Solution to phenylbutazone purity challenge

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The joint winners of the phenylbutazone purity challenge (published in volume 408 issue 12) are: Lorenzo Biancalana and Beatrice Campanella, University of Pisa, Italy.

The award entitles the winning group to select a Springer book of their choice up to a value of €100,-.

Our Congratulations!

Solution

In the last 15 years NMR has become a reference technique for the determination of purity for organic standards containing NMR active nuclei [1, 2]. The puzzle proposed for this *Analytical Challenge* was focused on the use of ¹H-NMR for the characterization of a phenylbutazone standard. Such material was purchased from Sigma-Aldrich and analyzed in parallel by HPLC-UV and ¹H-NMR. Initially, application of the two methodologies gave results which were not in agreement [3]. Whereas the HPLC-UV method found only two minor impurities that did not exceed the 1 % level, the NMR assay was less generous: integration of the proton signal at position 5 (Fig. 1), provided a phenylbutazone purity estimate of only $0.8119 \pm 0.0014_{k=1}$ g/g [3]. Such evidence could alert the analyst that an impurity, structurally related to phenylbutazone, is present. This

conclusion would also be supported by the presence of an unusual signal at 2.2 ppm in the ¹H-NMR spectrum of phenylbutazone (Fig. 2) that should not be there. Such explanation suggests that something went wrong during the synthesis of the standard.

In this case, however, the guilty party is not the organic chemist, but the chemical character of phenylbutazone. Figure 1 summarizes the problem and solution for our case. Phenylbutazone undergoes *keto-enol* tautomeric equilibrium that makes the interpretation of the ¹H-NMR data not very intuitive. The existence of this effect for phenylbutazone is reported in the literature [4] and is confirmed by the NOESY spectrum of phenylbutazone [3].

Since the *keto* form of phenylbutazone is in equilibrium with the *enol*, the proton in position 5 of the *keto* (chemical shift 3.9 ppm, Fig. 2) is shifted to the hydroxy group of the *enol*. This chemical effect makes the use of this proton unsuitable for quantitation purposes. Furthermore, the hydrogens in position 4 show a different chemical shift when the molecule is in a *keto* (1.9 ppm) or *enol* form (2.2 ppm).

Fig. 1 Phenylbutazone keto-enol tautomerism

This article is the solution to the Analytical Challenge to be found at <http://dx.doi.org/10.1007/s00216-016-9412-4>

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Fig. 2 ^1H -NMR spectrum of the phenylbutazone maleic acid mixture in DMSO-d_6 . When the phenylbutazone is in its *keto* form the hydrogens in position 4 can be seen at 1.9 ppm, whereas, when the molecule is in the *enol*, such hydrogens are detected as a triplet at 2.2 ppm

For the calculation of the purity, the analyst should opt for protons that are not affected by the tautomerism, such as those at 0.9 ppm (belonging to the methyl group in position 1):

$$w_A = \frac{I_A}{I_B} \cdot \frac{N_B}{N_A} \cdot \frac{M_A}{M_B} \cdot \frac{m_B}{m_A} \cdot w_B =$$

$$= \frac{9.537}{10.000} \cdot \frac{2}{3} \cdot \frac{308.373}{116.0719} \cdot \frac{13.802}{23.332} \cdot 0.9995 =$$

$$= 0.9987 \pm 0.0018 \text{ g/g}$$
(1)

where W_i , I_i , N_i , M_i , and m_i are respectively the purity (as mass fraction), NMR signal intensity, number of resonant nuclei, molar mass, and mass of the material i (the phenylbutazone material is indicated with letter A, whereas the maleic acid internal standard with B). As an alternative to this calculation scheme, the analyst can utilize the signal at 1.3 ppm which represents the four protons in positions 2 and 3:

$$w_A = \frac{12.705}{10.000} \cdot \frac{2}{4} \cdot \frac{308.373}{116.0719} \cdot \frac{13.802}{23.332} \cdot 0.9995 =$$

$$= 0.9979 \pm 0.0018 \text{ g/g}$$
(2)

In order to estimate measurement uncertainty, the JCGM guideline was applied [5]. NMR results are reported in

Table 1 Purity of phenylbutazone by ^1H -NMR

Signal ppm	I_A	N_A	$w_A \text{ g/g}$
0.9	9.537 ± 0.011	3	0.9987 ± 0.018
1.3	12.705 ± 0.013	4	0.9979 ± 0.018

Experimental data: $I_B = 10.000 \pm 0.010_{k=1}$, $N_B = 2$, $w_B = 0.9995 \pm 0.0010_{k=1} \text{ g/g}$, $m_A = 23.332 \pm 0.003_{k=1} \text{ mg}$, $m_B = 13.802 \pm 0.003_{k=1} \text{ mg}$, $M_A = 308.373 \pm 0.003_{k=1} \text{ g/mol}$, $M_B = 116.0719 \pm 0.0012_{k=1} \text{ g/mol}$.

Table 1 and they are in agreement with the purity obtained by HPLC-UV.

The apparent inconsistency between the two methodologies was simply due to the fast *keto* $\rightleftharpoons$ *enol* equilibrium (Fig. 1). Due to kinetics, the two tautomers could not be distinguished by conventional HPLC [6] and, therefore, only one major peak was detected. Despite this interesting chemical effect, the application of ^1H -NMR - *after a proper interpretation of the chemistry* - still produced a metrologically-sound purity estimation for phenylbutazone. The results presented in this *Analytical Challenge* further confirm that NMR is a powerful tool for purity assessment of organic compounds and a valuable asset for the production of Certified Reference Materials.

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