Quantitative NMR:

An essential tool for the certification of Organic Reference Materials

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Background:

Modern day chemical analysis continues to rely on the availability of fully certified reference materials (CRMs) for the validation of detection methods.

The Chemical Reference Materials team at NMI, Australia, currently assesses the purity of a ***"pure substance"*** organic compound using a combined approach comprising results from the following techniques, each analysing different components of a given material:

Organic: Analysis by GC-FID and/or HPLC with UV & ELSD detection.

Volatile(s): Analysis by TGA and Karl Fischer moisture.

Non-volatile residue: Analysis by TGA.

The sum of these is then taken as the certified purity.

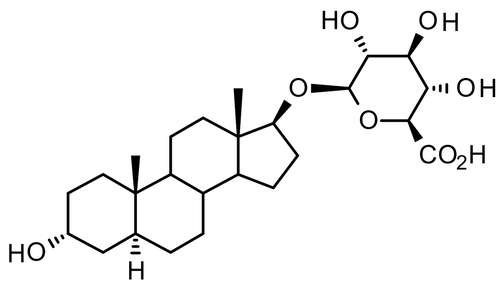
The Advantage of QNMR:

In order to improve the characterisation and purity assessment of organic compounds, we also use QNMR, which has the distinct advantage of directly assessing the analyte of interest, both in terms of ***quality*** i.e. structural consistency, and ***quantity***, the amount of analyte present.

Here we present examples where QNMR has complemented the above techniques, affording a more comprehensive and robust purity certification.

Reference: Wells, R J.; Cheung, J.; Hook, J.M. (2008) *NMR Spectroscopy in Pharmaceutical Analysis*, Holzgrabe, U., Wawer, I., Diehl, B., (Eds.) Elsevier, New York, pp. 219-315.

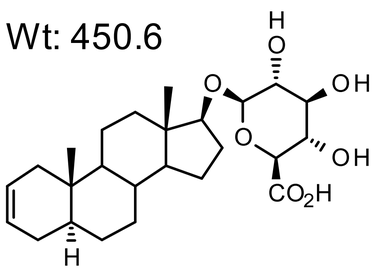
Case Study 1: *5α-Androstan-3α,17β-diol-17-β-glucuronide*



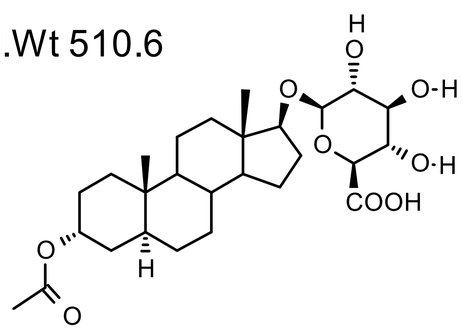
This metabolite of an androstan-diol is detected as an indicator for abuse of the performance enhancing drug, and is synthesised for use as a CRM.

HPLC-ESLD reveals the presence of two impurities, A and B, with apparent concentrations of 0.15% and 0.55%, respectively, and identified by LC-MS as likely

A, Mol Wt: 450.6



B, Mol.Wt 510.6

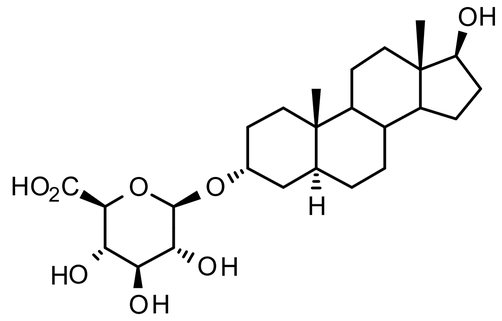


^{1}H QNMR in d4-MeOH shows two peaks at δ 4.87 and δ 5.84 consistent with an olefinic double bond in impurity A.

^{1}H NMR (d6-DMSO + 2 drops of D_2O) shows a peak at δ 1.97, assigned to the acetate group of impurity B.

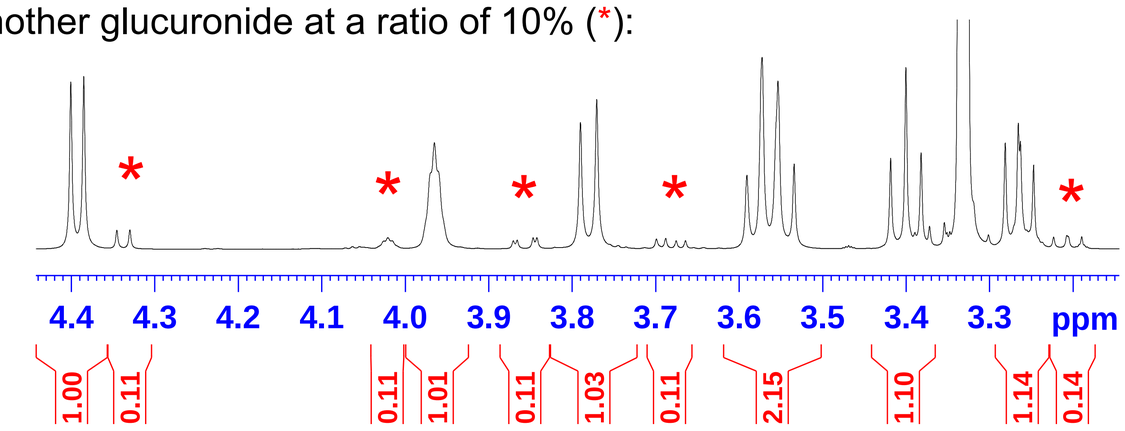
Integration of QNMR spectra also reveals that HPLC-ESLD has a non-uniform detector response to 1, A and B, with actual concentrations being 2.0 and 3.0 %, respectively.

Case Study 2: 5α-androstan-3α,17β-diol-3-β-glucuronide



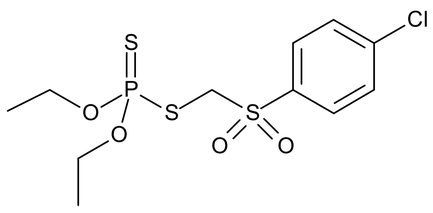
HPLC-ELSD indicates an apparent organic purity of 99.8%.

However, QNMR in d4-MeOH or d6-DMSO clearly reveals the presence of another glucuronide at a ratio of 10% (*):



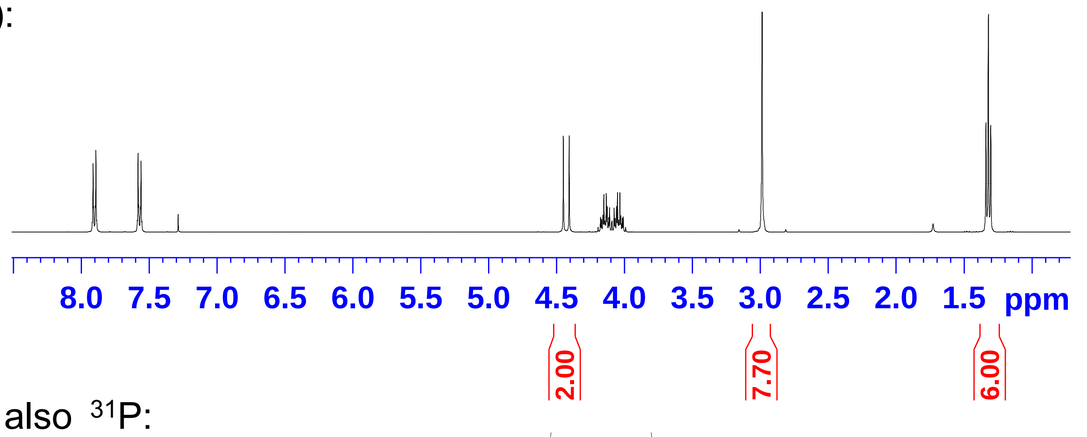
Furthermore, a pair of doublets at δ 5.1 is indicative of olefinic protons from a dehydration side-product (similar to A) , at a ratio of 1.4%.

Case Study 3: Carbophenothion sulfone

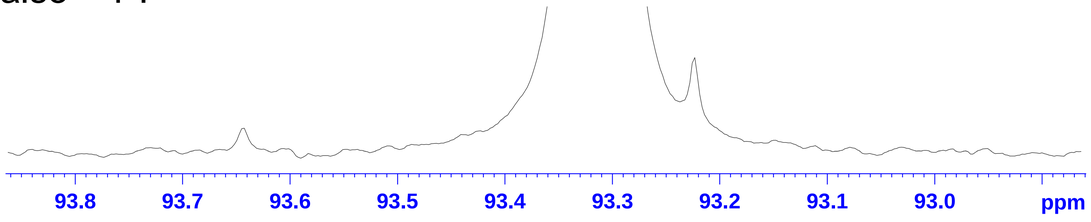


This is an oxidation product of the highly toxic organophosphate pesticide carbophenothion, and is a necessary CRM for the detection of carbophenothion in the environment.

The phosphorus enables QNMR not only using ^{1}H (with $DMSO_2$ internal std):



but also ^{31}P:



Purity from ^{1}H is 99.3% (m/m), purity from ^{31}P also 99.3% (P atom)