Testing the Stability of Pesticides in Stock Solutions by Quantitative NMR

Hubert Zipper, Maren Ilse, Ellen Scherbaum, Michelangelo Anastassiades

E-Mail: Hubert.Zipper@cvuas.bwl.de

Introduction

A significant source of error in pesticide residue analysis is the degradation of standards in stock solutions, working solutions and sample extracts. QC protocols require laboratories to ensure that this source of error remains insignificant. Currently LC-MS/MS and GC-MS(/MS) are the most widely employed techniques for testing the stability of pesticides. These techniques are sensitive and selective enough for the testing of mixtures, but measurement uncertainty requires multiple injections (typically ≥5) to achieve the accuracy required for reliable conclusions about compliance with the stipulated thresholds. An additional disadvantage is the unavailability of the instrument during measurements for routine pesticide analyses of samples and the need to prepare a new stock solution that is measured against the old one, which can be quite costly.

Is Quantitative NMR an Alternative?

Quantitative proton 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 mass spectrometry, qNMR yields relatively low-sensitivity measurements, but a major advantage is that the reference standard does not need to be the identical material, but only one universal standard unrelated to the target analyte. This considerably reduces the costs associated with the purchase and preparation of "new" standard stock solutions. Additionally, qNMR has been reported to be highly precise and accurate, thus reducing the number of replicate measurements required. The non-destructive nature of this technique makes it possible for samples to be kept for measurements over the course of several years, e.g. in flame-sealed NRM tubes. A comparison of the main characteristics of qNMR and chromatographic techniques is shown here (based on [1]):

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 & Specificity	Chromatographic separation = better specifity
Instrument independent	Reproducibility	Instrument-dependent

These merits and positive results from pre-tests with two model compounds (parathion-methyl, paraoxon-methyl) encouraged us to start exploring the suitability of qNMR for testing the stability of pesticides.

Experiments

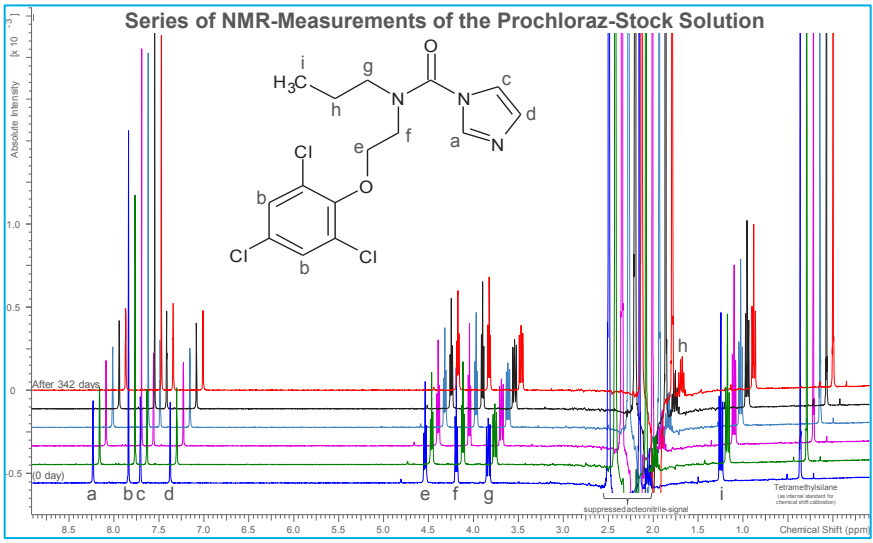
Stock solutions of 50 pesticides were prepared at 1 mg/mL in acetonitrile and/or formic acid-acidified (0.4 Vol.%) acetonitrile, filled in NMR-tubes, flame-sealed and stored at 8°C. 1H-NMR spectra were recorded at 400 MHz (Bruker Avance 400). As acetonitrile has a proton resonance, the acetonitrile-signal was reduced by solvent suppression techniques. The certified reference standard 3,5-dinitrobenzoic acid was used as an universal calibration standard. Over the course of one year, six qNMR-measurements were performed.

Reference: 1. Simmler Ch, Napolitano JG, McAlpine JB; Current Opinion in Biotechnology 2014, 25:51-59

Results

The NMR-spectra of the 50 compounds revealed that the neat standards of most compounds were of high purity as no detectable impurities of organic compounds could be identified in the spectra. After almost one year of storage, no degradation could be observed for most of compounds. An exemplary series of NMR-spectra is shown below for the prochloraz-stock solution. Neither new NMR-signals nor decreasing integrals could be observed in the spectra indicating that no degradation of prochloraz occurred.

No degradation was detected for these compounds:
Acetamiprid; Aldicarb; Aldicarb-Sulfon; Aldicarb-Sulfoxide; Bendiocarb; Boscalid; Captan; Tetrahydrophthalimide; Carb-aryl; Chlorpyrifos; Cyprodinil; Carbofuran; 3-Hydroxycarbo-furan; 4,4'-Dichlorobezophenon; DMST; Dioxacarb; Difeno-conazol; Fenhexamid; Fludioxonil; Phthalimide; Fosthiazate; Furathiocarb; Methiocarb; Methiocarb-Sulfoxid; Methiocarb-Sulfone; Methomyl; Methomyl-Oxime; Metolachlor; Oxamyl; Oxamyl-Oxime; Phenmedipham; Prochloraz; BTS 44595; BTS 44596; BTS 9608; Propamocarb; Propoxur; Prothioconazole-Desthio; Pyraclostrobin; Pyrimisulfan; Pyriofenone; Silthiofam; Thiodicarb



For each of the evaluated NMR-signals (a – g), the concentration of the stock solution determined by qNMR showed a good correlation with the concentration that was prepared using a balance (1 mg/mL ≙ 2.68 mmol/L; in acetonitrile). Regarding the NMR-measurements over a time course of almost one year, the individual NMR-signals revealed a good correlation to the concentration of prepared solution and very low relative standard deviations (RSD). Both results indicate a high level of reproducibility and correct quantification.

Prochloraz-Concentrations (mmol/L) determined by using different NMR-signals (ppm)

Storage Duration [d]	a 7.87	b 7.47	c 7.34	d 7.01	e 4.17	f 3.83	g 3.47	h(*) 1.68	i(*) 0.88	Average (mmol/L)	RSD (%)
0	2.687	2.668	2.672	2.697	2.674	2.657	2.717	(1.249)	(2.886)	2.682	0.756
14	2.692	2.670	2.687	2.702	2.689	2.664	2.723	(0.945)	(2.826)	2.690	0.736
31	2.661	2.645	2.659	2.681	2.637	2.654	2.684	(1.188)	(2.808)	2.660	0.664
94	2.691	2.685	2.687	2.702	2.690	2.689	2.737	(1.266)	(2.898)	2.697	0.687
183	2.661	2.652	2.661	2.689	2.653	2.652	2.692	(1.098)	(2.811)	2.665	0.653
342	2.706	2.690	2.686	2.711	2.699	2.687	2.743	(1.026)	(2.801)	2.703	0.737
Average (mmol/L)	2.683	2.668	2.675	2.697	2.673	2.667	2.716	(1.129)	(2.838)		
RSD (%)	0.685	0.661	0.506	0.390	0.907	0.623	0.875	(11.35)	(1.499)		

NMR-signals at 1.68 ppm (h) and 0.88 ppm (i) were excluded from statistics because of the negative influence of the solvent-suppression-signal on the integrals of these signals (see NMR-spectra above: suppressed acetonitrile-signal at ≈2 ppm).

Degradation was observed in case of folpet (degr. by ~2% to phthalimide), tolylfluanid (degr. by ~5% to DMST), bensultap (degr. by ~14%; degr. products not identified) and to a minor extend in case of dicofol (to p,p'-dichlorobenzophenone).

Summary

Although some drawbacks have to be considered (e.g. resonance overlapping, influence of the acetonitrile-suppression-signal on the integration of nearby proton-signals) the outcome of this study indicates that qNMR is a promising technique for the assessment of pesticide stability in stock solutions.

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