

## Analytical Methods

## Vortex- assisted liquid- liquid microextraction for the trace determination of potassium bromate in flour food products

Mehrnoosh Sadeghi<sup>a</sup>, Mohammad Saber Tehrani<sup>a</sup>, Hakim Faraji<sup>b,c,\*</sup><sup>a</sup> Department of Chemistry, Science and Research Branch, Islamic Azad University, Tehran, Iran<sup>b</sup> Department of Chemistry, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran<sup>c</sup> Research & Development Department, Behavand Darou-Animal Feed Supplements and Premixes Manufacturer, Alborz, Iran

## ARTICLE INFO

## Keywords:

Potassium bromate  
Flour and flour related food products  
Microextraction technique  
Chemometrics  
Green analytical chemistry

## ABSTRACT

Potassium bromate, also reported as a carcinogenic agent, commonly functions to improve flour in the baking industry to increase bread volume. In this study, a green and novel preconcentration and microextraction method, termed as vortex assisted liquid–liquid microextraction combined with UV–Vis spectrophotometry was developed and utilized for trace determination of Potassium Bromate in food samples. Furthermore, various chemometric methods have been used. Under optimum conditions, the linearity range was obtained in the range between 0.02 and 2 µg/mL. Using the proposed analytical approach, the detection limits and quantitation of KBrO<sub>3</sub> were 0.02 and 0.07 µg/mL, respectively. A pre-concentration factor of 22.2 was reported. The precision of the method was evaluated in the terms of repeatability and reproducibility and expressed by the relative standard deviation; the levels of them were considerably higher than 5.07 and 4.8%. The proposed approach was applied to the determination of trace bromate in different flour products.

## 1. Introduction

Bread is regarded as one of the main staple foodstuffs all over the world, particularly in southeast Asia and Africa (Shanmugavel, Santhi, Kurup, Kalakandan, Anandharaj, & Rawson, 2020). It is a rich source of carbohydrates and various minerals such as calcium, magnesium, and also iron with lower levels. Potassium bromate (KBrO<sub>3</sub>) is widely used as a food additive in the baking industry to improve flour properties (Ali et al., 2013). It is a tasteless and inodorous white powder that is often added to enhance the quality and structural properties of flour and its final products. Also, the Bromate ion acts as an oxidizing agent that plays a vital role in proofing the dough by oxidizing the sulfhydryl groups of gluten proteins into disulfide bonds (Aggrawal & Rohrer, 2020; Joye, Lagrain, & Delcour, 2009; Rahali et al., 2011; Shi, Liang, Cai, & Mou, 2006). However, the International Agency for Research on Cancer based on the clinical evidence, categorized potassium bromate as a potential carcinogen for humans (group 2B) that causes cancer in animal models (Rahali et al., 2011). Therefore, some countries have banned or limited KBrO<sub>3</sub> usage as a maturing agent in bakery products. According to the United States Food and Drug Administration (FDA), the maximum permissible concentration of bromate in bread is 0.02 µg/g (Ali et al., 2013). Generally, during the baking process, KBrO<sub>3</sub> converts

to non-hazardous bromide, but small amounts remain present in bread, if the baking is not performed at high temperatures or long enough. Therefore, monitoring the residual concentration of potassium bromate in bakery products is of utmost importance (Aggrawal et al., 2020).

Various methods such as spectrofluorometric, spectrophotometric approaches, ionic chromatography (IC), HPLC- UV, and also ICP with mass spectrometry have been applied for bromate measurements in flour and other related products (Afkhani, Madrakian, & Bahram, 2005; Arias, Li, Huggins, Keller, Suchanek, & Wehmeyer, 2010; De Borja, Rohrer, Pohl, & Saini, 2005; Gahr, Huber, & Niessner, 1998; Ketai, Huitao, Jian, Xingguo, & Zhide, 2000; Bartakova, Dvorackova, Chromcova, & Hrdlicka, 2020; Michalski & Lyko, 2013). However, no pre-concentration method has been reported to date for bromate determination in food samples with complex matrices. Miniature extraction methods are easier and more economical than conventional solid and liquid phase extraction methods that use only a few milliliters of organic solvent and adsorbent to extract analytes from aqueous solutions. In recent years, dispersive liquid- liquid microextraction (DLLME) has been applied as a powerful approach for pretreatment and pre-concentration of the goal analytes in biological and inorganic samples. In the liquid-liquid micro-extraction method, two extracting and dispersing solvents (acetonitrile, tetrahydrofuran, methanol, and acetone) are used. After

\* Corresponding author at: Naghsh-e-Jahan Sq., 338177489, Pishva, Varamin, Iran.

E-mail address: [hakimfaraji@yahoo.com](mailto:hakimfaraji@yahoo.com) (H. Faraji).<https://doi.org/10.1016/j.foodchem.2022.132109>

Received 23 July 2021; Received in revised form 6 January 2022; Accepted 6 January 2022

Available online 10 January 2022

0308-8146/© 2022 Elsevier Ltd. All rights reserved.

the solvents are added to the sample, a cloud-like solution is formed, after being centrifuged, the extracting organic solvent which is now rich in analyte is separated from the solution and placed in a separate and transparent phase at the bottom of the test tube. The advantages of this method include high pre-concentration factor, having high accuracy results, and as mentioned earlier, easy and cost-effective application (Aydin, Yilmaz, & Soyvak, 2018; Soyvak & Karaca, 2016; Yamini, Rezazadeh, & Seidi, 2019). Nonetheless, this method has a number of drawbacks such as the limitation of using toxic organic solvents, the generation of hazardous waste, and the centrifugation step which is not in accordance with Green Analytical Chemistry objectives (Rezaee, Yamini, & Faraji, 2010).

To eliminate these conventional dispersive and extracting solvents and centrifuging steps, Auxiliary energies such as mechanical, electrical, and also electromagnetic energies have been employed in liquid base microextraction methods (Rezaee et al., 2010). According to these approaches, ultrasonic-assisted liquid-liquid microextraction (UA-LLME), vortex assisted liquid-liquid microextraction (VA-LLME) and pulsed electrically assisted liquid-phase microextraction techniques were developed (Campillo, Vinas, Šandrejová, & Andruch, 2017). Vortex agitation of a solution generates an empty center by using mechanical energy (Ojeda & Rojas, 2014). Vortex may form because of vigorous liquid agitation and could effectively affect the analyte mass transfer. VA-LLME was firstly introduced by Yiantzi et al (Yiantzi, Psillakis, Tyrovolas, & Kalogerakis, 2010).

In order to overcome DLLME limitations, toxic organic solvents used in classic DLLME were substituted with a green solvent and vortex stirring was employed instead of using a dispersing solvent. In this study, for the first time, a green and sustainable microextraction method termed "vortex assisted liquid-liquid microextraction" combined with UV-Vis spectrophotometry to determine trace amounts of potassium bromate in flour and related flour products. Further, using chemometrics methods, the main effects of the independent variables on extraction efficiency were investigated. A validation study was performed according to certified guidance.

## 2. Experimental background

### 2.1. Reagents, standards and real samples

Potassium bromate ( $\text{KBrO}_3$ ), hydrochloric acid (37%), decanol, 1-undecanol, and dodecanol were purchased from Merck Chemicals GmbH (Darmstadt, Germany). Promethazine hydrochloride (PMZ) was obtained from Sigma-Aldrich Chemicals Company (also known as Millipore Sigma, owned by Merck KGaA, Darmstadt, Germany). All the reagents and solvents used in this study were of analytical grade. A standard solution of bromate (500  $\mu\text{g}/\text{mL}$ ) was achieved by dissolving 0.05 g of  $\text{KBrO}_3$  in deionized water. To prepare the stock standard solution of promethazine hydrochloride, the proper amount of PMZ was completely dissolved in deionized water and then stored in a dark place at 4 °C. Aqueous working solutions of the analyte were prepared on the day of analysis by diluting the stock solution with deionized water to achieve appropriate concentrations (0.2–60  $\mu\text{g}/\text{mL}$ ). Four samples were used in this study. Spaghetti (Zarmakaron Co., Alborz, Iran) and Baguette (Cenan Co., Alborz, Iran), and flour (Zar Co., Alborz, Iran) were obtained from a local supermarket (Alborz, Iran). The Lavash bread was bought from a local bakery (Alborz, Iran).

#### 2.1.1. Sample preparation

First, the bread samples were divided into smaller pieces and then dried in an oven at 75 °C. The samples were completely pulverized and 5 g of the powder was dissolved in 20 mL of water. The samples were then centrifuged at 3000 rpm and the solution was passed through filter paper. After adding the optimal amounts of Promethazine and HCl, the solution was diluted to a volume of 5 mL. The extraction solvent was added to it and finally, the solution was subjected to a vortex for one

minute. Finally, the sample absorbance was read at 496 nm using a UV-Vis device. The spaghetti samples were also divided into small pieces and powdered with a mixer. VA-LLME extraction steps were performed under optimal conditions. Finally, the results were reported as a percentage of the recovery. As for the flour, the steps were the same as the previous ones.

### 2.2. Apparatus

A Mekasys Optizen 3220UV double beam UV-Vis (spectrophotometer Korea) which has a measuring range of 190–1100 nm and a micro quartz cuvette black wall in the dimensions of 45 × 12.5 × 12.5 mm with the length and inner duct of 10 and 2 mm, respectively, was used for absorbance measurements. A small vortex agitator (IKA Vortex 4 Basic) was applied during VA-LLME procedure. Design of experiments and data processing were conducted and evaluated using Minitab, version 17.

### 2.3. Reaction mechanism

This reaction is based on redox reactions. Promethazine is easily oxidized with  $\text{KBrO}_3$  in an acidic medium and forms colored compounds. The sulfur atoms in PMZ are sensitive to oxidation and lead to producing colored free radicals. Bromate ion oxidizes PMZ to a colored free radical or semiquinone. Hence, a red-pink color with absorption maxima at 500 nm is appeared due to the heterocyclic centered radical cations (Kojlo et al., 2001; Mahmud, Moni, Imran, & Foyez, 2021).

### 2.4. Vortex assisted liquid-liquid microextraction procedure

The VA-LLME procedure used for potassium bromate was as follows: Firstly, different concentrations of bromate ion working standard were mixed with a sample solution that contained the proper amount of promethazine hydrochloride (0.06 mol/L) and 12 mol/L hydrochloric acid. Subsequently, the redox reaction occurred between bromate and KBr in HCl medium the color of the solution was changed to pink. In order to obtain fine droplets, 0.5 mL of 1-undecanol (as the extracting phase) was slowly added and the mixture was shaken vigorously using a vortex shaker (IKA Vortex 4 Basic) for 1 min at 3000 rpm to form fine droplets. The two phases were separated by centrifugation at 3000 rpm for 3 min. Finally, the organic layer containing the redox products was withdrawn by a suitable microsyringe and the analysis was performed using a UV-Vis spectrophotometer at 496 nm.

## 3. Result and discussion

### 3.1. Optimization of VA-LLME procedure

Sample preparation is considered as a substantial step to determine trace levels of analyte in a complex sample. Therefore, the initial step in the optimization process was to select the appropriate variables. In this study, seven independent parameters were investigated: volume and concentration of HCl, volume and concentration of PMZ, extracting solvent volume, vortex speed, and time. Primarily, a classical univariate approach was adopted to identify the optimum type of extracting solvent. Afterward, a two-step multivariate procedure including Plackett-Burman design (PBD) for screening and Central Composition Design (CCD) for optimization purposes were applied to designate the remaining optimal variables.

#### 3.1.1. Selection of extracting solvent

The present study is largely aimed that reducing or eliminating the use of organic and toxic solvents as well as obtaining high extraction efficiency. Therefore, it is of great significance to choose a suitable extracting solvent. An appropriate extracting solvent should adequately extract the desired analyte in addition to having a low water solubility.

In this regard, three water-immiscible solvents including decanol, 1-undecanol, and dodecanol were evaluated. Initially, 2 mL of each solvent was added to a 5 mL aqueous sample spiked with different bromate ion concentrations. Next, using a vortex agitator (3000 rpm), the mixture was stirred vigorously and subsequently centrifuged at 3000 rpm for 2 min. Among two different solvents, 1-undecanol had the best ability to retain its microdroplet shape and was easily collected for UV-Vis analysis (Fig. S1).

### 3.1.2. Screening design

In the following step, the PBD was employed as a screening approach to evaluate the significant parameters (including, volume and concentration of HCl, volume and concentration of PMZ, extracting solvent volume, vortex speed, and time) and to assess the principal effects of the aforementioned variables on the extraction efficiency using a multivariate approach. Next, a total number of 15 tests (three replicates at the central point to estimate the experimental error) were conducted at random. Table 1 illustrates further details of the experimental design, comprising the corresponding codes, also the high and low levels of each parameter. Moreover, in order to estimate the significance of the model, ANOVA was run on the experiment design. The obtained ANOVA results suggested that the model terms were significant (see supplementary elements, Table S1). The findings obtained from the Pareto chart obviously, revealed that the volume and concentration of PMZ exert substantial effects on bromate ion absorbance ( $p < 0.05$ ) while the other parameters do not play a major part in the VA-LLME extraction efficiency of bromate ion Fig. 1.

### 3.1.3. Optimization design

The two-level design could be applied only for the linear response models and does not provide information on the optimal values or non-linear relationships. Therefore, it is essential to adopt a multi-level design. At last, the parameters with significant effect achieved by PBD analysis (i.e. volume and concentration of PMZ were investigated further.

To optimize the concentration (A) and volume of PMZ (B), the researchers employed a response surface methodology design (RSD) on the basis of central composite design (CCD). All the factors and corresponding levels, symbols, and design matrix for CCD are presented in

**Table 1**  
Experimental variables, codes, levels and absorbance in the PBD design.

Variables	Coded	Levels		
		Low (-1)	Centre (0)	High (+1)
HCl concentration (M)	A	8	10	12
HCl volume ( $\mu\text{L}$ )	B	50	100	150
PMZ concentration (mol/L)	C	0.001	0.01	0.100
PMZ volume ( $\mu\text{L}$ )	D	250	500	750
Extraction phase volume( $\mu\text{L}$ )	E	300	400	500
Vortex speed (rpm)	F	1000	2000	3000
Vortex duration (min)	G	1	3	5

run	order	A	B	C	D	E	F	G	Abs
4	1	1	-1	1	1	-1	1	-1	0.514
14	2	0	0	0	0	0	0	0	0.243
15	3	0	0	0	0	0	0	0	0.229
2	4	1	1	-1	1	-1	-1	-1	0.127
5	5	1	1	-1	1	1	-1	1	0.108
7	6	-1	1	1	1	-1	1	1	0.527
11	7	-1	1	-1	-1	-1	1	1	0.086
6	8	1	1	1	-1	1	1	-1	0.483
13	9	0	0	0	0	0	0	0	0.251
10	10	1	-1	-1	-1	1	1	1	0.119
1	11	1	-1	1	-1	-1	-1	1	0.119
9	12	-1	-1	-1	1	1	1	-1	0.154
12	13	-1	-1	-1	-1	-1	-1	-1	0.076
3	14	-1	1	1	-1	1	-1	-1	0.475
8	15	-1	-1	1	1	1	-1	1	0.489

Table 2 (also see supplementary elements, Table S2). In this research, the CCD matrix contained 14 experiments (4 cube points, 3 center points in cube, and 4 axial points), which were randomly conducted. Moreover, to assess the statistical significance of the model, the analysis of variance (ANOVA) was used. As the results suggested, while the lack of fit (LOF) was insignificant, the regression of the model was significant ( $p < 0.05$ ). Additionally, as reflected in the results, for the linear coefficients (A and B), quadratic term coefficient ( $A^2$ ), and cross product coefficients (AB) ( $p < 0.05$ ), while the quadratic term coefficient ( $B^2$ ) has no significant effect ( $p > 0.05$ ). Furthermore, a second-order polynomial equation is regarded as the response regression model which could be presented by Eq. (1) as follows:

$$Y = 0.2330 + 2.0039 A + 0.000487B - 3.2977 A^2 - 0.00 B^2 - 0.0014AB \quad (1)$$

In this study, the value of the determination coefficient ( $R^2 = 99.84\%$ ) as a scale of the variation around the mean depicted a positive correlation between the experimental data and the fitted response model. Based on Eq. (1) three dimensional (3D) two-parameter response surface plots were obtained to determine the optimal conditions for achieving the maximum extraction efficiency of potassium bromate Fig. 2. As illustrated in Fig. 1, the optimum conditions were obtained in about 1104  $\mu\text{L}$  of PMZ (0.06 mol/L). According to the results, 1104  $\mu\text{L}$  of PMZ and up to 0.06 mol/L PMZ concentration was chosen as the optimum condition for the following experiments.

### 3.2. Method validation

The primary goal of the present study was to devise a novel analytical method that could determine trace amounts of  $\text{KBrO}_3$  in bread samples using VA-LLME combined with UV-Vis spectrophotometry. Validation of the proposed analytical approach is one of the leading features to support the reliability of the results. Validation of the proposed method was statistically conducted according to the International Conference on Harmonization (ICH) guidance (ICH, 2005) and based on the validation process described in our previous study (Mostafavi, Feizbakhsh, Kono, & Faraji, 2019). The calibration model, linearity range, detection and quantitation limit (LOD and LOQ respectively), precision (repeatability and reproducibility), and accuracy were investigated by employing a matrix-matched strategy.

To reduce the effect of the sample matrix on the analysis results the calibration curve was obtained by preparing flour samples that did not contain the analyte. They were spiked with stock potassium bromate ion solution in the concentration range (0.02–2  $\mu\text{g}/\text{mL}$ ) under optimal conditions. The coefficient of determination ( $R^2 = 0.9983$ ) clearly indicated the positive relationship between response and each parameter. The linearity range for  $\text{KBrO}_3$  quantification was obtained in the range of 0.02–2  $\mu\text{g}/\text{mL}$ .

Also, the researchers quantified the limit of detection (LOD) and the limit of quantitation based on the IUPAC's new recommendation as a measure of analytical sensitivity. Accordingly, LOD and LOQ were obtained 0.02 and 0.07  $\mu\text{g}/\text{mL}$ , respectively (Table 3).

The proposed method's accuracy was evaluated by comparing the relative recovery (RR %) of  $\text{KBrO}_3$  in the sample spiked at three different levels of analyte (0.02, 0.04, and 0.06  $\mu\text{g BrO}_3^-$ ). The results were statistically analyzed using a  $t$ -test at a 95% level of confidence, expressing the null hypothesis as:  $H_0$  and the alternative hypothesis:  $R_{\text{exp}} = 100\%$  (Faraji & Helalizadeh, 2017). The results demonstrated no statistical difference ( $p > 0.05$ ); therefore,  $t_{\text{exp}} < t_{(0.025, N-1)}$ , and the accuracy of the VA-LLME/UV-Vis procedure for determining the trace of  $\text{KBrO}_3$  was confirmed (Table 3). Thus, the proposed approach had a reliable efficiency for pre-concentration and determination of potassium bromate in different flour food products.

Furthermore, the precision was assessed by repeatability and reproducibility and was indicated through a relative standard deviation percentage (RSD %). The repeatability was evaluated by performing 10

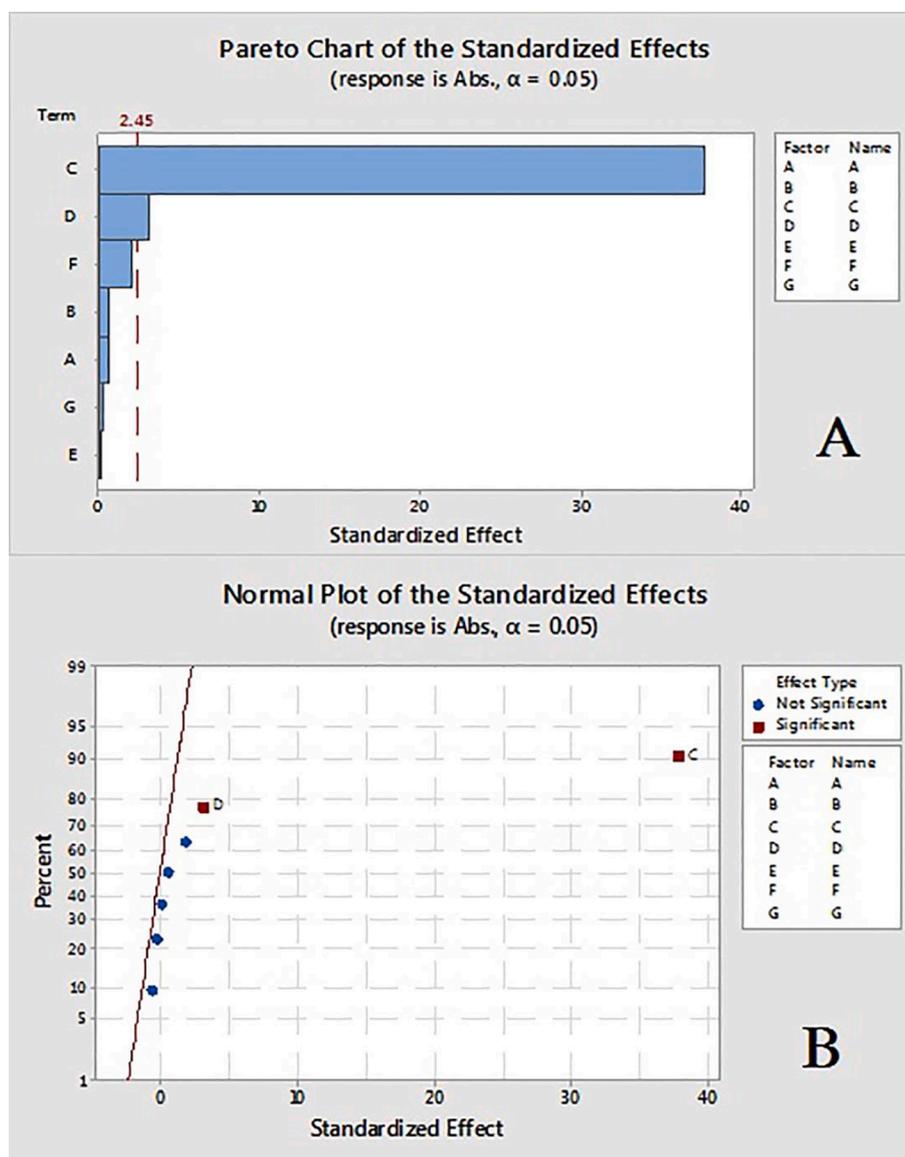


Fig. 1. (A) Pareto charts of the main effect achieved from the PBD, (B) Normal probability plot of standardized effects.

experiments on the same day at low, medium, and high concentration levels of  $\text{KBrO}_3$  spiked in the samples. In order to determine the reproducibility, 21 experiments were conducted during three days (three concentration levels, triplicates). The precision was desirable because all obtained RSD values were below the maximum mentioned limit. As presented in Table 4, the repeatability and reproducibility values ranged between 4.8 and 6.7% and 5.07–7.4%, respectively. Furthermore, ANOVA was run to assess the reproducibility during three consecutive days. In accordance with the results, the  $p$ -value was obtained smaller than 0.05 at the 95% confidence level.

### 3.3. Specificity

The specificity was measured by investigating the impacts of common interfering ions on VA-LLME of bromate ions. For this purpose, the sample solution was spiked with various concentrations of different ions and determined under the optimized process. The relative error  $\leq 5$  was employed as the tolerance limit, revealing the maximum concentration of interfering ion leading to a 5% variation in the analytical signal of bromate ion. As displayed in Table S3, the ions do not interfere with the pre-concentration and determination of bromate ions.

The results show that PMZ is relatively selective, and as it does not reduce different ions, it can be used selectively along with VA-LLME /UV-Vis for the determination of bromate ions in flour-based samples.

### 3.4. Analysis of real samples

Applicability of the proposed procedure was examined for the analysis of the bromate ion under optimum conditions in Lavash bread, Baguette, flour, and spaghetti samples fortified with different amounts of potassium bromate. The concentration of analyte identified in various real flour-based samples was represented in Table 4. Also, the recovery experiments were carried out by using the standard addition method in 0.02, 0.04 and 0.06  $\mu\text{g BrO}_3^-$ . Hence, the recovery was considered as percent recovery which assayed as the difference between the mean and the accepted value. Ultimately, the statistical evaluation of the obtained results was carried out using a  $t$ -test at 95% level of confidence. The obtained results (Table 4) revealed no statistical difference and the accuracy of the proposed VA-LLME approach was confirmed in accordance to  $t_{\text{exp}} < t_{(0.025, N-1)}$ .

**Table 2**  
Experimental variables, codes, level and absorbance in the CCD.

Variable	Coded	Levels			Star point	
		High (+1)	Center (0)	Low (-1)	+a	-a
PMZ Concentration (mol/L)	A	0.1	0.05	0.01	0.123	0.008
PMZ volume ( $\mu\text{L}$ )	B	1000	750.0	500.0	1103.553	396.477
Run	Order	A	B	Abs.		
10	1	-a	0	0.547		
8	2	0	-a	0.293		
14	3	0	0	0.528		
9	4	0	+a	0.526		
13	5	0	0	0.534		
12	6	0	0	0.528		
11	7	+a	0	0.512		
2	8	-1	1	0.550		
4	9	1	1	0.521		
3	10	1	-1	0.677		
6	11	0	0	0.541		
5	12	0	0	0.546		
7	13	0	0	0.579		
1	14	-1	-1	0.561		

#### 4. Conclusion

To overcome the environmental concerns of the conventional DLLME method, a green, simple, fast, and economical approach based on VALLME coupled with UV-Vis spectrophotometry was developed to pre-concentrate and determine the trace amount of  $\text{KBrO}_3$  in flour and related foods. In this regard, Promethazine hydrochloride was used as a reactant undergoing oxidation in the identification and quantification of bromate. A green and sustainable solvent (1-undecanol) was used as extraction phase and the vortex shaker was applied instead of dispersing agent this method complies with a number of principles of green analytical chemistry, including the replacement of toxic solvents with safer ones, reduction of organic waste, reduction of sample volume, ease of process automation, operator safety, which is recommended by Galuska et al. (Galuska, Migaszewski, & Namieśnik, 2013)

Additionally, the chemometric methods such as PBD, CCD and RSM were utilized for optimization of the independent factors. The figures of merit of the proposed method including linearity, sensitivity, accuracy, and calibration curve were statistically acceptable and reliable. Finally, the applicability of the proposed method was evaluated by using the proposed method to assess the bromate ion in flour and flour related foods.

**Table 3**  
Results of method validation (sensitivity, accuracy and precision).

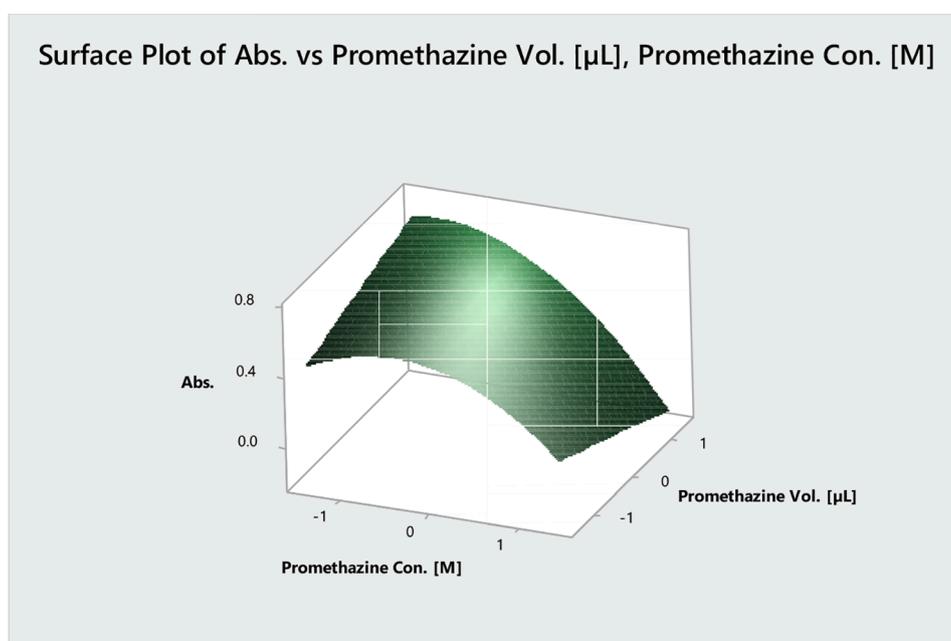
Accuracy		$t_{\text{exp}}^a$	Precision	
Spiked ( $\mu\text{g BrO}_3^-$ )	RR% n = 3		Repeatability n = 10	Reproducibility n = 7
0.02	97.00	2.006	6.10	7.40
0.04	98.50		6.70	6.40
0.06	91.20		4.80	5.07

<sup>a</sup>  $t(0.025, 8) = 2.30$ .

**Table 4**  
The accuracy of proposed procedure for bromate analysis in flour- based food samples.

Sample	Spiked ( $\mu\text{g BrO}_3^-$ )	Found ( $\mu\text{g/mL}$ )	Recovery (%)	RSD% (n = 3)	$t_{\text{exp}}^a$
Flour	0.00	Nd. <sup>b</sup>	-	-	1.76
	0.02	0.02 <sup>c</sup>	89.90	4.40	
	0.04	0.04	98.30	5.90	
	0.06	0.06	97.70	4.07	
Lavash bread	0.00	Nd.	-	-	1.05
	0.02	0.02	91.00	5.64	
	0.04	0.04	98.00	4.83	
	0.06	0.06	101.3	4.70	
Baguette	0.00	Nd.	-	-	1.74
	0.02	0.02	90.00	5.20	
	0.04	0.04	93.00	4.50	
	0.06	0.06	100.6	4.50	
Spaghetti	0.00	Nd.	-	-	-2.00
	0.02	0.02	98.00	5.07	
	0.04	0.04	91.00	4.60	
	0.06	0.06	97.50	5.10	

<sup>a</sup>  $t(0.025, 8) = 2.30$ . <sup>b</sup> Not detection. <sup>c</sup> The numbers were rounded.



**Fig. 2.** Response surface plots of analytical signal vs PMZ volume and PMZ concentration (mol/L).

## CRediT authorship contribution statement

**Mehrnoosh Sadeghi:** Investigation, Conceptualization, Methodology, Writing – original draft, Formal analysis, Resources, Validation, Visualization. **Mohammad Saber Tehrani:** Supervision, Project administration. **Hakim Faraji:** Supervision, Project administration, Conceptualization, Methodology, Software, Writing – review & editing.

## 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

The authors thank Dr. Koorosh Kazemzadeh, Behavand Darou–Animal feed supplements and premixes manufacturer and Razi Lab in Islamic Azad University. We would also like to show our gratitude to Ghazal Ardalan, Bachelor of Science, Department of Public Health, University of California, Irvine, USA, for writing assistance, language editing, and proofreading.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2022.132109>.

## References

- Afkhami, A., Madrakian, T., & Bahram, M. (2005). Simultaneous spectrophotometric determination of iodate and bromate in water samples by the method of mean centering of ratio kinetic profiles. *Journal of Hazardous Materials*, *123*(1-3), 250–255.
- Aggrawal, M., & Rohrer, J. S. (2020). Selective and sensitive determination of bromate in bread by ion chromatography-mass spectrometry. *Journal of Chromatography A*, *1615*, 460765. <https://doi.org/10.1016/j.chroma.2019.460765>
- Alli, A., Nwegbu, M., Inyang, B., Nwachukwu, K., Ogedengbe, J., Onaadebo, O., & Onifade, E. (2013). Assessment of bread safety: Determination of potassium bromate in selected bread samples in Gwagwalada, Abuja. *International Journal of Health and Nutrition*, *4*, 15–20.
- Arias, F., Li, L., Huggins, T. G., Keller, P. R., Suchanek, P. M., & Wehmeyer, K. R. (2010). Trace analysis of bromate in potato snacks using high-performance liquid chromatography–tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, *58*(14), 8134–8138.
- Aydin, F., Yilmaz, E., & Soylak, M. (2018). Vortex assisted deep eutectic solvent (DES)-emulsification liquid-liquid microextraction of trace curcumin in food and herbal tea samples. *Food Chemistry*, *243*, 442–447.
- Bartakova, M., Dvorackova, E., Chromcova, L., & Hrdlicka, P. (2020). Simple phenols in tropical woods determined by UHPLC-PDA and their antioxidant capacities: An experimental design for Randall extraction using environmentally friendly solvents. *Journal of Forestry Research*, *31*(3), 819–826.
- Campillo, N., Viñas, P., Sandrejšová, J., & Andrich, V. (2017). Ten years of dispersive liquid–liquid microextraction and derived techniques. *Applied Spectroscopy Reviews*, *52*(4), 267–415.
- De Borja, B. M., Rohrer, J. S., Pohl, C. A., & Saini, C. (2005). Determination of trace concentrations of bromate in municipal and bottled drinking waters using a hydroxide-selective column with ion chromatography. *Journal of Chromatography A*, *1085*(1), 23–32.
- Faraji, H., & Helalizadeh, M. (2017). Lead quantification in urine samples of athletes by coupling DLLME with UV-vis spectrophotometry. *Biological Trace Element Research*, *176*(2), 258–269.
- Gahr, A., Huber, N., & Niessner, R. (1998). Fluorimetric determination of bromate by ion-exchange separation and post-column derivatization. *Microchimica Acta*, *129*(3-4), 281–290.
- Gatuszka, A., Migaszewski, Z., & Namieśnik, J. (2013). The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices. *TrAC Trends in Analytical Chemistry*, *50*, 78–84.
- ICH, H. (2005). Validation of analytical procedures: text and methodology, Q2 (R1). Current Step 4 Version, Parent Guidelines on Methodology Dated November 6 1996. Incorporated in November 2005. *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland*.
- Joye, I. J., Lagrain, B., & Delcour, J. A. (2009). Use of chemical redox agents and exogenous enzymes to modify the protein network during breadmaking—A review. *Journal of Cereal Science*, *50*(1), 11–21.
- Ketai, W., Huitao, L., Jian, H., Xingguo, C., & Zhide, H. (2000). Determination of bromate in bread additives and flours by flow injection analysis. *Food Chemistry*, *70*(4), 509–514.
- Kojlo, A., Karpinska, J., Kuzmicka, L., Misiuk, W., Puzanowska-Tarasiewicz, H., & Tarasiewicz, M. (2001). Analytical study of the reaction of phenothiazines with some oxidants, metal ions, and organic substances. *Journal of Trace and Microprobe Techniques*, *19*(1), 45–70.
- Mahmud, S. S., Moni, M., Imran, A. B., & Foyez, T. (2021). Analysis of the suspected cancer-causing potassium bromate additive in bread samples available on the market in and around Dhaka City in Bangladesh. *Food science and nutrition*, *9*(7), 3752–3757.
- Michalski, R., & Lyko, A. (2013). Bromate determination: State of the art. *Critical Reviews in Analytical Chemistry*, *43*(2), 100–122.
- Mostafavi, B., Feizbakhsh, A., Konoz, E., & Faraji, H. (2019). Hydrophobic deep eutectic solvent based on centrifugation-free dispersive liquid–liquid microextraction for speciation of selenium in aqueous samples: One step closer to green analytical chemistry. *Microchemical Journal*, *148*, 582–590.
- Bosch Ojeda, C., & Sánchez Rojas, F. (2014). Vortex-assisted liquid–liquid microextraction (VALLME): Applications. *Chromatographia*, *77*(11-12), 745–754.
- Rahali, Y., Benmoussa, A., Ansar, M., Benziane, H., Lamsaouri, J., Idrissi, M., ... Taoufik, J. (2011). A simple and rapid method for spectrophotometric determination of bromate in bread. *Electronic Journal of Environmental, Agricultural & Food Chemistry*, *10*(1).
- Rezaee, M., Yamini, Y., & Faraji, M. (2010). Evolution of dispersive liquid–liquid microextraction method. *Journal of Chromatography A*, *1217*(16), 2342–2357.
- Shanmugavel, V., Komala Santhi, K., Kurup, A. H., Kalakandan, S., Anandharaj, A., & Rawson, A. (2020). Potassium bromate: Effects on bread components, health, environment and method of analysis: A review. *Food Chemistry*, *311*, 125964. <https://doi.org/10.1016/j.foodchem.2019.125964>
- Shi, Y., Liang, L., Cai, Y., & Mou, S. (2006). Determination of trace levels of bromate in flour and related foods by ion chromatography. *Journal of agricultural and food chemistry*, *54*(15), 5217–5219.
- Soylak, M. (2016). Vortex-assisted dispersive liquid-liquid microextraction of Pb (II) as 2-hydroxypyridine-3-carboxylic acid chelates from food and water samples prior to flame atomic absorption spectrometric determination. *Atomic Spectroscopy*, *37*(3), 108–113.
- Yamini, Y., Rezazadeh, M., & Seidi, S. (2019). Liquid-phase microextraction—The different principles and configurations. *TrAC Trends in Analytical Chemistry*, *112*, 264–272.
- Yiantzi, E., Psillakis, E., Tyrovola, K., & Kalogerakis, N. (2010). Vortex-assisted liquid–liquid microextraction of octylphenol, nonylphenol and bisphenol-A. *Talanta*, *80*(5), 2057–2062.