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Utilizing nuclear magnetic resonance (NMR) spectroscopy for assessing nadolol racemate composition

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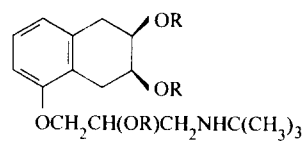
Abstract: NMR methods were developed for the determination of the racemate composition in nadolol raw materials. With high-field instruments (400 MHz or greater) the racemate ratio may be determined by the relative heights of the *t*-butyl peaks, which are well enough resolved for this determination. For lower field spectrometers, the *t*-butyl peaks are not resolved. An NMR method has been developed which involves preparation of the tribenzoate derivative of the drug. Seven lots of nadolol raw material, as well as several standards, were analysed for their racemate content. Three lots of raw material did not meet the USP limits of 40–60% for racemate A. Of these, two were granular in appearance and were found to vary markedly in racemate composition in successive analyses. The results for all the materials of uniform content agree very well with those from the HPLC method, as well as for the USP IR method using the absorbance at the corrected wavelength.

Keywords: *Nadolol; nadolol tribenzoate; ^{1}H-NMR; racemate composition.*

Introduction

There has been a great deal of interest during the last few years in the determination of the isomeric composition of drugs utilizing a variety of techniques. Recently, a problem was encountered in our laboratories in the determination of the racemate composition of nadolol raw materials. When analyses by the USP method using infrared spectroscopy and an HPLC method gave different results [1], NMR methods were developed to determine the actual racemate compositions.

Nadolol, *cis*-5-{3-[1,1-dimethylethyl)-amino]-2-hydroxypropoxy}-1,2,3,4-tetrahydro-2,3-naphthalenediol, is a β-adrenergic receptor blocking agent (β-blocker) which is used in the treatment of angina pectoris and hypertension. There are three chiral carbon centres in its structure. However, because the ring hydroxyls are in the *cis*-configuration, only four enantiomers are possible. These may be viewed as a mixture of two racemic diastereomers in which the +−/−+ (RS/SR) mixture is designated racemate A and the ++/−− (RR/SS) mixture racemate B (Fig. 1). These racemates have been reported to be readily separated by fractional crystallization. Racemate B has been shown to possess three times more β-blocking and antiarrhythmic activity than racemate A. Due to this difference in activity the USP [2] has set limits



R = H - nadolol

Optical Rotation		Racemate
Centre 1	Centre 2	
+	+	B
−	−	B
+	−	A
−	+	A

R = C(O)C$_6$H$_5$ - nadolol tribenzoate

Figure 1
Structures for nadolol and nadolol tribenzoate.

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for the racemate composition of the drug material to be 40–60% racemate A. A number of publications have discussed various methods for the quantitation of the racemates by chromatographic techiques [1, 3–5], infrared (IR) spectroscopy [1, 6], powder X-ray diffraction [6] and by nuclear magnetic resonance (NMR) spectroscopy [6]. The NMR method is based on the chemical shift difference of the *t*-butyl groups of the tetrabenzoate derivatives for the two racemates. In this report we present results from NMR studies which provide two different methods for determining the racemic composition of nadolol. The method of choice is determined by the field strength of the NMR spectrometer available to the user, as discussed below.

There are a number of potential NMR methods for determination of the racemate composition of a drug such as nadolol. Besides the direct measurement of heights or areas for peaks arising from the different racemates, alternative methods include the use of lanthanide shift reagents or solvating agents and the formation of suitable derivatives of the nadolol alcohol and/or amine groups. The results of this study for nadolol racemate composition are reported.

Experimental

Equipment and supplies

^{1}H-NMR spectra were recorded on either a Bruker AM400 (400.13 MHz) or a Bruker AC-F200 (200.13 MHz) spectrometer. Nadolol raw materials were obtained from the manufacturers or were submitted to the Branch in connection with New Drug Submissions. Racemates A and B were provided by E.R. Squibb & Sons Inc. (Princetown, NJ, USA).

Methods

Direct determination. Proton spectra of nadolol raw materials at 400.13 MHz were obtained with 32K data points and a sweep width of 5618 Hz. The data was zero-filled to 64K before processing, using a line-broadening of −1.5 and a Gaussian window-function equal to the spectral resolution (Hz/point). The ratio of the peak heights for the *t*-butyl resonance (at 1.1–1.3 ppm depending on drug concentration) then gives a measure of the racemate A content — [height of the high-field peak (*A*)/(sum of the heights)]. As the concentration of the drug is increased, the *t*-butyl signal gradually moves to higher field. This is illustrated in Fig. 2, where the USP sample is at a lower concentration (ca. 0.4 mg/0.5 ml) than the Y1 sample (1.1 mg/0.5 ml). However, there are no resonances from any other species present which would interfere with the measurements on the *t*-butyl signals.

Derivatization. The tribenzoate derivative of nadolol was prepared in the NMR tube by adding the drug (2 mg), 4-dimethylaminopyridine (12 mg) and benzoic anhydride (22 mg) to 0.5 ml $CDCl_3$ and allowing the tube to stand at room temperature for 2 h. The proton spectra were then acquired and processed as above. Completeness of the reaction may be checked by simply looking for resonances from the drug starting material. Once again the racemate A composition is determined from the heights of the *t*-butyl peaks (1.13 ppm). In this case, the lower field resonance arises from racemate A (Fig. 3).

Results and Discussion

The various alternative methods for racemate composition described above were evaluataed. For high field spectrometers, direct measurement of peak heights is obviously the preferred method. When the use of solvating agents was investigated, it was found that non-chiral reagents did not produce the desired separation of the racemates. On the other hand, chiral solvating agents gave three separate signals, instead of the expected four. One signal was due to a combination of two *t*-butyl resonances, with one isomer from each racemate. The other two signals could thus be used to determine the ratio of the racemates. This procedure would use only one-half the *t*-butyl signal for the drug and would, therefore, have less sensitivity than the other methods. It was also found that better separation of *t*-butyl signals could be obtained from the benzoylated drug. Thus for lower field strength spectrometers, derivatization to the tribenzoate analogs of the drug racemates was the method of choice. Differentiation of NMR signals for the *t*-butyl groups of the tribenzoate diastereomers was sufficient for quantitation on a spectrometer operating at 200.13 MHz.

Direct measurement

When the spectrum of nadolol is recorded in

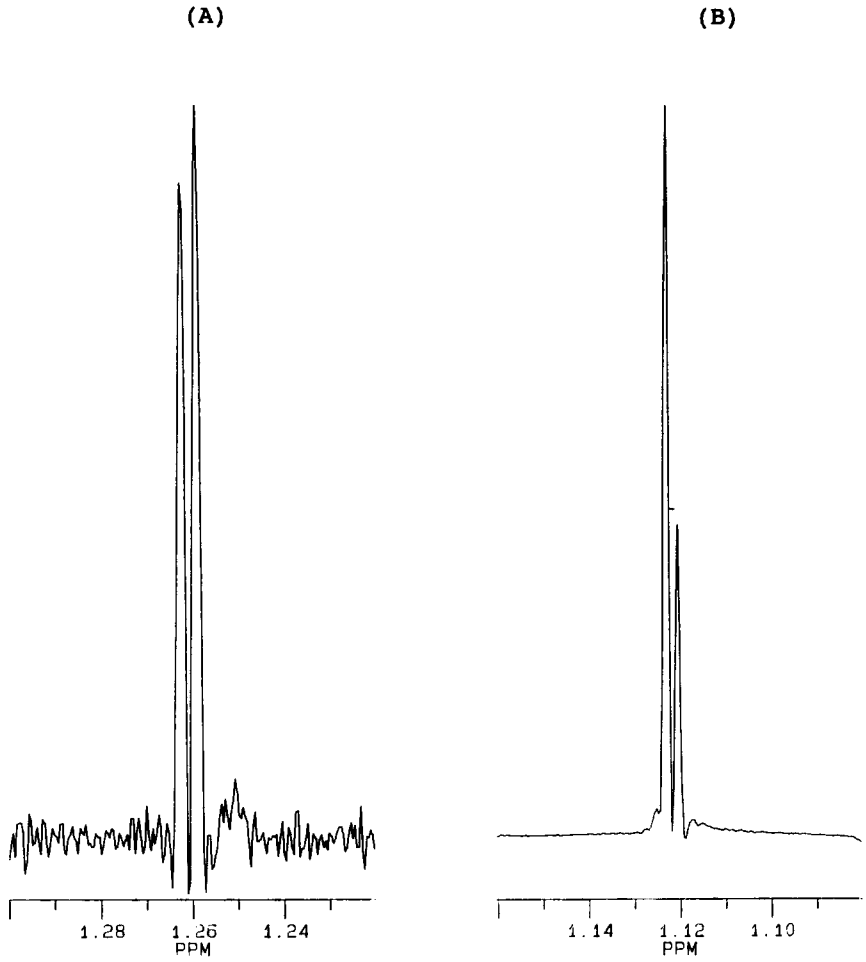

Figure 2
Spectra at 400 MHz of the *t*-butyl group for nadolol. (a) USP Lot F-1; (b) Lot Y1.

deuterochloroform at 400 MHz (and presumably at higher fields) the signals for the *t*-butyl groups for the two racemates give rise to separate resonances (Fig. 2). Thus, if a high field instrument is available, simply recording the spectrum and measuring the relative peak heights will give the ratio of the racemates directly.

However, when the ^{1}H-NMR spectrum of nadolol is recorded at 200 MHz (or less), the signals for the *t*-tutyl protons for both racemates appear as a single peak. If only lower field spectrometers are available, the method described below may be used to determine the racemate content.

Derivatization

It was previously reported [6] that the *t*-butyl groups for the nadolol tetrabenzoate racemates gave well separated resonances. However, these workers did not report their method for preparing the tetrabenzoate derivative. Attempts to prepare the tetrabenzoate by use of the usual benzoylating agents led to the formation of a tribenzoate derivative (Fig. 1), (as evidenced by mass spectral characterization of the product). Fortunately, the resonances for the *t*-butyl groups for the tribenzoate racemates were also sufficiently well separated (even on lower field spectrometers) to be used for the determination of the nadolol racemate ratio.

This derivatization procedure appears to be the method of choice for lower field instruments, giving excellent separation of signal resonances for the *t*-butyl groups of nadolol racemates A and B (Fig. 3). Furthermore, the reagents required are cheap, readily available and easy to work with since they are stable solids.

In order to verify the linearity of the peak height responses, a calibration curve was created. Pure racemate A was available for this experiment. However, sufficient racemate B

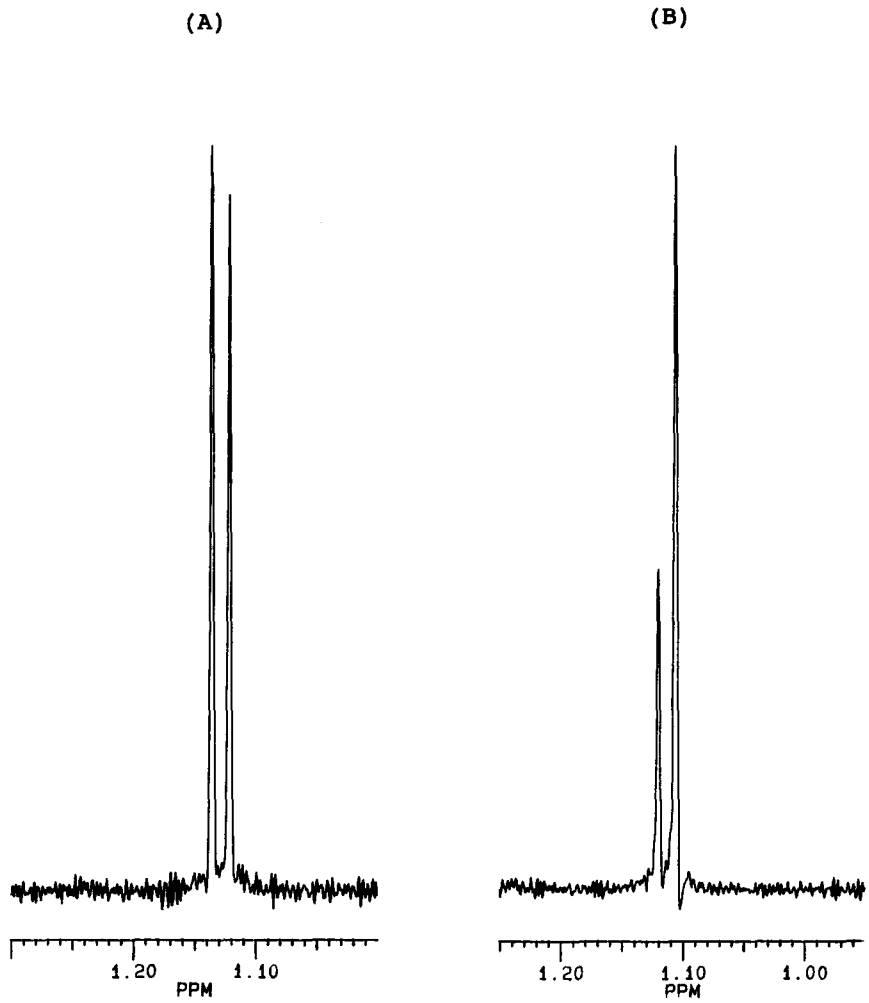

Figure 3
^{1}H-NMR at 200 MHz of the *t*-butyl group for the tribenzoate derivative of nadolol. (a) USP Lot F-1; (b) Lot Y1.

was not available, and when tested was found to contain approximately 15% racemate A. Thus it was decided to use one of the raw materials — LOT Y1 — for which the racemate A content was the lowest for all available raw materials (30%). Excellent agreement between calculated and measured racemate ratios was found using both peak heights (r = 0.999) and integrals (r = 0.998) over the range of 30–100% racemate A. These calculations were performed assuming the racemate A was pure (no B detected) and that the raw material contained 30% racemate A. The linear responses, with slopes of 1.0 and intercepts close to zero, obtained in this study confirm that either peak heights or peak areas may be used to determine the relative racemate concentrations in nadolol raw materials.

The precision of the method was determined using lot X3. Six replicate analyses on 1 day gave a result of 46.6% racemate A (SD = 0.4, RSD = 0.8%) and for duplicate analyses on six other days 46.2% (SD = 0.24, RSD = 0.5%). The intra-day precision was also determined for lot Y1. The results (30.1% racemate A, SD = 0.27, RSD = 0.9%), show excellent precision for a racemate A composition which is 10% lower than the range allowed in the USP.

The ruggedness of the method was tested by varying the concentrations of the drug and reagents, changing the time for analysis after mixing the reagents, etc. It was found that varying the concentrations of the drug or reagents did not change the analytical result, as long as the molar amounts of reagents were in excess of that of the drug. If the solutions were allowed to stand over night at room temperature, after the benzoylating agents had been added, some decomposition occurred. Thus, although it is necessary to wait at least 2 h after

adding these reagents in order to ensure complete reaction, the samples must be analysed on the same day. There was no detectable decomposition of the tribenzoate 8 h after addition of the reagents. The chloroform solution of nadolol by itself was found to be stable for at least 3 days. In order to verify that the method also works at different field-strengths, one lot of material was analysed for its intra-day precision. Six assays of Lot X3 gave 44.6% racemate A (SD = 0.21, RSD = 0.5%). This result indicates that this lot of raw material is also non-uniform in content since it does not agree with the result for a different aliquot obtained on the other instrument. In order to verify that there is no discrepancy between results obtained for the two spectrometers, three samples of another lot of material were analysed on both machines. The ratios obtained for lot X2 were virtually the same (AC200 51.7, 51.7, 51.5 and AM400 52.0, 51.7, 51.6).

Racemate A content in nadolol raw materials

Excellent agreement was obtained for the racemate A content between both of the NMR methods, and those from the IR and HPLC methods previously reported by other workers in our laboratories [1]. The results from the NMR study are given in Table 1. From the results, it can be seen that three lots of raw material fail to meet the USP racemate limit criterion. Lot Y1 has only 30% of racemate A. The other two which fail (lots X4 and X5) were granular materials and it was found that the racemate composition varied greatly in successive analyses. It should be noted that NMR provides an excellent method for determination of the homogeneity of these raw materials. Other methods, which require larger amounts of drug, would tend to give an "averaged" racemate content. Except for the two lots from company X, all raw materials appeared to be uniform in their racemate content (although Lot X3 showed some minor deviation from uniformity, as previously discussed).

Table 1
Racemate composition of nadolol raw materials*

Source	Lot number	% Racemate A
Squibb	02-770-508076	50
X	1	48
X	2	52
X	3	46
X	4	30–77†
X	5	25–35†
Y	1	30
Y	2	52
USP	F-1	52

* Results obtained for the tribenzoate derivatives on the Bruker AC200.

† Granular material with racemate composition varying between successive analyses.

Conclusions

The results in this study show the usefulness of NMR spectroscopy as a method for the determination of the racemate composition of nadolol. NMR methods such as those described here may be used for the validation of other methodology or it can be used as a stand-alone technique. NMR offers some advantages over other methods currently being used. It is fast and accurate. Usually a method may be developed within a few days and generally will have sufficient precision, accuracy and ruggedness to meet Pharmacopoeial requirements. NMR only requires either a non-racemic standard of known composition, or a racemic standard and a small amount of one optically pure isomer, for the original method development. After the method has been developed in one laboratory, it can be applied anywhere with no need for standards. Another major advantage of NMR is that it is non-specific (i.e. signals are observed for all proton bearing species present, not just for the drug being studied). If an impurity contains protons which are not overlapped with those of the drug, it can usually be detected down to levels of approximately 0.1% of the drug. Thus, NMR may detect impurities which would be missed by other techniques. For example, impurities may be missed in HPLC methods if they contain no UV chromophore, have very weak absorbance at the monitored wavelengths or have the same retention time as the drug or another known impurity. The main disadvantage of NMR spectroscopy is that it is currently not available in all laboratories.

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