



# Synthesized of a novel xanthate functionalized polypropylene as adsorbent for dispersive solid phase microextraction of caffeine using orbital shaker in mixed beverage matrices

Nail Altunay<sup>a</sup>, Mustafa Tuzen<sup>b,c,\*</sup>, Baki Hazer<sup>d,e</sup>, Adil Elik<sup>a</sup>

<sup>a</sup> Department of Chemistry, Sivas Cumhuriyet University, Sivas, Turkey

<sup>b</sup> Department of Chemistry, Tokat Gaziosmanpaşa University, 60250 Tokat, Turkey

<sup>c</sup> King Fahd University of Petroleum and Minerals, Research Institute, Center for Environment and Marine Studies, Dhahran 31261, Saudi Arabia

<sup>d</sup> Department of Aircraft Airframe Engine Maintenance, Kapadokya University, Nevşehir, Turkey

<sup>e</sup> Zonguldak Bülent Ecevit University, Department of Chemistry, 67100 Zonguldak, Turkey

## ARTICLE INFO

### Keywords:

Xanthate functionalized chlorinated polypropylene  
Orbital shaker  
Dispersive solid phase microextraction  
Spectrophotometer  
Caffeine  
Beverage and food samples

## ABSTRACT

A newly synthesized xanthate functionalized chlorinated polypropylene (PP-Xa) was used as adsorbent for the orbital shaker based on dispersive solid phase microextraction (OS-DSPME) of caffeine from several tea, coffee, energy drink, coca-cola and chocolate samples using UV-vis. spectrophotometer. Synthesized PP-Xa was characterized using Fourier Transform Infrared spectroscopy (FTIR-ATR) and nuclear magnetic resonance spectroscopy (<sup>1</sup>H NMR). Various parameters like pH, PP-Xa amount, extraction time, type of eluent and its volume were optimized. Linear range, detection limit (LOD), limit of quantification (LOQ), relative standard deviation (RSD), recovery values, and enrichment factor (EF) were found 90–1000 µg L<sup>-1</sup>, 27.3 µg L<sup>-1</sup>, 90 µg L<sup>-1</sup>, 1.9–2.6%, 98 ± 2%, and 167, respectively. Adsorption capacity of PP-Xa was found 271.9 mg g<sup>-1</sup>. Standard addition and reference method were used for confirm the accuracy of present method.

## 1. Introduction

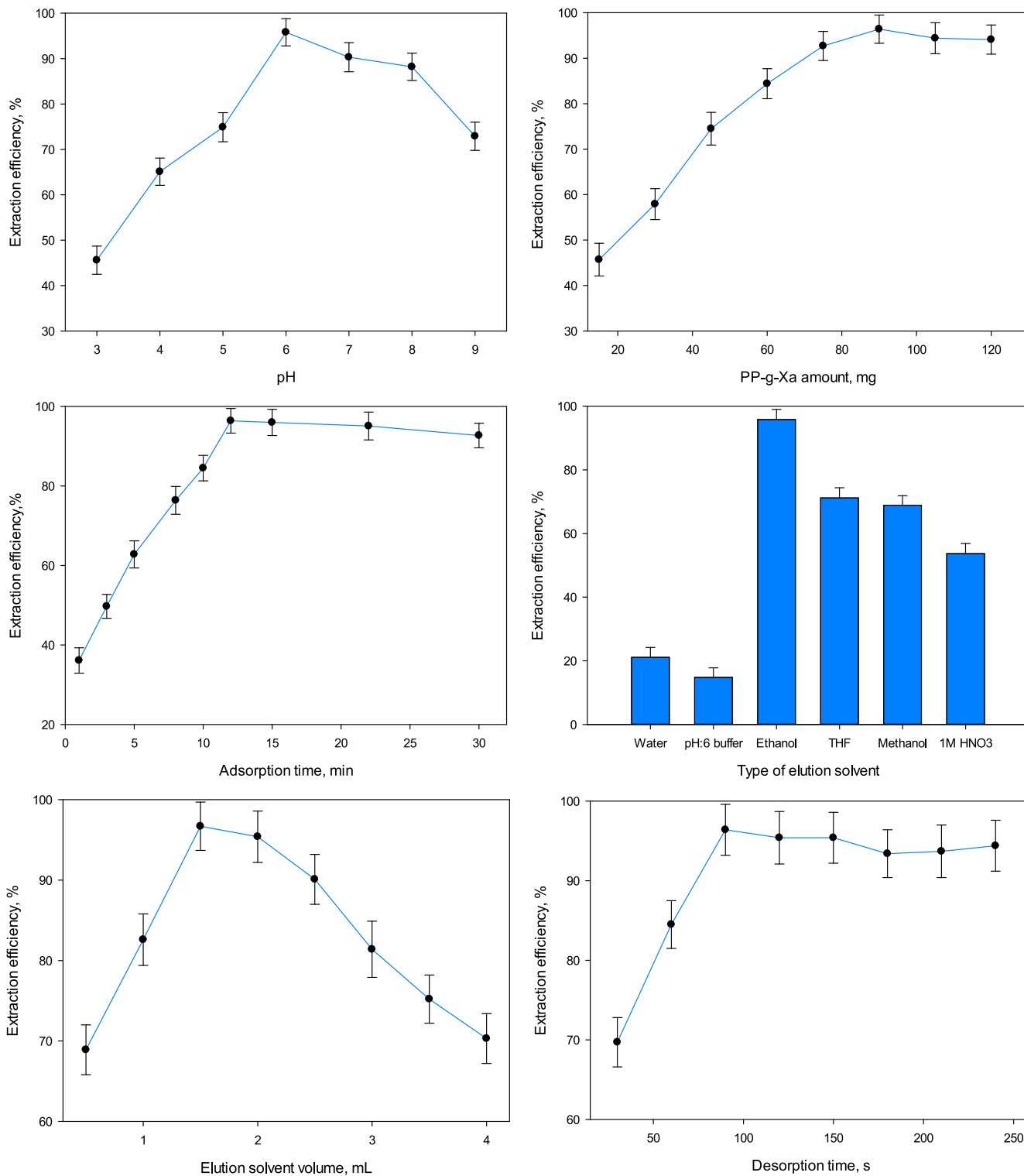
Caffeine is found as natural alkaloid in some plants such as coffee, tea, nesface, coca-cola, energy drinks, cacao and it is affected the central nervous system, gastric secretion and diuresis in human (Pradhan, Biswasroy, Kapil, & Jatin, 2017; Cauli, & Morelli, 2005). However, when consumed in excess, it might cause adverse mutagenic effects such as inhibition of DNA repair and also cause to cancer, diseases of heart and complications in ageing and pregnant women (Tyszczyk-Rotko, & Beczkowska, 2015; Sun, Huang, Wei, Wu, & Ren, 2011; Tyszczyk, Skalska-Kaminska, & Wozniak, 2011). Tea and coffee are popular beverages in the world. Therefore, it has great social and commercial importance (Zougagh, Rios, & Valcarcel, 2005). Coffee drinks are prosperous in bioactive substances such as caffeine, tannic acid, nicotinic acid, trigonelline, pyrogallollic acid, and quinolinic acid (Salinas-Vargas, & Cañizares-Macías, 2014). The caffeine consumption at high amounts may cause mood changes and caffeinism syndrome and resulting some problems such as irritability, headache, anxiety and insomnia (Lancu, & Strous, 2006; de Paula Lima, & Farah, 2019).

Caffeine is legally permission additives in soft drinks with an upper tolerance limit of 0.015% (w/w) (Ministerio de Agricultura, 1982). It is recommended daily caffeine consumption up to 300 mg for healthy adults (Shishov, Volodina, Nechaeva, Gagarinova, & Bulatov, 2019). So, the monitoring of caffeine contents in beverages and food samples is significant for the quality control of foods. The precise and accurate determination of caffeine contents in coca-cola, tea, coffee, and energy drinks samples are important because of high amount caffeine consumption in the world and its potential physiological effects.

Many analytical method such as UV visible spectrophotometer (Frizzarin, Maya, Estela, & Cerdà, 2016; Singh, & Sahu, 2006), gas chromatography with mass spectrometry (GC-MS) (Amini, & Hashemi, 2018), voltammetry (Fekry, Shehata, Azab, & Walcarius, 2020), Raman spectroscopy (Armenta, Garrigues, & de la Guardia, 2005), high performance liquid chromatography (HPLC) with ultraviolet detection (Al-Othman, Agel, Alharbi, Badjah-Hadj-Ahmed, & Al-Warthan, 2012) have been used caffeine determination in different beverage and food samples. Uv-vis. spectrophotometer has some advantages such as cheap, easy operation, cost effective and can be found in each laboratory.

\* Corresponding author at: Department of Chemistry, Tokat Gaziosmanpaşa University, 60250 Tokat, Turkey.

E-mail address: [mustafa.tuzen@gop.edu.tr](mailto:mustafa.tuzen@gop.edu.tr) (M. Tuzen).



**Fig. 1.** a. Effect of pH of sample solution on extraction efficiency of caffeine. Conditions: PP-Xa amount, 90 mg; adsorption time, 12 min; desorption solvent type and volume, 1.5 mL ethanol; desorption time, 90 s. **b.** Effect of PP-g-Xa amount on extraction efficiency of caffeine. Conditions: pH, 6.0; adsorption time, 12 min; desorption solvent type and volume, 1.5 mL ethanol; desorption time, 90 s. **c.** Effect of adsorption time on extraction efficiency of caffeine. Conditions: pH, 6.0; PP-Xa amount, 90 mg; desorption solvent type and volume, 1.5 mL ethanol; desorption time, 90 s. **d.** Effect of eluent type on extraction efficiency of caffeine. Conditions: pH, 6.0; PP-Xa amount, 90 mg; adsorption time, 12 min; desorption time, 90 s. **e.** Effect of eluent volume on extraction efficiency of caffeine. Conditions: pH, 6.0; PP-Xa amount, 90 mg; adsorption time, 12 min; desorption time, 90 s. **f.** Effect of desorption time on extraction efficiency of caffeine. Conditions: pH, 6.0; PP-Xa amount, 90 mg; adsorption time, 12 min; desorption solvent type and volume, 1.5 mL ethanol.

**Table 1**  
Selectivity of the OS-DSPME procedure for caffeine.

Matrix species	RSD (%)	Recovery (%)	Tolerable limit
Cl <sup>-</sup>	2.3	99 ± 2	2000
SO <sub>4</sub> <sup>2-</sup>	2.6	99 ± 2	2000
Mg <sup>2+</sup>	2.3	99 ± 1	1000
NO <sub>3</sub> <sup>-</sup>	2.2	98 ± 1	1000
Al <sup>3+</sup>	2.7	98 ± 2	1000
Ca <sup>2+</sup>	2.0	97 ± 2	500
Ni <sup>2+</sup>	2.1	96 ± 4	500
CO <sub>3</sub> <sup>2-</sup>	2.3	98 ± 4	500
Melamine	2.6	97 ± 3	500
Vanillin	2.4	97 ± 3	500
Acetaminophen	2.3	95 ± 4	250
Paracetamol	2.0	94 ± 5	250
Theophylline	2.8	93 ± 5	250
Nicotinamide	2.5	93 ± 4	50
Xanthine	2.1	91 ± 3	50
All matrix species	3.2	90 ± 4	250

**Table 2**  
The analytical performance of the presented method.

Parameters	Analytical value
Correlation coefficient ( $R^2$ )	0.9986
Linear range ( $\mu\text{g L}^{-1}$ )	90–1000
LOD ( $3S_b/m$ ) $\mu\text{g L}^{-1}$	27.3
LOQ ( $10S_b/m$ ) $\mu\text{g L}^{-1}$	90
RSD (N = 5, 100 and 200 $\mu\text{g L}^{-1}$ )	1.9 and 2.6
Extraction recovery (N = 5, 100 and 200 $\mu\text{g L}^{-1}$ )	96 ± 3 and 98 ± 2
Enrichment factor	167

**Table 3a**  
Repeatability and reproducibility, precision and accuracy data for extraction and determination of caffeine.

Added ( $\mu\text{g L}^{-1}$ )	Repeatability (N = 5)			Reproducibility (N = 3 × 5)		
	Calculated ( $\mu\text{g L}^{-1}$ )	RSD (%)	Recovery (%)	Calculated ( $\mu\text{g L}^{-1}$ )	RSD (%)	Recovery (%)
175	168.8	3.8	96.5	164.9	4.6	94.2
400	389.2	3.1	97.3	386.8	3.7	96.7
800	790.4	2.4	98.8	780.0	2.9	97.5

**Table 3b**  
Testing the matrix effect in the analysis of real samples for recovery of caffeine (N = 3).

Samples	Recovery of caffeine (%)		
	Low (175 $\mu\text{g L}^{-1}$ )	Middle (300 $\mu\text{g L}^{-1}$ )	High (600 $\mu\text{g L}^{-1}$ )
Green tea	93 ± 3	95 ± 4	97 ± 3
Black tea	94 ± 2	96 ± 2	98 ± 3
Tea bag	95 ± 3	97 ± 3	98 ± 2
Dark tea	95 ± 3	97 ± 3	98 ± 2
Coffee	93 ± 3	94 ± 3	97 ± 3
Nescafe	92 ± 2	93 ± 5	96 ± 3
Cola	96 ± 2	97 ± 4	99 ± 2
Energy drink 1	95 ± 3	96 ± 4	98 ± 1
Energy drink 1	95 ± 4	97 ± 2	98 ± 3
Espresso	91 ± 3	95 ± 3	97 ± 2
Red bull	95 ± 2	96 ± 3	98 ± 3
White chocolate	96 ± 3	96 ± 3	98 ± 2

Because of the low analyte levels and complex matrices of samples, analytical method development is necessary for sensitive and selective determination of caffeine using UV-vis. spectrophotometer. Several extractions and preconcentration methods have been used in order to solve these problems such as liquid–liquid microextraction (Shishov et al., 2019), magnetic solid phase extraction (Rahimi, Zanjanchi, Bakhtiari, & Dehsaraei, 2018), stir-bar sorptive extraction (Ghani, Ghoreishi, &

**Table 4**  
Application results of the presented method to the same real samples (N = 3, P = 0.95; tk = 2.78).

Samples	Presented method Calculated (mg L <sup>-1</sup> )	Reference method (Sereshti, & Samadi, 2014)	**t-value
		Calculated (mg L <sup>-1</sup> )	
Green tea	45 ± 3	47 ± 2	0.98
Black tea	97 ± 4	100 ± 3	1.05
Tea bag	75 ± 4	70 ± 4	1.54
Dark tea	118 ± 5	123 ± 4	1.36
Coffee	62 ± 2	65 ± 3	1.47
Nescafe	124 ± 3	129 ± 4	1.55
Cola	144 ± 6	152 ± 5	1.34
Energy drink 1	340 ± 15	356 ± 13	1.40
Energy drink 1	281 ± 12	298 ± 14	1.61
Espresso	316 ± 10	301 ± 12	1.67
Red bull	388 ± 17	362 ± 19	1.78
White chocolate	55 ± 2	58 ± 2	1.84

\*Mean ± SD,

\*\*The criterion t-value established by two paired ANOVA analysis for 6-degree of freedom at 95% confidence limit where  $t_{\text{exp}} = (m_a - m_b)/S_{\text{pooled}} \times [(n_1 + n_2)/(n_1 \times n_2)]^{1/2}$  and  $S_{\text{pooled}} = [(n_1 - 1)S_{m,1}^2 + (n_2 - 1)S_{m,2}^2/(n_1 + n_2 - 2)]^{1/2}$ .

Azamati, 2018), ultrasound-assisted emulsification microextraction (Rahimi, Khorshidi, & Heydari, 2020), dispersive liquid–liquid microextraction (Sereshti, & Samadi, 2014), hollow fiber liquid phase microextraction (Xiong, Chen, He, & Hu, 2010). Orbital shaker based on dispersive solid phase microextraction (OS-DSPME) method has some advantages such as simple, cheap, simultaneous extraction of analytes including ten different test tube and efficiency operational extraction of analytes.

Chlorinated polypropylene (PP-Cl) is an elastomer widely used to the modification reactions. The chlorinated moieties of PP-Cl can be reacted with functional organic substances. For example, an amphiphilic polymer can be obtained by the reaction of PP-Cl with polyethylene glycol (Balci, Alli, Hazer, Guven, Cavicchi, & Cakmak, 2010). Imidazol functionalized PP-Cl was used as a biofuel cell (Kilic, Korkut, & Hazer, 2015). Very recently, the antioxidant property of the tannic acid functionalized PP-Cl has been reported (Hazer, & Ashby, 2021).

In this study, a novel and newly synthesized xanthate functionalized PP-Cl was used as adsorbent for orbital shaker based on dispersive solid phase microextraction (OS-DSPME) of caffeine. This PP-Cl derivative contains dithio carbonate units and sulfur atoms can associate hydrogen bonds with the carboxylic acid moieties of the caffeine. Developed method was successfully applied to several tea, coffee, energy drink, coca-cola and chocolate samples for the determination of their caffeine contents.

## 2. Experimental

### 2.1. Instrumentation

A double beam UV–Visible spectrophotometer (Shimadzu UV-1800 PC model, Tokyo, Japan) was used for absorption measurements. For mixing and centrifugation processes Multi Bio RS-24 (BioSan, Berlin, Germany) model orbital rotator and universal-320 Hettich (London, England) centrifuge were used. pH of solutions was measured using the HI 2211 pH/ORP meter. Ultra-pure water was obtained by a Milli-Q water purification system (Millipore, USA). Ultrasonic bath (SK5210LHC Kudos, Shanghai, China) was used to prepare the samples for analysis.

Table 5

Comparison of the presented method with other previously reported methods for the determination and extraction of caffeine in different samples.

Extraction procedure	Analysis method	Linear range ( $\mu\text{g L}^{-1}$ )	LOD ( $\mu\text{g L}^{-1}$ )	RSD (%)	Recovery (%)	EF	References
UAE	HPLC-UV	160–25000	50	<4	89–116.6	–	(Al-Othman et al., 2012)
A-HLLME-DES	HPLC-UV	100–2000	30	<5	101–103	18	(Shishov et al., 2019)
MSPME	SWV	97–3880	9.7	<4.4	95–103	–	(Filik, & Avan, 2018)
HLLME	GC-MS	160–5000	50	<4.8	99.1–104.9	11	(Amimi, & Hashemi, 2018)
UAEME	HPLC-UV	50–400	15.2	<3.6	96.8–101.8	–	(Rahimi et al., 2020)
UAE-MSPE	GC-MS	50–5000	10	<5.9	93.1–97.7	–	(Rahimi et al., 2018)
SBSE	HPLC-UV	0.2–200	0.1	<6.1	91.2–104	50	(Ghani et al., 2018)
SPME	UV-VIS spectrophotometer	90–1000	27.3	<2.6	96–98	167	Present method

UAE: Ultrasound assisted extraction; HPLC-UV: High performance liquid chromatography with ultraviolet spectroscopy; A-HLLME-DES: Automated homogeneous liquid–liquid microextraction using deep eutectic solvent; MSPME: Magnetic solid phase microextraction; SWV: Square wave voltammetry; GC-MS: Gas chromatography–mass spectrometry; HLLME: homogeneous liquid–liquid microextraction; UAEME: ultrasound-assisted emulsification microextraction; UAE-MSPE: Ultrasound assisted extraction-magnetic solid phase extraction; SBSE: Stir bar sorptive extraction.

## 2.2. Materials and chemicals

Molar mass of chlorinated polypropylene (PP-Cl) was 57 kDa with polydispersity (PDI) 3.57. PP-Cl has one chlorine atom in average in three repeating units with MW 147 Da and supplied from Sigma-Aldrich. Potassium-O-ethyl dithiocarbonate (potassium xanthogonate, KXa) was supplied from Merck, Germany. All chemicals were analytical grade, highly pure, and used without further purification. Caffeine pure substance (1,3,7-trimethyl-1H-purine-2,6 (3H,7H)-dione) was obtained from Sigma (St. Louis, MO, USA). 1000 mg L<sup>-1</sup> caffeine stock solution was prepared by dissolving 1000 mg of caffeine in 1 L distilled deionized water. Working solutions were freshly prepared by sequential dilution of the stock solution. Eluent solutions such as ethanol and methanol were purchased from Sigma, while nitric acid and tetrahydrofuran (THF) were purchased from Merck (Darmstadt, Germany). Citrate buffer (0.1 M, pH 6.0) solution was prepared with mixture of 2.43 g of sodium citrate dihydrate and 0.34 g of citric acid in 100 mL water.

## 2.3. Synthesis and characterization of PP-Xa

PP-Cl (1.47 g) was dissolved in 20 mL of tetrahydrofuran. KXa (2.24 g) was added in this solution and stirred at 40 °C for 24 h. Xanthate functionalized chlorinated polypropylene (PP-Xa) was isolated from the aqueous solution by pouring in excess water (300 mL). The precipitated polymer was washed with distilled water and dried under vacuum at 40 °C for 24 h (Fig. S1).

The characteristic signals of the obtained new polymer (PP-Xa) were observed. FTIR (cm<sup>-1</sup>): 2952 (C–H), 1100–1200, and 705 (C–S) (Fig. S2); 1H NMR ( $\delta_{\text{ppm}}$ ): 6.70 (–CH<sub>2</sub>S–), 4.0–4.6 (CH<sub>3</sub>–CH<sub>2</sub>–O–), 2.36 (CH<sub>3</sub>–CH<sub>2</sub>–O–), 0.80–1.60 (CH–, CH<sub>2</sub>– and CH<sub>3</sub>– main chain of polypropylene), 3.50 (unreacted –CH<sub>2</sub>Cl of PP-Cl) (Fig. S3) (Postma, Davis, Li, Moad, & O'Shea, 2006).

## 2.4. Sample preparation

In order to investigate the applicability of the OS-DSPME procedure, green tea, black tea, tea bag, dark tea, coffee, nescafé, cola, energy drink, espresso, red bull and white chocolate were collected from markets in Sivas/Turkey. These samples were prepared according to the following procedure (Sereshti, & Samadi, 2014). Solid samples were firstly ground into homogeneous powder with a laboratory blender. Then, 1 g of the solid samples was accurately weighed using analytical balance and poured into 50 mL conical tubes. Liquid samples (3 mL) were taken with the help of a micropipette and transferred to 50 mL conical tubes. About 15 mL of boiled water was added to the conical tubes, and then the tubes were placed in the ultrasonic bath and sonicated for 15 min at room temperature to facilitate the removal of caffeine from the samples into the aqueous solution. In order to separate the solid particles from the aqueous solution, the tubes were centrifuged at 4000 rpm for 5 min and

then filtered with a membrane filter and stored at 4 °C until the OS-DSPME procedure is applied.

## 2.5. OS-DSPME procedure

The extraction of caffeine from the collected samples was carried out as follows. 10 mL of prepared samples were added to 50 mL conical tubes including 150  $\mu\text{g}$  caffeine. Then, the pH of the sample solutions was adjusted to pH 6.0 with citrate buffer. Next, 90 mg of the synthesized PP-Xa adsorbent was added to the tubes to adsorb the caffeine in the sample solution. In order to accelerate the interaction between the sample solution and the adsorbent, the tubes were placed on the orbital rotator and then mixed at 250 rpm for 12 min. Followed by centrifugation (4000 rpm 3 min), the PP-Xa adsorbent including caffeine was collected at the bottom of the tubes and the aqueous portion was then discharged by decantation. To elute the caffeine from the PP-Xa adsorbent, 1.5 mL of ethanol was added to the tubes and vortexed for 90 s. Then, the aqueous portion was transferred to microcuvettes, analyzed with a UV-vis spectrophotometer at lambda max (325 nm). All experimental steps were continued with the sample blank and performed in triplicate.

## 2.6. Calculation of extraction efficiency and validation assays

Extraction efficiency (E.E%) was evaluated as a reference to determine the appropriate values of experimental factors in the optimization studies. The E.E% was calculated according to the.

Equation 1.

$$E.E (\%) = 100 \times C_{\text{final}} V_{\text{final}} / C_0 V_0 \quad (1)$$

Where  $V_{\text{final}}$ ,  $V_0$ ,  $C_{\text{final}}$  and  $C_0$  refer to the volume of the final phase, volume of the sample solution, the amount of caffeine in the measured phase, and the initial amount of caffeine in the sample solution, respectively.

Standard deviation was calculated for blank samples (N = 10) under identical conditions. Detection limit (LOD) and quantification limit (LOQ) were calculated according to the Equation 2 and 3, respectively.

$$\text{LOD} = 3S_{\text{blank}}/m \quad (2)$$

$$\text{LOQ} = 10S_{\text{blank}}/m \quad (3)$$

Where  $S_{\text{blank}}$  is standard deviation of blank samples, m is the slope of the calibration curve.

Enrichment factor (EF) was calculated according to the Equation 4.

$$\text{EF} = m_1/m_2 \quad (4)$$

Where  $m_1$  is the slope for the calibration curve without the OS-DSPME procedure while  $m_2$  is the slope of the calibration curve after the OS-DSPME procedure.

The precision was investigated by the repeatability and reproducibility of the OS-DSPME procedure. Six identical samples with added standard caffeine solution were analyzed in five consecutive days for calculating relative standard deviation (RSD). The RSD was calculated according to the Equation 5.

$$\text{RSD (\%)} = 100 \times S_{\text{blank}}/X_{\text{average}} \quad (5)$$

Where  $S_{\text{blank}}$  is standard deviation of blank samples,  $X_{\text{average}}$  is mean value containing 100 and 200  $\mu\text{g L}^{-1}$  of 10 mL reagent blank solution.

### 3. Results and discussion

#### 3.1. Optimization of OS-DSPME conditions

##### 3.1.1. Effect of pH

pH plays a vital role in the OS-DSPME procedure and the E.E% is prominently influenced by the pH of the sample solution. The effect of pH on E.E% of caffeine was investigated in range of 3–9. Results show that E.E% of caffeine was obtained in slightly acidic medium at pH 6.0 while lower the E.E% was observed in alkaline medium. Low E.E% at lower pH may be due to caffeine' stability. Based on maximum E.E% pH 6.0 was selected as optimum pH. Results for different pH vs E.E% were given in Fig. 1a.

##### 3.1.2. Effect of amount of the PP-Xa

The selection of a suitable adsorbent was of prime importance in an OS-DSPME procedure. Selection criteria for the suitable adsorbent were based on its cost-effectiveness, availability, eco-friendly, high selectivity, and E.E%. In this procedure, a new of the adsorbent "PP-Xa" was synthesized for caffeine. The amount of adsorbent that must be added to the sample solution is very important in order for the caffeine in the sample solution to be adequately adsorbed to the PP-Xa. If the amount of the PP-Xa added is insufficient, quantitative analytical results cannot be obtained because full adsorption is not completed. The addition of excessive adsorbent both increases the cost and causes unnecessary chemical consumption. Considering these reasons, the effect of the PP-Xa amount on the E.E% of caffeine was investigated in the range of 10–120 mg. The results of the study show that maximum E.E% of caffeine was achieved with the addition of 90 mg of the PP-Xa. In addition, the E.E% remained stable in the PP-Xa additions after 90 mg. For these reasons, 90 mg of the PP-Xa was chosen as the optimum value for further studies. Results for different amount PP-Xa vs E.E% were given in Fig. 1b.

##### 3.1.3. Ionic strength of sample solution

In OS-DSPME studies, phase separation is facilitated by increasing the ionic strength of the sample solution. Therefore, the effect of ionic strength on both phase separation and E.E% of caffeine was investigated by adding different volumes of 5% (w/v) NaCl to the sample solution. There was no significant difference between the E.E% obtained (94.6%) in the absence of NaCl and the E.E% obtained (94.2–95.1%) in the added NaCl volumes. For this reason, ionic strength adjuster was not used in this study.

##### 3.1.4. Effect of adsorption time

After adding the PP-Xa to the sample solution, orbital shaker was used to increase the interaction between the PP-Xa and caffeine and to shorten the adsorption time. In this context, the effect of adsorption time on E.E% of caffeine was investigated in the range of 1–30 min at constant shaking speed. As the adsorption time increased from 1 min to 12 min, the E.E% of caffeine increased sharply and remained stable in the following adsorption times. This means that a shaking time of 12 min is sufficient to ensure full adsorption. Therefore, the adsorption time of 12 min was chosen as the optimum value for further studies. Results for different adsorption time vs E.E% were given in Fig. 1c.

##### 3.1.5. Effect of type of elution solvent

After the adsorption step is completed in OS-DSPME procedures, the analytes adsorbed on the synthesized PP-Xa must be taken back into the aqueous solution. In this context, the selection of suitable eluent solvents plays a key role. The eluent solvent to be selected should be environmentally friendly, cheap and a small amount should be sufficient for desorption. In this context, equal volumes of methanol, ethanol, water, pH:6 buffer solution, THF and 1 M  $\text{HNO}_3$  were investigated as desorption solvents. From the results (see Fig. 1d), it was seen that the best E.E% of caffeine was obtained when ethanol was used. It is very important for the selected chemical conditions that there is no desorption especially in water and pH 6 buffer. Therefore, ethanol was chosen as desorption solvent for further studies. Results for different elution solvents vs E.E% were given in Fig. 1d.

##### 3.1.6. Effect of elution solvent volume

The second most important variable in achieving full desorption is the volume of desorption solvent. Sufficient solvent must be added to the PP-Xa so that all caffeine can be taken into the aqueous solution. Therefore, the effect of ethanol volume on the E.E% of caffeine was investigated in the range of 0.5–4.0 mL. The low E.E% obtained when the ethanol volume is <1.5 mL can be attributed to the insufficient volumes added for the adsorption of caffeine on the PP-Xa. In particular, the reduction in E.E% of caffeine at volumes above 1.5 mL is due to the increase in the final volume. Therefore, 1.5 mL of ethanol was used as the desorption solvent volume for further studies. Results for different ethanol volumes vs E.E% were given in Fig. 1e.

##### 3.1.7. Effect of desorption time

After the ethanol was added to the PP-Xa, it must interact well with the PP-Xa to ensure complete desorption. If complete desorption is not achieved, some amount of caffeine remains on the PP-Xa. Therefore, both the E.E% is reduced and less caffeine is detected than expected. For these reasons, after adding ethanol on the PP-Xa, vortex was applied to this mixture to ensure effective interaction. It can be seen in the Fig. 1f that a 90 s vortex is sufficient for quantitative E.E% of caffeine. Therefore, 90 s vortex time chosen as the optimum desorption time for further studies. Results for different desorption time vs E.E% were given in Fig. 1f.

#### 3.2. Adsorption capacity

Adsorption capacity is one of the key factors to evaluate the E.E% of caffeine. The adsorption capacity of the PP-Xa for caffeine was investigated according to the batch adsorption approach. The related experimental includes the following steps. A 50 mL of model solution containing 150 mg of caffeine was spiked to 100 mL beaker containing 90 mg of the PP-Xa. Then, the obtained solution was vortexed for about 1 h. After separation of the PP-Xa from the aqueous solution, the possible amounts of caffeine in the final solution were determined by UV-vis spectrophotometer. Using the formula in the batch adsorption approach (Duran, Ozdes, Gundogdu, Imamoglu, & Senturk, 2011), we calculated the adsorption capacity as 271.9  $\text{mg g}^{-1}$  for caffeine.

#### 3.3. Stability of the PP-Xa

The stability of PP-Xa was investigated by testing the cycle number dependence of E.E% for 150  $\mu\text{g L}^{-1}$  of caffeine, using the same PP-Xa for subsequent cycles. The results showed that no significant reduction in E.E% of caffeine was observed even after 25 cycles of PP-Xa runs for the adsorption and desorption steps of caffeine from the complicated matrices, suggesting that PP-Xa are suitable for prolonged repetitive adsorption/desorption. This adsorbent, which maintains its stability even with high repeated use, is promising for effective extraction of caffeine.

### 3.4. Selectivity of the PP-Xa

Since the adsorption and desorption steps are performed on model solutions, the selectivity of the PP-Xa for caffeine should be tested in the presence of different matrix species. In this context, the selectivity of PP-Xa for caffeine was investigated by adding matrix ions in Table 1 to the model solutions. Tolerance limit, recovery and RSDs were calculated for each matrix ion. With the addition of matrix ions, the absorbance for caffeine were affected less than  $\pm 5\%$ , which shows that this procedure is tolerable to higher amounts of matrix ions and highly selective for caffeine analysis. As a result of the studies, high tolerance limits were obtained for caffeine in the presence of the studied matrix ions. In addition, quantitative recovery and low RSDs indicate that the synthesized adsorbent has high selectivity for caffeine.

### 3.5. Method validation

To evaluate and confirm the validity of the OS-DSPME procedure, some analytical features such as curve equation, linear range, correlation coefficient ( $R^2$ ), LOQ, LOD, EF, RSD and extraction recovery were investigated for extraction and determination of caffeine in complicated matrices. The standard curve equation was  $A = 0.0629[\text{Caffeine amount}] + 0.0072$  with  $R^2 = 0.9986$ . The linearity range was 90–1000  $\mu\text{g L}^{-1}$ . The LOD and LOQ were 27.3  $\mu\text{g L}^{-1}$  and 90  $\mu\text{g L}^{-1}$ , respectively. The RSD were in range of 1.9–2.6% for 100 and 200  $\mu\text{g L}^{-1}$  of caffeine. The EF was 167. The extraction recovery was  $96 \pm 3$ – $98 \pm 2\%$ . Results were shown in Table 2.

The precision of the method was evaluated by repeatability and reproducibility studies. In this regard, six identical samples with standard caffeine solution added were analyzed in five replicates in one day for the repeatability study, while the same samples were analyzed in five consecutive days for the reproducibility study. As a result of the study, the RSD for repeatability was in the range of 2.4–3.8%, while the RSD for reproducibility was in the range of 2.9–4.6%. In addition, high agreement between the added and calculated values is evident from the recoveries obtained (94.2–98.8%). These results showed that the OS-DSPME procedure has good precision. All results were presented in Table 3a.

In order to evaluate the accuracy of the OS-DSPME procedure and the matrix effect, three-level standard solutions of caffeine were added to the studied samples, and then the recovery values were calculated for each added concentration (175, 300 and 600  $\mu\text{g L}^{-1}$ ) by applying the OS-DSPME procedure. In the results in Table 3b, it is seen that quantitative recoveries ( $91 \pm 4$ – $99 \pm 2\%$ ) were obtained. These results indicate both low matrix effect and high accuracy of the method.

The robustness was evaluated by measuring the effect of small changes in the analytical parameters on the E.E% of caffeine. The pH, PP-Xa amount, adsorption time, eluent solvent volume, and desorption time were investigated, while all the other parameters were kept constant. RSD of the E.E% was calculated for each parameter. The RSD for pH, PP-Xa amount, adsorption time, eluent solvent volume, and desorption time were 1.7%, 1.5%, 2.4%, 2.8%, and 1.9% respectively. A lower RSDs shows the robustness of the OS-DSPME procedure.

### 3.6. Real sample analysis

After investigating the validation parameters of the method, the OS-DSPME procedure was applied for the extraction and determination of caffeine from complicated matrices such as green tea, black tea, tea bag, dark tea, coffee, nescafé, cola, energy drink, espresso, red bull and white chocolate. In order to evaluate the reliability of the results of the analysis of real samples, the same samples were also analyzed with the reference method. Then, with the help of the results obtained from both studies, the  $t$ -value was calculated and compared with the critical  $t$ -value. The criterion  $t$ -value generated by the two-paired ANOVA analysis for 6-degrees of freedom at the 95% confidence limit is 2.78. From the results in

Table 4, it is seen that all experimental  $t$ -values are lower than the critical value, which means that the results found with the OS-DSPME procedure have high accuracy. Caffeine levels in the studied samples ranged from  $45 \pm 3$  to  $388 \pm 17 \text{ mg L}^{-1}$ .

### 3.7. Comparing with other methods

The new OS-DSPME procedure was compared with already reported analytical methods in the literature. The reported methods (see Table 5) were associated with many limitations like using more toxic chemicals, more time-consuming, and sometimes more complicated steps were involved. This OS-DSPME procedure was found preferable over the reported method due to the use of a green adsorbent. The OS-DSPME procedure was performed without any heating step. The linear range and LOD values of the OS-DSPME procedure were comparable to those reported in the literature. In addition, the EF of the OS-DSPME procedure was higher than the other methods compared, and the RSD value was lower. Additional to the above-mentioned advantages, this new OS-DSPME procedure is highly selective and faster.

## 4. Conclusions

In the first step of this study, a new xanthate functionalized polypropylene (PP-Xa) was synthesized and characterized for the extraction of caffeine from complicated beverage matrices prior to its UV-vis spectrophotometric analysis. In the second step, for the extraction and determination of caffeine, the necessary OS-DSPME conditions using the PP-Xa adsorbent were determined and optimized. No heat step was required for this procedure. The OS-DSPME procedure was a promising method for the analysis of caffeine. The validation studies shown that the OS-DSPME procedure meets the requirements of linearity, precision and accuracy for the analysis of caffeine in the selected samples. This procedure can be a good alternative to conventional analytical methods for caffeine analysis due to simplicity, high sensitivity, low cost, and short extraction time. The OS-DSPME procedure can be successfully used for the extraction and determination of caffeine in complicated beverage matrices at low levels without any complicated instrumentation.

### CRediT authorship contribution statement

**Nail Altunay:** Investigation, Validation, Writing – original draft, Writing – review & editing, Software. **Mustafa Tuzen:** Investigation, Validation, Writing – original draft, Writing – review & editing, Software. **Baki Hazer:** Investigation, Writing – original draft, Writing – review & editing, Software. **Adil Elik:** Investigation, Supervision.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgments

This work was supported by the Kapadokya University Research Funds (#KÜN.2020-BAGP-001). Dr. M. Tuzen thanks to Turkish Academy of Sciences (TUBA) for partial supports. The authors would like to thank to Sivas Cumhuriyet University and Tokat Gaziosmanpaşa University.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2022.133464>.

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