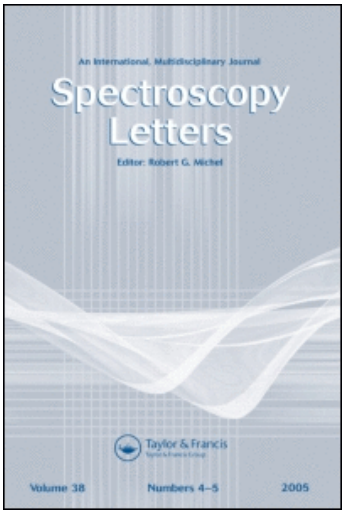

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Fluorescence Quenching Method For The Assay Of Three Cephalosporins Using The Combined Trigonometric Function Of Fuorier Series

Keywords: Spectrofluorometry, Cephalosporins, Fourier series, Fluorescence Quenching.

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Three cephalosporins (CS), namely, Cefadroxil, Cephalexin and Cephradine were assayed in their pharmaceutical preparations through the utility of mercurochrome fluorescence quenching phenomenon proceeding the alkaline degradation of cephalosporins. The decrease in mercurochrome fluorescence intensity was directly proportional to the degraded cephalosporins (DCS) in concentration range of 0.01-0.05 mg%. The parameters for the applications of the combined trigonometric function of Fourier series ($\Delta t'$

method) were investigated aiming at cancelling the systematic error appeared during the use of the differential spectrofluorometric method (ΔF-method). The results of $\Delta t'$ method was statistically compared with those of the orthogonal function method (ΔP_2 method). Both methods were found of equal potential in correcting for the interferences. The Stern-Volmer equation was here derived using ΔF, $\Delta t'$ and ΔP_2 in order to assess the quenching power of DCS to mercurochrome compared with CS. The quenching constant ratios of DCS to CS were extremely high and decreasing in the order of cefadroxil, cephradine and cephalexin.

Fluorescence quenching of halogenofluoresceins-mercuries and fluorescein-mercury compounds could be induced using sulphur compounds such as sulphide ion, thiourea and thioxine derivatives, and long chain alkylamine compounds[1-7]. Such phenomenon has proved to be useful in detecting and determining microamounts of thiols[8-10]. Containing β-lactam ring with sulphur atom, penicillins have been determined colorimetrically after reaction with different cations like iron, mercury, copper and vanadium[11-14]. Lately, penicillins have been determined through fluorescence quenching of mercurochrome in a weakly basic medium[15].

Generally, the ratios of fluorescence intensities in the absence (Fo) and presence (F) of a quencher (Fo/F) to the quencher concentration [Q] is presented by the Stern Volmer Equation[16]

$$Fo/F = 1 + K\,[Q] \qquad \text{------} \rightarrow \text{eq. (1)}$$

The mercurochrome (as indicator) is here utilized to assay 3 cephalosporins after alkaline hydrolysis (as quenchers). The obtained data, ΔF versus λ, have been treated by the combined trigonometric function of Fourier series. Such method has been compared with the orthogonal function method[17] and differential spectrofluorimetric method (ΔF-method) and proved to be of high potential in correcting for the systematic error appeared in the analytical results.

Analogous to the utility of Fourier series in UV spectrophotometric analysis[18], the emission curve could be convoluted to get the measured coefficient t' at the optimum λ_m. Such coefficient is linearly correlated to the analyte concentration.

$$\text{i.e.} \quad t'_j = \alpha'_j C \qquad \text{----------} \rightarrow \text{eq. (2)}$$

where α'_j is the corresponding constant.

In the presence of interfering substance, arrangement to minimize such interference is based on the nature of diverse substance and its contribution (positive or negative) to the exciting and emission spectra of the analyte. Parameters chosen for the Fourier function method applica-

tions include, the combined trigonometric function, number of points, intervals and analytical λ_m^{19} .

Experimental

Apparatus:

The fluorimetric measurements were carried out on a Perkin-Elmer Model 650-10S Spectrofluorimeter equipped with 1-cm quartz cells and a Perkin-Elmer Model 56 recorder. The instrument controls were set as follows: sensitivity range, 0.3; slit width, 10 nm for both excitation and emission; response and mode, normal.

Materials:

(A) Authentic powders : cefadroxil monohydrate (I), cephalexin monohydrate (II) and cephradine (III).

(B) Pharmaceutical Preparations: (1) Duricef capsules (Mead Johnson, Pharco Co., Egypt) 500 mg cefadroxil monohydrate each. (2) Keflex capsules (Lilly Co., Kahira, Egypt) 250 mg cephalexin monohydrate each. Keflex syrup; each 5 ml contains 125 mg cephalexin monohydrate. (3) Velosef capsules (Squibb Co., Egypt) 250 mg cephradine each. Velosef syrup: each 5 ml contains 125 mg cephradine.

(C) Mercurochrome solution: 1.0% w/v in water.

(D) McIlvaine's-Citric acid Phosphate Buffer pH. 7.0: 181.5 ml of 0.1 M citric acid monohydrate ($C_6H_8O_7.H_2O$) and 818.5 ml 0.2 M disodium monohydrogen phosphate dihydrate

($Na_2HPO_4.2H_2O$) were mixed in 1L-volumetric flask. The reagents were of analytical grade.

(E) Polyvinylpyrrolidone (PVP): 1.0% w/v solution in water.

(1) Preparation of the Alkali-induced degradation products (DCS)[20].

Transfer quantitatively an accurate weight (20.0 mg) of the cephalosporin (CS) into 100-ml volumetric flask using 10.0 ml of 1 N sodium hydroxide solution. Leave for 20 minutes at room temperature for complete degradation. Neutralize with 10.0 ml 1 N hydrochloric acid solution. Complete to the mark with water.

(2) Preparation of Calibration Graphs for the Degraded Cephalosporins:-

Dilute 10.0 ml of the prepared degradation product of cephalosporin to 100.0 ml with water. Transfer 1.0-5.0 ml portions (in 1.0-ml steps) of the diluted solution to separate 100-ml volumetric flasks. Add to each 2 ml of 1.0% w/v PVP solution, 5.0 ml of 1.0% w/v mercurochrome solution, and 20.0 ml buffer solution of pH 7.0 and complete to volumes with water. Prepare a reagent blank, and measure the fluorescence intensity of the blank (Fo) and of each solution (F) versus water at λ_{em} = 530 nm and λ_{ex} = 300 nm. Calculate (a) The ΔF values = (Fo-F). (b) The combined trigonometric coefficient ($\Delta \grave{t}$) using the following formula:

$$\Delta\grave{t}=[\Delta Fo(+1.5) + \Delta F_1(o) + \Delta F_2(-1.5) + \Delta F_3(-1.5) + \Delta F_4(o) + \Delta F_5(+1.5)]/3 \quad \text{eq. (3)}$$

(c) The orthogonal function coefficient (ΔP_2) from the following formula:

$$\Delta P_2=[\Delta Fo(+5) + \Delta F_1(-1) + \Delta F_2(-4) + \Delta F_3(-4) + \Delta F_4(-1) + \Delta F_5(+5)]/84 \quad \text{eq. (4)}$$

where:

- In case of $\Delta t\grave{}$, the values in paranethesis are the $T\grave{}$ values of $\cos x + \cos (x+ \frac{2\pi}{n+1})$
- The subscripts 0,1,2,.....,5 represents 520,525,530..... 545 nm. respectively.

(3) Determination of DCS constituting 3-50% in mixtures with CS.

Weigh accurately 20.0 mg of the cephalosporin. Transfer to 100-ml volumetric flask with water and adjust the volume. Transfer 1.0-6.0 ml portions (in 1.0-ml steps) of the prepared solution into separate 100-ml volumetric flasks. Add to each; 5.0 ml of the previously prepared and diluted solution of the alkaline degradation product ($\simeq$ 0.1 mg%); 2.0 ml 1.0% w/v PVP solution; 5.0 ml of 1.0% w/v mercurochrome solution and 20.0 ml buffer solution of pH 7.0. Complete to the mark with water. Prepare blank experiment, substituting sample solution with water. Complete as described under calibration curve preparation starting from (Measure the fluorescence intensity .....).

Application to Pharmaceutical Preparations:

(1) Assay of Cefadroxil, Cephalexin and Cephradine in Capsules through alkaline degradation and subsequent application of the proposed methods:

Transfer accurate weights (equivalent to 20.0 mg of the pure drug) from the contents of previously mixed 20 capsules into 100-ml volumetric flasks. Dissolve using 10.0 ml of 1 N sodium hydroxide solution. Leave for 20 minutes at room temperature. Neutralize with 10.0 ml 1 N hydrochloric acid solution. Adjust the volume to the mark with water. Complete as under preparation of calibration graphs for the degraded cephalosporins starting from "Dilute 10.0 ml of the prepared degradation product...".

(2) Assay of Cephalexin and Cephradine in Suspensions:

Reconstitute the bottle contents using 60.0 ml water (each 5 ml equivalent to 125 mg). Transfer a volume of the suspension (equivalent to 20.0 mg of the pure drug) into 100-ml volumetric flasks. Complete as under assay of capsules starting from "Dissolve using 10.0 ml of 1 N sodium hydroxide solution .....".

Results and Discussions

Chemical Quenching:

The addition of micrograms of degraded cephalosporins-hereafter DCS-(quenchers) to mercurochrome (MHg, indicator);

the halogenofluorescien mercury compound; changed the color of the latter.

Due to the production of hydrogen sulphide during degradation of cephalosporins in alkaline medium, the fluorescence quenching of mercurochrome was assumed to the formation of Hg-S bond[8 - 12]. The complex formed between degraded cephalosporin solutions and mercurochrome was referred as MHg-DCS.

The excitation maximum and emission maximum of mercurochrome solution in water are 300 and 530 nm, respectively (Fig. 1 (a),(b)). Addition of DCS to the mercurochrome solution resulted in the decrease of fluorescence intensity (Fig. 1(c)). The decrease in fluorescence intensity (due to quenching phenomenon) of the mercurochrome solution was directly proportional to the DCS concentration in buffer pH 7.0 solution (Fig. 2).

The Fourier Function Method:

According to the general rules previously mentioned[18,19] the $T^{`}$ function [where $T^{`} = \cos x + \cos (x+\frac{2\pi}{n+1})$] was chosen as it made a large contribution to the $\Delta F=(Fo-F)$ emission curves of the MHg-DCS over the xyz segments (Figs. 3a,4a& 5a). Hence the coefficient $\Delta t^{`}$ should afford a precise concentration estimate. On the other hand, the $\Delta F$ emission curves of MHg-CS give more or less linear curve under xyz region of DCS. Convolution of both types of curves were made through $\Delta t^{`}$ calculations using 6-points at 5 nm

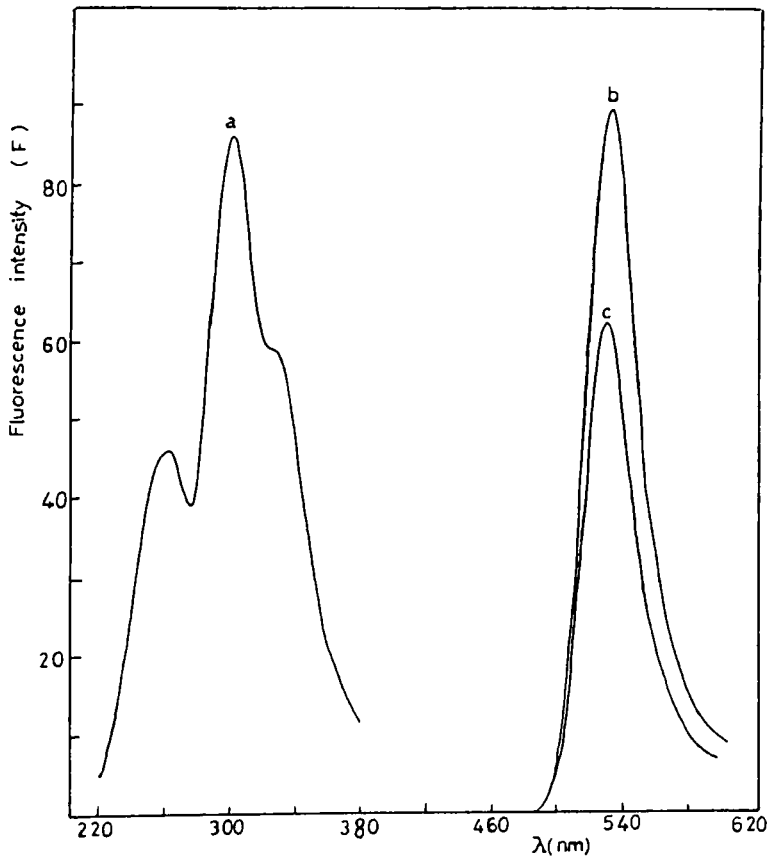

Figure 1. Excitation (a) and Emission (b) spectra of 0.05 mg% mercurochrome in buffer pH 7.0.(c) The emission spectrum of MHg-DCS corresponding to 0.04 mg% of cephalexin and 0.05 mg% mercurochrome.

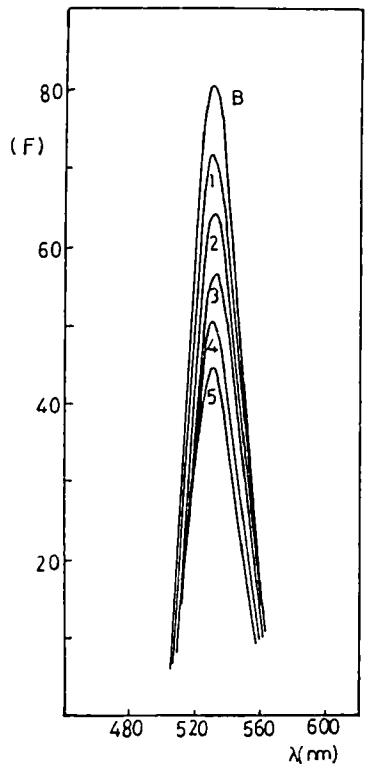

Figure 2. Quenching effect of different concentrations of the alkali-induced degradation products corresponding to 0.01-0.05 mg% cephalexin (curves 1-5) on the emission spectrum of 0.05 mg% mercurochrome(B).

intervals between wavelength range of 520-545 nm. The convolution curve for MHg-DCS exhibited optimum Δt at such λ_m (532.5 mm) where the corresponding MHg-CS showed no contribution (Figs. 3a,4a&5a).

Under the described experimental conditions a linear correlation was obtained between the combined trigonometric

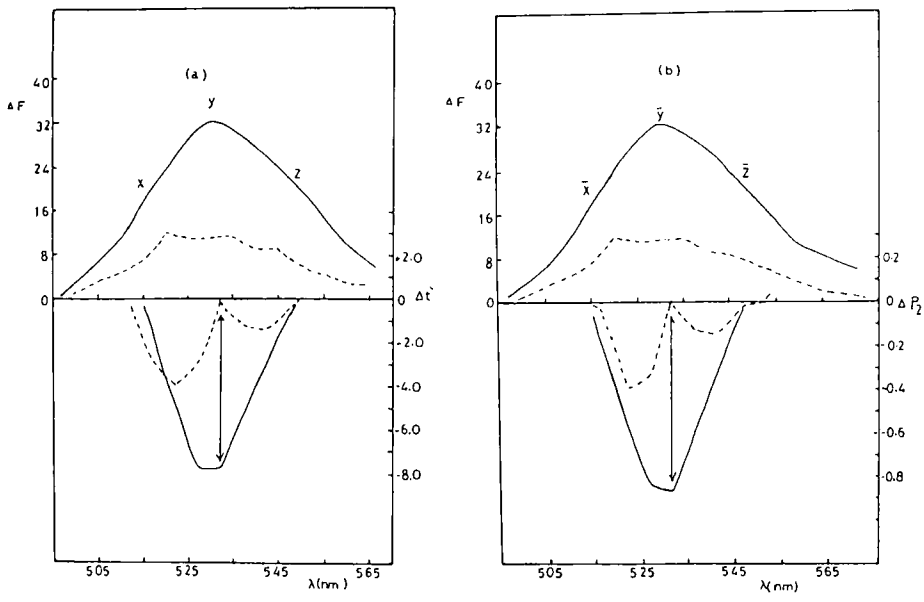

Figure 3. (a) The ΔF curves and their convoluted $\Delta t`$ curves (derived therefrom) for MHg-DCS (——) and MHg-CS (---), each corresponding to 0.05 mg% cefadroxil and 0.05 mg% mercurochrome in buffer pH 7.0. (b) The ΔF curves and their convoluted ΔP_2 curves (derived therefrom) for MHg-DCS (——) and MHg-CS (---), each corresponding to 0.05 mg% cefadroxil and 0.05 mg% mercurochrome in buffer pH 7.0.

function coefficient $\Delta t`$ and DCS concentrations corresponding to the range 0.01 - 0.05 mg% of the CS (in 0.01 mg% steps). The regression analysis was made for the slope (b), intercept (a) and correlation coefficient (r) for the three cephalosporins as presented in Table 1.

For the sake of the comparison, the orthogonal function and ΔF methods were also conducted.

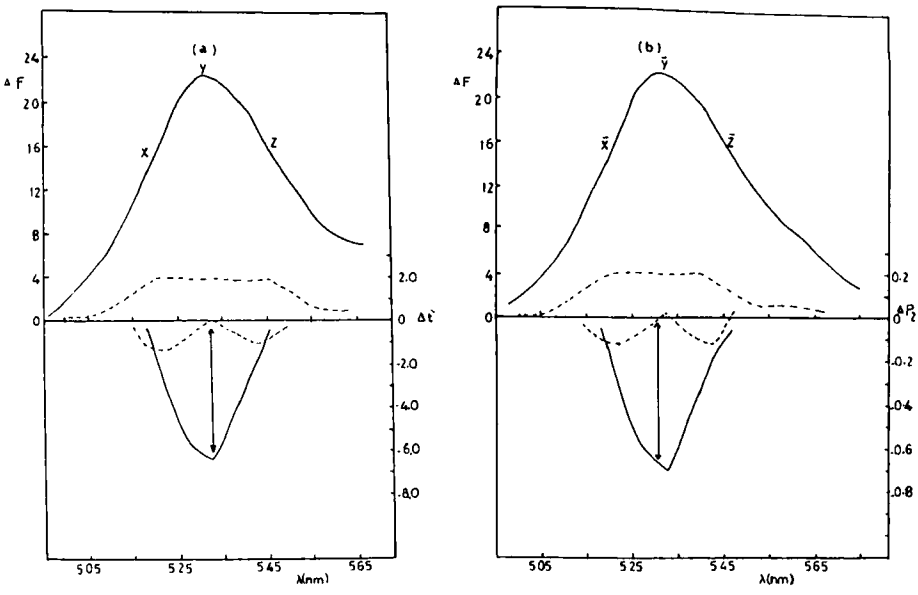

Figure 4. (a) The ΔF curves and their convoluted $\Delta t^{`}$ curves (derived therefrom) for MHg-DCS(——) and MHg-CS (---), each corresponding to 0.05 mg% cephalexin and 0.05 mg% mercurochrome in buffer pH 7.0.
(b) The ΔF curves and their convoluted Δp_2 curves (derived therefrom) for MHg-DCS(——) and MHg-CS (---), each corresponding to 0.05 mg% cephalexin and 0.05 mg% mercurochrome in buffer pH 7.0.

In the orthogonal function method, the $\bar{x}\bar{y}\bar{z}$ segment of the ΔF curve makes a large contribution to MHg-DCS curves, while the MHg-CS curves exhibit more or less linear contribution (Figs. 3b,4b&5b). Accordingly[17], the ΔP_2 coefficient of the quadratic polynomial might afford precise concentration estimate for DCS.

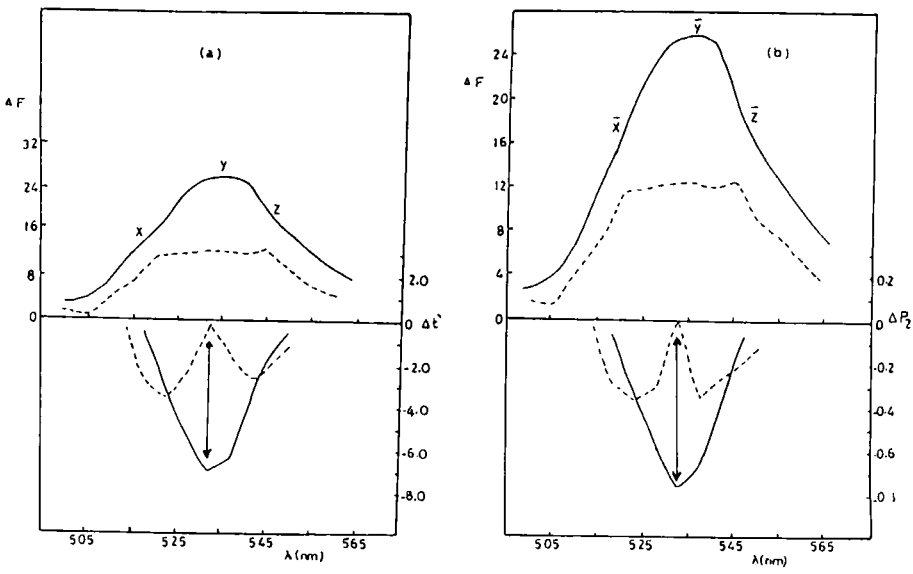

Figure 5. (a) The ΔF curves and their convoluted Δt curves (derived therefrom) for MHg-DCS (——) and MHg-CS (---), each corresponding to 0.05 mg% cephradine and 0.05 mg% mercurochrome in buffer pH 7.0.
(b) The ΔF curves and their convoluted Δp_2 curves (derived therefrom) for MHg-DCS (——) and MHg-CS (---), each corresponding to 0.05 mg% cephradine and 0.05 mg% mercurochrome in buffer pH 7.0.

The ΔP_2 values were calculated for 6 points at 5 nm intervals between the wavelength range of 520-545 nm using equation(4). The convoluted curve of MHg-DCS-thereby resulted-exhibited optimum ΔP_2 values at λ_m 532.5 nm, where nil contribution from MHg-CS curves (convoluted under the same parameters) was observed. Linear plots of ΔP_2 versus DCS concentration corresponding to the range 0.01-0.05 mg% of

Table 1: Regression analysis data for the calibration curves of cephalosporins (after alkaline degradation) using the three methods of assay.

Compound(*)	ΔF-method			Δt'-method			ΔP_2-method		
	(a)	(b)	(r)	(a)	(b)	(r)	(a)	(b)	(r)
Cefadroxil	0.370	443.0	0.9995	-0.29	122.0	0.9997	0.0015	16.35	0.9995
Cephalexin	0.170	291.0	0.9998	0.05	67.0	0.9997	0.0006	7.49	0.9996
Cephradine	0.740	294.0	0.9996	0.125	120.5	0.9997	0.0127	16.18	0.9999

(*) conc. range 0.01-0.05 mg% (in 0.01-mg% steps) of the corresponding CS.

(a) intercept, (b) slope, (r) regression coefficient.

the CS (in 0.01 mg% steps) was obtained. Regression analysis data for the slope (a), intercept (b) and regression coefficient (r) are presented in Table 1.

In the ΔF method, plots of ΔF values versus DCS concentration corresponding to the range 0.01-0.05 mg% (in 0.01-mg% steps) of the CS are linear with negligible intercepts. The regression analysis gave slopes (b), intercepts (a) and regression coefficients (r) values reported in Table 1.

Derivation of Stern Volmer Equations:

Stern Volmer equation[16] (eq.(1),P.3) could be used for the quantitation of quenchers (Q) and also to assess the quenching power of DCS in comparison to CS.

Accordingly, "Fo/F" could be measured as a function of quencher concentration [Q]. The Stern Volmer equation could be derived using $\tilde{t}$ or P_2 coefficients calculated from the Fo data ($(\tilde{t})_o$ or $(P_2)_o$) and the F data($(\tilde{t})$or(P_2)) using the same parameters previously described for $\Delta\tilde{t}$ or ΔP_2 coefficients calculation, respectively. The regression data for intercept (a), slope (b) and regression coefficient (r) of the derived Stern Volmer equation are presented in Table 2. Applying the 3 measured ratios (Fo/F ,$(\tilde{t})_o/(\tilde{t})$ and $(P_2)_o/(P_2)$), the corresponding high slopes ratios between DCS and CS indicated the high ability of the former as strong quencher. More important the quenching potential of the DCS to mercurochrome are in

Table 2: Data of Stern Volumer Equation (SVE) applying the proposed methods.

Compound	SVE applying ΔF method		Quenching constants ratios
	MHq-DCS*	MHq-CS	$K_S(DCS)/K_S(CS)$
Cefadroxil	Fo/F=0.977+7.32[Q]	Fo/F=0.998+0.0086[Q]	851.16
Cephalexin	Fo/F=0.984+5.81[Q]	Fo/F=0.979+0.0367[Q]	158.31
Cephradine	Fo/F=0.994+5.45[Q]	Fo/F=0.9798+0.0279[Q]	195.34
	SVE applying Fourier Function method		
Cefadroxil	$\frac{(\dot{t})_o}{(\dot{t})}$=0.947+9.08[Q]	$\frac{(\dot{t})_o}{(\dot{t})}$=0.999+0.0033[Q]	2751.50
Cephalexin	$\frac{(\dot{t})_o}{(\dot{t})}$=0.983+6.24[Q]	$\frac{(\dot{t})_o}{(\dot{t})}$=0.984+0.010 [Q]	624.00
Cephradine	$\frac{(\dot{t})_o}{(\dot{t})}$=0.955+10.71[Q]	$\frac{(\dot{t})_o}{(\dot{t})}$=1.003+0.0056[Q]	1912.50
	SVE applying orthogonal Function method		
Cefadroxil	$\frac{(P_2)_o}{(P_2)}$=0.953+9.07[Q]	$\frac{(P_2)_o}{(P_2)}$=0.996+0.0017[Q]	5335.30
Cephalexin	$\frac{(P_2)_o}{(P_2)}$=0.978+6.39[Q]	$\frac{(P_2)_o}{(P_2)}$=0.981+0.0097[Q]	654.70
Cephradine	$\frac{(P_2)_o}{(P_2)}$=0.949+11.46[Q]	$\frac{(P_2)_o}{(\dot{P_2})}$=1.002+0.0042[Q]	2728.60

* The calculated regression coefficients were in the range of 0.9922 to 0.9984.

decreasing order for cefadroxil, cephradine, cephalexin; regardless the method used.

The high ratio of Ks(DCS)/Ks(CS) of Fourier series and orthogonal function methods relative to the ΔF method indicated the high potential of the former methods in correcting for the CS interferences.

Assay of Different Preparations:

To assess the validity and applicability of the above proposed methods, 10 synthetic mixtures of the DCS (coexisted in a ratio range of 3-50%) with CS were prepared. Equally accurate (t-test) and equally precise results (F-test) were obtained applying the Fourier and orthogonal function methods (Table 3). Meanwhile positive systematic error with high coefficient of variation was obtained using the ΔF method. According to the data of the latter method the error appeared on adding CS in eight times concentration of DCS. Accordingly the Fourier and orthogonal method offered high potential in correcting for the CS interference during the DCS estimation. It is important therefore that the proposed methods could be applied for the determination of degraded cephalosporins coexisting in wide concentration range (3-50%) with the intact cephalosporins without the interference of the latter. On the other hand, the proposed methods were here applied for the assay of pharmaceutical preparations (cefadroxil, cephalexin and cephradine in capsules and suspensions) through the prior alkaline

Table 3: Assay results of DCS constituting 3-50% of CS in mixture

Compound	(a) Mean % Recovery & CV%		
	ΔF-method	Δt-method	ΔP_2-method
Cefadroxil	102.06&1.79	100.35&0.68	100.17&0.48
		(2.82)	(3.23)
		6.93	13.91
Cephalexin	105.32&4.76	100.44&0.68	100.01&0.84
		(3.21)	(3.47)
		49.0	32.11
Cephradine	103.52&3.33	100.34&0.70	99.69&0.59
		(2.95)	(3.58)
		22.63	31.86

(a) Mean of 10 determinations.

- Figures in parenthesis are the calculated t values for which the theoretical t (P=0.05) is 2.10. The underlined figures are the calculated F- values for which the theoretical F (95%) is 3.18.

degradation to the corresponding DCS and subsequent determination of the latter. The results obtianed are presented in Table 4. Compared with the official method[21] of assay, the accuracy (t-test) and the reproducibility (F-test) of both fourier and orthogonal function methods were compa-

Table 4: Assay results of cephalosporins in pharmaceutical preparations using fluorescence quenching techniques and subsequent application of the proposed methods

Compound	(a) Mean% Recovery & CV%			
	Δ F-method	Δ t-method	Δ P_2-method	Official[24] method
Cefadroxil capsules	100.79&1.29	99.65&0.85	99.41&0.85	99.77&0.55
	(1.63)	(0.26)	(0.79)	
	5.50	2.39	2.39	
Cephalexin capsules	100.08&1.36	100.15&0.94	99.75&0.43	99.84&0.60
	(0.36)	(0.62)	(0.27)	
	5.14	2.45	1.95	
Cephalexin suspension	100.35&1.20	99.62&0.82	99.98&0.94	99.64&0.54
	(1.21)	(0.05)	(0.70)	
	4.94	2.31	3.03	
Cephradine capsules	100.14&1.41	99.91&0.95	99.39&0.51	99.99&0.67
	(0.21)	(0.15)	(1.58)	
	4.43	2.01	1.73	
Cephradine suspension	100.13&1.36	99.94&0.86	100.27&0.92	99.49&0.94
	(0.87)	(0.79)	(1.33)	
	2.09	1.19	1.04	

- Figures in parenthesis are the calculated t-values for which the corresponding theoretical value (P=0.05) is 2.31.
- The underlined figures are the calculated F-values for which the corresponding theoretical value at 95% level is 6.39.

(a) Mean of 5 determinations.

rable. The proposed methods therefore could be recomended for the wide applicability in the routine and control analysis specially for those drugs which are usually carrying expiry date of use. The pharmaceutical preparations may be assayed before degradation to determine the amount of degraded products and also after alkaline hydrolysis to assay the total. The difference will give the real concentration of the intact cephalosporins.

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References:

1. Wronski M., Chem. Anal., 1968, 13,733.
2. Yanȳsheva V.S., Zavod. Lab., 1964, 30,23, Thru. Anal. Abstr. 1966, 12, 1688.
3. Goreeve G., Hard M., and Freiser H., Talanta, 1970, 17,272.
4. Wronski M., Chem. Anal., 1969, 14,29.
5. Gruelnsert A.T., Talanta, 1971, 18,881.
6. Johar G.S., Microchim. Acta., 1974,4,729, Thru. Anal. Abstr., 1975, 28,2B15.
7. Rosibois B., Ballin M., Bertrand J., Ann. Pharm. Fr., 1974, 32,309, Thru. Anal. Abstr., 1975, 27,407.
8. Wronski M., Talanta, 1968, 15,241.

9. Grünert, A., Ballschmiter K., and Tölg G., Talanta, 1968,15,451.

10. Colovos G., Haro M. and Freiser H., Talanta, 1970,17,273.

11. Stock F.G., Analyst, 1954,79,662.

12. Bundgaard H.,Ilver K., J. Pharm. Pharmacol,1972,24,790.

13. El-Sebai A.I., Beltagy Y.A., Abd El-Khalek M.M., Talanta, 1977,24,328.

14. Haginaka J., Wakai J., Yasuda H., and Uno T., Anal. Sci., 1985,1,73.

15. Mori I., Fujita Y., Fujita K., Kitano S., Kawabe H., Koshiyama Y., Tanaka T., Miyawaki S., Nagao Y. and Nagai K., Chem. Pharm. Bull., 1985,33,4629.

16. Wolfbeis, O.S., and Urbano, E., Anal. Chem., 1983, 55,1904.

17. El-Yazbi F.A. and Korany M.A., Spectroscopy Letters, 1985,18,543.

18. Wahbi A.M., Abdine H. and Korany M.A., Pharmazie, 1978, 33,278.

19. Korany M.A., Ph.D. Thesis, University of Alexandria, Egypt, 1974, pp. 30.

20. Wahbi A.M., Belal S., Bedair M., and Abdine., Egypt. J. Pharm. Sci., 1982,23,151.

21. The British Pharmacopoeia, the Pharmaceutical Press London (1973) pp. 88,90.

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