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CHROMATOGRAPHY OF CEPHALOSPORINS ON DEAE-SEPHADEX

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SUMMARY

A method is described for separating and quantifying new cephalosporin derivatives by means of column chromatography on DEAE-Sephadex A-25. The procedure has been used to determine some breakdown products of cefacetril.

INTRODUCTION

Cephalosporin C, 7-aminocephalosporanic acid (7-ACA) and their derivatives are usually separated by paper chromatography and determined quantitatively, after bioautographic detection, with *Bacillus subtilis*[1]. The chromatographic separation of cephalosporins can also be effected on thin layer of silica gel[2,3] and diethylaminoethyl-cellulose[4]. Column chromatography has been used[5] to study the relationship between the chromatographic behaviour of certain cephalosporins and their protein binding power.

In this paper, we describe a chromatographic method involving the use of an ion-exchange column of DEAE-Sephadex A-25; this method permits the separation and quantitative determination of some cephalosporins by UV spectrophotometry.

The technique has proved to be especially useful for the separation of certain breakdown products of the antibiotics.

EXPERIMENTAL

Apparatus and materials

DEAE-Sephadex A-25 (Cl^-) was obtained from Pharmacia (Uppsala, Sweden). A Beckman DU-2 and a Perkin-Elmer Model 124 twin-ray spectrophotometer, with a Perkin-Elmer Model 165 recorder, were employed.

The cephalosporins used were cephaloridine, sodium cefacetril, cephalexin, sodium cephalosporin C, 7-ACA, desacetoxycephalosporin C (7-DACA), sodium cephalothin, sodium cephapirin, cephradine and sodium cefazolin. These drugs were prepared in the Research Laboratories of Alfa Farmaceutici S.p.A. (Bologna, Italy) and purified to constant spectrophotometric and chromatographic values.

Preparation of the resin and the column

Amounts of 20 g of DEAE-Sephadex were washed repeatedly with distilled water, equilibrated with 100 ml of the solution employed for elution and then poured into a 1.5-cm diameter glass column so as to give a height of 20 cm after settling.

Elution of cephalosporins

A 1-ml volume of a solution containing 500 μg of each cephalosporin was placed on the column and elution was carried out with 0.2 *M* sodium acetate–acetic acid (pH 4.5 and 6.0), 0.2 *M* sodium acetate–0.1 *M* sodium hydroxide (pH 8.0) and 0.2 *M* sodium acetate–acetic acid containing 146 g/l of sodium chloride, (pH 3.5). Each 5-ml fraction was determined spectrophotometrically against a blank of the same eluent at wavelengths corresponding to the absorption peaks of each cephalosporin.

Degradation of cefacetril

The hydrolysis of cefacetril was studied at 100° by immersing test-tubes containing a solution (1000 μg/ml) in 0.2 *M* sodium acetate (pH 8.0) for 10 min in a thermostatically controlled water-bath.

Elution of degradation products of cefacetril

A 2-ml volume of a solution containing 2000 μg of degraded cefacetril was poured on to the column of DEAE-Sephadex and eluted with 0.2 *M* sodium acetate (pH 8.0), 35 fractions of 5 ml were collected and then eluted with 150 ml of 0.2 *M* sodium acetate–sodium chloride (pH 3.5). The fractions corresponding to the second eluent were yellow and red in colour.

RESULTS

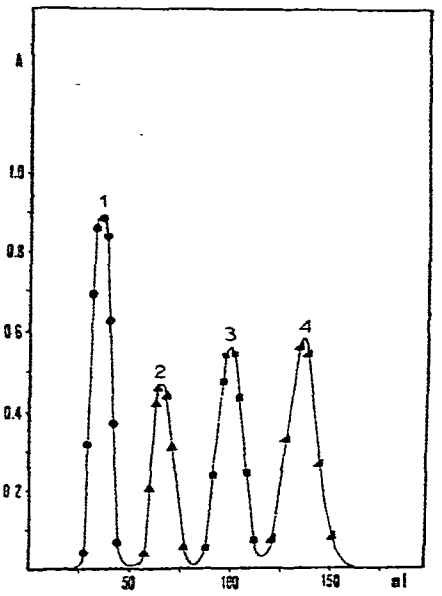
The elution volumes (V_e) obtained for each cephalosporin at different pH values are listed in Table I.

Figs. 1–3 show the separation of the cephalosporins on DEAE-Sephadex.

TABLE I

ELUTION VOLUMES OF CEPHALOSPORIN DERIVATIVES

Cephalosporin	*V_e (ml)*		
	pH 4.5	*pH 6*	*pH 8*
Cephaloridine	30	35	38
Cephradine	33	28	68
Cephalexin	35	35	78
Sodium cephapirin	41	90	103
7-ACA	48	80	74
7-DACA	53	105	77
Sodium cephalosporin	68	60	57
Sodium cefacetril	100	90	79
Sodium cephalothin	125	127	123
Sodium cefazolin	138	125	122



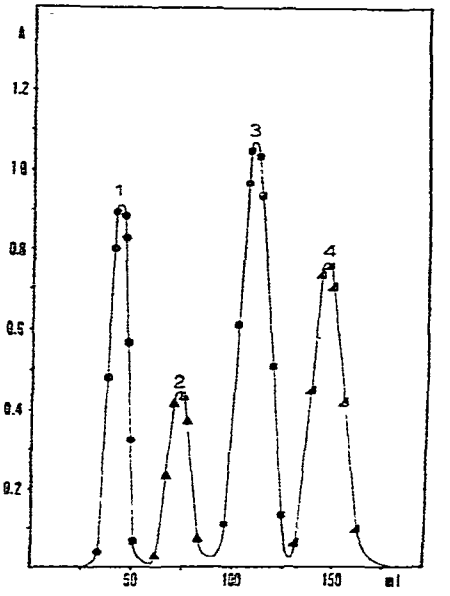


Fig. 1. Chromatographic separation of cephalosporin antibiotics on a 20 × 1.5 cm column of DEAE-Sephadex A-25 (Cl^-) eluted with 0.2 *M* sodium acetate (pH 4.5). The volume of each fraction was 5 ml. Absorbance measurements were performed at 262 nm for cephalexin (1), 269 nm for cephalosporin C (2), 258 nm for cefacetril (3) and 272 nm for cefazolin (4).

Fig. 2. Chromatographic separation of cephalosporin antibiotics on a 20 × 1.5 cm column of DEAE-Sephadex A-25 (Cl^-) eluted with 0.2 *M* sodium acetate (pH 6.0). The volume of each fraction was 5 ml. Absorbance measurements were performed at 259 nm for cephradine (1), 260 nm for cephalosporin C (2), 258 nm for cephapirin (3) and 237 nm for cephalothin (4).

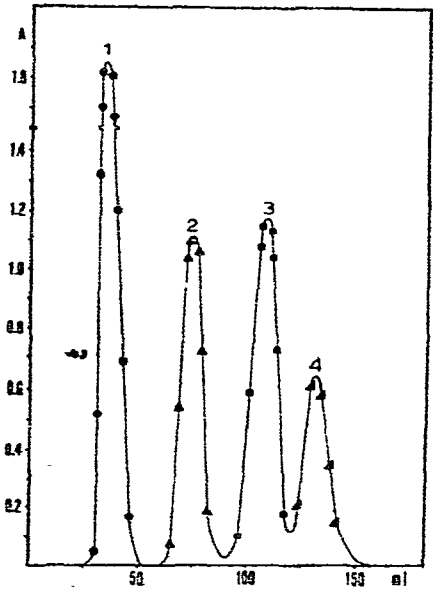


Fig. 3. Chromatographic separation of cephaloridine (1), 7-ACA (2), cephapirin (3) and cefazolin (4). Column: DEAE-Sephadex A-25 (Cl^-), 20 × 1.5 cm. Eluent: 0.2 *M* sodium acetate (pH 8.0). Samples of 5 ml collected. Absorbance measurement at 240 nm (1), 265 nm (2), 258 nm (3) and 272 nm (4).

Quantitative assay

In order to perform a quantitative spectrophotometric analysis of cephalosporin derivatives, we calculated their $E^{1\%}_{1cm}$ values. Table II shows the percentage recoveries and the extinction values of the antibiotics.

TABLE II

SPECTROPHOTOMETRIC CHARACTERISTICS AND RECOVERIES OF CEPHALOSPORINS

Cephalosporin	*pH*	*Wavelength of maximum absorption (nm)*	$E^{1\%}_{1\ cm}$	*Recovery ± S.D. (%)**
Sodium cephalosporin C[6]	4.5–6.0	260	200	98 ± 0.8
Sodium cephalothin[6]	6.0	237	336	97.5 ± 1.1
Cephaloridine[6]	8.0	240	381	102.5 ± 2.1
Cephalexin[6]	4.5	262	236	100 ± 0.5
Sodium cefazolin[7]	4.5	272	277	105 ± 1.7
7-ACA[2]	8.0	265	310	99 ± 2.3

* Standard deviation based on eight determinations.

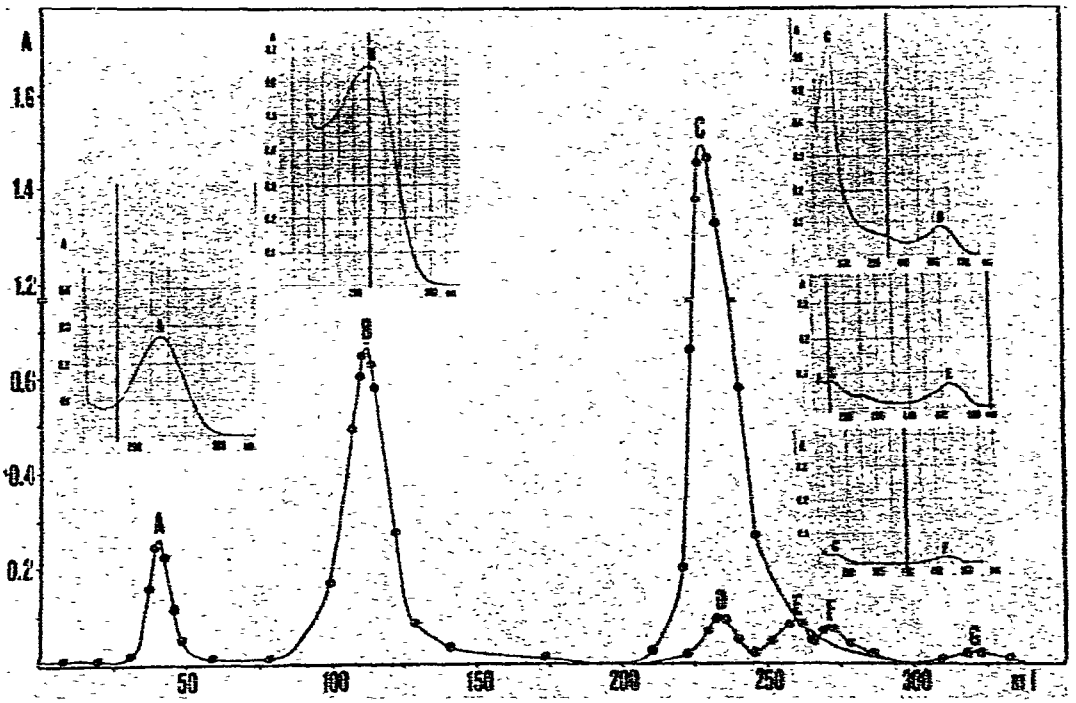


Fig. 4. Chromatographic separation of degradation products of cefacetril. Cefacetril (B) and degradation products (A, C) read at a wavelength of 260 nm; ○, degradation products read at a wavelength of 484 nm. The ultraviolet and/or visible spectrum of each compound was evaluated. Column: DEAE-Sephadex A-25 (Cl^-), 20 × 1.5 cm. Elution of cefacetril (B) and compound A with 0.2 *M* sodium acetate (pH 8.0); elution of C, D, E, F, G (uncharacterized degradation products) with 0.2 *M* sodium acetate (pH 3.5); 5-ml samples collected.

Assessment of the degradation products

The technique can also be used to assess the purity of commercial cephalosporins. Fig. 4 shows the chromatography of breakdown products of cefacetril on DEAE-Sephadex.

DISCUSSION

Chromatography on Sephadex anion exchanger is suitable for the separation of acidic substances such as cephalosporin antibiotics and enables the quantitative assay of each cephalosporin to be performed spectrophotometrically. The chromatograms in Figs. 1–3 show good separations of the cephalosporins, which were eluted as symmetrical peaks with few millilitres of eluent.

The method is suitable for the analysis of the products of the partial hydrolysis of cephalosporins, as an eluent system can usually be found that will separate the degradation products. The chromatogram shown in Fig. 4 illustrates the clear separation of the breakdown products of cefacetril. The results in Table II show that the technique is satisfactory for the quantitative determination of cephalosporin derivatives.

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