J. Edu. & Sci., Vol. 28, No. 2 , 2019 : 289-299

التقدير الطيفي للايبوبروفين والنستاتين بتكوين معقد المزدوج الايوني باستخدام كروموتروب 2R

انتصار عادل شهاب
قسم الكيمياء/ كلية التربية للبنات
جامعة الموصل
entesar@uomosul.edu.iq

ضياء نجم الصبحة
قسم الكيمياء/كلية التربية للعلوم الصرفة
جامعة الموصل
dr_theiaa@yahoo.co.uk

تاريخ الاستلام **2018/09/17**
تاريخ القبول **2018/10/02**

الخلاصة

تم تطوير طريقة طيفية بسيطة ومضبوطة في تقدير أدوية الأيبوبروفين والنستاتين بأشكالها النقية وفي مستحضراتها الصيدلانية. اعتمدت الطريقة على معقدات التجمع الأيوني بين الأدوية أعلاه وصبغة الكروموتروب 2R في وسط حامضي مكونة لون برتقالي محمر. امتلكت تلك المعقدات أقصى امتصاص عند الأطوال الموجية 562 و566 نانوميتر لكل من الأيبوبروفين والنستاتين على التوالي. لقد أمكن تطبيق قانون بير في مدى التراكيز 10–90 و2–40 مايكروغرام/مللتر وبامتصاصية مولارية 1.36×10^{3} و 7.59×10^{3} لتر.مول$^{-1}$.سم$^{-1}$ وبمعدل استرجاعية 99.77% و100.60% للدوائين أعلاه على التوالي. كما لوحظ عدم وجود تداخل من قبل المضافات الصيدلانية، وقد أمكن تقدير الدوائين في بعض مستحضراتهما الصيدلانية بنجاح.

الكلمات المفتاحية: التجمع الأيوني، مطياف فوتومتري، أيبوبروفين، نستاتين، كروموتروب2R

Spectrophotometric Determination of Ibuprofen and Nystatin Via Ion Pair Complex Formation Using Chromotrope 2R

Intisar A. Shihab
Department of Chemistry
College of Education for Girls
entesar@uomosul.edu.iq

Theia'a N. Al-Sabha
Department of Chemistry
College of Education for Pure Sciences
dr_theiaa@yahoo.co.uk

Received **Accepted**
17/09/2018 **02/10/2018**

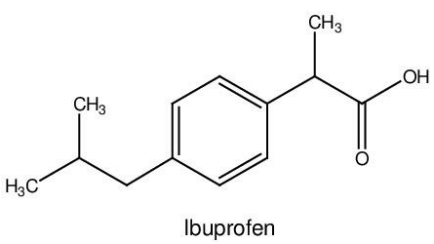
Abstract

This research paper tackles a developed method for Ibuprofen and Nystatin drugs determination both in their pure form and pharmaceutical formulations. The proposed method is based on the formation of binary complexes (ion association complexes) between the above drugs and Chromotrope 2R (C2R) in acidic medium forming a reddish orange colours .The complexes show maximum absorption at 562 nm and 566 nm for Ibuprofen and Nystatin respectively. The method was adhering to the Beer's law over concentration range 10-90 and 2-40 µg/ml with molar absorptivity values 1.36×10^3 and 7.59×10^3 l. mol^{-1}. $cm^{-1}$and average recovery 99.77% and 100.60 % for the above mentioned drugs respectively. No observed interferences appeared from the excipients commonly existed in pharmaceuticals. Successfully, application for both Ibuprofen and Nystatin was conducted in their pharmaceutical formulations.

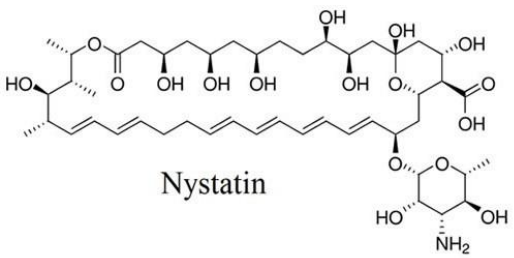
Keywords: Ion association; Spectrophotometry; Ibuprofen, Nystatin, Chromotrope 2R

Introduction

Ibuprofen [I] treats a lot of pains including fever and flu. It is widely known as nonsteroidal anti-inflammatory drug (NSAID) that can block producing some substances that cause inflammation. Nystatin [II]treats infections of different kinds. Basically, it is an antifungal drug that stops the growth of fungus [1,2].



Ibuprofen



Nystatin

[I] [II]

Different analytical methods have been proposed for the determination of Ibuprofen and Nystatin in pharmaceuticals. These methods included spectrophotometric [3-5], chromatographic [6−9], electrophoretic [10−12], potentiometric and [13−15] methods for the determination of Ibuprofen. Also; spectrophotometric [16-18], HPLC [19–21] and electrophoresis [22,23] are used for determination of Nystatin. Here, a spectrophotometric method which is new simple and sensitive for determining both of Ibuprofen and Nystatin was proposed. Actually it was applied for the pharmaceutical product analysis.

Experimental

Apparatus

Visible spectrophotometer (T92 UV) equipped with a 1.0-cm path has been used for spectral measurements as well as a length glass cell and RLO 60P For pH measurements portable, pH-meter with a combined glass electrode. Statistically, Excel 2010 software has been used.

Reagents

Products of BDH and Fluka companies have been used in this research.C2R (3×10^{-3}M) solution was prepared by dissolving 0.1405g in 100 ml distilled water in volumetric flask. Acetate buffer solution (pH 2.7) was prepared by mixing of 0.1 M of acetic acid and sodium acetate solutions and adjusted by pH meter. Ibuprofen (250 µg/ml) was used in the experiment manufactured by Samara (SDI, Samarra-Iraq). It is prepared by dissolving 0.025g of Ibuprofen as pure form in 100 ml distilled water in volumetric flask. Nystatin (100 µg/ml) is prepared by dissolving 0.01g of Nystatin as pure form in 100 ml distilled water in volumetric flask.

General procedure

Increasing volumes of 250 µg/ml Ibuprofen and 100 µg/ml Nystatin working solutions were transferred separately to a series of 25 ml volumetric flasks to cover the concentration range 10–90 µg/ml for Ibuprofen and 2-40 µg/ml for Nystatin. Then, a process of adding 2.5 ml of $3x10^{-3}$M C2R and 10 ml of acetate buffer solution for Ibuprofen and 3.0 ml of 0.1 M HCl for Nystatin. Dilution of the solutions was done to the mark via distilled water. The measurement of the absorbance was at 562nm and 566nm at room temperature against their respective reagent blank for above drugs.

Procedure for tablets

What have been weighed and pulverized accurately are five tablets of each Piofen (each tablet contains 400 mg Ibuprofen) and Mycodin (500000 IU=102.9 mg Nystatin). A part of the fine and homogenized powder equivalent to one tablet was weighed and dissolved as usual. It was mixed well and filtered with Whatmann filter paper No.1. The filtrate was diluted to the 100 ml with distilled water. A suitable volume was diluted, and the general procedure was followed.

Ampoule

Three pharmaceutical ampoules (Ketoprofen), each one contains 100 mg per 2 ml Ibuprofen, were mixed well. A volume equivalent to one ampoule of the content was diluted appropriately with water to 250 µg/ml. The concentration of each drug per

ampoule was determined using its respective calibration graph constructed for pure drug by following the general procedure**.**

Results and Discussion

A reddish orange ion-pair complexes in acidic medium with λ_{max} at 562 nm and 566 nm is shown in (Fig 1), as a result of the reaction of Nystatin and Ibuprofen respectively with C2R dye. Electrostatic interaction between the most basic center in the drugs molecules (hydroxyl groups) and the carboxylate anion of the dye formed complexes.

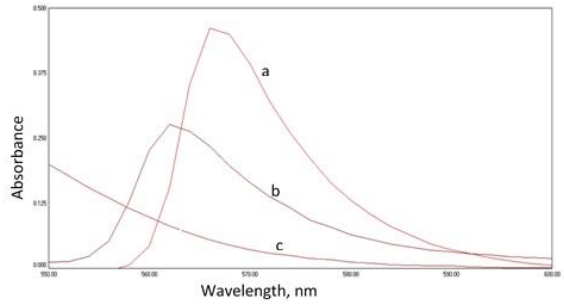


Figure 1. Absorption spectra of 60µg/ml Ibuprofen (a) and 8µg/ml Nystatin (b) with C2R against reagent blank (c) under optimum conditions.

Optimization of reaction conditions

High sensitivity was achieved via the different parameters influences such as pH, reagent concentration, temperature and developing time.

pH effect, acid and buffer solution

pH effect on the reaction of Ibuprofen and Nystatin with C2R was examined in acidic medium, by adding increasing amounts of 0.1M HCl. The maximum colour intensity was found in the presence of 3 ml HCl at pH 1.9 and 2.4 for Ibuprofen and Nystatin respectively (Fig.2). The effect of different buffer solutions of pH 1.9 and 2.4 were tested on the absorption of Ibuprofen and Nystatin ion pair complexes respectively. As shown in (Fig.3), 10 ml of acetate buffer solution gave maximum intensity for Ibuprofen, whereas this intensity decreased for Nystatin in comparison with the presence of HCl. However; different types of acid were examined for Nystatin-C2R complex (Fig.4). It has been found that HCl was the best acid that gave high intensity. Therefore, 10 ml acetate buffer and 3 ml HCl were used for Ibuprofen and Nystatin ion pair complexes in subsequent experiments.

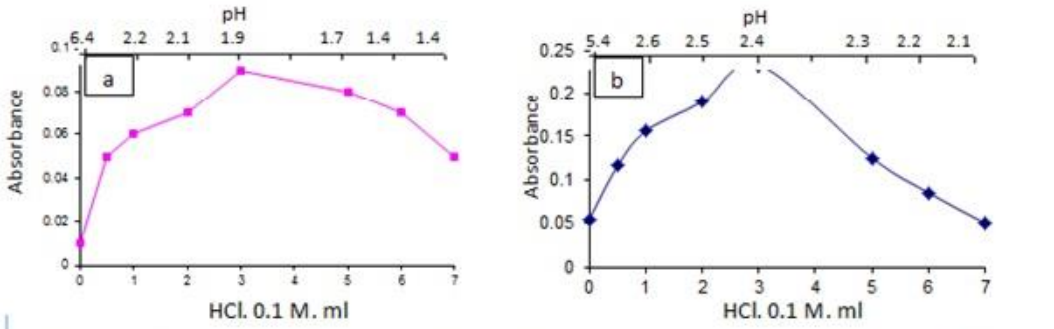


Figure 2: Effect of pH on the absorbance of 25µg/ml Ibuprofen (a) and 8µg/ml Nystatin (b) ion pair complexes with 2.5 ml of 3×10^{-3} M C2R dye.

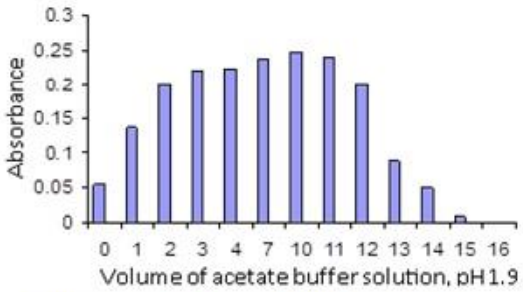


Figure 3: Effect of acetate buffer solution on the absorbance of 25µg/ml Ibuprofen-C2R ion pair complex

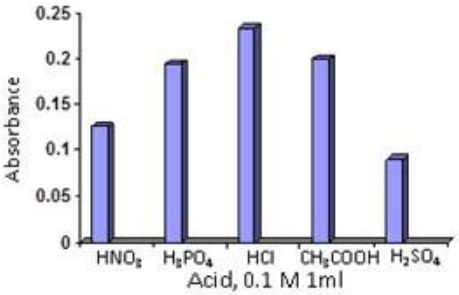


Figure 4: Effect of acids on the absorbance of 8µg/ml Nystatin-C2R ion pair complex

Effect of C2R concentration

The effect of C2R concentration on the absorbance of the ion-pair complexes was investigated by using different concentrations of reagent. As shown in (Fig. 5), it was found that 3×10^{-3} M solution gave maximum sensitivity. However, 2.5ml of

3×10^{-3} M solution gave maximum absorbance for both drugs Ibuprofen and Nystatin (Fig.6) that used in the subsequent experiments.

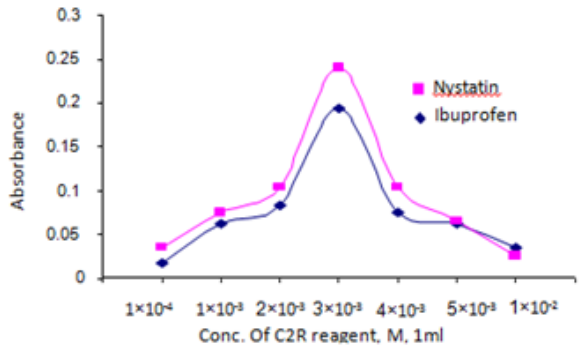


Figure 5: Effect of C2R reagent on the absorbance of 25µg/ml Ibuprofen and 8 µg/ml Nystatin

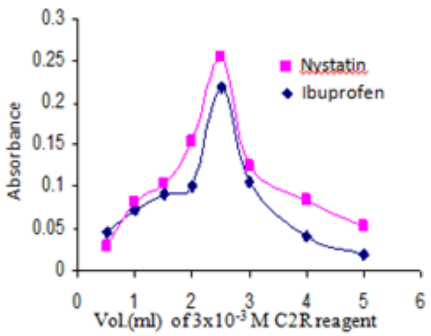


Figure 6: effect of 3×10^{-3} M C2R amount on the absorbance of 25µg/ml Ibuprofen and 8 µg/ml Nystatin

Effect of time and temperature

The effect of time on the colour development and stability of the ion-pair complexes was studied by measuring the absorbance of complexes by increasing time intervals at room temperature (20°C) and 40°C.The results showed that the ion-pair complexes gave maximum absorbance after 15 mins and remained constant about 50 mins for Ibuprofen. For Nystatin, absorbance remained constant about 30 mins at room temperature. However; the absorbance of both complexes was decreased at 40°C indicating the destruction of ion pair complexes, (Fig. 7). Then, 15 mins at room temperature has been considered the optimum quent experiments.

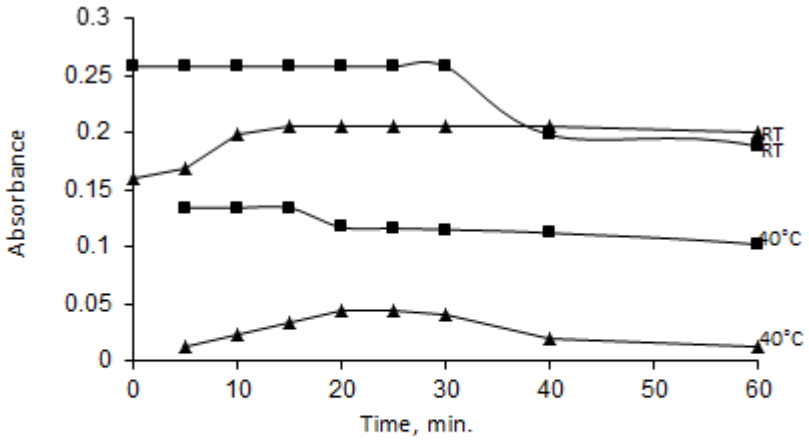


Figure 7: Effect of temperature and developing time on the Absorption of ion-pair complexes for 8 µg/ml Nystatin(■) and 25µg/ml Ibuprofen (▲) with C2R.

Composition and stability constant of the ion-pair complexes

The composition of the ion-pair was studied by Job's method of continuous variation [24] and slop ratio methods [25] using equimolar solutions of 1×10^{-4} M of drugs and C2R. The results shown in (Fig.8) indicated that the ion-pair complexes were formed in the ratio of 1:1. The apparent stability constant was estimated by comparing the absorbance of a solution containing stoichiometric amounts of the drug and C2R (As) to one that contains an excessive amount of C2R reagent (Am). The average conditional stability constant of the complexes was calculated, according to the 1:1 ratio, by the following equation:

$$Kc=1-\alpha / \alpha^2 C$$

$$\alpha=Am-As/Am$$

Where Kc is the stability constant ($l.mol^{-1}$), α is dissociation degree and C the concentration of the complex which is equal to the concentration of the drug. The stability constants for three different concentrations were found $4.2x10^5$ and 9.6×10^6 $l. mol^{-1}$ for Ibuprofen and Nystatin respectively indicating the good stabilities.

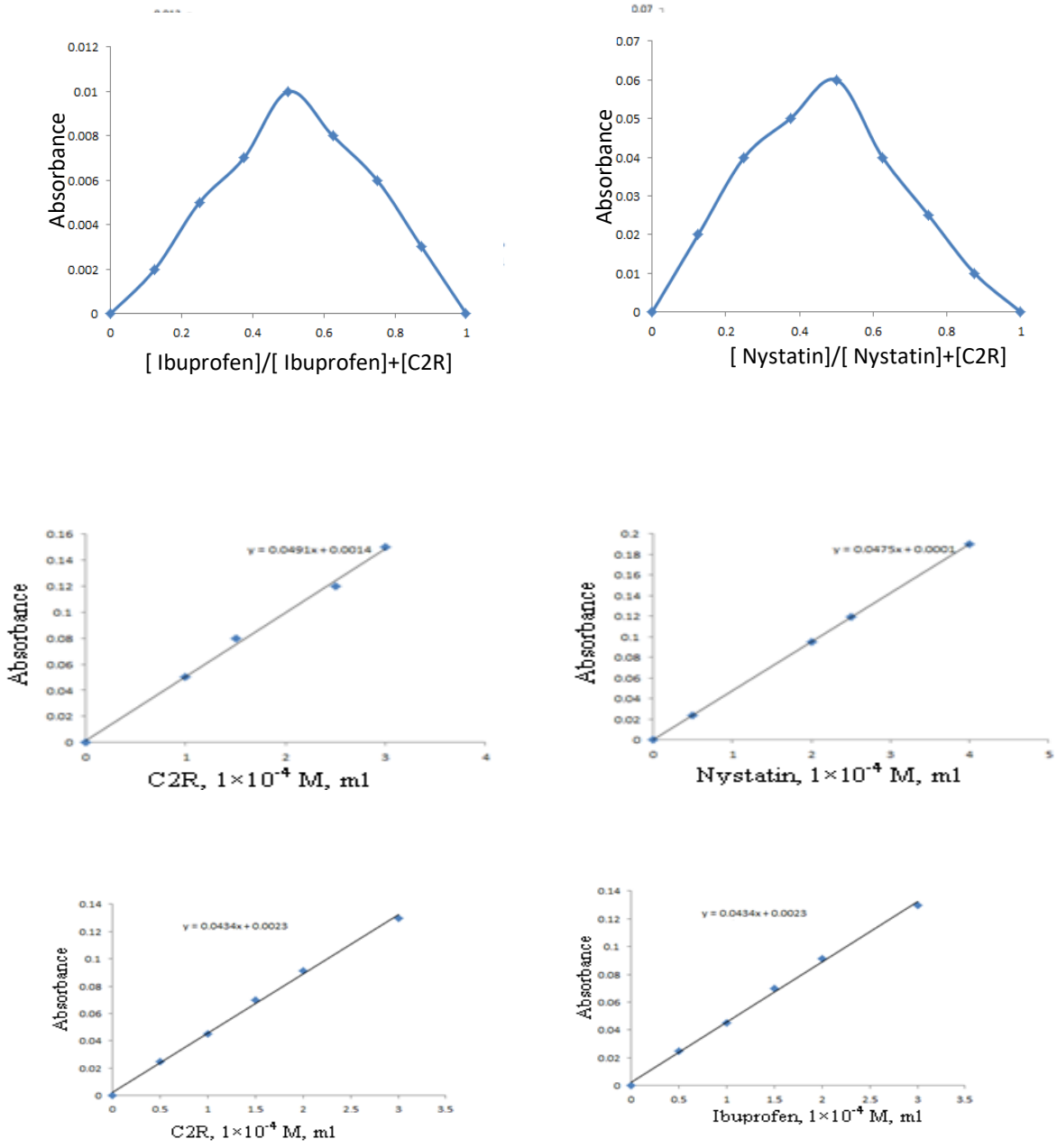


Figure 8: Job's and slope ratio methods for drugs-C2R ion-pair complexes

Linearity and range

Linear relationships were actually found between the absorbance and the concentration of the drugs (Fig. 9) in the ranges 10-90 and 2-40 µg/ml with molar absorptivity values $1.36x10^3$ and $7.59\ x10^3$ $l.mol^{-1}.cm^{-1}$ for Ibuprofen and Nystatin respectively. Five replicates of each three different concentrations for Ibuprofen and Nystatin by the relative standard deviation and accuracy indicated that the method was both precise and accurate. Limit of detection (LOD) and limit of quantitation (LOQ) were then calculated as seen in (Table 1).

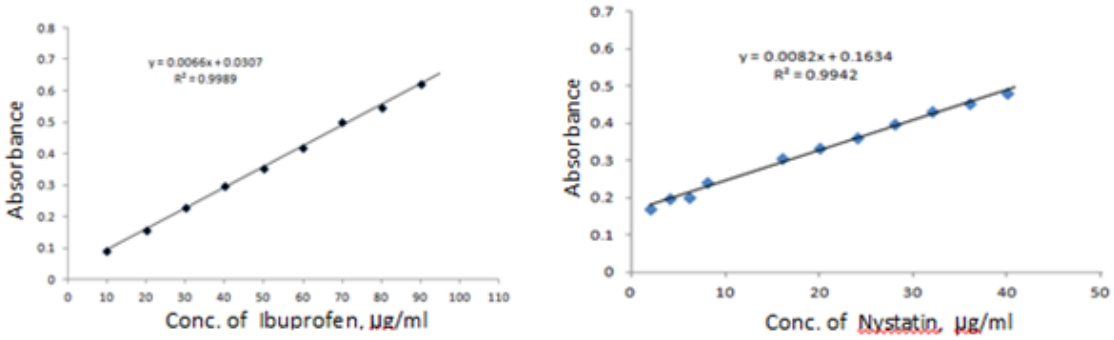


Figure 9. Calibration graphs for determination of Ibuprofen and Nystatin drugs

Table 1. Summary of optical characteristics and statistical data for the proposed method.

Parameter	Ibuprofen	Nystatin
λ_{max} (nm)	562	566
Linear range (µg/ml)	10-90	2-40
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	$1.36x10^3$	$7.59 x10^3$
LOD (µg/ml)	1.7	1,24
LOQ (µg/ml)	5.7	4.14
Average recovery (%)*	99.77	100.60
Regression equation (Y)**		
Slope, *a*	0.0066	0.0082
Intercept, *b*	0.0307	0.1634
RSD*	≤1.4	≤ 0.2

*Average of four determinations

**$Y = aX + b$, where X is the concentration of Ibuprofen and Nystatin in µg/ml

Specificity

The specificity of the method was investigated by the observation of any interference encountered from the common excipients of the pharmaceutical formulations by the measurement of the absorbance of solutions containing 50µg/ml Ibuprofen and 20 µg/ml Nystatin separately, and various amounts of different additives, up to 30 and 15-fold excess for Ibuprofen and Nystatin respectively, in a final volume of 25ml. It was found that the studied excipients did not interfere seriously (Table 2).

Table 2. Effect of excipients for assay of Ibuprofen and Nystatin.

Excipient	Recovery% of 50 µg/ml Ibuprofen per fold excess excipient				Recovery % of 20 µg/ml Nystatin per fold excess excipient			
	5	10	20	30	5	10	12.5	15
Glucose	**96.91**	**97.19**	**100.8**	**90.00**	**99.79**	**100.00**	**100.00**	**100.30**
Lactose	**99.01**	**102.5**	**98.03**	**98.84**	**99.69**	**100.30**	**99.39**	**96.06**
Starch	**98.03**	**99.71**	**100.8**	**100.00**	**100.30**	**99.69**	**10.00**	**90.09**
Arginine	**98.03**	**100.8**	**101.6**	**97.75**	**100.00**	**99.39**	**99.69**	**99.69**
NaCl	**101.12**	**100.56**	**97.75**	**87.07**	**99.69**	**100.00**	**99.39**	**91.39**

Analytical applications

The proposed method was successfully applied to determine Ibuprofen (tablet and ampoule) and Nystatin tablet in their pharmaceutical formulations, using three different concentrations for each formulation. The average recovery % was in the range 99.84-102.60 % for Ibuprofen and 98.95-100.50 % for Nystatin indicating that the method is accurate (Table 3). The obtained results of Ibuprofen tablet were compared statistically by a Student's t-test for accuracy and a variance ratio F-test for precision with the official method procedure [14] at the 95% confidence level with four degrees of freedom. The results showed that the experimental t-test =1.67 and F-test =4.42 were less than the theoretical value (t=4.30, F=9.28), that has indicated that there was no significant difference between the proposed method and official method. However, Nystatin has only biological assay the official method.

Table3: Assay of Ibuprofen and Nystatin in pharmaceutical preparations

Pharmaceutical formulation	Drug amount present(µg/ml)	Recovery* (%)	Average drug content found (mg)	Certified value (mg)
Piofen tablet [a]	**10** **40** **80**	**98.00** **105.02** **103.65**	**102.22**	**100**
Ketoprofen ampoule[b]	**10** **40** **80**	**97.40** **102.00** **101.00**	**100.1**	**100**
Mycodine tablet[c]	**6=3000IU** **20=10000IU** **40=20000IU**	**103.30** **102.0** **98.95**	**101=507050 IU**	**100=500000 IU [26]**

*Average of four determinations
[a] Pioneer for pharmaceutical industries, Iraq
[b]So.Se. PHARM, Industria Pharmaceutica, Made in Italy
[C] Antibiotice SA Romania

Conclusion

For the determination of Ibuprofen and Nystatin, a simple, accurate and precise spectrophotometric method was developed. The method depended highly on the formation of ion-pair complexes between C2R dye and the drugs. Statistics indicated the high reproducibility and accuracy of the suggested method. Analysis of samples showed that there is no interference from common additives and auxiliary substances. The advantage of the method is less time-consuming and requiring no variety of elaborative treatments and tedious extraction procedures as well as the capability of successful application of pharmaceutical preparations**.**

Acknowledgements

We here certify that this research paper is conducted at the laboratories of the college of Education for Pure Sciences and funded by the same college.

References

1. Huskisson E. C., Hart F.D., Shenfield G. M.and Taylor R.T., Practitioner, 207, 639-643. (1971),
2. Sweetman, S. C., "Martindale: The complete drug reference". 35th Ed. London: Pharmaceutical Press,. p.392, (2002).
3. Palabiyik I. M., Dinc E. and Onur F., J. Pharm. Biomed. Anal., 34:473-483 (2004).
4. Ivanović D., Medenica M., Marković S. and Mandić G., Arzneim. Forsch. (Drug Res.),50: 1004-1008 (2000),.
5. Mitic S. S., Miletic G. Ž., Pavlovic A. N., Arsic B. B. and Valentina V. Ž., J. Serb. Chem. Soc., 73:879–890 (2008).

6. Tan S. C., Jackson S. H. D., Swift C. G. and Hutt A. J., Chromatographia, 46:23-32 (1997).
7. Canaparo R. Muntoni, E., Zara G. P., Pepa C. D., Berno E., Costa M. and Eandi M., Biomed. Chromatogr. 14:219-226 (2000).
8. Wang P., Qi M., Liu L. and Fang L., J. Pharm. Biomed. Anal., 38:714-719 (2005).
9. Moedar M., Schrader S., Winkler M. and Popp P., J. Chromatogr. A, 873:95-106 (2000).
10. Hamoudova R. and Pospisilova M., J. Pharm. Biomed. Anal., 41:1463-1467 (2006).
11. Stubberud K. and O. Astrom, J. Chromatogr. A, 826:95-102(1998).
12. Sadecka J., Ckart M., Hercegova A., Polonsky J. and Skacanii I., J. Pharm. Biomed. Anal. 25:881-891(2001).
13. "European Pharmacopoeia", 5th Ed., Council of Europe, Strasbourg, 2002
14. "British Pharmacopoeia" 98/34/EEC, The Stationery Office, London, 2005, p. 1024.
15."The United States Pharmacopoeia", USP-27/NF-22, Authority of the United States Pharmacopoeial Convention, Inc., Rockville, 2004, p. 987.
16.Lupan L., Bandula R., Vasilescu M and Bercu C., Fresenius J. Anal. Chem, 355: 409-411(1996).
17. Botsoglou N.A. and Fletouris D.J., J. Agric. Food Chem., 44:1271-1274 (1996).
18. Gallego J.M. L. and Arroyo J. P., Anal. Chim. Acta, 460:85-97 (2002).
19. Cione A. P., Liberale M. J. and Silva P. M., Braz. J. Pharm. Sci., 46:305-310 (2010).
20. Thomas A.H., Pharm B. and Newlan P., G.J. Quinlan, J. Chromatogr., 216:367-373 (1981).
21. ShokranehF. et.al, Iran. J. Pharm. Res. 14:43-49 (2015).
22. GallegoJ. M. L. and Arroyo J. P. , Anal. Lett., 35:2105-2118 (2002).
23. Raith K., Althoff E., Banse J., Neidhardt H. and Neubert R., Electrophoresis 19:2907-2911(1998).
24. Job P.,"Spectrochemical Methods of Analysis", Wiley Intersience: New York, (1971), p 346.
25. Harvey A.E. and Manning D.L., J. Am. Chem. Soc., 72:4488–4493 (1950).
26. Public Assessment Report for paediatric studies submitted in accordance with Article 45 of Regulation (EC) No1901/2006, as amended, Nystatin, p 6/17, http://www.e-lactancia.org/media/papers/ Nystatin-DS2006.pdf