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TWO SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF CEFOPERAZONE AND CEFADROXIL IN THEIR PHARMACEUTICAL PREPARATIONS.

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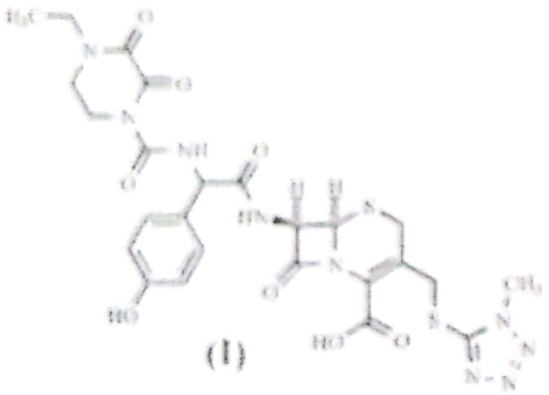
ABSTRACT

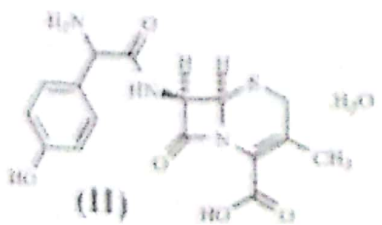
Two sensitive spectrophotometric methods were adopted for determination of two cephalosporins namely cefoperazone sodium and cefadroxil. The first method was based on coupling each of the two compounds with diazotized *p*-nitroaniline. The absorbance was measured at 472 nm, 474 nm for cefoperazone and cefadroxil, respectively. Beer's law was obeyed in concentration rang of 40-200 µg/ml, 8-40 µg/ml for cefoperazone and cefadroxil, respectively. Stoichiometry of the reaction was determined and the ratio of each diazo salt to both drugs was 2:1 in both cases. The second method was based on the application of differential spectrophotometry to the nitroso derivative of both drugs in acid and alkaline medium. Cefoperazone showed no chromic shift (335 nm) on alkalinization but exhibited marked hyperchromic effect, while cefadroxil showed both hyperchromic effect and bathochromic shift (300-349 nm). Calibration graphs of ΔA values versus concentration were linear in range 20-120, and 12-28 µg/ml of cefoperazone and cefadroxil, respectively. The two methods hold well their accuracy and precision when applied to the analysis of Cefobid vials and Duricef capsules.

INTRODUCTION

Many methods have been reported for quantitative determination of cefoperazone (I) and cefadroxil (II). Fluorimetric determination in alkaline degradation[1] of cefadroxil and coupling technique of synchronous[2] fluorimetry were described. Cefoperazone was determined using adsorptive stripping voltametric technique[3] and by single-sweep oscillopolarographic study[4]. First and second "zero crossing" derivative spectrophotometry was applied for mixture of cefoperazone and sulbactam[5] or with cefamandol naftate[6].



(I)



(II)

Cefadroxil was determined in tablet using different spectrophotometric method[7]: Iodine and wool fast Bl.[8] oxidative coupling reaction[9] and in dosage forms mixed with tetracycline[10]. Chromatographic determination of cefoperazone with each of vitamin C[11] and sulbactam by HPLC were reported[12], while cefadroxil was determined by column liquid chromatography[13] and by reversed phase HPLC[14].

Different phenolic compounds were determined depending on reaction of phenolic groups with nitrous acid to form coloured nitroso compounds[15,16] which were utilized in the present work for determination of the two drugs. To increase the selectivity of nitrosation methods, application of differential spectrophotometry[17] to the chromogen was proposed in this work. Selective spectrophotometric assay of these two drugs may also be developed using ΔA method.

EXPERIMENTAL

Apparatus:

Shimadzu 260 uv recording spectrophotometer.

Material and reagents:

All reagents were of analytical grade. Cefoperazone sodium and cefadroxil were obtained as gift from Pharco-Pharmaceuticals, Alexandria and were used as standards without further purification.

Stock solution of the two drugs were prepared by dissolving 0.1 gm of each in 100 ml of dist. water. The working standard solutions of drugs containing 20 µg ml^{-1} for cefoperazone and 4 µg ml^{-1} for cefadroxil were prepared by further dilution from the stocks.

p-Nitroaniline solution, 1 mg ml^{-1} in 1 N hydrochloric acid

Sodium nitrite solution 3%

Sodium hydroxide 1 N

Pharmaceutical preparations

Cefobid vials (Pfizer, Egypt) containing 0.5 gm cefoperazone sodium per vial.

Duricef capsules (Bristol-Mayers Squibb, Egypt) batch No 6279 labelled to contain 500 mg cefadroxil per capsule.

Procedures

A) Diazocoupling method:-

p-Nitroaniline solution (0.8 ml) was mixed with sodium nitrite solution (1.0 ml) in a 25 ml calibrated flask and the mixture was left to stand for 10 min. in ice bath. An aliquot of standard solution [cefoperazone sodium (1-5 mg), cefadroxil (0.1- 0.5mg)] was added to the diazo reagent followed by 2 ml of 1 N sodium hydroxide solution and the mixture was left to stand for 5 min. The solution was then diluted to volume with dist. water and the absorbance was measured at 474 and 472 nm for cefoperazone and cefadroxil respectively against a blank prepared in the same way but omitting the drug.

Stoichiometry studies:

Volumes of 0.5-4.5 ml of 2×10^{-3} M portions of *p*-nitroaniline (v_1) were diazotized and coupled according to previous procedure A with the corresponding complementary volume of 2×10^{-3} M aqueous solution of each of the two drugs (v_2) to give a total volume of 5ml for ($v_1 + v_2$).

B) ΔA method:

Two aliquot portions each of 1 ml (0.5 mg for cefoperazone or 0.1 mg for cefadroxil) were transferred to two 25 ml calibrated flask, 1 ml of HCl (1 mol.L^{-1}) were placed in a boiling water bath for 30 min (nitrosation step). One of the flask was treated with 2 ml 0.1 N NaOH (1) and the other with 2 ml water (2), left for 5 min and then completed to volume with dist water. The absorbance of flask (1) and (2) for cefoperazone was measured at 335 nm each against the corresponding reagent blank. The (A_2-A_1) value at 335 nm was used to calculate the cefoperazone concentration. For cefadroxil the absorbance A_1 at 300 nm and A_2 at 349 nm. The (A_2-A_1) value was used to calculate the cefadroxil concentrations.

C) Procedure for pharmaceutical formulations:

The content of 20 Duricef capsules or 6 Cefobid vials were thoroughly mixed and quantity equivalent to 0.1 mg for cefadroxil or 0.5 mg of cefoperazone was taken and proceed as in procedure A, B.

RESULTS AND DISCUSSION

Cefoperazone and cefadroxil being phenolic compounds were capable for diazocoupling using diazotized *p*-nitroaniline. Due to the blocking of para position diazocoupling would take place in the two ortho positions. The optimum condition for diazocoupling were studied and the stability of the complex were maintained by the use of 0.8 ml of 3% sodium nitrite solution, 0.8 ml 1 mg ml^{-1} *p*-nitroaniline in 1.5 N hydrochloric acid and the medium was made alkaline with 2 ml of 1 N sodium hydroxide solution. Reaction mixture was allowed to stand for 5 min. before dilution with dist. water and measuring the absorbance at 472 nm, 474 nm for cefoperazone and cefadroxil respectively [Fig. (1)].

The diazo compound was stable enough for the use in quantitative analysis of Cefobid vial and Duricef capsule.

To investigate the stoicheiometry of the reaction, Job's method of continuous variations[20] was employed. The maximum absorbance was attained when $\frac{V_2}{V_2 + V_1}$ was 0.640. This result would indicate that the ratio of the diazo salt to either cefoperazone or cefadroxil were 2:1. This was expected because of the two free ortho positions to the hydroxyl group in both drugs. The ΔA method was used for assay of these two drugs. The absorption spectra of nitro-sation reaction products in acid and alkaline media indicated that alkalinization could result in hyperchromic effect as well as bathochromic shift of λmax for cefadroxil (300-349) [Fig. (2)]. On the other hand, alkalinized nitroso cefoperazone showed no shift (335) but it exhibited marked hyperchromic effect [Fig. (3)].

Preliminary investigation of the effect of different reagent concentrations with respect to maximum sensitivity led to the optimum parameters for reproducible results. Adaptation of these spectral changes into ΔA method were successful.

Cefobid vial and Duricef capsules were quantitatively analyzed by application of these procedures.

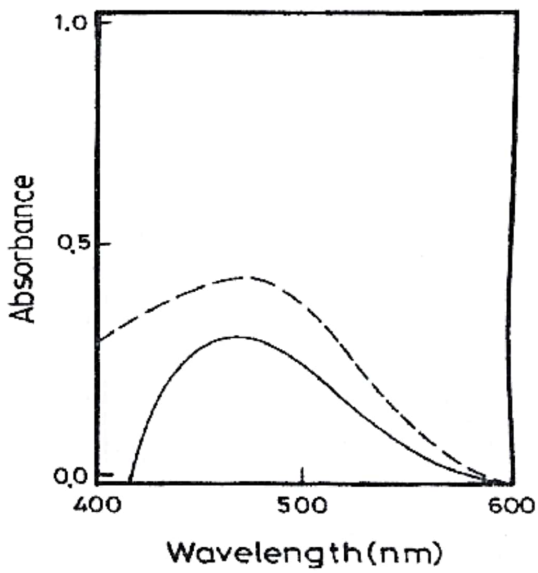


Fig. 1:

——— Absorption spectra of cefoperazone diazocoupling colour

--------- Absorption spectra of cefadroxil diazocoupling colour.

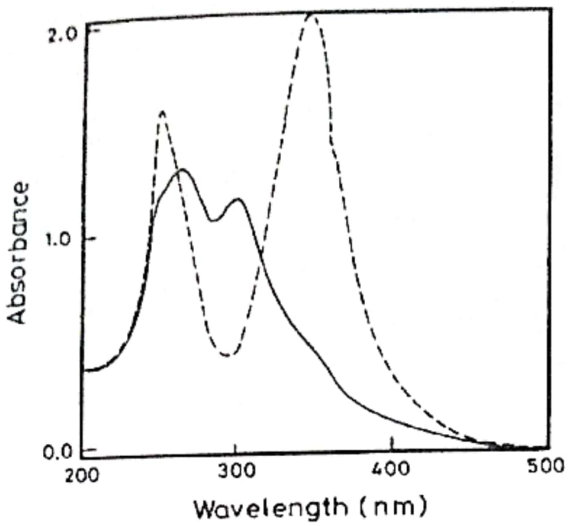


Fig. 2:
——— Absorption spectra of cefadroxil nitroso compound in acid medium.
---------- Absorption spectra of cefadroxil nitroso compound in alkaline medium.

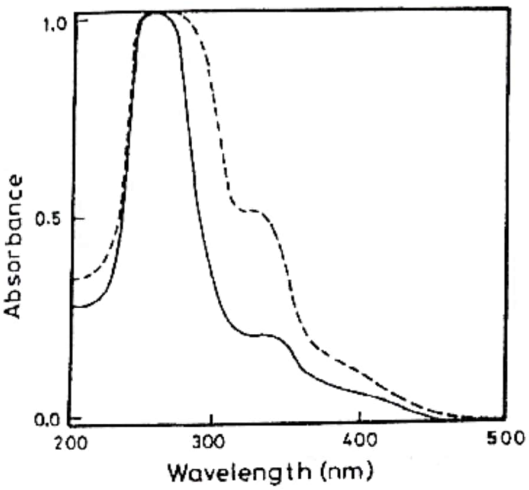


Fig. 3:
——— Absorption spectra of cefoperazone nitroso compound in acid medium.
---------- Absorption spectra of cefoperazone nitroso compound in alkaline medium.

Quantification, accuracy and precision:

A linear correlation between absorbance and concentration was found in the ranges 20-120, 12-28 μg ml^{-1} for cefoperazone and cefadroxil respectively. The correlation coefficient, intercepts and slopes for the calibration data of the two cited drugs were calculated using the least square method and represented by the regression equation, [Table 1].

The precision and accuracy of the two methods were tested by estimating five replicates of the two cited drugs within the Bear's law limits. The mean recovery, standard deviation and the relative standard deviation were given in Table 2.

The utility of each method was verified by means of replicate measurements of pharmaceutical formulations and recovery experiments. Recoveries were determined by adding standard drugs to the pre-analyzed mixture of pharmaceutical preparations by the proposed methods (Table 3).

The performance of the methods was assessed by calculation of t- and f-values compared with official methods[22]. The results obtained showed that the calculated t- and f-values did not exceed the theoretical values (95% confidence limits for five degree of freedom), (Tables 2 and 3). From these findings it could be concluded that, the proposed methods did not differ significantly from official methods.

CONCLUSION

The data given above would reveal that the proposed methods were simple, accurate and sensitive with good precision and accuracy. The proposed methods could be used for the routine determination of cefoperazone sodium and cefadroxil in the pure form and in pharmaceutical formulations.

Table 1: Regression equation and correlation coefficients of cefoperazone and cefadroxil using diazometric and ΔA methods.

	Cefoperazone sodium		Cefadroxil	
Method	Diazometric	ΔA	Diazometric	ΔA
Bear's law limits μg ml^{-1}	40-200	20-120	8-40	12-28
Regression equation Intercept (a)	0.4095	0.0102	0.022	0.054
Slope (b)	0.0026	0.0033	0.0249	0.0241
correlation coefficients (v)	0.9999	0.9997	0.9996	0.9996

A = a + bc
C = concentration in μg ml^{-1}

Table 2: Statistical analysis of the results obtained using proposed methods with the official methods in the analysis of cefoperazone sodium and cefadroxil.

	Cefoperazone sodium			Cefadroxil		
Method	Diazometric	AA	official	Diazometric	AA	official
Mean	100.69	100.73	100.70	100.14	100.07	100.21
SD	2.05	1.31	1.39	0.62	0.22	0.52
N	5	6	5	5	5	3
Variance	4.24	1.73	3.73	0.39	0.05	0.27
t-test	0.611 (2.36)	0.632 (2.26)		0.184 (2.44)	0.565 (2.44)	
f-test	1.134 (6.39)	2.161 (5.19)		1.455 (6.94)	5.458 (19.05)	

Table 3: Statistical analysis of the results obtained by analysis of cefoperazone sodium and cefadroxil in pharmaceutical dosage form using the proposed methods compared with the official methods.

	cefoperazone sodium in cefibid vial			Cefadroxil in Duricef capsules		
Method	Diazometric	AA	official	Diazometric	AA	official
Mean recovery%	100.526	100.7	100.18	100.345	100.071	100.23
SD	2.077	1.226	1.729	0.556	0.534	0.8529
N	5	6	5	5	5	3
Variance	4.314	1.5030	2.989	0.396	0.2856	0.727
t-test	0.162 (2.36)	0.564 (2.26)		0.396 (2.44)	0.417 (2.44)	
f-test	1.1585 (6.39)	1.988 (5.19)		1.144 (6.94)	1.0565 (19.05)	

Values in parenthesis are the tabulated values of t and f at p = 0.025.
N is the number of experiments, where each result is the average of triplicate measurements.

REFERENCES

1. Yang, j; Zhou, G.J.; M,Q.L.; Dong, j and Qli Dong, **j. Anal. Lett.**, 31 (6): 1047(1998).
2. Yang, J.H.; Zhou, G.J.; Jie, N.Q.; Han, R.J.; Lin, C.G. and Hu, J.T. **Anal. Chim. Acta**, 325 (3), 195 (1996).
3. Ali, A.M.M.; El-Maali, N.A. and Ghandour, M.A. **Electroanalysis** 5 (1), 85 (1993).
4. Hu, J.B; Xie, G.Q.; Li, Q.L.; Mao, Y.N. and Huang, Q.Q. Fenxi Shiyanshi, 18 (2), 51 (1999), **Through analytical abstract** 61(8).
5. Parra, A.; Garica-Villanova, J.; Rodemas, V. and Gomez, M.D. **J. Pharm. Biomed. Anal.**, 12 (5); 653 (1994).
6. Morelli, B. **Anal. Lett.**, 21 (5), 759 (1988).
7. Mishra, P.R.; Jain, S. and Jain, N.K. **Indian Drug**, 35 (9); 600 (1998).
8. Sastry, C.S.P.; Rao, S.G.I.; Naidu, P.y. and Srinivas K.R. **Talanta**, 45 (6): 1227 (1998).
9. Sastry, C.S.P.; Rao, K.R. and Spasad, D.S. **Mikrochim. Acta.**, 126 (1-2): 167 (1997).
10. Alyi, F.A.; Walash, M.I. and Belal, F. **Anal. Lett.**, 27 (14): 2677 (1994).
11. Sun, S.Y. and Pan. M.C. Sepu., 11 (4) 259 (1993), **Through analytical abstract** 56 (3) (1994).
12. Zeng, L.M.; Huang, Y.D.; Tang, Y. and Yaowu Fenxi Zazhi, 17 (5), 291 (1997), **Through analytical abstract** 60 (5) (1998).
13. Hsu, M.C.; Chang, Y.W. and Lee, Y.T.; **J. Chromatogr.**, 604 (1-2), 181 (1992).
14. Shinde, V.M. and Shabadi, C.V. Indian Drugs, 34 (7), 399 (1997), **Through analytical abstract** 60(2) 1998.

15. Abou-Ouf, A.; Walsh, M.I. and Hassan, S.M. **Analyst**, 105, 169 (1980).

16. Amer, M.M.; Elldhy, M. and El Tarasse, M.F.; **Pharmazie**, 37, 182 (1982).

17. Belal, S.F.; El Sayed, M.A. and El-Wahly, A.E.; **Analyst**, 104, 919 (1979).

18. Wahbi, A.M.; Abdine, H. and Abdel Hay, M.H.; **J. Assoc. Off. anal. Chemist.**, 61, 1113 (1978).

19. Belal, S.; Abou El Kheir, A. and El Shanawani, A.A.; **Analyst**, 110, 205 (1985).

20. Rose, J.; "Advanced Physico-Chemical Experiments", "Pitman, London, p. 54, 1964"

21. Abou El Kheir, A.; Belal, S. and El Shanwani, A.A., **Pharmazie**, 40, 4, 1 (1985).

22. United States Pharmacopia XXIV, NF US Pharmacopial convention, Rockville, MD, pp. 326,337 (2000).

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طريقتان طيفيتان لتقدير سيفوبيرازون و سيفادروكسيل في المستحضرات الصيدلية.

لبنى محمد عبد العزيز
قسم الكيمياء الطبية - كلية الصيدلة - جامعة الزقازيق – الزقازيق - مصر.

تم في هذا البحث استخدام طريقتين لتقدير مركبي السيفوبيرازون والسيفادروكسيل واعتمدت الطريقة الأولى على تزاوج المركبين مع ديازونيتروزوانيلين وتم قياس الامتصاص عند طول موجه ٤٧٢ نم و ٤٧٤ نم لكل من سيفوبيرازون و سيفادروكسيل بالتناظر وكانت العلاقة بين التركيز والامتصاص خط مستقيم خلال حدود ٤٠-٢٠٠ ميكروجرام/ مل ، ٨-٤ ميكروجرام / مل لكل من سيفوبيرازون و سيفادروكسيل بالتتابع. وكانت النسبة المولاريه للتفاعل ولملح الديازونيتروزوانيلين لكلا المركبين ١:٢ في الحالتين. واعتمدت الطريقة الأخرى على الفرق الطيفي لقياس مركب النيتروزو للعقارين في الوسط الحمضي والقلوي. وأوضحت طريقة الفرق الطيفي في الوسطين أنه ليس للسيفوبيرازون أى تأثير على الطول الموجي ولكن تزيد قيمة الامتصاص بينما اظهر السيفادروكسيل ازاحه لطول الموجي الأعلى وأيضا زيادة في الامتصاص. تم تطبيق الطريقة على حقن السيفوبيد و كبسولات الديوراسيف وقد أثبتت الطريقة الحساسية والإتقان.