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Short communication

Quantitative proton nuclear magnetic resonance for the structural and quantitative analysis of atropine sulfate

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ABSTRACT

This study assessed a general method of quantitative nuclear magnetic resonance (qNMR) for the calibration of atropine sulfate (Active Pharmaceutical Ingredient, API) as reference standard. The spectra were acquired in D_2O using maleic acid as the internal standard. Conformational behaviors of tropane ring were observed and studied by means of NMR and ROESY experiments at different temperature, which showed that the azine methyl group was at equilibrium for axial and equatorial conformations at room temperature. Signal delay and monitor signals of qNMR experimentation were optimized for quantification. The study reported here validated the method's linearity, range, limit of quantification, stability and precision. The results were consistent with the results obtained from mass balance approach.

1. Introduction

As a part of drug quality control process, the reference standard is widely used for qualitative and quantitative analysis. Unlike chromatography, quantitative ^{1}H NMR does not require a high purity reference standard for accurate quantification of the test compound of interest, because selected functional group(s) being observed, e.g. the nucleus of a hydrogen atom, has a molar response coefficient of 1 regardless of the compound, assuming that the proton does not exchange with the deuteriums of the solvent [1]. Therefore, QNMR is especially applicable for content determination of substances lack of ultraviolet adsorption [2–4] and highly suitable to evaluate the purity determination of primary reference standards [5–7] as well as the quality of drugs [3,8].

Atropine sulfate is a type of tropane alkaloids with description of benzene acetic acid α-(hydroxyl methyl)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, sulfate (2:1) mono-hydrate, as shown in Fig. 1, and it is a competitive antagonist for the muscarinic acetylcholine receptors and classified as an anticholinergic drug. Sharma et al. [9] have reported a quantitative ^{1}H NMR method for analysis of atropine sulfate using *N-methyl* as monitor signal for quantification. In the study reported in this article, we found that atropine sulfate does not exist in the single unique conformation, but in conformational equilibrium observed from the variable temperature NMR spectra. Our finding suggests that Sharma's method have limitation in the choice of monitor signals for quantification. In this report, we studied various conformational structures of atropine sulfate using methods of 1D and 2D spectroscopic experiments, such as VT-NMR, APT and ROESY techniques. Here, we describe a modified quantitative ^{1}H NMR method to determine the purity of atropine sulfate. The results are consistent with the result from mass balance approach. The method further confirms that the qNMR is a rapid, convenient and accurate technique for the value assignment of atropine sulfate as a reference standard.

2. Experimental

2.1. Materials

Atropine sulfate 97.1% (determined by mass balance approach) was provided by Puri Pharmaceutical Factory, Henan, China (Batch No. 20110603); maleic acid 99.78% (standard for quantitative NMR) was purchased from Fluka Analytical, USA (Lot. BCBB7987V); deuterated solvent, D_2O (99.8%) was purchased from J&K Chemical, Japan.

2.2. Sample preparation

Calibrated Gilson™ syringes (1 mL and 100 μL) were used for the volume measurements and Micro-balance Mettler Toledo MX5 (Mettler-Toledo GmbH, Switzerland) was used for weight measurement.

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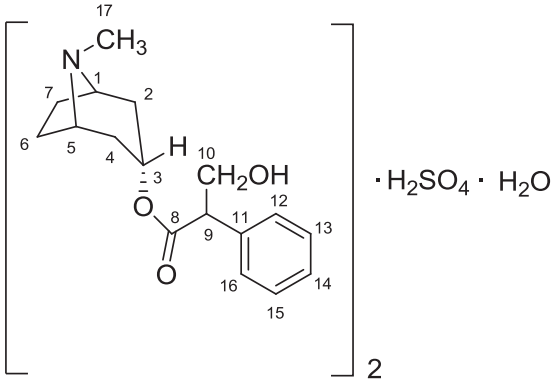

Fig. 1. Structure of atropine sulfate.

For conformational analysis, i.e. ^{1}H NMR, APT, VT-NMR and ROESY experiments, approximately 10 mg of atropine sulfate was dissolved in 600 μL D_2O. For determination of the performance, i.e. linearity and range of 6 analytes containing from 6.988 to 56.104 mg atropine sulfate were dissolved in 1.0 mL D_2O. Sample weights for qNMR were approximately 27.00 mg (0.04 mol/L). The internal standard of maleic acid, 4.64 mg (0.04 mol/L) was added to each analyte.

2.3. NMR spectroscopy

All of the ^{1}H, APT NMR spectra and the two-dimensional experiments, i.e. ^{1}H, ^{13}C heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC), ^{1}H–^{1}H correlation spectroscopy (COSY) and rotating-frame nuclear Overhauser effect correlation spectroscopy (ROESY) were performed at 298 K using Bruker Avance spectrometer at 500.13 MHz proton frequency with 5 mm dual-core probe and BVT23000 temperature control unit. Variable-temperature NMR (VT-NMR) experiments

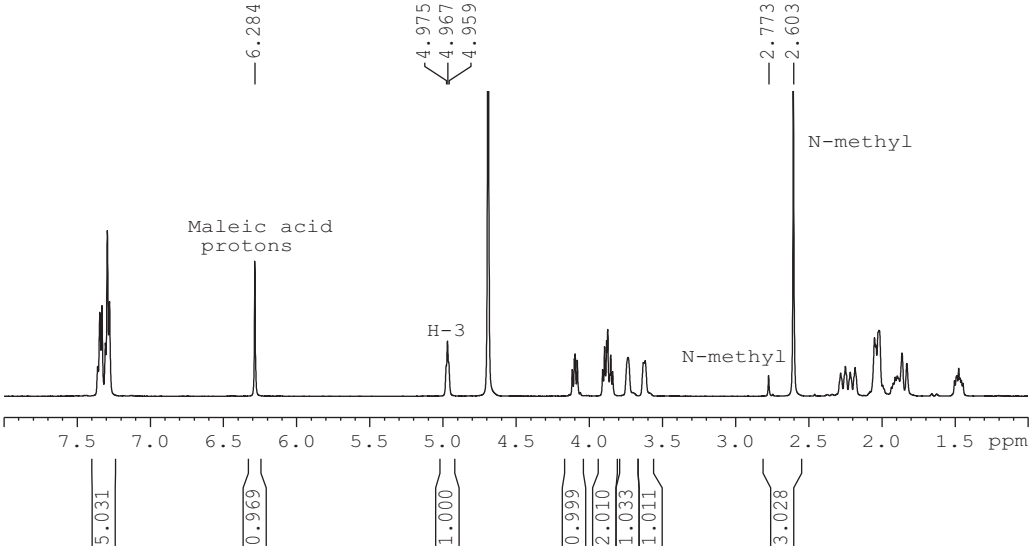

Fig. 2. ^{1}H NMR of mixture of atropine sulfate and maleic acid in D_2O (500 MHz, 298 K).

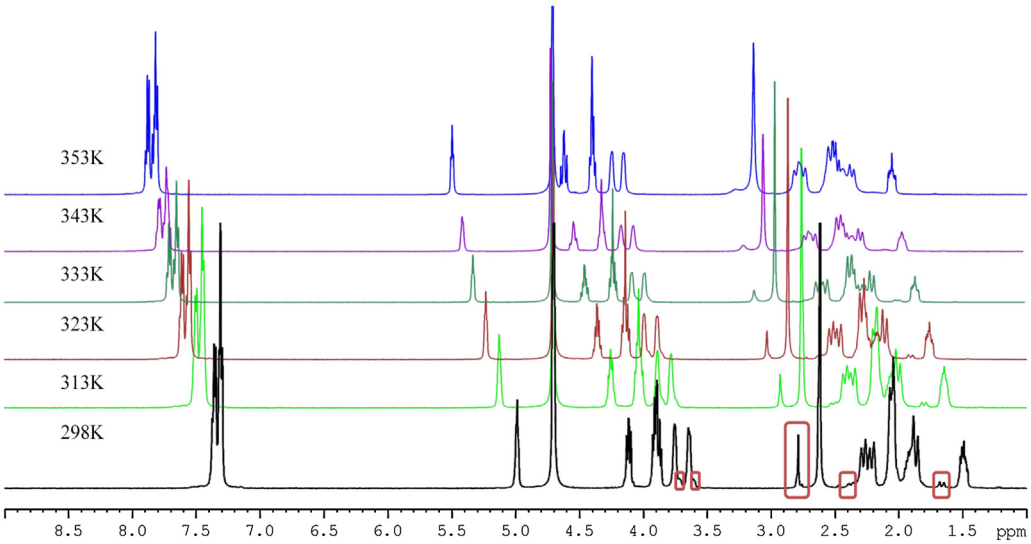

Fig. 3. The variable-temperature ^{1}H NMR spectra of atropine sulfate in D_2O (the red rectangle indicates the signals of non-dominant conformation parts). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)