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Colorimetric Determination of Certain Cepbalosporins in Pure Form and in Pharmaceutical Formulations

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Summary. A simple and reproducible spectrophotometeric method for the assay of cefotaxime sodium, cefuroxime sodium and ceffriaxone disodium with methyl red and rose bengal reagents has been developed. The procedure is based on ion pair complex formation in buffer medium of pH 5.6 and 9.00, respectively. Beer's law is obeyed in the range 2.0-68 μg ml at Amax 521 and 566 nm using methyl red and rose Bengal, respectively. For more accurate analysis, Rngbom optimum concentration range is found to be l5-65 p.g ml. The molar absorptivity and Sandell sensitivity were calculated. Six replicate analysis of solutions containing seven different concentrations of the examined drugs were carried out and gave a mean correlation ; coefficient < 0.9988; the factors of the regression line equation for the three ' cephalospqrins were calculated. The proposed method was applied to the ' determination of the examined drugs in pharmaceutical formulations and the results demonstrated that the method is equally accurate, precise and reproducible as the official methods.

Introduction

Cefotaxime sodium, cefuroxime sodium and ceftriaxone disodium are some of the third-generation cephalosporin antibiotics characterized by a broad antibacterial spectrum and a resistance to beta-lactamase-producing organisms. In addition to its antimicrobial activity (streptococci, staphylococci, pneumo-cocci, etc. Cephalosporin's are distributed widely into tissues and body fluids, including pleural, pericardial and synovia! fluids. However, while the earlier cephalosporin's

failed to penetrate the central nervous system and were unsuccessful in the treatment of meningitis, the third-generation cephalosporin's enter the central nervous system and reach therapeutic concentrations, there sufficient for treatment of meningitis caused by aerobic gram-negative bacteria. These characteristics are of considerable clinical and hence, analytical interest.

Several analytical procedures are available in the literature for the analysis of cephalosporin's, via spectrophotometric polarographic stripping voltammetric fluorimetric and HPLC methods.

The aim of this work is to develop a simple and reproducible colorimetric procedure for the determination of cefotaxime sodium, cefuroxime sodium and ceftriaxone disodium.in acidic medium by ion pair chemical reaction with methyl red and rose Bengal as counter ions.

Experimental

Material and reagents

All chemicals and reagents used were of analytical grade and all solutions were prepared in doubly distilled water. A freshly prepared $5xl0^{-3}$ M aqueous solution of methyl red and rose bengal was prepared by dissolving appropriate weight in warm . water. Buffer solution. A mixture of 250 ml of 0.2 M potassium hydrogen phthalate and different volume of 0.1 M HC1 was diluted to one liter with water to produce different pH values.

Cefotaxime sodium (I) was obtained from Hoechst Orients Egypt, Cairo, under license from Hoechst AG, Frankfurt/Main, Germany, whereas cefuroxime sodium (II) was obtained from Glaxo Welcome, Egypt, Cairo, under license from Glaxo Welcome group Ltd., England. Ceftriaxone disodium (III) was obtained from EIPICO under license from Roche (Switzerland). Stock solutions were prepared by accurately weighing 100 mg of the examined drug into a 100 ml calibrated flask, dissolved in

warm water and kept in dark to avoid any degradation of the drugs.

Instrumentation

Spectral and absorbance measurements were made with Perkin-Elmer Lambda 5B spectrophotometer UV/Vis with 10-mm quartz cells. The pH of solutions was checked using an Orion Research Model 601A/ digital analyzer.

General Procedure

Pipette a 1.5 ml aliquot of the examined drug solution (concentration range as indicated in Table 1) in a 10 ml calibrated flask. Add 3.0 ml of buffer solution of pH 5.6 and 9.0 using methyl red and rose bengal, respectively, 1.0 ml of 5 x 10"3M reagent solution (freshly prepared) and 2.0 ml of ethanol. Allow the mixture to stand at 50 ± 2 C for 5.0 min and then dilute to volume with water. Measure the absorbance at 520 and 566mm using methyl red and rose bengal, respectively, against a reagent blank in a similar manner. The examined drug concentration was read from a calibration curve.

Formulations

The following commercial formulations were subjected to the analytical procedure Claforan vials (Hoechst Orient Egypt, Cairo) containing 524 mg cefotaxime sodium equivalent to 500 mg cefotaxime per vial, Claforan vials (Laboratorires Roussel 97, rue-de Vaugrirared- Paris) containing 524 mg cefotaxime sodium equivalent to 500 S cefotaxime per vial, and 1048 mg cefotaxime sodium equivalent to 1000 mg cefotaxinl per vial, Primocef (Julphar, Gulf Pharmaceutical Industries, Ras Al-Khaimah, UAE) Containing 250, 500, 1000 and 2000 mg cefotaxime sodium per vial, Zinnat vials (Glaxo Wellcome, Egypt) containing 263 mg, equivalent to 250 mg cefiiroxime'1 Rocephen vials (EIPICO) containing ceftriaxone disodium equivalent to ceftriaxone per vial, Rocephen vials (Roche, F. Hoffinann-La-Roche Ltd, Base! containing ceftriaxone

disodium equivalent to 500, and 1000 mg ceftriaxone per vi were used.

Procedure for vials

The contents of each vial was transferred into separate 500-ml calibrated flask and made up to volume with water. Suitable aliquots of the standard drug solutions were mixed with 0.5 ml of the solution prepared above in 50 ml calibrated flask and diluted to the mark with water. The assay was completed as described above under general procedure. The recovery of the drug was computed from the corresponding regression, equation.

Results and discussion Optimization

Preliminary investigations revealed that the studied drugs react with each of reagents to yield soluble ion-pair complexes exhibiting absorption maximal at 521, and 566 nm using methyl red and rose Bengal, respectively. Under the experimental conditions, the corresponding reagent blank showed a negligible absorbance.

Investigations were carried out to establish the most favourable conditions for ion-pair reaction of reagents with the studied drugs to achieve maximum colour development in their determination. The influence of these variables has been tested and summarized in Table (1) and shown in Figs. (1 and 2).

Composition of the complex

The stoichiometry of the complexes formed was investigated at the optimum pH I applying the molar ratio and continuous variation methods. The results indicated the formation of 1:1 ion-pair (Table 1). The presence of the ion-pair may be supported by the bathochromic shift observed from 485 nm for methyl orange reagent to 521, 520 and 522 nm for cefotaxime sodium, cefuroxime sodium and ceftriaxone disodium, ming rose bengal reagent, shift from 522 nm to 566, 567 and 565 nm, respectively, was obtained for the above mentioned drugs.

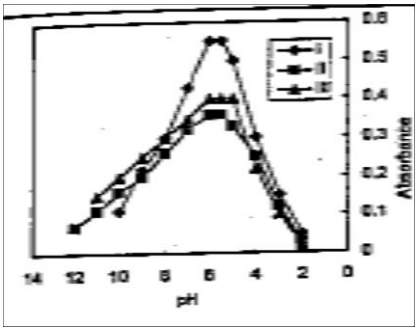

Fig. 1 : effect of pH values on the absorbance of the ion pairs of drug I , II and III.

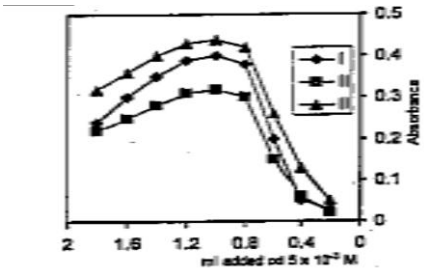

Fig. 4 : effect of rose bengal concentration on absorbance of formed ion pairs with the studied drugs

Analytical features

Regression plots showed that there was a linear dependence of absorbance on concentration over the Beer's law ranges. The optimum conditions were those used in the procedure. The molar absorptivity, Sandell sensitivity, slope, intercept, correlation coefficient, detection and quantification limits were obtained by a linear least-squares treatment of the results for ion pairs 'in solution. For more accurate analysis, Ringbom optimum concentration ranges were calculated (Table1). The reproducibility of the proposed procedure was determined by analyzing ten replicate samples of each drug (40 ugTnT1). The relative standard deviations and ranges of error obtained are given in Table 1.

Table 1: Quantitative Parameters for the proposed procedure

Parameter	Methyl red			Rose bengal		
	I	II	III	I	II	III
pH	5.6	5.5	5.6	9.00	9.00	9.00
λ_{max}	521	520	522	566	567	565
Reagent nm/M	$5.0x10^{-4}$	$5.0x10^{-4}$	$5.0x10^{-4}$	$5.0x10^{-4}$	$5.0x10^{-4}$	$5.0x10^{-4}$
Reaction time/min	5.0	5.0	5.0	5.0	5.0	5.0
Beer's range/µg ml^{-1}	2.0–64.0	2.0–68.0	2.0– 59.0	2.0 –62.0	2.0 –60.0	2.0 –65.0
Temperature/°C	25	25	25	25	25	25
Ringbom range/µg ml^{-1}	3.5-60.0	4.0 –65.0	4.08 –56.5	3.0 –57.0	4.0 –55.0	3.0 –61.4
Detection limit/µg ml^{-1} (22)	0.34	0.45	0.37	0.34	0.36	0.35
Quantification limit/µg ml^{-1} (22)	1.98	2.08	2.02	1.97	2.05	1.90
Molar absorptivity/L mol^{-1} cm^{-1}	$8.32x10^4$	$5.3x10^4$	$2.97x10^4$	$3.21x10^4$	$2.83x10^4$	$3.29x10^4$
Sandell sensitivity/ng cm^{-2}	4.17	6.67	10.26	10.81	12.50	9.30
Stoichiometric ratio	1: 1	1: 1	1: 1	1: 1	1: 1	1: 1
Stability constant	5.69	5.89	5.50	9.06	8.64	9.65
Stability/h	15	15	18	15	18	18
Regression equation [a]						
Slope	0.14	0.09	0.10	0.09	0.08	0.11
Intercept	-0.037	0.056	0.040	0.067	-0.025	-0.030
Correlation coefficient (r)	0.9996	0.9992	0.9988	0.9994	0.9998	0.9996
RSD% of slope	$3.45x10^{-4}$	$6.13x10^{-4}$	$5.27x10^{-4}$	$7.32x10^{-4}$	$5.34x10^{-4}$	$8.09x10^{-4}$
RSD% of intercept	$1.67x10^{-4}$	$2.98x10^{-4}$	$2.67x10^{-4}$	$3.45x10^{-4}$	$2.65x10^{-4}$	$4.12x10^{-4}$
Range of error	1.20	1.15	1.45	1.00	1.35	1.50
RSD%	0.91	0.78	0.85	1.15	1.07	0.98
Student t-value (2.57)[b]	0.35	0.71	0.75	0.76	0.35	0.56
Variance ratio F-test (5.05)[b]	1.72	4.07	2.92	1.02	3.20	1.49

[a] A = a + bC, where C is the concentration in µg ml^{-1}

[b] Values in parentheses are the theoretical values for t- and F- values.

Interference studies

The effects of common excipients that often accompany the studied drugs in various dosage forms were tested for possible interference in the assay. An attractive feature of the procedure is its relative freedom from interference by the usual tablet diluents and excipients. Amounts far in excess of their normal occurrences in dosage forms w ere added, and no effect due to these excipients was noted.

Table 2: Assaying of the studied drugs in dosage forms applying the standard addition technique

Pharmaceutical Formulation	Taken Added	Found jignil' UR ml" ug ml"		
		(MR)±SD	fRB)+SD	Official+SD
Zinnat .	10 0	9.8510.53	9.80 ± 0.42	9.90 ± 0.75
(250 mg of II)	10	19.80 ± 0.40	19.75 + 0.38	19.90 ±0.99
	20	29.70 ± 0.64	29.60 + 0.65	30.25 ±1.03
	30	39.60 + 0.58	39.50 + 0.67	40.25 ±1.34
Claforan	5.0 0	4.84 + 0.34	4.75 ± 0.32	4.85 + 0.64
(500 mg of I)	10	14.80 + 0.41	14.70 ± 0.45	14.85 + 0.41
	30	34.70 + 0.81	34.65 + 0.43	34.90 ±1.07
	50	54.50 + 0.66	54.45 ± 0.44	54.60 ±0.78
Claforan	7.5 0.0	7.25 + 0.95	7.20 ± 0.67	7.15 + 0.54
(500 mg of I)	15	22.15 ±0.83	22. 15 ±0.78	22.05 + 0.86
	30	37.05 + 0.67	37.00 ± 0.92	37.00 ±0.96
	45 •	51.80 + 0.97	51.70 + 1.08	51.60±1.11
Primocef	5.0 0.0	4.80 + 0.76	4.75 + 0.58	4.75 + 0.47
(500 mg of I)	7.5	12.25 ±0.56	12.30 ± 0.46	12.20 + 0.67
	22.5	26.60 ±0.48	26.65 ± 0.62	26.70 ± 0.87
	37.5	41.95 ±0.75	41.90 + 0.71	42.00 ±1.01
Primocef	10 0.0	9.70 + 0.85	9.65 ± 0.90	9.60 ±0.97
(1000 mg of I)	20	29.50 + 1.01	29.60 + 1.08	29.40 ±1.13
	40	49.25 ± 0.90	49.30 ± 0.91	49.20 ± 0.86
Primocef	15 0.0	14.50 ±1.12	14.55 + 0.98	14.50 + 0.86
(2000 mg of I)	7.5	22.00 ±1.20	21.95 + 1.05	21. 90 ±0.96
	22.5	3 1.80 ±0.97	31.75 ±1.11	31.75 ±1.22
	37.5	51.55 ±1.22	51.50 + 1.17	51.40±1.33
Rocephen	20 0.0	19.45 ±0.55	19.40 + 0.75	19.25 + 1.25
(lOOOmgofm)	15	24.30 ± 0.82	34.25 + 0.96	34.35 ± 0.97
	30	48.85 ±0.56	48.80 + LOS	48.75 + 1.37
	37.5	56.60 ±0.74	56.70 ±0.66	56.55 ±1.71
Rocephen	15 0.0	14.50 ±0.66	14.60 + 0.85	14.50 ±1.11
(lOOOmgofm)	10	24.45 ±0.97	24.50 + 0.78	24.40 ±1.21
	30	44.25 ±1.04	44.30 + 1.14	44.30 ±1.25
	50	64.00 ±1.20	64.00 ± 1.23	64.05 ±0.87

Average of six determinations.

Analytical applications

The proposed procedure was further applied to the analysis of certain dosage forms containing the studied drugs. The results in Table 2 are in accordance with those obtained by the official methods.™ Statistical analysis of the results using Student test and the variance ratio F- test showed no significant difference between the performance of the proposed and official methods as regards to accuracy and precision .

Conclusion

The proposed procedure is fairly simple, less time-consuming and more sensitive than the official methods/211 The principal advantage of the proposed procedure is suitable for the determination of the studied drugs in their dosage forms without interference from excipients and additives such as starch, glucose, magnesium stearate or from common degradation products suggesting application in bulk drug analysis.

Statistical comparisons for the results of the proposed procedure with the official methods indicate that there is no significant difference with regard to accuracy and precision. In comparison with the existing photometric methods the proposed procedure especially with methyl red are simpler, more sensitive, cheaper and accurate.

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