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Quantitative analysis of β-adrenergic blocking agents by NMR spectroscopy

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Abstract: The quantitative analysis by ^1H NMR of labetalol, oxprenolol and four other β-adrenergic blocking agents is described. The method depends on the integration of selected resonances of the analyte and an internal reference which do not overlap. Procedures for the extraction of the analyte from tablets are given and corrections due to the NMR features of excipients outlined in some cases. The NMR method is reasonably precise and rapid, and the assay results compare favourably with those obtained by a UV procedure.

Keywords: *Quantitative ^1H NMR spectroscopy; β-adrenergic blocking agents.*

Introduction

During an analytical survey of antihypertensive drugs, conducted by our Institute, we were concerned with the analyses of many pharmaceuticals, generally formulated as tablets, containing β-adrenergic blocking agents (Table 1). Since all these drugs possess, in their molecular structure, methyl groups which resonate in the upfield region of ^1H NMR spectra and were well isolated from protons of a different nature, it seemed interesting to apply the NMR technique to the quantitation of these drugs, taking advantage of the fact that they were present in tablets at high dosage levels.

The technique of NMR spectroscopy has found general application and has become the method of choice for the elucidation of structure and stereochemistry of new compounds, but has received less attention for quantitative applications. However, it has been used increasingly for quantitative evaluation of active ingredients in pharmaceuticals after the large number of papers devoted to this technique by Turczan *et al.* of the U.S. Food and Drug Administration [1–3]. An NMR spectroscopic method was also reported for the determination of alprenolol and propranolol, both in mixture with isosorbide dinitrate [4]. Gas chromatographic and high-performance liquid chromatographic techniques were developed for atenolol and nadolol, respectively, and extended to the quantitation of other β-adrenergic blocking agents [5–6]. HPLC methods were also described for the analysis of oxprenolol in pharmaceuticals [7] and of propranolol [8].

The present paper describes a quantitative method for the evaluation of some β-adrenergic blocking agents (Table 1) by ^1H NMR spectroscopy, giving details of the

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Table 1
Proton groups chosen for quantitative analysis of β-adrenergic blocking agents in the presence of an internal standard*

Compound †	Structure	E.W. ‡	Internal standard	E.W. ‡	Solvent for NMR
Labetalol–HCl §	$C_6H_3(OH)(CONH_2)$–$CHOHCH_2NHCH(CH_3)CH_2CH_2$–$C_6H_5$	364.45/3	$C_6H_5CONHCH(CH_3)_2$	163.21/6	Pyridine
Oxprenolol–HCl	$CH_2{=}CHCH_2O$–C_6H_4–$OCH_2CHOHCH_2NHCH(CH_3)_2$	301.80/6	$C_6H_5NHCOCH_3$	135.16/3	Pyridine
Sotalol–HCl	CH_3SO_2NH–C_6H_4–$CHOHCH_2NHCH(CH_3)_2$	308.81/6	$CH_3NHCONH_2$	74.08/3	Pyridine
Atenolol	H_2NCOCH_2–C_6H_4–$OCH_2CHOHCH_2NHCH(CH_3)_2$	266.34/6	$C_6H_5NHCOCH_3$	135.16/3	Pyridine
Celiprolol–HCl	$(CH_3)_3CNHCH_2CHOH$–O–C_6H_3–$NHCON(CH_2CH_3)_2$	415.96/15	$CH_3COONa \cdot 3H_2O$	136.08/3	H_2O
Bevantolol–HCl	CH_3–C_6H_4–$OCH_2CHOHCH_2NHCH_2CH_2$–C_6H_4–OCH_3	381.90/3	CH_3–C_6H_4–COOH	136.14/3	$CHCl_3$ ‖

*Dotted lines indicated the protons selected for the analysis.
†Indicated in the form (base or salt) present in the tablets.
‡Equivalent weight = M.W./number of protons under integration.
§From four different manufacturers.
‖Chloroform of analytical grade was washed with water and dried over sodium sulphate to remove 0.75% ethanol used as stabilizer.

extraction procedures of the active ingredient from tablets, the proposed internal standards and the selected regions of the spectra for integration. All determinations were carried out in non-deuterated solvents.

Experimental

Extraction procedures of drugs from tablets

Labetalol–HCl, oxprenolol–HCl and sotalol–HCl. Ten tablets were weighed and finely powdered. A portion of powder, equivalent to 200 mg of labetalol–HCl, was accurately weighed into a 50 ml Erlenmeyer flask, 25 ml methanol was added and the mixture shaken for 30 min. After that, 11 ml of 0.05 methanolic solution of $AgNO_3$ (equivalent amount plus 1–2% excess for neutralization of the hydrochloric salt) was added, the mixture shaken for 2 min and then left standing for 10 min. After addition of *N*-isopropylbenzamide (*ca* 45 mg, accurately weighed) as internal standard, the suspension was filtered quantitatively under low pressure, through a pad of Celite, and the solution was taken to dryness on a rotavapor. The residue was dried in a vacuum oven (30°C, 2 h) and then dissolved in 4 ml of pyridine. About 0.5 ml of this solution was

transferred into a 5 mm NMR tube and the spectrum was measured. Four or more independent experiments were carried out for each pharmaceutical formulation. In a similar way, at least once, or in parallel if necessary, a sample was prepared without addition of internal standard to verify the baseline of the spectrum in the region of analytical interest for the internal standard.

This procedure was used to extract the following preparations: (a) labetalol–HCl (100 and 200 mg) tablets obtained from three different manufacturers and labetalol–HCl with indapamide (0.9 mg) from one manufacturer; (b) oxprenolol–HCl (160 mg) formulated with chlorthalidone (20 mg) as coated tablets. The amount of powder equivalent to the average weight of one tablet was used for extraction and acetanilide (*ca* 150 mg accurately weighed) was added. The final volume in pyridine was 3 ml; (c) sotalol–HCl (160 mg) formulated with xipamide (10 mg): the amount of powder equivalent to the average weight of one tablet was used for the extraction and *N*-Methylurea (*ca* 70 mg accurately weighed), as internal standard, was added. The final volume in pyridine was 3 ml.

Atenolol. The amount of powdered tablets, equivalent to 100 mg of atenolol (one tablet) was suspended in 25 ml of chloroform and stirred for 1 h. The suspension was quantitatively filtered under low vacuum through a pad of Celite after the addition of acetanilide (*ca* 100 mg accurately weighed). The solution was taken to dryness in a rotavapor and then placed in a vacuum oven (30°C, 2 h). The residue was dissolved in 1–2 ml of pyridine and 0.5 ml used for the spectrum.

Celiprolol–HCl and oxprenolol–HCl. An aliquot of powdered tablets corresponding to 200 mg of celiprolol–HCl and sodium acetate trihydrate (*ca* 300 mg accurately weighed), as internal standard, was placed in a glass-stoppered 10 ml centrifuge tube. After the addition of 5 ml distilled water, the suspension was centrifuged for 20 min. The clear supernatant (*ca* 0.5 ml), filtered if necessary, was used for the spectrum.

In a similar way a sample of powdered tablets was treated corresponding to 160 mg of oxprenolol–HCl to which sodium acetate trihydrate (*ca* 140 mg accurately weighed) was added before centrifugation. Each tablet contained oxprenolol–HCl (160 mg), and chlorthalidone (20 mg).

Bevantolol–HCl. The amount of powdered tablets corresponding to 500 mg of drug was suspended in 6–7 ml of chloroform and occasionally shaken. After 1 h, the suspension was filtered into a 10 ml volumetric flask and brought to volume after the addition of 160–170 mg (accurately weighed) of *p*-toluic acid as internal standard; 0.5 ml of this solution was used for the spectrum.

Sotalol–HCl calibration curve. The amount of powdered tablets corresponding to 160 mg of drug (one tablet) was placed in a glass-stoppered 10 ml centrifuge tube and 5 ml of water (accurately measured) was added. The sample was shaken for 5 min and centrifuged for 20 min. The clear supernatant (*ca* 0.5 ml) was used for the spectrum. Three aqueous solutions of pure sotalol–HCl, whose concentration ranged between 24 and 40 mg ml^{-1}, were used for the calibration curve. Each spectrum was run with the instrument set at the same parameters and consecutively. Integrals were measured at different integral amplitudes and the best-fitted line [integral height (mm) vs concentration (mg ml^{-1})] was used as the standard curve: $y = 0.33x$; $r = 1.00$.

Materials

All reference substances were of analytical grade purity. When chloroform, containing 0.75% ethanol as stabilizer, was used for recording the spectra, it was washed five times with water (5 × 5 ml for 100 ml of chloroform), and then dried over sodium sulphate. In this condition, the NMR spectrum of the solvent alone (spectrum amplitude 50, RFP 0.05) did not show the presence of ethanol.

NMR spectra

A 60 MHz spectrometer (Varian T-60) was used. The spectra were recorded in the established solvents (Table 1) in 5 mm tubes. All the precautions to assure accuracy of integration were taken, including high signal-to-noise ratio, correct phasing, adjustment of magnetic field homogeneity and spin rate so that spinning side bands were minimized in the operating region and might be neglected [9, 10]. The analytical signals were integrated four times and the values averaged. This operation was also repeated at different integral amplitudes.

In pyridine and chloroform the δ scale was referenced to TMS, in water to DSS, adjusted to 0 ppm. Once the chemical shifts of the protons of each drug had been calibrated, there was no need to add TMS or DSS to all the solutions under examination.

The selected internal reference substances were solids, soluble in the operative solvents, and with analytical signals in a region free from other signals.

The spectrum of a mixture of pure drug and internal standard was always compared with that of tablet-derived material to observe the interference of extracted excipients on the signals of analytical interest.

The amount of each active ingredient was calculated as follows:

$$\frac{\text{mg X}}{\text{tablet}} = \frac{I_X}{I_{std}} \cdot \frac{EW_X}{EW_{std}} \cdot \frac{\text{mg std}}{\text{mg powder}} \cdot \text{average tablet weight}$$

where I_X and I_{std} were the integral value of the signal (or signals) representing X (drug) and the internal standard, respectively; EW_X and EW_{std} were the M.W. divided by the number of protons under integration of the drug and the standard, respectively.

UV spectroscopy

Absorbance values were measured in 1 cm silica quartz cells, using a Perkin–Elmer 402 UV-visible recording spectrophotometer.

The amount of powdered tablets, equivalent to 100 mg of labetalol–HCl, was transferred to a 50 ml volumetric flask. After the addition of water (25 ml), the flask was shaken for 30 min and diluted with water to 50 ml. The solution was filtered and, after discarding the first 10 ml of filtrate, was diluted to obtain two concentrations of 4 mg% and 8 mg% (w/v) whose absorbance was measured (λ_{max} 305 nm). Solutions of pure labetalol–HCl in water, containing from 2 to 12 mg% (w/v), were used to construct a calibration curve: $y = 0.08x$; $n = 7$; $r = 1.00$. Bevantolol–HCl was extracted from tablets in a similar way. The calibration curve, obtained by plotting the absorbance at 280 nm against concentration (2–10 mg%, w/v) was linear: $y = 0.04 + 0.08x$; $n = 5$; $r = 1.00$. Atenolol was extracted from tablets with methanol (the amount of one tablet corresponding to 100 mg of drug in 50 ml of methanol). The absorbances of methanolic solutions containing 6, 8 and 10 mg% (w/v) were measured. The calibration curve,

obtained by plotting the absorbance at 278 nm against concentration (4–15 mg%, w/v) was linear: $y = 0.02 + 0.05x$; $n = 5$; $r = 1.00$.

Least-squares regression analysis was used to determine the intercept, the slope and the correlation coefficient for each calibration curve.

An aliquot of powdered tablets corresponding to 160 mg of oxprenolol–HCl and 20 mg of chlorthalidone (one tablet) was transferred to a 100 ml volumetric flask, and 10 ml of methanol and 10 ml of 0.1 N HCl were added. The suspension was heated at 50–60°C for 20 min and after cooling, the solution was made up to volume with 0.1 N HCl. An aliquot of 5 ml of clear solution was then diluted to 100 ml with 0.1 N HCl to obtain a solution containing 8 mg% of oxprenolol–HCl and 1 mg% of chlorthalidone. Two standard solutions containing, separately, oxprenolol–HCl and chlorthalidone at the same concentration (8 mg% and 1 mg%, w/v, respectively) were prepared and the UV spectrum recorded from 190 to 300 nm. The amount of oxprenolol–HCl (mg/tablet) is given by:

$$\frac{\mathrm{Abs}_{276}\,\mathrm{Oxpr(tablet)} - \mathrm{Abs}_{276}\mathrm{Chlort(std)}}{\mathrm{Abs}_{276}\mathrm{Oxpr(std)}} \cdot 160$$

At 276 nm the absorbance of a 1 mg% solution of chlorthalidone was 0.05.

Results and Discussion

Many combined factors, such as a suitable extraction procedure together with a good choice of integration signal, internal standard and solvent for the NMR measurement, can help in minimizing the interference due to the excipient material occasionally extracted with the active ingredient.

The extraction of labetalol–HCl from tablets with chloroform, after alkalinization with ammonia, or directly as hydrochloride with water, gave poor results due to the low solubility of the base in chloroform and of the hydrochloride in water. The reported extraction procedure with methanol and neutralization of the hydrochloride with methanolic silver nitrate was very satisfactory. The spectrum of labetalol in pyridine revealed (Fig. 1a) a small peak at δ 1.25, due to the excipient (probably magnesium stearate) solubilized by methanol. This peak occurred in the region of the isopropylic methyl doublet of *N*-isopropylbenzamide used as internal standard (Fig. 1b). A correction can be made on the integral value of the latter signal by subtracting the integral value of the peak at δ 1.25 evident in the spectrum without internal standard.

Oxprenolol–HCl was also extracted from tablets with methanol–silver nitrate, and the spectrum recorded in pyridine with acetanilide as internal standard. In this condition, the peak at δ 1.25, due to the excipient, appeared near the upfield peak of the analytical doublet of oxprenolol (Fig. 2b). The integration of the analytical signals can be calculated, but the presence of this interference made the integration less accurate. To overcome this difficulty, oxprenolol–HCl was also extracted from tablets directly as the hydrochloride. The spectrum in water, using sodium acetate trihydrate as internal standard, is shown in Fig. 2a.

Sotalol–HCl can be extracted quantitatively from tablets with water (the amount of powder corresponding to one tablet in 5 ml of water with the addition of *ca* 140 mg of accurately weighed sodium acetate trihydrate). After centrifugation, 0.5 ml of clear solution (filtered if necessary) was used for the spectrum. The strong water signal at δ

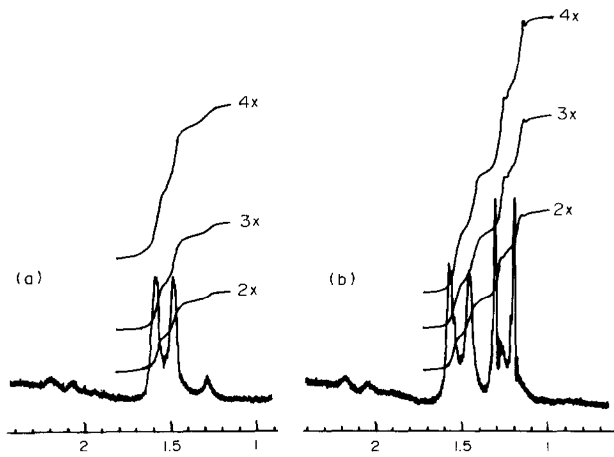

Figure 1
NMR signals used for quantitation of labetalol–HCl from tablets: (a) without, (b) with *N*-isopropylbenzamide (δ 1.28), as reference compound, in pyridine.

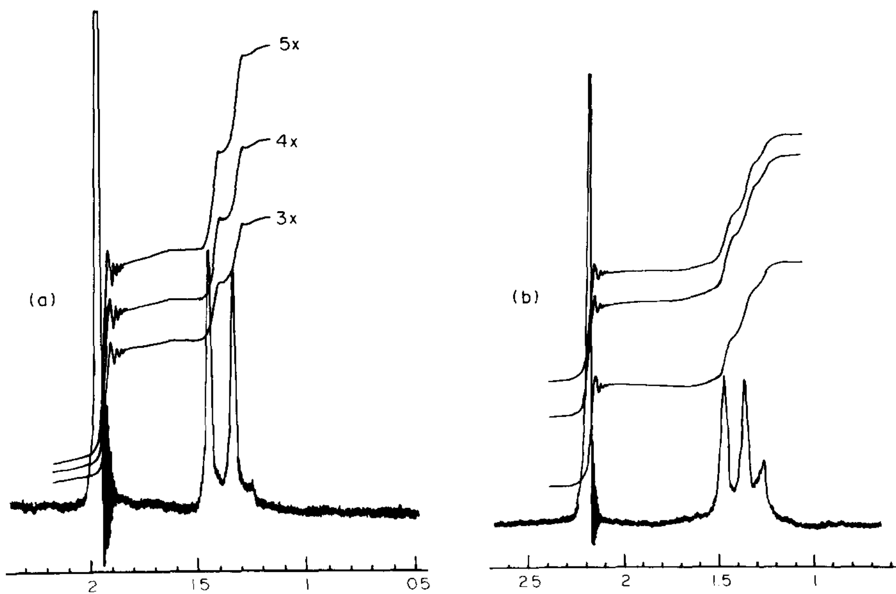

Figure 2
NMR signals used for quantitation of oxprenolol–HCl from tablets: (a) in the presence of sodium acetate trihydrate (δ 1.92), as reference compound, in water; (b) in the presence of acetanilide (δ 2.20), as reference compound, in pyridine; at δ 1.25 there is a strong peak due to the excipient.

4.6, with its spinning side bands, did not interfere in the upfield region of analytical interest, but the base line showed a slope in the region of the internal standard, so that accurate integration became impossible. By substituting sodium acetate with DSS (2,2-dimethyl-2-silapentane-5-sulfonic acid sodium salt), another inconvenience occurred since, at the concentration used (*ca* 75 mg of DSS for 160 mg of sotalol–HCl), the absorption of the methylene protons β to the sulfonyl group was visible in the region of sotalol integration. By using *N*-methylurea, as internal standard, and the extraction procedure described in the Experimental section, reproducible and accurate results were obtained (Fig. 3).

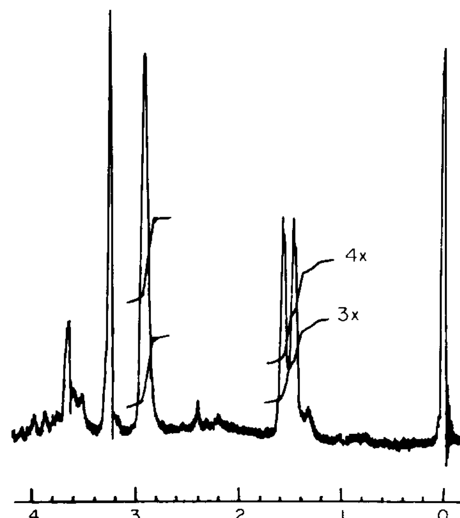

Figure 3
NMR signals used for quantitation of sotalol–HCl from tablets in the presence of *N*-methylurea (δ 2.93), as reference compound, in pyridine.

No difficulties were met in the quantitation of atenolol (Fig. 4), celiprolol–HCl (Fig. 5) and bevantolol–HCl (Fig. 6) with the extraction procedure, internal standard and solvent for spectra as reported in the Experimental section and in Table 1.

The analytical data obtained by the NMR determinations are listed in Table 2 and many of them are compared with data obtained by UV spectroscopy. Indapamide, chlorthalidone and xipamide, when present, did not interfere in the integration region of labetalol, oxprenolol and sotalol, respectively. Per cent standard deviations ranged from 1.0 to 4.15 and are generally larger than those found in the UV method.

The presented data allow some considerations to be made. In the case of oxprenolol, by using a more appropriate internal standard, namely, sodium acetate, the S.D. decreased; The mean % value and its S.D. were in good agreement with values employing UV spectroscopy. In the case of sotalol, the lowest S.D. was obtained using *N*-methylurea as internal standard. With DSS as internal standard, the integral value of the analytical signal of sotalol experienced the contribution of one methylene group ($-CH_2$ β to sulfonyl) of DSS resonating in the same region. As a consequence, the % found increased 1.1% over that result employing *N*-methylurea as internal standard. The sotalol concentration in tablets was also determined without an internal standard, by using a calibration curve obtained by plotting integral heights against three concen-

Figure 4
NMR signals used for quantitation of atenolol from tablets in the presence of acetanilide (δ 2.20), as reference compound, in pyridine.

Figure 5
NMR signals used for quantitation of celiprolol–HCl from tablets in the presence of sodium acetate trihydrate (δ 1.92), as reference compound, in water.

Table 2
Determination of β-adrenergic blocking agents in commercial tablets by ^{1}H NMR

Drug	mg/tablet declared	Internal standard	n*	mg/tablet (S.D.) found	% found (S.D.)	UV method % found (S.D.)
Labetalol–HCl (I)	200	*N*-Isopropylbenzamide	7	207.3 ± 2.7	103.6 ± 1.35	96.0 ± 2.0†
Labetalol–HCl (II)	200	*N*-Isopropylbenzamide	8	202.0 ± 6.3	101.0 ± 3.25	101.15 ± 1.5
Labetalol–HCl (III)	100	*N*-Isopropylbenzamide	9		99.95 ± 4.15	100.2 ± 3.7
Labetalol–HCl (IV)‡	100	*N*-Isopropylbenzamide	6		100.3 ± 1.1	100.1 ± 08
Oxprenolol–HCl§	160	Sodium acetate.3 H_2O	8	163.4 ± 2.1	102.1 ± 1.0	102.3 ± 1.2‖
Oxprenolol–HCl§	160	Acetanilide	8	165.1 ± 2.7	103.2 ± 2.0	
Sotalol–HCl¶	160	*N*-Methylurea	6	167.7 ± 4.95	104.8 ± 3.1	—
Sotalol–HCl¶	160	DSS	6	169.5 ± 6.8	105.9 ± 4.3	
Sotalol–HCl¶	160	External standard	6	167.0 ± 8.5	104.4 ± 5.1	
Atenolol	100	Acetanilide	5		98.2 ± 0.5	104.1 ± 3.8**
Celiprolol–HCl	200	Sodium acetate.3 H_2O	4	206.4 ± 5.1	103.2 ± 2.55	—
Celiprolol–HCl	400	Sodium acetate.3 H_2O	4	398.1 ± 9.9	99.5 ± 2.5	—
Bevantolol–HCl	100	*p*-Toluic acid	4		101.7 ± 3.7	99.0 ± 3.2††
Bevantolol–HCl	200	*p*-Toluic acid	4	193.6 ± 1.9	96.8 ± 0.95	92.9 ± 1.05

*Number of independent experiments.
†At 305 nm.
‡Plus indapamide, 0.9 mg.
§Plus chlorthalidone, 20 mg.
‖At 276 nm.
¶Plus xipamide, 10 mg.
**At 278 nm.
††At 280 nm.

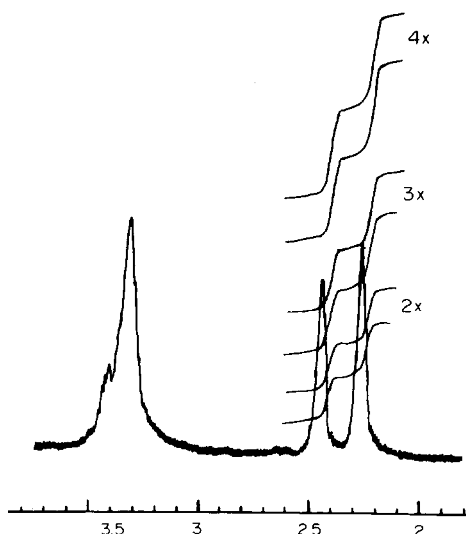

Figure 6
NMR signals used for quantitation of bevantolol–HCl from tablets in the presence of *p*-toluic acid (δ 2.45), as reference compound, in chloroform.

trations of sotalol–HCl, one corresponding to the declared amount of drug/tablet in 5 ml of water and the other two to ±20% of the theoretical value. The mean % value agreed well with that found with the same extraction procedure and internal standard; the S.D., however, was twice as large (Table 2). The greater variation in measurements emphasized the importance of a good internal standard for the precision of the method.

In conclusion, the results obtained showed that the NMR technique can be applied to the quantitation of many β-blocking agents from tablets. The method, as found previously, is reasonably precise and rapid and can compete with other analytical techniques. Furthermore, the use of non-deuterated solvents in this investigation, makes this method an economical one. Better precision and accuracy of measurements should be obtained with NMR spectrometers of higher resolution.

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