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Note

Determination of cefsulodin sodium [D(—)-SCE-129] by high-performance liquid chromatography

LEE ELROD, Jr.*, LANDY B. WHITE, DAVID C. WIMER and ROBERT D. COX
Analytical Research Department, D.417, Abbott Laboratories, North Chicago, IL 60064 (U.S.A.)
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Cefsulodin sodium or D(−)-SCE-129 (I, Fig. 1) is a unique semisynthetic cephalosporin antibiotic having pronounced antipseudomonal activity[1–4]. The drug substance is chemically [6R-[6α,7β(R*)]]-4-(aminocarbonyl)-1-[[2-carboxy-8-oxo-7-[(phenylsulfoacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl[methyl]-pyridinium hydroxide inner salt, monosodium salt. This paper describes the analysis of cefsulodin sodium in 500 mg (potency) vials using reversed-phase high-performance liquid chromatography (HPLC). Assay results obtained by HPLC are compared with those obtained by the currently accepted hydroxylamine and microbiological agar diffusion methods.

Procedures reported in the literature for the quantitation of β-lactam antibiotics use various microbiological[5], polarographic[6–8], gas–liquid chromatographic[9] and chemical techniques. Included in the chemical assays for these compounds are iodometric titration[10,11], fluorometry[12,13] and colorimetric procedures after reaction with either hydroxylamine[14–16], ninhydrin[17] or nicotinamide[18]. The two official procedures specified most often for cephalosporins are the microbiological agar diffusion[19] and the hydroxylamine[20] methods.

In this work HPLC is used because it offers the desired combination of speed, accuracy and sensitivity. The HPLC procedure is specific and stability indicating, since cefsulodin sodium is resolved from its various manufacturing impurities and/or degradation products (II–V, Fig. 1). Procedures using both ion-exchange and reversed-phase HPLC are reported in the literature for the quantitation of β-lactam antibiotics in bulk drug[21,22], dosage forms[23–25], fermentation broths[26,27] and biological matrices[28–32].

EXPERIMENTAL

Apparatus

The HPLC system consisted of a model M-6000A pump and U6K injector (Waters Assoc., Milford, MA, U.S.A.), a SF 770 variable-wavelength UV detector (Schoeffel, Westwood, NJ, U.S.A.) and a SP-4100 data handling system (Spectra-Physics, Santa Clara, CA, U.S.A.). The chromatographic separations were achieved using a Zorbax® C_8 (4.6 mm I.D. × 25 cm) column (DuPont, Wilmington, DE, U.S.A.) fitted with a RP-8 (4.6 mm I.D. × 3 cm) pre-column (Brownlee, Santa Clara,

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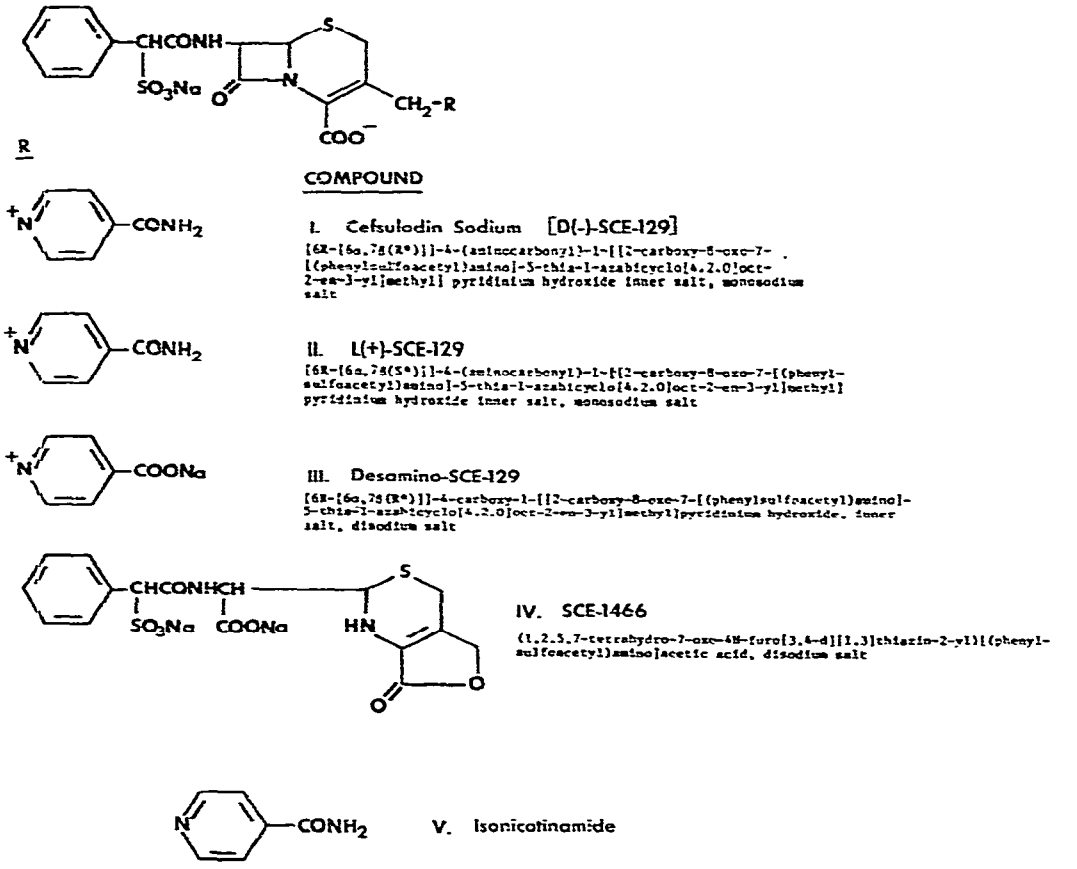

Fig. 1. Chemical structures of cefsulodin sodium (I) and manufacturing impurities and/or degradation products (II–V).

CA, U.S.A.). The HPLC eluent and solutions chromatographed were filtered through 0.45-μm polycarbonate membranes (Nuclepore, Pleasanton, CA, U.S.A.).

Reagents

Acetonitrile and methanol used were distilled-in-glass UV grade from Burdick and Jackson (Muskegon, MI, U.S.A.). Ammonium acetate was ACS grade from J. T. Baker (Phillipsburg, NJ, U.S.A.). Cefsulodin sodium reference standard and 500 mg (potency) vials were from Takeda (Osaka, Japan). Phenoxy acetic acid was 98% minimum purity from Aldrich (Milwaukee, WI, U.S.A.).

Chromatographic conditions

HPLC Eluent. 0.02 *M* ammonium acetate in water–methanol–acetonitrile (950:35:15). A 1.54-g portion of ammonium acetate was dissolved in 1 liter of aqueous solution containing 35 ml of methanol, 15 ml of acetonitrile and 4 ml of glacial acetic acid. If necessary, the eluent was adjusted to pH 4.1 by addition of more acetic acid.

Flow-rate. 1.5 ml/min.

Pressure. 1700 p.s.i.

Detector. 254 nm at 0.04 a.u.f.s., attenuation at 16 (with 1 mV integrator output).

Injection volume. 10 μl.

Analytical procedure

Cefsulodin sodium 500 mg (potency) vials were reconstituted and diluted with distilled water to obtain drug concentrations of approximately 2 mg/ml. A 5-ml portion of each sample was mixed with 5 ml of internal standard solution [16 mg/ml of phenoxy acetic acid in water–acetonitrile (9:1)], diluted to 100 ml with distilled water and chromatographed. The peak area response for each sample preparation was compared to that of a 100 μg/ml solution of cefsulodin reference standard prepared in a similar manner.

RESULTS AND DISCUSSION

The HPLC conditions described in this procedure give optimum separation of the drug substance and various impurities while maintaining a reasonable assay time. In preliminary work, cefsulodin sodium was quantitated using a reversed-phase ODS

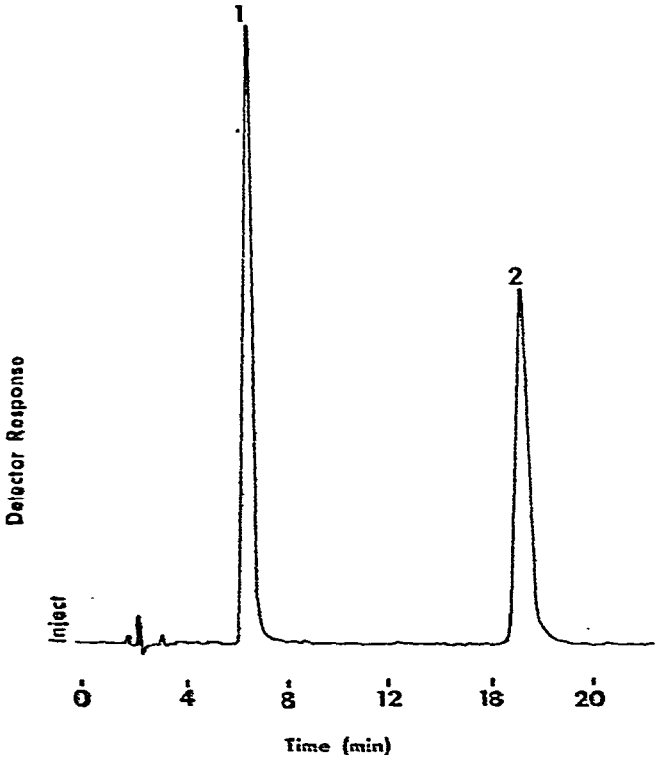

Fig. 2. Chromatogram of a typical cefsulodin sodium vial preparation. Conditions stated in text. Peaks: 1 = cefsulodin sodium, 2 = internal standard.

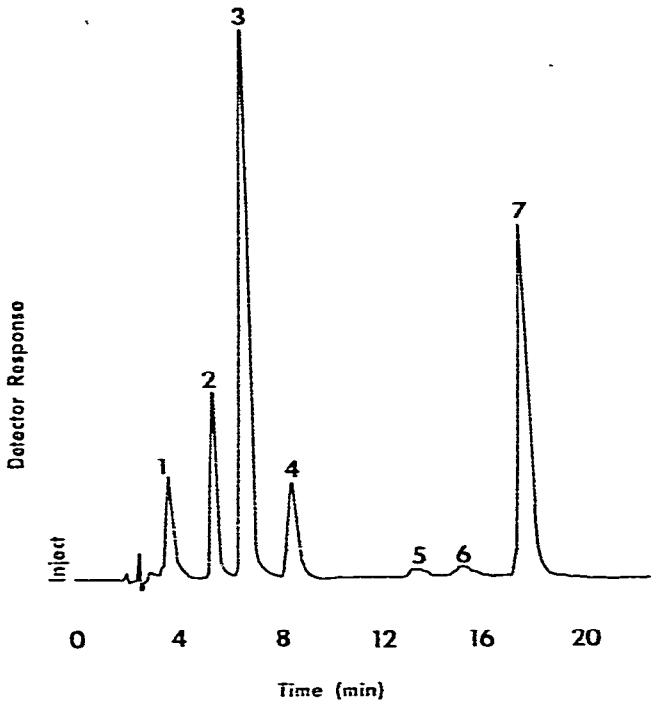

Fig. 3. Chromatogram of cefsulodin sodium standard preparation spiked with impurities (approx. 10% level). conditions stated in text. Peaks: 1 = desamino-SCE-129; 2 = L(+)-SCE-129; 3 = cefsulodin sodium; 4 = isonicotinamide; 5, 6 = SCE-1466 [possibly D(−) and L(+) isomers]; 7 = internal standard.

column with a 0.02 *M* ammonium acetate buffer–acetonitrile (98:2) eluent. This system, while equivalent to that described here for potency determination, failed to completely resolve the minor impurities from each other.

Presented in Fig. 2 is a typical chromatogram of a cefsulodin sodium vial preparation described in the text, while Fig. 3 is a chromatogram of the cefsulodin sodium reference standard preparation which was spiked (at about the 10% level) with possible degradation products and manufacturing impurities. Linearity of the detector response was demonstrated for cefsulodin sodium free acid concentrations

TABLE I

POTENCY DETERMINATION (PERCENT LABEL CLAIM) FOR CEFSULODIN SODIUM 500-mg VIALS BY VARIOUS METHODS

Sample	*HPLC*	*Hydroxylamine*	*Microbiological*
1	104.0	103.4	104.8
2	106.2	103.8	103.4
3	101.8	105.4	103.0
4	107.2	106.8	103.6
5	104.6	104.8	101.4
6	104.2	102.4	101.0

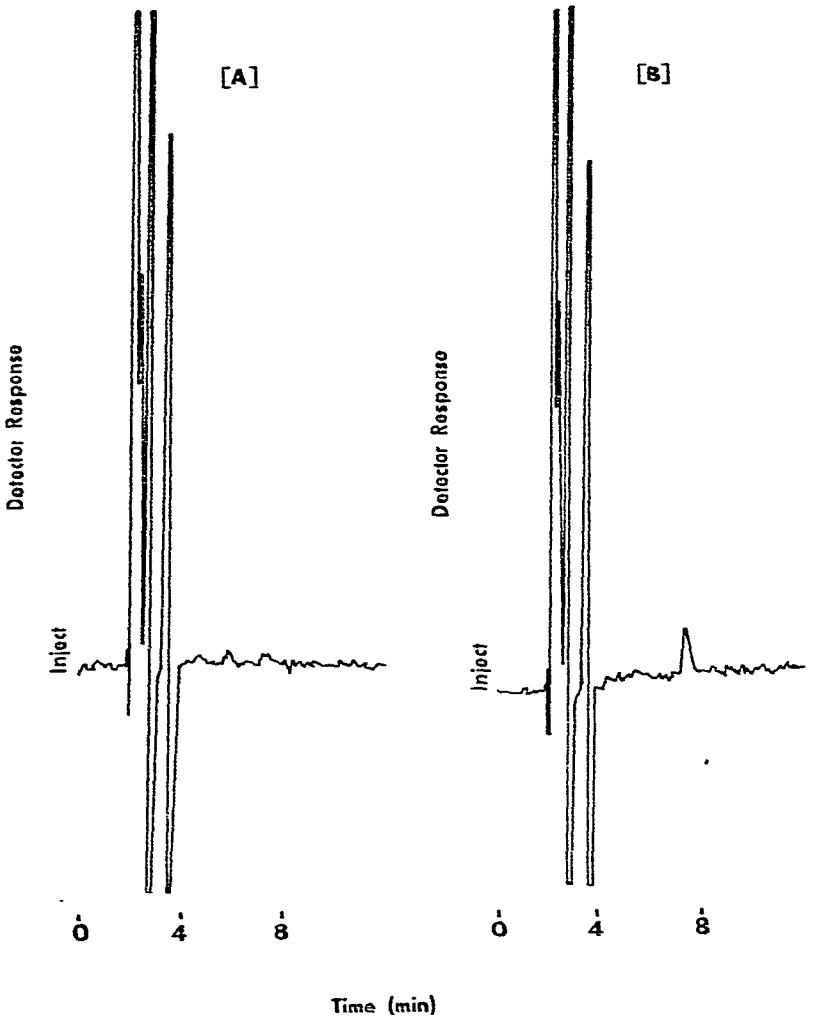

Fig. 4. Chromatograms of [A] solution blank and [B] 0.10 μg/ml cefsulodin standard. Conditions stated in text; using 75 microliter injection volume, UV detector at 261 nm, sensitivity 0.04 AUFS at attenuation 2.

of 29 to 145 μg/ml. The plot of free acid concentration *vs.* peak area ratios of drug to internal standard essentially intersected the origin and had a correlation coefficient of 0.9999.

In this work, the HPLC detector was operated at 254 nm since this allows the use of fixed-wavelength instruments. Cefsulodin sodium exhibits a wavelength maxima at 261 nm in aqueous solution ($\varepsilon = 1.52 \times 10^4$ l mole^{-1} cm^{-1}). Using a UV detector operated at the maxima and larger injection volumes cefsulodin sodium is detectable and may be quantitated to 0.10 μg/ml. In Fig. 4 a 0.10 μg/ml cefsulodin sodium solution and solution blank are chromatographed under conditions described in the text.

To compare the HPLC procedure for cefsulodin sodium with official chemical

TABLE II

POTENCY DETERMINATION (PERCENT LABEL CLAIM) FOR DEGRADED CEFSULODIN SODIUM 500-mg VIALS BY VARIOUS METHODS

Sample	*HPLC**	*Hydroxylamine*	*Microbiological*
Heat	103.4	102.6	104.6
UV	103.8	103.0	104.4
Water–heat	84.2	91.0	87.8
pH 8 Buffer–heat	43.0**	76.2	50.4

* Solutions were chromatographed on an ODS column using a 0.02 *M* ammonium acetate–acetonitrile (98:2) eluent.

** The HPLC assay showed 22% label claim of the L(+) isomer of cefsulodin sodium.

and microbiological assays, six lots of vials were assayed by HPLC, by the hydroxylamine[20] procedure and by the agar diffusion[19] method. As shown in Table I, potencies (as percent label claim) obtained by the three methods agreed well. As an additional comparison of the potency methods, individual cefsulodin sodium vials (Sample 6, Table I) were stressed under the following conditions: (a) dry heat at 110°C for 1.25 h, (b) intensive UV radiation for 1.25 h, (c) diluted to 50 ml with water and heated at 70°C for 30 min and (d) diluted to 50 ml with pH 8.0 phosphate buffer and heated at 70°C for 30 min. Each sample was assayed by the three methods described above and the results are summarized in Table II. For normal stability situations (heat, light and simple solution degradation), the methods are comparable. When cefsulodin is degraded in base, the chemical method shows a high bias, probably resulting from the conversion of the drug to its L(+) isomer.

Precision data for the HPLC procedure are summarized in Table III. Assays were performed by three analysts over several days and as shown the relative standard deviation was ±1.4%.

TABLE III

PRECISION DATA FOR THE HPLC ASSAY OF CEFSULODIN SODIUM 500-mg VIALS

Day	*Analyst*	*Percent Label Claim*
1	1	104.6
1	1	106.0
2	2	102.8
2	2	108.2
3	3	105.2
3	3	104.8
3	3	104.2
4	1	105.0
4	1	106.4
Mean		105.2
Standard deviation		±1.5
Relative standard deviation		±1.4%

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REFERENCES

1 H. Nomura, T. Fugono, T. Hitaka, I. Minami, T. Azuma, S. Moimoto and T. Masuda, *J. Med. Chem.*, 17 (1974) 1312.
2 H. Nomura, I. Minami, T. Hitaka and T. Fugono, *J. Antibiot.*, 29 (1976) 928.
3 U. Ullmann, *J. Antimicrob. Chemother.*, 5 (1979) 563.
4 S. R. Durham and R. Wise, *J. Antimicrob. Chemother.*, 6 (1980) 743.
5 J. V. Bennett, J. L. Brodie, E. J. Benner and W. M. M. Kirby, *Appl. Microbiol.*, 14 (1966) 170.
6 E. C. Rickard and G. G. Cooke, *J. Pharm. Sci.*, 66 (1977) 379.
7 J. A. Squella, L. J. Nunez-Vergara and E. M. Gongalez, *J. Pharm. Sci.*, 67 (1978) 1466.
8 A. G. Fogg and N. M. Fayad, *Anal. Chim. Acta.*, 108 (1979) 205.
9 T. Nakagawa, J. Haginaka, M. Masada and T. Uno, *J. Chromatogr.*, 154 (1978) 264.
10 J. F. Alicino, *Anal. Chem.*, 33 (1961) 648.
11 H. Zia, M. Tehrani and R. Zargarbashi, *Can. J. Pharm. Sci.*, 9 (1974) 112.
12 A. B. C. Yu, C. H. Nightingale and D. R. Flanagan, *J. Pharm. Sci.*, 66 (1977) 213.
13 R. H. Barbhaiya, R. C. Brown, D. W. Payling and P. Turner, *J. Pharm. Pharmacol.*, 30 (1978) 224.
14 G. E. Boxer and P. M. Everett, *Anal. Chem.*, 21 (1949) 670.
15 W. M. Holl, M. O'Brien, J. Filan, T. R. Mazeika, A. Post, D. Pitkin and P. Actor, *J. Pharm. Sci.*, 64 (1975) 1232.
16 J. W. Munson, D. Papadimitriou and P. P. DeLuca, *J. Pharm. Sci.*, 68 (1979) 1333.
17 L. P. Marrelli, *J. Pharm. Sci.*, 57 (1968) 2172.
18 L. P. Marrelli, in E. H. Flynn (Editor), *Cephalosporins and Penicillins*, Academic Press, New York, 1972, pp. 617–619.
19 *Code of Federal Regulations, Title 21*, U.S. Government Printing Office, Washington, DC, 1980, Part 436.105.
20 *Code of Federal Regulations, Title 21*, U.S. Government Printing Office, Washington, DC, 1980, Part 436.205.
21 E. R. White, M. A. Carroll and J. E. Zarembo, *J. Antibiot.*, 30 (1977) 811.
22 M. G. Young, *J. Chromatogr.*, 150 (1978) 221.
23 J. M. Indelicato, B. A. Steward and G. L. Engel, *J. Pharm. Sci.*, 69 (1980) 1183.
24 V. D. Gupta and K. R. Stewart, *J. Pharm. Sci.*, 69 (1980) 1264.
25 M. A. Carroll, E. R. White, Z. Jancsik and J. E. Zarembo, *J. Antibiot.*, 30 (1977) 397.
26 J. H. Kennedy, *J. Chromatogr. Sci.*, 16 (1978) 492.
27 R. D. Miller and N. Neuss, *J. Antibiot.*, 29 (1976) 902.
28 R. J. Mehta, M. K. Fox, D. J. Newman and C. H. Nash, *J. Antibiot.*, 30 (1977) 1132.
29 J. S. Wold and S. A. Turnipseed, *Clin. Chem. Acta.*, 78 (1977) 203.
30 T. Nakagawa, J. Haginaka, K. Yamaoka and T. Uno, *J. Chromatogr.*, 147 (1978) 509.
31 F. Salto, *J. Chromatogr.*, 161 (1978) 379.
32 L. A. Wheeler, M. De Meo, B. D. Kirby, R. S. Jerauld and S. M. Finegold, *J. Chromatogr.*, 183 (1980) 357.