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HPLC analysis of γ-irradiated β-lactam antibiotics

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Summary

γ-irradiated cephalosporins and semi-synthetic penicillin powders previously examined by us using different analytical techniques have been subjected to HPLC analysis. The HPLC data support the earlier conclusions, thus confirming the possibility of irradiating cefoxitin, flucloxacillin and nafcillin at a 2.5 Mrad dose level to effect sterilization, and cephalothin and ticarcillin at a 1 Mrad dose level.

Introduction

The feasibility of the radiation sterilization of a wide range of β-lactam antibiotics has been examined by us (Jacobs 1979, 1980a, b and c, 1981, 1983).

The increased application of high-performance liquid chromatography (HPLC) as a reliable analytical tool has prompted us to apply this technique for analysis of some of the irradiated β-lactam antibiotics (cephalosporins and penicillins) that were earlier examined by other means. It is envisaged that this approach may consolidate earlier findings, add weight to the conclusions thus derived, and it is hoped, advance the acceptance by the appropriate regulatory authorities of the sterilization of these drugs by γ-irradiation. A number of drugs have already been approved by the FDA and other authorities following their radiation-sterilization (for example see, Diding, 1975; Nash, 1974).

This study has focused on five antibiotics which cannot be sterilized by conventional means such as autoclaving, and, as such, their sterilization at present involves highly demanding and costly aseptic techniques. Thus, they are potential candidates for radiation-sterilization, in which their irradiation treatment can take place after the drugs have been packaged in their outer and shipment containers.

Materials and methods

The β-lactam antibiotics tested were the cephalosporins — cefoxitin sodium (Merck & Co, West Point, PA) and cephalothin (Lark, Milan, Italy), and the semi-synthetic penicillins—flucloxacillin sodium (Beechams, Brentford, U.K.), nafcillin sodium (Bristol Laboratories, Syracuse, NY), and ticarcillin di-sodium (Ticar, Beechams Laborato-

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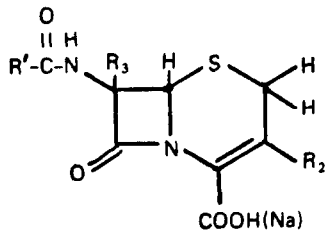
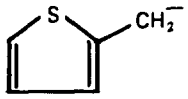
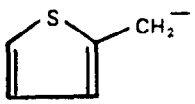
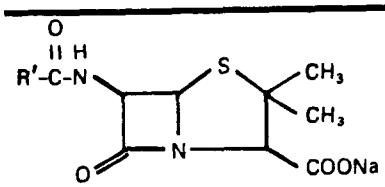


	R'	R_2	R_3
Cephalothin		$-CH_2O.CO.CH_3$	H
Cefoxitin Na		$-CH_2O.CO.NH_2$	OCH_3



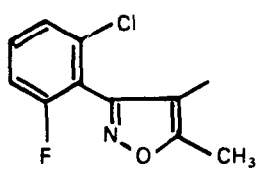
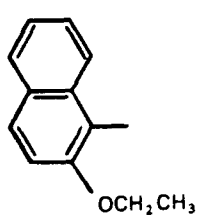
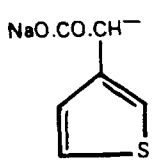
	R'
Flucloxacillin Na	
Nafcillin Na	
Ticarcillin di-Na	

Fig. 1. Chemical structures of β-lactam antibiotics tested.

ries, Bristol, TN). Their chemical structures are depicted in Fig. 1.

γ-irradiation at 18.8°C of 1 g samples in open glass vials was undertaken using a Gammacell 220 ^{60}Co source (A.E.C.L., Canada). Doses delivered were 1, 2.5 and 5 Mrad (10, 25 and 50 kGy, respectively) based on an approximate dose rate of 17 krad · min^{-1}.

The HPLC methodology for cephalothin was as follows: the liquid chromatograph (Waters Associates HPLC 244) was equipped with a reverse-phase micro-Bondapak phenyl column (30 cm × 3.9 mm i.d.), a 254 nm fixed UV detector and a Schoeffel variable wavelength UV detector set at 270 nm connected in series. The mobile phase was 0.01 M ammonium acetate in either 25% or 35% aqueous methanol solution. Samples were eluted at a flow rate of 1.8 ml/min at room temperature. Routinely, 20 or 50 μl aliquots of solutions of the irradiated and unirradiated drugs, at suitable concentrations, were used.

For cefoxitin, nafcillin and ticarcillin, a reverse-phase micro-Bondapak C_{18} column (30 cm × 3.9 mm i.d.) was used. The variable UV detector was set at 235 nm for cefoxitin, and at 220 nm for nafcillin and ticarcillin. The mobile phase was either a 15% or 20% aqueous solution of acetonitrile containing 1% acetic acid for cefoxitin; either a 43% or 53% solution of methanol in 0.01 M aqueous ammonium acetate for nafcillin; and for ticarcillin a 25%, 20% or 15% solution of methanol in 0.1 M aqueous ammonium acetate, to which was added 0.5% of phosphoric acid (85%).

For flucloxacillin, a reversed-phase Partisil PXS 10/25 C_8 column was used. The variable wavelength UV detector was set at 220 nm. The mobile phase was either a 42%, 35% or 28% aqueous solution of methanol containing 0.01% sodium bicarbonate.

All reagents were of analytical grade.

The modification and variation of the mobile phases was an attempt to improve separation between the various radiolysis product peaks. The choice of the different wavelengths was generally a compromise between the λ_{max} and a wavelength where degradation products could be detected and where interference by the mobile phase components would not be problematic.

Results and Discussion

Presented in Table 1 are the percentage recoveries (that is, comparison of the area under the curve of the major peak with that of the unirradiated control) for the 5 Mrad irradiated samples of the drugs, as well as the magnitude (%) of the different radiolysis product peaks.

Any discrepancy between the % recovery value and that for the total radiolysis, following 5 Mrad radiation treatment, is attributed to the 1–2% inherent error in the methodology and to the fact that, in general, molar detection sensitivity need not necessarily be the same for the parent compound and its products. Furthermore, there may be peaks that have remained undetected, because only a limited number of wavelengths were examined and certain radiolysis products may not absorb in the UV region of the spectrum. The purity of the antibiotic peak was routinely determined by absorbance ratioing, in order to be sure that this peak was not masking radiolysis products that have the same retention time and that also absorb in the UV region. This analysis indicated that each of the antibiotic peaks was representative of a single entity.

In order to relate the HPLC-generated data to those previously obtained, a summary of these earlier findings is presented in Table 2.

Cephalothin

From the earlier data it is apparent that γ-irradiation somewhat reduces the potency of cephalothin. This reduction, on the basis of UV absorbance, chemical assay, and specific optical rotation measurements, is around 3.1% following a 5 Mrad dose, and 2.2% (or 97.8% purity) as determined by UV absorbance alone. HPLC data indicate a percentage recovery of 94.88%. The free acid was examined in the present study (Fig. 2) and the sodium salt in the earlier study, but this fact is not thought to contribute significantly to any variation in response.

A number of peaks that are present in both irradiated and unirradiated samples are larger in the former, presumably as a result of interaction between hydrolysis and radiation effects. The phenomenon was not characteristic of cephalothin alone.

Interestingly, the 20.20-min retention time peak observed on the 2.5 Mrad irradiated sample was not apparent in the 5 Mrad sample (Fig. 2).

Cefoxitin sodium

Comparison of the chromatograms for the irradiated and unirradiated cefoxitin (Fig. 3) indicates the presence of at least 3 or 4 radiolysis product peaks with retention times shorter than that for the cefoxitin peak, and one large peak with a longer retention time. Nevertheless, the HPLC data support the earlier conclusions as to the high radiation stability of cefoxitin sodium. A contributory factor to the radiation stability of

TABLE 1

RADIOLYSIS PRODUCT PEAKS AND PERCENTAGE RECOVERIES AS DETERMINED BY HPLC

Antibiotic	Peak (RT) (min)	1.0 Mrad (%)	2.5 Mrad (%)	5.0 Mrad (%)	% recovery at 5.0 Mrad
Cephalothin	1.89	0.46	0.54	0.88	
	4.70	0.24	0.40	0.39	
	20.20	n.d.	0.01	n.d.	
	Total	0.70	0.95	1.27	94.88
Cefoxitin Na	2.28	n.d.	n.d.	0.10	
	2.41	0.05	0.06	0.07	
	2.61	0.10	0.15	0.26	
	7.18	n.d.	n.d.	0.26	
	17.16	0.08	0.15	0.33	
	Total	0.23	0.36	1.02	97.86
Flucloxacillin Na	1.91	0.03	0.07	0.12	
	3.39	n.d.	n.d.	0.04	
	4.31	0.01	0.01	0.03	
	5.14	0.07	0.13	0.28	
	6.12	0.01	0.02	0.02	
	8.41	n.d.	n.d.	0.08	
	8.59	0.01	0.03	0.08	
	11.29	0.01	0.01	0.02	
	12.45	0.01	0.04	0.07	
	Total	0.15	0.31	0.74	96.66
Nafcillin Na	3.6	0.04	0.05	0.09	
	4.9	0.02	0.02	0.06	
	6.2	0.00	0.02	0.06	
	7.6	0.03	0.02	0.04	
	13.9	n.d.	n.d.	0.01	
	21.7	0.01	0.05	0.23	
	Total	0.10	0.16	0.48	98.49
Ticarcillin di Na	4.17	0.23	0.22	0.22	
	8.22	0.18	0.34	0.29	
	10.07	0.04	0.10	0.20	
	10.84	0.14	0.28	0.34	
	16.05	0.05	0.05	0.34	
	21.54	0.06	0.11	0.34	
	28.71	0.12	0.14	0.13	
	51.00	0.14	0.20	0.42	
	55.00	0.04	0.03	0.09	
	Total	1.00	1.47	2.37	95.80

n.d. = not detected.

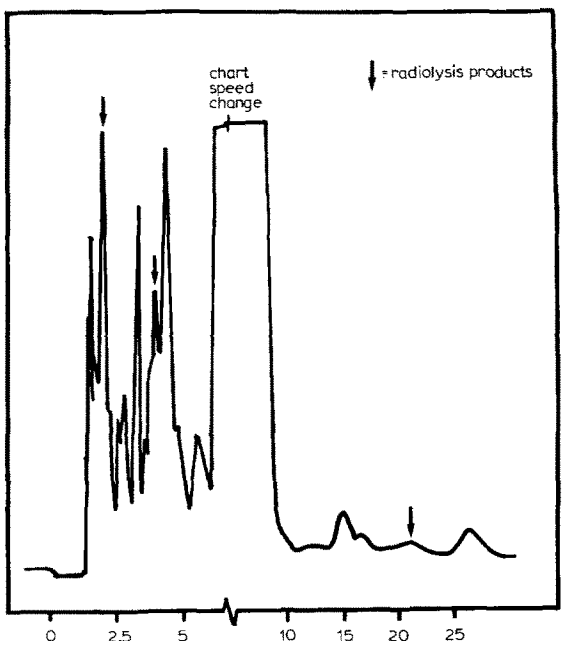
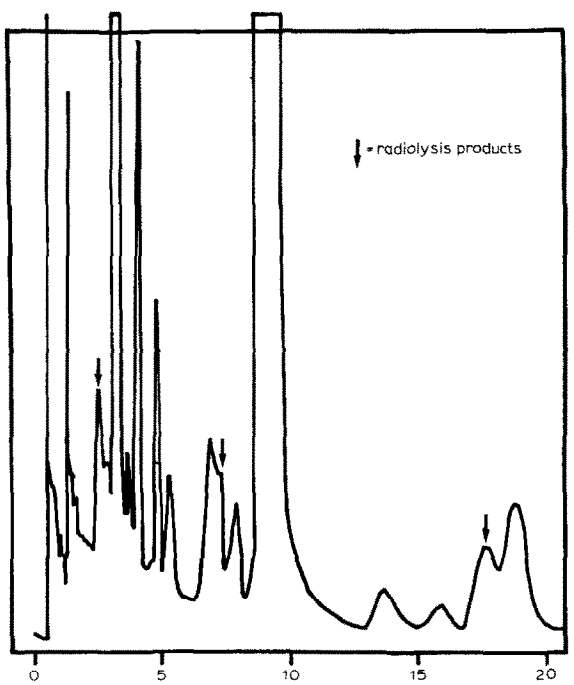


Fig. 2. Typical HPLC chromatogram for 5 Mrad irradiated cephalothin (4.4 mg/ml). Separation conditions: mobile phase, 25% methanol in 0.01 M ammonium acetate; wavelength, 270 nm; injection volume, 50 μl; sensitivity, 0.1 AUFS.



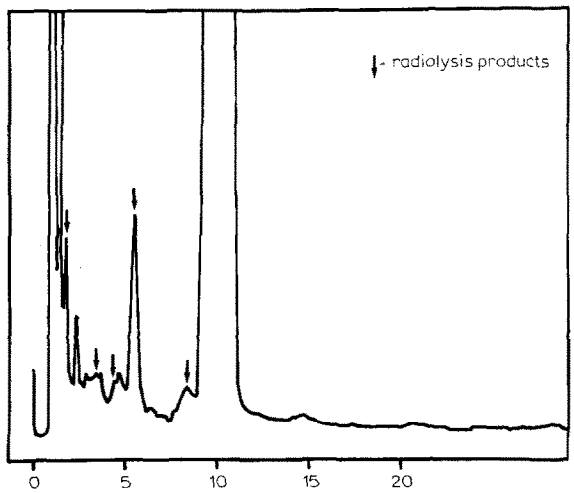


Fig. 4. Typical HPLC chromatogram for 5 Mrad irradiated flucloxacillin sodium (2.1 mg/ml). Separation conditions: mobile phase, methanol/1% sodium bicarbonate solution/water 280:10:710; wavelength, 254 nm; injection volume, 50 μl; sensitivity, 0.005 AUFS; chart speed, 40 cm/h.

this compound may be its low water content, which has been found to be less than 0.5% (Jacobs, unpublished data), whereas with most antiobiotic powders in our hands, this value is closer to 4%. Furthermore, it has previously been suggested (Jacobs, 1981) that cefoxitin's chemical structure, with a 3-carbamoyloxymethyl moiety substituted into the dihydrothiazine ring, may play an important role in this compound's radiation chemistry.

Flucloxacillin sodium

Examination of the chromatograms for the irradiated and unirradiated drug (Fig. 4) indicates the presence of about 7 radiolysis-product peaks in addition to the control peak. Both the earlier findings and the present HPLC data confirm the radiation stability of flucloxacillin sodium.

Nafcillin sodium

In Fig. 5, some 6 peaks are observed in addition

←

Fig. 3. Typical HPLC chromatogram for 5 Mrad irradiated cefoxitin sodium (4.5 mg/ml). Separation conditions: mobile phase, acetonitrile/water/acetic acid 15:84:1; wavelength, 254 nm; injection volume, 50 μl; sensitivity, 0.05 AUFS; chart speed, 1 cm/min.

to the nafcillin peak. Again the data indicate the high stability of this compound to γ-irradiation, with close correlation between the HPLC and earlier data.

Ticarcillin di-sodium

In the case of irradiated ticarcillin di-sodium, the HPLC analysis (Fig. 6) indicates the presence of about 9 peaks in addition to the control peak. Comparison of all the data indicates that this compound is hardly affected by irradiation. This might be attributed to its unique structure, in which the phenyl group on the α-carbon atom of the N-acyl side chain is replaced by a 3-thienyl ring.

It will be noted that for all the antibiotics tested, the HPLC assay (% recovery at 5 Mrad — Table 1) is always 2% or 3% less than that observed at a similar dose when determined by a chemical assay (Table 2) or, in most cases, by the microbiological assay. Both the chemical assay (either by an iodometric or hydrolysis method) and the microbiological assay essentially reflect any change induced in the β-lactam ring of the penicillin or

TABLE 2

EARLIER DATA ON γ-IRRADIATED ANTIBIOTICS

Antibiotic [supplier]	Radiation dose (Mrad)	M.Pt. [a]	Microbiological assay [b] (%, ± S.D.)	Chemical assay	UV absorbance [a,e]	Specific optical rotation (±1.5%)
Cephalothin Na[1] [Glaxo]	0.0	210	(100) [f]	(100) [c,f]	0.653	(+) 114°
	1.0	206	93 ± 2	101.3 [a]	0.645	(+) 114°
	2.5	205	91 ± 3	100.0 [a]	0.630	(+) 111°
	5.0	202	90 ± 2	97.8 [a]	0.635	(+) 119°
Cefoxitin Na[2]	0.0	260	(100) [f]	100) [c,f]	0.550	(+) 195°
[M.S.D.]	2.5	260	97 ± 4	101.7 ± 1.2	0.550	(+) 188°
	5.0	260	100 ± 1	100.0 ± 0.1	0.550	(+) 187°
Flucloxacillin Na[4]	0.0	165	(100) [f]	97.3 ± 0.4 [d]	0.500	(+) 149°
[Beechams]	1.0	165	96 ± 3	100.5 ± 0.1	0.517	–
	2.5	163	97 ± 2	98.0 ± 4.6	0.526	–
	5.0	162	99 ± 5	100.7 ± 3.1	0.527	(+) 139°
Nafcillin Na[3]	0.0	166	(100) [f]	(100) [f,g]	not	(+) 200°
[Wyeth]	2.5	165	99 ± 1	101.0 ± 1.9	det'd.	(+) 200°
	10.0	165	99 ± 1	98.4 ± 2.1		(+) 195°
Ticarcillin di-Na[5]	0.0	212	(100) [f]	(100) [f,g]	0.520	(+) 148°
[Beechams]	2.5	212	–	100.7 ± 0.9	–	(+) 124°
	5.0	213	101 ± 2	97.9 ± 0.4	0.520	(+) 129°

[a] Means of < 2 determinations within ±1%.

[b] Using a 2 dose cylinder plate method on Difco Antibiotic No. 1 Medium with 0.1 ml *Staphylococcus aureus* (Teva 20).

[c] B.P. Iodometric method.

[d] B.P. Hydrolysis method.

[e] Optical density values of aqueous solutions in 10 mm quartz cells at λ_{max}.

[f] Unirradiated sample used as standard for assay and taken as 100%.

[g] Methodology of Bundgaard and Ilver (1972).

References:

[1] Jacobs, 1980a.

[2] Jacobs, 1981.

[3] Jacobs, 1980b.

[4] Jacobs, 1979.

[5] Jacobs, 1980c.

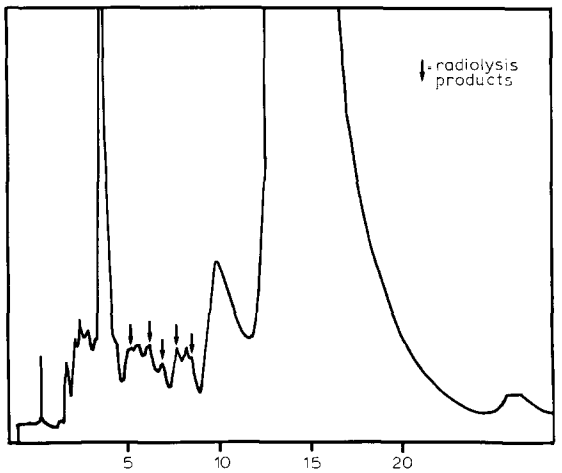


Fig. 5. Typical HPLC chromatogram for 5 Mrad irradiated nafcillin sodium (2.5 mg/ml). Separation conditions: mobile phase, 0.01 M ammonium acetate in 43% aqueous methanol; wavelength, 254 nm; sensitivity, 0.005 AUFS; injection volume, 50 μl; chart speed, 1 cm/min.

cephalosporin. They will not be sensitive to a change in any other moiety of the antibiotic, such as removal, in the case of penicillins, of the 6-amino substituent. Similarly, the other analytical methods, such as specific optical rotation or UV absorbance, will be indicative of very specific changes in the drugs examined. This may not, however, be the case in HPLC analysis, where radiolysis products are separated from the parent compound prior to UV detection and thus any reasonable structural change could be discerned above the threshold sensitivity of the detector.

In conclusion, therefore, we see that the HPLC data support the earlier conclusions regarding the feasibility of radiation sterilization of those β-lactam antibiotics considered, and that it would be possible to sterilize cefoxitin, flucloxacillin and nafcillin at doses of around 2.5 Mrad, and cephalothin and ticarcillin at somewhat lower doses (say 1 Mrad) provided that initial contamination (bioburden) was low.

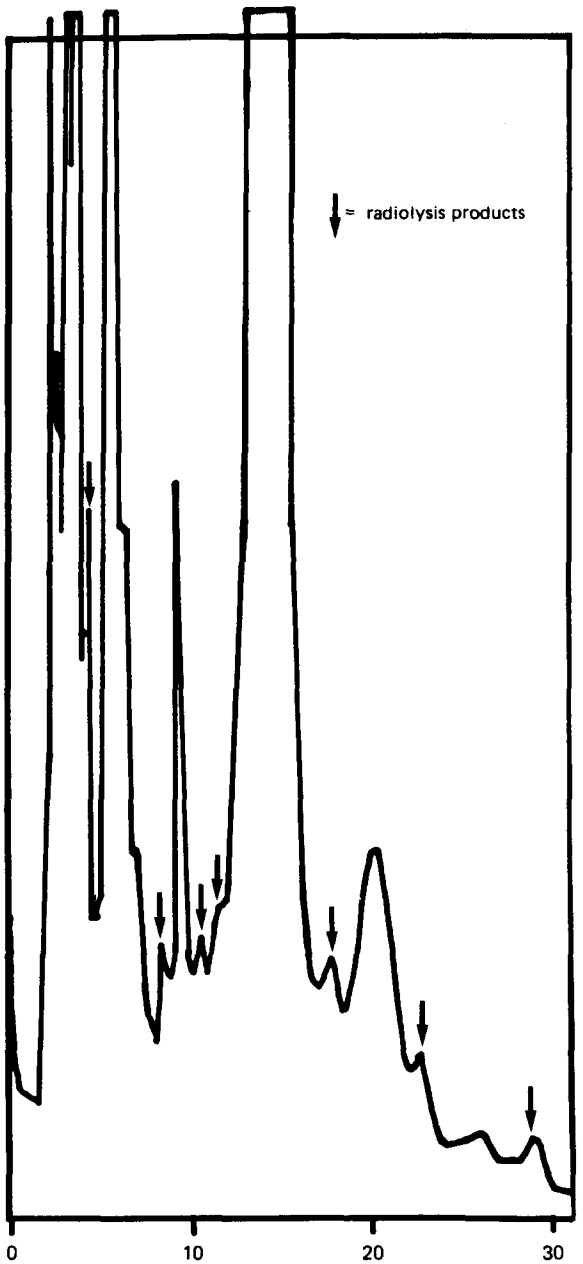


Fig. 6. Typical HPLC chromatogram for 5 Mrad irradiated ticarcillin di-sodium (3.7 mg/ml). Separation conditions: mobile phase, 20% methanol in 0.1 M ammonium acetate, pH 4.0: wavelength, 250 nm; injection volume, 50 μl; sensitivity, 0.005 AUFS; chart speed, 20 cm/h; guard column, CO:PELL ODS 30–38 μm glass beads.

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