calculated and the correction equation for our tablet machine was obtained from the formula

$$Y = 0{\cdot}00915\ X^{0{\cdot}368}$$

For practical purposes the mean value of the upper and the lower punch forces can be used as X in the previous equation. This approximation is valid if the percentage of the die wall friction is not high. In the case of high friction the entire system would have to be analysed to study the effects of the great force differences between the upper and lower punch.

When account is taken that the distance resolution in our tablet press is 0·02 mm, the accuracy of the correction equation can be regarded as sufficient for this system.

In conclusion, the mounting of the displacement transducer system far from the surface of the upper punch causes a relatively great error in measured distance values and hence significant differences may be obtained in compaction studies. Using the proposed empirical correction equation, it is possible to obtain more precise values which approach those obtained with more complicated mounting systems.

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The quantitative determination of cephalexin by proton magnetic resonance spectroscopy

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For cephalexin [7-(D-2-amino-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0.] oct-2-ene-2-carboxylic acid] the official methods of analysis (British Pharmacopoeia 1973; Code of Federal Regulations 1977) are the microbiological agar diffusion assay and/or the iodometric assay. Other assays include i.r. spectrophotometry (Casu & Ventura 1974), column chromatography (Tortolani & Romagnoli 1976), u.v. spectroscopy (Wahbi & Unterhalt 1977), fluorometry (Yu et al 1977), high performance liquid chromatography (Hartmann & Rodiger 1976; Carroll et al 1977), and gas-chromatography (Nakagawa et al 1978). The nuclear magnetic resonance method presented here provides a specific, rapid quantitative analysis for this compound, which compares well with the official microbiological and iodometric assays.

Materials. Cephalexin formulations were obtained from commercial sources in Italy (Glaxo, Lilly, Dukron), chemicals and intermediates were Pierrel's products. Deuterium oxide, 20% deuterium chloride (99·9% isotopic purity for both), 40% sodium hydroxide—d_1 (99% isotopic purity) were purchased from E. Merck, Darmstadt, Germany and sodium 3-trimethylsilylpropionate-2,2-3,3-d_4 was obtained from Merck, Sharp & Dohme, Montreal, Canada. 3-picoline and 36% hydrogen chloride were regent grade (C. Erba, Milan, Italy).

Method. Approximately 80–150 mg of cephalexin (drug substance) or 150–250 mg of capsule contents were accurately weighed into a 5 ml flask, then 1 ml of the internal standard solution (approximately 30 mg ml^{-1} of 3-picoline in deuterium oxide or distilled water) was added. Deuterium chloride (or hydrogen chloride) was added until a complete solution was obtained by gentle swirling. For intermediates, samples were treated as before but were dissolved by careful addition of 10% sodium hydroxide-d_1 (in deuterium oxide). If an opalescence was present, samples were centrifuged, pipetted into a 5 mm i.d. n.m.r. tube and sodium 3-trimethylsilylpropionate-2,2-3,3-d_4 was added.

Spectra were recorded on a Varian T-60 n.m.r. spectrometer using a 250 s scan time and a sweep width of 100 Hz between 80 and 180 Hz. The methyl peaks of 3-picoline and of cephalexin (about 150 and 126 Hz respectively) were carefully integrated five times. The amount (%) of cephalexin in a sample was calculated as follows, using the average integral value:

$$\%\ \text{Cephalexin} = \frac{Ac}{As} \times \frac{Ws}{Wc} \times \frac{MWc}{MWs} \times 100$$

Ac = integral value for cephalexin; As = integral value for standard; Ws = weight of internal standard in 1 ml; Wc = weight of cephalexin; MWc = molecular weight of cephalexin; MWs = molecular weight of standard.

The n.m.r. spectrometry has been applied to the identi-

* Correspondence.

Table 1. Comparison between n.m.r., microbiological and iodometric results for the quantitative determination of cephalexin.

Sample	% Cephalexin[a]			
	N.m.r. method	Microbiological[b] method	Iodometric[b] method	Water content %
Cephalexin bulk drug				
1	90·7[c]	90·8	89·5	2·44
2	91·5[c]	92·0	92·3	5·9
3	90·5[d]	89·6	93·0	6·1
4	92·0[c]	93·0	92·7	7·1
5[e]	83·7[c]	82·1	84·5	7·5
6[e]	78·8[c]	79·6	78·5	8·2
Cephalexin capsules				
7	91·4[f]	90·9	90·4	6·2
8	60·4[c]	62·4	62·7	6·3
9	88·8[f]	88·6	88·3	6·1
10[e]	83·3[c]	84·1	82·7	7·7
Cephalexin sulfosalicylates				
11	45·2[d]	45·7	48·1	7·1
12	36·2[d]	40·9	42·0	6·3
13	33·0[b]	35·6	37·0	6·7
Average	74·3	75·0	75·5	

[a] On as is basis.
[b] Mean of two determinations vs Pierrel working standard (930 μg ml^{-1} assayed vs FDA reference standard).
[c] Mean of four determinations.
[d] Mean of five determinations.
[e] Aged samples.
[f] Mean of eight determinations.

fication of β-lactam antibiotics (Wilson et al 1974); it has been used quantitatively only for the determination of the diastereoisomer ratio in phenethicillin (Wilson et al 1977).

The methyl resonance* (125·6 Hz, 2·09 δ) of the dihydrothiazinyl moiety of cephalexin was chosen as the signal for its quantitative determination, because it is a sharp singlet, distant from other proton signals in the molecule, and is specific, being adequately separated from peaks of likely impurities and potential degradation products.

Thus the signal of the analogous methyl group of 7-ADCA, the starting material of the synthesis is at 136 Hz (2.26 δ), while the methyl peak of Δ^2-cephalexin, eventually present following a possible bond isomerization (Cocker et al 1966) by action of a base on its ester intermediate, occurs at 108 Hz (1·8 δ).

The degradation products do not interfere in the analysis as can be inferred from the comparison with other assays on the aged samples (see Table 1). All the chemical shifts were measured under the same conditions of solvent (D_2O) and acidity (DCl).

The internal standard method was used in all determinations (Kasler 1973). 3-Picoline was preferred to the 2-isomer because its methyl peak (150 Hz) was nearest to that of cephalexin.

Two samples of cephalexin were each analysed eight times by different operators on a period of days. For sample A the mean value was 87·1% ± 0·57 (c.v. 0·66), for sample B 87·3% ± 0·71 (c.v. 0·82). Water can be a satisfactory solvent in this analysis because its protons have no resonance in the region of analytical interest.

A sample analysed in deuterium oxide (with DCl) gave an assay figure (mean of 4 determinations) of 90·7% ± 0·62 (c.v. 0·68); in H_2O (with HCl) the corresponding figures were 90·9% ± 1·06 (c.v. 1·16).

Table 1 compares the results obtained by the n.m.r. method with those of the official microbiological assay and the iodometric assay.

Samples include drug substance, cephalexin sulphosalicylates (an intermediate), and cephalexin capsules.

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* Chemical shifts in Hz refer to an operative frequency of 60 MHz.

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