

Analysis of acetanilide herbicides and their rotational isomers by picoSpin 80 NMR

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Application benefit

Thermo Scientific™ picoSpin™ 80 Series II NMR is a powerful analytical tool to qualitatively and quantitatively analyze acetanilides and their isomers. Compared to the traditional methods using chromatography and high-field NMR, the NMR method using picoSpin 80 is cost-effective, time-efficient and straightforward.

Abstract

The picoSpin 80 Series II NMR spectrometer can be used as a low-cost alternative to a high-field instrument for the qualitative and quantitative analysis of acetanilide herbicides. The picoSpin offers adequate resolution to differentiate between acetanilide isomers and a facile means to determine the isomeric ratio by simply integrating the corresponding signals.

Introduction

Acetanilides are a class of preemergent herbicides that inhibit the growth of unwanted grasses and weeds associated with the production of agricultural crops like corn, soybeans, cotton and peanuts.¹ The general structure of acetanilide herbicides is shown in Figure 1.

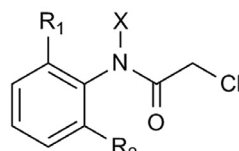


Figure 1: General Structure of Acetanilide Herbicides

Acetanilides represent some of the most widely used pesticides for both crop and non-crop use. In 2007, metolachlor and acetochlor, two representative acetanilides, were the 4th and 5th most used pesticides in the United States, with an approximated use of around 30 million pounds each.²

Due to the widespread use of these herbicides, it is important to understand the metabolic pathways of these molecules and identify their transformation products. Special attention has to be paid to stereoisomers, as they could have vastly different reactivity and degradation pathways.³⁻⁴ For acetanilides, amide *cis-trans* isomerization has been well established where the rotation of the amide bond from the substituted aromatic ring is hindered.³⁻⁶

Quantitative determination of the isomeric ratio in a mixture presents a particular analytical challenge. Traditionally, a number of techniques including Gas Chromatography-Mass Spectrometry (GC-MS), Capillary Zone Electrophoresis (CZE), High-Performance Liquid Chromatography (HPLC) and high-field Nuclear Magnetic Resonance (NMR), have been used to analyze the acetanilide isomers.⁴⁻⁵ Chromatographic techniques including GC-MS, HPLC and CZE often require time-consuming method development. The chromatography runs are generally on the order of 10-15 min, often with non-baseline resolution, giving rise to the uncertainty in isomeric ratio. High-field NMR has proven to be a powerful tool for the analysis of acetanilide isomers by integrating their signals. However, the associated intensive capital and space investment often prohibits their use in smaller facilities or in factories where pesticide production is taking place.

This work demonstrates the qualitative and quantitative analyses of a group of acetanilides with varying structural complexity using an inexpensive and portable picoSpin 80 Series II NMR spectrometer. NMR spectra were obtained in as little as a few seconds compared to the longer analysis times of other techniques. Additionally, a facile means to determine the isomeric ratio is also presented. This methodology was applied to investigate the impact of solvent polarity on the ratio of *cis-trans* rotational isomers of the acetanilide.

Experimental

A picoSpin 80 Series II NMR spectrometer was used to acquire the spectra of acetanilide herbicides. The spectrometer is an 82 MHz, pulsed, Fourier transform ^1H NMR. The instrument contains a 2 Tesla temperature controlled permanent magnet and is fitted with a 40 microliter capillary cartridge used for sample introduction into the spectrometer.

All samples were purchased from Sigma Aldrich and were used as received. Both solid and liquid samples were analyzed in deuterated solvent and injected into the capillary cartridge using 1 mL slip-tip polypropylene syringes and 22 gauge blunt-tipped needles. Back-to-back sample injection and data acquisition was separated by a solvent/air/solvent/air flush to thoroughly clean the capillary.

All spectra were acquired using the following acquisition parameters: Between 1 and 64 scans, 90° excitation pulse, 750 ms acquisition time and 8 second recycle delay. Spectral data was processed using the Mnova™ NMR analysis software with a standard set of processing parameters.

Results and discussion

Figure 2 depicts the ^1H NMR spectrum of propachlor as a 1.8M solution in deuterated DMSO. The signals corresponding to each proton can be readily assigned. The doublet resonating at δ 1.0 ppm corresponds to the two methyl groups of six protons, the singlet at δ 3.8 ppm originates from the two methylene protons alpha to the carbonyl, the septet at δ 4.8 ppm is from the one isopropyl methine proton, and the five protons from the aromatic ring are located between δ 7.1-7.6 ppm.

Substitutions adjacent to the amide bond on the aromatic ring could hinder the rotation of the amide bond, resulting in rotational isomers (*cis-trans* isomers). This phenomenon has been extensively studied using high-field NMR spectrometers; although the ^1H NMR spectrum of alachlor

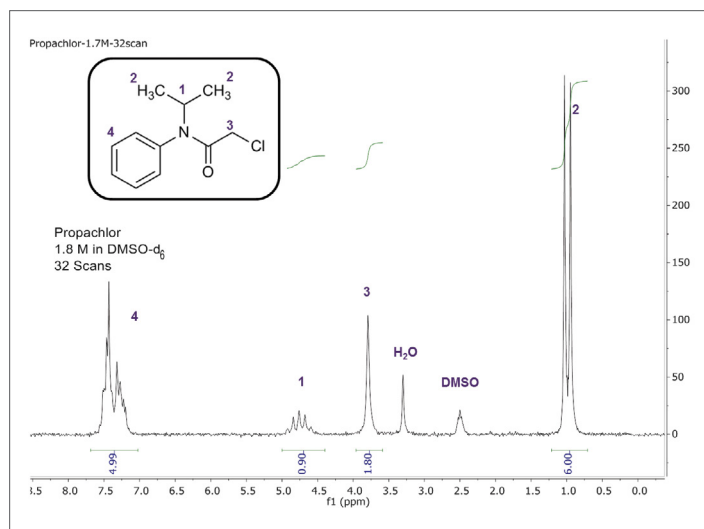


Figure 2: ^1H NMR spectrum of a 1.8M solution of propachlor in DMSO-d_6 acquired in 5 minutes using 32 scans. The residual DMSO signal and water present in the deuterated solvent are identified.

in Figure 3 clearly indicates that the resolution of the picoSpin 80 Series II is sufficient to resolve the signals corresponding to each isomer. In the case of alachlor, ethyl groups are located at the 2 and 6 position of the aromatic ring. The orientation of the carbonyl group away from the aromatic ring corresponds to the *trans*- isomer, and the one aligning toward the aromatic ring corresponds to the *cis*- isomer. In Figure 3, the three methoxy protons from the two isomers can be seen as two singlets at δ 3.3 and 3.4 ppm. The two methylene protons next to the carbonyl show a significant difference in chemical shift due to their change in proximity to the aromatic ring in the two isomers. They are present as singlets at δ 3.8 ppm (*trans*-) and δ 4.7 ppm (*cis*-). The two methylene protons located between the amide nitrogen and the methoxy oxygen resonate at δ 4.9 ppm for both isomers, although a small shoulder ascribed to the minor *cis*- isomer can be observed, indicating a subtle difference caused by the isomerism.

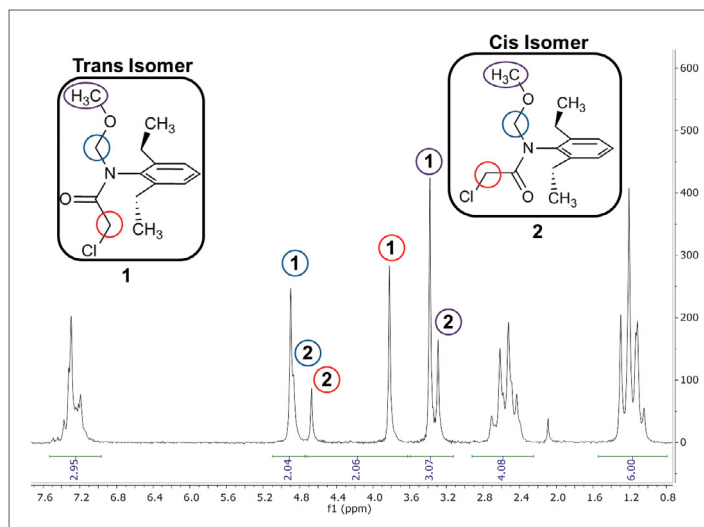


Figure 3: ^1H NMR spectrum of a 3.7M solution of alachlor in DMSO-d_6 acquired using 32 scans. Signals labeled as **1** correspond to the *trans* isomer and **2** correspond to the *cis* isomer. Unlabeled multiplets represent an overlap of *trans* and *cis* signals.

The protons corresponding to the ethyl functional groups resonating between δ 1.0-1.3 ppm and δ 2.4-2.7 ppm and the aromatic signals located at δ 7.1-7.5 ppm cannot be differentiated between the two isomers.

Figure 4 shows the NMR spectra of three acetanilides: butachlor, alachlor and acetochlor. All three structures have substitution at the 2- and 6- position of the aromatic ring, leading to *cis*-/*trans* isomerism. Compared to alachlor, both butachlor and acetochlor have longer alkyl side chains attached to the oxygen. In addition, acetochlor has unsymmetrical ring substituents. These structural differences in butachlor and acetochlor compared to alachlor are annotated in their respective ^1H NMR spectra (Figure 4.)

Figure 5 shows the NMR spectra of alachlor in three different solvents. As the solvent polarity decreases from DMSO-d_6 to CDCl_3 , the isomeric ratio (*trans*/*cis*) increases. The results are also summarized in Table 1. Using the most nonpolar solvent CDCl_3 , the *trans* to *cis* ratio was 92.2% to 7.8%, which was similar to previously reported data in CDCl_3 .⁴

The dependence of alachlor isomer distribution on solvent polarity has been studied previously using high-field NMR instruments, and similar trends have been reported.³⁻⁴ For alachlor and other similar molecules with amide *cis*-*trans* isomerization, the transition state between the two isomers is characterized by a partial C-N bond rotation. This transition state for amide bond rotation is less polar compared to its *cis* and *trans* isomers. In more non-polar solvents, the energy of the transition state decreases; thus, promoting the isomerization. Since the *trans* isomer is more stable than the *cis* isomer,^{3, 6} the *trans*/*cis* ratio increases as solvent polarity decreases.

This data confirms that the choice of solvent is critical to the isomer distribution of acetanilide herbicides in solution, and that the picoSpin 80 Series II NMR spectrometer can easily examine this ratio quantitatively.

Conclusions

The picoSpin 80 Series II NMR spectrometer can be used as a low-cost alternative to a high-field instrument for the qualitative and quantitative analysis of acetanilide herbicides. Some acetanilide molecules are present as rotational isomers due to hindered rotation of the amide bond.

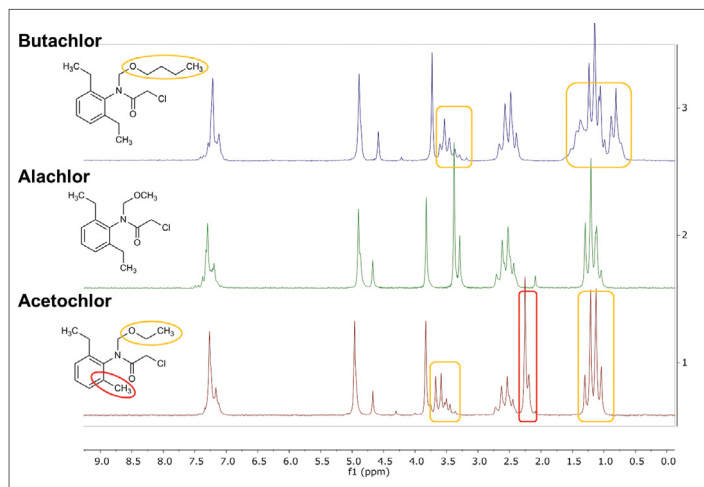


Figure 4: Stacked ^1H NMR spectra of butachlor, alachlor and acetochlor in DMSO-d_6 . The structural changes of butachlor and acetochlor compared to alachlor are highlighted.

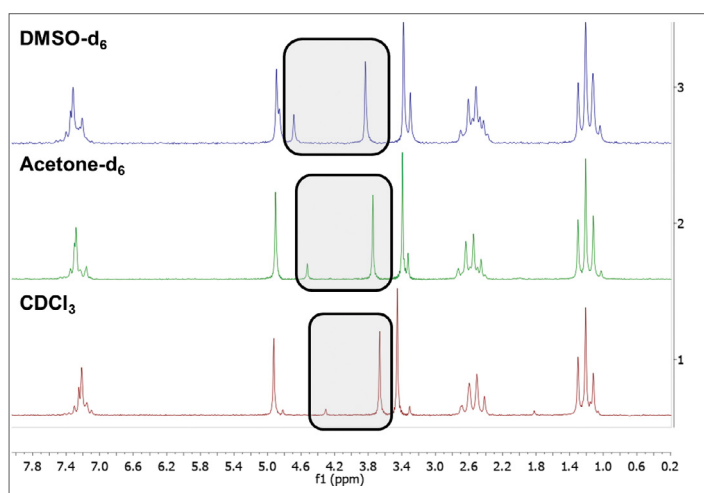


Figure 5: ^1H NMR spectra of a 1M solution of alachlor using three different deuterated solvents.

Solvent	Relative Polarity	% Trans Isomer	% Cis Isomer
DMSO-d_6	0.444	73.7	26.3
Acetone- d_6	0.355	83.6	16.4
CDCl_3	0.259	92.2	7.8

Table 1: The effect of solvent polarity on isomer distribution in alachlor. All samples were analyzed in triplicate and the average values are reported.



The picoSpin offers adequate resolution to differentiate the isomers and a facile means to determine the isomeric ratio by simply integrating the corresponding signals. The results clearly suggest that solvent polarity has a profound impact on the isomeric distribution in solution, and great caution should therefore be exercised when analyzing and interpreting the isomeric ratio.

References

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