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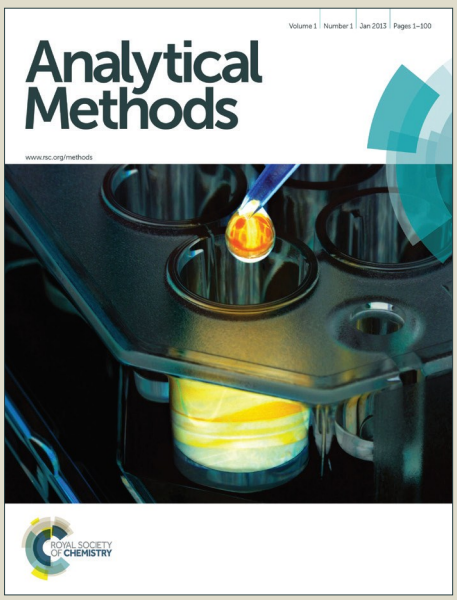

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Spectrophotometric determination of cyclamate in artificial sweeteners and beverages after ultrasound-assisted emulsification microextraction

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Abstract

A new method has been developed for determination of cyclamate using ultrasound-assisted emulsification microextraction (USAE-ME) procedure coupled with UV-Vis spectrophotometry. The method is based on the protonation of cyclamate ions in acidic medium and extraction of the formed cyclamic acid into fine droplets of chloroform as an extraction solvent that contain Rhodamine B (RhB) reagent. The extracted cyclamic acid can further react with RhB for formation of a highly colored ion-pair complex of [cyclamate][$RhBH^+$], which used for subsequent spectrophotometric determination of cyclamate. One variable at a time optimization and response surface methodology (RSM) based on central composite design were used to obtain optimum conditions for microextraction procedure and nearly same experimental conditions were obtained using both optimization methods. Under the optimum conditions the calibration graph was linear over the range 50-900 ng mL^{-1} (R^2=0.9994) and the limit of detection (S/N=3) was estimated to be 10 ng mL^{-1}. Relative standard deviation for a 200 ng mL^{-1} of cyclamate was 2.3% (n=5). The purposed method was successfully applied for determination of cyclamate in beverages and sweetener tablets. The average recovery of spiked samples was 99.7%. The results demonstrated that the developed method is simple, rapid, inexpensive, accurate and remarkably free from interference effects.

Keyword: Ultrasound-assisted emulsification microextraction, Spectrophotometric determination, cyclamate, Rhodamine B

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1. Introduction

Artificial sweeteners are widely used as additives in food, beverages and pharmaceutical products as a non-caloric alternative to sugars. Cyclamate (cyclohexylsulphamic acid, monosodium salt) is an artificial sweetener that is 35 times sweeter than sugar. It has been widely used in low-calorie foods and beverages. It has been reported that cyclamate might increase the risk of bladder cancer in humans when it is converted into cyclohexylamine in the gastrointestinal tract. Although recent animal studies fail to demonstrate that cyclamate is a carcinogen or a co-carcinogen, other issues must be resolved before cyclamate can be approved for commercial use as a food additive.[1]

Nowadays, cyclamate is approved for use in more than 50 countries worldwide. The acceptable daily intake value for cyclamate has been set at 11 mg/kg body weight by the Joint Expert Committee on Food Additives (JECFA) and at 7 mg/kg body weight by the Scientific Committee for Foods (SCF).[2] The permitted levels of use vary from 250 to 1500 mg/kg depending on food category.[3] Because the safety of cyclamate to human is not clear completely; the restricted content level in foods and beverage is different in different countries. Therefore, it is necessary to develop sensitive and reliable method for the determination of cyclamate in a wide range of food and beverage samples.

Standard methods for cyclamate determination are Kjeldahl method,[4] gravimetric analysis[5] and redox titration.[4] These methods are tedious, time consuming and suffer from multiple interferences. Flow injection methods have also been proposed, involving detection by chemiluminescence,[6] flame atomic absorption spectrometry,[7] turbidometry[8] and biamperometry.[9] All procedures involve previous sample treatment, continuous reagent addition in several steps and generating considerable amount of waste. Several chromatographic methods such as gas

chromatography, [10-14] high performance liquid chromatography (HPLC), [15,16] HPLC-mass spectrometry,[17,18] capillary electrophoresis[19,20] and ion chromatography[21] have also been reported for cyclamate determination. However, these methods require complex sample preparation procedures, extended cleanup steps and chemical derivatization in order to overcome interference effects and improve the characteristics of cyclamate for chromatographic separation and detection system. Also, these methods require the involvement of skilled personnel, well equipped laboratories and expensive instrumentation.

Various spectrophotometric techniques have been developed for cyclamate determination. Cyclamate has poor absorbance in ultraviolet region and therefore, a chemical derivatisation is often performed in order to provide suitable sensitivity and selectivity. The treatment of cyclamate with nitrous acid followed by diazotization and coupling with 2-aminoethyl-1-naphthyamine,[22] hydrolysis of cyclamate to cyclohexylamine and the subsequent reaction with 1,2-naphtoquinine-4-sulphonate[23] and reaction of cyclamate with chlorine for formation of N-N-dichlorcyclohexylamine[24] have been used for generation of spectrophotometrically active derivatives. Also the reaction of cyclamate with an excess of nitrite solution and determination of unconsumed nitrite using Griess reaction[25] or with safranine as a reagent[26] has been described.

Since the matrices of food and beverages samples are often complex, sample preparation plays an important role in the analytical procedures. Recently ultrasound-assisted emulsification microextraction (USAE-ME) has been introduced as an efficient liquid phase microextraction, and applied for extraction and preconcentration of different analytes.[27-30] This technique is based on the emulsification of micro-volume of organic extraction solvent in aqueous phase by ultrasound radiation and further separation of two phases. The application of ultrasound radiation facilitates the emulsification phenomenon and accelerates the mass-transfer process between two

immiscible phases, which together with the large surface of contact between the phases leads to an increment in the extraction efficiency in a minimum amount of time.[31, 32] This method has certain advantages including high enrichment factor, low consumption of organic solvent, ability of combination with different determination methods and low cost. Up to now, this method has been successfully applied for determination of organic and inorganic compounds in many fields, but most of its applications were focused on the couplings with advanced analytical instruments. In this work, USAE-ME coupled with UV–Vis spectrophotometry has been applied for preconcentration and quantitative determination of the cyclamate.

UV-Vis spectrophotometry is used extensively for determination of various inorganic and organic species, and is available easily in most laboratories. It has the advantages of significant precision and accuracy, low cost, and easy handling. However, the application of this technique for analysis of different real samples is limited by its poor sensitivity and selectivity. Hyphenation of it with some advanced microextraction methods can overcome these problems.[33] Ion pair formation of ionic species with different colorants have aroused considerable interest in extractive spectrophotometry. Microextraction of such colored ion associate for subsequent spectrophotometric determination can provide sensitive, relatively simple and fast approach to routine analysis. In continuation of our previous research work on application of USAE-ME for spectrophotometric determination of some compounds, [34,35] the present paper describes the successful application of USAE-ME procedure for extractive spectrophotometric determination of cyclamate using RhB reagent. The method is based on the USAE-ME of cyclamate in acidic media and subsequent formation of an ion-pair complex for spectrophotometric determination of cyclamate. To the best of our knowledge no studies for USAE-ME of cyclamate and its spectrophotometric determination with RhB have been reported. The main parameters

influencing extraction and determination were investigated in details. The results of this study show that hyphenation of USAE-ME procedure with ordinary UV–Vis spectrophotometer equipped with a quartz microcell can significantly improve the sensitivity of measurements. Analytical characteristics of the method are evaluated and compared with other methods.

2. Experimental

2.1. Chemicals and standards

All chemicals were of analytical high grade. Carbon tetrachloride, chloroform, nitrobenzene and chlorobenzene, as extraction solvent, rhodamine B (RhB) as a cationic dye, sodium cyclamate, sodium chloride, sulfuric acid (98%) and nitric acid (65%) were purchased from Merck (Darmstadt, Germany). Doubly distilled deionized water was used throughout. Cyclamate working solutions were prepared daily by stepwise dilution from standard stock solution (1000 mg L^{-1}) in double distilled water. Solution of the RhB dissolved in chloroform was prepared daily. All test tubes cleaned with 0.1 M nitric acid, deionized water and acetone.

2.2. Instrumentation

A UV-Vis Spectrophotometer Model T80 (PG Instruments Ltd., Korea) with a 100 µL quartz microcell (Fisher Co., Germany) was used for the spectrophotometric determination. A 40 kHz ultrasonic water bath Model Parsonic 2600s (Parsnahand Co, Iran) was applied for emulsification process and phase separation was achieved via a centrifuge Model 16105 (Farayand Co., Iran) in 10 mL calibrated conical glass tubes (Isolab Co., Germany). Vortex mixer Model L46 (LABIN Co., Netherlands) was used for better combining and accelerating reaction between reagents.

2.3. USAE-ME procedure

A 5 mL aliquot of the sample solution containing cyclamate and 1.0×10^{-1} M H_2SO_4 was placed in a 10 mL screw cap glass test tube with conical bottom. The tube was immersed into ultrasonic bath in such a way that the levels of both liquids (in bath and sample tube) were the same. Then, 200 µL of chloroform (extraction solvent) containing RhB (2×10^{-4} mol L^{-1}) was injected rapidly into the sample solution using a 250 µL syringe. Emulsification and extraction was performed at 40 kHz of ultrasonic frequency for 20 s at 25 ± 1◦C. As a result, oil-in-water (O/W) emulsions of chloroform (dispersed phase) in water (continuous phase) were formed. After equilibrium time (1 min), emulsion disrupted by centrifugation at 3500 rpm for 5 min, which resulted in the sedimentation of colored organic phase at the bottom of the conical test tube. 100 µL of the settled down phase was quantitatively transferred to quartz microcell using a syringe for the spectrophotometric analysis.

2.4. Preparation of real samples

All samples, including tablet sweetener, soft drink and fruit juice drink were purchased from local market. They were pretreated by the relevant procedures as follows. The final solutions were filtered through 0.45 µm nylon filters and the filtrates were further diluted to obtain desired concentration of cyclamate before the analysis.

2.4.1. Sweetener tablet

Sweetener tablets (n = 10) were placed in a mortar and ground to a fine powder. Then, 50.0 mg of powder was dissolved in water and diluted to 50 mL.

2.4.2. Soft drink

The soft drink was degassed for 5 min in an ultrasonic bath, before being diluted by water. Then a 10 mL of soft drink was diluted to 50 mL in a calibrated flask.

2.4.3. Fruit juice drink

A 10 mL volume of the fruit juice drink was directly diluted to 50 mL in a calibrated flask.

2.5. Experimental Design

Central composite design (CCD) was used for efficient optimization of the microextraction conditions. CCD is one of the most frequently used response surface methodology (RSM), is affected by a combination of several factors. RSM plays an important role in designing, formulating, developing and analyzing new scientific research, as well as improving existing studies and products. Three independent variables, namely concentration of RhB (X1), concentration of H_2SO_4 (X2) and sonication time (X3), were studied at five levels with four replicates at the central point, using a CCD method. For each of the three studied variables, high and low set points were selected to construct an orthogonal design as shown in Table 1. The design matrix for 18 experimental sets and the observed values of the corrected absorbance for cyclamate are shown in Table 2.

For an experimental design with three factors, the model including linear, quadratic, and cross terms can be expressed as Eq. (1)

$$Response = b0 + b1X_1 + b2X_2 + b3X_3 + b4X_1 \times X_1 + b5X_2 \times X_2 + bX_3 \times X_3 + b7X_1 \times X_2 + b8X_1 \times X_3 + b9X_2 \times X_3 \quad (1)$$

Within Eq. (1), X_1 to X_3 are the variable parameters, and b0 to b9 are the coefficient values obtained through multiple linear regressions (MLR). The response surface plots were obtained through a statistical process that describes the design and the modeled CCD data. Response surface methodologies graphically illustrate relationships between parameters and responses and are the way to obtain an exact optimum.[36, 37] The Design-Expert software (Trial Version 8.0.0, Stat-Ease Inc., Minneapolis, MN, USA) was employed to analyze the data and experimental design.

In order to show the fitness of the model, the squared regression coefficient (R^2) is used. However, the adjusted regression coefficient (R^2_{adj}) and the prediction regression coefficient (R^2_{pred}) are better criteria than absolute regression coefficient (R^2). Since R^2 always decreases when a regression variable is eliminated from the model in statistical modelling, the R^2_{adj}, which takes the number of regression variables into account, is usually selected.[38, 39] In addition, R^2_{pred}, which indicates the predictive power of the model, is chosen for the same reason. This parameter was approximated using prediction error sum of squares (PRESS) that is calculated from residuals. Therefore, R^2, R^2_{adj}, and R^2_{pred} altogether are very convenient to get a quick impression of the overall fit and the prediction power of a constructed model.[40]

3. Result and discussion

In this study, USAE-ME coupled with UV–Vis spectrophotometry has been applied to determination of trace amounts of cyclamate ion. Presented method is based on extracting an acidic solution of cyclamate ion in chloroform and subsequent protonation of RhB dissolved in chloroform to form a highly colored ion-pair complex which is readily soluble in organic solvents. The absorption spectrum of formed ion-pair shows a maximum absorbance at 560 nm (Fig. 1) which can be used as the wavelength for the analytical determination. The reagent blank at this wavelength shows a low absorption. The remarkable color difference after USAE-ME procedure between sample solution containing cyclamate ion (pink color) and blank solution (colorless) is the key factor contributed to the high sensitivity of method for cyclamate ion determination. This technique is very rapid and efficient but there are many factors that should be optimized before its application. In this work, optimization was performed both via one variable at a time and CCD methods. The corrected absorbance was selected as the extraction

efficiency under different experimental conditions and all results were average of three replicate measurements.

3.1. One variable at a time method

3.1.1. Selection of type and volume of the extraction solvent

The selection of an appropriate extraction solvent is critical to the USAE-ME process since its physicochemical properties not only affect the emulsification phenomenon but also the extraction efficiency. A suitable extraction solvent should have higher density than water, low solubility in water, high extraction capability for the formed ion-pair, least tendency to the reagent blank and good emulsification efficiency. On the basis of these considerations, carbon tetrachloride, chloroform, nitrobenzene and chlorobenzene were selected as potential extraction solvents for the study. Among studied solvents, chloroform showed higher extraction efficiency, considerably lower absorbance for reagent blank and better emulsification efficiency in shorter times, therefore, chloroform was chosen as extraction solvent for the subsequent studies. In order to examine the effect of the extraction solvent volume, different volumes of chloroform (170-250 μL) were used as the extraction solvent for the same USAE-ME procedure. The volumes smaller than 170 μL were avoided due to dissolution of organic phase in aqueous phase and because of the difficulty of sample manipulation which led to a reduction in precision. It was observed that the sensitivity increased gradually by increasing the volume of chloroform up to 200 μL and then decreased with further increases in chloroform volume due to dilution effects. Hence a volume of 200 μL was used for further experiments.

3.1.2. Effect of sulfuric acid concentration

The sulfuric acid concentration is a key parameter on the formation of the cyclamic acid and its effective extraction into chloroform droplets. In the present study, the extraction of cyclamate

was examined under different sulfuric acid concentration in the range of 1.0×10^{-5} - 5.0×10^{-1} mol L^{-1}. According to the obtained results, sensitivity increased by increasing concentration of H_2SO_4 up to 1.0×10^{-2} mol L^{-1} and then was nearly constant by increasing concentration of H_2SO_4. Therefore, 1.0×10^{-1} mol L^{-1} H_2SO_4 was selected as optimum amount of acid for further studies.

3.1.3. Effect of the RhB concentration

RhB was selected due to its ability to form a colored ion-pair complex with the cyclamate ion. The effect of the RhB concentration on the measured absorbance was studied in the range of 2.0×10^{-5} – 3.5×10^{-4} mol L^{-1}. As Fig. 2 shows, the highest extraction efficiency was obtained over the RhB concentration range 1.5×10^{-4}- 2.5×10^{-4} mol L^{-1}. Thereby, a concentration of 2×10^{-4} mol L^{-1} RhB was selected for subsequent experiments.

3.1.4. Effect of salt addition

The effect of salt addition on the performance of USAE-ME was investigated by adding different amounts of sodium chloride (0–3%, w/v) in aqueous solution. The results (Fig.3) revealed that the extraction efficiency decreases with the increasing of NaCl concentration. This could be considered as the result of two major competitive effects: salting-out effect and viscous resistance effect.[41] Based on the experimental results, no addition of salts was chosen in the subsequent studies.

3.1.5. Effect of sonication time

The time of sonication plays an important role in the emulsification and mass transfer phenomena. Sonication produces fine droplets of organic solvent in the aqueous bulk which results in a great contact area between two phases and therefore, provides better mass transfer and higher extraction efficiency. However, long sonication time may result in the increasing of the solubility of formed ion-pair and organic solvent in aqueous phase. These can reduce the

extraction efficiency. Sonication time was examined in the range of 15–50 s under constant ultrasound power. As shown in Fig. 4, by increasing of sonication time, the absorption of the formed ion-pair complex remained nearly constant up to 25 s and decreased gradually up to 50 s. Therefore, the sonication time of 20 s was selected for further studies. This sonication time was sufficient to ensure that effective emulsification was occurred without any possible analyte loss due to increased solubility.

3.1.6. Effects of equilibrium and centrifugation time

In USAE-ME, Equilibrium time was defined as time interval between the formation of homogeneous cloudy solution and phase separation by centrifugation. The effect of the equilibrium time was investigated in the range of 0.5–5 min. The results showed that the variations of complex absorbance versus extraction time are not remarkable. In fact, the surface area between microdrops of organic phase and aqueous sample solution is infinitely large and consequently, the mass transfer from sample solution to extraction solvent is very fast. Therefore, the equilibrium state is achieved quickly and extraction time is very short. This is the most important advantage of this method. Thus, the time of 1 min was selected as equilibrium time for subsequent experiments.

Centrifugation was required to break down the emulsion and accelerate the phase separation process. The effect of centrifuging time was evaluated in the range of 2–10 min at 3500 rpm. The results showed that the best extraction efficiency was achieved with a centrifuging time of 5 min. When the centrifuging time was longer than 5 min, the absorbance remained constant.

3.2. Experimental design and response surface modeling

Experimental design provides the optimal values of significant variables which give the maximum responses. After performing some preliminary experiments, the extraction conditions were optimized using the CCD. Effective parameters such as concentration of RhB, concentration of H_2SO_4, and sonication time were included in the design. A full quadratic model including all terms of Eq. (1) for cyclamate was used in the first step. Then to evaluate the significance of each factor and interaction terms, analysis of variance (ANOVA) was used and the insignificant terms ($p > 0.05$) were eliminated from the model through a 'stepwise elimination' process. Table 3 contains the regression coefficients for each term in the model and the analysis of variance (ANOVA) of the effects. By the elimination of insignificant terms of Eq. (1) from the constructed model, calibration R^2 decreased to 0.976 but adjusted R^2, and R^2 of prediction increased to 0.964 and 0.927, respectively. From the ANOVA results (Table 4), the model was found to be significant, with a p-value less than 0.0001 and F-value of 76.14. The lack-of-fit (LOF) P-value of 0.126 implies that the LOF was not significant relative to the pure error. Fig. 5 shows the predicted values versus the observed values of responses. Most plots were scattered monotonously around the line; this indicates a good correlation between predicted and actual responses, and thus a good fit for the proposed quadratic model.

In order to gain insight about the effect of each variable, the three dimensional (3D) plots for the predicted responses were formed based on the model function. The response surface plots are represented in Fig. 6, which show the 3D plots of absorbance of samples (560 nm) versus pairs of variables while one of the variables is considered to be constant at its optimum point. As shown in Fig. 6, there was a non-linear relation between the response and the variables X_1–X_3, because the surface plots of the response are curvature.

From the constructed models (the results of Table 3 and the response surfaces of Fig. 6), the following results were concluded: concentration of H_2SO_4 (X_2) affects the model by only linear variables while concentration of RhB (X_1), and sonication time (X_3) affect by both linear and quadratic terms. Also the response surface showed that there is an interaction between concentration of H_2SO_4 and sonication time. With increasing of acidity, the ionic strength increases and this can reduce the intensity of ultrasound wave for affection emulsification, due to absorption of ultrasound wave in viscous media. After the analysis of results, the following conditions were selected to evaluate the performance of the extraction procedure: concentration of RhB 2×10^{-4} mol L^{-1}, concentration of H_2SO_4 1.0×10^{-1} and sonication time 20 s.

3.3. Analytical parameters

Analytical characteristics of the presented method were evaluated under optimized conditions. The calibration graph was linear between 50 and 900 ng mL^{-1}, with the linear regression equation A = 0.0012 C + 0.056 (C, ng mL^{-1} cyclamate) and correlation coefficient of 0.9994 (number of calibration points, n = 11). The limit of detection (LOD), based on a signal-to-noise ratio of 3 was 10 ng mL^{-1}. The obtained LOD is lower than the permitted level imposed by SCF for soft drinks (250 mg L^{-1})[3], indicating the suitability of the method for determination of cyclamate in real samples. The precision of the method was investigated by determining intra-day precision and inter-day precision (expressed at RSD%). The inter-day precision was evaluated over five replicates spiked at concentration level 200 ng mL^{-1} of cyclamate within one day. The intra-day precision was evaluated over five daily replicates, spiked at same level per work day, over period of three days. The inter-day and intra-day precisions were 2.3% and 2.9%, respectively. The preconcentration factor (PF) was calculated as the ratio between the volumes of settled down phase (V_{sed}) and aqueous sample (V_o). Under experimental conditions, the collected organic

phase from the bottom of test tube was 175µL. With a 5mL final sample solution, an enrichment factor of 28.6 was found for cyclamate determination. Comparison of the analytical features and general characteristics of proposed method and other methods for determination of cyclamate are presented in Table 5 and 6. The presented method has distinct advantages in terms of low limit of detection, better RSD, ease of operation, high selectivity and simplicity.

3.4. Interference study

A study of interferents was performed with samples containing 200 ng $mL^{-1}$of cyclamate and 50-fold excesses of potential interferents under the optimized conditions. No interference (<5%) was caused from the presence of large amounts of common substances present in soft drinks and artificial sweeteners mixtures: saccharin, fructose, aspartame, lactose, dextrose, glucose, sodium citrate, ascorbic acid, acetic acid and caffeine. This investigation showed that the method is remarkably free from interference effects.

3.5. Analysis of real samples

The applicability of the presented method to the real samples was investigated by determination of cyclamate in soft drinks and sweetener tablets. Recovery studies were carried out by adding known concentration of cyclamate at three levels to dilute solution of samples that did not contain any cyclamate. For each concentration level, three replicate experiments were made and the results obtained were compared with the added concentrations. The obtained relative recoveries (Table 7) varied from 92.3 to 104.2%, evidencing the absence of matrix effect on the performance of proposed method. Accuracy of the method was checked with the AOAC official method.[5] The results are presented in Table 8. The statistical analysis of the results using Student's t- test showed that there are no significant difference between results obtained by two methods at 95% confidence level.

4. Conclusion

In this research, ultrasound-assisted emulsification microextraction coupled with UV–Vis spectrophotometry has been successfully applied for preconcentration and quantitative determination of the cyclamate in different actual samples including: artificial sweeteners and beverages samples with acceptable recoveries (92.3-104.2%) and RSD (<5%). Low consumption of organic solvent, high enrichment factor, low cost, simplicity, short analysis time, good precision, and no matrix interference compared to other methods are clear advantages of the proposed method. The developed method was successfully used for the quantitative analysis of cyclamate in real samples. Analytical characteristics of the method are evaluated and compared with other methods.

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Figure captions

Fig. 1. Absorption spectra for the ion-pair of [Cyclamate$^-$][RhBH$^+$] (a) and blank solution after USAE-ME(b) conditions; sample volume: 5.0 mL, Cyclamate: 550ng mL^{-1}, RhB: 2×10^{-4} mol L^{-1}, H_2SO_4: 1.0×10^{-1} mol L^{-1}, extractant: **,** 200 µL of chloroform, sonication time: 20 s, equilibrium time: 1 min, centrifuging time: 5 min at 3500 rpm.

Fig. 2. Effects of RhB concentration on ion-pair complex absorption. conditions; sample volume: 5.0 mL, Cyclamate: 500ng mL^{-1}, H_2SO_4: 1.0×10^{-1} mol L^{-1}, extractant: , 200 µL of chloroform, sonication time: 25s, equilibrium time: 1 min, centrifuging time: 5 min at 3500 rpm. Error bars represent the standard deviation for three experiments.

Fig. 3. Salt addition effect on ion-pair complex absorption, conditions; sample volume: 5.0 mL, Cyclamate: 500ng mL^{-1}, RhB: 2×10^{-4} mol L^{-1}, H_2SO_4: 1.0×10^{-1} mol L^{-1}, extractant: **,** 200 µL of chloroform, sonication time: 25s, equilibrium time: 1 min, centrifuging time: 5 min at 3500 rpm. Error bars represent the standard deviation for three experiments.

Fig.4. Effects of sonication time on the ion-pair complex absorption, conditions; sample volume: 5.0 mL, Cyclamate: 500ng mL^{-1}, RhB: 2×10^{-4} mol L^{-1}, H_2SO_4: 1.0×10^{-1} mol L^{-1}, extractant: **,** 200 µL of chloroform, equilibrium time: 1 min, centrifuging time: 5 min at 3500 rpm. Error bars represent the standard deviation for three experiments.

Fig. 5. The predicted response vs. the observed response.

Fig. 6. The Response surface plots for the effects of variables on response.

Table 1

The variables and values used for central composite design (CCD).

Coded factor levels						
Variable name		-1.67(low)	-1	0	1	1.67(high)
X_1	RhB($\times10^{-5}$mol L^{-1})	7	11	16	21	25
X_2	-Log H_2SO_4 (mol L^{-1})	1	1.8	3	4.2	5
X_3	Sonication time (s)	20	26	35	44	50

Table 2

Design matrix and responses for the central composite design.

Run	RhB($\times10^{-5}$mol L^{-1})	-Log H_2SO_4 (mol L^{-1})	Sonication time(s)	Corrected Absorbance
1	25.0	3.0	35.0	0.650
2	16.0	3.0	35.0	0.671
3	16.0	3.0	50.0	0.700
4	11.0	4.2	26.0	0.524
5	11.0	1.8	44.0	0.568
6	16.0	3.0	35.0	0.691
7	21.0	1.8	26.0	0.971
8	21.0	4.2	44.0	0.658
9	11.0	4.2	44.0	0.494
10	16.0	3.0	35.0	0.660
11	21.0	4.2	26.0	0.708
12	16.0	1.0	35.0	0.881
13	16.0	5.0	35.0	0.442
14	16.0	3.0	20.0	0.950
15	11.0	1.8	26.0	0.769
16	16.0	3.0	35.0	0.650
17	7.0	3.0	35.0	0.302
18	21.0	1.8	44.0	0.818

Table 3

Estimated regression coefficients and analysis of variance (ANOVA) for response surface quadratic model.

Model terms	Coefficient estimate	Mean square	p-value
Intercept	0.67	-	-
X_1	0.099	0.140	<0.0001
X_2	-0.11	0.160	<0.0001
X_3	-0.063	0.053	0.0002
X_1X_2	-0.013	1.355E-03	0.3314
X_1X_3	3.51E-03	9.870E-05	0.7873
X_2X_3	0.035	9.378E-03	0.0262
X_1^2	-0.055	0.048	0.0003
X_2^2	3.41E-03	1.421E-04	0.7464
X_3^2	0.062	0.047	0.0003

Table 4

ANOVA results for the obtained models of cyclamate

Source	Sum of Squares	df	Mean square	F Value[a]	p-value	prob>F
Model	0.49	6	0.081	76.14	<0.0001	significant
Residual	0.012	11	1.067E-03			
Lack of fit	0.011	8	1.351E-03	4.38	0.1260	not significant
Pure error	9.260E-04	3	3.087E-04			

[a] Test for comparing model variance with residual (error) variance.

Table 5

Comparison of analytical features of diverse methods for the determination of cyclamate.

Method	LOD[a] (ng mL^{-1})	LR[b] (ng mL^{-1})	RSD%	Ref.
FI-chemiluminescence	400	1000-50000	-	[6]
FI–spectrophotometry	1540	<201200	3.5	[23]
AAS	250	1000-90000	3.1	[7]
HPLC-UV	110	300-30000	-	[15]
HPLC-MS	5	50-5000	-	[17]
HS-SDME–GC	890	5340-178000	4	[13]
GC-ECD	50	5000–250000	0.28	[14]
USAE-ME-spectrophotometry	10	50-900	2.3	This work

[a] Limit of detection.

[b] Linear range.

Table 6

Comparison of the general characteristics of different methods for the determination of cyclamate

Method	Derivatization/ sample pretreatment	Sample through-put	Recovery (%)	Cost of equipment	Remarks	Ref.
FI-chemiluminescence	Sensitizing of chemiluminogenic oxidation of sulphite by cerium(IV) in acidic media	100 h^{-1}	-----	Moderate	Waste volume: High Selectivity: Low Complexity:Moderate	[6]
FI-Spectroph-otometry	Hydrolysis by H_2O_2 in 80 ^oC and derivatization by sodium 1,2 -naphthoquinon-4-sulfonate	-----	-----	Moderate	Waste volume: High Complexity: Low Selectivity: High	[23]
AAS	Oxidation by Sodium nitrate in acidic media and precipitation by lead ion in ethanol	35 h^{-1}	95-100	High	Waste volume: High Complexity:Moderate Selctivity: High	[7]
HPLC-UV	Hydrolysis by H_2O_2 in 100^o C and derivatization by trinitro-benzenesulfonic acid	4 h^{-1}	95-99	High	Waste volume:High Complexity: High Selectivity:High	[15]
HPLC-MS	Ion pair formation by tris (hydroxymethyl)aminomethane	-----	97-102	High	Waste volume: Low Selectivity: High Complexity: High	[17]
SDME-GC-FID	Oxidation by sodium nitrate in acidic media followed by HD-SDME into n-dodecane	5 h^{-1}	96-98	Relatively high	Waste volume:Mild Selectivity: High Complexity:Moderate	[13]
GC-ECD	Oxidation by sodium hypo-chlorite in acidic media followed by LLE into n-hexan	-----	86-97	Relatively high	Waste volume: High Selectivity: High Complexity: High	[14]
USAE-ME-Spectrophotometry	USAE-ME of cyclamate into chloroform and ion pair formation with RhB	10 h^{-1}	92-104	Low	Waste volume: Low Selectivity: High Complexity: Low	This work

Table 7

Determination of cyclamate (mean ± SD, n=3) in artificial sweeteners and beverages.

Sample	Added (ng mL^{-1})	Found (ng mL^{-1})	Recovery (%)
artificial sweeteners	-	ND [a]	-
	100	95 ± 3	95.0
	200	209 ± 4	104.5
	350	342 ± 5	97.7
Soft drink	-	ND	-
	100	102 ± 3	102.0
	200	192 ± 5	96.0
	350	343 ± 6	98.0
Fruit juice drink	-	ND	-
	100	103 ± 4	103.0
	200	194 ± 3	99.0
	350	359 ± 7	102.5

[a] Not detected.

Table 8

Determination of cyclamate in real samples by proposd method and official method

Sample [a]	concentration of cyclamate	
	Proposed method	Official method[5]
Artificcial swetner (mg g^{-1})		
Sample A	195 ± 4	200 ± 6
Sample B	159 ± 4	162 ± 5
Soft Drink (mg L^{-1})		
Sample C	743 ± 5	739 ± 6
Sample D	ND	ND

[a] sample composition: (A) sodium cyclamate, sodium saccharin and fructose; (B) sodium cyclamate, sodium sacchrin, dextrose and aspartame; (C) carbonated water, caramel, caffeine; sacchrin, sodium cyclamate, essence, sodium benzoate and citric acid; (D) water, orange juice, sugar, natural orangeflavour, citric acid and witamine C

[b] All results: Mean ± stand ard deviation of three replicate determinations

Fig 1

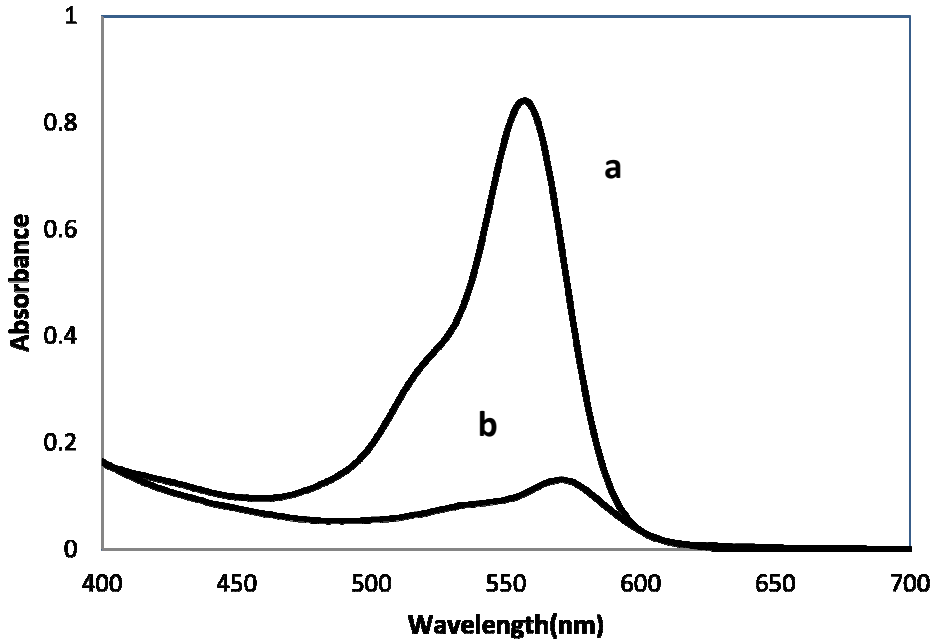

Fig 2

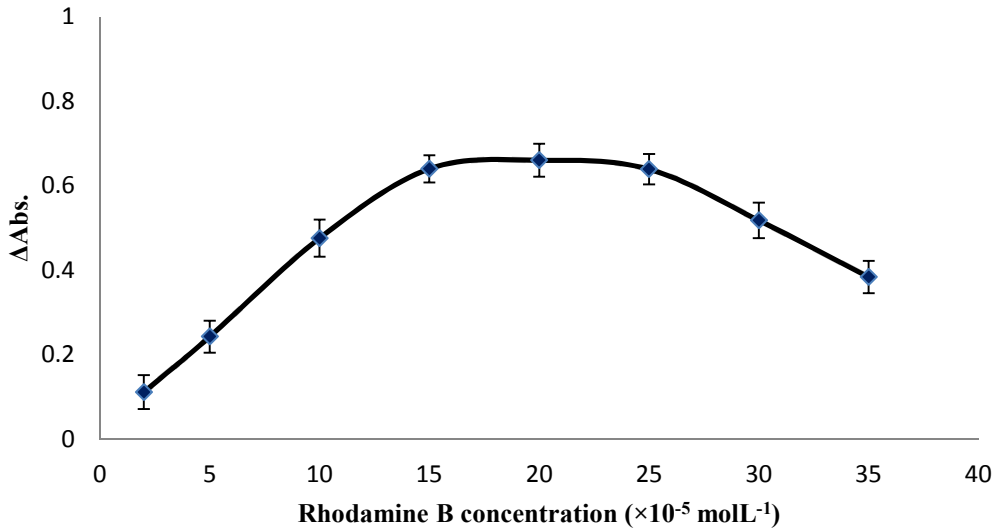

Fig 3

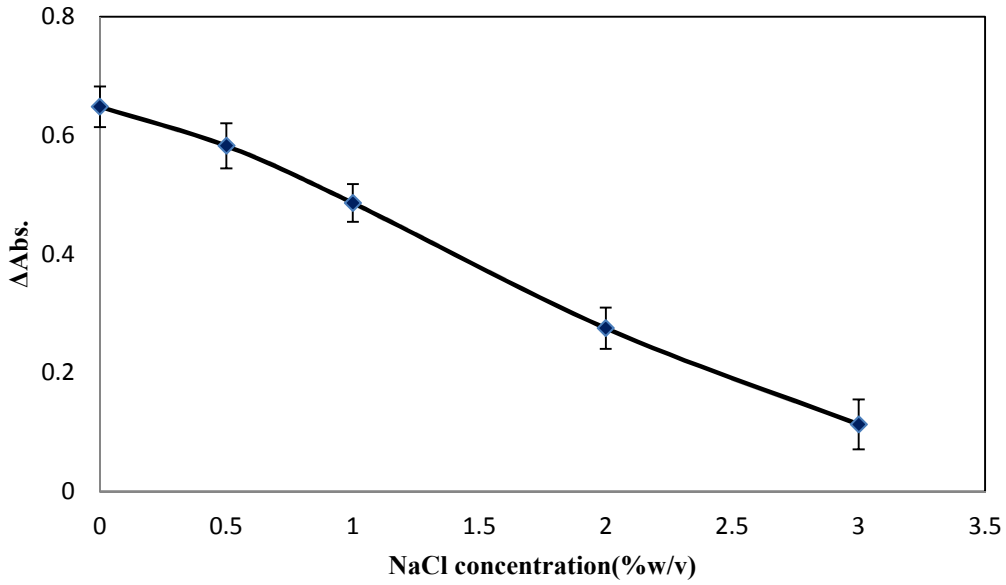

Fig 4

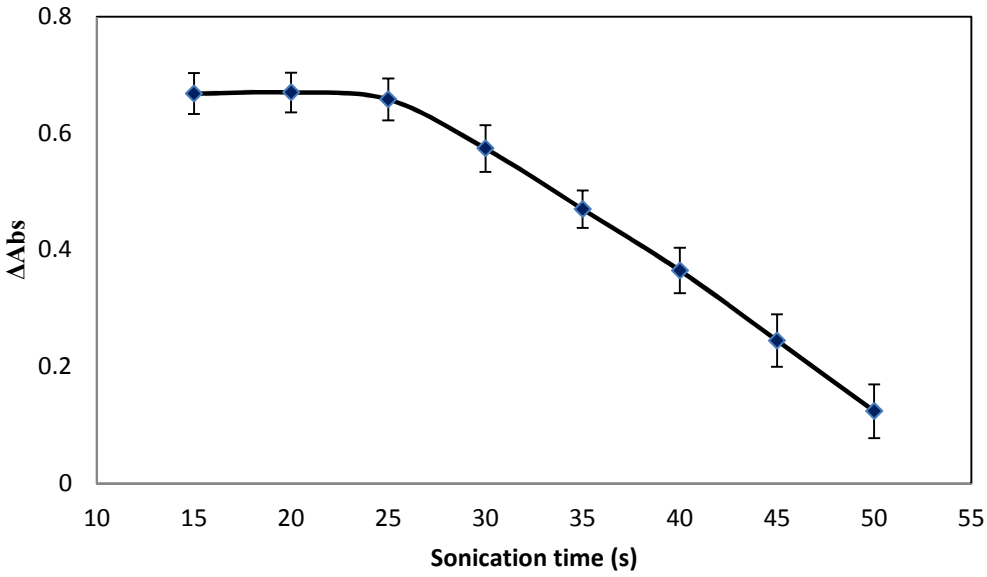

Fig 5

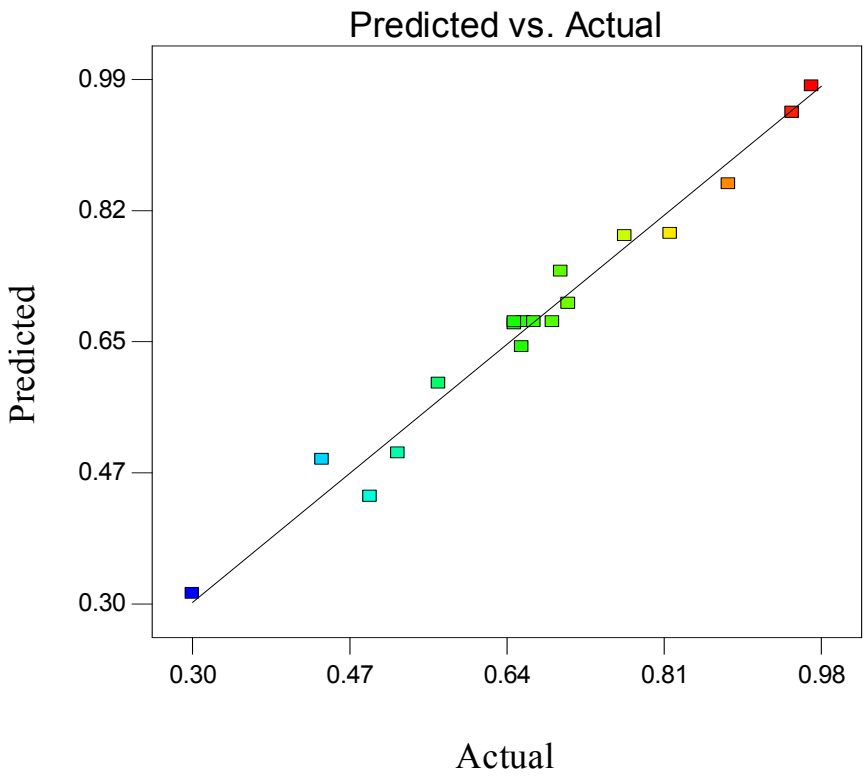

Fig 6