2. Experimental

2.1. Chemicals

Sodium salts of cefotaxime, ceftazidime and ceftriaxone (Sigma Co, USA) were used as received. Other chemicals and reagents were of analytical grade and the solvents were of HPLC grade.

2.2. Instruments

A DU 640 spectrophotometer (Beckman Inc., USA) connected to a color video graphics display and a dot-matrix printer (Epson LQ 1170) was used. The spectrophotometer was equipped with a software package for multicomponent analysis by FSQ. All spectrophotometric analyses were made using 1 cm quartz cells. The HPLC chromatograph comprised an isocratic HPLC pump (Schimadzu LC-10AD) attached to a 50 μl injector loop, a diode array detector (Schimadzu SPD-M 10A), a communication bus module (Schimadzu CBM 10-A), an Acre view 33D video display and a dot matrix printer. Chromatographic separations were made at ambient temperature using (150 mm × 6 mm ID) Schimpack GLC-ODS, 5 μm column and mobile phase composed of acetonitrile and 0.1 M ammonium acetate solution in a ratio 10:90. The pH of the mobile phase was adjusted to 7.5 using ammonia solution. The effluent was monitored at 270 nm, flow rate 1.5 ml/min and sensitivity range AU/FS [mAbs]: 0–80.

2.3. Procedures

2.3.1. HPLC analysis

Separate standard solutions of cefotaxime, ceftazidime and ceftriaxone (1 mg/ml) were freshly prepared in deionized water. Working standard solutions were prepared in the mobile phase in the concentration range 5–20 μg/ml. A 50 μl aliquot of each solution was injected onto the column. Quantitative analysis was based on peak area measurements. The unknown concentration of antibiotic in samples was calculated from the linear regression equation relating the peak area to concentration.

2.3.2. Preparation of antibiotic alkali-induced degradation product

An accurate weight of 10 mg of the antibiotic powder (cefotaxime, ceftazidime and ceftriaxone) was transferred into a 10 ml volumetric flask, dissolved in 8 ml phosphate buffer solution (pH 10) and heated at 60°C in a water bath for 30 min. The solution was cooled and diluted to volume with phosphate buffer solution. Complete degradation of the antibiotic was confirmed from HPLC chromatograms.

2.3.3. Calibration standard mixtures of antibiotic and its alkali-induced degradation products (FSQ analysis)

Accurate volumes of 100–220, 150–350 and 100–180 μl of cefotaxime, ceftazidime and ceftriaxone solutions (1 mg/ml) were separately transferred into 10 ml volumetric flasks and mixed with 50 μl of cefotaxime, ceftriaxone alkali-induced degradation products or 75 μl ceftazidime alkali-induced degradation products (1 mg/ml). The solutions were diluted to volume with acetonitrile–water 10:90 vol./vol. and were scanned over the spectrum range 265–320 nm using the FSQ technique.

2.3.4. Comparison of HPLC and FSQ (laboratory-made samples)

Accurate volumes of 100–300 μl of cefotaxime, ceftazidime and ceftriaxone solutions (1 mg/ml) were mixed with 50 μl of the corresponding alkali-induced degradation product (1 mg/ml) and diluted to 10 ml with mobile phase. The concentration of the antibiotic in the prepared samples was determined using the stored calibration matrix (FSQ) or linear regression equation of a standard curve run simultaneously with the samples.

2.3.5. Analysis of commercial injections

An accurate weight of 100 mg of the powder injection was transferred into a 100 ml volumetric flask, dissolved and diluted to 100 ml with mobile phase. An aliquot of the prepared solution was suitably diluted and analyzed using FSQ and HPLC.

2.3.6. Stability studies

Accurate volumes (1 ml) of aqueous solutions of cefotaxime or ceftazidime or ceftriaxone (1 mg/ml) were transferred into separate 10 ml volumetric flasks. The solutions were diluted to volume with the corresponding buffer solutions (pH 2–10). The flasks were placed in a water bath at 40 ± 2°C and 50 μl aliquots were drawn at 30 min intervals and analyzed using HPLC. The % drug remaining was calculated by referring to standard curves prepared simultaneously during analysis. The kinetic parameters including the degradation rate constant and degradation half-life were computed from first-order plots of cefotaxime, ceftazidime and ceftriaxone at different pH values. The rate constant–pH profiles were established by plotting the logarithmic values of the observed degradation rate constants versus pH. The values of K_{deg}, $t_{1/2}$ and t_{90} for the examined antibiotics at pH 5 at 25 and 4°C were extrapolated from Arrhenius plots.

3. Results and discussion

3.1. Spectrophotometry

Cefotaxime, ceftazidime and ceftriaxone are cephalosporin antibiotics that are highly sensitive to hydrolysis. For content uniformity testing and stability studies, it is necessary to establish stability-indicating assays that can be applied to determine the antibiotic in the presence of its degradation products. Fig. 1 displays the UV–Vis absorption spectra of cefotaxime, ceftazidime and ceftriaxone and their alkali-

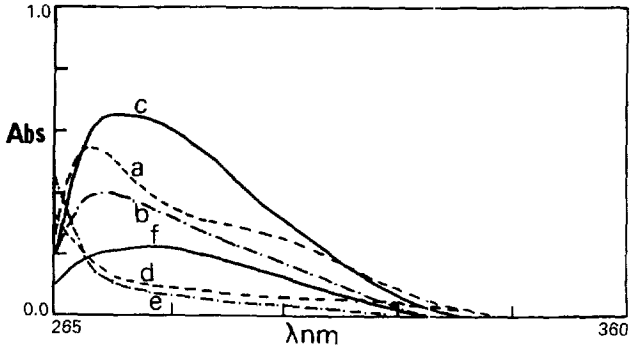

Fig. 1. UV–Vis absorption spectra of cefotaxime (a), ceftazidime (b) and ceftriaxone (c) and their alkali-induced degradation products (d, e, f) in acetonitrile–water 10:90 vol./vol. (1 mg/100 ml).

induced degradation products in phosphate buffer solution at pH 10. The degradation compounds exhibited strong parasitic absorbance in the wavelength range 265–320 nm that hindered the application of the conventional spectrophtometric analysis. Full spectrum quantitation (FSQ) for multicomponent analysis constitutes a rapid and effective spectrophotometric technique for analysis of complex mixtures. Thus, FSQ was applied to quantify cefotaxime, ceftazidime and ceftriaxone in the presence of their alkali-induced degradation products and in commercial injections. Mathematically, FSQ is a spectrophotometer self-built software that uses B-matrix calculations and principal component analysis (PCA) to analyze simultaneously mixtures of cefotaxime or ceftazidime or ceftriaxone in the presence of their alkali-induced degradation products. Experimentally, FSQ applies Fourier pre-processing of the entire absorption spectra of the individual antibiotics with their alkali-induced degradation products at variable concentrations to calculate matrix calibration coefficients. The latter are stored and used to compute simultaneously the unknown concentrations of antibiotics in samples. Standard calibration mixtures, containing cefotaxime or ceftazidime or ceftriaxone in the concentration range 1.0–2.2, 1.5–3.5, 1.0–1.8 mg/100 ml and alkali-induced degradation products at concentration 0.50 or 0.75 mg/100 ml, were scanned over the wavelength range 265–320 nm. In order to minimize matrix effects and optimize quantification of the antibiotics, FSQ calibration parameters such as starting term, term number and vector number were specified. The selected parameters were: 0, [64], 4 for cefotaxime, 0, [16], 4 for ceftazidime and 0, [16], 3 for ceftriaxone. The calculated concentrations of the examined antibiotics using FSQ are listed in Tables 1–3. Under the selected conditions, the values of the standard error of estimate (SEE) were 0.09, 0.08 and 0.01 for cefotaxime, ceftazidime and ceftriaxone. The values of the total SEE were 0.07, 0.04 and 0.02, whereas the average % residual values were 0.04, 0.03 and 0.02, respectively. The computed values indicated the high accuracy of the FSQ technique as the calculated concentrations were close to the actual values. Moreover, the results showed

Table 1
Standard calibration mixtures of cefotaxime (comp 1) and its alkali-induced degradation products (comp 2) for FSQ analysis

Std no.	% Resid.	Component	Concentration (mg/100 ml)		
			Actual	Calculated	Difference
Read 1	0.009	comp 1	1.0000	1.0061	0.0061
		comp 2	0.5000	0.4969	−0.0031
Read 2	0.048	comp 1	1.5000	1.5571	0.0571
		comp 2	0.5000	0.4709	−0.0291
Read 3	0.055	comp 1	1.7000	1.6413	−0.0587
		comp 2	0.5000	0.5299	0.0299
Read 4	0.041	comp 1	1.9000	1.9291	0.0291
		comp 2	0.5000	0.4852	−0.0148
Read 5	0.033	comp 1	2.2000	2.1747	−0.0253
		comp 2	0.5000	0.5129	0.0129

Table 2
Standard calibration mixtures of ceftazidime (comp 3) and its alkali-induced degradation products (comp 4) for FSQ analysis

Std no.	% Resid.	Component	Concentration (mg/100 ml)		
			Actual	Calculated	Difference
Read 1	0.028	comp 3	1.5000	1.4688	−0.0312
		comp 4	0.7500	0.7557	0.0057
Read 2	0.050	comp 3	2.0000	2.0695	0.0695
		comp 4	0.7500	0.7373	−0.0127
Read 3	0.030	comp 3	2.5000	2.4626	−0.0374
		comp 4	0.7500	0.7568	0.0068
Read 4	0.034	comp 3	3.0000	3.0208	0.0208
		comp 4	0.7500	0.7462	−0.0038
Read 5	0.045	comp 3	3.5000	3.4802	−0.0198
		comp 4	0.7500	0.7536	0.0036

Table 3
Standard calibration mixtures of ceftriaxone (comp 5) and its alkali-induced degradation products (comp 6) for FSQ anaysis

Std no.	% Resid.	Component	Concentrated (mg/100 ml)		
			Actual	Calculated	Difference
Read 1	0.020	comp 5	1.0000	0.9964	−0.0036
		comp 6	0.5000	0.5085	0.0085
Read 2	0.023	comp 5	1.2000	1.2062	0.0062
		comp 6	0.5000	0.4907	−0.0093
Read 3	0.010	comp 5	1.4000	1.4023	0.0023
		comp 6	0.5000	0.4991	−0.0009
Read 4	0.021	comp 5	1.6000	1.5899	−0.0101
		comp 6	0.5000	0.4858	−0.0142
Read 5	0.018	comp 5	1.8000	1.8049	0.0049
		comp 6	0.5000	0.5147	0.0147

that FSQ was able to eliminate matrix effects due to the alkali-induced degradation products. Replicate analysis of standard samples containing cefotaxime, ceftazidime and ceftriaxone at concentrations 1.0, 2.0 and 1.0 mg/100 ml gave RSD % of 1.27, 3.11 and 0.10 (Table 4). The results proved the high precision and reproducibility of the FSQ procedure.

Table 4
Precision study for the determination of cefotaxime, ceftazidime and ceftriaxone by FSQ

Compound	Actual conc. (mg/100 ml)	Calculated conc. (mg/100 ml)
Cefotaxime		
	1.0000	1.0066
		1.0357
		1.0275
		1.0315
		1.0314
Mean ± SD		1.0253 ± 0.013
RSD (%)		1.27
Ceftazidime		
	2.0000	2.0784
		2.0797
		2.1986
		2.0385
		2.0439
Mean ± SD		2.0878 ± 0.065
RSD (%)		3.11
Ceftriaxone		
	1.0000	0.9974
		0.9999
		0.9984
		0.9978
		0.9975
Mean ± SD		0.9982 ± 0.001
RSD (%)		0.100

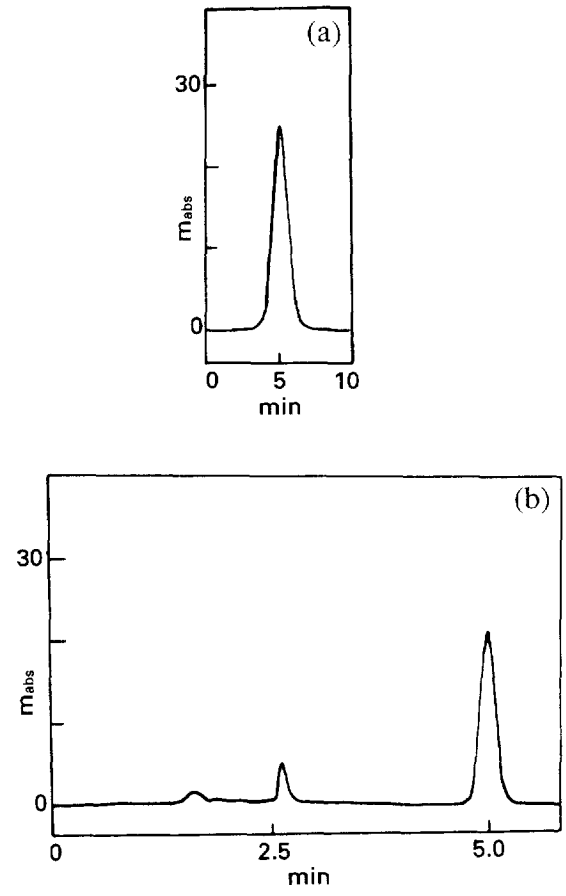

Fig. 2. HPLC chromatograms of standard (a) and degraded (b) solutions of cefotaxime (20 μg/ml).

3.2. Liquid chromatography (HPLC)

Figs. 2–4 display the HPLC chromatograms of standard and degraded solutions of cefotaxime, ceftazidime and ceftriaxone in phosphate buffer solution (pH 10) at 40°C. Under the selected chromatographic conditions, optimum resolution of the antibiotic from its corresponding degradation products could be recognized. The resolved compounds appeared as sharp and well-defined peaks at 5.0, 2.5 and 2.0 min, respectively. Rapid elution of ceftazidime and ceftriaxone may be attributed to the high polarity of the molecules compared to cefotaxime. The pH as well as the composition of the mobile phase influenced the peak formation and position. Decreasing of the pH of the mobile phase to 4–5 resulted in very broad and poorly developed peaks with an increase of retention time of the resolved compounds, particularly cefotaxime (15 min). Moreover, increasing the percentage of acetonitrile in the mobile phase up to 30% resulted in the rapid elution of ceftazidime and ceftriaxone (0.5 min). Adjustment of the pH of the mobile phase to 7.5 and the % of acetonitrile to 10% resulted in well-defined peaks at reasonable retention times. Unlike FSQ, HPLC differentiated various degradation products of the examined antibiotics at varying pH values. These products were attributed to the hydrolysis of the acetoxy ester grouping and cleavage of the β-lactam ring [19]. Quantitative analysis of cefotaxime, ceftazidime and ceftriaxone was based on peak area (PA) measurements which were automatically computed by the instrument software at 270 nm and AU/FS [mAbs] of 0–80. Linearity was obtained for the individual antibiotics over the concentration range 5–20 μg/ml. The linear regression equations were:

$PA \times 10^{-4} = -0.27 + 2.45C$ (cefotaxime) ($r = 0.9979$)

$PA \times 10^{-4} = 2.07 + 1.92C$ (ceftazidime) ($r = 0.9899$)

$PA \times 10^{-4} = 0.92 + 5.87C$ (ceftriaxone) ($r = 0.9947$)

where C is the concentration of the antibiotic (μg/ml).

The lowest concentration of an antibiotic that could be detected under the chromatographic conditions was 0.25 μg/ml and the lowest volume of samples that could be injected was 20 μl. The accuracy and precision of HPLC were evaluated by replicate analysis of samples containing 10 μg/ml (cefotaxime and ceftriaxone) and 20 μg/ml (ceftazidime) (Table 5). The calculated concentrations were in good correlation with the actual values with RSD % of 0.91, 2.66 and 1.83 for cefotaxime, ceftazidime and ceftriaxone. The validity of FSQ and HPLC for the analysis of the investigated antibiotics in the presence of alkali-induced degradation products and in commercial injections was examined. Recov-

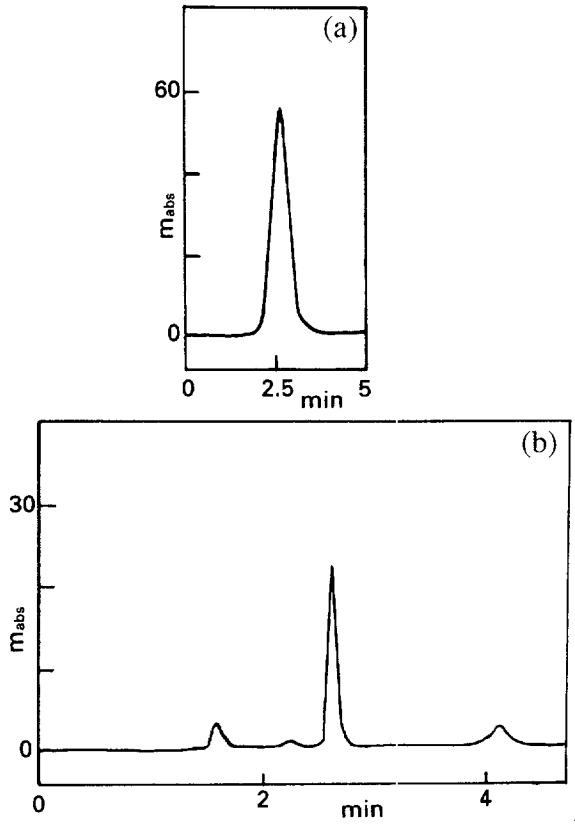

Fig. 3. HPLC chromatograms of standard (a) and degraded (b) solutions of ceftazidime (20 μg/ml).

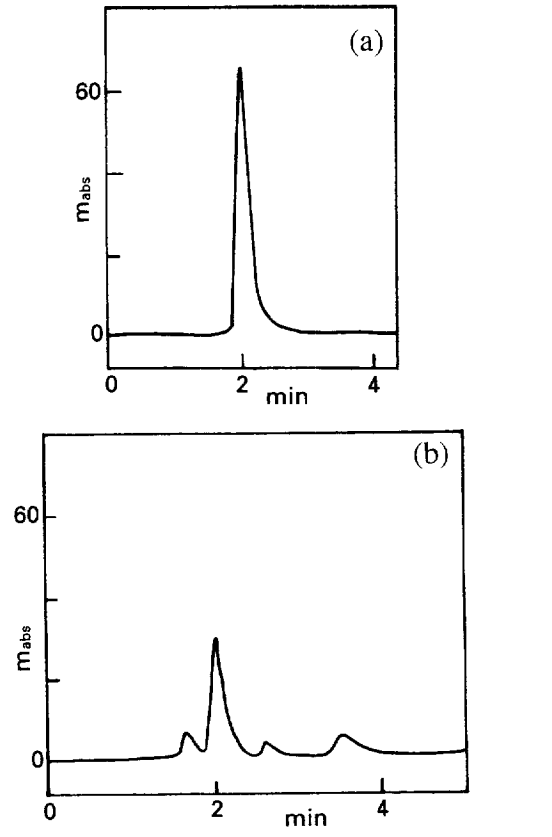

Fig. 4. HPLC chromatograms of standard (a) and degraded (b) solutions of ceftriaxone (5 μg/ml).

ery studies and statistical analysis using student *t*-test and *F*-test indicated insignificant differences between the proposed methods as the calculated concentrations were found to be in good agreement with the actual values or the claimed contents (Tables 6 and 7).

3.3. Stability studies

The stability of the examined antibiotics in aqueous solutions of varying pH 2–10 were studied using the developed HPLC procedure due to high sensitivity and selectivity. The results revealed that the degradation of antibiotics followed pseudo-first-order kinetics. The kinetic parameters representing the degradation of cefotaxime, ceftazidime and ceftriaxone at different pH values are listed in Table 8. Furthermore, the rate constant–pH profiles for the degradation of the antibiotics are shown in Fig. 5. As can be seen, the rate of degradation was very fast at pH 2 and 10, whereas in the pH range 4–6, the antibiotics exhibited relatively slow degradation with optimum stability at pH 5. The higher rates of degradation at pH 2 and 10 are due to hydrogen ion and hydroxyl ion catalysis. The calculated parameters indicated that aqueous solutions of cefotaxime were relatively more stable than those of ceftazidime or ceftriaxone. Accelerated stability studies of cefotaxime, ceftazidime and ceftriaxone

Table 5
Precision study for the determination of cefotaxime, ceftazidime and ceftriaxone by HPLC

Compound	Actual conc. (μg/ml)	PA [a]	Calculated conc. (μg/ml)
Cefotaxime	10.0000	242728	10.0111
		238917	9.8556
		244011	10.0634
		240725	9.9294
Mean ± SD			9.9648 ± 0.091
RSD (%)			0.91
Ceftazidime	20.0000	400212	19.7689
		420333	20.8170
		397878	19.6473
		410412	20.3000
Mean ± SD			20.1333 ± 0.536
RSD (%)			2.66
Ceftriaxone	10.0000	575146	9.6480
		591235	9.9222
		600176	10.0747
		593422	9.9595
Mean ± SD			9.9011 ± 0.181
RSD (%)			1.83

[a] PA: peak area.

Table 9
Predicted kinetic parameters for the degradation of cefotaxime, ceftazidime and ceftriaxone at pH 5 at 25 and 4°C

	Cefotaxime			Ceftazidime			Ceftriaxone		
	K_{deg} (h^{-1})	$t_{1/2}$ (h)	t_{90} (h)	K_{deg} (h^{-1})	$t_{1/2}$ (h)	t_{90} (h)	K_{deg} (h^{-1})	$t_{1/2}$ (h)	t_{90} (h)
25°C	0.016	43.31	6.56	0.049	14.14	2.14	0.118	5.87	0.88
4°C	0.007	99.00	15.00	0.029	23.89	3.62	0.049	14.14	2.14

of cefotaxime, ceftazidime and ceftriaxone in the presence of degradation products and in commercial injections. For routine analysis of a large number of samples, FSQ can be used as a viable alternative to HPLC as the technique is simple, non-tedious and gives results within seconds. For stability studies, HPLC is recommended, since the method has a higher sensitivity and selectivity and uses a small volume of samples.

Acknowledgements

The author is grateful to the Public Authority for Applied Education and Training (PAAET), Kuwait for financial support. The valuable technical assistance of Miss Amel Al-Hamdan is greatly appreciated.

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