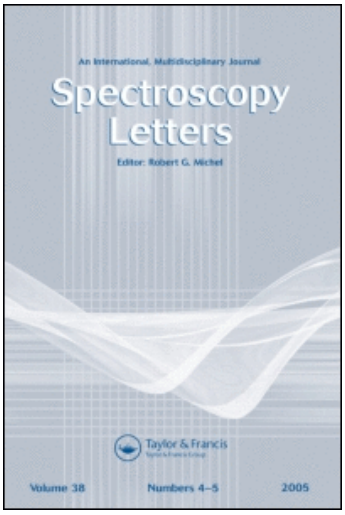

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PMR Assay of β-Lactm Antibiotics 1: Assay Of Cephalosporins

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PMR ASSAY OF β-LACTAM ANTIBIOTICS I : ASSAY OF CEPHALOSPORINS

Key Words:

NMR Analysis, Cephalosporins, PMR Assay, β-Lactam antibiotics, Cephalotin, Cephaloridine, Cephradine, Cefoxitin, Cefotaxime.

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Abstract:

A PMR method for quantitative determination of cephalosporins namely cephalotin, cephaloridine, cephradine, cefoxitin and cefotaxime in bulk drugs and pharmaceutical formulation are presented. The determination is based on the integration of the 6-H and/or 7-H of the β-lactam ring system relative to that of the nine protons of t-butanol. The method is rapid, accurate and precise, with an average standard deviations of ± 1.51 in standard mixtures and ± 1.15 in pharmaceutical formulation. The procedure furnishes a specific means of identification of each individual cephalosporin as well as detection of the commonly encountered impurites.

Introduction:

Cephalosporins constitute a group of β-lactam antibiotics, several of which are clinically used. Many methods have been described for their quantitative analysis. Among these iodometric titrations, one of which is utilizing alkali for opening the β-lactam ring (1). This method is officially adopted by the B.P. 1980 (2). In the other method opening the β-lactam ring is achieved by cephalosporinase (3). Other methods including non-aqueous titration (4,5), colorimetric methods using either hydroxylamine (6,7) or 5,'5-dithiobis-(2-nitrobenzoic acid) (8) reagents. Spectrophotometric methods were reported as a stability indicating assays (9). For the determination of blood level concentrations, a fluorometric assay was also described (9). Microbiological techniques have been reported (10-11) and currently adopted by the U.S.P. (XX) 1980 (12). Chromatographic methods particularly high performance liquid chromatography has been used (4, 9). An NMR procedure for quantitative determination of cephalexin present as an impurity in cephradine has been recenty developed (13).

The fact that cephalosporins have a unique and similar molecular structures and by virtue of their high dosage, has attracted our attention of developing a general PMR assay for their quantitative determination as drug intities and in pharmaceutical formulations.

Experimental:

All spectra were recorded at 37° on a Varian T-60A, 60-MHz NMR spectrometer using deutrium oxide as the solvent and t-butanol as

the internal standard. Chemical shifts were measured relative to DSS (sodium-2,2-dimethyl-2-silapentane-5-sulphonate) at 0 ppm.

Chemicals: Deutrium oxide[1], t-butanol[2], standard cephalotin[3], cephaloridine[3], and cephradine[3], mefoxin[4], and cefotaxime[5]. Pharmaceutical formulation[6-7] were also obtained.

1. Koch-Light Laboratories Ltd., Colnbrook Buck, England.
2. B.D.H. Poole, England.
3. British Pharmacopoeia Commision Lab., Stanmore, U.K.
4. Merck Sharp and Dohme, Westpoint, Pa., U.S.A.
5. Rossel Uclaf, France.
6. Eli Lilly & Co., Indianapolis, Ind. U.S.A.
7. Squibb, Greece.

Assay of Cephalosporins:

Weigh accurately a portion of the powder equivalent to 100 mg of the cephalosporin into a glass stoppered sample tube. Add 1.0 ml accurately measured D_2O containing an accurate weight of t-butanol, stopper and shake for 3 min. Transfer about 0.5 ml of the resulting solution into a NMR tube and record the spectrum, adjusting the spin rate to reduce the spinning side bands as much as possible. Integrate the peaks of interest (The 6- or 7-H of the β-lactam ring appearing at 4.86 - 5.80 ppm and the 9 protons of t-butanol appearing at 1.23 ppm) at least three times and determine the average integrals.

The amount of cephalosporin is then calculated as follows:-

$$\text{mg of cephalosporin} = \frac{A_c}{A_b} \times \frac{E.W_c}{E.W_b} \times \text{mg of t-butanol}$$

Where A_c is the integral value of the cephalosporin signal, A_b that of the t-butanol signal, $E.W_c$ is the molecular wieght of cephalosporin and $E.W_b$ is one ninth of the molecular weight of t-butanol (= 8.24).

Results and Discussion:

The PMR spectra of some clinically used cephalosporins namely, cephalotin, cephaloridine, cephradine, cefoxitin and cefotaxime in D_2O exhibited among other peaks two distinct signals appearing at 4.90 - 5.80 ppm. The lowest field signal is attributed to the 7-H while the other signal is assigned to 6-H of the β-lactam ring (Fig. 1). The multiplicity of these protons are usually doublets with a very small coupling constant, except if either 6-H or 7-H is substituted, the multiplicity of the remaining proton is a singlet as the case with cefoxitin, where the 7-H is replaced by a methoxyl function (Fig. 2). In order to assure uniformity of the quantitative determinations, the 6-H and 7-H proton signals were chosen, also the integration of any of these signals gives the largest area for measurement and free from any interference. The chemical shift values for 6-H and 7-H of the said cephalosporins are listed in Table 1.

t-Butanol in D_2O exhibiting a 9-protons singlet at 1.23 ppm assigned to its 3 methyl groups, is employed as internal standard. Its PMR proton singlet is widely separated from those of cephalosporins which allows facile and accurate determination.

Since all the used cephalosporins and t-butanol are freely soluble in D_2O it becomes the solvent of choice. Moreover, the

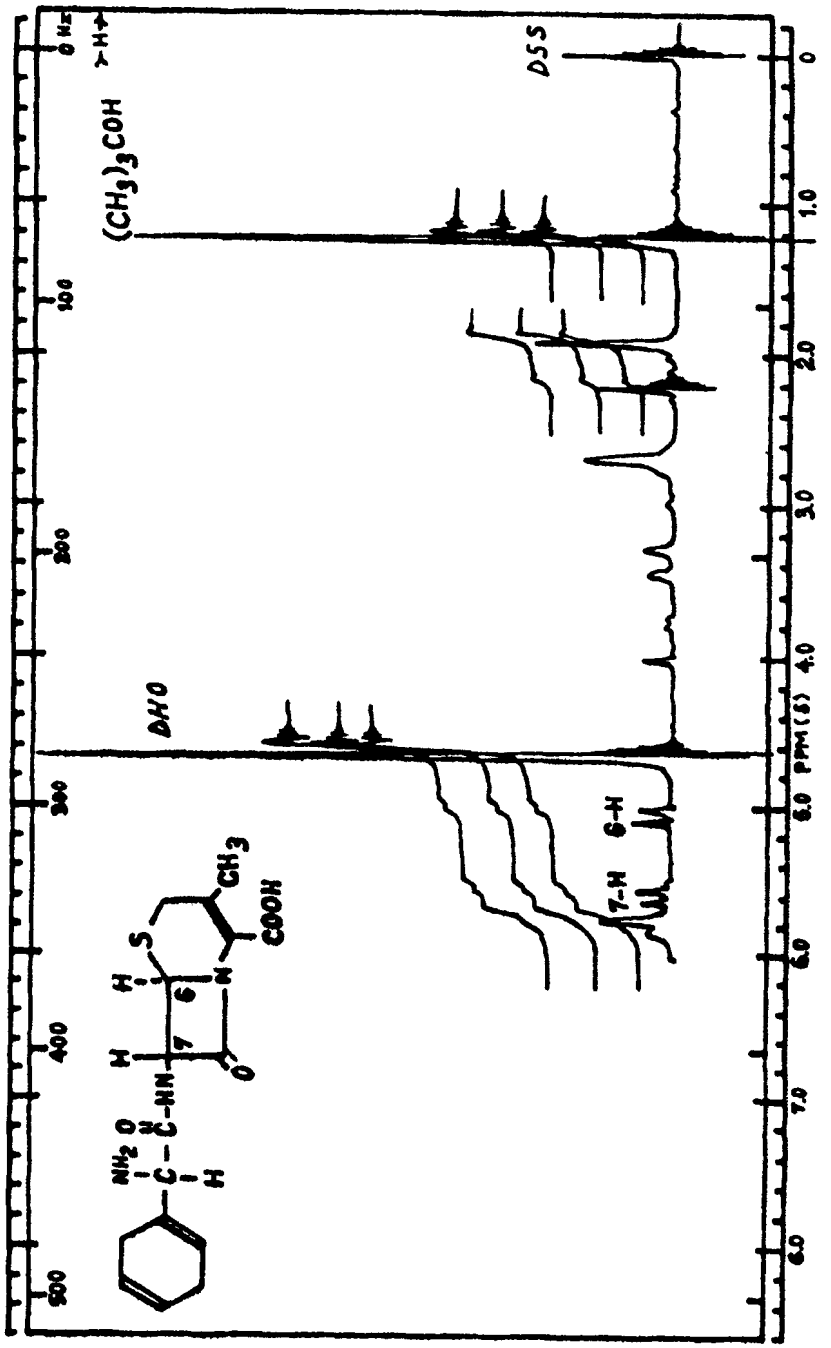

FIG 1: Part of the PMR spectrum of cephradine and t-butanol in D_2O.

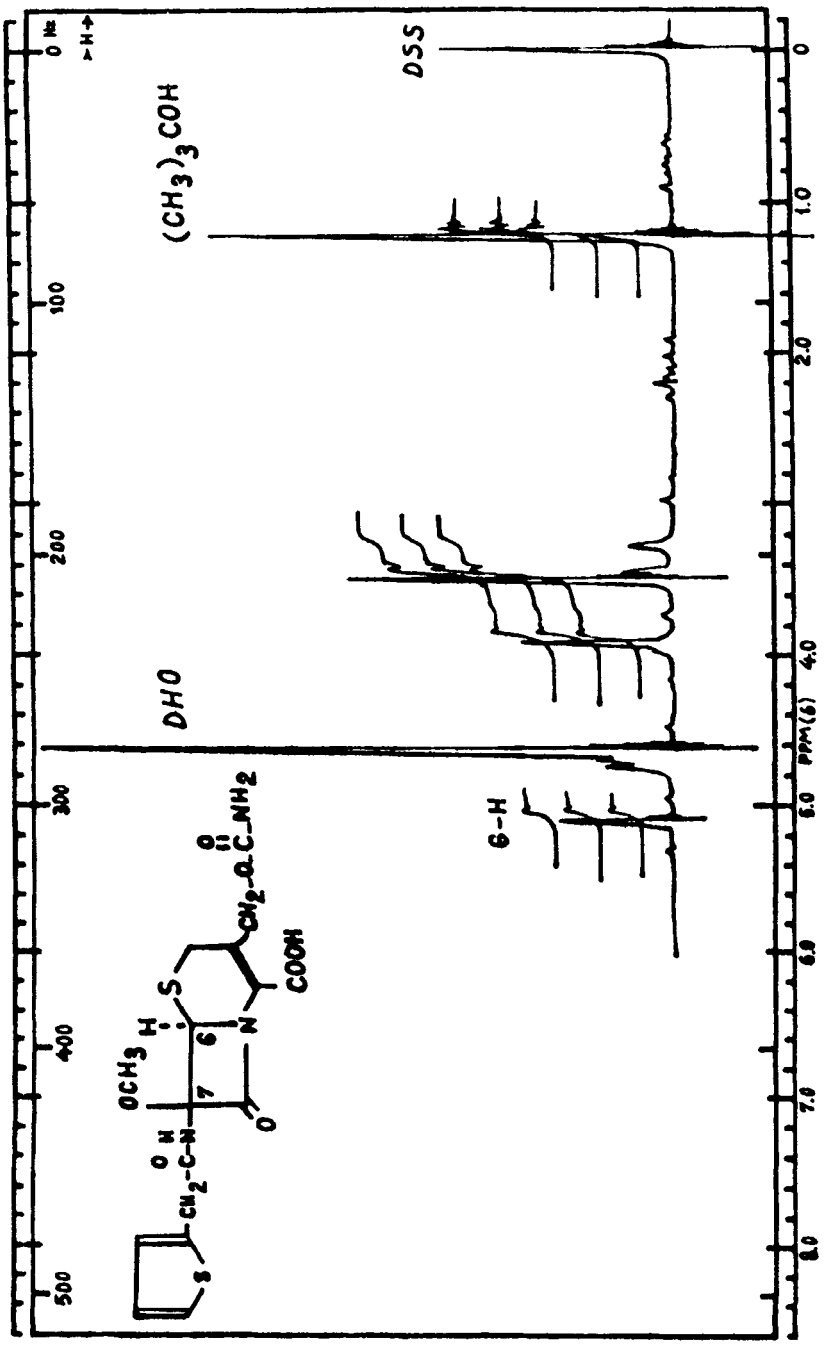

FIG 2: Part of the PMR spectrum of cefoxitin and t-butanol in D_2O.

Table 1: The chemical shift values for 6-H and 7-H of the β-lactam ring.

Cephalosporin	6-H	7-H	M.W.
	δ p p m		
Cephalotin	4.96	5.20	396.44
Cephaloridine	5.06	5.66	415.5
Cephradine	5.00	5.38	349.41
Cefoxitin	5.11	-	449.44
Cefotaxime	5.23	5.80	477.23

DHO proton singlet at 4.66 ppm does not interfere with the proton signals of both compounds.

Assay of a series of known standard mixtures of cephalosporins and t-butanol (Table 2) established the accuracy and presession of the method with standard deviations of 1.40, 1.59, 1.42, 1.93 and 1.11 for cephalotin, cephaloridine, cephradine, cefoxitin and cefotaxime respectively (Table 2).

The results of estimation of cephalosporins in their pharmaceutical formulations (injectables and capsules) by the method under investigation are in good agreement with the declared amounts (Table 2).

Obviously this PMR technique has distinct advantage over other methods previously described for their determination, being rapid, accurate and specific.

Table 2: PMR Assay of Cephalosporins in Standard Mixtures and Dosage Forms.

Cephalosporin	Standard mixtures		Dosage Forms	
	Average Recovery %	Standard Deviation	Average Recovery %	Standard Deviation
Cephalothin sodium.	100.45	± 1.40	99.36	± 1.26
Cephaloridine	100.21	± 1.59	100.47	± 2.00
Cephradine	99.78	± 1.42	100.29	± 1.39
Cefoxitin	100.36	± 1.93	100.0	± 0.91
Cefotaxime	100.83	± 1.11	101.65	± 0.11
Average*		± 1.51		± 1.15

* - of 8 replicates

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