Testing the Stability of Pesticides in Stock Solutions by Quantitative NMR

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Introduction

A significant source of errors in pesticide residue analysis is the degradation of standards in stock solutions, working solutions (e.g. mixtures) and sample extracts. QC protocols require from laboratories to ensure that this source of errors remains insignificant. Currently LC-MS/MS and GC-MS(/MS) are the most widely employed techniques to test the stability of pesticides in pesticide residue labs. These techniques are sensitive and selective enough to allow the testing of mixtures but the relatively high measurement uncertainty requires multiple injections (typically ≥5) to achieve the accuracy required for safe judgment about the compliance with stipulated thresholds. An additional disadvantage is that stability tests occupy measurement time required for routine pesticide analyses. The need to prepare a new standard to be measured against the old one increase the costs of the current approach.

Nuclear magnetic resonance (NMR) spectroscopy is usually applied for structure elucidation and purity assessment of organic compounds. Quantitative ^{1}H-NMR (qNMR) has been gaining popularity e.g. in drug analysis, and quality control applications as it produces qualitative and quantitative information simultaneously. Compared with MS, NMR yields relatively low-sensitivity measurements but it provides high precision and accuracy [1], which allows reducing the number of replicate measurements. A major advantage is that the reference standard does not need to be the identical material, but only an universal standard unrelated to the target analyte. This considerably reduces the costs associated with purchasing of standards and preparation of stock solutions. Additionally, qNMR provides information about the purity of neat standards, which is a major gap in current QC-schemes. The non-destructive nature of this technique makes it possible that samples are kept for measurements over the course of several years (e.g. in flame sealed NRM tubes). The following table compares the main characteristics of qNMR and chromatographic techniques (based on [2]):

Quantitative NMR		LC-MS/MS GC-MS(/MS)
Weight/dilution Non-destructive analysis	Sample preparation	Weight/dilutions destructive analysis
Structural properties (restriction: e.g. ^{1}H)	↓ Detection	Physical properties (restriction: e.g. ionization)
Certified reference material (one universal calibrant)	↓ Calibrant	Structurally identical reference needed
Internal/external standard (with/without calibration curve)	↓ Quantitation	Internal/external standard (with/without calibration curve)
Low µM	↓ Sensitivity	Low nM - pM
Resonance overlapping	↓ Selectivity & Specifity	Chromatographic separation = better specifity
Instrument independent	↓ Reproducibility	Instrument-dependent

The numerous merits of the NMR-technique led us to start exploring the suitability of qNMR for pesticides stability testing in stock solutions.

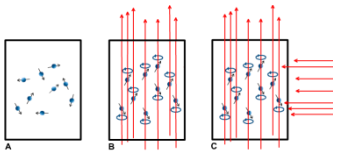
Reference

[1] Pauli GF, Gödecke T, Jaki BU, Lankin DC; Quantitative 1H NMR. Development and potential of an analytical method: an update; J Nat Prod. 2012 Apr 27;75(4):834-51

[2] Simmler Ch, Napolitano JG, McAlpine JB; Universal quantitative NMR analysis of complex natural samples; Current Opinion in Biotechnology 2014, 25:51-59

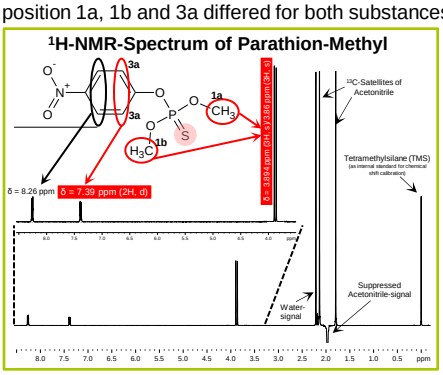
Experiments

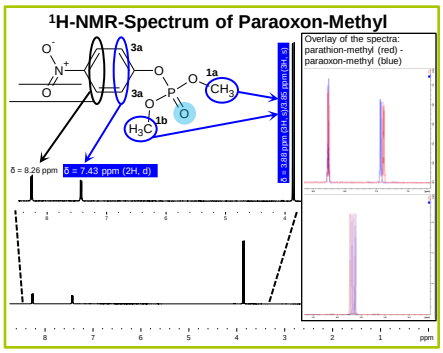
To start with, parathion-methyl and paraoxon-methyl were chosen as model compounds. ^{1}H-NMR spectra were recorded at 400 MHz (Bruker Avance 400). To avoid that acetonitrile (solvent of stock solutions) would saturate the dynamic range of the NMR receiver, its signal was reduced by solvent suppression techniques. This resulted in NMR spectra which could be used for purity assays and quantitative experiments. 1,2,4,5-tetrachloro-3-nitrobenzene was used as external calibrant for qNMR.



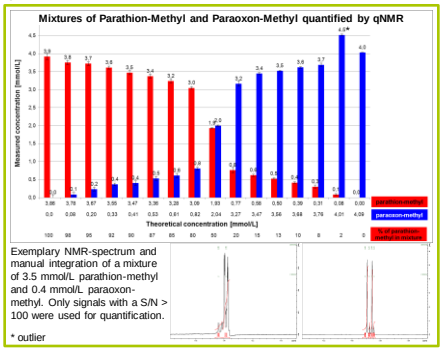
Results

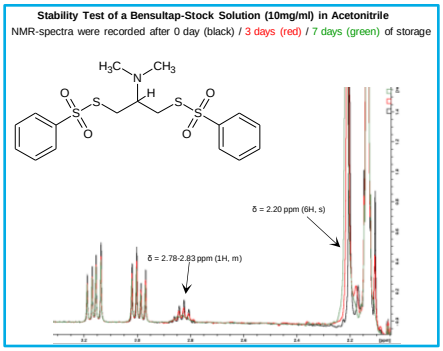
The NMR-spectra of the individual model compounds revealed that the neat standards were of high purity as no impurities of organic compounds could be identified in the spectra. The chemical shifts for the protons in position 1a, 1b and 3a differed for both substances:





Mixtures of the model compounds were prepared in varying concentrations in order to simulate the degradation of parathion-methyl in a stock solution (1 mg/ml; 3.86 mmol/L) to paraoxon-methyl and measured by qNMR:





A stability test was performed with bensultap stored in acetonitrile at RT in a tightly closed NMR-tube and measured by NMR each day. After 7 days of storage the signal at δ = 2.78-2.83 ppm (1H, m) lost the multiplet character and the signal at δ = 2.20 ppm (6H, s) significantly increased in intensity indicating a degradation of bensultap.

Summary

The outcome of this pilot study indicates that qNMR is a promising technique to assess the stability of pesticides in stock solutions. The applicability of qNMR in checking and comparing the purity of neat standards will also be assessed.

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