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Improved solid-state NMR quantifications of active principles in pharmaceutical formulations

Stéphanie Sanchez [a], Fabio Ziarelli [b,*], Stéphane Viel [a], Corinne Delaurent [a], Stefano Caldarelli [a]

[a] Aix-Marseille Université, ISM2-UMR-6263, Equipe CES, case 512, av. Escadrille Normandie Niémen, 13397 Marseille Cedex 20, France
[b] Aix-Marseille Université, Fédération des Sciences Chimiques de Marseille, Spectropole (CNRS-FR1739), case 511, av. Escadrille Normandie Niémen, 13397 Marseille Cedex 20, France

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ABSTRACT

The facility of implementation reached by solid-state nuclear magnetic resonance (ssNMR) spectroscopy makes this technique increasingly popular in pharmaceutical sciences, and more specifically for the dosage of active principles in pharmaceutical formulations, since about 80% of the formulations currently available on the market are present in the solid form. In this case, analysis by MAS NMR allows faster and simplified protocols, as a solubilization step is not required. However, the specificity of the ssNMR experiments should be explicitly taken into account when designing an accurate measurement procedure. In this work we show that, by using a combination of external concentration referencing and a properly designed sample preparation optimized for quantitative determinations, quantification of active principles in pharmaceutical formulations can be performed with both speed and precision. The method is illustrated by reinvestigating the dosage of Meprobamate, an anxiolytic agent typically prescribed in case of anxiety or muscular soreness, present in a commercial formulation (Equanil®). Specifically, with respect to previously proposed analytical protocols, the procedure outlined here allows fast quantification with excellent precision.

1. Introduction

Solid-state nuclear magnetic resonance (ssNMR) has recently attracted growing interest for the characterization of pharmaceutical compounds in the solid state [1,2]. This is primarily due to considerable improvements in the instrumentation and to the fact that about 80% of the drugs currently available on the market are present in the solid form. ssNMR allows not only the chemical structure [3] but also the physical properties (polymorphism, multiple molecules per asymmetric unit cell, disorder) of the pharmaceutical substances to be investigated [4,5]. These properties are of the utmost importance because they influence not only the solubility but also the biocompatibility and physico-chemical stability of the drugs.

In parallel, many studies have reported on the use of NMR for quantitative analyses [6,7], showing that NMR is a very linear and robust technique that allows both for precise and accurate quantifications [7,8]. In this context, the use of ssNMR for quantification bears many advantages [9], as the technique is fully non-destructive and requires no preliminary sample manipulations [10]. In addition, the analysis is performed on the bulk on significant amounts of sample, which allows the effects of possible heterogeneities to be averaged out.

A few ssNMR quantitative studies have already been reported [11], devoted for instance to the relative quantification of various crystalline or amorphous forms [9,12], or to the quantification of active principles in pharmaceutical formulations [11,13,14]. In this latter case, the methods described in the literature have almost exclusively relied on the use of an internal standard.

The use of an internal standard reduces the effects of instrumental instabilities and allows direct quantifications to be achieved [15–17]. The most significant drawbacks are the difficulty of preparing homogeneous mixtures of sample and standard, an especially critical step in the solid state, and the reduction in sensitivity caused by sample dilution. This point is especially important for NMR because of its relatively low sensitivity with respect to other analytical techniques.

Recently, we have shown that it is possible to increase the sensitivity, and hence the precision, of ssNMR quantifications based on the external standard method, by using a rotor packing optimized specifically for quantitative determinations [18].

In this work, we will show an application of this method for the precise quantification of active principles in pharmaceutical formulations. As an illustration, we chose to work on Meprobamate

* Corresponding author. Fax: +33 491 282 897.
E-mail address: fabio.ziarelli@univ-cezanne.fr (F. Ziarelli).

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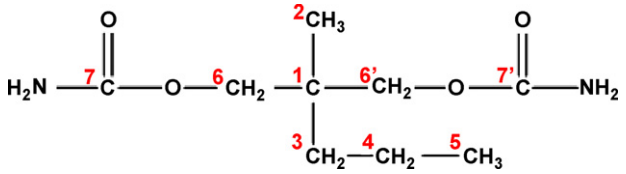

Fig. 1. Molecular structure and atom numbering of Meprobamate ([2-(carbamoyloxymethyl)-2-methyl-pentyl] carbamate).

(Fig. 1), an anxiolytic agent prescribed in case of anxiety or reflexive muscular contractions, by reinvestigating its dosage in commercial formulations; in fact, Meprobamate is commercially available as a solid formulation (Equanil®) containing other excipients such as starch, talc, magnesium stearate, etc.

While a few distinct analytical protocols have indeed been proposed for the dosage of Meprobamate, based either on chromatographic [19] or spectroscopic techniques [15], a trusted method has not been singled out. The sole official suggestion is to be found in the United States Pharmacopeia monograph which recommends the use of reversed phase liquid chromatography at 200 nm to assay Meprobamate in tablets [20]. As a matter of fact, typical chromatographic methods cannot be used in this case because this aliphatic compound does not exhibit any useful UV absorption. On the other hand, gas chromatographic methods are faced with the difficulty of thermal degradation as well as other experimental artifacts (peak tailing), which dramatically reduce the detection limit and decrease the analytical reliability.

In any case, the main drawback of most previously reported methods is the required sample manipulations (*e.g.* solubilization, extraction, derivatization, etc.) which, in addition to being both time- and reagent-consuming, may potentially introduce additional sources of errors.

In contrast, the method proposed here does not require any sample treatment. Overall, the proposed method may routinely yield relatively fast measurements (from 20 to 60 min depending upon the available NMR instrumentation) with a precision higher than 1%.

2. Experimental

2.1. Samples

High-purity Meprobamate ([2-(carbamoyloxymethyl)-2-methyl-pentyl] carbamate) was provided by the Council of Europe as a Chemical Reference Substance (CRS). Its molecular structure together with the corresponding atom numbering used in this study, are shown in Fig. 1. Equanil® 400 mg and 250 mg were obtained from SANOFI-AVENTIS. While Meprobamate is commercially available as a fine powder, Equanil® is provided in the form of tablets, which were finely grounded with a mortar and pestle prior to analysis. For the ssNMR analysis, 10 tablets were used giving an averaged weight of 615.9 and 398.5 mg per tablet for Equanil® 400 and 250, respectively.

Two synthetic mixtures of Meprobamate with starch (Sigma–Aldrich, starch from potato suitable for electrophoresis) were also prepared by grounding precisely known amounts of both substances (determined by weight) with a mortar and pestle. Mixture A was obtained by mixing 300.0 mg of Meprobamate and 150.3 mg of starch, whereas mixture B was obtained by mixing 60.0 mg of Meprobamate and 40.2 mg of starch. The proportion of these mixture components were chosen to respect those found in the commercial formulations. In addition, to perform the liquid-state NMR assignment, Meprobamate was dissolved in deuterated acetone purchased from Eurisotop. All materials were used as received.

2.2. Liquid-state NMR

All liquid-state NMR experiments were conducted at 300 K on a BRUKER AVANCE400 DPX spectrometer operating at 400 MHz for the ^{1}H Larmor frequency, and equipped with a BRUKER 5 mm liquid-state ^{1}H/X BBI probe. The liquid-state NMR assignment was performed in acetone-d_6 using a combination of conventional 1D (^{1}H, ^{13}C{^{1}H}) and 2D (^{1}H–^{13}C HSQC and HMBC) experiments, for which typical acquisition parameters were used as summarized in Ref. [21].

2.3. Solid-state NMR

All ssNMR experiments were conducted at 300 K on a BRUKER AVANCE400 DSX spectrometer operating at 400 MHz for the ^{1}H Larmor frequency, and equipped with a BRUKER 4 mm ^{1}H/X solid-state CP MAS probe. The spinning rate was set to 10 kHz. For the ^{13}C CP MAS experiments, a ramped ^{1}H pulse starting at 100% power and decreasing until 50% was used during the contact time (4 ms) in order to circumvent Hartman–Hahn mismatch [22,23]. ^{1}H decoupling during signal acquisition was ensured by the GT8 scheme [24]. The recycle delay was set to 13 s, five times the largest T_1. The number of scans was adjusted to ensure a signal-to-noise ratio of at least 150 [8].

In addition, to facilitate the assignment of the solid-state ^{13}C chemical shifts of Meprobamate, a CPPI (cross-polarization polarization inversion) experiment [25] was performed with a pulse inversion length of 40 μs. With this pulse length, CH_3 and quaternary carbons appear positive, CH_2 negative, and CH signals are nearly zero.

As evidenced later on in the text (Section 3.2), when using the external reference method, the quantitative coil volume of the probe must be known. The probe used here was equipped with a 12-turn coil and its quantitative volume was 30 μl. Accordingly, all ssNMR quantitative measurements were performed using a 4 mm rotor prototype provided by ROTOTEC–SPINTEC [18]. Importantly, because the quantitative coil volume is only probe-dependent, it can be used for the quantification of all types of solid samples provided that the same probehead is used. Finally, note that the integrals were measured and compared from one spectrum to another by using the INTERSCALE function of the XwinNMR 3.5 software from Bruker. This function allows the value of a signal integral in a given spectrum to be used as an intensity reference for all the other spectra.

3. Results and discussion

3.1. Assignment of the ^{13}C NMR spectra of Meprobamate

To the best of our knowledge, although Meprobamate is a rather well known active principle, the assignment of its ^{13}C NMR spectrum has not been reported yet. Therefore, to ease the assignment of the solid-state NMR spectrum, we first assigned the liquid-state ^{13}C spectrum (Table 1).

Specifically, the latter assignment was performed in acetone-d_6 using conventional experiments (see experimental section). This assignment served as a basis to interpret the ^{13}C CP MAS spectrum of the solid form of Meprobamate, shown in Fig. 2a. The solid-state NMR assignment was further confirmed using a CPPI experiment recorded on pure Meprobamate (Fig. 2b). Note that, in this spectrum, signals due to CH_3 and quaternary carbons are positive whereas CH_2 signals appear negative.

Overall, the CPPI experiment allows the solid-state assignment derived from liquid-state data to be confirmed, with the notable exception of the C-1 and C-3 groups, whose ^{13}C chemical shifts