A New Ultrasonic Assisted Cloud Point Extraction and Preconcentration Procedure for the Spectrophotometric Oxalate Determination in Beverages

İçeceklerde Spektrofotometrik Okzalat Belirlenmesi için Yeni Ultrasonik destekli Bulutlanma Noktası Ekstraskisyonu ve Zenginlerştirme Yöntemi

Research Article

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ABSTRACT

A new ultrasonic-assisted cloud point extraction procedure (UA-CPE) was developed for preconcentration of oxalate in beverages prior to determination via spectrophotometry. At optimal conditions, the calibration curve was highly linear in a working range of 0.4–170 μg L^{-1}. The limits of detection and quantification were 0.12 and 0.40 μg L^{-1}. the precision as the percentage relative standard deviation, RSD% (n: 5; 10, 15, 30 μg L^{-1}) was lower than 3.5%. The method was successfully applied to the determination of soluble and total oxalate in beverage samples using two different acidic extraction approaches under ultrasonic power. Finally, the method was validated by comparing the results obtained with those of the reference method.

Key Words

Pyronine B, Clouding point, Ultrasound assisted extraction.

ÖZ

Spektroforomere aracılığıyla okzalatın belirlenmesi öncesi onun önderiştirilmesi için yeni ultrasonik destekli bulutlanma noktası özütleme yöntemi geliştirildi. Optimum koşullarda, kalibrasyon eğrisinin 0.4–170 μg L^{-1} çalışma aralığında yüksek doğrusalığa sahip olduğu görüldü. Seçme ve tayin sınırları sırasıyla 0.12 ve 0.40 μg L^{-1} olarak bulundu. Yüzde bağıl standart sapma olarak kesinlik, %BSS (n: 5; 10, 15, 30 μg L^{-1}), %3.5 den daha düşük gözlendi. Ultrasonik koşullar altında, yöntem iki farklı asidik özütleme yaklaşımı kullanarak içecek örneklerindeki çözünür ve toplam okzalatın belirlenmesi için başarılı bir şekilde uygulandı. Son olarak, yöntemin geçerliliği elde edilen sonuçların referans yöntem ile karşılaştırılması ile doğrulandı.

Anahtar Kelimeler

Pyronin B, bulutlanma noktası, Ultrasonik destekli özütleme.

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INTRODUCTION

Oxalate is not an essential nutrient and is found in many kinds of edible plants with variable concentrations [1,2] and if consumed in large amounts, may be harmful to human health [1]. It binds with dietary calcium in human urine, so that it will form insoluble oxalates, CaC2O4, accumulating in the body and lead to a number of maladies [3] such as kidney stones [4] and urinary stones [5]. Also, it may cause hazes and sediments in beverages such as beer and wine. An intake of large amounts of soluble oxalate can increase the risk of kidney stone development because of the increased concentration of oxalate in the urine. As consumption of additional oxalate in the diet can cause the development of kidney stones in susceptible people, it is important to identify high oxalate containing foods and, if possible, to reduce these levels by processing. In this context, the oxalate fractionation in food and beverages is of great importance because of its harmful effect on human health, in which the daily consumption of oxalate is 50-200 mg day^{-1}. The detailed knowledge of oxalate content in foods and beverages is necessary to develop a good analytical strategy for the stone formation control.

There are a number of analytical methods for the determination of oxalate in foods and beverages in literature. These methods are mainly based on enzymatic analysis with arylamine bound oxalate oxidase and peroxidase [6], spectrochemical [7,8], electrochemical [9,10] and chromatographic separations [11-21]. Determination of oxalate by the sensitive methods such as GC-MS, LC-MS, CE-MS and flow injection spectrofluorimetry are expensive and time consuming. Also, a wide variety of other techniques, based on liquid chromatography (LC) and capillary electrophoresis (CE) interfaced with techniques of mass spectrometry, known as hyphenated techniques, which in situ/on line separation and detection, GC-MS, LC-tandem MS [19] and CE-MS [20] are considerably more difficult to use, have been proposed for the separation and determination of oxalate from interfering matrix ions. Thus, we described a spectrophotometric method with low detection limit, which is simple, low cost, rapid, efficient and moreover sensitive and selective with a suitable chromogenic reagent for analyte in visible region.

Despite improvements in modern analytical instruments, determination of oxalate species at low concentrations by this technique is often difficult due to the complexity of sample matrix. The ultrasonic assisted cloud point extraction (UA-CPE) as a preconcentration tool offers many advantages such as low cost, rapidity, simplicity, high preconcentration factor and low toxicity because of usage of eco-friendly solvents when compared with other preconcentration techniques such as liquid–liquid extraction (LLE) and solid phase extraction (SPE). The UA-CPE was successfully coupled to spectrophotometry greatly to improve its detection limit and the selectivity. The UA-CPE was successfully applied to preconcentration of organic and inorganic species like 5-hydroxymethylfurfural (5-HMF) in different honey and acidic beverage samples [22], sulfite as food additive [23], total iodine in milk based products [24] and also oxalate with a reasonable sensitivity difference in vegetable matrices [25] by our research group.

The objective of the exist study was to establish a method for quantifying oxalate in beverage matrices in terms of fractionation of total and soluble oxalate with and without two different acidic extractions. The method is based on sensitive and selective ion-association of anionic complex, $VO_2(Ox)_2^{3-}$ produced by the reaction of oxalate with V(V), with xanthene derivative dye, Pyronine B (H_2In^{2+}), and then extraction into micelles of polyethylene glycol tert-octylphenyl ether (Triton X-114) at pH 5.0. The variables affecting complex formation and UA-CPE efficiency were extensively investigated and optimized. The accuracy of the method was confirmed by using a reference method as well as recoveries from spiked samples. The method was successfully employed for analysis of the soluble, insoluble and total oxalate contents in various beverage samples with satisfactory results.

MATERIALS and METHODS

Reagents and Standard Solutions

All reagents were prepared using high-purity

water with resistivity of 18.2 MΩ, using water obtained from a milli-Q plus water purification system (Millipore, Bedford, MA, USA). Standards, buffer solutions, calibration solutions and dilutions were prepared with this water. Before starting the experiment, all the plastic and glassware were cleaned by 10% (w/v) HNO3 solution, and were rinsed with the water. The stock solution of 500 mg L^{-1} V(V) were prepared using NH_4VO_3 (≥ 99.0% ACS reagent, Sigma-Aldrich Co., St. Louis, MO, USA) with water, and was diluted daily for obtaining working solutions. A 1.0×10^{-3} mol L^{-1} aqueous solution of Pyronine B was also prepared fresh daily by dissolving suitable amount of the reagents supplied from Merck (Darmstadt, Germany) in ethanol and diluting with the water. 1000 mg L^{-1} of oxalate solution was prepared by adding 1.43 g of $H_2C_2O_4\times2H_2O$ (≥ 99.0%, Sigma-Aldrich) to a 1 L volumetric flask, dissolving and diluting in water. The calibration solutions of oxalate in range of 0.4-170 μg L^{-1} were daily prepared by the serial dilution of a 1000 μg L^{-1} stock solution with water. The recovery experiments from spiked beverage samples were performed by adding the oxalate concentrations of 5 and 15 μg L^{-1} including 10, 15 and 30 μg L^{-1} for precision of the method. The nonionic surfactants, Ponpe 7.5, Triton X-45, 100 and 114 (Sigma-Aldrich) were used without further purification to prepare the 5.0% (v/v) aqueous solutions. The 100 mL of 0.1 mol L-1 at pH 5.0 citrate buffer solution was daily prepared by mixing 20.5 mL of 0.1 mol L-1 citric acid (≥ 99.5%, Sigma-Aldrich) and 29.5 mL of 0.1 mol L-1 sodium citrate (≥ 99.0%, Sigma-Aldrich) solutions, and was diluted to 100 mL with the water.

Instrumentation

In the current study, the apparatus used in experimental studies are as follows; a Shimadzu Model UV-Visible 1601 PC spectrophotometer (Kyoto, Japan) equipped with a 1 cm quartz cell was used for absorbance measurements. A centrifuge (Universal-320, Hettich Centrifuges, and Buckinghamshire, England) was used to facilitate the phase separation. A pH meter (pH-2005 model, JP Selecta, Barcelona, Spain) was used for pH measurements. An ultrasonic bath (UCS-10 model, Seoul, Korea) was used to maintain the temperature in UA-CPE experiments, and used to assist the fast and efficient extraction of analyte from beverage matrices.

Sampling and Sample Preparation

All of samples selected for analysis were supplied from local markets in Sivas, Turkey.

In case of pretreatment of alcoholic beverages (10 mL), 5.0 mL of 0.1% (v/v) 2-octanol solution and potassium metabisulfite (8 mg L^{-1}, 1 mL) were added to the beer samples to prevent foaming and destroy any protein, and they were degassed for 15 min using an ultrasonic power. Wine samples (50 mL) were de-alcoholized at 80 °C using a reduced pressure evaporator until the total volume was approximately a quarter of the initial volume. The samples were kept cool, filtered with a membrane filter (0.45-mm pore size) and diluted to a volume 50 mL with the water. The beverages without alcohol were centrifuged for 5 min at 4000 rpm and filtered using a membrane filter.

In case of pretreatment of milk samples, the suitable volumes of milk samples (10 mL) after homogenization by stirring in a vortex for 2 min, were deproteinized, defatted and thoroughly clarified with trichloroacetic acid (TCA) (2.0 mL of 5.0%, w/v) to reduce approximately pH to 4.5 and separate the protein, casein and fat, the slurries were transferred to a volumetric flask of 100 mL and diluted with water, then filtrated by using a membrane filter after centrifugation for 5 min at 4000 rpm.

The soluble and total oxalate levels of selected beverage and milk samples without and with acid extraction were monitored by a slight modification of the method as described by Savage et al [2]. To determine the total oxalate, all the samples were carefully pretreated and extracted by two different acidic extraction approaches under ultrasonic power:

(1) Diluted H_2SO_4 for 10 min at 80°C,
(2) diluted H_3PO_4 containing 400 mg L^{-1} NaCl for 10 min at 60°C.

For the extraction of the total oxalate, aliquots of 2.0 mL of homogenized/pretreated samples were placed into two separate flasks of 100 mL. Then, 20 mL of 0.1 mol L^{-1} H_2SO_4 at 80°C was

added to the one, and 20 mL of 0.2 mol L^{-1} H_3PO_4 containing 400 mg L^{-1} NaCl at 60 °C was added to the other. After that, the mixtures in each flask were separately sonicated and extracted under ultrasonic power for 10 min. The mixtures were allowed to cool, and then filtered by using a membrane filter of 0.45 μm into a flask of 100 mL and the final volume was diluted to 100 mL with water before analysis.

To determine the soluble oxalate fraction, the same procedure was followed as in the extraction for total oxalate, except that the water was used to extract the soluble oxalates under ultrasonic power for 10 min at 80°C and 60°C, respectively. To control the matrix effect and signal fluctuations caused by temperature and other factors, the soluble and total oxalate fractions of all the samples were determined by using standard addition method before submission to UA-CPE procedure, and then spectrophotometric detection at 553 nm. With this purpose, the signal intensity of the sample solution initially was measured, then the portions of a solution of the analyte at a known concentration (1.0, 1.5 and 3.0 mL of 0.5 mg L-1) were added to samples and the signal intensity was measured after each addition. The fractions of insoluble oxalate of the samples were calculated from difference between soluble and total oxalate fractions. All samples were run in batches that included blanks and samples externally spiked at levels of 5 and 15 μg L^{-1} for oxalate as another indicator of the method accuracy. All samples were prepared in triplicate run, and the results were indicated with error bars.

The procedure

A preconcentration experiment was carried out according to the following procedure. 2.0 mL aliquots of the sample or standard solutions containing oxalate in the range of 0.4–170 μg L^{-1}, 0.75 mL of citrate buffer at pH 5.0, 2.0 mL of 10 mg L-1 V(V), 1.5 mL of $1.0x10^{-3}$ mol L^{-1} Pyronine B (H_2In^{2+}), 0.4 mL of 20% (w/v) KCl and 0.75 mL of 5.0% (v/v) Triton X-114 solution, respectively, were added to a 50 mL volumetric flask and diluted to the mark with the water. The solutions were mixed well, and incubated in the ultrasonic bath (300 watt, 40 kHz) at 50°C for 15 min to start the process of extraction and preconcentration of oxalate in the surfactant-rich phase. The phase separation was then accelerated by centrifuging at 3500 rpm for 10 min. Then, the volumetric flask has been held in a refrigerator to facilitate phase separation, which is coacervated to the bottom of the vial. The viscosity of the surfactant-rich phase increased, so the aqueous phase was separated by a simple decantation. The surfactant-rich phase was dissolved and diluted to 0.5 mL with acetonitrile and transferred into a quartz cell. The absorbances of sample solutions were measured at 553 nm. A blank solution was prepared in a similar way, except that the water was used instead of oxalate.

The Reference Method

For comparison purposes, due to the lack of a certified reference material (CRM) related to oxalate in beverage matrices, we decided to use an independent kinetic method [26] with a slight modification, which is based on catalytic effect of oxalate in order to ensure the reliability of the results. The details related to modification are given in our first study [25], in which the oxalate fractions of vegetable samples are monitored and determined with a similar analytical approach. In this context, the samples, which similarly were pretreated and extracted, were submitted to a fixed time of 3 min and an induction period of 12 min in ultrasonic bath (300 watt, 40 kHz) at 35°C under the optimal reagent conditions, and then their oxalate levels were kinetically determined by spectrophotometry at 525 nm.

RESULTS and DISCUSSIONS

GeneralAspectS Related to method development

From literature investigations before method development step, the formation of a stable complex ion between V(V) and oxalate was observed in previous studies, which are based on two separate electroanalytical approaches, DP-ASV [27] and sequential AdSV [27]. In another study [29], it was observed that stable anionic complexes at ratios of 1:1 and 1:2 similarly formed in pH range of 2.0-8.0 according to Equation (1a-b).

$VO_2(OH)_2^- + HOx^- \rightarrow VO_2(OH)(Ox)^{2-} + H_2O$ (1a)

$VO_2(OH)_2^- + 2HOx^- \rightarrow VO_2(Ox)_2^{3-} + 2H_2O$ (1b)

Pyronin B (H_2In^{2+}, HIn^+ and In) selected as ion-pairing reagent in detection of oxalate by spectrophotometry, is a fluorescence sensitive xanthene derivative dye with pKa values of 6.9 and 10.1, which is planar and cationic in nature [31]. Also, it is a highly aggregating dye in microheterogenous media at lower pHs than 7.0, so as to give H- and J-types aggregates. Depending on dye concentration, polarity and medium acidity, it has been observed that the dye at $3.0x10^{-6}$ mol L^{-1} can be able to aggregate with a KD value of $4.71x10^5$ at 20°C, in which monomer-dimer equilibrium is observed with a blue shift of 16 nm [31]. Due to these properties of the dye, it is clear that the ion-pairing reagent, which is predominantly H_2In^{2+} or HIn^+ in environment, tends to give a stable hydrophobic complex with anionic $VO_2(Ox)_2^{3-}$ complex, produced in the presence of V(V) at pH 5.0, so that it will form ion-association complex. Because of its high solubility in aqueous micellar media, from prior studies, it was observed that the ion-pairing complex could easily be extracted into micellar phase of the nonionic surfactant, Triton X-114. The mechanism proposed can be postulated as follows:

$VO_2(Ox)_2^{3-} + 3H_2In^{2+} \rightarrow (H_2In^{2+})3[VO_2(Ox)_2]_2$ (2)

or

$VO_2(Ox)_2^{3-} + 3HIn^+ \rightarrow (HIn^+)3[VO_2(Ox)_2]_2$ (3)

$(HIn^+)_3[VO_2(Ox)_2]_{(aqueous\ phase)} \leftrightarrow (HIn^+)_3[VO_2(Ox)_2]_{(micellar\ phase)}$ (4)

Optimization of Analytical Variables

The optimization was carried out by the univaried method at fixed oxalate concentration of 15 μg L-1. The working range and optimal values obtained for each parameter in optimization step were presented in Table 1.

Under the optimized conditions, the absorption spectra of ternary complex as a function of absorption wavelength in absence and presence of trace oxalate at levels of 10, 15 and 30 μg L^{-1} is given in Fig. 1, in which there is a linear relationship in a concentration range falling in linearity region with increasing oxalate concentration against analyte blank.

From studies conducted pHs ranging from 2.0 to 6.5, the maximum absorbance was obtained at pH 5.0 with citrate buffer system. The complexation at pHs lower than 5.0 is quantitatively incomplete due to protonation of oxalate and aggregation of dye. At lower pHs

Table 1. Optimization of analytical parameters affecting complex formation and UA-CPE efficiency.

Parameters affecting complex formation	Working range	Optimal value
pH	2.0-6.5	5.0
Buffer concentration, mmol L^{-1}	0.2-4.0	1.5
Pyronine B concentration, μmol L^{-1}	4-40	30
Vanadium cocentration, μg L^{-1}	50-550	400
Nonionic surfactants concentration, % (v/v)	0.025–0.25	0.075
Salting out agent concentration, % (w/v)	0.08-0.8	0.2
Parameters affecting UA-CPE efficiency		
Equilibrium temperature, °C	25–70	50
Incubation time, min	2–40	15
Centrifugation rate, rpm	1000–4000	3500
Centrifugation time, min	2–20	10
Cooling time in refrigerator, min	3-25	5
Acetonitrile as diluent, mL	0.2-2.0	0.5

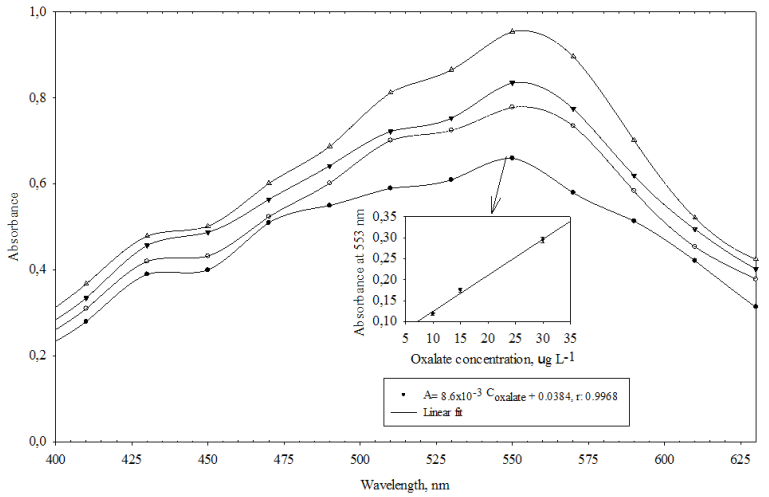

Figure 1. The absorption spectra of ternary complex as a function of absorption wavelength, nm in absence and presence of trace oxalate at levels of 10, 15 and 30 μg L^{-1}, Linearity relationship of analytical signal with increasing oxalate concentration for three replicate measurements at peak maximum.

than 3.0 and low concentrations, V(V) (with pKa values of 3.4 and 8.0) exists as the relatively stable pervanadyl ion, VO_2^+. While it is available in form of neutral $VO_2(OH)$ at pHs near to 4.0, in the region of pH close to neutral conditions (5.0–8.0), V(V) predominantly acts as an anion, $VO_2(OH)_2^-$ and VO3$(OH)_2^-$ as follows [32]. Perhaps, the cause of decrease in signal may be either oxidation of ion-pairing reagent by the reduction of V(V) in presence of oxalate or aggregation of dye at acidic pHs. Also, at pHs higher than 5.0, it can be either the deprotonation of ion-pairing reagent at low concentrations or formation of anionic hydroxy pervanadyl complexes, $VO_2(OH)_2$- and $VO_3(OH)_2^-$ as a result of hydrolysis of V(V). Also, the effect of buffer concentration to signal was investigated in range of 0.2-4.0 mmol L^{-1}, and the best signal was obtained at buffer concentration of 1.5 mmol L-1. At higher concentrations than 1.5 mmol L^{-1}, the signal gradually was decreased due to dissociation of anionic $VO_2(Ox)_2^{3-}$ complex depending on increasing citrate concentration. Therefore, a buffer concentration of 1.5 mmol L^{-1} was adopted to be enough for quantitative extraction.

One factor that increases the signal of the oxalate is also the effect of the ion-pairing reagent concentration, Pyronine B (H_2In^{2+}) in range of 4-40 μmol L^{-1}. H_2In^{2+} is a xanthene derivative dye showing strong fluorescence (λex: 553 nm, λem: 580 nm). In the present study, H_2In^{2+} as ion-pairing agent with a concentration of 30 μmol L^{-1} due to give a maximum signal was selected for oxalate species in presence of V(V) and Triton X-114 at pH 5.0.

From studies conducted in range of 50-550 μg L^{-1} for V(V), it was observed that the signal intensity linearly increased with increasing vanadium concentration in range of 50-400 μg L^{-1}, and then gradually decreased at the higher concentrations than 400 μg L^{-1}. This decrease in signal may be due to ion-association of anionic $VO_2(OH)_2^-$ or $VO_3(OH)_2^-$ species formed as a result of hydrolysis of V(V) with H_2In^{2+} in absence of oxalate, so as to cause increase in blank signal.

Generally, the addition of salt increases extraction of analyte into the surfactant-rich phase. From studies conducted in range of 0.08-0.8% (w/v) of electrolytes, a high extractability and signal for oxalate was also obtained using 0.2% (w/v) of KCl as salting out agent. The signal considerably decreased with increasing KCl concentration in range of 0.2-0.8% (w/v). This effect might be explained by the additional surface charge when the KCl concentration is very high, thus changing the molecular architecture of the surfactant and consequently the micelle formation process. It is necessary to emphasize that different blank solutions were also evaluated and a significant signal difference between signals of sample and blank was not obtained.

In UA-CPE choosing a suitable nonionic surfactant is important, because the temperature corresponding to cloud point is related with the hydrophilic property of a surfactant. From studies conducted in range of 0.025-0.25% (v/v) of the selected nonionic surfactants, the best extraction efficiency was obtained using 0.075% (v/v) of Triton X-114. Therefore, Triton X-114 was preferably chosen as extracting agent for further studies. Also, this value corresponds to a maximum concentration of 3.94 mmol L^{-1} above the critical micelle concentration of 0.20 mmol L^{-1} for Triton X-114.

For the completion of the complex formation and efficient separation of phase in the UA-CPE, It is desirable to have the lowest equilibration temperature and shortest incubation time. As a result of experimental studies conducted in range of 25-70°C and 2-40 min respectively, the solutions became turbid as soon as the solutions were put into the water bath at 50°C and incubation time at 15 min due to give the best extraction efficiency.

After preconcentration, the surfactant-rich phase obtained is very viscous. Thus, its viscosity must be reduced using diluting agents prior to spectrophotometric determination. In order to investigate the selectivity and extraction efficiency of the method, it is very important to choose the suitable diluting agent. Thus, different diluting agents such as methanol, ethanol, acetone, acetonitrile, THF, acidic methanol and ethanol were used and the best analytical sensitivity, m/sm (in which m and sm are the slope of calibration curve and standard deviation of slope), from calibration curves obtained for oxalate concentrations at levels of 25, 50 and 75 μg L^{-1} was provided using acetonitrile. Also, from a serial sensitivity study conducted in range of 0.2-2.0 mL of acetonitrile, the best signal was obtained using 0.5 mL of acetonitrile.

Analytical Features, Precision and Effect of Concomitants

Analytical features of the method (regression equation, linear range, the limit of detection, limit of quantitation, precision, sensitivity enhancement factor (is often used for signal enhancement) and preconcentration factor) at 553 nm were spectrophotometrically achieved by measuring the difference between signals of sample and blank as a function of oxalate concentration in a wide concentration range after preconcentration with UA-CPE under optimum reagent conditions. The calibration curves were highly linear in a

Table 2. Analytical figures of merit of the proposed method.

Parameters	Analytical features	
	After preconcentration at 553 nm	Before preconcentration at 553 nm
Analytical species	Oxalate	Oxalate
Linear working range, μg L^{-1}	0.4-20, 20-170	75-2500
Slopes (m)	0.0245, 6.19×10^{-3}	3.16×10^{-4}
Intercepts (b)	0.1486, 0.5363	0.156
Regression coefficient, r^2	0.9968, 0.9970	0.9945
Precision as RSD % (n: 5; 10, 15, 30 μg L^{-1})	2.35-3.50	-
Recovery % (n: 5; 10, 15 and 30 μg L^{-1})	97.3-102.6	-
*The limit of detection (n: 10, LOD, $3\sigma_{blank}/m$), μg L^{-1}	0.12	22.4
*The limit of quantification (n: 10, LOQ, $10\sigma_{blank}/m$), μg L^{-1}	0.40	74.6
**Sensitivity improvement factor	77.5	-
***Preconcentration factor	100	-

*The limits of detection and quantification corresponding ratio of three- and ten-fold of standard deviations of ten replicate blank analysis to slope of calibration curve obtained after preconcentration with UA-CPE.

**The value calculated as ratio of slopes of calibration curves obtained with and without preconcentration with UA-CPE.

***The value calculated as analyte concentration giving same analytical signal with and without preconcentration by means of UA-CPE.

working range of 0.4-170 μg L^{-1} with their slope and intercepts, which could be divided into two linear sections for low and high oxalate concentrations respectively. The calibration equations obtained by regression analysis are as follows:
Abs.= 0.0245[Oxalate, μg L^{-1}] + 0.1486 in range of 0.4-20 μg L^{-1} and Abs.= 6.19x10^{-3}[Oxalate, μg L^{-1}] + 0.5363 in range of 20-170 μg L^{-1} with a better correlation coefficient than 0.995.

As can be seen from Table 2, the limits of detection (LOD, 3σblank/m) and quantification (LOQ, 10σblank/m), defined as the ratio of three and ten times the standard deviation of the blanks (σblank) to the slope of the calibration graph (m), were determined by analysis a blank solution. The LOD and LOQ were obtained 0.12 and 0.4 μg L^{-1}, respectively. When considered linear working range of 0.4-20 μg L^{-1}, the sensitivity enhancement factor (EF), which is described as ratio of slopes of calibration curves constructed with and without preconcentration, and preconcentration factor (PF), which is defined as the ratio of analyte concentration in the surfactant-rich phase to the initial concentration in the aqueous phase or analyte concentration giving same analytical signal with and without preconcentration by UA-CPE, have been determined to be 77.5 and 100, respectively. The precision as the percentage relative standard deviation (RSD%) was generally found to be lower than <3.5% (n: 5; 10, 15, 30 μg L^{-1}). The accuracy of the method was also investigated through the recovery test in the samples by five replicate measurements of standard oxalate solutions at levels of 10, 15 and 30 μg L^{-1}, and their recovery values varied from 97.3 to 102.6%.

Matrix effect was evaluated by considering concomitants, which can potentially be found in the real samples. The tolerance limit of an interfering ion was identified as the concentration of added oxalate causing a relative error smaller than ±5.0%, which are related to the preconcentration and determination steps of oxalate. The results obtained are given in Table 3. The results showed that interferences possess no obvious influence on the signal intensity of the analyte. But the effects of interfering ions such as Fe^{3+}, $Sn^{2+}$and Zn^{2+} can be suppressed up to a tolerance limit of 30-fold by the addition of 1.0 mL of 0.01 mol L^{-1} NH_4F to the solution prior to preconcentration. In a similar way, the interference effect of Mo^{6+} can efficiently be controlled up to a tolerance limit of 20-fold by adding 250 μL of 0.02 mol L-1 tartaric acid solution to the solution prior to preconcentration while the effect of nitrate interference is greatly suppressed up to a tolerance limit of 15-fold by adding 250 μL of 0.02 mol L-1 alkaline hydroxylamine hydrochloride solution containing 20 mg L^{-1} Cu^{2+} ions to reduce NO_3^- to NO_2^-. As will clearly understood from results, the application of the method permits to interference-free determination of oxalate in the selected samples. Finally, when compared with analytical features of our previous study [25], it is clear that the present study shows lower detection limit, good precision and higher preconcentration factor with a reasonable sensitivity enhancement in linearity range of 425-fold.

Table 3. The effect of potential interfering species on determination of 15 μg L^{-1} of oxalate.

Interfering species	Tolerance limits	Recovery (%)
CH_3COO^-, and HPO_4^{2-}	>1000	97.5-103.7
NH_4^+ and Mn^{2+}	750-1000	98.1-102.5
Mg^{2+}, Pb^{2+} and Co^{2+}	400-750	96.5-101.9
Br^-, S^{2-}, and NO_2^-	300-400	97.5-103.4
SO_4^{2-}, Ca^{2+} and Citrate	200-300	97.8-103.1
Ag^+, Fe^{2+}, and Tartrate	100-200	96.3-104.2
Ni^{2+}, Bi^{3+} and Sb^{3+}	50-100	96.4-103.8
Fe^{3+}, Sn^{2+} and Zn^{2+}	25-50	97.5-103.5
Mo^{6+} and NO_3^-	10 and 25	98.2 and 102.9

The Analytical Applications

The accuracy of the method was verified in two ways: firstly through the spiked recoveries and secondly by analysis of similarly pretreated, extracted and diluted beverage samples with the reference method. The results can be seen in Table 4. It has been observed that the results obtained from five replicate measurements of samples by the proposed method are statistically in good agreement with those of reference method. It can be concluded that the method is accurate and free from systematic error. In order to control the method accuracy, a recovery study was performed by externally adding the known amounts of oxalate into aqueous solutions of all samples (5 and 15 μg L^{-1}) before submission to UA-CPE procedure. From the results, it has been found that the recoveries of spiked samples are highly quantitative in range of 97.3–102.6% with a RSD ranging from 2.0 to 3.5% for total oxalate. Moreover, as can be seen from Table 4, the Student's t-test for comparison of the mean values and their RSDs indicated that there was not a significant difference between the mean values obtained by both the proposed method and reference method at the significance level of 0.05. Because the calculated t-values in range of 0.89-2.26 and 1.00-1.56 for soluble oxalate and total oxalate, respectively, are lower than the tabulated t-value of 2.78, it can be concluded that there statistically isn't a significant difference between the mean values obtained by both methods.

Also, we investigated the applicability of the methodology by determining of soluble, insoluble and total oxalate species in beverage samples. Aliquot of 2.0 mL of the pretreated and extracted sample solutions were transferred into volumetric tubes of 50 mL, individually. Then, the method was successfully applied to determine the amounts of total oxalate in samples after comparatively two separate pretreatments with (i) 20 mL of 0.1 mol L^{-1} H_2SO_4 at 80°C and (ii) 20 mL of 0.2 mol L^{-1} H_3PO_4 containing 400 mg L^{-1} NaCl at 60°C under the ultrasonic power by adding the portions of a solution of oxalate at a known concentration (1.0, 1.5 and 3.0 mL of 0.5 mg L-1) at a known concentration (1.0, 1.5 and 3.0 mL of 0.5 mg L^{-1}), in which 20 mL of water for soluble oxalate is used instead of diluted acid under the same conditions. The insoluble oxalate level was calculated from the difference between soluble and total oxalate levels. The results are given in Table 4. It can be seen that the recoveries from spiked solutions at levels of 5 and 15 μg L^{-1} are quantitatively varied in the range of 97.3-102.6% for vegetable samples. The precision of five replicate determinations is typically lower than 3.5% for both soluble oxalate and total oxalate.

The analytical features of method such as linear working range, detection limit, precision, waste generation, cost and time of analysis, preconcentration and sensitivity enhancement factors were also compared with those of other detection methods reported in the literature. When analyzing the Table 5, it can clearly be seen that the proposed method has some advantages such as wider linearity range of 425-fold, low detection limit, good precision, quantitative recovery, and high preconcentration factor with a good sensitivity enhancement for the spectrophotometric determination of soluble, insoluble and total oxalate in selected beverages. Because of these properties, the proposed method can be considered as an alternative tool to sensitive but expensive, poor precision and recovery especially in low concentrations, time-consuming and experienced user-requiring analytical techniques such as IC, HPLC, CE, CZE and GC with conductivity, UV, fluorescence and flame ionization detection, which is hyphenated with MS.

CONCLUSIONS

In the existing study, a simple, accurate and reliable analytical method was developed for pre-concentration, fractionation and determination of total and soluble oxalate from beverages by coupling UA-CPE to spectrophotometry with and without acidic extraction. The proposed method has good repeatability, low cost, safety and reproducibility with low LOD (0.12 μg L^{-1}) and PF (100), and could be applied to analysis of other complex samples as well as the determination of oxalate in different beverages. Moreover, ultrasonic bath was successfully used to assist the fast (less than 15 min) and efficient extraction of analyte from sample matrix before

Table 4. Determination of soluble, insoluble and total oxalate contents of alcoholic and nonalcoholic beverage and milk samples, and recoveries of spiked samples (n: 5).

Samples	Added oxalate, (μg L^{-1})	After extraction with 20 mL of 0.1 mol L^{-1} H_2SO_4 for 10 min at 80°C under ultrasonic power	After extraction with 20 mL of 0.2 mol L^{-1} H_3PO_4 containing 400 mg L^{-1} NaCl/ for 10 min at 60°C under ultrasonic power	With independent kinetic method	**The calculated statistical t- and F- values								
		[a1]Soluble Oxalate, (μg L^{-1})	[c]Insoluble Oxalate, (μg L^{-1})	[a2]Total Oxalate, (μg L^{-1})	[a1, a2]RSD (%)	*Recovery (%)	[b1]Soluble Oxalate, (μg L^{-1})	[c]Insoluble Oxalate, (μg L^{-1})	[b2]Total Oxalate, (μg L^{-1})	[b1, b2]RSD (%)	*Recovery (%)	Total oxalate, (μg L^{-1})	
Added-sugar beer	-	15.0±0.6	8.4	23.4±1.0	3.5- 3.3	-	14.6±0.5	8.1	22.7±0.8	3.4- 3.5	-	23.0±0.9	0.89-1.56
	5	19.6±0.8	-	28.1±1.0	3.4-3.1	92-94	19.3±0.7	-	27.3±1.0	3.3-3.2	94-92	-	
	15	29.6±1.2	-	37.4±1.3	3.5-3.3	97.3-93.3	29.4±1.0	-	37.3±1.3	3.4-3.1	98.7-97.3	-	
5.0% alcohol beer	-	10.6±0.4	11.7	22.3±0.8	3.5-3.3	-	10.2±0.5	11.5	21.7±1.0	3.4-3.5	-	21.8±0.8	1.40-1.00
	5	15.2±0.6	-	27.0±1.0	3.4-3.3	92-94	14.8±0.6	-	26.4±1.0	3.5-3.3	92-94	-	
	15	25.3±1.0	-	36.9±1.2	3.5-3.2	98-97.3	24.8±1.0	-	36.4±1.2	3.4-3.3	97.3-98	-	
Red wine	-	15.3±0.6	23.0	38.3±1.3	3.5-3.4	-	15.1±0.6	22.5	37.6±1.3	3.2-3.1	-	37.5±1.2	1.38-1.17
	5	19.8±0.8	-	43.0±1.5	3.3-3.5	90-94	19.7±0.7	-	42.3±1.5	3.2-3.5	92-94	-	
	15	30.0±1.2	-	52.8±1.8	3.4-3.3	98-96.7	29.8±1.0	-	52.4±1.8	3.4-3.4	98-98.7	-	
White wine	-	30.3±1.1	7.4	37.7±1.3	3.0-3.1	-	29.7±1.1	7.7	37.4±1.3	3.3-3.5	-	37.2±1.2	0.86-1.17
	5	34.8±1.1	-	42.3±1.4	2.7-2.6	90-92	34.3±1.2	-	42.0±1.5	3.5-3.4	92-92	-	
	15	44.8±1.5	-	52.2±1.6	3.3-3.1	96.7-96.7	44.2±1.5	-	51.8±1.6	3.4-3.1	96.7-96	-	
Apple juice	-	3.4±0.13	1.10	4.5±0.2	3.4- 3.4	-	3.3±0.12	1.1	4.4±0.2	3.5-4.5	-	4.7±0.2	2.23-1.00
	5	8.1±0.3	-	9.3±0.3	3.5-3.0	94-96	8.0±0.3	-	9.1±0.3	3.2-3.3	94-94	-	
	15	18.1±0.4	-	19.2±0.4	2.2-2.1	98-98	18.0±0.4	-	19.1±0.4	2.2-2.1	98-98	-	
Cherry juice	-	2.6±0.1	0.42	3.0±0.2	3.1- 3.3	-	2.6±0.1	0.44	3.0±0.2	3.1- 3.3	-	2.8±0.1	2.23-4.00
	5	7.4±0.3	-	7.8±0.3	3.5-3.3	96-96	7.3±0.3	-	7.9±0.3	3.1- 3.0	94-98	-	
	15	17.3±0.4	-	17.7±0.5	2.3-2.8	98-98	17.4±0.4	-	17.6±0.5	2.3-2.8	99-97	-	

Pear juice	-	8.2±0.3	11.3	19.2±0.7	3.4- 3.5	-	8.1±0.3	10.9	19.0±0.7	3.3- 3.5	-	18.7±0.6	1.60-1.36
	5	13.0±0.4	-	24.1±0.7	3.1-2.9	96-92	12.8±0.4	-	23.7±0.7	3.1-2.9	94-94	-	
	15	22.8±0.7	-	34.2±0.9	3.1-2.6	97-98	22.7±0.7	-	33.6±0.9	3.1-2.7	97-97	-	
Grape juice	-	5.0±0.2	4.0	9.3±0.3	3.4- 3.2	-	4.9±0.2	4.20	9.2±0.3	3.0- 2.9	-	9.0±0.3	2.24-1.00
	5	9.7±0.3	-	14.0±0.4	3.1-2.9	94-94	9.7±0.3	-	13.8±0.4	3.1-2.9	96-92	-	
	15	19.5±0.6	-	23.8±0.7	3.1-2.9	97-97	19.6±0.6	-	23.7±0.7	3.1-2.9	98-97	-	
Apple vinegar	-	6.7±0.2	1.85	8.6±0.3	3.0- 3.5	-	6.6±0.2	1.90	8.5±0.3	3.0- 3.5	-	8.3±0.3	2.24-1.00
	5	11.4±0.3	-	13.3±0.4	2.6-3.0	94-94	11.3±0.3	-	13.2±0.4	2.7-3.0	94-94	-	
	15	21.4±0.6	-	23.3±0.7	2.8-3.0	98-98	21.3±0.6	-	23.2±0.7	2.8-3.0	98-98	-	
Grape vinegar	-	6.7±0.2	1.85	8.6±0.3	3.0- 3.5	-	6.5±0.2	1.90	8.5±0.3	3.0- 3.5	-	8.3±0.3	2.24-1.00
	5	11.4±0.3	-	13.3±0.4	2.6-3.0	94-94	11.3±0.3	-	13.2±0.4	2.7-3.0	96-94	-	
	15	21.5±0.6	-	23.3±0.7	2.8-3.0	99-98	21.3±0.6	-	23.1±0.7	2.8-3.0	99-97	-	
Kiwi juice	-	18.5±0.6	41.1	59.6±1.6	3.2- 2.8	-	18.2±0.5	41.0	59.2±1.5	2.8- 2.7	-	60.3±1.7	0.98-1.13
	5	23.1±0.7	-	64.3±2.0	3.0-3.1	92-94	22.8±0.7	-	63.7±2.0	3.1-3.1	92-90	-	
	15	33.0±1.0	-	74.1±2.3	3.0-3.1	97-97	32.7±1.0	-	73.6±2.3	3.1-3.1	97-96	-	
Semi-skimmed milk	-	5.2±0.2	2.16	7.4±0.3	3.5- 3.4	-	5.2±0.2	2.15	7.3±0.3	3.5- 3.5	-	7.1±0.3	2.24-1.00
	5	9.8±0.3	-	12.1±0.4	3.1-3.3	92-94	9.8±0.3	-	12.0±0.4	3.1-3.3	92-94	-	
	15	19.7±0.5	-	22.0±0.6	2.5-2.7	97-97	19.8±0.6	-	21.8±0.6	3.0-2.8	97-97	-	
Whole milk	-	10.2±0.4	3.40	13.6±0.5	3.5- 3.3	-	10.1±0.4	3.20	13.3±0.5	3.4- 3.2	-	13.1±0.4	2.26-1.56
	5	14.7±0.5	-	18.2±0.6	3.2-3.0	90-92	14.6±0.5	-	17.8±0.6	3.2-3.4	90-90	-	
	15	24.7±0.7	-	28.3±0.8	2.8-2.8	97-98	24.6±0.7	-	28.0±0.8	2.9-2.7	97-98	-	

[a1]The average plus standard deviation of five replicate measurements for soluble oxalate after extraction with 20 mL of water at 80 oC for 10 min.

[a2]The average plus standard deviation of five replicate measurements for total oxalate after extraction with 20 mL of 0.1 mol L^{-1} H_2SO_4 at 80°C for 10 min.

[b1]The average plus standard deviation of five replicate measurements for soluble oxalate after extraction with 20 mL of water at 60°C for 10 min.

[b2]The average plus standard deviation of five replicate measurements for total oxalate after extraction with 20 mL of 0.2 mol L H_3PO_4 containing 400 mg L^{-1} NaCl at 60°C for 10 min.

[c]The insoluble oxalate values obtained by calculating the analytical difference between total oxalate and soluble oxalate contents of beverage samples.

[*]The percent recoveries obtained for five replicate measurements of spiked at two different oxalate concentration levels of 5 and 15 μg L^{-1}

[**]In order to compare the mean total oxalate levels of beverage samples with equal sample size obtained by using both the proposed UA-CPE-spectrophotometric method and independent kinetic comparison method, in which the statisticalt- and F-variance ratio values for four or eight degrees of freedom at probability level of 0.05 is 2.78 and 6.59 respectively.

T**able 5.** Comparison of analytical features of the proposed method with those of other detection methods reported in literature.

Sample	Detection tool	Linear range	LOD	RSD (%)	References
Soft drinks, foodstuffs	Enzymatic analysis	4.5-108 mg L^{-1}	4.5 mg L^{-1}	3.5-7.5	[6]
Two synthetic samples	Spectrofluorimetry	1-8 μmol L^{-1}	0.1 μmol L^{-1}	3.2	[7]
Vegetables	Chemiluminescence	0.17-8.21 mg L^{-1}	0.05 mg L^{-1}	1.3	[8]
Beer and urine	IC with chemiluminescence detection	0.035-10 mg L^{-1}	0.025 mg L^{-1}	3.1	[13]
Different beers	CZE	2 –25 μmol L^{-1}	0.5 μmol L^{-1}	<3.4	[15]
Foods	HS-GC	0-30 μmol L^{-1}	1.95 μmol L^{-1}	<3.8	[17]
Urine	LC-tandem MS	100-2212 μmol L^{-1}	3.0 μmol L^{-1}	≤6.0	[19]
Green and black tea	IC	0.1-20 mg L^{-1}	0.03 mg L^{-1}	1.0	[21]
Vegetables	Spectrophotometry	1.2-240 μg L^{-1}	0.36 μg L^{-1}	<5.3	[25]
Human serum	CZE after on column preconcentration	0.5-1000 mg L^{-1}	0.1 mg L^{-1}	3.5-10.5	[33]
Different anion mixtures	Indirect FIA-FAAS	10-170 μmol L^{-1}	8 μmol L^{-1}	1.3	[34]
Foods	CE and IC	2-40 mg kg^{-1}	0.6 mg kg^{-1}	6.3	[35]
Vegetables and waters	Spectrophotometry	1.0-70 μg L^{-1}	0.8 μg L^{-1}	<4.0	[36]
Spinach, beet and mushroom	Catalytic spectrophotometry	2-180 mg L^{-1}	0.7 mg L^{-1}	<4.7	[37]
Corks, beer and spinach	DPP	0.2-20 μmol L^{-1}	0.05 μmol L^{-1}	-	[38]
Beverages	Spectrophotometry	0.4-170 μg L^{-1}	0.12 μg L^{-1}	<3.5	The present study

IC: ion chromatography, CZE: capillary zone electrophoresis, HS-GC: headspace gas chromatography, DPP: differential pulse polarography.

preconcentration. The proposed method is eco-friendly, because the Triton x-114 has lower toxicity than those using organic solvents. The power of the detection technique can also provide improvements in sensitivity and selectivity when Pyronine B is selected as a suitable photometric probe in visible region.

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