Int. J. New. Chem., 2025, Vol. 12, Issue 5, pp. 950-971.

International Journal of New Chemistry

Published online 2025 in http://www.ijnc.ir/.

Open Access

Print ISSN: 2645-7236

Online ISSN: 2383-188x

Original Research Article

β-Cyclodextrin Induced Coacervative Extraction of Erythromycin in Chicken Tissue Samples followed by HPLC Determination and Experimental Design for Optimization

Mohammad Hassannejad[1], Kamal Alizadeh[1*], Mahboob Nemati[2], Amir Abbas Matin[3], Roya pourmohammad[4]

[1]*Department of Chemistry, Lorestan University, Khorramabad 6813717133, Iran*

[2]*Department of Pharmacology, Tabriz University of Medical Sciences, Tabriz 1476651664, Iran*

[3]*Department of Chemistry, Faculty of Sciences, Azarbaijan Shahid Madani University, Tabriz, Iran*

[4]*Department of Pathology, Tabriz University of Medical Sciences, Tabriz, Iran*

Received: 2025-02-01 *Accepted: 2025-04-09* *Published: 2025-04-10*

ABSTRACT

As a medium-spectrum macrolide antibiotic, erythromycin is widely utilized in veterinary medicine to treat a wide range of infections. A coacervative extraction method was designed and optimized for the extraction of erythromycin residues prior to their liquid chromatographic analysis. The proposed approach was based on a water-induced coacervative extraction. Erythromycin was reacted with β-cyclodextrin to form a complex. The ERY-β-CD complex improved the stability of erythromycin in acidic environment. Capric acid in THF as a surfactant molecule was responsible for vesicle formation. Also, a Central Composite Design as a chemometric method was applied to perform a multivariate optimization of the impacts of five different parameters on the extraction efficiency of the proposed approach. After optimizing the method, the parameters were successfully used to determine erythromycin residues in edible meat. The linear range and limit of detection were 60-480 µg Kg^{-1} and 27.20 µg Kg^{-1}, respectively.

Keywords: Erythromycin-β-cyclodextrin; Coacervative extraction; Central Composite Design; Chicken tissue; Liquid chromatography

*Corresponding author email address: Alizadehkam@yahoo.com

1. Introduction

Macrolide antibiotics are a group of antibacterial compounds, which are active against gram-positive and gram-negative bacteria, as well as non-classical pathogens and share common chemical structures, such as erythromycin (ERY) with one or more amino sugars (Fig. 1). These antibiotics are widely used in human and veterinary medicine. In addition, this class of antibiotic compounds can be utilized as feed additives to promote animal growth [1-4].

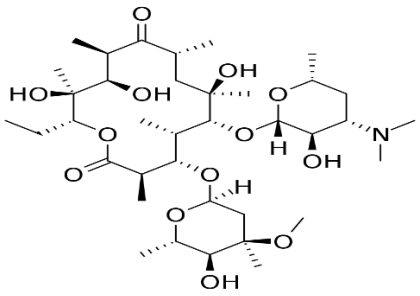

Fig. 1. Chemical Structure of Erythromycin

To ensure consumer protection against veterinary drug residues, their harmful effects on human health as an important issue must be controlled. Application of veterinary drugs in the foods originating from animal species has been strictly regulated by the European Union. They can be allowed to be used only for specific therapeutic purposes under a veterinarian's strict control of administration [5-6]. As a representative of macrolide antibiotics, ERY can be produced by Saccharopolyspora erythraea. Those people who have an allergy to penicillin are often treated with ERY since they have nearly similar antimicrobial spectra [7]. This macrocyclic antibiotic consists of a lactone ring with 14 members, 10 asymmetric centers, and the 2 sugars of L-cladinose and D-desosamine in its structure. Hence, it is very difficult to synthetically produce this compound. To prevent animal disease outbreaks and losses during transportation or dehydration in any disease cases, ERY is sometimes used in veterinary practice by administering it through feed additives or drinking water since showing efficient biological effects against gram-positive bacteria and mycoplasma [8-9]. Unfortunately, antibiotic residues left in foods due to the non-observance of their withdrawal times after animal treatment potentially endanger human health. Therefore, ERY detection in meat samples is quite important [10]. The analytical methods employed for ERY determination include Stripping Square Wave Voltammetry

(SSWV) [11], High-Performance Liquid Chromatography (HPLC) [12], liquid chromatography-tandem mass spectrometry [13], liquid chromatoghraphy [14], electrochemistry [15], chemiluminescence [16], and spectrophotometry [7].

These kinds of determinations need efficient sample preparation methods prior to analysis because of the presence of trace concentrations of residual drugs and a complex sample matrix like meat. Conventional sample preparation methods are time-consuming due to the need for multistage operations, which are harmful to human health and environment regarding the consumption of large amounts of toxic organic solvents with low preconcentration factors [17].

Most of the methodologies report the use of conventional techniques, such as liquid-liquid extraction (LLE) [18], solid phase extraction (SPE) [19] and liquid phase extraction [20] for extraction and isolation of ERY from the matrices.

Despite the huge advancement in modern analytical instruments for monitoring the analytes of interest at low levels, an ideal preconcentration step is urgently required compared to the common approaches which utilize large solvent volumes and sometimes provide low efficiencies [21]. To remedy, a superb extraction technique known as coacevate extraction was introduced and successfully applied for a vast number of analytes [22].

Coacervative extraction (CAE) was first introduced by De Jung et al. in 1930. It is a fast, cheap, and safe sample preparation method based on phase separation occurring between an aqeous phase and supramolecullar assemblies called coacervates (CA) [23]. A dehydrating agent like salt, non-solvent, pH, or temperature is employed to create low-volume water-immiscible liquid coacervate phases in colloidal solutions. The most common coacervates are those based on surfactant micelles, which are frequently utilized for analytical extractions [24-25]. To improve the ecofriendly nature of extraction, coacervative extraction (CAE) can be applied [26].

The principle of CAE is similar to that of Liquid–Liquid Extraction (LLE), which involves analyte partitioning between the two aqueous solution and the surfactant-rich phases. CA as a reverse micelles was dissolved in THF in a test tube for the formation of reverse micelles [21-22].

CAE is similar to cloud point extraction (CPE) procedure. but it should be noted that CPE involves the phase separation of neutrally charged (nonionic or zwitterionic) surfactants induced by the high temperature and this may affect stability of the compounds of interest, while CAE involves phase separation of ionic amphiphiles induced by other parameters (e.g., addition of electrolytes, pH change, addition of organic co-solvents, or simple mixing of oppositely charged amphiphiles). that this procedure had no effect on the stability of the compounds [27-28].

Several advantages are provided using anionic surfactant for CAE:

(a) to develop a rapid, simple and sensitive analytical method, (b) high preconcentration factors (c) the extraction can be carried out in a short time since equilibration time is not required (rapidity), (d) lowering the energy usage, (e) eliminating toxic reagents and solvents, (f) to increase the safety for the operator, (g) green analytical chemistry and (h) these surfactants are absorbance-free in the UV–Vis region. [26-29]

Determination of erythromycin by coacervation extraction has limited due to its instability and conversion under acidic conditions via an intramolecular dehydration reaction.

β-Cyclodextrin (β-CD) can be used to improve erythromycin stability in the acid environment (Fig. 2). Cyclodextrin monomer (CDm), such as β-cyclodextrin (β-CD), are cyclic oligomers of glucose that are molecular "carriers" with a hydrophilic exterior and a hydrophobic cavity. β-Cyclodextrin possess a unique ability to form complexes with specific drug molecules via noncovalent interactions in their hydrophobic cavities. The most common pharmaceutical applications of β-CD and their chemical derivatives are to increase solubility of the complexed species, reduce bitterness, enhance stability, and decrease tissue irritation upon dosing [30-31].

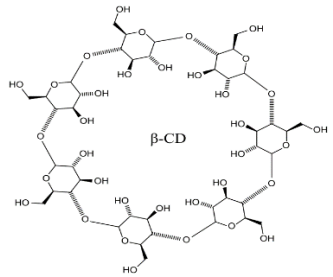

Fig. 2. Chemical Structure of β-Cyclodextrin.

Complex formation between β-Cyclodextrin and erythromycin via dynamic noncovalent interactions using the host-guest model has been well described. This model commonly includes the use of β-Cyclodextrin as host molecules and attracting hydrophobic guest molecules, which are incorporated into the hydrophobic cavity of cyclodextrin through the formation of a hydrogen bond [32].

In this work the effects of various experimental parameters such as coacervate composition, pH, concentration of chelating agent, ionic strength and extraction time were for preconcentration and determination of ERY were investigated. Central composite design (CCD) and response surface methodology (RSM) were applied for the optimization of influential parameters affecting the extraction efficiency of the method. Finally, the extracted phase sedimented at the bottom of the test tube underwent high-performance liquid chromatography with ultra violet detecton.

2. Experimental

1-2. Instrumentation

The HPLC system (KNAUER, Germany) was equipped with D-7000 interface, K-1000 model quarternary pump, L-2500 UV–Vis detector, and a manual injector (20 µl) for ERY determination. The separation was performed on XTerra ODSH-Optimal with the specifications of 150×4.6 mm and 5 µm (Waters, Ireland). A centrifuge (Beckman, United States) was utilized as a phase separation device.

2-2. Reagents

HPLC-grade methanol (MeOH), acetonitrile (MeCN), tetrahydrofuran (THF), n-Hexane, glacial acetic acid (HOAc), and sodium acetate were purchased from Merk (Darmstadt, Germany). Citric acid monohydrate ($H_3Cit\text{-}H_2O$) and trisodium citrate dihydrate ($Na_3Cit\cdot 2H_2O$)

with analytical grades were purchased from Beijing Chemical Reagent Company (Beijing, China). Sodium chloride (NaCl) was purchased from Welch Co. (Shanghai, China). Erythromycin and β-cyclodextrin were purchased from Sigma-Aldrich (St Louis, MO). 100 mg L^{-1} of ERY stock solution was prepared by dissolving an appropriate amount of the drug in acetonitrile. All the stock solutions were stored in the dark at -4 ^{o}C.

3-2. Chromatographic separation of ERY

A mixture of 75 mM of acetate buffer, acetonitrile, methanol, and THF (8:86:3:3, v/v/v/v) with a pH of 4.1 and flow rate of 1.0 mL min^{-1} was applied as a mobile phase in the isocratic elution mode. The injection volume was 20 µL for all the samples and the detection was performed at 210 nm.

4-2. CAE procedure for ERY extraction from chicken tissues

The extraction was performed on 10 g of drumstick without skin and fat, which was previously crushed to obtain a homogeneous paste. The resulting paste was spiked with 50 µgL^{-1} of ERY and β-CD with molar ratio (1:1). 15 mL of acetonitrile was added to the sample and homogenized by vortex shaking for 2 min. The resulting mixture was centrifuged at 1500 rpm for 5 min. Subsequently, the supernatant was filtered through a membrane filter with a pore size of 0.22 µm. The liquid phase was collected and evaporated to dryness under a stream of N2 gas. The dried residue was dissolved in 20 mL of citrate buffer (0.1 M, pH 6.0) and defatted with 15 mL of hexane through LLE. Centrifugation was necessary to avoid excessive emulsion. The aqueous phase was separated. Then, 1 mL of 0.1 M HCl was added to it and its pH was adjusted to 1. Afterwards, it was transferred into a 30-mL extraction vessel and followed by the rapid injection of 105.5 mg of decanoic acid solution into THF (3.32 mL). The resulting cloudy solution was dilluted to the mark with distilled water.

To enhance ERY extraction rate and accelerate the coacervate phase segregation from the bulk solution, the mixture of sonication and centrifugation at 4000 rpm were both performed for 10 min. Eventually, the aliquots of the coacervate were withdrawn by using a microsyringe to be directly injected into the HPLC-UV system for analysis.

5-2. Experimental design

According to the literature and based on the preliminary experiments, the most effective parameters on the performance of CAE process were selected to be CA weight (A), THF volume (B), pH (C), salt amount for ionic strength (D), and extraction time (E). High and low set-points were chosen to obtain an orthogonal design for each variable. A CCD consisting of 32 treatments for 5 factors in 5 levels and 6 center points was utilized to achieve the best response by optimizing the values of the factors. In the CCD, random experiments were conducted to minimize the effects of uncontrolled variables and the respective design matrix as shown in Table 1.

Table 1. The factors included in the central composite design and the levels for each of them

Parameter	**Abbreviation**	**Factors' levels**	
		Low	**High**
Capric acid weight (mg)	CA	10	200
Tetrahydrofuran (V/V%)	THF	1	20
pH	pH	1	4
Salt addition (M)	Salt	0	1
Extraction time	Ext. Time	0	30

For the assessment of the extraction efficiency (α=2), ERY peak area was applied as the HPLC response. To predict the dependent variable response for ERY extraction, a model of quadratic polynomials was obtained as displayed by Equation 1:

$$Y = bo + \sum_{i=1}^{4} bixi + \sum_{ij=1(i\neq1)}^{6} bijxixj + \sum_{i=1}^{4} biixi^{2} \qquad \text{Eq. 1.}$$

where Y and xi are the dependent and independent variables; b0 represents the constant coefficient; and bi, bij, and bii indicate the coefficients of linear, interaction, and squared effects, respectively [33].

3. Results and discussion

1-3. Effect of βCD concentration

The effect of different molar ratios of ERY-β-CD (1:1, 1:2, 1:3 and 1:4) were investigated through Fixing the amount of erythromycin. although the large macrocyclic lactone ring structure and the two sugar units of ERY did not enter the β-CD cavity, the results indicated that there was obvious interactions between the two sugar units as well as the diagonal position on the macrocyclic ring of ERY and β-CD. The results indicated that the maximum extraction was achieved with the increase in the molar ratios of β-CD to ERY from 1:1 to 3:1, while a further ratio increase up to 4:1 decreased the extraction efficiency (Fig. 3). It means that three β-CD molecules may interact to ERY by Waals force to form a packed complex. This result supported the consensus that high functional monomer to template molar ratios would increase nonspecific adsorption, while low molar ratios produce insufficient recognition sites.

Thus, further optimization was carried out with molar ratio 1:3 ERY-β-CD complex.

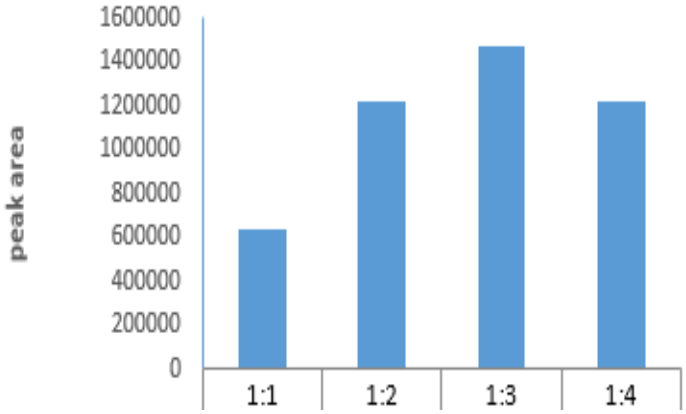

Fig. 3. Effect of β-CD concentration on the ERY extraction, Conditions: 105.5 mg capric acid, THF = 11.07 %; pH = 1; NaCL 1M; extraction time: 30 min.

2-3. Optimization of other parameters by the CCD

The extraction conditions were optimized via the proposed CAE method and a half-fraction of the CCD. The 5 factors of CA weight (mg), THF amount (V/V%), pH, salt concentration (M), and extraction time (min) were included in the design (see Table 1). A quick injection of CA and THF mixture into the liqiud phase was carried out with an adjusted pH for each experiment. After the cloudy solution was formed, it was centrifuged to subsequently analyze the directly injected aliquots of the coacervate phase into the HPLC column.

To model the extraction efficiency of ERY obtained from the aqueous sample by using micellar nanoparticles (coacervates), a regression equation with input-coded variables was constructed and presented in Equation 2 as follows:

Res = -785016 +10603CA+314483THF-231989pH +284214Salt+9133Ext. Time-28.99CA*CA-12164THF*THF-175628Salt*Salt-455.5CA*THF+504.2CA*pH+11358THF*pH-280.5THF*Ext. Time -8328Salt*Ext. Time Eq. 2.

The CCD could explain the effects of the interaction with its quadratic variables, as well as the linear impacts of the factors on the response. To assess the significance of each interaction factor and term, Analysis of Variance (ANOVA) was employed in this investigation (Table 2). A coefficient of determination (R^2) could express the polynomial model's quality. The model's high potential for predicting the response was demonstrated by an R^2 of 0.9982, which further indicated a good relationship between the fitted model and the experimental data. The model's significance was confirmed with an F-value of 1640.33 and a p-value of less than 0.000 as represented by the ANOVA summary results. The P-value of 0.768 obtained through the lack-of-fit test was implicative of the insignificance of the mentioned test relative to the pure error. The parameters of CA (A), THF volume (B), pH (C), salt amount for ionic strength, (D) and time extraction (E) had significant linear effects on the response.Moreover, the interactive effects of the mentioned parameters on the response were found to be significant. The predicted vs. actual

responses are exhibited in Figure 4(a). Most of the plots showed monotonous scattering around the line, thus indicating a good correlation between the predicted and actual responses, as well as the proposed quadratic model's good fit. The residuals vs. the predicted responses are shown in Figure 4(b). The random scattering of the residual plots demonstrated the constant variance of the experimental measurements for all the values of Y. The next step was to get the maximum response by finding an optimum value for each factor. Now, the model's 3D graphs could be used in the assessment of the interactive effects of 2 variables on the response.

Table 2 Analysis of variance (ANOVA) for response surface quadratic model

Source	d.f.[a]	Adj SS	Adj MS	F-Value[b]	P-Value[c]
Model	13	1.86385E+12	1.43373E+11	1640.33	0.000
Linear	5	5.93537E+11	1.18707E+11	1358.13	0.000
CA	1	37864679733	37864679733	433.21	0.000
THF	1	4.81679E+11	4.81679E+11	5510.90	0.000
pH	1	48253220817	48253220817	552.07	0.000
Salt	1	399905376	399905376	4.58	0.048
Ext. Time	1	5526856401	5526856401	63.23	0.000
Square	3	1.43520E+12	4.78400E+11	5473.39	0.000
CA×CA	1	66631071604	66631071604	762.33	0.000
THF×THF	1	1.17303E+12	1.17303E+12	13420.68	0.000
Salt×Salt	1	3436555074	3436555074	39.32	0.000
2-Way Interaction	5	2.05837E+11	41167410971	471.00	0.000

CA×THF	1	1.68978E+11	1.68978E+11	1933.28	0.000
CA×pH	1	5161991409	5161991409	59.06	0.000
THF×pH	1	26198012164	26198012164	299.73	0.000
THF×t.ex	1	1598040600	1598040600	18.28	0.001
Salt×Ext.Time	1	3900876849	3900876849	44.63	0.000
Error	16	1398476855	87404803		
Lack of fit	11	803398745	73036250	0.61	0.768
Pure Error	5	595078110	119015622		
Total	29	1.86524E+12			

[a] Degrees of freedom.

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

[c] Probability of seeing the observed F-value if the null hypothesis is true.

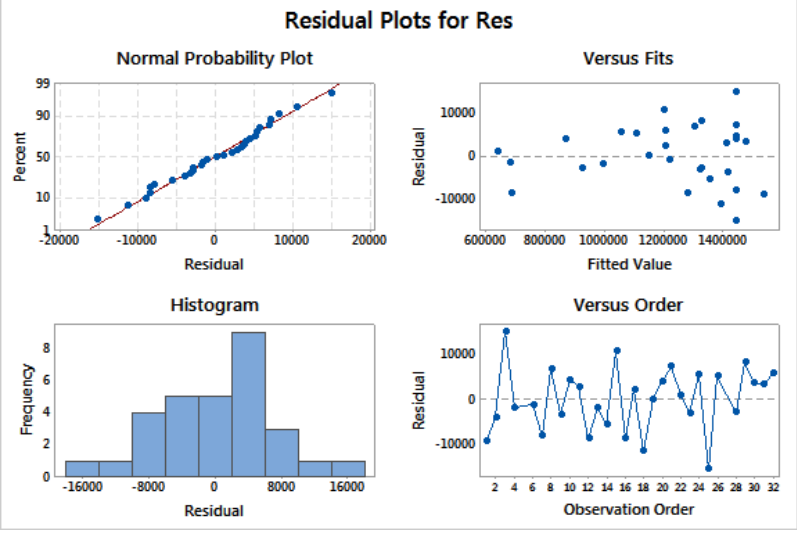

Fig. 4. (a) Normal probability plot of residuals for ER%; (b) plot of residuals versus predicted response for ER%.

To optimize the response with all its factors, a response surface model was utilized with the estimated regression coefficients. The Optimization plot of the conditions predicted for the optimum point and the suitability of the prediction were presented in figures 5 and 6.

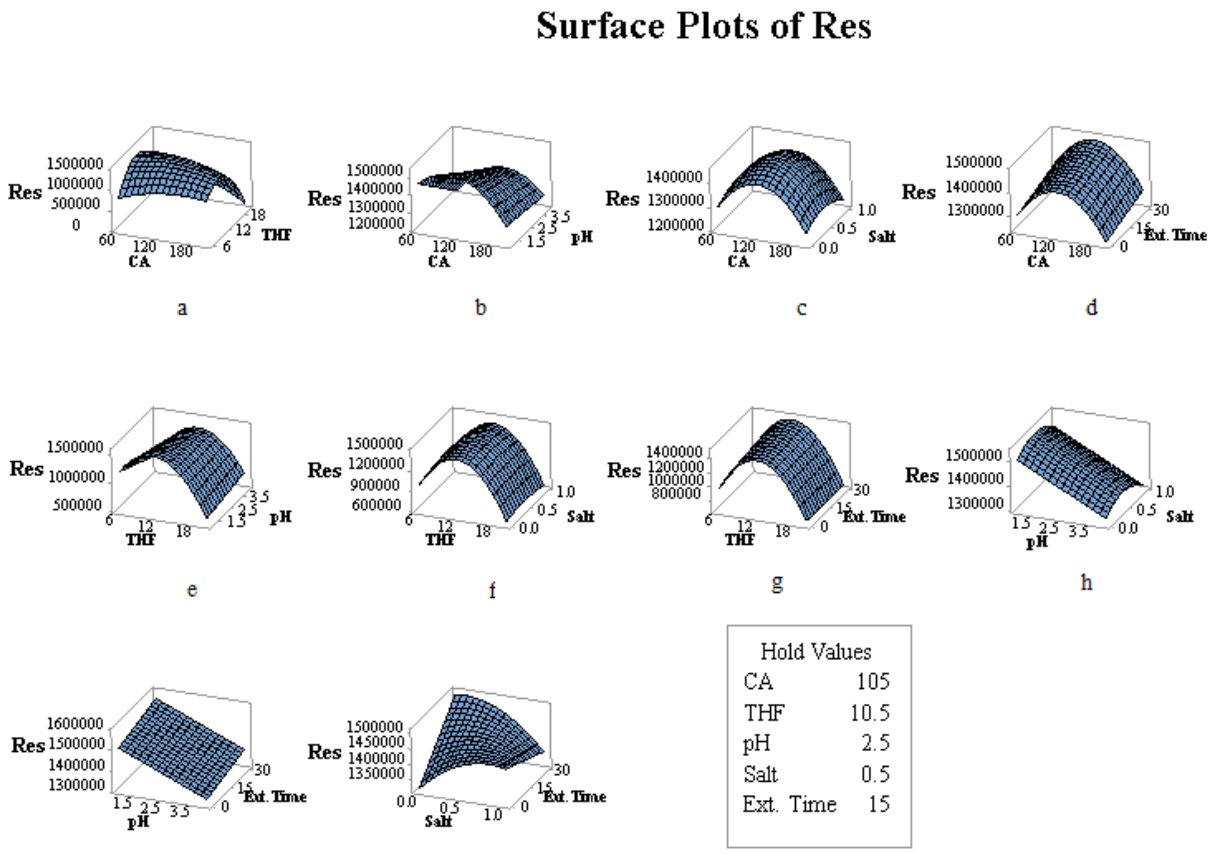

Fig. 5. Surface plots of response of predicted Erythromycin peak area as a function of (a) CA versus THF; (b) CA versus pH; (c) CA versus Salt; (d) CA versus Ext. Time; (e) THF versus pH; (f) THF versus Salt; (g) THF versus Ext. Time; (h) pH versus Salt; (i) pH versus Ext. Time and (j) Salt versus Ext. Time.

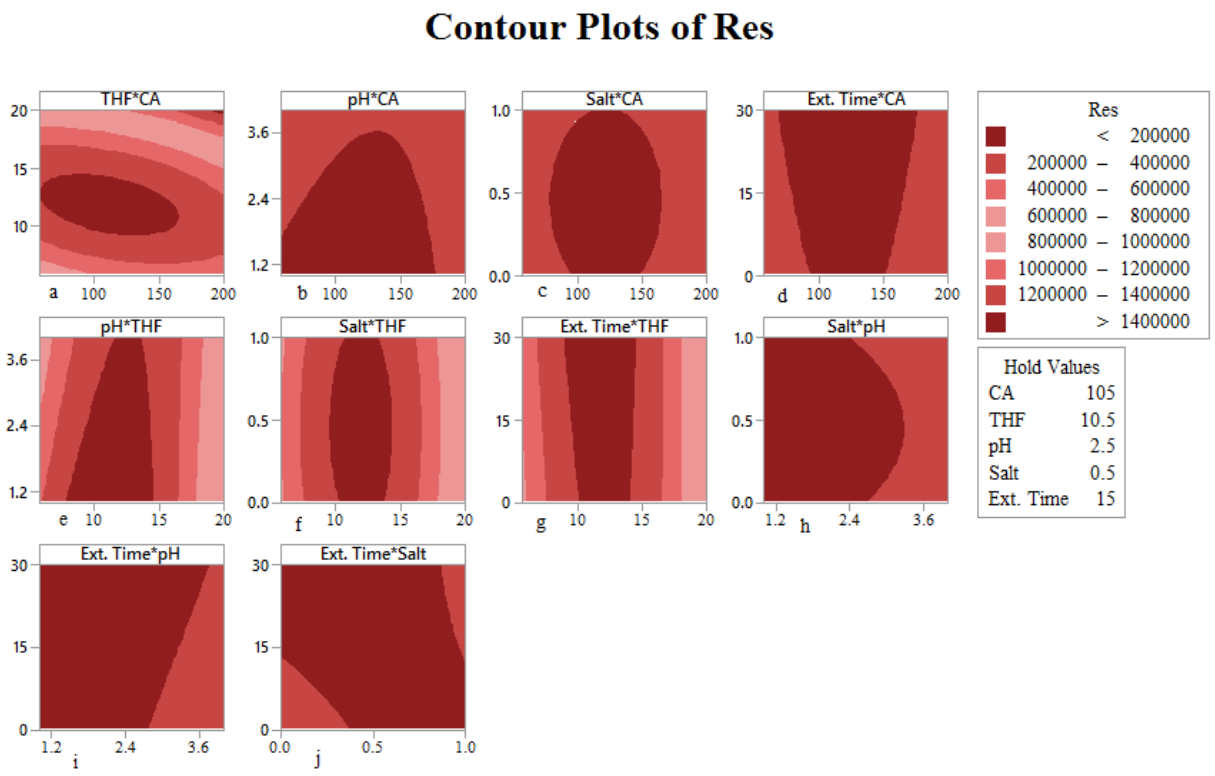

Fig. 6. Contour plots of recovery response of Erythromycin. The area of the highest acceptance is slightly located on the right upper hand of the plots.

Coacervation was merely obtained in solvents capable of dissolving alkyl carboxylic acids allowing the self-assembly of the amphiphiles that were miscible in water. Hence, the effect of the coacervate composition on extracting Erythromycin was assessed using various composition of the binary solution of decanoic acid in tetrahydrofuran. The quantity of decanoic acid and the amount of the solvent in the colloidal solution significantly affects both the volume of extractant obtained and extraction efficiency. Considering the non-solvent properties for the decanoic acid reverse micelles, water is only a minor coacervate component.

The influence of the decanoic acid quantity on the extraction recovery of erythromycin was assessed. Through dissolving various quantities of decanoic acid in 3.32 mL of tetrahydrofuran, a set of solutions were prepared. Based on Figure 5, by increasing the quantity of decanoic acid the extraction efficiency improved and got a maximum in 105.5 mg of decanoic acid, then it reduced at a higher quantity. The erythromycin extraction efficiency was low at

lower amounts of decanoic acid, possibly as a result of the fact that low coacervate volumes were not capable of entrapping the hydrophobic ERY-β-CD complex quantitatively. Consequently, in further investigations, 105.5 mg of decanoic acid was utilized.

Furthermore, the impact of the tetrahydrofuran volume was investigated on formating the coacervate phase and erythromycin extraction recovery. The experimental conditions were constant including various volumes of tetrahydrofuran: 1-20 (% v/v) with 105.5 mg of decanoic acid. The coacervate stage was formed in all mixtures, while the solution is turbid. According to Figure 4, the maximal extraction efficiencies were found within the value of 11.07 (% v/v) that recoveries gradually reduced over it. Under this value, only a portion of the decanoic acid contributed to the coacervate, consequently, the recovery reduced. The dissolution of a fraction of the coacervate and ERY-β-CD complex in tetrahydrofuran-water bulk solution is the possibly the reason for the reduction in the extraction recovery at higher volumes of tetrahydrofuran. Hence, as the optimal volume, 11.07 (% v/v) of tetrahydrofuran was chosen.

Coacervation procedure takes place only in solutions with protonated capric acid molecules (pKa = 4.8 ± 0.2). To improve the stability of erythromycin in an acidic environment, the β-cyclodextrin are well known cyclic oligosaccharides with the ability to form complexes with erythromycin via noncovalent interactions

It was demonstrated that the pH with an exclusive role in the formation of ERY-β-CD complex and succeeding extraction is the main parameter for CAE. Based on the initial study, it was indicated that within the coacervate step, the ERY-β-CD is extracted successfully in acidic conditions. ERY coacervative extraction was investigated within the pH range of 1.0-4.0. Based on the findings (Figure 4), it was indicated that the recovery of ERY is near to 100%, for pH of Low values. Therefore, for the succeeding experiments, pH =1.0 was selected.

Different experiments were carried out to investigate the impact of ionic strength on the CAE performance by addition of various quantities of NaCL (0.0-1.0 mol L^{-1}). Other experimental conditions were retained constant. The results showed that the extraction efficiency increased with the addition of the NaCl concentration. The addition of salt results in a greater efficiency of surfactant in reducing the tension of the ERY-water interface. This indicates that the salt has a positive effect on reducing the tension of the ERY-water interface, the more

effective surfactant by addition of salt is presumably due to the modification of the ERY-water interface by the added salt. Another reason may be the compression of the electrical double layer of the interface by the added salt, which can reduce the interface film thickness and rigidity and lead to the reduction of interfacial tension. Therefore, the NaCl of 1M was selected for further study.

The effectiveness of micelle-mediated extractions on the basis of the Reverse Micelle was reported in terms of the time spent for interacting the analytes with micelles and getting into their core. The following test was performed to determine the influence of this parameter on the recovery and enrichment factors of ERY. Using Ultrasonic water, the mixture with the coacervate phase and the bulk sample solution were mixed (Fig 5). By altering the extraction time within 0 and 30 min, a slight fluctuation is found on enrichment factors and recoveries for Erythromycin. Therefore, in the present work, the 30-min time was selected as the optimal extraction time.

3-3. Analytical figures of merit

Using the response surface model, 5 experiments were replicated at the predicted optimal conditions of 105.5 mg of CA (A), 3.32 mL of THF (B), pH=1 (C), 1 mL of 0.1 M NaCl (D) and 30 min of the extraction time (E). An extraction recovery of 81% was obtained with an enrichment factor of 305 after 50 µg L^{-1} of ERY was extracted under the mentioned optimum conditions. It could be concluded that the model's prediction was desirable with a quantitative recovery. A linear calibration curve over a range of 60-480 µg Kg^{-1} of ERY was achieved with an R^2 of 0.9982. The limit of detection for the determination of ERY in the meat samples was obtained to be 27.198 µg Kg^{-1} through this method.

4-3. Application of the developed method for the analysis of real samples

To evaluate the application of the proposed method for real sample analysis, it was used to determine ERY residues in chicken liver, drumstick and breast tissues that were purchased from the local markets. The collected samples were prepared according to the procedure described and analyzed. The results are summarized in Table 3. To study the matrix effect of the different meat samples on the current method, relative recoveries were determined for each analyte by spiking

them with erythromycin at three different concentration levels. Typical chromatogram of a spiked chicken meat sample with 50 µg L^{-1} ERY was presented at Fig. 7. Figure 7 indicate that erythromycin could be determined selectively without interference from meat samples. Figure 8 shows the chromatograms obtained for chicken Liver tissue. Figure 9 shows the chromatograms obtained for drumstick and breast tissues (samples spiked with 300µg/Kg of erythromycin). The results are summarized in Table 4.

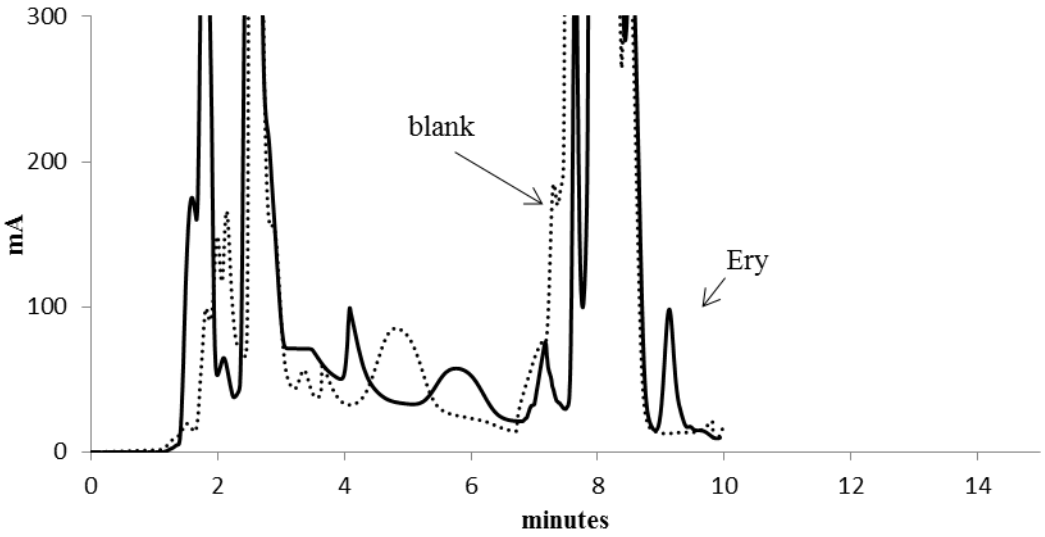

Fig. 7. HPLC chromatogram of ERY in spiked meat sample (spiked ERY = 50 µgL^{-1}, (1:3) molar ratio ERY with β-cyclodextrin,105.5 mg of CA, 3.32 mL of THF, pH=1, 1 M NaCl and 30 min of the extraction time).

Table 3. Application of the developed method for the analysis of real sample

Sample	Concentration (µg/Kg)	Recovery (%)	Enrichment	RSD (%)
Liver	85.43	85.0	365	7.66
Drumstick	Below detection limit	-	-	-
Breast	Below detection limit	-	-	-

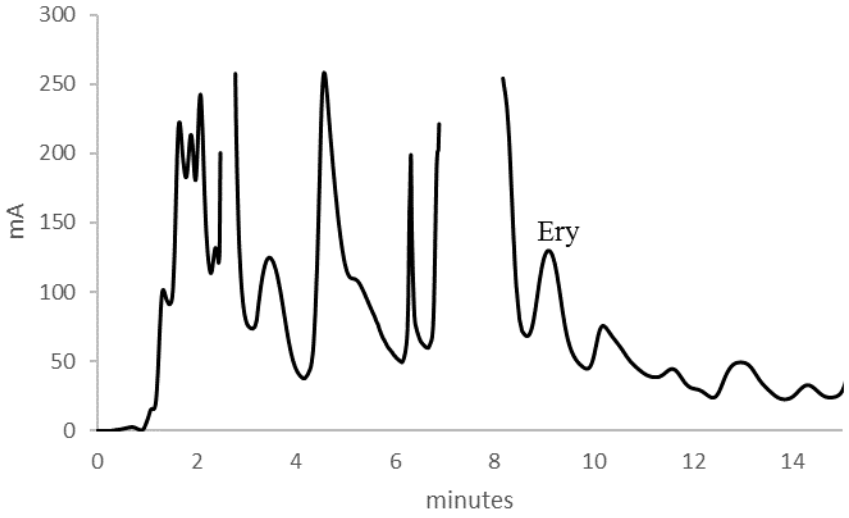

Fig. 8. Chromatograms of chicken Liver tissue under optimum conditions

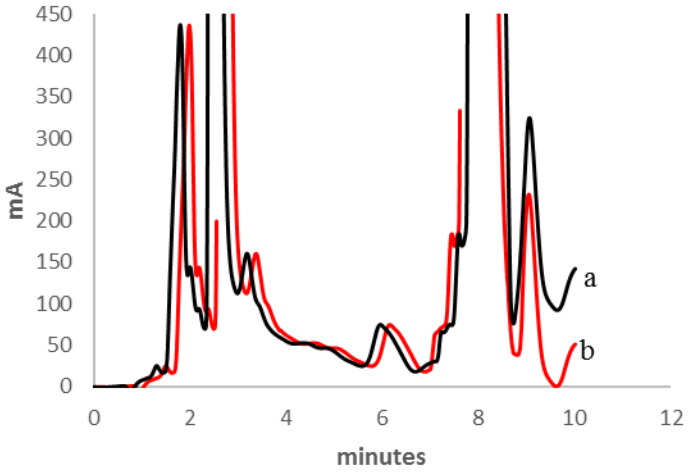

Fig. 9. Typical HPLC chromatograms of: (a) Breast tissue, (b) Drumstick tissue spiked with 300 µg Kg^{-1} of erythromycin, under optimal conditions ((1:3) molar ratio ERY with β-cyclodextrin,105.5 mg of CA, 3.32 mL of THF, pH=1, 1 M NaCl and 30 min of the extraction time).

Table 4. Obtained accuracy of erythromycin for spiked plasma samples using CAE method.

Sample	Concentration (µg/Kg)	added (µg/Kg)	Recovery (%)	Enrichment	RSD (%)
Drumstick	247.08	300	82	308	7.55
Breast	249.36	300	82.6	311	5.94

5-3. Comparison with other works

In order to show the analytical advantages of the proposed method for the determination of erythromycin, A summary of the analytical performance of the developed method compared to those of the other methods reported in the literature is presented in Table 5.

Table 5 Comparison of some characteristics of proposed method with other methods reported for determination of ERY

Method	Linear range (μgKg^{-1})	LOD	RSD (%)	Determination in real sample	Recoveries %)	Reference
MISPE-HPLC	24.2- 482.6	24.1	2.0	milk	98.0	[34]
LC-MS	30.0-500.0	10.0	11.3	meat	79.0	[35]
LC-MS	25.0-400.0	41.0	15.0	meat	58.0	[36]
LC-MS	0.01-1.0	0.01	10	liver	75.1	[37]
LC-MS	0.07- 2.2	0.07	0.2	drug	98.8	[15]
CAE-HPLC	60-480	27.19	5.4	chicken tissue	85.03	This work

As it is shown in Table 5, the proposed method and the other methods are similar in terms of RSDs; sometimes, RSDs of the proposed method are even better. The proposed method outperforms other methods in terms of the LODs, the linear ranges and analysis time. Based on

these results, it could be concluded that the proposed CAE method is a sensitive, repeatable and simple technique furthermore the selected drug in different samples can be preconcentrated and determined successfully by this technique.

4. Conclusions

Determining Erythromycin in chicken tissue samples was effectively carried out through cooperative extraction. The suggested process is simple, fast, easy to use, safe, and low-cost. Using reverse micelle for the CAE technique involves some considerable benefits compared to extraction mediated by nonionic surfactant micelle. Our study results indicate that the hydrophobic erythromycin molecule can interact with β-cyclodextrin to form a nonhost-guest β-CD-ERY complex.

The process is performable at low temperature with no impact on the stability of the considered compounds. At higher temperatures, the stability of most chemicals reduces results in not making possible extracting these components through traditional CPE procedures requiring the temperature to be incremented. Through the offered technique low detection limit, high recovery as well as uses of salt and avoids become possible via large quantity for extracting concentrated ERY. In this paper, it is shown that using experimental strategy a succeeding benefit is enabled based on the working time and number of knowledge in optimizing the circumstances of tests for CAE and determining ERY. Utilizing central composite plan and consequently response surface methodology the impact of independent variables like CA (A), THF volume (B), pH (C), salt amount for ionic strength, (D) and time extraction (E) on determining ERY were studied to obtain the ideal conditions. Moreover, the potential interactions were indicated between variables. Analyzing real samples with good findings implies the properness of this process in determining ERY in the real specimens.

References

[1] G. Balizs, A. Hewitt, Analytica Chimica Acta, 492, 105 (2003).

[2] S. Wang, H.Y. Zhang, L. Wang, Z.J. Duan, I. Kennedy, 26, 362 (2006).

[3] L. Clarke, T.L. Fodey, S.R. Crooks, M. Moloney, J. O'Mahony, P. Delahaut, R. O'Kennedy, M. Danaher, Meat Science. 97, 358 (2014).

[4] L. Chen, H. Wang, Q. Zeng, Y. Xu, L. Sun, H. Xu, L. Ding, Analytica Chimica Acta, 634, 215 (2009).

[5] M. Reig, F. Toldra, Meat Science, 78, 60 (2008).

[6] R. Cazorla-Reyes, R. Romero-González, A.G. Frenich, M.A.R. Maresca, J.L.M. Vida, Journal of Pharmaceutical and Biomedical Analysis, 89, 203 (2014).

[7] T. Sonia, E.H. Awatef, F.F. Elkady, Bulletin of Faculty of Pharmacy, 49, 81 (2011).

[8] B. Song, Y. Zhoua, H. Jin, T. Jing, T. Zhou, Q. Hao, Y. Zhou, S. Mei, Y. Lee, Microchemical Journal, 116, 183 (2014).

[9] I. Pala-Ozkok, A. Rehman, E. Ubay-Cokgor, D. Jonas, D. Orhon, Biochemical Engineering Journal, 90, 195 (2014).

[10] A. Esposito, L. Fabrizi, D. Lucchetti, L. Marvasi, E. Coni, E. Guandalini, Antimicrobial Agents and Chemotherapy, 51, 1043 (2007).

[11] N.P. Minh, T.B. Lam, N.T. Giao, International Food Research Journal, 18, 387 (2011).

[12] P. Edder, L. Coppex, A. Cominoli, C. Corvi, Food Additives and Contaminants, 19, 232 (2002).

[13] A. Deubel, A.S. Fandino, F. Sorgel, U. Holzgrabe, Journal of Chromatography A, 1136, 39 (2006).

[14] A. Deubel, U. Holzgrabe, Journal of Pharmaceutical and Biomedical Analysis, 43, 493 (2007).

[15] W. Lian, S. Liu, J. Yu, X. Xing, J. Li, M. Cui, J. Biosensors and Bioelectronics, 38, 163 (2012).

[16] J.S. Ridlen, D.R. Skotty, P.T. Kissinger, T.A. Nieman, Journal of Chromatography B, 694, 393 (1997).

[17] G Jia, Ch. Bi, Q. Wang, J. Qiu, W. Zhou, Zh. Zhou, Analytical and Bioanalytical Chemistry, 384, 1423 (2006).

[18] D. Lucchetti, L. Fabrizi, A Esposito, E. Guandalini, M.D. Pasquale, E. Coni, Journal of Agricultural and Food Chemistry, 53, 9689 (2005).

[19] V. Licul-Kucera, M. Ladanyi, G. Hizsnyik, G. Zaray, V.G. Mihucz, Microchemical Journal, 148, 480 (2019).

[20] F. Kamarei, H. Attar, S. Nikjah, M. Goodarzi, Arabian Journal of Chemistry, 7, 292 (2014).

[21] Sh. Abbasi, S.A. Haeri, Chromatographia, 78, 971 (2015).

[22] J. Milanovi, L. Petrovi, V. Sovilj, J. Katona, Food Hydrocolloids, 37, 196 (2014).

[23] E.K. Paleologos, D.L. Giokas, M.I. Karayannis, Trends in Analytical Chemistry, 24, 426 (2005).

[24] F. Ruiz, S. Rubio, D. Perez-Bendito, Journal of Chromatography A, 1163, 269 (2007).

[25] A. Melnyk, J. Namiesnik, L. Wolska, Trends in Analytical Chemistry, 71, 282(2015).

[26] Ch. Chen, F. Liao, Y. Lin, T. Hsieh, J. Weng, Ch. Feng, Talanta, 199, 464 (2019).

[27] R. Farhoosh, Journal of Food Science and Technology, 18, 1 (2018).

[28] M. Bahram, L. Shokri, N. Mohseni, Analytical and Bioanalytical Chemistry Research, 3, 19 (2016).

[29] R. Jannesar, F. Zare, M. Ghaedi, A. Daneshfar, Ultrasonics Sonochemistry, 32, 380 (2016).

[30] G. Chalumot, C. Yao, V. Pino, J.L. Anderson, Journal of Chromatography A, 1216, 5242 (2009).

[31] F. Mady, A.E. Abou-Talebc, K.A. Khaleda, K. Yamasakid, D. Ioharad, K. Taguchib, M. Anrakue, F. Hirayamad, K. Uekamad, M. Otagirib, International Journal of Pharmaceutics, 397, 1 (2010).

[32] W. Song, X. Yu, S. Wang, R. Blasier, D.C. Markel, G. Mao, T. Shi, W. Ren, International Journal of Nanomedicine, 6, 3173 (2011).

[33] A. Mohammadi, R. Tavakoli, M. Kamankesh, H. Rashedi, A. Attaran, M. Delavar, Analytica Chimica Acta, 804, 104 (2013).

[34] M.A. Garcia-Mayor, G. Paniagua-Gonzalez, B. Soledad-Rodriguez, R.M. Garcinuno Martinez, P. Fernandez-Hernando, J.S. Durand-Alegria, Food and Chemical Toxicology, 78, 26 (2015).

[35] C. Juan, J. Carlos Molto, J. Manes, G. Font, Food Control, 21, 1703 (2010).

[36] H. Berrad, F. Borrull, G. Fonta, R.M. Marce, Journal of Chromatography A, 1208, 83 (2008).

[37] M. Horie, H. Takegami, K. Toya, H. Nakazawa, Analytica Chimica Acta, 492, 187 (2003).

HOW TO CITE THIS ARTICLE

Mohammad Hassannejad, Kamal Alizadeh, Mahboob Nemati, Amir Abbas Matin, Roya pourmohammad, "**β-Cyclodextrin Induced Coacervative Extraction of Erythromycin in Chicken Tissue Samples followed by HPLC Determination and Experimental Design for Optimization**" International Journal of New Chemistry., 2025; 12(5), 950-971.