Sensitive Spectrophotometric Method for Determination of Chloramphenicol in Pharmaceutical Preparations Using 7,7',8,8'-tetracyanoquinodimethane reagent

Theia'a N. Al-Sabha
Department of Chemistry
College of Education
University of Mosul

Hayfa M. Al-Hammoshi
Department of Chemistry
College of Science
University of Mosul

Received 13 / 10 / 2011

Accepted 14 / 12 / 2011

الخلاصة

تم وصف طريقة طيفية بسيطة وحساسة وسريعة لتقدير الكلورامفينيكول بصيغته النقية وفي مستحضراته الصيدلانية. تعتمد الطريقة على تفاعل الكلورامفينيكول المختزل مع الكاشف 7،7'،8،8'-رباعي سيانو كوينوثنائي الميثان عند دالة حامضية 10 لينتج معقد برتقالي اللون يمتلك أقصى امتصاص عند الطول الموجي 475 نانوميتر. أمكن تطبيق قانون بير بحدود 0.05−5 مايكروغرام/مللتر وبامتصاصية مولارية 1.51×10^5 لتر/مول.سم وذلك تحت الظروف المثلى للتفاعل. لقد كان حد الكشف والحد الكمي 0.028 و 0.323 مايكروغرام/مللتر على التوالي، كما وجدت دقة الطريق وتوافقها مرضيتين، حيث بلغت نسبة الاسترجاعية 100.12% والانحراف القياسي النسبي أفضل من 0.7. تم دراسة طبيعة المعقد المتكون وتم اقتراح ميكانيكية التفاعل. طبقت الطريقة بنجاح في تقدير الكلورامفينيكول في مستحضراته الصيدلانية على شكل كبسول وشراب ومرهم بدقة وتوافق جيدين وتم مقارنة الطريقة المقترحة مع الطريقة القياسية في دستور الأدوية البريطاني وطرائق أخرى.

Abstract

A simple, sensitive and rapid spectrophotometric method for the determination of chloramphenicol in pure as well as in dosage form is described. The method is based on the reaction of reduced chloramphenicol with 7,7',8,8'-tetracyanoquinodimethane (TCNQ) at pH10 to form an orange colored complex of maximum absorption peak

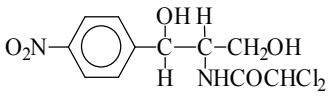
(λ_{max}) at 475 nm. Under the optimized reaction conditions, Beer's law correlating the absorbance with chloramphenicol concentration was obeyed in the range of 0.05-5 μg ml^{-1}. The molar absorptivity was 1.51×10^5 L. $mol^{-1}cm^{-1}$. The limits of detection and quantitation were 0.028 and 0.323 μg ml^{-1}, respectively. The accuracy and precision of the method were satisfactory; the average recovery was 100.12 % and relative standard deviation ≤0.7. The stoichiometry of the reaction was studied, and the reaction mechanism was postulated. The proposed method was successfully applied to the determination of chloramphenicol in its pharmaceutical capsule, syrup and ointment with good accuracy and precisions. The results obtained by the proposed method are compared with those obtained by the official method and other reported methods.

Keywords: Spectrophotometry; Chloramphenicol; TCNQ; Pharmaceutical formulations

Introduction

Chloramphenicol [2,2 - dichloro - N - [(1R, 2R - 2-hydroxy - 1 - (hydroxymethyl) – 2 - (4-nitrophenyl)ethyl] acetamide, (Scheme 1), is a broad-spectrum antibiotic active against Gram-positive and Gram-negative bacteria. It is produced naturally by the soil bacterium *Streptomyces Venezuelan*, but is presently mainly produced by chemical synthesis[1–3]. It has been used in veterinary practice for prevention and treatment of many bacterial infections because of its efficiency, availability and low cost[4,5]. Moreover, Due to its genotoxic effect and severe side effects, such as anemia, leucopenia, agranulocytosis and a plastic anemia in some people, its use is limited to the therapy of serious infections (*e.g.* typhoid fever and meningitis). Furthermore, its use in food production, such as aquaculture farming, has been banned worldwide [1,6]. Various analytical methods have been used for the determination of chloramphenicol, which include official method[7,8], high-performance liquid chromatography[9–11], gas chromatography[12], displacement chromatography[13], ion-selective electrode technique[14], electrogenerated chemiluminescence[15], titrimetry[16–18], electrochemical techniques[19], flow-injection biamperometric method[20], bioluminescence micro method[21], enhanced chemiluminescence method employing an online photochemical reaction[22], atomic absorption spectrometry[23]. Many Spectrophotometric methods, depending on reduction of nitro group, have been reported for determination of chloramphenicol using various reagents such as isonicotinic acid hydrazide[24], N-(1-naphthyl)ethylenediamine[25], trisodium pentacyanoaminoferrate[26], Ninhydrin[27], iminodibenzyl, 3-aminophenol and pyrocatechol molybdate[28], orthogonal polynomials[29], ammonium molybdate[30] and p-dimethylaminobenzaldehyde[31]. However; some of these methods

suffer from disadvantages such as low sensitivity and narrow range of determination, tedious and needing extraction, heating and either require a long time for stable color development or exhibit instability of the colored product. The aim of the present work was to provide simple, high sensitive, and rapid spectrophotometric method for determining of chloramphenicol in pure form as well as in pharmaceutical preparations.



$C_{11}H_{12}Cl_2N_2O_5$
M.wt= 323.1 g/mol

Scheme 1: Chemical structure of chloramphenicol

Experimental

Apparatus

Shimadzu UV-1650 PC UV-Visible spectrophotometer equipped with a 1.0-cm path length silica cell**,** Philips PW (9421) pH-meter with a combined glass electrode was used for pH measurements**.** All calculations in the computing process were done in Microsoft Excel for Windows.

Chemicals

Chloramphenicol and its pharmaceutical formulations (capsule, eye drops and ointment) were kindly provided by state company for Drug Industries and Medical appliance-(SDI) Sammara-Iraq. TCNQ and other chemicals were obtained from Fluka and BDH companies. All solvents were analytical reagent grade and water was distilled.

Standard solution*:* 500 μg ml^{-1} reduced chloramphenicol (RCAP) solution was prepared by dissolving of 50 mg of its pure form in 20 ml of distilled water and was reduced using 0.5 g zinc powder and 1 ml of conc. hydrochloric acid and kept for 30 min with stirring for complete reduction. The reduced solution was filtered and diluted with water to 100 ml in a calibrated flask and kept protected from sun light in ambient bottle.

Reagent solution*:* 4.9×10^{-3} M TCNQ solution was prepared freshly by dissolving 0.05 g in acetonirile and diluted to 50 ml in a calibrated flask.

Buffer solution*:* 0.025 M hydrated sodium tetraborate ($Na_2B_4O_7.10H_2O$) was prepared by dissolving 0.9525 g in distilled water and diluted to 100 ml in a calibrated flask. The pH of 10 value was adjusted with pH meter by addition of 0.1 M NaOH solution.

Recommended procedure

Aliquots of the working solution of RCAP (0.05–5 μg ml^{-1}) were transferred into a series of 5 ml calibrated flasks. Then, 0.5 ml of 4.9×10^{-3}

M TCNQ and 0.5 ml of borate buffer solutions were added and diluted to the mark with acetonirile. The solutions were left for 10 min at room temperature and the absorbance was measured at 475 nm against reagent blank.

Procedure for chloramphenicol assay in capsules, injection and ointment.

Ten capsules (250 mg each) were emptied, pulverized, and dissolved in distilled water with vigorous stirring. The solution was diluted to 1 liter. An aliquot equivalent to 50 mg of chloramphenicol was taken and reduced using zinc and HCl. The solution was filtered, and subjected to the recommended procedure described above for pure chloramphenicol. For injections (0.5% each), a suitable volume was diluted, and the above procedure was followed. For ointment (1%), a portion of the ointment sample equivalent to 50 mg of chloramphenicol was dissolved in 50 ml of petroleum ether and extracted with distilled water. It was further extracted with three 25 ml of distilled water. The combined extracts were filtered and made up to 100 ml with distilled water, a suitable volume was diluted, and the above procedure was followed.

Results and Discussion

Spectral characteristics

The proposed method involves the reduction of chloramphenicol and reaction with TCNQ reagent in the presence of borate buffer of pH 10 to form an orange colored complex having maximum absorption at 475 nm. This wavelength was used for all subsequent measurements. The absorption spectra of the reaction product are shown in Figure 1. The corresponding reagent blank has low absorbance at this wavelength.

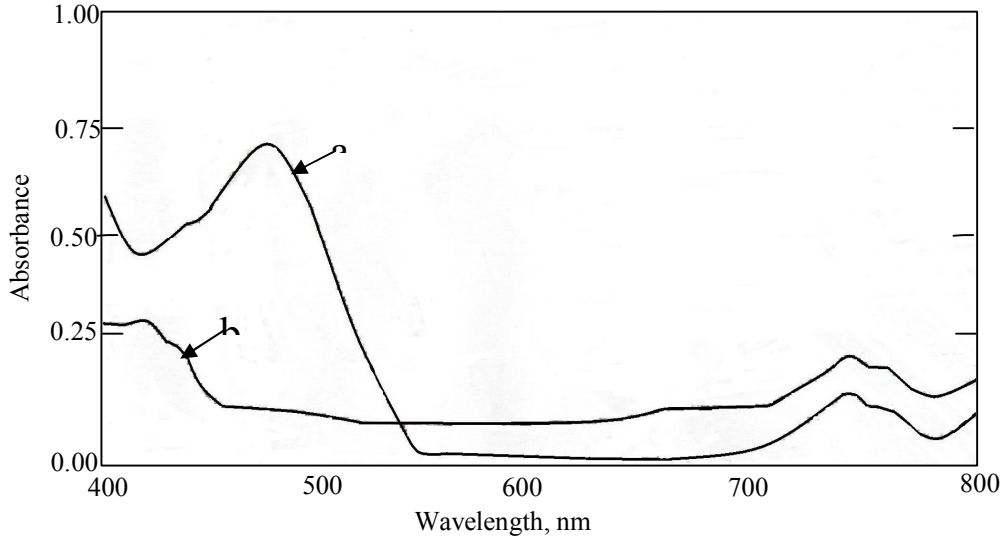


Figure 1: Absorption spectra of (a) RCAP (1.5 µg ml^{-1}) complex with TCNQ reagent (4.9×10^{-3} M) in the presence of pH 10 against reagent blank and (b) reagent blank against acetonirile.

Optimization of reaction conditions

The optimum conditions for the formation of orange colored complex were investigated as a function of pH and the type of the buffer, reaction temperature, time development and the reagent amount.

Effect of pH, buffer solutions and bases

The effect of pH on the absorption of the complex was studied in the range 7-12 using borate buffer solution (adjusted with pH meter by addition of NaOH or HCl solution). It was found that pH 7 gave negative absorbance and the solution became turbid in using pH 8 and 9 where as pH 12 shows no absorbance. However; 0.5 ml of pH 10, which was selected, gave clear solution with maximum absorbance value (Figure 2).

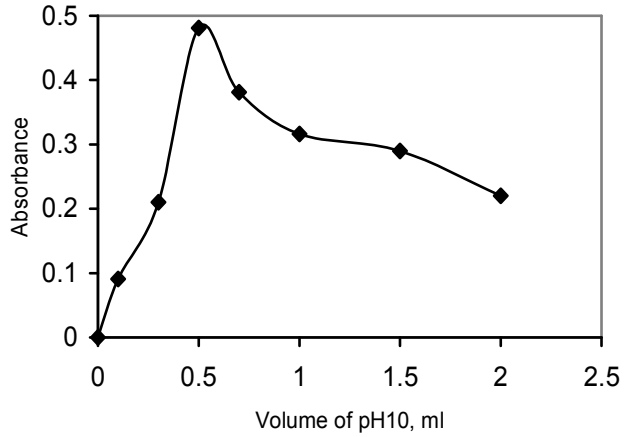


Figure 2. Effect of pH10 amount on the absorption of 1.0 µg ml^{-1} RCAP with TCNQ reagent

Effect of TCNQ concentration

The effect of changing the TCNQ concentration on the absorbance of solution containing a fixed amount of chloramphenicol was studied. It is evident that the absorbance increases with increasing TCNQ concentration and reached maximum on using 0.5 ml of 4.9×10^{-3}M TCNQ (Figure 3).

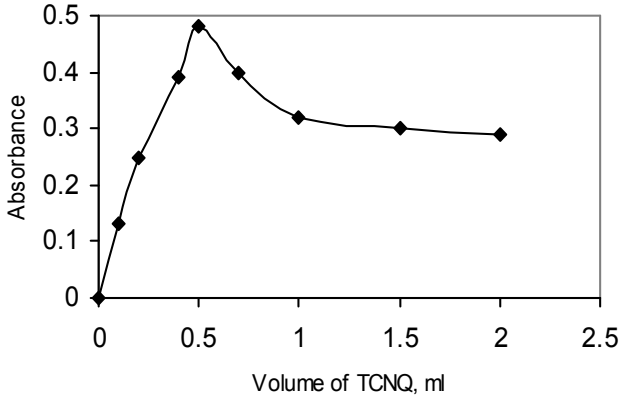


Figure 3. Effect of 4.9×10^{-3}M TCNQ volume on the absorption of 1.0 µg ml^{-1} reduced chloramphenicol in the presence of 0.5 ml pH10

Effect of temperature and time on the color product

The reaction time was determined by following the color development at room temperature and in thermostatically controlled water-bath at different temperatures. The absorbance was measured against reagent blank treated similarly. It was observed that the sensitivity reached maximum after 15 min at room temperature and was stable for a further 25 min (Figure 4). As seen in Figure 4, the absorbance was decreased at higher temperatures, indicating dissociation of the complex.

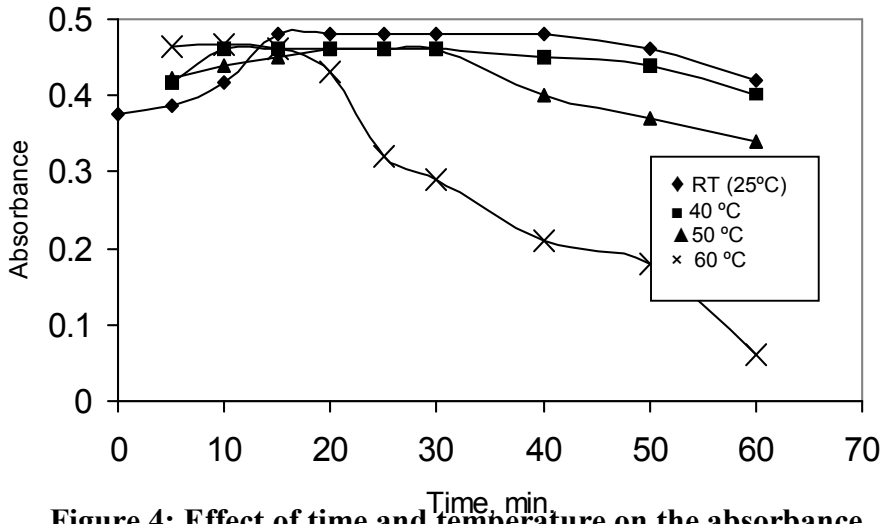


Figure 4: Effect of time and temperature on the absorbance of 1.0 µg ml^{-1} reduced chloramphenicol

Quantification

Under the experimental conditions described above, standard calibration curve of CT-complex for RCAP with TCNQ was constructed by plotting absorbance versus concentration, The correlation coefficient is 0.9982 indicating a good linearity. Beer's law is obeyed in the ranges as cited in Table 1, and the molar absorptivity values indicating the high sensitivity of the method.

Table 1: Summary of optical characteristics and statistics for the proposed method.

Parameter	Values of method
Beer's law limits (µg ml^{-1})	0.05 - 5.0
Molar absorptivity (l.mol^{-1}. cm^{-1})	1.514×10^{5}
LOD (µg.ml^{-1})	0.028
LOQ (µg.ml^{-1})	0.323
Average recovery (%)**	100.13
Correlation coefficient	0.9982
Regression equation (*Y*) *	
Slope, *a*	0.4687
Intercept, *b*	0.0018
RSD**	≤ 0.7

* $Y = a\,X + b$, where X is the concentration of chloramphenicol in µg ml^{-1}.
** Average of six determinations.

Interference

The extent of interferences by some excipients which often accompanied pharmaceutical preparations were studied by measuring the absorbance of solutions containing 1.0 μg ml^{-1} of chloramphenicol and various amounts of diverse species in a final volume of 5.0 ml. It was found that the studied excipients do not interfere in the determination of chloramphenicol in its dosage forms. An error of ± 5 % in the absorbance reading was considered tolerable. Typical results are given in Table 2.

Table 2: Effect of excipients for assay of chloramphenicol

Excipient	Recovery%* of 1.0 μg ml^{-1} chloramphenicol per μg ml^{-1} excipient added		
	25	50	100
Glucose	99.89	98.66	100.80
Lactose	102.00	99.13	96.34
Starch	100.89	101.15	103.72
NaCl	99.72	103.80	104.80
Glycogen	100.70	99.60	97.30
Diphenylamine**	99.27	103.20	103.50

* Average of three determinations
** Prepared in ethanol

Analytical applications

The proposed method was successfully applied to determine chloramphenicol in pharmaceutical preparations. The obtained results were compared statistically by a Student's *t*-test for accuracy and a variance ratio *F*-test for precision with the official method[7] at the 95% confidence level with six degrees of freedom, as cited in table 3. The results showed that the experimental *t*-test and *F*-test were less than the theoretical value (t=2.45, F=6.39), indicating that there was no significant difference between the proposed method and official method. The proposed method is compared favorably with other reported methods as shown in table 4.

Table 3: Assay of chloramphenicol in pharmaceutical preparations using the proposed method and comparison with the official method.

Procedure applied	Pharmaceutical preparation	Drug amount present (μg ml^{-1})	Recovery[a] (%)	Drug content found (mg)	Average recovery (mg)	Certified value (mg)
Proposed NQS method	Capsule	0.5 2 5	97.00 97.30 97.30	248 248 249	248.33 (2.13,3.1)[b]	250
	Eye drops	0.5 2 5	100.00 100.30 100.90	0.47 0.44 0.47	0.46% (1.81,2.16)	0.5 %

	Ointment	0.5 2 5	99.30 100.50 98.80	4.92 5.12 4.93	4.99 (2.23,2.72)	5.0
British Pharmacopoeia	Capsule	10	99.60	249	-	250
	Eye drops	10	98.40	0.493	-	0.5%

[a] Average of three determinations.
[b] Figures in parenthesis are the calculated values for *t*, and *F*.

Table 4: Comparison of spectrophotometric methods for RCAP determination

No. Reagent	λ_{max} (nm)	Linearity (μg ml^{-1})	ε (l/mol.cm)	Remarks	Ref.
1. Diazotization and coupling with NEDA	550	20 – 200	-	Not sensitive	[25]
2. Reduction and coupling with trisodium pentacyano amino ferrate	540	4 – 32	-	Freshly prepared reagent required	[26]
3. Ninhydrin – $SnCl_2$	570	10 – 100	1.7×10^3	Tedious hydrolysis and heating	[27]
4. Aminodibenzyl 3-aminophenol sodium molybdate and pyrocatechol	590 470 490	0.25 – 14 0.2 – 10 0.5 – 10	3.1×10^4 3.1×10^4 3.2×10^4	Several reagents, boiling, organic solvent and strong acid medium	[28]
5. TCNQ	475	0.05 - 5.0	1.51×10^5	Proposed method	

Stoichiometry and stability constant of chloramphenicol -TCNQ complex

The stoichiometry of the reaction of RCAP with TCNQ was studied by the Job method [32], using solutions of equimolar of each reduced chloramphenicol and TCNQ reagent. The results obtained in Fig.7 show that 1:1 reduced chloramphenicol to reagent was formed. This indicates that the nitrogen atom in amino group present in reduced chloramphenicol, which has more electron density and less sterically hindered, is responsible for the formation of the n-π charge transfer complex was formed.

The stability constant (K_{st}) of the product was determined according to the previous ratio and found 6.6205×10^4 l. mol^{-1}.

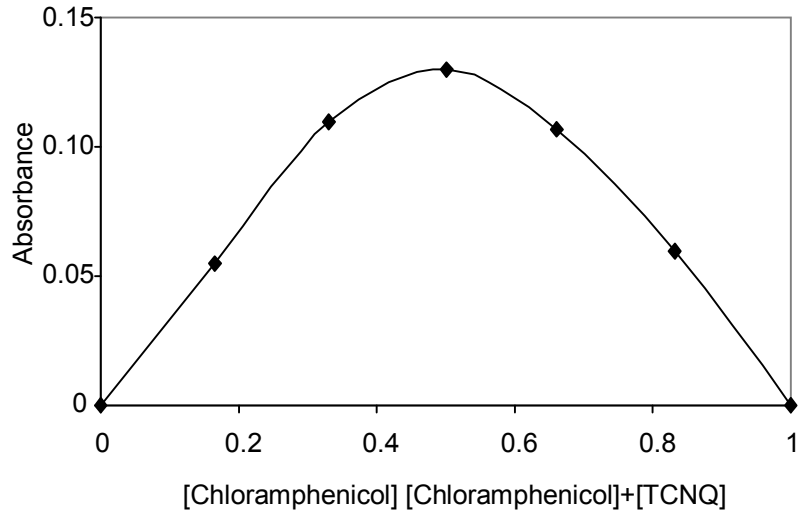
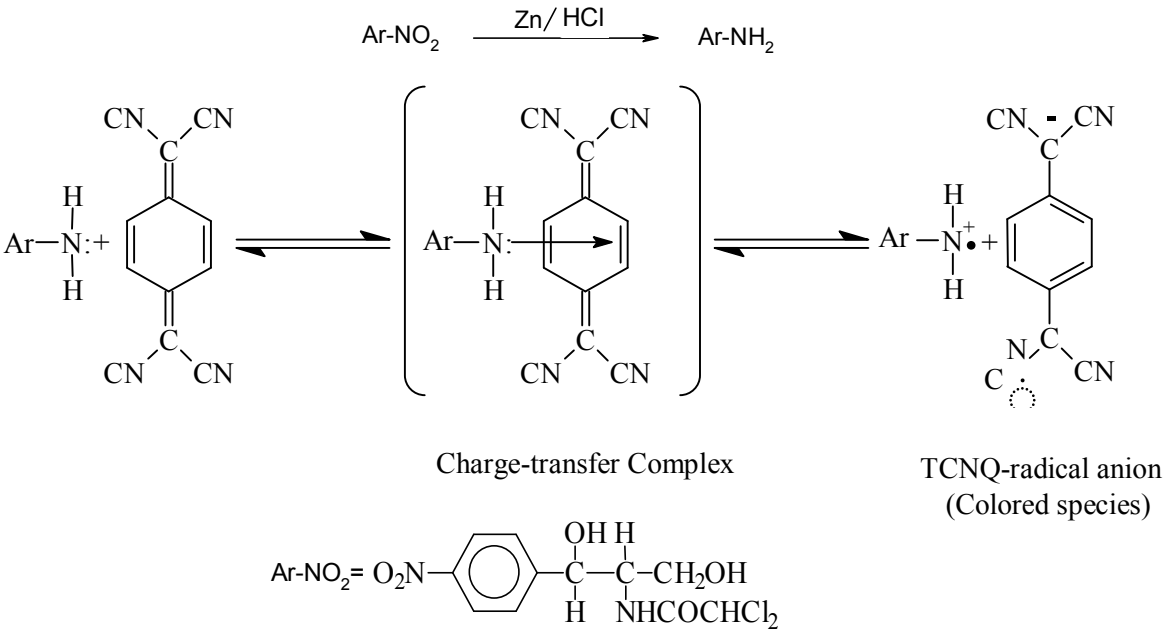


Fig. 2: Continuous variation plot for reaction of chloramphenicol (3.09×10^{-4} M) with TCNQ reagent.

Reaction mechanism

A solution of RCAP and TCNQ in the presence of pH10 yields an intense yellowish-green color, having a new absorption band has been appeared with maximum absorption at 475 nm in electronic spectrum (Fig.1). This band may be attributed to the formation of the radical anion $TCNQ^{\cdot -}$, which was probably formed by the dissociation of an original donor-acceptor (D-A) complex with reduced chloramphenicol. The dissociation of the complex was promoted by the high ionizing power of acetonitrile. On the basis of a literature background search[33] and our experimental findings, a reaction mechanism is proposed (scheme 1).



Scheme 2: Probable mechanism for reduced chloramphenicol -TCNQ complex formation

Conclusion

The proposed method is simple and more sensitive than most of the previously reported spectrophotometric methods. The statistical parameters and the recovery test data indicate the high reproducibility and accuracy of the proposed method. Analysis of authentic samples containing chloramphenicol showed no interference from common additives and auxiliary substances in general. Hence, this method could be considered for the determination of chloramphenicol both in pure form and in pharmaceutical preparations.

References

[1] Database of National Toxicology Program (NTP), U. S.Department of Health and Human Services, Substance Profiles Report on Carcinogens, 11th ed., http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s032chlo.pdf.

[2] Guya, P. A.; Royerb, D.; Mottiera, P.; Gremauda, E.; Perisseta, A.; Stadlerc, R. H., *J.Chromatogr*.,2004,1054, 365-371.

[3] Impens, S.; Reybroeck, W., Vercammen, J.; Courtheyn, D.; Ooghe, S.; De Wasch, K.; Smedts, W.; De Brabander, H., *Anal. Chim. Acta*, 2003, 483, 153-163.

[4] Agüí, L.; Guzmán, A.; Yáñez-Sedeño P.; Pingarrón, J. M., *Anal. Chim. Acta*, 2002, 461, 65-73.

[5] Viñas, P.; Balsalobre, N.; Hernández-Córdoba, M., *Anal.Chim.Acta*, 2006, 558,11-15.

[6] Shakila, R. J.; Vyla, S. A.; Kumar, R. S.; Jeyasekaran, G.; Jasminem, G. I., *Food Microbio*., 2006, 23, 47-51.

[7] British Pharmacopoeia, HM Stationery Office, London (1998), Vol. I, pp. 1553 – 1556.

[8] United States Pharmacopoeia (XXIV Ed.), USP Convention Inc., Rockville (2000), pp. 373–376.

[9] Ashton, M., *J. Liq. Chromatogr*., 1989, 12, 1719 – 1732.

[10] Sadhana, G. S.; Ghogare, A. B., *J. Chromatogr*., 1991, 542, 515 – 520.

[11] Khalil, S. A. H.; Al Shareff, A. H., *Anal. Lett.*, 1993, 26, 1163 – 1179.

[12] Munns, R.K.; Holland, D.C.; Roybal, J.E.; Storey, J.M.; Long, A.R.; Stehly, G.R. and Plakas, S.M., *J. Assoc. Anal. Chem*., 1994, 77, 596 – 601.

[13] Mc Niven, S.; Kato, M.; Levi, R., Yano K. and Karube, I., Anal. Chim. Acta., 1998, 365, 69–74.

[14] Pentari, J. G.; . Efstathiou, C. E, *Anal. Chim. Acta*, 1983, 153, 161 – 168.

[15] Lindino, C. A.; Bulhoes, L. O. S., *Braz. Chem. Soc.*, 2004, 15,. 178-182.

[16] Jayaram, B.; Mayanna, S. M., *Talanta*, 1983, 30, 798 – 800.

[17] Paned, P. N.; Chandra1, U., Fresenius', *J. Anal. Chem*., 1967 231, 35-36.

[18] Dessouky, Y. M.; Toth, K.; Pungor, E., *Analyst*, 1970, 95, 1027 – 1031.

[19] Morales, A.; Toral, M. I.; Richter, P., *Analyst*,1984,109,633– 636.

[20] Bautista, J. A.G.; Mateo, V. G.; Martinez, J., *Anal. Chim. Acta*, 2000, 404,141– 150.

[21] Boeckx, R. L.; Brett, E. M., *Clin. Chem*., 1981, 27, 819 – 822.

[22] David, V. Saez, R. M.; Mateo, J. V.; Martinez, J., *Analyst*, 2000,125, 1313 – 1319.

[23] Montero, R.; Gallego, M.; Valcarcel, M., *Talanta*, 1990, 37, 1129 – 1132.

[24] Mason, E. 0.; Kaplan, S. L.; Baker C. J.; Ritter, D. A., *Ant. Agen. Chem*., 1979, 15, 544-546.

[25] Hassan, S. S. M.; Eldesouki, M. H., *Talanta*, 1979, 26, 531–537.

[26] Devani, M.; Shishoo, C. J.; Doshi, K. J.; Shah, A. K., *J. Assoc. Anal. Chem*., 1981, 64, 557–563.

[27] Hiremath, R. C.; Mayanna, S. M., *Microchim. Acta*, 1984, 2, 175 – 179.

[28] Naik, S. D.; Nagaraja, P.; Yathirajan, H. S.; Hemanthakumar, M. S.; Mohan, B. M., *Pharm. Chem*. J., 2006, 40, 576-581.

[29] Abdel Hamid, M. E.; Abuirjeie, M.A., *J. Pharm. Biomed. Anal.*,1987,5,577 – 583.

[31] Eboka, C. J.; Smart J.; Adelusi, S. A., *Trop. J. Pharm. Res.*, 2003, 2, 215-221

[32] Hargis, L.G.. "Analytical Chemistry, Principles and Techniques", Prentice- Hall Inc., New Jersey. (1988) pp. 424- 427.

[33] Nagaraja, P.; Murthy, K.C. S.; Yathirajan, H. S., *Talanta*, 1996, 43, 1075-1080.