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NUCLEAR MAGNETIC RESONANCE ANALYSIS OF PHENYTOIN AND SODIUM PHENYTOIN IN SOLID DOSAGE FORMS

by

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ABSTRACT

A rapid and specific nuclear magnetic resonance (NMR) spectroscopic method was developed for determining phenytoin and its sodium salt in capsules and tablets. Acetamide was used as the internal standard and 0.5% sodium deuteroxide in deuterium oxide served as the NMR solvent. The concentration of drug per unit dose was calculated from the integration values for the resonance signals of phenytoin at about 7.40 ppm and of the internal standard at about 1.97 ppm. The average recovery value of phenytoin added to synthetic samples, in concentrations ranging from 84 to 122 mg, was 99.9 $\pm$ 0.2% (SD) with a coefficient of

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variation of 0.2%. The method using commercial products gave results comparable to those obtained by the titrimetric and gravimetric methods of USP XX. Excipients of tablets and capsules such as sucrose and lactose did not interfere with the determinations. The proposed method was found suitable for measuring the content uniformity of capsules and tablets, and offered a positive means of identification on phenytoin in these dosage forms.

INTRODUCTION

Phenytoin (5,5-diphenyl-2,4-imidazolidinedione) is the drug of choice for the prevention of convulsive seizures associated with epilepsy, particularly of those seizures unresponsive to bromide or barbiturate treatments (1).

Methods used for the assay of phenytoin in biological and pharmaceutical samples have included titrimetry (2), colorimetry (3), ultraviolet spectrophotometry (4-8), and thin-layer chromatography coupled to colorimetry (9). In the USP XX (10) the assay of phenytoin in dosage forms relies on a non-aqueous titration, whereas its sodium salt is measured gravimetrically. The compendial testing for content uniformity of these two drugs in capsules and tablets is done spectrophotometrically. All these methods are often non-specific, lengthy, and requiring extensive sample preparation. Gas chromatographic methods for phenytoin (11-20) are more specific but in all cases they require

an initial derivatization. A complicating feature of derivatization is that phenytoin can yield more than one derivative since its imidazolidinedione ring contains more than one reactive center (19). High-pressure liquid chromatography (HPLC) has also been used for the analysis of phenytoin (21-27), but although more direct than gas chromatography it still necessitates an extraction step and may not be conclusive enough for identification purposes.

The NMR method presented here simply involves the dissolution of the powdered sample in the spectroscopic solvent, addition of the internal standard, and recording of the spectra. One assay can be completed in less the 20 minutes. The proposed method is selective enough to permit the assay of phenytoin in the presence of certain excipients of tablets and capsules, and it possesses the required sensitivity for measuring the content uniformity of these dosage forms.

EXPERIMENTAL

Apparatus - All NMR spectra were recorded on a 90 MHz spectrometer[1] at an ambient probe temperature of 35°, using a sweep time of 5 minutes and a sweep width of 10 ppm. All chemical shifts are reported in parts per million (ppm) relative to DSS at 0 ppm.

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1. Varian Model EM-390, Varian Associates, Inc., Palo Alto, CA.

Chemicals and Samples - Phenytoin powder (working standard), sodium phenytoin (working standard), acetamide[2] (internal standard), sodium-2,2-dimethyl-2-silapentane sulfonate[3] (DSS, reference compound), deuterium oxide[4] (D_2O, + 99.7 atom % D isotopic purity, solvent), sodium deuteroxide[5] (NaOD, 40 wt. % solution in D_2O, + 99 atom % D isotopic purity, solvent), phenytoin tablets 50 mg, phenytoin capsules 100 mg, and sodium phenytoin capsules 100 mg were obtained from various commercial sources.

Assay of Tablets and Capsules - Weigh and finely powder not less than 20 tablets, or weigh and combine the contents of not less than 20 capsules. Weigh accurately a portion of powder equivalent to about 92 mg of phenytoin (or about 100 mg of sodium phenytoin) and transfer it to a glass-stoppered graduated 15 ml centrifuge tube. Add about 72 mg of acetamide, accurately weighed, and fill the tube to the 2 ml mark with 0.5% NaOD in D_2O. Effect solution by means of a vortex mixer, keeping the solution from touching the stopper, and centrifuge. Using a capillary pipet, transfer about 0.4 ml of the supernatant to an analytical NMR tube that contains a few crystals of DSS.

Place the tube in an NMR spectrometer and record the spectra

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2. Fisher Scientific Co., Fairlawn, NJ.
3. N.M.R. Specialties, Inc., New Kensington, PA.
4. Merck & Co., Inc., Rahway, NJ.
5. Aldrich Chemical Company, Inc., Milwaukee, WI.

using a spin rate that will produce no interfering spinning side bands between 1.3 and 2.6 ppm and between 6.6 and 8.0 ppm. Integrate the singlet at about 1.97 ppm and the singlet at about 7.40 ppm at least five times. Obtain the average height of the integral steps, and calculate the amount of phenytoin (or sodium phenytoin) per unit dose using the equation given below.

Calculations - The amount of phenytoin (as $C_{15}H_{12}N_2O_2$) or sodium phenytoin (as $C_{15}H_{11}N_2O_2Na$) per unit dose was obtained from

$$\text{mg/unit dose} = A_{ph}/A_{st} \times EW_{ph}/EW_{st} \times C \times U/S$$

where A_{ph} is the average height of the integral step for the aromatic protons of phenytoin absorbing at 7.40 ppm, A_{st} is the average height of the integral step for the methyl protons of acetamide absorbing at 1.97 ppm, EW_{ph} is the formula weight of phenytoin divided by the number of absorbing protons (252.3/10 = 25.23; sodium phenytoin 274.3/10 = 27.43), EW_{st} is the formula weight of acetamide divided by the number of absorbing protons (59.07/3 = 19.69), C is the weight of acetamide used in the assay, mg, U is the average weight of the unit dose, mg, and S is the weight of sample taken for the assay, mg.

RESULTS AND DISCUSSION

The preparation of the sample solution for NMR analysis was simplified by selecting solvents that readily dissolved both the analyte and the internal standard.

Although water promptly dissolved sodium phenytoin, this solvent cannot be used in NMR work because its strong resonance peaks will obscure the region where the phenyl protons of phenytoin resonate. No such problem was encountered using D_2O even though this solvent contains traces of HDO. A 0.5% solution of NaOD in D_2O was found a better NMR solvent for both phenytoin and sodium phenytoin since phenytoin is not very soluble in D_2O.

The use of acetamide as an internal standard offered several advantages. First, its methyl protons gave a strong singlet at about 1.97 ppm, away from the signals produced by phenytoin and by certain tablets and capsules excipients. Second, when it is mixed with phenytoin, the solubility of the latter in the NMR solvent is enhanced. Third, acetamide has a low proton equivalent weight. Furthermore, this compound remained stable in solution for at least 4 hours.

Figure 1 shows the 90 MHz spectra of phenytoin and of acetamide in 0.5% NaOD in D_2O. The ten phenyl protons of phenytoin absorb as a singlet at about 7.40 ppm. The two aromatic rings, one above and the other below the plane of the imidazolidinedione ring, appeared equivalent. Evidently, the heterocyclic ring is positioned flat on the NMR time scale and the fast inversion of the lone pair of electrons on the non-bridged nitrogen next to the sp^3 carbon causes the spin of the N-H proton to influence both aromatic rings equally.

It is also apparent that the various aromatic protons differ

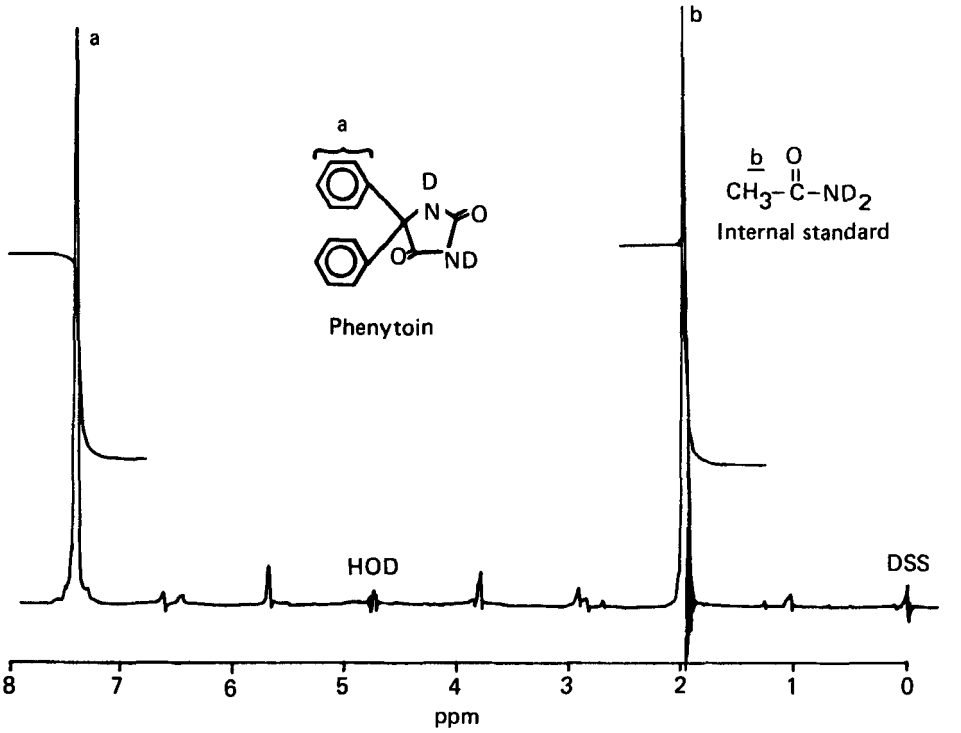

Figure 1. 90 MHz NMR spectra of (a) sodium phenytoin capsule and (b) acetamide, the internal standard. Solvent: 0.5% NaOD in D_2O. Reference compound: DSS.

little from each other. Thus, the signal at 7.40 ppm shows no splitting from the various spin-spin couplings and rather, it emerges as a singlet which, however, is considerably broader than the singlet of benzene.

Water-soluble excipients such as sucrose and lactose found in tablets and capsules, respectively, were easily recognized in the spectra by the signals of their anomeric protons and from the peak pattern for the non-hydroxylic protons, all of which appeared in the middle part of the spectra, well separated from

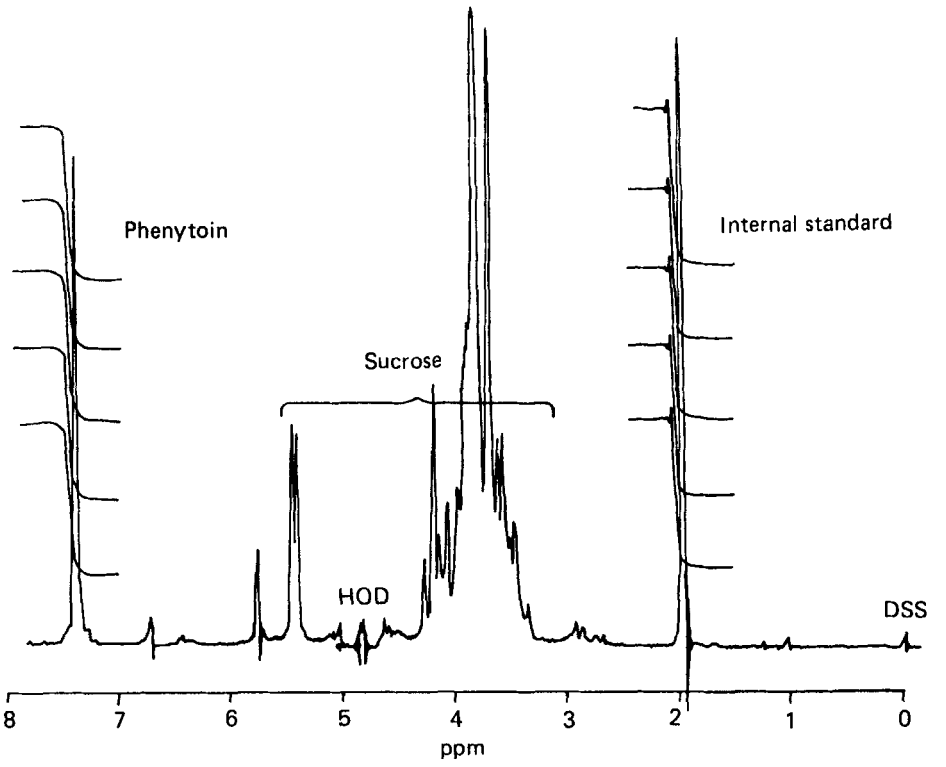

Figure 2. 90 MHz NMR spectra of (a) phenytoin tablet and (b) acetamide, the internal standard. The tablet contains sucrose as an excipient. Solvent: 0.5% NaOD in D_2O. Reference compound: DSS.

the peaks of phenytoin and acetamide (Figure 2). All remaining hydroxylic protons were exchangeable with the NMR solvent.

Table 1 summarizes the results of the NMR analysis of several mixtures of phenytoin or its sodium salt with various amounts of acetamide. For amounts of drug added ranging from 84.2 to 121.8 mg the average recovery value was 99.9 $\pm$ 0.2% (SD), with a corresponding CV of 0.2%. The accuracy of the determination was not significantly affected by varying the relative proportions of

Table 1 - Determination of Phenytoin and Sodium Phenytoin in Standard Mixtures by NMR.

Standard Mixture	Acetamide Added, mg[a]	Amount Added, mg	Amount Found, mg	Recovery %
1[b]	80.9	111.8	111.8	100.0
2[b]	80.9	99.0	99.2	100.2
3[b]	55.7	102.0	101.7	99.7
4[c]	89.2	99.1	99.0	99.9
5[c]	62.1	100.1	99.9	99.8
6[c]	68.5	84.0	84.2	100.2
7[c]	83.5	122.1	121.8	99.8
Av.				99.9
SD				0.2
CV, %				0.2
USP XX		500.0	500.5	100.1

[a]Internal standard.
[b]Sodium phenytoin.
[c]Phenytoin.

internal standard to phenytoin within the range shown in Table 1.

Table 2 summarizes the results of the analysis of various lots of commercial 50 mg tablets and 100 mg capsules by the NMR and compendial methods. Overall, the assay values by the NMR method differed by one percent or less from those obtained by the compendial method.

Table 2 - Determination of Phenytoin and Sodium Phenytoin in Commercial Dosage Forms by Proposed NMR and USP XX Methods.

	NMR Method		USP XX Method	
Sample	Amount Found, mg	Amount Found, %	Amount Found, mg	Amount Found, %
		Phenytoin tablets, 50 mg		
1	50.70	101.4	50.21	100.4
2	50.96	101.9	50.43	100.9
3	50.76	101.5	50.35	100.1
4	51.00	102.0	50.71	101.4
5	49.72	99.4	ND[a]	ND
6	50.86	101.7	ND	ND
7	50.87	101.7	ND	ND
		Phenytoin capsules, 100 mg		
1	102.9	102.9	101.9	101.9
2	102.0	102.0	101.2	101.2
3	102.2	102.2	102.0	102.0
4	102.1	102.2	102.0	102.0
5	101.5	101.5	ND	ND
6	102.5	102.5	ND	ND
7	102.1	102.1	ND	ND
8	100.0	100.0	ND	ND

[a]ND: not done.

Table 3 presents the results of the assay for content uniformity of sodium phenytoin capsules. Comparing these results with those for composite samples, it can be seen that the NMR method is well suited for this type of assay. An estimate of the good reproducibility of the NMR method can be gained from examining the series of integration traces seen in Figure 2.

Table 3 - Determination of Content Uniformity of Sodium Phenytoin Capsules by NMR.

Capsule, 100 mg	Found, % of declared
1	99.7
2	101.1
3	101.1
4	101.1
5	101.1
6	99.7
7	98.4
8	97.8
9	99.5
10	99.5
Av.	99.9
Range	97.8 - 101.1
Composite, 100% of declared	
1	100.0[a]
2	100.0[a]

[a]Average of two determinations

In summary, the NMR method described here is simple, rapid, and specific. It is sufficiently sensitive and rapid to be of utility in assaying individual tablets and capsules, and also can serve as an identification test for phenytoin.

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