EXTRAÇÃO DE PONTO DE NÉVOA ECO-AMIGÁVEL ACOPLADA COM UM MÉTODO ESPECTROFOTOMÉTRICO PARA A DETERMINAÇÃO DO HIDROCLORETO RANITIDINA EM AMOSTRAS FARMACÊUTICAS

ECO-FRIENDLY CLOUD POINT EXTRACTION COUPLED WITH A SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF RANITIDINE HYDROCHLORIDE IN PHARMACEUTICAL SAMPLES

الاستخلاص بنقطة الغيمة مع طريقة طيفية لتقدير الرانيتيدين هيدروكلوريد في النماذج الصيدلانية

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RESUMO

O método simples, rápido, econômico e ambientalmente amigável foi desenvolvido para a determinação espectrofotométrica do cloridrato de ranitidina (R-HCl) em amostras farmacêuticas após extração pelo método do ponto de névoa. O método baseado na redução de Fe(III) pelo cloridrato de ranitidina em Fe(II), que posteriormente reagiu com o ferricianeto para formar produtos coloridos em (pH 4,0), o surfactante Triton X-114 foi usado como um extrator do cloridrato de ranitidina. A linearidade da curva de calibração foi mantida a partir de concentrações entre 0,5-60,0 µg/mL na absorção máxima de 693 nm. Fatores necessários para condições de reação, incluindo pH, FeCl₃ e K₃[Fe(CN)₆], volume de surfactante, temperatura, tempo e ordem de adição foram investigados. A análise de regressão indica que o coeficiente de correlação foi de 0,9998 e a capacidade de absorção molar foi de 0,46·10⁴ L/mol.cm. Os limites de detecção e quantificação foram de 0,475 e 1,567 µg/mL, respectivamente. O limite de confiança do declive e o limite de confiança do intercepto a 95% foram 0,0147 ± 0,00015 e 0,0642 ± 0,01033. A sensibilidade de Sandell também foi calculada e foi encontrado 0,0680 µg/cm². O fator de pré-concentração foi de 50,0%. Os estudos de validação para três concentrações diferentes (5,0, 10,0 e 30,0) µg/mL de cloridrato de ranitidina deram desvios padrão relativos entre 0,142-0,728 e a porcentagem de recuperações variou de 98,780 ± 0,719 – 99,840 ± 0,142. O método proposto foi aplicado com sucesso para a determinação do cloridrato de ranitidina em alguns de seus produtos farmacêuticos, com recuperação entre 99,108–99,808 e RSD% entre 0,012– 0,031. Os resultados obtidos com o método proposto o tornam adequado para uso na determinação do cloridrato de ranitidina em sua forma de dosagem a granel e em comprimidos.

Palavras-chave: *Ponto de névoa; Ranitidina; Espectrofotometria.*

ABSTRACT

The simple, rapid, economical, and environmentally friendly method was developed for spectrophotometric determination of ranitidine hydrochloride (R-HCl) in pharmaceutical samples after extraction by the cloud point method. The method based on the reduction of Fe(III) by ranitidine hydrochloride to Fe(II), which subsequently reacted with ferricyanide to form colored products at (pH 4.0), then Triton X-114 surfactant was used as an extractant for ranitidine hydrochloride. The linearity of the calibration curve was maintained from concentrations between 0.5–60.0 µg/mL at the maximum absorption 693 nm. Factors required for reaction conditions including pH, FeCl₃, and K₃[Fe(CN)₆] concentration, the volume of surfactant, temperature, time, and order of addition were investigated. Regression analysis indicates that the correlation coefficient was 0.9998 and the molar absorptivity was 0.46·10⁴ L/mol.cm. Detection and quantification limits were 0.475 and 1.567 µg/mL, respectively. The confidence limit of slope and the confidence limit of the intercept at 95% were 0.0147 ± 0.00015

and 0.0642 ± 0.01033 . Sandell's sensitivity was also calculated and it was found $0.0680 \mu\text{g}/\text{cm}^2$. The preconcentration factor was 50.0%. Validation studies for three different concentrations (5.0, 10.0 and 30.0) $\mu\text{g}/\text{mL}$ of ranitidine hydrochloride gave relative standard deviations between 0.142–0.728 and the percentage recoveries ranged from 98.780 ± 0.719 – 99.840 ± 0.142 . The proposed method was successfully applied for the determination of ranitidine hydrochloride in some of its pharmaceutical products with recovery between 99.108–99.808 and RSD% between 0.012– 0.031. The results obtained from the proposed method make it suitable to use in the determination of ranitidine hydrochloride in its bulk and tablet dosage form.

Keywords: Cloud point; Ranitidine; Spectrophotometry.

المخلص

تم تطوير طريقة بسيطة، سريعة، اقتصادية وصديقة للبيئة لتقدير الرانيتيد هيدروكلورايد في الأقراص الدوائية بعد استخلاصه بطريقة نقطة الغيمة. الطريقة تعتمد على اختزال الحديد الثلاثي بواسطة الرانيتيد هيدروكلورايد إلى الحديد الثنائي والذي بدوره يتفاعل مع فروسانييد الحديد لتكوين ناتج ملون عند دالته حامضية 4 بعدها استخدم تريتون X-114 لاستخلاص الرانيتيد هيدروكلورايد. الخطية لمنحنى المعايرة تم الحصول عليها عند تراكيز تراوحت بين 0.5 و 60.0 مايكروكروم /مل عند امتصاصية عظمى 693 نانومتر. تم اختيار العوامل المطلوبة لظروف التفاعل بما في ذلك الدالته الحامضية، تركيز FeCl_3 و $\text{K}_3[\text{Fe}(\text{CN})_6]$ ، حجم مخفض الشد السطحي، درجة الحرارة، الوقت وتسلسل الإضافات. يشير تحليل الانحدار إلى أن معامل الخطية 0.9998 والامتصاص المولاري 0.46×10^4 لتر /مول. سم. حد الكشف وحد الكشف الكمي كانت 0.0147 و 0.0642 ± 0.01033 و 0.475 و 1.567 مايكروكروم / مل على التوالي. حد الثقة للميل والقطع عند حدود ثقة 95% كانت 0.00015 ± 0.0147 و 0.0642 ± 0.01033 على التوالي. تم أيضاً دراسة حساسية ساندل وقد وجد أنها تساوي 0.0680 مايكروكروم /سم². عامل التركيز المسبق كان 50.0%. الدراسة التقييمية لثلاث تراكيز من الرانيتيد هيدروكلورايد (5.0، 10.0 و 30.0) مايكروكروم /مل أعطت انحراف قياسي نسبي منوي يتراوح بين (0.142 و 0.728) والاستيعابية المئوية بين 98.780 ± 0.719 - 99.840 ± 0.142 . الطريقة المقترحة يجعل منها طريقة مناسبة لاستخدامها في تقدير الرانيتيد هيدروكلورايد في المستحضرات الصيدلانية.

الكلمات الرئيسية: غيمة. رانيتيد. قياس الطيف الضوئي.

1. INTRODUCTION

Ranitidine hydrochloride (R-HCl) has the chemical name, according IUPAC ((E)-1-N'-[2-[[5-[(dimethylamino)methyl]furan-2-yl]methylsulfanyl]ethyl]-1-N-methyl-2-nitroethene-1,1-diamine hydrochloride and the trade name is Zantac. The structure of ranitidine with the formula ($\text{C}_{13}\text{H}_{22}\text{N}_4\text{O}_3\text{S} \cdot \text{HCl}$) explained in Figure 1 (Keith, 2000).

Figure 1. Structure of ranitidine hydrochloride.

R-HCl is classified under the medication that has the ability to act as histamine H_2 -blocker on parietal cells in a stomach leading to decrease secretion of acid by these cells. Generally, ranitidine used to duodenal ulcer treatment and also in the management of the hypersecretory condition. R-HCl, in many cases, used in the treatment of gastro esophageal reflux and peptic ulcer diseases (Zhang *et al.*, 2015; Narayana *et al.*, 2010; Darwish *et al.*, 2008).

R-HCl has the ability to coordinate with the transition metals and also to form ion-pair

complexes with dyes in the acidic medium due to the presence of amine group and thioether sulphur in its structure (Kiszkziel *et al.*, 2015; El-Yazbi *et al.*, 2003; Pérez-Ruiz *et al.*, 2001).

In brief, CPE (*cloud point extraction*) at optimized temperature (above the cloud point temperature) cloudy aqueous solutions contain non-ionic surfactant will have formed lowering the solubility of surfactant in water, thereby formation of two phases, aqueous poor phase and a small volume of organic-rich phase. The analyte will be partitioning between these two phases related to its hydrophobicity (Bai *et al.*, 2001; Takagai and Hinze, 2009; Hinze and Pramauro, 1993; Stalikas, 2002; Delgado *et al.*, 2004).

In CPE, phase separation was maintained due to the formation of the micellar phase by using appropriate surfactant under optimized conditions. These micelles will aggregate at the CMC (*critical micelles concentration*), which is the minimum concentration of surfactant. CPE was considered environmentally friendly due to the use of a small amount of solvent and non-volatile surfactant and non-toxic. In addition to that in CPE the analyte can be determined and the matrix can be removed in one step (Jia *et al.*, 2008).

Many analytical methods were reported for determination of R-HCl, some of these methods include spectrophotometry, flow injection, voltammetry, potentiometry, conductometric, HPLC, Redox reaction, ESI-LC-MS/MS and TLC

(Baqir *et al.*, 2014; Moldovan and Aboul-Enein, 2012; Basavaiah and Nagegowda, 2004; Elbashir and Merghani, 2018; Issa *et al.*, 2005; Frag *et al.*, 2011; Ahamed *et al.*, 2006; Vicentini *et al.*, 2016; Tang *et al.*, 2007; Merghani and Elbashir, 2018; Bindewald *et al.*, 2018; Sharma *et al.*, 2011; Alamgir *et al.*, 2017; Kantariya *et al.*, 2013; Amin *et al.*, 2003; Bellorio *et al.*, 2013).

The present study aimed to develop a new, economic, friendly, and rapid cloud point extraction coupled with a spectrophotometric method for extraction and determination of ranitidine hydrochloride from pharmaceutical preparations.

2. MATERIALS AND METHODS

2.1. Reagent and materials

The standard of ranitidine hydrochloride (99.0%) supplied from Merck was prepared by dissolving 0.01 g in distilled water, then complete the volume to 100 mL in a volumetric flask. Triton X-114 (99.9%) purchased from Sigma-Aldrich company was prepared by diluting 10 mL in distilled water with gently heating at 30 °C for complete dissolving, then complete the volume to the mark with 100 mL distilled water in a volumetric flask.

Ferric chloride (99.9%) obtained from the BDH company was prepared by dissolving 0.0162 g in 1 mL concentrated HCl and then made up to 100 mL volumetric flask with distilled water.

Sodium hydroxide (99.0%) supplied from BDH company was prepared by dissolving 0.4 g in distilled water and complete to the mark in 100 mL volumetric flask with distilled water. Potassium ferricyanide (99.0%) provided from Sigma-Aldrich was prepared by dissolving 0.0329 g in distilled water and complete to the mark in 100 mL volumetric flask with distilled water.

HCl supplied from BDH was prepared by diluting 0.877 mL in distilled water and complete to the mark in 100 mL distilled water in a volumetric flask.

2.2. Apparatus

In SHIMADZU double-beam spectrophotometer (UV-1800) was used for absorbance measurements, pH-meter, (HANNA™) for pH measurement, shaking water bath (JULABO™) was used for temperature control throughout carrying the experiments of cloud point extraction.

2.3. Sample preparation

Ten tablets of each different product that contain 150 mg of R-HCl were grinded and mixed well to obtain bulky homogenous powders from each product. Weight equivalent to 0.01 g of pure R-HCl has been taken and dissolved in distilled water, then filtered and transferred to 100 mL volumetric flask and complete the volume to the mark to prepare 100 µg/mL. From these stock solutions of different products dilute solutions were prepared for applying the proposed method (Basavaiah and Nagegowda, 2004).

2.4. General procedure for cloud point extraction

Different volumes of standard solutions were added in 10 mL centrifugal screw tubes ranging from 0.05–6.0 mL of 100 µg/mL which corresponding to 0.5–60.0 µg/mL of ranitidine-HCl. Giving sequence, 0.8 mL of $8.0 \cdot 10^{-3}$ M FeCl_3 solution were added for each tube followed by addition of 0.6 mL of $6.0 \cdot 10^{-5}$ M $\text{K}_3\text{Fe}(\text{CN})_6$ solution and adjusted the pH of solutions (pH 4.0) by using 0.1 M NaOH and/or 0.1 M HCl.

For cloudy solutions, 0.7 mL of Triton X-114 was added to the final solution and completed to the mark with 10 mL distilled water, then incubated for 20 min in water bath at 50 °C, then cooled. The aqueous layer was removed by microsyringe, and the organic-rich phase was diluted with 0.5 mL ethanol. The absorbance of each solution was measured at λ_{max} 693 nm against a blank solution (Khammas and Ahmad, 2016).

3. RESULTS AND DISCUSSION

3.1. Absorption spectra

R-HCl (50 µg/mL), FeCl_3 ($1.0 \cdot 10^{-3}$ M), $\text{K}_3[\text{Fe}(\text{CN})_6]$ ($1.0 \cdot 10^{-3}$ M) solutions and the colored complex were scanned in the UV-Vis from 190–1100 nm against the blanks for spectral study. It was found that ranitidine R-HCl, FeCl_3 , $\text{K}_3[\text{Fe}(\text{CN})_6]$ and Triton X-114 gave maximum absorption at 308 nm, 290 nm, 303 nm, and 224 nm respectively. While the colored solution for R-HCl after addition of FeCl_3 and $\text{K}_3[\text{Fe}(\text{CN})_6]$ in the presence of Triton X-114 gave maximum absorption at 693 nm. This absorption for the colored product was adopted for further optimization in Figure 2.

3.2. Optimization of experimental conditions

Cloud point extraction affected by a various factor that can improve the extraction thereby the efficiency of the proposed method, for those such parameters like pH, incubation time, the temperature of equilibrium, Triton X-114 volume, FeCl_3 , and $\text{K}_3[\text{Fe}(\text{CN})_6]$ concentrations were investigated by the classical optimization. Triton X-114 selected due to its low cloud point temperature (22 to 23 °C) and low critical micelles concentration (~0.2 mM) (Santaladchayakit, and Srijaranai, 2012).

3.2.1. Effect of pH

Extraction efficiency was widely affected by pH value, thereby affecting the absorbance of organic rich-phase. Thus, pH of the solution was study in the range of 2.0–10.0 at 50 °C for 20 min at R-HCl within concentration of 50 µg/mL. All other parameters kept constant as in the described procedure. From the results in Figure 3, was chosen pH 4.0 due to the highest response.

Change in the pH generally affects the ionization form of the analyte leading to change water solubility and the extractability of analyte. pH adjustment should be optimized to ensure that the neutral molecular form of the drug was present before the CPE step also. The pH value affects the partitioning of analyte in the CPE. It was clearly explained in Figure 3 that in pH 4.0 had the highest response, and afterthought, this point the pH decreases. Thus, pH 4.0 was selected for optimization.

3.2.2. Effect of ferric chloride concentration

Fe (III) salt like FeCl_3 can be used as oxidant agent for the determination of some compound to form oxidizing colored product. Fe (II) was used as a reduced form of Fe(III), for that the concentration of FeCl_3 on the formation of the colored product complex was investigated by adding the different volume of 1.0×10^{-3} M FeCl_3 solution ranged from (0.1–1.0) mL at pH 4 and keeping the other condition constant (Figure 4). R-HCl reduce Fe (III) and forming Fe (II) which is subsequently reacted with $\text{K}_3[\text{Fe}(\text{CN})_6]$ to form the blue product which measured at 693 nm. From Figure 4, the highest absorption was maintained when the concentration of FeCl_3 was 8.0×10^{-5} M (0.8 mL of $1.0 \cdot 10^{-3}$ M in 10 mL final aqueous solution). So this concentration was chosen for further optimization.

3.2.3. Effect of potassium ferricyanide

Some of the oxidizing agents have an important effect on the coupling reaction between ligand and the metal. To this reason, effect of potassium ferricyanide was tested by the addition of various volumes of $\text{K}_3[\text{Fe}(\text{CN})_6]$ ranged from (0.1–1.0) mL (Figure 5).

The maximum signal obtained was at volume 0.6 mL, which is related to the concentration of 1.0×10^{-5} M $\text{K}_3[\text{Fe}(\text{CN})_6]$ in 10 mL final solution, for that this concentration was adopted for optimization.

3.2.4. Effect of surfactant amount

The efficiency of extraction in CPE highly depended on the concentration of surfactant which is also affected the preconcentration factor, Thus, different volume of surfactant ranged from (0.1–2.0) mL of 10% Triton X-114 was tested. We can see the results in Figure 6.

The optimized volume of the surfactant was 0.7 mL, as explained in Figure 6. Below this point, it was difficult to separate the surfactant rich phase due to the small volume of rich phase relative to the total volume of solution. The other hand, above this point the volume of surfactant led to a decrease in the preconcentration factor and to interfere with the back-extraction.

Generally, the volume of surfactant added to the sample solution in CPE was low due to the high viscosity of surfactant ($\eta \sim 20$ cP) and low CMC for Triton X-114 (0.20–0.35 mM) (Sirimanne *et al.*, 1998).

However, the efficiency of extraction was strongly affected by the amount of surfactant used. Maximum extraction efficiency maintained when the ratio of volume of rich phase to that of aqueous phase (V_s/V_a) is minimum leading to improve the preconcentration factor (Khammas and Mubdir, 2014).

3.2.5. Effect of temperature and time

Cloud point extraction is a type of equilibrium extraction. The optimal extraction efficiency was obtained once the equilibrium temperature and incubation time were established. The two factors were studied in the range of 20.0–80.0 °C and 5.0–70.0 min, respectively, as shown in Figures 7 and 8.

According to the results (Figures 7 and 8), maximum absorption was achieved at a

temperature 50 °C, and at time 20 min for that, these two factors were recommended for the CPE procedure.

3.2.6. Effect of order addition

Several experiments have been tried for the purpose of testing the order of reactants addition. According to the highest absorption maintained number 1 was chosen for the CPE procedure. This can be explained due to the reduction of Fe^{+3} to Fe^{+2} ion by R-HCl, then reacted with ferricyanide to produce a Prussian blue color product. These experiments sorted in Table 1.

3.2.7. Calibration curve

Under optimization conditions, linearity was achieved at a concentration between (0.5–60.0) $\mu\text{g/mL}$. The calibration curve was maintained by plotting absorption versus concentrations of R-HCl at $\lambda_{\text{max}} = 693 \text{ nm}$ (Figure 9). The analysis of data obtained from the calibration curve, as illustrated in Table 2.

3.3. Method Validation

3.3.1. Accuracy and precision

Accuracy and precision regarded as the most requirements for testing the method reliability and quality of the results obtained. For that accuracy was studied in term of percentage recovery by subjected three different concentrations within the calibration curve (5.0, 10.0 and 30.0 $\mu\text{g/mL}$) to CPE procedure. Precision also studied in terms of repeatability for five times. All results of accuracy and precision were illustrated in Table 3. The results indicated that the proposed method has a good precise, and small systematic error, which can be useful for analysis the of R-HCl in pharmaceutical preparations.

3.4. Application

Two products contain R-HCl (Reptidine 150 mg and Ranimac 150 mg) were chosen from a local pharmacy for application study. Ten tables of each product were weighted, grind and mix well to form bulky homogenous powders. Then 0.1 g of pure R-HCl from each product was dissolved in distilled water and complete the volume to the mark in 100 mL volumetric flask to prepare 1000

$\mu\text{g/mL}$. From these two solutions 50 $\mu\text{g/mL}$ was prepared for the application study for the proposed method.

4. CONCLUSIONS

A newly developed method was preceded by a combination of cloud point extraction (CPE) and spectrophotometry to determine ranitidine-HCl in the pharmaceutical dosage form. This method could be a good alternative for the liquid-liquid extraction (LLE), method which consumes a large volume of solutions and also more useful than solid-phase extraction (SPE) due to the high cost of SPE columns.

The developed CPE–spectrophotometry method was economical, rapid, and simple, due to the analytical parameters obtained (percentage recovery of extraction, LOD, LOQ, and RSD%), it can be used for monitoring R-HCl in pharmaceutical preparations.

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Figure 2. Absorption spectra of $R-HCl$ (A), $K_3[Fe(CN)_6]$ (B), $FeCl_3$ (C), Triton X-114 (D) and colored product complex (E).

Figure 3. Effect of pH on the absorption of $R-HCl-(Fe^{+2})$ complex.

Figure 4. Effect of FeCl_3 concentration on the absorption of $\text{R-HCl}-(\text{Fe}^{+2})$ complex.

Figure 5. Effect of potassium ferricyanide on the absorption of $\text{R-HCl}-(\text{Fe}^{+2})$ complex.

Figure 6. Effect of surfactant volume on the absorbance of $R\text{-HCl}-(\text{Fe}^{+2})$ complex.

Figure 7. Effect of temperature on the absorption of $R\text{-HCl}-(\text{Fe}^{+2})$ complex.

Figure 8. Effect of time on the absorption of R-HCl –(Fe²⁺) complex.

Figure 9. Calibration curve of the Ranitidine hydrochloride complex.

Table 1. Effect of order addition of reactants.

No.	Addition	Abs.
1	R-HCl + FeCl ₃ + K ₃ [Fe(CN) ₆] + Buffer (4) + Triton X-114	0.775
2	R-HCl + K ₃ [Fe(CN) ₆] + FeCl ₃ + Buffer (4) + Triton X-114	0.698
3	R-HCl + Buffer (4) + FeCl ₃ + K ₃ [Fe(CN) ₆] + Triton X-114	0.595

Table 2. Data Analysis for Ranitidine-HCl determination.

Parameter	Data Analysis
$\lambda_{\max}$ (nm)	693
Regression equation	$y = 0.0147x + 0.0642$
Standard deviation of regression line ($S_{y/x}$)	0.0045
Correlation coefficient (r)	0.9998
C.L for the slope ($b \pm ts_b$) at 95%	0.0147 ± 0.00015
C.L for the intercept ($a \pm ts_a$) at 95%	0.0642 ± 0.01033
Linearity range ($\mu\text{g/mL}$)	0.5–60.0
Limit of detection ($\mu\text{g/mL}$)	0.475
Limit of quantification ($\mu\text{g/mL}$)	1.567
Sandell's sensitivity ($\mu\text{g/cm}^2$)	0.0680
Molar absorptivity (L/mol.cm)	0.46×10^4
Preconcentration factor*	50.0%

*Preconcentration factor was the ratio of the original volume of sample to that of extracted volume or rich surfactant-phase

Table 3. Accuracy and precision for the determination of Ranitidine-HCl.

Amount of R-HCl ($\mu\text{g/mL}$)		Recovery% (mean $\pm$ SD)	RSD% (n=5)	$E_{\text{rel}}\%$
Taken	Found at 95% C.L $\left(\bar{x} \pm t \frac{s}{\sqrt{n}}\right)$			
5.0	4.966 ± 0.014	99.320 ± 0.228	0.230	0.680
10.0	9.878 ± 0.089	98.780 ± 0.719	0.728	1.220
30.0	29.952 ± 0.053	99.840 ± 0.142	0.142	0.160

Table 4. Application of the proposed method for the determination of Ranitidine-HCl.

Name of product	Amount of R-HCl ($\mu\text{g/mL}$)		Recovery% (mean $\pm$ SD)	RSD% (n=5)	$E_{\text{rel}}\%$
	Taken	Found at 95% C.L $\left(\bar{x} \pm t \frac{s}{\sqrt{n}}\right)$			
Reptidine	50	49.904 ± 0.0068	99.808 ± 0.012	0.012	0.192
Ranimac	50	49.554 ± 0.0183	99.108 ± 0.310	0.031	0.892