) higher than a drug concentration in the used buffer solution (10 mL of B–R buffer, pH 3, containing 2×10^{-5} M HCT). However, the presence of very high concentrations (20-fold) of starch decreased the anodic current by 10% of its original signal. No obvious effect on the voltammetric response of the analyzed HCT was recorded with all additions of sucrose interference.

3.5. Analytical applications

The developed ASV method was applied to determine HCT in some real samples, such as pharmaceutical tablets and biological fluids. The HCT content of commercially available tablets (Monozide 25, consisting of 25 mg of hydrochlorothiazide; Jordanian Pharm., Mfg.co., Jordan) was analyzed directly by the developed ASV method after the dissolving and filtration steps. Five aliquots of the dissolved real sample were diluted to the required concentration level and measured by

Table 2 – Voltammetric determination of hydrochlorothiazide (HCT) in biological fluids by the anodic stripping voltammetry method.

	Added HCT (5×10^{-6} M)	
	Spiked urine (recovery %)	Spiked plasma (recovery %)
	91	89
	91	88
	90	88
	90	88
	88	87
Mean	90%	88%
SD (standard deviation)	±1.22	±0.71

the standard additions approach. The results of this study show a recovery mean of 95% with a standard deviation of ±0.71%, as can be seen in Table 1.

By contrast, the applicability of the ASV technique for analysis of HCT in biological fluids was also evaluated by obtaining its recovery from spiked human urine and plasma. A fast and simple pretreatment process [35], which is actually considered a slight modification of the sample preparation method for the determination of some drugs, was used. It was carried out by adding a small amount of 5% $ZnSO_4.7H_2O$ solution, NaOH, and methanol to the biological samples and centrifuging the mixture to remove and eliminate most of interfering substances by precipitation. The determination of HCT in spiked urine and plasma resulted in mean recoveries of 90% ± 1.22 (standard deviation) and 88% ± 0.71, respectively, as can be seen from Table 2.

Conflicts of interest

The author declares no conflicts of interest.

Acknowledgments

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