

5.2: Determining Enantiomeric or Isomeric Purity of Active Pharmaceutical Ingredients

This document contains two separate applications that discuss the use of quantitative NMR measurements for the analysis of enantiomeric or isomeric purity of pharmaceutical compounds.

Application 1. Q-NMR for Determination of the Enantiomeric Purity of Pharmaceutical Ingredients

Enantiomeric purity (EP) is important in the development of active pharmaceutical ingredients (API) by the pharmaceutical industry. For some drugs, API enantiomers can produce dramatically different pharmacological responses. Enantiomeric purity is commonly determined by effecting a chiral separation however, chiral separations can be time consuming and typically involve the use of expensive chiral columns. Although it is not possible to distinguish enantiomers directly with NMR, derivatization to form diastereomers produces molecules with distinct NMR spectra.¹ Determination of EP by NMR using chiral solvating agents (CSAs) alleviates the need for chemical derivatization or standards. CSAs interact with the enantiomers in solution, in effect forming transient diastereomers. The use of CSAs in NMR was first reported in 1966 by Pirkle.² The CSA used in this study was 1,1'-binaphthol shown as compound 1 in Figure 1. This compound is known to resolve chiral amines such as the compounds 2-5 shown below. Some of the chiral compounds in Figure 1 are prescribed clinically to treat such disorders as depression and anxiety (e.g., 2, Zoloft® and 3, Paxil®). Compound 5, fenfluramine, was a component of the anti-obesity drug Fen-Phen, which was withdrawn from the US market after reports linked it to heart damage.

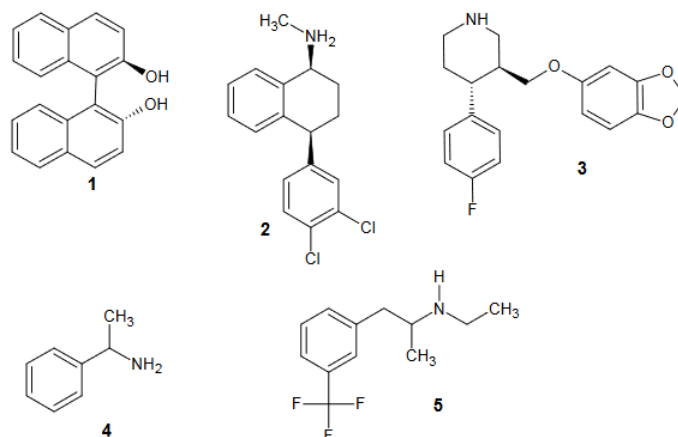


Figure 1. Structures of (1) the chiral solvating agent (R)-1,1'-bi-2-naphthol, (2) (+)-sertraline HCl, (3) (-)-paroxetine HCl, (4) racemic methylbenzylamine, and (5) racemic fenfluramine HCl.

Enantiomeric separation by NMR is based on the intrinsic differences in the diastereomeric complexes formed and/or differences in the association kinetics of the equilibria below:



where S represents the CSA molecule while E and E' represent the different solute enantiomers.³

In experiments by Salsbury et al. to determine enantiomeric purity of the APIs, fenfluramine, sertraline and paroxetine, and the model compound, methylbenzylamine (MBA, compound 4 in Figure 1), the analytes were dissolved in CDCl₃ and chemical shifts referenced to tetramethylsilane (TMS).¹ The ¹H NMR spectra used to determine the limits of detection and quantitation were measured with 64 transients, tip angle of 30°, relaxation delay of 1s and line broadening of 0.3 Hz. Proton chemical shift assignments were confirmed using COSY. Standards were weighed (1-4 mg) and CDCl₃ solutions containing an appropriate molar ratio of the analyte and 1,1'-binaphthol were prepared. The ability of the CSA interactions to resolve the mixtures of enantiomers was evaluated using MBA mixtures. Using standards of methylbenzylamine at different concentrations to obtain a calibration curve, the limit of quantitation was determined to be below 1% of the minor component. Analysis of racemic fenfluramine revealed that it contained 50.2 ± 0.4% the S-enantiomer. Although chiral HPLC could not be performed for fenfluramine or sertraline

without derivatization, the analysis of paroxetine enantiomers was carried out by both NMR and HPLC yielding results of $7.5 \pm 0.3\%$ and 8.5% , respectively.

References

1. Salsbury, J. S. and Isbester, P. K. *Magn. Reson. Chem.* **2005**, 43, 910-917
2. Pirkle, W. H. *J. Am. Chem. Soc.* **1966**, 88, 1837
3. Pirkle, W. H. and Hoover, D. J. *Top. Stereochem.* **1982**, 13, 263

Application 2. Q-NMR for the Quantitation of the E/Z Isomer content of Fluvoxamine

Fluvoxamine is an antidepressant with two possible isomeric structures as shown in Figure 1 below. The activity of fluvoxamine resides in the E-isomer (Figure 1A). However, the Z-isomer (Figure 1B) occurs in all the synthesis pathways. Transport proteins can discriminate between the E- and Z-isomers. The British Pharmacopeia limits the content of Z-isomer to 0.5%. The QNMR method described measures the Z-isomer to the 0.2% level in 15 mg of the drug substance.

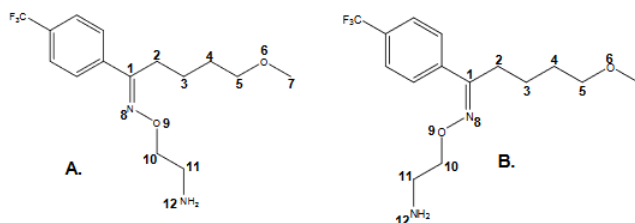


Figure 1. Structures of (A) (E)-fluvoxamine and (B) (Z)-fluvoxamine. The pharmaceutical formulation is available as the maleate salt of fluvoxamine. The numbering of the atoms correlates with the NMR spectrum reported in the reference.

An advantage of Q-NMR for determining the Z-isomer content is the minimum sample preparation required. In this example, 15 mg of material was dissolved in deuterated methanol which was also used as the ^1H (3.31 ppm) chemical shift reference.¹ ^1H NMR spectra were acquired by coaddition of 128 transients over a spectral width of 4595 Hz. FIDs were apodized by multiplication with an exponential function equivalent to 0.3 Hz line broadening. For quantitation, the C-2 (Z) and C-2 (E) proton resonances of the Z- (2.62 ppm) and E- (2.90 ppm) fluvoxamine isomers were manually integrated and the values compared.

Before performing quantitative measurements, it was necessary to determine the limits of quantification and detection for each isomer. Although the pure E-isomer was commercially available, the pure Z-isomer was not. Instead the authors had access only to a 1:1 (E/Z) mixture. Therefore a stock solution containing 5.13% (Z) fluvoxamine was prepared by mixing appropriate amounts of the pure E and E/Z mixture. Serial dilutions were made from this stock solution for NMR analysis. With each dilution, the concentrations of the E- and Z-isomers decreased, but the %Z content remained at 5.13%. For the spectrum measured at each concentration, manual integration of the C-2(Z) and C-2(E) proton resonances was performed three times. The difference between calculated and determined values of the Z-isomer content was less than 5% at concentrations down to 0.07 mg/L. Greater deviation from calculated values was observed at lower Z-isomer concentrations. Based on these experiments, the limits of quantitation and detection were determined to be 0.07 mg/L and 0.018 mg/L, respectively. To determine linearity, a set of mixtures containing 0-10% Z-isomer were measured in triplicate. The correlation coefficient of the calibration plot was found to be 0.9999 with a coefficient slope of 0.9923. Since the British Pharmacopeia tests requires the content of Z to be less than 0.5%, solutions were prepared containing 0.15-1.01% of the Z-isomer. NMR spectra were measured in triplicate and each spectrum integrated three times. Linear regression analysis yielded a correlation coefficient of 0.994 with a slope of 1.042, indicating that the Q-NMR assay is linear over this concentration. The Q-NMR method was found to be an accurate, sensitive, and timesaving method for the determination of Z- fluvoxamine content.

Reference

1. Deubner, R. and Holzgrabe, U. *Magn. Reson. Chem.* **2002**, 40, 762-766

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