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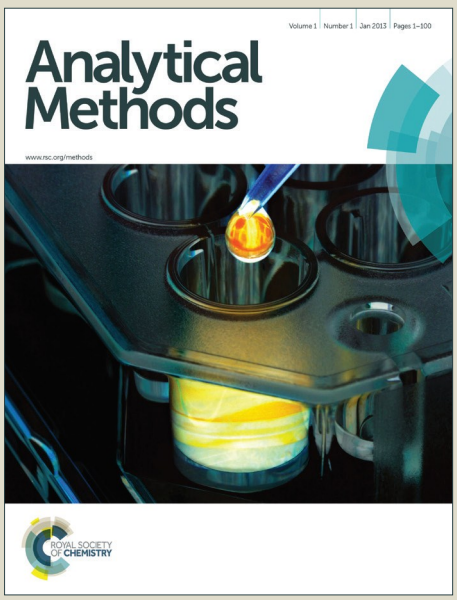

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A new simple UV–VIS spectrophotometric method for determination of sulfite species in vegetables and dried fruits using preconcentration process

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Abstract

A new eco-friendly, selective and sensitive method was developed for preconcentration and determination of trace levels of sulfite by ultrasonic-assisted cloud point extraction (UA-CPE). The method is based on the selective ion-association of anionic complex, $Cu(SO_3)_2^{2-}$, produced depending on sulfite concentration in presence of excess Cu(II) ions with Toluidine blue (TB^+) at pH 7.5, and then extraction of the formed ion-associate complex into surfactant rich phase of Triton X-45 micelles. In the optimized reagent conditions, the calibration curve is linear in range of 2.5–350 μg L^{-1}, and the limits of detection and quantification of the method (LOD and LOQ) ($3\sigma_{blank}$/m and $10\sigma_{blank}$/m) are 1.15 and 3.82 μg L^{-1} with sensitivity enhancement factor of 95. The results demonstrated that the method achieved acceptable quantitative recoveries of 95.7 to102.9 % with relative standard deviations (RSDs) of 2.1-4.8 %. The method showed good selectivity, and was successfully applied to the quantification of sulfite species in vegetables and dried fruit samples with satisfactory results. The results were compared with those of the standard 5,5′-dithio-bis(2-nitrobenzoic acid (DTNB) method, and the two paired t-test was used to determine whether the results obtained by the two methods differ significantly.

Keywords: Vegetables, Dried Fruits, Sulfite, Food additives, Food Safety, Ultrasonic-Assisted Cloud Point Extraction, Spectrophotometry

Highlights

► Ultrasonic assisted CPE was used for the preconcentration of sulfite.

► The effect of foreign ions may be present actual samples were investigated.

► The method was applied to the quantification of sulfite in vegetables and dried fruits.

►The analytical variables affecting UA-CPE efficiency were optimized.

► The enrichment factor 95 was obtained.

1. Introduction

Sulfites are commonly used as an additive in a wide variety of foods and beverages, because they inhibit development of both enzymatic and non-enzymatic browning in a variety of processing and storage situations.[1,2] The ingestion of foods containing large concentrations of sulfites is associated with asthmatic reactions and food intolerance symptoms.[3,4] The ADI of sulfite (expressed as SO_2) is 0.7 $mgkg^{-1}$ body weight. The US FDA requires a sulfite warning on the label of foods containing concentrations of ≥10 $mgkg^{-1}$ sulfite.[5] This obligation is also compulsory for any food sold in the European Union and Korea. Sulfite and sulfating agents as food additives are easily detected by various analytical methods. However, sulfites may undergo reactions with certain food components such as aldehydes, ketones or polyphenols, and become reversibly or irreversibly bound.[6,7] Whereas the reversibly bound adducts are decomposed only slowly upon acidification, the decomposition takes place more rapidly when heated to boiling temperature or in alkaline media. In the analytical quantification of sulfites, this behavior is used to determine the free, as well as the bound sulfites. Especially the bound sulfites may account for too low analytical findings, if they are not–or not completely– released. Some of it added to foods often disappears as a result of reversible and irreversible chemical reactions. Thus, it is often important to measure both free and reversibly bound forms of sulfite that are present in foods.

Recently, many methods in literature have been developed for determining sulfites speciation in various beverage and foods. The determination methods of sulfite species are mainly based on inductively coupled plasma–atomic emission spectrometry (ICP-AES),[8,9] diffuse reflectance fourier transform infrared spectroscopic (DRS-FTIR) analysis (DRS-FTIR),[10] spectrofluorimetry,[11] spectrophotometry,[12] vapor generation–inductively coupled plasma–optical emission spectrometry (VG-ICP-OES),[13] high-resolution continuum source

flame atomic absorption spectrometer (HR-CS-FAAS),[14,15] surface-enhanced raman scattering (SERS),[16] chemiluminescence,[17] flow injection analysis (FIA) with chemiluminescence detection,[18] HPLC with UV detection,[19] ion chromatography,[20] headspace gas chromatography and electron capture detection (HS-GC-ECD),[21] and automatic FIA with voltammetric detection,[22] until now. Although the detection techniques such as ICP-AES and VG-ICP-OES have very high analytical sensitivity, they can only determine total sulfite. Also, a wide variety of other techniques, based on capillary electrophoresis (CE) [23] and liquid chromatography (LC) [24] interfaced with techniques of atomic spectroscopic, known as hyphenated techniques, CE-HG-AFS, CE-ICP-MS, HPLC-ICP-MS, LC-ICP-MS, IC-HG-AFS, and HPLC-HG-AFS have been proposed for the identification, determination of sulfite species with and without hydride generation. The primary advantage of this approaches is the unequivocal species separation and specific on line detection. Unlike all of them, the UV-Vis spectrophotometry is still widely used in analytical chemistry. Moreover, the device has advantages such as simplicity, inexpensive, accuracy, selectivity, rapidity and no need expert user than others. Despite improvements in modern analytical instruments, determination of trace sulfite species at low concentrations by this technique is often difficult due to the complexity of sample matrix. To overcome this problem, ultrasonic-assisted cloud point extraction (UA-CPE) step is used for separation and preconcentration for sulfite from these samples. The use of the procedure as a preconcentration technique has the following advantages such as relatively low toxicity, high preconcentration factor, inexpensive compared to organic solvents and nonvolatile according to other preconcentration techniques such as dispersive liquid–liquid microextraction (DLLME),[25,26] headspace single-drop microextraction (HS-SDME),[27] solid phase extraction (SPE),[28] and liquid–liquid extraction (LLE).[29] Also, the UA-CPE was efficiently coupled with spectrophotometry, and successfully used in order to enhance its low detection limit as well as the selectivity of the technique.[30]

Moreover, the technique has successfully been applied to determination of essential and toxic species such as 5-HMF,[31] fluoride[32] nitrite,[33] and total iodine[34] in the monitoring of foods and beverages safety and environmental pollution by our research group.

The objective of this study was to develop a simple, sensitive, accurate and reliable method for the determination of trace amounts of sulfite in vegetables and dried fruit samples by using UA-CPE technique combined with UV-Vis spectrophotometry. The proposed UA-CPE method was based on the selective ion-association of anionic complex, $Cu(SO_3)_2^{2-}$, produced depending on sulfite concentration in presence of excess Cu(II) ions with Toluidine blue (TB^+), which is named as 3-amino-7(dimethyl amino)-2-methyl phenothiazin-5-ium, at pH 7.5, and then extraction of the formed ion-associate complex into surfactant rich phase of Triton X-45 micelles.

2. Materials and Methods

2.1. Apparatus and Reagents

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. This spectrophotometer has a wavelength accuracy of ±0.2 nm and a bandwidth of 2 nm in the wavelength range of 190–1100 nm. The absorbance measurements at 630 nm after preconcentration were made for quantification of sulfite in vegetables and dried fruit samples. An ultrasonic cleaner (UCS-10 model, Seoul, Korea) was used to maintain the desired temperature within ±1.0 °C and digest the samples. The pH measurements were done with a pH meter (pH-2005, JP Selecta, Spain). Eppendorf vary-pipettes in range of 10–100 and 200–1000 μL were used to deliver accurate volumes. A vortex mixer (12 watt, 60 Hz) was used for thorough mixing of solutions (VM-96B model, Seoul, Korea). A centrifuge (Universal-320, England) was used to accelerate the phase

separation process. A refrigerator was used to keep the samples fresh, and cool up to the analysis.

Ultra-pure water with a resistivity of 18.2 MΩ cm was prepared using a Labconco (USA) water purification system. All solutions were prepared with this ultra-pure water. Fresh sodium sulfite stock solutions (500 mg L^{-1}) were prepared by weighing sodium sulfite from Merck (Darmstadt, Germany) and dissolving it in ultra-pure water and adding 0.2 % (v/v) glycerol to stabilize the solution. The 500 mg L^{-1} of $CuCl_2$ stock solutions (Sigma, St. Louis, MO, USA) were prepared by dissolving solid $CuCl_2$ salt at suitable amount in water and diluting to 500 mL with water. A 1.0×10^{-4} mol L^{-1} of Stock TB^+ (Sigma) solution was prepared fresh daily by dissolving the reagents in ethanol (Merck) and diluting with water. All working solutions were prepared by a serial dilution of the stock solutions at suitable proportions. Solutions of 2.5 % (v/v) of Triton X-45 (Sigma) were prepared by dissolving 2.5 mL of surfactant in 100 mL of water. A 0.04 mol L^{-1} of Britton-Robinson (BR) buffer was used to keep pH of the solutions. The buffer consists of a mixture of 0.04 mol L^{-1} H_3PO_4 (Merck), 0.04 mol L^{-1} H_3BO_3 (Merck) and 0.04 mol L^{-1} CH_3COOH (Merck) that has been titrated to the desired pH with 0.2 mol L^{-1} NaOH. The electrolyte solutions at equal molar concentrations (5.0 (w/v) %) potassium chloride (KNO_3), sodium sulfate (KCl), sodium chloride (NH_4Cl) and sodium chloride (NaCl) were prepared by dissolving an appropriate amount of chemicals (Sigma) in 50 mL of water. Acidic extraction solution was prepared by dissolving 1.82 g of D-mannitol in 800 mL of the degassed water in a 1 L volumetric flask, adding 1.92 g conc. methanesulfonic acid, and bringing to volume with the degassed water. Filter through using a membrane filter 0.45 mm pore size. Alkaline extraction solution was prepared by dissolving 2.84 g of sodium phosphate dibasic and 1.82 g of D-mannitol in 900 mL of the degassed water in a 1 L volumetric flask, then bringing to volume with the

degassed water. All the prepared stock solutions were stored in polyethylene bottles in a refrigerator at 4 °C.

2.2. Sample collection and preparation

All of the vegetables and dried fruit samples selected for analysis were supplied from greengrocers and local supermarket in Sivas, Turkey. Before starting the experiment, all the glassware and other mineralization containers used were washed in 10 % (v/v) HNO_3 to avoid contamination. Primarily, all of the selected vegetables was carefully washed with ultra-pure water. Then, the edible parts such as leaves and roots were cut and crushed, and homogenized in a blender at high speed. The samples were kept in a freezer at -20 ^{o}C until the extraction moment.

Sample preparation can be very important in sulfite determination since sulfite can easily be oxidized to sulfate. To overcome this event, D-mannitol solution as a stabilizer was used to reduce the oxidation of sulfite, and the sulfite solutions were prepared fresh daily. The vegetables and dried fruit samples were prepared to analysis with four successive analytical steps like homogenization, extraction, deproteinization and reduction.

The steps of firstly preparation process to determine free sulfite are as follows; 10 mL of acidic extraction solution were added to approximately 2-3 g of the sample in a homogeneous slurry into beaker of 100 mL. Then, the beakers were covered with watch glasses and left overnight for the pre-digestion of samples. After, the sample solutions were degassed and digested for 10 min using an ultrasonic bath under ultrasonic power (300 watt, 50 Hz) at 30 ^{o}C. After centrifugation at 4000 rpm for 2 min, the digested samples were filtered using a membrane filter (0.45 μm pore size) into a 50 mL volumetric flask and the final volume was diluted to 50 mL with ultra-pure water before analysis. To determine total sulfite, 10 mL of alkaline extraction solution were added to approximately 2-3 g of the sample

in a homogeneous slurry into beaker of 100 mL. Then, the beakers were covered with watch glasses and left overnight for the pre-digestion of samples. After, the sample solutions were degassed and digested for 10 min using an ultrasonic bath under ultrasonic power (300 watt, 50 Hz) at 45 ^{o}C. After centrifugation at 4000 rpm for 2 min, the digested samples were filtered using a membrane filter (0.45 μm pore size) into a 50 mL volumetric flask and the final volume was diluted to 50 mL with ultra-pure water before analysis.

The steps of secondly preparation process to determine free sulfite are as follows; approximately 2-3 g of the sample in a homogeneous slurry is weighted into 50 mL volumetric flask, 2.0 mL of 2-mercaptoethanol and approximately 45 mL ultra-pure water are added. After, the sample solutions were degassed and digested for 5 min using an ultrasonic bath under ultrasonic power (300 watt, 50 Hz) at 35 ^{o}C. After centrifugation at 4000 rpm for 2 min, the digested samples were filtered using a membrane filter (0.45 μm pore size). The total sulfite was determined by the following procedure. A approximately 2-3 g of the sample in a homogeneous is weighted into 50 mL volumetric flask, 3.0 mL of 2-mercaptoethanol, 40 mL the water and 5–7 mL of 0.2 mol L^{-1} disodiumtetraborate are added. After, the sample solutions were degassed and digested for 5 min using an ultrasonic bath under ultrasonic power (300 watt, 50 Hz) at 50 ^{o}C. After centrifugation at 4000 rpm for 2 min, the digested samples were filtered using a membrane filter (0.45 μm pore size).

2.3. Statistical analysis

A five replicate blank analysis were also carried out in order to correct for any analyte contaminants from the reagents used for sample preparation. The sulfite contents of all samples were determined by using three pointed-standard addition approaches in order to suppress the matrix effect by direct spectrophotometry after separation and preconcentration with UA-CPE under the optimized reagent conditions. Each point in optimization step and

calibration curves with and without preconcentration was run in triplicate, and the results were indicated with error bars. The one- and two-paired ANOVA tests in optimization step and analysis step of samples were conducted for statistical comparisons.

2.4. The general UA-CPE procedure

For UA-CPE of free and total sulfite levels of samples, 3.0 mL aliquots of the sample or standard solutions containing in the range of 2.5–350 μg L^{-1} sulfite were placed in 50 mL volumetric centrifuge tubes and 0.75 mL 0.04 mol L^{-1} BR buffer at pH 7.5, 1.25 mL of 1.0×10^{-4} mol L^{-1} TB^{+}, 0.7 mL of 5.0 mg L^{-1} Cu(II), 1.25 mL of 5.0 % (w/v) KCl, 1.75 mL of 2.5 % (v/v) Triton X-45, respectively were added and submitted to sonication under ultrasonic power (300 watt, 40 Hz) for 5 min at 40 ^{o}C. To accelerate the extraction, the mixture was vigorously shaken using a vortex agitator for 2 min at 3000 rpm (maximum setting) leading to the formation of fine droplets. Then, the resulting mixture was separated to two separate phases by centrifugation of 5 min at 4000 rpm. The mixtures were cooled in a refrigerator for 2 min to increase the viscosity of the surfactant-rich phase and to facilitate the extraction of the aqueous phase. After that, the aqueous phase was easily separated from surfactant-rich phase by inverting the tube. The surfactant-rich phase was dissolved in 0.5 mL of methanol containing 1.0 mol L^{-1} HNO_3 to decrease the viscosity and transferred into a quartz cell prior to spectrophotometric detection at 630 nm.

3. Results and Discussions

The method is based on ion-associate complex formation of anionic complex, $Cu(SO_3)_2^{2-}$, produced by the complexation of sulfite with excess Cu(II) ions with Toluidine blue, TB^{+} at pH 7.5, and then CPE of ion-associate complex formed from aqueous solution using Triton X-45 micelles above critical micelle concentration (CMC). The extracted surfactant-rich phase is diluted with THF, and its absorbance of hydrophobic complex, which is linearly proportional to sulfite concentration, is spectrophotometrically measured at 630 nm with a wavelength shift

of 12 nm in the presence of KCl as sensitivity enhancement or salting out agent. Therefore, as a result of the sensitive and selective coordinatively bonding of sulfite to both Cu^{2+} ions and active hetero S-atom in aromatic ring and electron-donor $–NH_2$ and $–N(CH_3)_2$ groups of ion-pairing reagent, TB^+, due to soft acid-soft base interactions, the ion-pairing complex assisted by Triton X-45 micelles can be extracted by CPE procedure. Moreover, the proposed mechanism is clearly supported by a kinetic study based on reaction of TB^+ with sulfite at pH 7.2, in which Cu^{2+} ions play an active role as a promoter or catalytic activator.[35] In the relevant kinetic study, it was also observed that Cu-TB, Cu-sulfite and Cu-TB-sulfite species based on binary and ternary interactions have complex formation constants of 7.168, 6.367 and 14.291 with a standard deviation ranging from 0.003 to 0.008 at pH 7.2. Thus, for further studies, the different analytical variables affecting CPE efficiency was optimized in order to achieve the maximum sensitivity.

3.1. Effect of pH and buffer volume

The perconcentration of sulfite by UA-CPE involves previous formation of a stable complex, which needs to present sufficient hydrophobicity to be extracted into a small volume of the surfactant-rich phase. The choice of the most appropriate working pH was carried out by comparing the analytical signal for sulfite solution. Thus, in this part of experiment, the effect of different buffers were extensively investigated for the determination of sulfite in the surfactant-rich phase in the range pH 5.5-10.5. It can be seen in Fig. 1(a) that the highest analytical signal is recorded at pH 7.5 of BR buffer system, so it was considered as optimal pH value for further studies. At lower pHs than 7.5, extraction efficient is very low because of complex formation is inadequate as a result of protonation of sulfite with pK_a values of 1.91 and 7.18 and ion-pairing reagent, TB^+ with a pK_a value of 7.9 in water [36] due to conversion of sulfite to bisulfite ($SO_3^{2-} + H^+ \leftrightarrow HSO_3^-$) and monocationic form of dye to dicationic form ($TB^+ + H^+ \leftrightarrow TBH^{2+}$) as well as its dimerization (to give dimer and higher oligomers with

hypsochromic shift) depending on pH of environment and concentration within 10^{-5} to 10^{-6} mol L^{-1}. The dye can also subject to disproportionation as a result of dimerization in normal and reverse micellar media in acidic pHs as follows, so as to give the oxidized and reduced forms of redox sensitive ion-pairing reagent, TB^+.[37-40]

$$TB^+ + H^+ \rightarrow TBH^{2+}, \text{ protonation in acidic pHs} \quad (1)$$

$$2TBH^{2+} \rightarrow (TBH)_2^{4+}, \text{ dimerization depending on concentration and pH} \quad (2)$$

$$(TBH)_2^{4+} + H_2O \rightarrow TBOH_{(oxidized)} + TBH_2^{2+}{}_{(reduced)} + 2H^+, \text{ disproportionation} \quad (3)$$

Thus, a pH value of 7.5 was selected as an optimal value due to give the highest sensitivity. Furthermore, the volume of this buffer solution at fixed concentration on the analytical signal was also investigated in range of 0.2–2.5 mL in Figs. 1(b). According to the results obtained, 0.75 mL of 0.04 mol L^{-1} BR buffer at pH 7.5 was added to the aqueous micellar solutions to maintain this pH in further studies.

3.2. Effect of reagent concentrations on UA-CPE

In the current study, Toluidine blue (TB^+) was selected as ion-pairing reagent for sulfite in presence of excess Cu(II) ions and Triton X-45 as extracting agent at pH 7.5. TB^+ is a basic metachromatic dye with resonance stabilized planar geometry, especially due to its chromogenic phenothiazine ring containing hetero-N and S atoms including oxochromic–NH_2 and –$N(CH_3)_2$ groups. The UA-CPE efficiency depends on the hydrophobicity of the ion-pairing reagent and the complex formation. The extraction efficiency of sulfite by Triton X-45 micelles from the aqueous solution was calculated as follows:

$$Extraction\ efficiency(\%) = \frac{C_c.V_c}{C_i.V_i}100 = \frac{C_i.V_i - C_s.V_s}{C_i.V_i}100$$

Where C_i symbolize the concentration of sulfite in the initial sample of volume V_i, C_c symbolize the concentration of sulfite in the aqueous phase of volume V_c and C_s symbolize the concentration of sulfite in the surfactant rich phase of volume V_s. One factor that increases the extraction efficiency also is the effect of the complexing agent.

To obtain the best extraction efficiency, the volume of TB^+ at fixed concentration of 1.0×10^{-4} mol L^{-1} on the analytical signal was investigated in range of (0.1–2.5) mL, and the results were shown in Figs 2(a). As can be seen, the analytical signal for sulfite increased with increasing ion-pairing reagent volume and reached a maximum value at 1.25 mL. At higher volumes than 1.25 mL, the reason of the decrease in signal may be concentration dependent aggregation of dye, so as to give dimer and further oligomers. Another reason may be conversion of dye, TB^+ to TBH at high concentrations by means of redox reaction proceeded as follows:

$$TB^+_{(\text{oxidized form})} + SO_3^{2-} + H_2O \rightarrow TBH_{(\text{colorless reduced form})} + SO_4^{2-} + H^+$$

Therefore, a 1.25 mL at fixed concentration of 1.0×10^{-4} mol L^{-1} TB^+ was selected as optimal value for further studies.

The variation of the analytical signal as a function of the volume of the Cu(II) at fixed concentration of 5 mg L^{-1} in the presence of 20 μg L^{-1} sulfite was investigated in range of (0.0-2.5) mL. The results in Figs. 2(b) indicated that the signal intensity of the analyte linearly increases with Cu(II) volume up to 0.7 mL. The maximum signal intensity gradually decreased with increasing slope at the higher volumes. The cause of this decrease in signal may be either hydrolysis of Cu(II) with hydrolysis constant of pK_h: 7.9 to give dissolved $Cu(OH)^+$ and $Cu(OH)_2$ species or coordinatively complexation of Cu^{2+} ions, which is a soft Lewis acid and acts as acceptor, with basic electron donor groups of TB^+ like hetero S atom in ring and $-NH_2$ and/or $-N(CH_3)_2$ based on donor-acceptor mechanism in absence of sulfite due

to increase in blank signal.[41,42] So, 0.7 mL of 5 mg L^{-1} Cu(II) was selected as optimal value for further studies.

The existence of chemically active groups in the nonionic surfactants such as Triton X-45, 100 and 114, PONPE 7.5 and Tween 20 can be evaluated to be advantageous under certain conditions when electrostatic interactions are suitable. The nonionic surfactants are those most widely employed to perform CPE experiments. A successful preconcentration process by UA-CPE should maximize the extraction efficiency by minimizing the phase volume ratio ($V_{surfactant\text{-}rich\ phase}$ /$V_{aqueous}$), thus improving its preconcentration factor. The variation of the analytical signal as a function of volume of the nonionic surfactants at fixed concentration of 2.5 % (v/v) in the range of 0.1–3.0 mL was also investigated. Figs. 3(a) also shows that the best quantitative extraction was observed with 1.75 mL of 2.5 % (v/v) Triton X-45. At lower volumes than 1.75 mL, the extraction efficiency of ternary complex was low because there are few molecules of the surfactant to entrap the ligand-metal complexes quantitatively. At higher volumes than 1.75 mL, there will be an increase in volume of surfactant phase obtained after centrifugation. This will cause to a decrease in preconcentration factor due to a greater quantity of solvent used in the dissolving step. Therefore, the optimum nonionic surfactant volume used for the sulfite analysis was adopted as 1.75 mL of 2.5% (v/v) Triton X-45, in order to achieve the highest possible extraction efficiency and good preconcentration factor.

Generally, the amount of electrolyte solution increases distribution of analyte into the surfactant-rich phase. Also, studies on the effects of electrolyte intensity on the cloud point behavior of non-ionic surfactants have been reported. It was observed that the presence of electrolytes decreases the cloud point, resulting in a more efficient extraction. The lower cloud point is attributed to electrolytes promoting dehydration of the poly (oxyethylene) chains. Because of all these reasons, in the range 0.0 to 3.0 mL of equal molar concentrations

(5.0 (w/v) %) KNO_3, KCl, NH_4Cl and NaCl electrolyte solutions were added to investigate the effect of electrolyte solution on the extraction recovery. As can be seen from Figs. 3(b), it was found that the phase separation ability of salts followed; KCl >NaCl>NH_4Cl> KNO_3. Therefore, 1.25 of 5.0 (w/v) %) KCl was selected as optimal value for further studies.

3.3. Effects of other experimental conditions

The equilibrium temperature and time are two important parameters for an easy phase separation and preconcentration in UA-CPE. The shortest equilibration time and the lowest possible equilibration temperature are desired for efficiently separation of phases. Hence, the dependency of the absorbance on equilibrium temperature was investigated in the temperature range of 25-55 ^{o}C with sonication under ultrasonic power (300 watt, 40 Hz). As a result of experimental studies, it was found that the complex formation equilibrium remained a maximum and constant value in the temperature range of 25-40 ^{o}C. The results showed that an equilibrium temperature of 40 ^{o}C is appropriate for the UA-CPE. Keeping the equilibrium temperature of 40 °C, the influence of incubation time on the UA-CPE was investigated in range of 2–10 min. It has been seen that 5 min is sufficient to achieve a quantitative extraction of analyte. Thus, 40 °C and 5 min under ultrasonic power (300 watt, 40 Hz) were selected as optimal value the equilibrium temperature and incubation time, respectively.

The effect of vortex extraction time was investigated for the quantitative response of the detection tool as a function of extraction time. After standing in an ultrasonic bath, the centrifuge tubes are immediately placed in the vortex device. The dependency of maximum sensitivity on vortex time was investigated in the time range of 0-10 min at fixed 3000 rpm. The experimental procedures clearly shows that after agitating the mixture for 2 min, the concentration of sulfite in micellar phase reached equilibrium. It has been seen that the fine droplets formed during the preconcentration procedure are able to extract the target analyte

towards equilibrium faster because of the shorter diffusion distance and larger specific surface area. Thus, a vortex extraction time of 2 min at 3000 rpm was chosen as optimum value.

Also, centrifuge time and rates are very necessary to preconcentrate low levels of sulfite with high efficiency in a short time. Thus, under optimal conditions obtained, the effect of the centrifuge time and rate were investigated in rage of 1-10 min and 500-4000 rpm, respectively. The results have showed that centrifugation for 5 min at 4000 rpm and cooling for 2 min in an ice-bath leads to the maximum recovery and sensitivity for sulfite.

The volume of the surfactant-rich phase acquired is very viscous and small for detection by UV-Vis spectrophotometry. So, it must be diluted with an appropriate solvent before detection. The various solvents such as methanol, acetone, acetonitrile, ethanol and THF in range of 0.25-1.0 mL were added to surfactant-rich phase after phase separation. From a serial replicate studies conducted, the best absorbance as a measure of analytical sensitivity was obtained in the presence of 0.5 mL of methanol containing 1.0 mol L^{-1} HNO_3.

3.4. Matrix effect

Cations and anions that may react with sulfite, ion-pairing reagent or Cu(II) ions can be found in the real samples. An ion was considered as an interfering ion when it caused an error greater than ± 5.0 % in the determination step. Thus, the selectivity and utility of the method for determination of sulfite were further investigated in the presence of interfering matrix components under the optimum conditions. The results are shown in Table 1. As it understood from the results, only few ions interfere with the method at 10-fold tolerance ratios. Before determination by means of UV-Vis spectrophotometry, the interference of the ions can be improved up to a tolerance limit of 350 as described in the Table 1. So, in terms of selectivity, it was confirmed that extraction efficiency would not be affected by the presence of high concentrations of matrix cations.

4. The analytical figures of merit

Characteristics properties of the proposed method were obtained by preconcentration 3 mL of sulfite solution in aforesaid optimum conditions. The calibration graph were constructed by measuring the difference between absorbance of sample and blank for surfactant-rich phase as a function of sulfite concentration. The resulting calibration equation is as follows:

$\Delta A=(5.8\pm0.05)\times10^{-3}C_{sulfite,\ \mu g\ L^{-1}} + (0.35\pm0.02)\times10^{-2}$ with correlation coefficient of 0.9981; in range of 2.5-350 μg L^{-1}

Where ΔA is the analytical signal as absorbance change expressed, r is the linear correlation coefficient and C is concentration of the analyte. Also, Table 2 shown other analytical performance properties of the method with and without preconcentration UA-CPE. The limits of detection (LOD) and quantification (LOQ) defined as $3\sigma_{blank}/m$ and $10\sigma_{blank}/m$ (n: 12) (in which σ_{blank} is the standard deviation of twelve replicate measurements of the blank and m is the slope of the calibration graph) were found to be 1.15 and 3.82 μg L^{-1} respectively. The sensitivity enhancement and preconconcentration factors have been 95 and 83.3 after preconcentration with UA-CPE. As a result of five replicate measurements, the precision as a relative standard deviation (RSDs) was in range of 2.1-4.8 %.

5. The accuracy and precision of the method

The accuracy and precision of the method was verified in two ways: firstly through the standard DTNB method as well as recoveries of spiked samples and secondly with inter-day and intra-day study. The precision, which is the closeness of agreement between independent test results obtained under stipulated conditions, was estimated as the relative standard

deviation (RSD %). The accuracy, which is the closeness of agreement between the result of a measurement and the true value of the measured, was estimated as the recovery percentage (%). The proposed method was also validated by evaluating analytical curves, linearity, matrix effect, limits of detection (LOD) and quantification (LOQ) in accordance to FDA guidelines for five replicate determinations at four different concentration levels of sulfite over two days during a single week by spectrophotometric analysis after preconcentration with UA-CPE. The obtained inter-day and intra-day results are shown in Table 3 in detail, and show sufficient repeatability of the measurements. For accuracy of the method, the recovery values obtained by with inter-day and intra-day study were changed from 96.5 to 102.7 % with a RSD ranging from 2.4 to 4.4%.

In order to control of the accuracy of the method, the sulfite levels of samples similarly pretreated at pH 2.0 and 9.5 were measured and comparatively evaluated by the standard 5,5′-Dithio-bis(2-nitrobenzoic acid (DTNB). The analysis of the samples by standard DTNB method [43] was carried out as follows: A known amount of the samples was placed in a volumetric tube of 10 mL and diluted with water approximately to 8 mL. Then, 1 mL of DTNB solution (0.060 g of DTNB per 100 mL of 10% ethanol) was added and the solution was diluted to the 10-mL mark line with water. The absorbance was measured at 412 nm against ultra-pure water as analyte blank after 15 min reaction at 20 ^{o}C. In order to reduce the absorbance of analyte blank and suppress the interference effect of potential ions present in selected samples such as Cu^{2+}, Fe^{2+}, Mn^{2+}, Cr^{3+}, VO^{2+} and MoO_2^{+}, the pH of sample solution was initially adjusted to 6.5 with 0.2 mol L^{-1} phosphate buffer containing 250 µl of 0.02 mol L^{-1} oxalic acid. When a regression analysis (n: 6, independently) is conducted for a serial standard sulfite solution in range of 0.2-4.0 mg L^{-1} in presence of oxalic acid at pH 6.5, according to standard method, a good improvement in regression data was obtained as follows:

Abs.: 0.265±0.012 [sulfite, mg L^{-1}] + 0.0132±0.0011 with a correlation of coefficient of r^2: 0.9985

Linear range was 0.04-3.5 mg L^{-1} with limits of detection and quantification of 0.012 and 0.04 mg L^{-1} respectively. When necessary, to prevent possible nitrite interference in analysis of selected samples, 150 µL of 0.01 mol L^{-1} sulfamic acid was added to the matrix environment before CPE. The results obtained are shown in the Table 4.

5. The analysis of vegetables and dried fruit samples

The preconcentration procedure was applied to the determination of free, total and reversible organic matrix-bound sulfite species in vegetables and dried fruit samples, using the obtained optimum conditions. The preconcentration procedure was performed with 3 mL of prepared samples as stated in Section "Sample collection and preparation". Determination of free and total amount of sulfite was expressed in sample preparation step for two digestion processes. The reversible organic matrix bound sulfite level was calculated from the difference between free sulfite and total sulfite after pre-treatment based on two different approaches. The recovery studies were also applied to the corresponding samples by the determination of two different concentrations of sulfite added to the samples. It can be seen that the results of the recovery study are in the range of 95.4– 104.7 % with RSDs of 1.2–4.8% for the firstly preparation process, whereas they are in the range of 95.7–104.1 % with RSDs of 1.0–4.15 for secondly preparation process. The more detailed results for the vegetables and dried fruit samples analyzed are presented in Table 4.

Table 5 shows the comparison of analytical performance properties of the method with other methods reported in the literature. When examining the Table 5, the limit of detection and preconcentration factor of the method with a good sensitivity improvement are better or comparable to those reported in some references. Also, the method gives wide linear range,

minimum solvent consumption and good selectivity/precision, which is much better than the previous some references reported based on direct determination. The results of this CPE process clearly show the potential and versatility of the method, which could be applied to determination of sulfite species in vegetables and dried fruit samples.

6. Conclusions

The results obtained with the method applied to monitoring of free, total and reversibly bound sulfite in vegetables and dried fruit samples show a good agreement with those obtained by using a standard DTNB method. The ultrasonic-assisted-micellar extraction procedure has greener, economical, simple, rapid, high preconcentration, sensitivity enhancement factor and less reagent consumption. Also, The UV-Vis device is a comparatively toward easy, simple, inexpensive routine analyses and easy-to-operate analytical technique that is readily available in most laboratories. In this method, the amounts of sulfite in the selected samples were selectively and sensitively monitored using UV-Visible spectrophotometry at 630 nm. The method allows sulfite detection at levels of 1.15 μg L^{-1}. As a result, the method represents an alternative procedure for the reliable quantification of the sulfite species in the samples when compared with the high cost and poor precise techniques such as LC-ICP-MS, FMAS, VG-ICP-OES, ICP-AES and IC-HG-AFS requiring hard conditions and expert-user in his/her field.

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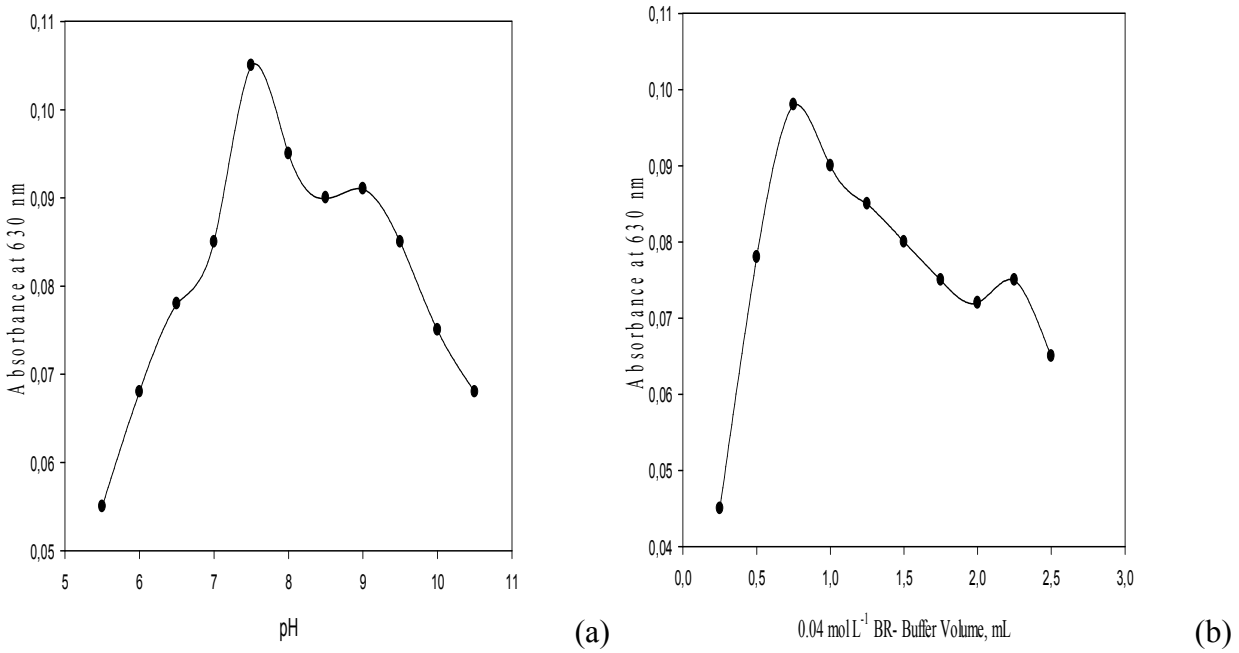

Figure 1 Effect of (a) pH and (b) buffer volume of 0.04 mol L^{-1} BR on UA-CPE efficiency. Optimal conditions: 20 µg L^{-1} SO_3^{2-}, 1.25 mL of 1.0×10^{-4} mol L^{-1} TB^+, 0.7 mL of 5.0 mg L^{-1} Cu (II), 1.25 mL of 5.0% (w/v) KCl, 1.75 mL of 2.5% (w/w) Triton X-45 under ultrasonic power (300 watt, 40 Hz) at 40 ^oC for 5 min, vortex agitator of 2 min at 3000 rpm and centrifugation time of 5 min at 4000 rpm

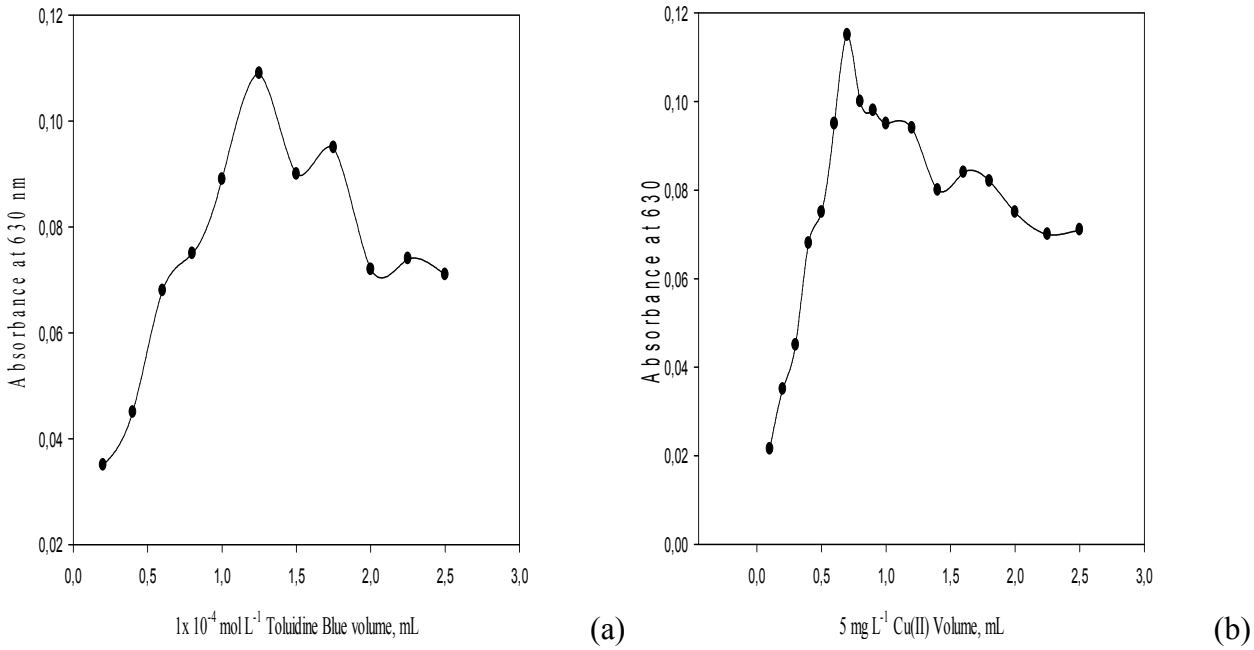

Figure 2 Effect of (a) TB^+ and (b) Cu (II) volume on UA-CPE efficiency. Optimal conditions: 20 µg L^{-1} SO_3^{2-}, 0.75 mL 0.04 mol L^{-1} BR buffer at pH 7.5, 1.25 mL of 5.0% (w/v) KCl, 1.75 mL of 2.5% (w/w) Triton X-45 under ultrasonic power (300 watt, 40 Hz) at 40 °C for 5 min, vortex agitator of 2 min at 3000 rpm and centrifugation time of 5 min at 4000 rpm

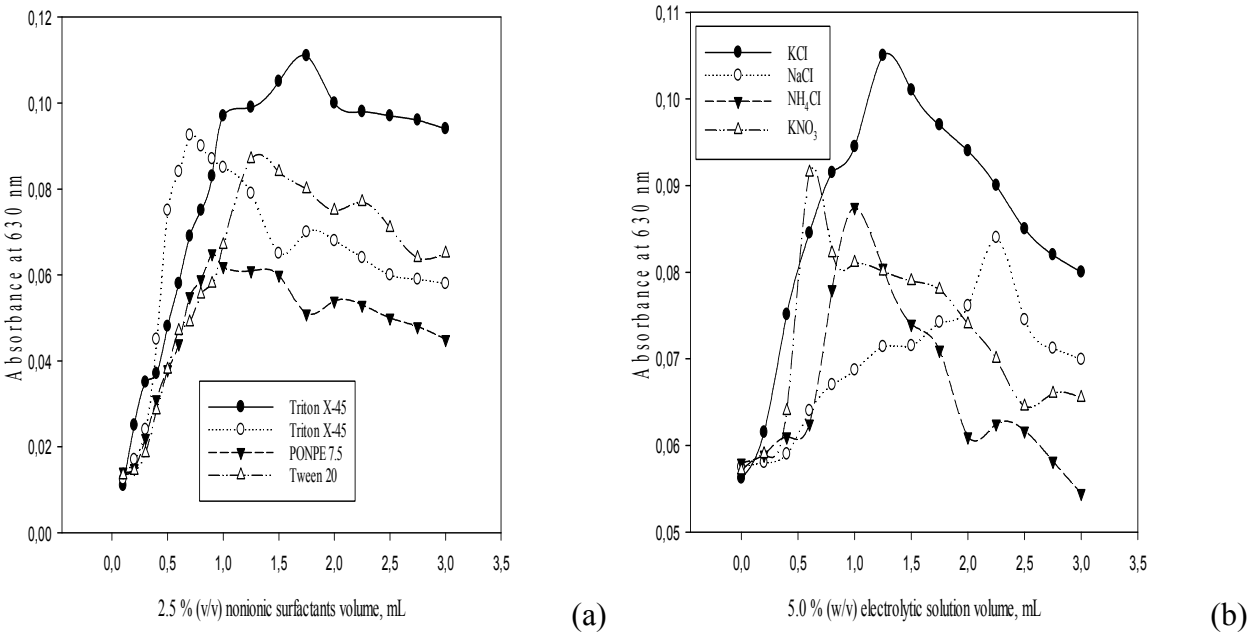

Figure 3 Effect of (a) electrolytic solution and (b) nonionic surfactant volumes on UA-CPE efficiency. Optimal conditions: 20 µg L^{-1} SO_3^{2-}, 0.75 mL 0.04 mol L^{-1} BR buffer at pH 7.5, 1.25 mL of 1.0×10^{-4} mol L^{-1} TB^+ and 0.7 mL of 5.0 mg L^{-1} Cu (II) under ultrasonic power (300 watt, 40 Hz) at 40 °C for 5 min, vortex agitator of 2 min at 3000 rpm and centrifugation time of 5 min at 4000 rpm

Table 1 Tolerance limits of interfering ions in the determination of 20 µg L^{-1} SO_3^{2-}

Interfering ions	*Tolerance limit, $W_{interfering\ species}/W_{Sulfite}$	Recovery %
Na^+, NH_4^+, HCO_3^- and Ca^{2+}	>1000	98.1-101.2
Cl^-, Br^-, Cr^{3+} , Hydrazine, Citrate, Cd^{2+} and Co^{2+}	500-750	97.8-102.8
Fe^{2+}, Mg^{2+}, Ag^+, Pb^{2+}, and Se^{4+}	300-500	96.5-103.4
Mo^{6+}, V^{5+}, CN^- and Sb^{5+}	200-300	96.1-102.0
S^{2-}, Ni^{2+}, and Tartrate	100-200	95.7-103.8
Oxalate, Ascorbic acid, SCN^-, CO_3^{2-},	50-100	96.7-98.5
[a] Cu^{2+}, [b]Zn^{2+} and [d](Fe^{3+}, Al^{3+})	30-50 ([a]250, [d]350)	95.9-102.4
[c]NO_3^- and [d](SiO_3^{2-})	10-25 ([c]150, [d]200)	97.4 and 102.7

* Concentration ratios of interfering ions and sulfite at fixed concentration of 20 µg L^{-1}

[a] After pre-treatment with 50 µL of 1.0×10^{-3} mol L^{-1} thiourea solution

[b] After pre-treatment with 25 µL of 0.025 % (w/v) formaldehyde solution

[c] After pre-treatment with 25 µL of 0.025 % (w/v) hydrazine hydrochloride solution

[d] After pre-treatment ant heating with 0.1 mL of 1.0×10^{-3} mol L^{-1} NaF at 80 °C

Table 2 Analytical characteristics of the proposed method

Parameters	Analytical features	
	After preconcentration, 630 nm	Before preconcentration, 644 nm
Analytical species	SO_3^{2-}, µg L^{-1}	SO_3^{2-}, µg L^{-1}
Linear range	2.5-350	120-1200
Regression equation	$\Delta A=(5.8\pm0.05)\times10^{-3}C_{sulfite,\ \mu g\ L^{-1}} + (0.35\pm0.02)\times10^{-2}$	$\Delta A=(7.5\pm0.5)\times10^{-3}C_{sulfite,\ \mu g\ L^{-1}} + (6.3\pm0.3)\times10^{-4}$
Regression coefficient, r^2	0.9981	0.9859
RSD (%) (5, 10 and 25 µg L^{-1}, n: 5)	2.1-4.8	3.5-6.2
Detection limit, LOD (n:12, $3\sigma_b/m$)	1.15	37.6
Quantification limit, LOQ (n: 12, $10\sigma_b/m$)	3.82	125.5
*Sensitivity enhancement factor	95	-
**Preconcentration factor	83.3	-

* The sensitivity enhancement factor is calculated as the ratio of slopes of the calibration curves obtained with and without preconcentration by means of UA-CPE

** The preconcentration factor, which is calculated as the concentration ratio of analyte in the final diluted surfactant rich extract ready for UV-VIS determination and the initial solution was averagely 50 mL for sulfite

Table 3 Intra-day and inter-day precision and accuracy data for the five replicate measurements of different concentrations of sulfite (n: 5)

Sulfite taken, µg L^{-1}	Repeatability (intra-day)			Intermediate (inter-day)		
	*Found, Sulfite µg L^{-1}	RSD %	Recovery %	*Found, Sulfite µg L^{-1}	RSD %	Recovery %
5	4.9±0.08	4.9	101.9	4.8±0.07	4.8	102.5
10	10.3±0.09	3.7	100.8	9.8±0.08	3.5	98.9
20	20.8±0.1	2.5	99.0	21.0±0.1	2.8	101.4
40	39.1±0.2	1.3	98.7	41.1±0.1	1.6	98.3

* $\bar{x} \pm \mu = \bar{x} \pm s\frac{t}{\sqrt{n}}$ (t: 2.78, P: 0.05); t-Student coefficient for n-1 degrees of freedom

Table 4 The analytical results obtained for the determination of sulfite in some dried fruit and vegetable samples (n: 5)

Samples	By first digestion approach						By second digestion approach						
	Added, Free Sulfite (μg L^{-1})	Found (μg kg^{-1})			RSD %	Recovery %	Found (μg kg^{-1})			RSD %	Recovery %	[a]Found by the modified standard DTNB method	[b]The experimental t- and F-values
		*Found, Free, Sulfite	***Found, Reversibly bound Sulfite	**Found, Total, Sulfite			*Found, Free Sulfite	***Found, Reversibly bound Sulfite	**Found, Total Sulfite				
Dried fruit samples													
Dried apricot[1]	-	5.7±0.1	3.8	9.5±0.1	4.7	-	5.8±0.08	3.9	9.7±0.1	3.4	-	9.7±0.3	0.45, 1.35
	5	10.4±0.1	4.1	14.5±0.2	3.5	97.3	10.5±0.1	4.1	14.6±0.2	2.1	97.2	-	-
	20	25.4±0.2	3.5	28.9±0.3	1.8	99.0	25.4±0.2	3.3	28.7±0.3	1.3	98.7	-	-
Dried raisin[1]	-	3.3±0.1	4.4	7.7±0.1	4.2	-	3.4±0.08	4.2	7.6±0.2	3.7	-	7.6±0.2	0.27, 1.85
	5	8.6±0.2	4.0	12.6±0.2	2.4	103.7	8.1±0.1	4.3	12.4±0.2	2.4	96.2	-	-
	20	23.6±0.2	4.3	27.9±0.2	1.3	101.4	23.0±0.2	4.8	27.8±0.3	1.2	98.1	-	-

	-	4.3±0.1	4.0	8.3±0.1	4.4	-	4.4±0.09	4.1	8.5±0.1	4.0	-	8.5±0.3	0.70,
Dried apple[1]	5	9.1±0.1	4.5	13.6±0.2	3.2	97.2	9.0±0.1	4.7	13.7±0.2	3.1	95.8	-	1.30
	20	23.9±0.2	4.6	28.5±0.3	1.6	98.3	23.9±0.2	5.0	28.9±0.3	1.9	97.9	-	-
													-
	-	3.9±0.09	4.2	8.1±0.1	3.9	-	4.0±0.1	4.2	8.2±0.1	3.7	-	8.0±0.2	0.15,
Dried fig[1]	5	8.9±0.1	3.8	12.7±0.2	2.4	96.1	9.3±0.1	3.1	12.4±0.2	2.5	103.5	-	1.15
	20	23.5±0.2	3.9	27.4±0.3	1.3	98.5	24.5±0.2	2.8	27.3±0.2	1.4	102.1	-	-
													-
	-	2.1±0.08	4.4	6.5±0.1	3.3	-	2.2±0.09	4.2	6.4±0.1	3.4	-	6.7±0.2	0.60,
Dried	5	6.9±0.1	4.8	11.7±0.2	2.0	97.0	7.4±0.1	3.7	11.1±0.2	2.1	102.4	-	1.53
apricot[2]	20	21.8±0.1	4.3	26.1±0.2	1.2	98.3	22.2±0.2	3.7	25.9±0.3	1.3	98.6	-	-
													-
	-	1.8±0.09	3.8	5.6±0.1	3.7	-	1.9±0.09	3.8	5.7±0.1	3.3	-	5.7±0.2	0.27,
Dried raisin[2]	5	6.6±0.1	4.4	11.0±0.2	2.5	97.1	7.2±0.1	4.0	11.2±0.2	2.4	103.5	-	1.48
	20	21.6±0.2	3.6	25.2±0.3	1.4	98.7	21.4±0.2	4.3	25.7±0.2	1.5	97.9	-	-
													-
	-	1.3±0.09	3.3	4.6±0.1	3.4	-	1.4±0.09	3.3	4.7±0.1	3.3	-	4.5±0.1	0.38,
Dried apple[2]	5	6.2±0.1	3.5	9.7±0.2	2.2	98.0	6.1±0.1	3.8	9.9±0.1	2.1	95.8	-	1.20
	20	21.7±0.2	2.6	24.3±0.3	1.3	101.7	21.2±0.2	3.2	24.4±0.3	1.4	98.7	-	-
													-

	-	2.0±0.09	3.7	5.7±0.1	3.7	-	2.1±0.1	3.7	5.8±0.1	3.8	-	5.7±0.1	0.40,
Dried fig[2]	5	6.8±0.1	4.1	10.9±0.2	2.5	96.9	7.3±0.1	3.9	11.2±0.3	2.6	102.9	-	1.05
	20	21.7±0.2	3.7	25.4±0.3	1.6	98.5	22.4±0.2	3.5	25.9±0.3	1.5	101.5	-	-
													-
Vegetables samples													
	-	3.5±0.09	5.6	9.1±0.2	3.1	-	3.7±0.09	5.6	9.3±0.2	3.3	-	4.2±0.1	0.75,
Spinach	5	8.2±0.1	6.6	14.8±0.2	2.4	95.8	8.4±0.1	6.3	14.7±0.2	2.1	97.1	-	1.70
	20	23.1±0.2	6.8	29.9±0.3	1.3	98.1	23.3±0.2	7.0	30.3±0.3	1.5	98.5	-	-
													-
	-	2.9±0.08	2.7	5.6±0.1	3.0	-	3.0±0.09	2.7	5.7±0.1	3.1	-	5.7±0.1	0.90,
Lettuce	5	7.6±0.1	3.1	10.7±0.3	2.3	96.7	7.7±0.1	3.2	10.9±0.2	2.6	96.2	-	1.85
	20	23.2±0.2	2.1	25.3±0.3	1.5	101.2	22.6±0.2	1.8	25.4±0.2	1.4	98.5	-	-
													-
	-	3.7±0.1	3.2	6.9±0.1	2.7	-	3.7±0.09	3.3	7.0±0.1	2.8	-	6.7±0.1	0.55,
Cabbage	5	8.9±0.1	2.8	11.7±0.3	1.5	102.4	8.5±0.1	2.7	11.2±0.2	1.6	98.0	-	1.63
	20	23.3±0.2	3.1	26.4±0.3	1.1	98.5	23.5±0.2	3.4	26.9±0.3	1.2	99.1	-	-
													-
Yellow	-	5.8±0.1	3.4	9.2±0.1	2.9	-	5.7±0.1	3.6	9.3±0.2	2.8	-	9.3±0.2	0.80,
pepper	5	11.1±0.2	3.6	14.7±0.2	1.8	102.1	11.0±0.2	3.3	14.3±0.2	1.9	103.1	-	1.90
	20	26.0±0.3	3.8	29.8±0.2	1.1	101.0	26.1±0.2	3.4	29.5±0.3	1.1	101.7	-	-
													-

	-	3.1±0.09	4.4	7.5±0.1	3.4	-	3.2±0.09	4.4	7.6±0.1	3.5	-	7.7±0.1	1.05,
Corn	5	7.8±0.1	4.9	12.7±0.3	2.7	95.9	7.9±0.1	5.1	13.0±0.3	2.6	95.7	-	1.90
	20	22.6±0.2	4.4	27.0±0.3	1.9	97.8	22.8±0.2	4.0	26.8±0.3	1.7	98.3	-	-
													-

[1] Treated with SO_2

[2] The non-treated with SO_2

*The average plus standard deviation of five replicate measurements for free sulfite after pre-treatment with mannitol and methanesulphonic acid at pH 2.0

**The average plus standard deviation of five replicate measurements for total sulfite after pre-treatment with mannitol/Na_2HPO_4 at pH 9.5

***The reversible organic matrix bound sulfite level calculated from the difference between free sulfite and total sulfite after pre-treatment based on two different approaches

[a] The modified standard DTNB method, which is based on detection of anionic degradation product at 412 nm using pH 6.5 phosphate buffer containing oxalic acid after stabilization of sulfite with mannitol for monitoring of total sulfite at pH 9.5 in order to slow down and control sulfite oxidation

[b] In order to compare the mean values and their standard deviations for independent two samples t- and F-tests with equal sample size the statistical t- and F-critical values at 95% confidence level and 8 degrees of freedom are 2.31 and 6.39, respectively

Table 5 Comparison of the proposed method with the other analytical methods in literature

Sample matrix	Detection method	Linear range	Detection limit	RSD %	Enrichment factor	References
Dried Fruits	Anion Exchange Column and Conductivity Detection	1.588-50.8 μgmL^{-1}	0.143 μgmL^{-1}	lower than 2.88%	50	20
Wine	Square-wave voltammetry	10-250 mgL^{-1}	2.7 mgL^{-1}	lower than 6%	45	22
Drinking water and food samples.	UV–Vis Fiber Optic Linear Array Spectrophotometry	2–100 $\mu g\ L^{-1}$	0.2 $\mu g\ L^{-1}$	2.0-2.8%	133	26
Vegetable and fruit samples	UV-VIS	0.004-0.100 mgL^{-1}	0.004mgL^{-1}	lower than 5.13%	350	27
Wine samples	Amperometric detection	0.2-20 mgL^{-1}	0.05 mgL^{-1}	1.0-4.1 %	-	44
Mineral water, sugar and white wine	Fluorescent detection	0.5–150 µM	0.2 µgM	2.4-5.6%	-	45
Vegetables and dried fruit matrices	UV-VIS	2.5-350 μgL^{-1}	1.15 μgL^{-1}	2.1-4.8%	90	The current work