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Original Research Article

Selective extraction and enrichment of 5-hydroymethylfurfural from honey, molasses, jam and vinegar samples prior to sensitive determination by micro-volume UV-vis spectrophotometry

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ABSTRACT

In this study, a new method was developed for the extraction/enrichment of 5-hydroxymethylfurfural (5-HMF) from sample matrix prior to analysis. The method is based on the selective formation of the imine adduct and ion-pair between 5-HMF and phenosafranine (weak base, PSF^+) in the presence of sodium dodecyl sulfate (SDS) at pH 5.5 by the base induced disproportionation. Then, the complexes were enriched into the micellar phase of Triton X-114, diluted with ethanol, and detected at 532 nm by micro-volume UV–vis spectrophotometry. The variables affecting extraction efficiency were optimized. In optimal conditions, the calibration curves were over the range of 2–100 and 2–200 μg L^{-1} with the detection limits of 0.53 and 0.75 μg L^{-1} using 0.2 and 3.0 mL of 1.0×10^{-3} mol L^{-1} SDS. From enrichment of 15-mL sample, an enrichment factor of 37.5-fold was obtained. The accuracy/ precision studies after spiking were performed, and observed to be in range of 97.3–102.3 % and 2.5–3.8% (10, 25 and 75 μg L^{-1}, n: 5). After validation, the method was applied to the analysis of the selected foods. From the results, it was observed that 5-HMF levels were in the range of 1.05–18.10 mg kg^{-1} with a RSD% of 3.0–4.2 % and recovery of 95.5–98.0 % by sample extraction with sonication while they ranged from 1.15–18.05 mg kg^{-1} with a RSD% of 3.0–4.2 % and recovery of 95–99 % without sonication. Finally, it was observed that the results obtained were in agreements with those of the modified White method, statistically validating the method.

1. Introduction

The Maillard reaction is a chemical reaction between amino acids and reducing sugars and occurs in food storage at low temperature as well as during cooking conditions (Wagner et al., 2007). The reaction takes place in all the foods that are baked, fried and heat-treated during and/or after production throughout the shelf life (Mlotkiewicz, 1998). 5-Hydroxymethylfurfural (5-HMF) is important Maillard reaction product which is present in many foodstuffs at high levels. 5-HMF is an indicator of quality in several food products, and there is a 5-HMF content limitation for some foods such as molasses and honey because of its adverse effects on human health like cytotoxic, mutagenic, genotoxic and carcinogenic consequences (Ruiz-Matute et al., 2010). Therefore, Turkish Food Codex and Codex Alimentarius Standard Commission have set the maximum limit for 5-HMF in honey at 40 mg kg^{-1} (with a higher limit of 80 mg kg^{-1} for honeys originating from tropical regions) to ensure that the product has not undergone extensive heating during processing and is safe for consumption (Codex Alimentarius Commission Standards, 2001; Alimentarius, 1982).

All this information reveals that analytical determination of 5-HMF amount, which is an important parameter for honey quality, is an important issue. For these reasons, a pre-separation and enrichment tool is required before determining with these techniques in order to get rid of the matrix effect and lower the detection limit. Some separation-enrichment methods generally reported for solid phase extraction (SPE) for 5-HMF with a suitable chromatographic and electrophoretic techniques such as gas chromatography (GC), liquid chromatography (LC), capillary electrophoresis (CE) and micellar electrokinetic chromatography (MEKC) with ultraviolet (UV), diode array detector (DAD), pulsed amperometric detector (PAD), and mass spectrometric (MS)

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detection are based on tedious, time-consuming and complex analytical processes such as solid phase microextraction without and with head space (SPME and HS-SPME), vortex-assisted liquid-liquid microextraction (VA-LLME) (Teixidó et al., 2006; Costa et al., 1999; Nozal et al., 2001; Spano et al., 2006; Garcia-Villanova et al., 1993; Yuan and Chen, 1998; Wong et al., 2012; Teixidó et al., 2011; Edris et al., 2007; Spano et al., 2009; Xu et al., 2015; Tsai and Kao, 2012; Abu-Bakar et al., 2014; Pérez-Palacios et al., 2013). However, these techniques have reservations such as more expensive, more or less time-consuming processes, irreversible adsorption problem of substances in capillary columns, high operating pressure for effective separation, unsatisfactory enrichment factor, expert user requirement, large amounts of expensive organic materials and waste.

Unlike all these time consuming and laborious separation techniques, ultrasonic assisted cloud point extraction (UA-CPE) can be efficiently used to separate and enrich the analyte from the sample matrix. The main parameters for surfactants to be used in this enrichment method are cloud point temperature (CPT) aggregation number (N_{agg}) of and critical micelle concentration (CMC) of the micelle or mixed micellar systems. When a nonionic surfactant solution in absence and presence of ionic surfactant is heated to a certain temperature, it will become cloudy due to partial solubility. This temperature at which cloudiness is observed is called cloud point temperature. Where the surfactant concentration is close to the CMC above the cloud point temperature, the low volume surfactant-rich phase and dilute bulk aqueous phase are easily separated by centrifugation. The UA-CPE is an eco-friendly and organic toxic solvent-free enrichment method, which is often used in trace organic and inorganic analysis (Jalbani and Soylak, 2015; Duran et al., 2011). The method has been successfully applied to the separation and enrichment of non-toxic and toxic species such as sulfite, proline, formaldehyde and bisphenol A as well as 5-HMF in complex food and beverage matrices by our research group (Altunay and Gürkan, 2015; Dagdeviren et al., 2018; Temel and Gürkan, 2018a;, 2018b; Gürkan and Altunay, 2015).

Different methods have been used to determine 5-HMF in different food and beverages matrices. Determination of trace levels of 5-HMF have been generally conducted by spectrometric methods (Iglesia et al., 1997; Chernetsova and Morlock, 2012), LC (Tomasini et al., 2012), GC (Teixidó et al., 2006), by chromatographic techniques such as ion exclusion chromatography (IEC) (Kim and Richardson, 1992); and CE (Chen and Yan, 2009) and MEKC (Costa et al., 1999) using CE techniques with detection of UV, DAD, PAD, and MS. Analysis of 5-HMF in food production is usually carried out in the routine analysis laboratory to determine critical limits. Contrary to large research laboratories, it is very difficult to provide the necessary financial support for such laboratories. Unlike all of these, micro-volume UV–vis spectrophotometer can be used directly in organic trace analysis, especially for use in narrow-budget research laboratories. The UA-CPE can be an even simpler, easier-to-use, lower-cost, faster and more effective analytical tool when combined with a micro-volume UV–vis spectrophotometer with a selection of suitable chromophore for analyte.

The main aim of the present study is to establish a new ion-pair UA-CPE procedure for selective extraction and enrichment of trace amounts of 5-HMF from the sample matrix prior to determination by micro-volume UV–vis spectrophotometry. The method is based on the formation of imine adduct and ion-pair by base-induced disproportionation as a result of interaction between 3,7-diamino-5-phenylphenazin-5-ium chloride (weak base, phenosafranine, PSF^+) and 5-HMF in presence of sodium dodecyl sulfate (SDS) at pH 5.5; SDS below and above CMC was used as synergistic agent. The formed ion-pair was then extracted into the micellar phase of Triton X-114. After dilution of the surfactant-rich phase with ethanol, the 5-HMF levels of samples were monitored at 532 nm by micro-volume UV–vis spectrophotometer. There are three special features in the center of the study in term of the novelty of the method: (i) the application of ultrasound energy shortens the extraction time and increases the extraction efficiency; (ii) the facility of direct monitoring of 5-HMF by micro-volume UV–vis spectrophotometer without need to a further detection tool; and (iii) the synergistic use of PSF^+ and SDS in the extraction process provides a significant improvement in both the sensitivity and selectivity of the method. In addition, the use of cells with micro-capacity in determination step results in low consumption of samples and reagents, and so negligible generation of wastes. In order to fully characterize the proposed UA-CPE process, the main parameters affecting extraction efficiency were evaluated and optimized.

2. Experimental

2.1. Reagents and solutions

Ultra-pure water (resistivity of 18.2 MΩ cm) obtained from a water purification system (Labconco, Kansas City, USA) was used throughout this study. All reagents including analyte were of analytical purity, and were purchased from Sigma-Aldrich (St. Louis, MO, USA). The PSF^+, SDS, and polyethylene glycol *tert*-octylphenyl ether (Triton X-114), including diluting solvents such as ethanol, methanol, acetone, acetonitrile, tetrahydrofuran (THF) were provided from Sigma-Aldrich. The solutions of 3.0×10^{-3} mol L^{-1}, 3.0×10^{-3} mol L^{-1} and 5.0 % (v/v), respectively, were prepared by dissolving the appropriate amount of each reagent in water, mixing with vortex to obtain a homogeneous solution if necessary (1200 rpm, 30 s), and completing it to 100 mL with water. Stock solution of 5-HMF (250 mg L^{-1}) was prepared by dissolving its appropriate amount in methanol and diluting with water. Subsequently, the working standard solutions were obtained by sequential dilution of the stock solution with aqueous methanol (9:1, v/v). Carrez-I solution was prepared by dissolving 21.9 g $Zn(Ac)_2.2H_2O$ (Merck, Darmstadt, Germany) in water, adding 3 g glacial acetic acid and diluting to 100 mL with water. Carrez-II solution was prepared by dissolving 10.6 g $K_4Fe(CN)_6.3H_2O$ (Merck, Darmstadt, Germany) in water and diluting to 100 mL. Citrate buffer solution of 0.1 mol L^{-1} was prepared by dissolving 2.401 g Na-citrate dihydrate and 0.347 g citric acid (Merck, Darmstadt, Germany) in enough water, adjusting the pH to 5.5 by dilute HCl or NaOH (each one, 2.0 and / or 0.2 mol L^{-1}) using a pH meter, and adding water until the volume is 100 mL. All solutions were kept in the refrigerator at 4 °C before analysis. All test equipment's used in the extraction step were washed with dilute nitric acid (2.0 % (w/v) and rinsed three times before use with water.

2.2. Instrumentation

A micro-volume UV–vis spectrophotometer with 1.0-cm quartz cells (Shimadzu UV-1800 model, Kyoto, Japan) was used for all absorbance measurements. The maximum absorbance of the sample extracts in the cells with micro-capacity, 0.35−0.70 mL was measured at 532 nm with a red shift of 12 nm against a blank (ethanol). The calibration curves were plotted for the amounts of 5-HMF against its relative absorbance at pH 5.5. These curves were used to determine the 5-HMF contents of samples. A programmable ultrasonic bath operating at 40 kHz, 300 W (UCP-10 model, Seoul, Korea) was used for extraction of 5-HMF from samples in temperature range of 0−80 °C. A vortex mixer operating at 50 Hz, 12 W (VM96-B model, Seoul, Korea) was used for homogenization of the selected samples. A centrifuge (Universal 320 Hettich model, London, UK) was used to accelerate phase separation. The pH of the solutions was adjusted with a pH meter equipped with a glass electrode (a JP Selecta brand, Barcelona, Spain).

2.3. Samples, and sample preparation for analysis

All samples were obtained from local markets in Sivas. Before the sample preparation step, liquid, semi-liquid and solid samples were thoroughly homogenized with a vortex mixer after mixing and dilution with some water.

The extraction processes with and without sonication was as follows

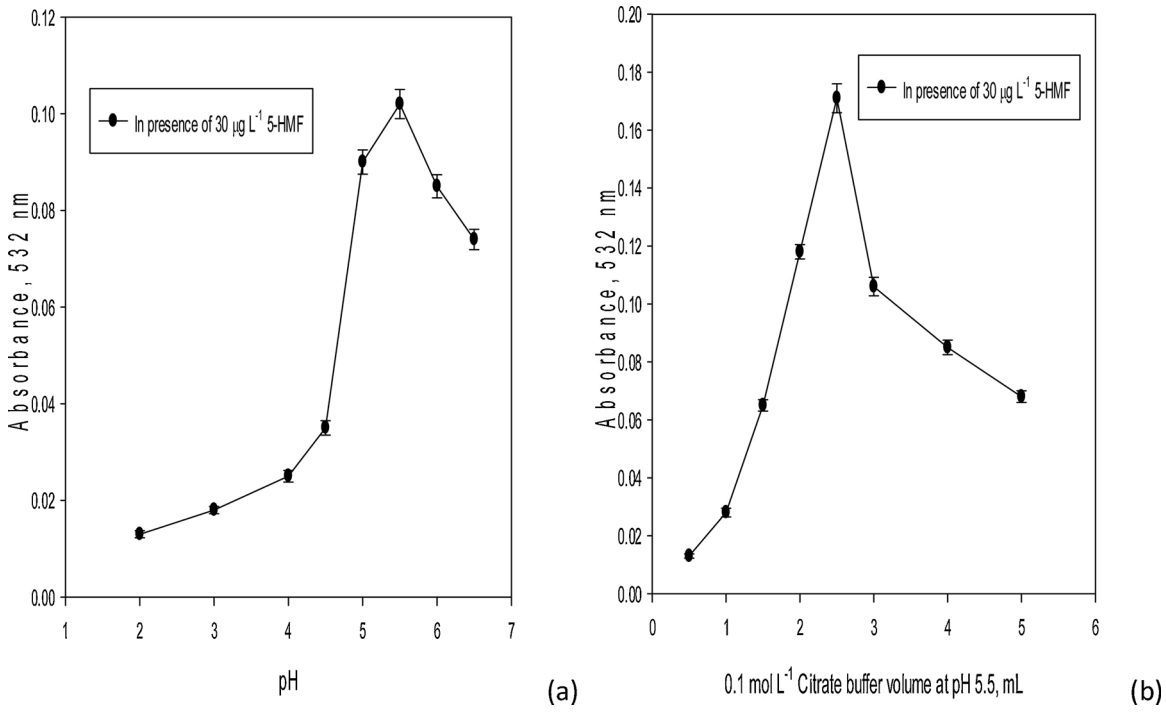

Fig. 1. Effect of (a) pH and (b) 0.1 mol L^{-1} Citrate buffer volume on absorbance, corrected against analyte blank at 532 nm (n: 3). Conditions: 0.4 mL of 3.0×10^{-3} mol L^{-1} PSF^{+}; 0.2 (or 3.0) mL of 3.0×10^{-3} mol L^{-1} SDS; 1.2 mL (or 0.8) mL of 5.0 % (v/v) Triton X-114; sonication for 12 min at 45 °C below CMC of SDS (or 7 min at 55 °C above CMC of SDS); and centrifugation for 10 min at 3000 rpm for triplicate measurements of 30 μg L^{-1} 5-HMF.

(Gürkan and Altunay, 2015):

(i) Aliquots of 2.0 *g* or 5.0 mL of semi-solid and liquid samples were transferred to 50 mL flasks in a similar manner. Next, 2.0 mL of 0.2 mol L^{-1} $HClO_4$ was independently added to all samples, followed by Carrez-I (2.0 mL of 1.123 mol L^{-1}) and Carrez-II (2.0 mL of 0.288 mol L^{-1}), respectively. The final volume of the mixtures was diluted to 50 mL with water. The prepared mixtures were sonicated and dissolved in an ultrasonic bath (300 W, 40 kHz) for 5 min at 40 °C. The pHs of the mixtures were adjusted to 7.0 with dilute NaOH solution (0.15 mol L^{-1}). After centrifugation at 3500 rpm for 10 min, the dissolved samples were filtered through a membrane filter (0.45 μm pore-size).

(ii) The same procedures were in parallel carried out without sonication. The only difference was to use 2.0 mL of 2.0 mol L^{-1} $HClO_4$ for 15 min at 45 °C in a temperature-controlled water bath for extraction.

In addition, with and without dissolving under ultrasonic power to check whether or not the acid concentration and heating between 20−60 °C have an effect on both the formation and conversion of levulinic acid/formic acid and the polymerization of 5-HMF, the analysis of the selected samples with spiking at 5, 10, 25 and 50 μg L^{-1} levels were carried out, and the slopes of calibration curves (in calibration ranges of 5–100 and 5−200 μg L^{-1} prepared from pure solvent and sample extracts) for control of matrix effect were obtained. No significant difference with signal suppression lower than ±8.5 % was observed between slopes. As a result, the formation of 5-HMF and conversion to levulinic acid did not occur under dilute acid conditions at temperatures lower than or equal to 40 °C.

2.4. The UA-CPE procedure

All extraction steps were carried out in 50-mL centrifuge tubes and parallel to the sample blank solution. First, aliquots of the standard aqueous solution containing 30 μg L^{-1} 5-HMF in optimization step or 15.0 mL of sample solution in enrichment step were added to these tubes and the pHs of the solutions were adjusted to pH 5.5 with 2.5 mL 0.1 mol L^{-1} citrate buffer, and then 0.4 mL 3.0×10^{-3} mol L^{-1} PSF^{+}, 0.2 (or 3.0) mL of 3.0×10^{-3} mol L^{-1} SDS, and 1.2 mL (or 0.8) mL of 5.0 % (v/v) Triton X-114 were sequentially added to the solution medium for formation of the ion-pair complex and sonicated in an ultrasonic bath for 12 min at 45 °C below CMC of SDS (or 7 min at 55 °C above CMC of SDS) to obtain two calibration curves with significant sensitivity difference. After the ion-pair formation, centrifugation was carried out for 10 min at 3000 rpm. After centrifugation, the imine adducts and ion-pair complex were trapped in the surfactant-rich phase under the tube. The aqueous phases were removed by decantation and the volumes of the surfactant-rich phase containing 5-HMF were diluted approx. to 0.4 mL with ethanol, and monitored at 532 nm by micro-volume UV–vis spectrophotometer for analysis against the sample blank.

In order to ensure the reliability of the results obtained, the 5-HMF contents of the samples were measured for 40 min at 40 °C under pretreated samples with a modified spectrophotometric White method (White, 1979), which is also known as an differential UV-photometry with and without bisulfite-reduction of 5-HMF, and evaluated comparatively. Analysis of the selected samples by the reference comparison method was carried out as follows:

2.0 g or 5.0 mL of the semi-solid and liquid samples were quantitatively transferred to a 50 mL flask, dissolved in 25 mL of water, and then aliquots (0.5 mL) of Carrez-I and Carrez-II solutions (each one) respectively were added and completed to 50 mL with water. Sample solutions were filtered through a 0.45-μm membrane filter. After discarding the first 10 mL of the filtrate, 5 mL portions of the remained solutions were placed in two separate test tubes; 5 mL of water or sample solution was added to one; to the second, 5 mL of 0.2 % (w/v) $NaHSO_3$ solution was added. The absorbance of the solutions at 284 and 336 nm were measured using a spectrophotometer. Their 5-HMF contents were measured using the formula reported by the International Honey Commission (IHC) and recommended for the original White method (Bogdanov, 2002):

$$6\text{-HMF (mg kg}^{-1}) = (A_{284} - A_{336}) \times 149.7 \times 5 / W \quad (1)$$

Here, W is the mass of the sample and the factor of 149.7 is a theoretical value associated with the molar absorption coefficient of 5-HMF at 284 nm. When performing regression analysis (for n: 6) for a range of 5-HMF solutions in the range of 0.1−5 mg L^{-1} under ultrasonic power, a good improvement in the regression data for the honey matrix was achieved according to the standard method as follows:

$$\text{Abs.} = 7.15 \times 10^{-3}\ \text{C (mg kg}^{-1}) + 0.0213,\ r^2\text{: } 0.9965 \quad (2)$$

The linear working range was 0.06−5 mg L^{-1}, with detection and quantitation limits of 0.018 and 0.06 mg kg^{-1}, respectively. The difference was only a signal fluctuation ranging from 2.7 % to 5.1 %, considering the slopes of the calibration curves according to the matrix type. Therefore, it can be concluded that this difference is not significant. Nevertheless, in the analysis of the samples, 250 μL of 1.0×10^{-3} mol L^{-1} thiourea solution was added to the medium before analysis to suppress the possible interference of similar aldehyde species.

In the optimization and the analysis steps of the samples, all the studies were sequentially carried out in three and five replicates. The averages and their standard deviations were calculated for each study. A 95 % confidence level was statistically adopted for all calculations. The paired Student's *t*-test at the 95 % confidence level was conducted to control whether or not there is a significant difference between the 5-HMF levels obtained by using the two methods in sample extracts.

3. Results and discussion

3.1. Optimization of analytical variables

The efficiency of UA-CPE process depends on various factors such as ultrasonic bath conditions, surfactant concentration, concentration of derivative and ion-pair forming reagents, pH, buffer concentration, diluent type and volume. All the above-mentioned variables were evaluated in detail and optimized. The optimization for analysis of 5-HMF at 30 μg L^{-1} at 532 nm with a red shift of 12 nm as indicator of complex formation was realized by using one-variable-at-a-time method. In the optimization step, each point was performed with three replicate measurements, and their averages plus standard deviations were considered as error bars.

3.1.1. The effect of pH and buffer concentration

Extraction of 5-HMF by the UA-CPE requires the formation of an ion-pair with sufficient hydrophobicity to be extracted into the small volume of the surfactant-rich phase. The pH plays a unique role in ion-pair formation, and later in UA-CPE yield. Therefore, pH is the first parameter to evaluate the effect on the determination of 30 μg L^{-1} 5-HMF. The effect of pH on extraction of ion-pair complex was studied in the range of 2.0–6.5 due to instability of 5-HMF at pH ≥ 8.0 in Fig. 1(a). Quantitative extraction efficiency, which gives maximum sensitivity, was obtained at pH 5.5. At lower and higher pHs, the sensitivity gradually decreased. This decrease may due to protonation of nucleophilic and electrophilic centers both on furan ring and phenazine skeletal of PSF^+ at low pHs or irreversibly further degradation of 5-HMF by base-induced Cannizzaro disproportionation at high pHs (Godoy-Alcántar et al., 2005; Subbiah et al., 2013). Here, the aldehyde acts as a hydride donor while the PSF^+ as an acceptor, resulting in a carboxylate ion and an alcohol, respectively. In fact, it is clear that a pH-controlled stable imine adduct or charge transfer complex, based on interaction between functional aldehyde group of 5-HMF and nucleophilic amines and electrophilic hetero N^+-atoms of PSF^+ at pH 5.5 by donor-acceptor mechanism, formed by base-induced disproportionation (herein, redox sensitive weak basic phenazine group dye with a pK_a value of 8.3 in the semi-reduced form) in presence of SDS as both counter-ion and sensitive enhancer. Where SDS is self-aggregated so to have a change in CMC and aggregation number ($N_{aggr.}$) depending on pH, buffer type, temperature, solvent polarity and types of the microenvironment (Dutta and Bhat, 1996; Thongngam and Mcclements, 2005; Surashree et al., 2008; Gawandi et al., 2002), this case is also assisted with the presence of an electron acceptor aldehyde and electron donor hydroxyl-methyl groups on the furan ring allowing to decrease the energy gap between the 5-HMF and PSF^+, and stabilizes the ion-pair complex, which facilitates nucleophilic and electrophilic addition reactions. Moreover, even if the hydroxymethyl group exhibits a higher reactivity than the aldehyde group, especially in nucleophilic addition to unsaturated hetero-N atom of PSF^+ with positive charge (5-HMF), it is clear that aldehyde group in terms of imine adduct formation (herein, with PSF^+) is more reactive than hydroxmethyl group at pH 5.5 by condensation actually progressing with acid catalyzed reaction. Considering the concentration-dependent dimerization and disproportionation of PSF^+, ion-pair formed by base-induced disproportionation will also be protected against matrix effect with increase in selectivity of the process as a function of SDS concentration. For all these reasons, a pH of 5.5 was optimally preferred and used for the next processes.

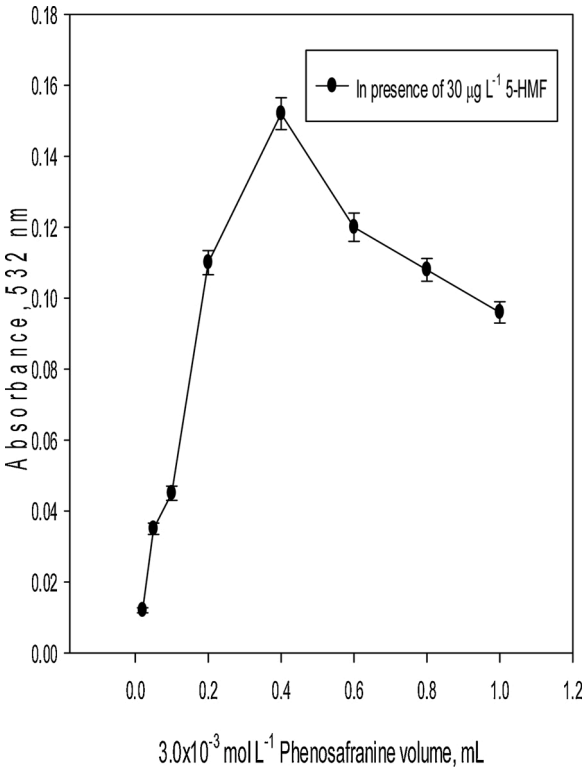

Fig. 2. Effect of 3.0×10^{-3} mol L^{-1} PSF^+ volume on absorbance, corrected against analyte blank at 532 nm (n: 3). Conditions: 2.5 mL of 0.1 mol L^{-1} citrate buffer at pH 5.5; 0.2 (or 3.0) mL of 3.0×10^{-3} mol L^{-1} SDS; 1.2 mL (or 0.8) mL of 5.0 % (v/v) Triton X-114; sonication for 12 min at 45 °C below CMC of SDS (or 7 min at 55 °C above CMC of SDS); and centrifugation for 10 min at 3000 rpm for triplicate measurement of 30 μg L^{-1} 5-HMF.

In addition, the effect of 0.1 mol L^{-1} citrate buffer solution volume at pH 5.5 in Fig. 1(b) was studied in the range of 0.5–2.5 mL to increase the sensitivity. The maximum absorbance was obtained with 2.5 mL of buffer. The sensitivity was significantly decreased at lower and higher buffer volumes due to increase in blank signal as a result of ion-pair formation between citric acid and PSF^+.

3.1.2. Effect of concentrations of derivatizing agent (PSF+)

The effect of 3.0×10^{-3} mol L^{-1} derivatizing reagent, PSF^+ as both nucleophile and electrophile at pH 5.5 in Fig. 2 was examined in a 50 mL centrifuge tube in the range of 0.0–1.0 mL. It could be seen that that the sensitivity was linearly dependent onto a volume of 0.4 mL, and decreased at higher volumes than 0.4 mL where the basicity and reactivity of PSF^+ with pK_a value of 8.3 changed with change in SDS

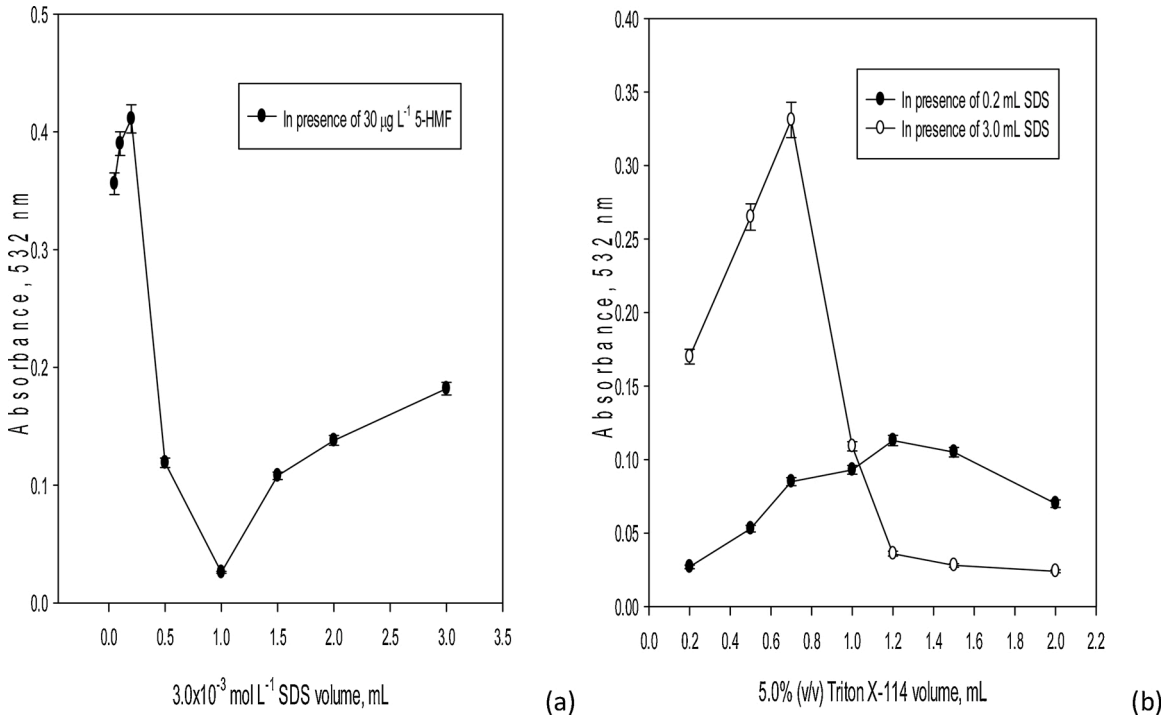

Fig. 3. Effect of (a) 3.0 × 10^{-3} mol L^{-1} SDS volume and (b) 5.0 % (v/v) Triton X-114 vol on analytical signal at 532 nm (n: 3). Conditions: 2.5 mL of 0.1 mol L^{-1} citrate buffer at pH 5.5; 0.4 mL of 3.0 × 10^{-3} mol L^{-1} PSF^{+}; sonication for 12 min at 45 °C below CMC of SDS (or 7 min at 55 °C above CMC of SDS); and centrifugation for 10 min at 3000 rpm for triplicate measurement of 30 µg L^{-1} 5-HMF.

concentration below and above CMC so as to form ion-pair in sub-micellar and micellar regions sequentially with CMC of 8.30, 2.51 and 0.079 mmol L^{-1} in water, buffer and buffer-phenazine group dye, neutral red (NR^{+}), respectively (Dutta and Bhat, 1996). A similar trend, except a red shift of 12 nm as indication of selective ion-pair formation at pH 5.5, was observed with a CMC value of 2.60 mmol L^{-1} in interaction of SDS with PSF^{+}, passing a minimum at 520 nm (Surashree et al., 2008; Gawandi et al., 2002). A change in the electron density at both functional primary amines and hetero N-atom on phenazine ring brings about the increase in the basicity and reactivity of substance as a result of strong interaction via hydrogen bonding and π-π stacking between PSF^{+} and 5-HMF. As a result, it is believed that the derivatizing agent, PSF^{+} will be affected from polarity of solvent, pH, temperature of the environment as well as buffer, electrolyte and surfactants types and concentrations for ion-pair or charge transfer complex formation with 5-HMF. Because of all these reasons, it is concluded that a derivatizing reagent volume of 0.4 mL is sufficient for further studies.

3.1.3. The effect of ionic (SDS) and non-ionic (Triton X-114) surfactant concentrations

The effect of 3.0 × 10^{-3} mol L^{-1} SDS on sensitivity was examined in the range of 0.0–3.0 mL in Fig. 3(a). With increasing surfactant volume up to 0.2 mL, the sensitivity increased and reached the maximum value. In range of 0.2–1.0 mL, it was observed that the sensitivity linearly decreased with increasing SDS volume and reached a minimal value at 1.0 mL due to participate in ion-pair formation, so as to lead an increase in sample blank. However, sensitivity in volumes greater than 1.0 mL increased linearly again. This phenomenon is thought to result from the concentration-dependent interaction of SDS with PSF^{+} to form an ion-pair, competitively with 5-HMF (Dutta and Bhat, 1996; Thongngam and Mcclements, 2005; Surashree et al., 2008; Gawandi et al., 2002). Therefore, with aim of establishing two calibration curves, it is concluded that aliquots of 0.2 and 3.0 mL of SDS respectively, so as to fall in submicellar and micellar regions, is sufficient for maximum sensitivity.

Triton X-114 was selected for an efficient phase separation in extraction process due to its useful physicochemical properties like low cloud point temperature, commercial availability, low toxicity, low cost and high density, which facilitates phase separation by centrifugation. The effect of Triton X-114 vol on the sensitivity was studied in the range of 0.2–2.0 mL of 5.0 % (v/v) in Fig. 3(b). The results showed that the best signal was obtained with 1.2 and 0.8 mL of 5.0 % (v/v) Triton X-114 in presence of 0.2 and 3.0 mL of SDS, respectively. In fact, these concentrations (12.37 and 1.58 mmol L^{-1}) were higher of 11.9 and 7.9-folds than CMC of 0.2 mmol L^{-1}. At higher volumes than 0.8 and 1.2 mL, the signal was significantly decreased. This reduction in the signal can be attributed to the presence of a large amount of surfactant, resulting in an increase in the volume of the surfactant-rich phase and thus a decrease in the pre-concentration factor.

3.1.4. Salting-out effect

According to Komaromy-Hiller et al. (1996), the salting-out phenomenon is directly related to desorption of ions to the hydrophilic parts of the micelles, increasing inter-attraction between micelles and consequently leading to the precipitation of surfactant molecules. Based on this discussion, the salting-out effect was studied in both the absence and presence of different concentrations of $NaNO_3$, Na_2SO_4 and NaCl (0.05–1.0 mol L^{-1}, each one) at 25 °C. It was found that NaCl resulted in the best signal, and the recoveries increased with increase in the salt concentration until reach a maximum at concentration of 0.25 mol L^{-1}. This effect may be due to the enhanced hydrophobic interactions among the surfactant aggregates and the analyte as well as the decrease in the cloud point temperature of Triton X-114 in the presence of NaCl. At higher concentrations than 0.25 mol L^{-1}, the signals decreased considerably. High concentration of salt can increase the density of water drops accompanied by the surfactant rich phase, and hence disturb the phase separation. On the other hand, the absence of salt decreased the signal by about 35 % especially in presence of 3.0 mL SDS (data, not shown). This may be due to an increase in cloud point temperature leading to incomplete phase separation. Hence, a concentration of 0.25 mol L^{-1}

Table 1
Analytical features of the proposed preconcentration method under optimized reagent conditions.

Parameters	By the matrix-matched calibration curves	
	Using 0.2 mL of 3.0×10^{-3} mol L^{-1} SDS	Using 3.0 mL of 3.0×10^{-3} mol L^{-1} SDS
Regression equations	$A_1 = 5.18 \times 10^{-3}$C (5-HMF, μg L^{-1}) + 0.053, $A_2 = 1.01 \times 10^{-3}$C (5-HMF, μg L^{-1}) + 0.0883	A_1= -0.0215C (5-HMF, μg L^{-1}) + 0.378, A_2= -1.31 × 10^{-3}C (5-HMF, μg L^{-1}) + 0.203
Correlation coefficient, r^2	0.9866, 0.9915	−0.9992, -0.9856
Linear working ranges, μg L^{-1}	2−20, 20−200	2−15, 15−100
LOD, μg L^{-1}	0.75	0.53
LOQ, μg L^{-1}	2.51	1.76
Recovery % (10, 25 and 75 μg L^{-1}, n: 5)	97.5−102.3	97.3−101.6
Repeatability (as RSD %, 10, 25 and 75 μg L^{-1}, n: 5 for same day)	2.6−3.7	2.5−3.8
Reproducibility (as RSD % 10, 25 and 75 μg L^{-1}, n: 5 for three consecutive days)	3.1−5.3	3.7−6.1
[a]Sensitivity enhancement factor	60	65
[b]Preconcentration factor	37.5	37.5
Sample volume, mL	15	15
Measurement wavelength, nm	532	532

[a] The ratio of slopes of calibration curves with and without the UA-CPE.
[b] The ratio of the aqueous bulk solution to the surfactant-rich phase volumes before and after the UA-CPE.

was chosen as the optimal.

3.1.5. Effect of diluents

In order to facilitate the detectability of the sample solution by micro-volume UV–vis spectrophotometry, it was necessary to decrease the viscosity of the surfactant-rich phase. Different polarity solvents such as THF, acetone, acetonitrile, ethanol, and methanol were tried to select the one producing the best results regarding sensitivity, reproducibility, and stability of the signal. The best result was obtained with ethanol. After phase separation some ethanol was added to the surfactant-rich dense phase; where the micellar phase is diluted to about 0.4 mL for an enrichment factor of 37.5 times from the enrichment of the optimal 15-mL sample (in range of 5−30 mL). This amount of ethanol was chosen to ensure a sufficient volume of the sample solution for maximum sensitivity. For smaller volumes, the reproducibility of the signals was very poor, whereas for higher volumes, there was a gradually decrease in the signal due to excess dilution.

3.1.6. Effect of equilibrium temperature and time

The UA-CPE technique relies on the properties of many nonionic surfactants that become cloudy and form micelles when heated to cloud point temperature. The effect of the equilibrium temperature on the cloud point temperature was investigated in the range of 30–60 °C. From the results, temperatures of 45 and 55 °C for 0.2 and 3.0 mL of SDS were independently found to be sufficient to carry out quantitative extraction. Incubation time is also an important parameter to consider. From the results, it was observed that optimal equilibrium times of 12 and 7 min were sufficient to obtain good extraction efficiency for the equilibrium time ranging from 3 to 30 min at 45 and 55 °C, respectively (data, not shown).

3.1.7. Effect of centrifugation rate and time

The effect of centrifugation rate and duration on the sensitivity of the method and the phase separation method at different speeds (1500−4000 rpm) in the interval of 3−20 min were investigated. From a series of repeated measurement results, a centrifugation time of 10 min at 3000 rpm was found to be sufficient for complete quantitative phase separation (data, not shown).

Table 2
Results of replicate measurements of 5-HMF at 100 μg L^{-1} in the presence of some interfering species (n: 3).

Interfering species	Tolerance limit, [Interfering]/[5-HMF]	Recovery %	RSD %
Inorganic cationic species			
Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, Sr^{2+}	1000	96.5−101.5	3.2−4.8
Zn^{2+}, Al^{3+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Fe^{2+}	500	93.5−102.2	2.8−5.7
Mo^{6+}, Pb^{2+}, Cd^{2+}, V^{5+}, V^{4+}	350	94.5−98.2	3.2−5.5
Se^{4+}, Cr^{3+}, Sb^{3+}, As^{3+}, As^{5+}	250	95.5−98.5	3.1−5.3
Sn^{2+}, Cu^{2+}, Fe^{3+}	100	93.5−97.2	2.5−6.5
Inorganic anionic species			
Cl^-, SO_4^{2-}, NO_3^-, $H_2PO_4^-$, HCO_3^-, Br^-, F^-	1000	94.0−98.0	3.0−5.0
SCN^-, NO_2^-	500	95.5−98.5	3.2−5.5
I^-, Oxalate	250	93.5−97.5	2.5−5.0
HSO_3^-	25−100	92.5−96.5	3.5−5.5
[a]HSO_3^-	>100	97.5−101.5	2.5−4.0
Organic species			
Valine, Throsine, Glycine, Alanine,	750	96.5−102.0	3.0−4.5
Phenylalanine, Glutamine, Methionine	500	95.5−102.0	2.5−5.0
Ethionine, Tryptophane	250	94.5−98.0	2.5−4.0
Phenol, Histidine	150	95.5−99.5	3.0−5.2
Ascorbic acid	100	93.5−98.5	2.0−4.5
Formaldehyde, [b]Acetaldehyde	>100	95.5−103.5	2.5−5.8

[a] It must be removed by either pre-heating the reaction mixture around pH 2.0 or pre-treating the mixture with 1.0 mL of 0.2 mol L^{-1} H_2O_2 before UA-CPE.
[b] Its concentration dependent interfering effect may be efficiently removed after agitating the pretreated samples in ultrasonic bath for 10 min at 80 °C prior to preconcentration with UA-CPE.

3.2. Analytical figures of merit

Under optimum conditions, the analytical features of the method were examined. The calibration curves, established using 0.2 and 3.0 mL of 3.0×10^{-3} mol L^{-1} SDS in submicellar and micellar regions were highly linear between 2−200 and 2−100 μg L^{-1}, respectively, with better correlation coefficient than 0.9856. Table 1 summarizes the analytical features of the method, including regression equation, linear working range, limits of detection/quantification, enrichment factor, and repeatability/reproducibility within and between days. The detection limits defined as $C_{L,min} = 3 \times s_{blank} / m$ (herein, $C_{L,min}$, s_{blank} and m detection limit, standard deviation of 10 blank replicate analysis and slope of calibration curves) were 0.75 and 0.53 μg L^{-1}. The enrichment factor (15/0.4 = 37.5) was calculated as the ratio of the sample volume (15-mL) to the surfactant-rich phase volume (0.4 mL) diluted with methanol prior to analysis. After enrichment of the 15-mL sample, sensitivity enhancement factors of 60 and 65 were obtained from the matrix-matched calibration curves prepared by spiking to sample extracts. The recovery rates, intraday and inter-day precision data were between 97.3–102.3 % and 2.5–6.1 % (as RSDs), respectively, as a measure of accuracy and precision for the five replicate measurements of 10, 25 and 75 μg L^{-1} 5-HMF on the same day and three consecutive days.

3.3. The matrix effect

To evaluate the selectivity of the method, the effects of different ionic and non-ionic organic/ inorganic species on three replicate

Table 3

(a) Results of precision, trueness and expanded uncertainty (k: 2) studies (n: 9).

Sample	Added 5-HMF (μg kg^{-1})	[a]Expanded uncertainty (U %)	[b]Intermediate precision (RSD %)	[c]Trueness (Recovery %)
Pine honey	10	10.4	6.5	103.5
Strawberry jam	15	8.2	4.5	94.5
Grape molasses	20	6.4	3.5	101.5
Apple vinegar	25	7.2	3.6	102.5

(b) The analysis results of 5-HMF levels present in honey, molasses, jam and vinegar samples, and percent recoveries of spiked samples (n: 5).

Samples	After extraction with mixture of 2 mL of 2.0 mol L^{-1} $HClO_4$, and Carrez-I and II solutions (sequentially each one, 2 mL) for 15 min at 45 °C				After extraction with mixture of 2 mL of 0.2 mol L^{-1} $HClO_4$, and Carrez-I and II solutions (sequentially each one, 2.0 mL) under ultrasonic power for 5 min at 40 °C				[**]The experimental t-values	[***]Found by the modified White method mg kg^{-1}
	Added, mg kg^{-1} 5-HMF	[*]Found, mg kg^{-1} 5-HMF	RSD %	Recovery %	Added, mg kg^{-1} 5-HMF	[*]Found, mg kg^{-1} 5-HMF	RSD %	Recovery %		
Grape molasses	–	7.65 ± 0.30	3.9	–	–	7.70 ± 0.30	3.9	–	0.53, 0.26	7.75 ± 0.30
	15	22.30 ± 0.80	3.7	98.0	15	22.40 ± 0.80	3.6	98.0	–	–
Carop molasses	–	18.05 ± 0.70	3.9	–	–	18.10 ± 0.70	3.9	–	0.12, 0.24	18.00 ± 0.60
	10	27.80 ± 1.0	3.6	97.5	10	27.70 ± 1.0	3.6	96.0	–	–
Pomegranate	–	12.60 ± 0.50	4.0	–	–	12.65 ± 0.50	4.0	–	0.35, 0.17	12.70 ± 0.40
	10	22.30 ± 0.80	3.6	97.0	10	22.20 ± 0.80	3.6	95.5	–	–
Apricot jam	–	4.30 ± 0.15	3.6	–	–	4.30 ± 0.15	3.5	–	0.53, 0.53	4.35 ± 0.15
	15	18.80 ± 0.60	3.2	97.3	15	18.90 ± 0.60	3.2	97.3	–	–
Strawberry jam	–	1.15 ± 0.04	3.5	–	–	1.05 ± 0.04	3.8	–	1.98, 1.98	1.10 ± 0.04
	15	15.70 ± 0.60	3.8	98.2	15	15.60 ± 0.60	3.8	97.5	–	–
Cherry berry	–	2.20 ± 0.07	3.2	–	–	2.25 ± 0.07	3.1	–	2.26, 1.13	2.30 ± 0.07
	15	17.00 ± 0.50	2.9	99.0	15	16.90 ± 0.40	2.4	98.0	–	–
Fig jam	–	9.55 ± 0.35	3.7	–	–	9.65 ± 0.35	3.6	–	0.23, 0.23	9.60 ± 0.35
	10	19.10 ± 0.70	3.7	96.0	10	19.20 ± 0.70	3.6	96.0	–	–
Flower honey	–	4.75 ± 0.20	4.2	–	–	4.85 ± 0.20	4.2	–	0.40, 0.40	4.80 ± 0.20
	15	19.20 ± 0.70	3.6	96.7	15	19.40 ± 0.70	3.6	97.3	–	–
Pine honey1	–	8.60 ± 0.30	3.5	–	–	8.65 ± 0.30	3.5	–	0.53, 0.26	8.70 ± 0.30
	10	18.10 ± 0.60	3.3	95.0	10	18.20 ± 0.60	3.3	95.5	–	–
Pine honey2	–	13.60 ± 0.50	3.7	–	–	13.70 ± 0.50	3.6	–	0.17, 0.17	13.65 ± 0.40
	10	23.40 ± 0.70	3.0	96.0	10	23.30 ± 0.70	3.0	96.0	–	–
Grape vinegar	–	3.10 ± 0.10	3.2	–	–	3.20 ± 0.10	3.2	–	0.79, 0.79	3.15 ± 0.10
	15	17.70 ± 0.60	3.4	97.7	15	17.80 ± 0.60	3.4	97.7	–	–
Apple vinegar	–	2.20 ± 0.07	3.1	–	–	2.25 ± 0.07	3.1	–	2.26, 1.13	2.30 ± 0.07
	15	16.75 ± 0.50	3.0	97.3	15	16.70 ± 0.50	3.0	97.3	–	–

[a] Acceptance criterion: U_{max} <2 * %RSD Horwitz function according to (Horwitz, 1982; Thompson et al., 2002).

[b] Acceptance criterion: % RSD < 2/3 Horwitz–Thomson function (Horwitz, 1982) and is (in % RSD): 14.7 % for values ≤100 μg kg^{-1} and 13.6 % for 200 μg kg^{-1} (Fryš, et al., 2011).

[c] Acceptance criterion: Rec = 85–115 %. CODEX criterion: 60–115 % for 10 μg kg-1 and 80–110 % for 0.1–10 mg kg-1 (Joint FAO/WHO Expert Committee on Food Additives, 2010).

[*] The average plus standard deviation of five replicate measurements for 5-HMF after pretreatment with two wet digestion approaches with and without sonication.

[**] In order to compare two mean values for independent two sample 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.

[***] The average plus standard deviation of five replicate measurements of 5-HMF obtained by using the modified spectrophotometric White method for samples similarly pre-treated with sonication of 5 min at 40 °C under ultrasonic power (300 W, 40 kHz).

measurements of trace 5-HMF were investigated in UA-CPE. A known concentration of 5-HMF (100 μg L^{-1}) with different inorganic/organic interfering concentrations at ratios ranging from 25 to 1000 was taken into account and the general extraction method was followed. From the results in Table 2, it can be concluded that the proposed method is relatively selective for the determination of 5-HMF at trace levels. The interfering effect of formaldehyde and acetaldehyde may be efficiently removed after agitating the pretreated samples in ultrasonic bath for 10 min at 80 °C prior to preconcentration with UA-CPE while The interfering effect of bisulfite was greatly suppressed by either pre-heating the reaction mixture around pH 2.0 or pre-treating the mixture with 1.0 mL of 0.2 mol L^{-1} H_2O_2 before UA-CPE (Mohamed et al., 2008). Moreover, in case of possible interference, besides diluting the sample solution, the matrix-matched calibration approach, to suppress the matrix effect, can be effectively used where the direct calibration approach is insufficient.

3.4. Analytical applications

In order to evaluate the applicability of the method, the precision, trueness and expanded uncertainty studies by analysis of the selected four quality control samples (n: 9), representing sample matrix, were carried out after spiking with 10, 15, 20 and 25 μg kg^{-1}. Analysis results are given in detail in Table 3(a). The recovery rates as a measure of accuracy; the RSDs as a measure of precision were taken into consideration. From the nine replicate measurement results, an intermediate precision with a RSD% ranging from 3.5–6.5% and recovery rates ranging from 94.5–103.5% were obtained where the expanded-uncertainty (U%) ranged from 6.4%–10.4%. These results show that the method is quantitatively accurate and precise (Fryš et al., 2011; FAO/WHO, 2010).

After concluding that the method is applicable to the analysis of real time samples, the method was applied to analysis of the selected samples, statistically checked in parallel with two different sample preparation steps with and without sonication to take into account the sample matrix, and the results were compared with those of independent modified White method. At the same time, the accuracy and precision of sample preparation procedures were comparatively evaluated in Table 3 (b) with and without spiking (10 and 15 mg kg^{-1}) after pretreatment. From the analysis results, while they ranged from 1.15–18.05 mg kg^{-1} with a RSD% of 3.0–4.2 % and recovery rate of 95–99 % in the selected samples by sample extraction without sonication; it was observed that 5-HMF levels were in the range of 1.05–18.10 mg kg^{-1} with a RSD% of 3.0–4.2 % and recovery rate of 95.5–98.0 % by sample extraction with sonication. By independent modified White method, the results found

Table 4
Amounts of 5-HMF in the foodstuffs analyzed by the existing method, and comparison with other detection methods.

Matrix type	Sample name	Detection method	Derivatizing agent, Extractant, Separation columns	Linear working range	Detection limit	Concentration, μg g^{-1}		Published data, μg g^{-1}	References
						Average (n: 3)	RSD %		
Honey	Multi-floral A Multi-floral B	HPLC or RP-HPLC	RP column (Spherisorb ODS II C-18 column)	0.13–100 mg L^{-1}	0.04 mg L^{-1}	38.3 4.6	3.0 10.0	0.8–138	(Nozal et al., 2001; Costa et al., 1999; Spano et al., 2006)
Breakfast cereals	Honey rings Corn flakes	LC	RP C-18 column (Spherisorb S5 ODS2)	10–150 μg g^{-1}	0.03 μg g^{-1}	24.7 46.8	3.0 4.0	4–193	(Garcia-Villanova et al., 1993)
Beverages	Without alcohol	HPLC/PAD	Cation-exchange resin based column (Bio-Rad Aminex HPX-87H hydrogen form)	0.1–100 mg L^{-1}	3 μg L^{-1} at 280 nm	8.5	4.0	4–22, 1–3	(Yuan and Chen, 1998)
Biscuits Jam Honey and vegetable oils	With Alcohol Honey biscuits Plum Honey	GC-MS MEKC	BSTFA SDS, a bare fused-silica capillary	25–700 μg kg^{-1} 1–25 mg L^{-1}	6 μg kg^{-1} 0.43 mg L^{-1}	6.3 7.0 12.7 3–25	7.0 5.0 2.0 0.6–4.0	– 11–1145	(Teixidó et al., 2006) (Wong et al., 2012)
Foodstuffs	Honey, Fruit juice	MEKC	SDS, uncoated fused-silica capillary	2.5–250 mg kg^{-1}	0.7 mg kg^{-1}	40	3.0–10.0, 7.0–12.0	3.3–42.3, 2.9–10.6	(Teixidó et al., 2011)
Heated processed foods, treacle	Honey	HS-SPM-HPLC	DVB/CAR/PDMS, HP5(cross-linked 5% methylsiloxane) capillary column	–	7 μg L-1 at 280 nm	200 μg L^{-1}	1.4	66.1–179	(Edris et al., 2007)
Honey	Different botanic origin honey samples	RP-HPLC	RP C18 column	0.01–100 μg L^{-1}	3 μg L-1 at 284 nm	–	0.2–1.5 % as CV%	5.90–155.8	(Spano et al., 2009)
Beverages	Cola and soft drinks	SPE-AMTC-PAD	–	1–100 mg L^{-1}	0.1 mg L-1	1.0 mg L-1	4.7	1.07–4.47	(Xu et al., 2015)
Foods	Infant formulas, beers and vinegars	SPME-GC-MS	PFBHA, PDMS/DVB fibre	0.1–50 mg L^{-1}	0.023 mg L^{-1}	0.23 mg L^{-1}	1.3–4.7	0.91–46.40, 0.023–27.87	(Tsai and Kao, 2012)
Beverages	Fruit juices	Salting out-VALLME-HPLC-DAD	1-Hexanol, ODS Hypersil C18 column	1–5000 μg L^{-1}	0.40–0.45 μg L-1	2–5000 μg L^{-1}	1.5–3.2	0.71–28	(Abu-Bakar et al., 2014
Foods	Coated deep fried products	HPLC-DAD-Full factorial CCD	Ethyl acetate-hexane (4:1, v/v)	0.8–56 mg L^{-1}	7.6 μg kg^{-1}	–	4.1–16.0	1.25	(Pérez-Palacios et al., 2013)
Honey/Beverages with and without alcohol	Different honey samples, nonalcoholic and alcoholic beverages	UA-CPE-Spectrophotometry	p-NPH/SDS	6.5–275 μg L^{-1}	1.96 μg L^{-1}	2575 and 150 μg L^{-1}	2.8–5.3, 3.8–5.6	0.903–1.555, 0.129–0.758 and 0.206–0.840	(Gürkan and Altunay, 2015)
Foodstuffs	Honey, Jam, molasses and vinegar samples	UA-CPE-Spectrophotometry	PSF^{+}/SDS	2–100, 2–200 μg L^{-1}	0.53, 0.75 μg L^{-1}	10, 25 and 75 μg L^{-1}	2.6–3.7, 2.5–3.8	0.91–13.80	The current study

RP-HPLC: Reverse phase-high performance liquid chromatography; LC: Liquid chromatography; HPLC / PAD: High performance liquid chromatography / photodiode array detection; GC–MS: Gas chromatography-mass spectrometry; MEKC: Micelle electrokinetic chromatography; HS-SPME: Solid phase microextraction with head space; SPE: Solid phase extraction; SPME: Solid phase microextraction; DRS: Diffuse reflection spectrophotometer; VA-LLME: Vortex-assisted-liquid-liquid microextraction; AMTC-PAD: Amine trapped column chromatography combined with pulse amperometric detection; Full factorial CCD: Designing a full factor central composite; CPE: Cloud point extraction; UA-CPE: Ultrasound assisted-cloud point extraction; BSTFA: N,O-bis-trimethylilyltrifluoroacetamide; DVB/CAR/PDMS: Divinylbenzene/Carboxen/Polydimethylsiloxane; PFBHA: o-2,3,4,5,6-(pentafluorobenzyl)hydroxylamine hydrochloride; PDMS/DVB: Poly(dimethyliloxane)/divinylbenzene; p-PNPH: p-Nitrophenylhydrazine; PSF^{+}: Phenosafranine.

were in range of 1.10–18.0 mg kg^{-1} of 5-HMF level in all the selected samples. It could be concluded that the results found by both sample preparation procedures were statistically in agreement with those of independent modified White method according to two paired Student's *t*-test. Similar results as a function of heating and storage time were also found in honey samples collected from Hatay provinces and Eastern Anatolian region in Turkey, with values of 5.73 and 3.3–19.1 mg kg^{-1}, respectively by two author groups (Sahinler and Gul, 2005; Yılmaz and Küfrevioglu, 2001). Finally, it could be seen that these results were lower than the permitted limit values (40 and 80 mg kg-1) prescribed by the European Union (EU) and Codex (Codex Alimentarius Commission Standards, 2001; Alimentarius, 1982) for the formation of 5-HMF during processing in selected food matrices, at a dose that does not pose a risk to human health.

3.5. Comparison with other related methods

A method comparison with other in literature was made to evaluate the effectiveness of the method. Thus, the efficiency of the method was evaluated by comparing the achieved analytical features with those of other extraction and detection techniques reported in the literature. As can be seen from Table 4, the method has a good accuracy, and intra-day /inter-day precision in linear working ranges of 100- and 50-folds using 0.2 and 3.0 mL of 3.0×10^{-3} mol L^{-1} SDS. In addition, the detection limits and the pre-concentration factor of the method were generally better than those of other chromatographic and capillary electrophoretic techniques at different elution and detection modes, which are often used in separation and detection of 5-HMF in food and beverage matrices (Teixidó et al., 2006; Costa et al., 1999; Nozal et al., 2001; Spano et al., 2006; Garcia-Villanova et al., 1993; Yuan and Chen, 1998; Wong et al., 2012; Teixidó et al., 2011; Edris et al., 2007; Spano et al., 2009; Xu et al., 2015; Tsai and Kao, 2012; Abu-Bakar et al., 2014; Gürkan and Altunay, 2015), except requiring a further separation/enrichment procedure such as VALLME (Tsai and Kao, 2012). In terms of operating parameters, the UA-CPE procedure was carried out using low-cost, simple devices and eco-friendly chemicals. Finally, the detection step was selectively realized with better recovery and reproducibility by using micro-volume UV–vis spectrophotometer, which is simple, easy to use, cost-effective and fast measurement capabilities according to tedious, time-consuming and complex chromatographic and electrophoretic techniques. Also, these separation techniques need expensive polymeric or cross-link co-polymeric column packaging materials/capillary fibres with amine functional group as well as hydrazine, hydroxylamine and amide functional derivatizing agents in order to improve the selectivity of the separation by gradient (or isocratic) elution at RP mode. Considering all this, UV–vis spectrophotometer is easily accessible in almost any analytical research lab, and does not require expert user in her/his area to conduct 5-HMF analysis, unlike sensitive but more complex and expensive instrumental methods such as LC- or GC with MS detection.

4. Conclusions

In the present study, Triton X-114 was chosen for the formation of the surfactant-rich phase due to its excellent physicochemical characteristics, low cloud point temperature, high density of the surfactant-rich phase, which facilitates phase separation easily by centrifugation, and commercial availability and relatively low price and low toxicity. The method is a promising alternative for the determination of 5-HMF in combination with micro-volume UV–vis spectrophotometry. From the results obtained, it can be considered that phenazine dye, PSF^+ is selective and efficient binder for UA-CPE of 5-HMF in presence of SDS as both sensitizer and counter-ion under and above its CMC. The simple accessibility, the formation of stable ion-pair, and consistency with the UA-CPE method are the major advantages of the use of pH-sensitive ion-pairing, PSF^+ in UA-CPE of 5-HMF. UA-CPE has been shown to be a practicable and versatile method, being adequate for analysis of low levels 5-HMF in selected food matrices. UA-CPE, which is assisted by ultrasound energy for acceleration of the mass transfer in the extraction process, is an easy to use, safe, rapid, inexpensive, and eco-friendly methodology for fast and efficient separation and preconcentration of trace 5-HMF in aqueous solutions. The complex in surfactant-rich phase can be directly detected in visible region using a simple spectrophotometry after dilution with ethanol. The proposed UA-CPE method incorporating PSF^+ as ion-pairing agent permits effective separation and preconcentration of trace 5-HMF, and finally micro-volume UV–vis spectrophotometer provides a novel route for trace determination of toxic 5-HMF in quality control of foodstuffs. A low-cost surfactant was used in extractive enrichment step, thus toxic organic solvent extraction generating waste disposal problems was greatly avoided. Also, the use of cells with micro-capacity in analysis step results in low consumption of samples and reagents, and so negligible generation of wastes. Finally, the method requires sample preparation time of nearly 30 min per sample. However, total nine samples including independently six samples and standards addition of three increasing concentrations around quantification limit can be prepared for a batch analysis simultaneously.

Ethical approval

This article does not contain any studies with human participants or animals carried out by any of the author himself.

Informed consent

Informed consent is not applicable to this study.

CRediT authorship contribution statement

S. Dağdeviren Baş: Conceptualization, Data curation, Investigation, Formal analysis. **R. Gürkan:** Methodology, Supervision, Validation, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The author declares that he has no conflict of interest.

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Appendix A. Supplementary data

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