JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Quantitative Nuclear Magnetic Resonance (QNMR) Spectroscopy for Assessing the Purity of Technical Grade Agrochemicals: 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Sodium 2,2-Dichloropropionate (Dalapon Sodium)

ROBERT J. WELLS,† JAMES M. HOOK,*,‡ TAREQ SAED AL-DEEN,§ AND D. BRYNN HIBBERT§

Artfern Agrochemical Development Pty. Ltd., 34 Merriwa Street, Gordon, NSW, Australia 2072, and NMR Facility and School of Chemical Sciences, University of New South Wales, Sydney, NSW, Australia 2052

Comparison of quantitative NMR spectroscopy (QNMR) with chromatographic methods such as gas chromatography (GC) or high-pressure liquid chromatography (HPLC) for the determination of the purity of and impurities in technical grade agrochemicals, 2,4-dichlorophenoxyacetic acid (2,4-D), **1**, and Dalapon sodium (sodium 2,2-dichloropropionate), **10**, has revealed that QNMR is more precise and accurate than the chromatographic methods. Quantitative impurity profiling of technical grade **1** is rapid and accurate using 600 MHz ^{1}H NMR. Extra dispersion at the relatively high frequency allowed full assignment of the NMR spectrum of **1** and its related organic impurities in technical samples. The percentage purity of **1** was measured by the difference QNMR method, which involves summing the amounts of impurities and subtracting from 100%. Results are superior in consistency to those obtained by chromatographic methods. The percentage purity of Dalapon sodium, **10**, in technical grade batches is readily obtained by ^{1}H QNMR, using either the difference method or the internal standard method, using dimethyl sulfone (DMSO2) internally as a reference material, that is chemically unrelated to the analyte. The latter method also allows the simultaneous identification and quantification of impurities, many of which are either not accessible to or detectable by the chromatographic methods. Uncertainty budgets for the QNMR method are presented and demonstrate that the major contributors to uncertainty lie in the weighing of the chemicals and in purity of the standard reference material prior to the QNMR experiment.

KEYWORDS: Quantitative NMR; QNMR; impurity profiling; 2,4-dichlorophenoxyacetic acid (2,4-D); Dalapon sodium; sodium 2,2-dichloropropionate; dimethyl sulfone (DMSO2); primary analytical ratio method; internal standard; uncertainty budget

INTRODUCTION

Chemical analysis of an agrochemical for approval by government for commercial use is not an exact science. The variation in analysis of 95% technical grade material for such registration that is accepted by the Australian National Registration Authority or the Food and Agriculture Organization of the United Nations may be between ±1.5 and 2%. In many countries, six or more repeat analyses of the same batch by a validated method are mandatory, as is the identification and quantification of all impurities in excess of 0.1% as required by government registration authorities as a matter for chemical auditing.

The analytical costs associated with these requirements are far from trivial, and the results are often open to question; for example, the sum of all detected constituents is often <98% or >102%, or a single value from one analytical laboratory will not be within 0.5% of the true value. In addition, there is no guarantee that the analytical methods used detects all impurities present. The quality of analytical data gathered by the most commonly used techniques, high-performance liquid chromatography (HPLC) and gas chromatography (GC), is more often dependent on the physical properties of a pure substance than on its structural complexity and can suffer from drawbacks of difficulties with sample preparation and the suitability and standardization of the detector. Without access to a standard of every impurity detected, it is virtually impossible to accurately quantify a minor constituent, and the relative uncertainty associated with the result can be very large.

The potential offered by quantitative NMR spectroscopy (QNMR) as a viable alternative to chromatographic methods

* Author to whom correspondence should be addressed (fax 61 29 38 56 141; e-mail j.hook@unsw.edu.au).
† Artfern Agrochemical Development Pty. Ltd.
‡ NMR Facility, University of New South Wales.
§ School of Chemical Sciences, University of New South Wales.

10.1021/jf0114379 CCC: $22.00
Published on Web 05/03/2002

for major component analysis, however, is considerable, but surprisingly underutilized (*1*−*13*). QNMR is particularly suitable for the simultaneous determination of the percentage of active constituent and impurities in organic chemicals such as pharmaceuticals (*1, 2*), agrochemicals (*3*−*5*), and natural products (*6*−*9*), as well as vegetable oils (*10, 11*), fuels (*12*), and solvents (*13*), to cite some recent applications. The method is substance specific given that each substance has an NMR spectrum readily assigned to a structure from the wealth of well-established chemical shift and spin−spin coupling data and correlation methods (*15, 20*) available for many nuclei, together with the fact that peak area is proportional to the number of nuclei being measured (*14*−*16*). QNMR is a valuable concept for the analyst (*6*) that meets the requirements of a primary ratio analytical method (*16*) because the ratio of peak areas of analyte and reference material are used directly to calculate the amount of analyte present as shown in

$$N_{\mathrm{anl}} = N_{\mathrm{std}}\left(\frac{I_{\mathrm{anl}}}{I_{\mathrm{std}}}\right)\left(\frac{\rho_{\mathrm{std}}}{\rho_{\mathrm{anl}}}\right)\mathrm{P}_{\mathrm{std}} \qquad (1)$$

where anl is the analyte, std is the standard reference material, and N is the number of moles; I is the peak area; ρ is the number of nuclei of peak measured; and P is the purity.

One immediate advantage of QNMR is the ease of sample preparation. A reference material, unrelated to target analyte, is carefully selected so that its NMR peaks do not overlap or obscure those of the target analyte (*3*) and thereby simplify peak integration. This reference material may be mixed with the analyte and measured simultaneously, for what is commonly called the internal standard QNMR method (*1*−*14*), or it may be used in a sealed capillary (*8*). It should also be possible to establish the purity of a chemical by difference, that is, by quantifying all of the impurities (as well as moisture and ash) and subtracting the sum of these impurities from 100%, without recourse to adding a reference material. We will call this the difference method.

Limits to accuracy and precision for QNMR have been estimated overall to be ±1% (*17*), but in a detailed metrological comparison between the use of chromatographic techniques and QNMR (*3*), Maniara et al. demonstrated that the experimental precision of ^{1}H and ^{31}P QNMR was of the order of 0.5%, which rivaled results obtained by current chromatographic techniques. QNMR therefore provided a very reliable determination of chemical purity in major component analysis and was found to be universal, rugged, highly specific, and linear over a wide concentration range (*3*).

In this paper, we compare the use of ^{1}H QNMR with the chromatographic techniques of HPLC and GC that are frequently used for the determination of the percentage of active ingredient and all impurities >0.1% in two well-established agrochemicals: the widely used herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), **1**, and sodium 2,2-dichloropropionate (Dalapon sodium), **10** (*4*), a selective systemic herbicide used for the control of annual and perennial grasses. The study of **1** will show how the purity of the target analyte may be established by the QNMR difference method, whereas the analysis of **10** demonstrates that the use of a reference material of known purity, dimethyl sulfone (DMSO2), as an internal standard allows calculation of the purity of the target analyte and its impurities directly. In addition, uncertainty budgets for both of these methods were prepared to ascertain the major contributors to uncertainty in the measurements.

MATERIALS AND METHODS

Chemicals. Technical grade 2,4-D, **1**, was supplied as an off-white powder by Artfern Pty. Ltd. Technical grade Dalapon sodium, **10**, was obtained as pale gray/green powders from two different sources, denoted in the text as A (samples A1−A5) and C (samples C1−C5). 2,2-Dichloropropionic acid (99%) was obtained from Dr. Ehrenstorfer GmbH, Ausberg, Germany. DMSO2 (98%) and deuterium oxide (D_2O, 99.9 atom % D) were both used as supplied by Aldrich Chemical Co., Sydney, Australia. DMSO2 was determined to be 99.95% pure by differential scanning calorimetry. Deuteriochloroform ($CDCl_3$, 99.8 atom % D) and hexadeuteriodimethyl sulfoxide (dmso-d_6, 99.9 atom % D) were products of Cambridge Isoptopes Laboratory, Andover, MA. All samples were measured in Wilmad 5 mm o.d. 507pp tubes.

Instrumentation. NMR spectra were acquired with Bruker AVANCE 600 and 300 NMR spectrometers operating at 600.13 and 300.13 MHz for proton NMR, respectively, using 5 mm probes. All data was processed using Brukers' XWINNMR software, unless otherwise stated. The following parameters were employed for acquisition of spectra of **1**: 600 MHz; spectral width, 12 ppm; acquisition time, 4.56 s; relaxation delay, 6 s; 30° pulse width, 3 μs; time domain, 64K data points; 32 scans; temperature, 298 K. An exponential line broadening window function of 0.3 Hz was used in the data processing. After Fourier transformation of the free induction decays, the spectra were phased, baseline corrected, and integrated in the appropriate region. Integration was also carried out by first deconvoluting the spectra in the methylene region to determine relative peak areas, especially where peak overlap occurred using WINNMR-1D from Bruker. Because the relaxation behavior of ^{1}H nuclei of **10** was longer than that of **1**, the acquisition for spectra of **10** at 300 MHz was adjusted accordingly: spectral width, 8 ppm; acquisition time, 13.7 s; relaxation delay, 6.3 s; 90° pulse width, 6.8 μs; time domain, 64K data points; 16 scans; temperature, 298 K. The FIDs were processed as above for **1**. The peaks for the analyte and the internal standard were integrated inside, that is, excluding, the ^{13}C satellites.

Samples and standards were weighed on an OHAUS model Explorer electronic balance to ±0.1 mg.

Methods. *Difference Method.* **1** (~10 mg) was dissolved in a mixture of $CDCl_3$ (0.5 mL) and dmso-d_6 (0.15 mL) and transferred to an NMR tube. ^{1}H spectra were measured on the Bruker DMX-600 at 600.13 MHz, and the ^{1}H spin−lattice relaxation times, T_1, of the methylene peaks were 1−2 s, as measured by the inversion recovery (180°−relaxation delay−90°−acquire) pulse sequence (*5, 20*). After assignment of the spectra of the various components, the quantification of impurities was carried out by integration (and/or deconvolution) of minor signals in the methylene region of the spectrum (see **Figure 3A,B**) and calculation relative to one of the ^{13}C−H satellite signals, each 0.55% of the major component (*2*), **1**, using

$$\%\ \mathrm{impurity} = \frac{I_{\mathrm{imp}}}{I_{\mathrm{ref}}} \times \frac{\mathrm{MW}_{\mathrm{imp}}}{\mathrm{MW}_{\mathrm{ref}}} \times 0.55 \qquad (2)$$

where ref denotes main component; imp, impurity; MW, molecular weight; and I, peak area.

All percent values derived from the NMR data in the tables were corrected for the molecular weights of each of the organic impurities. The amounts present were then summed, and this subtotal was subtracted from 100% to give the percentage of **1** in each sample. The amounts of moisture, sulfated ash, and triethanolamine insolubles were determined separately by the supplier (**Table 1**, footnote).

Internal Standard Method. As a necessary prelude to QNMR measurements, **10** and DMSO2 were analyzed qualitatively by routine ^{1}H and ^{13}C NMR experiments. After assignment of the subspectra of the impurities, representative samples of **10** and DMSO2 were examined by ^{1}H NMR to determine the longest spin−lattice relaxation time, T_1, among peaks of interest using the inversion recovery sequence (*5, 20*).

10: δ_{H} (HOD, 4.6 ppm) 2.14($T_1 \sim 3$ s);
δ_{C} (ppm) 34.5 ($-CH_3$), 85.9 ($-CCl_2-$), 172.4 (C=O)

DMSO2: δ_{H} (HOD, 4.6 ppm) 3.05 ($T_1 \sim 4$ s)

Table 1. Content (Percent) of Substituted Chlorophenoxyacetic Acids in Five Technical Grade Samples of 2,4-D, **1**, Determined by HPLC, GC-MS,[a] and ^{1}H QNMR[b] Measurements

		substitution pattern of phenoxyacetic acid				
batch	analytical method[c]	2,4-dichloro[d]	2-chloro	4-chloro	2,6-dichloro	2,4,6-trichloro
5	HPLC (M)	99.3	0.50	NR[e]	ND[f]	0.17
	HPLC (A)	98.2	0.89	NR	NR	NR
	GC-MS[g]	98.2	0.80	ND	0.13	0.22
	QNMR	98.8	0.67	0.10	0.22	0.20
19	HPLC (M)	99.3	0.52	NR	0.02	0.17
	HPLC (A)	97.4	0.91	NR	NR	NR
	GC-MS	97.4	0.82	ND	0.12	0.30
	QNMR	98.9	0.74	0.04	0.11	0.18
50	HPLC (M)	99.3	0.56	NR	ND	0.11
	HPLC (A)	98.1	0.94	NR	NR	NR
	GC-MS	97.6	0.86	ND	0.10	0.23
	QNMR	99.0	0.63	0.06	0.13	0.20
110	HPLC (M)	99.4	0.31	NR	0.02	0.22
	HPLC (A)	96.2	0.76	NR	NR	NR
	GC-MS	98.0	0.66	ND	0.12	0.22
	QNMR	99.2	0.45	0.05	0.12	0.17
126	HPLC (M)	99.0	0.75	NR	ND	0.17
	HPLC (A)	97.7	2.00	NR	NR	NR
	GC-MS	97.0	1.65	0.17	0.10	0.24
	QNMR	98.1	1.51	0.16	0.11	0.16

[a] GC-MS values were calculated from the ratios of total ion current to the sum of total ion current for all relevant peaks and were not corrected for molecular weight differences. [b] Quantitative results obtained by the difference method (see text) and reported for impurities from NMR experiments at 600 MHz were corrected for molecular weight differences. [c] (M) = manufacturer's laboratory; (A) = Australian commercial laboratory. [d] 2,4-D percent purity values derived from GC-MS and ^{1}H QNMR results were calculated by subtracting the sum of all other organic impurities from 100%. Values used for free phenol, sulfated ash, moisture, and triethanolamine insoluble material were supplied by the manufacturer: 0.68, 1.48, 1.23, 0.97, and 0.88% for batches 5, 19, 50, 110, and 126, respectively. [e] NR = not reported [f] ND = not detected. [g] 0.11% 2,4-dichlorophenol also detected.

Other peaks in the ^{1}H NMR spectrum of technical grade Dalapon sodium had the following T_1 values: δ_H (HOD, 4.6 ppm) 4.17 ($T_1 \sim 3$ s); 4.29 ($T_1 \sim 9-10$ s); 5.7−6.1 ($T_1 \sim 9-10$ s).

These values were used to set acquisition parameters and allow for full relaxation, generally $5T_1 > 99.5\%$ recovery of peaks area of the major components during acquisition of the NMR data for quantitation. QNMR analysis required seven replicates of each sample of five batches of **10** from the two sources (A1−A5 and C1−C5), that is, 70 samples in all. Seven replicates were chosen to give a mean value for which the standard error encompassed the 95% confidence interval. Each sample of **10** (~50 mg; 0.3 mmol) was weighed individually into a sample tube followed by an aliquot of the internal standard, DMSO2, as a stock solution in D_2O (22.94 mg/g) to obtain a molar ratio of ~2:1 analyte to standard, ensuring that the NMR peaks from the respective methyl groups of both analyte and internal standard would be of similar intensities. The solution was then transferred to an NMR tube and diluted further with D_2O to a constant volume (0.7 mL) for all repeat measurements so that magnetic field homogeneity could be efficiently attained for each sample during the QNMR experiments. All samples of the analyte and internal standard were measured using the optimized parameter ^{1}H NMR set, and peak areas obtained were then used to derive percent purity using eq 1.

HPLC analytical results for **1** were supplied by the manufacturer, denoted (M) in **Table 1**, and repeated by a commercial laboratory in Sydney, denoted (A), according to the published method of the Collaborative International Pesticides Analytical Council (CIPAC) (*18*). **10** was analyzed according to the published chromatographic CIPAC method (*19*). The methyl esters of technical grade **10** were prepared for GC-MS analysis by dissolving in H_2O (2 mg in 60 μL), acidifying with 1% acetic acid in methanol (200 μL), and treating with ethereal diazomethane until the faint yellow color persisted. The excess of diazomethane was removed in a stream of nitrogen (volume decrease by ~50%). The remaining solution was dried (Na_2SO_4) and used directly. For both HPLC and GC-MS, peak area ratios for the impurities were used and are uncorrected for detector response and molecular weight of species.

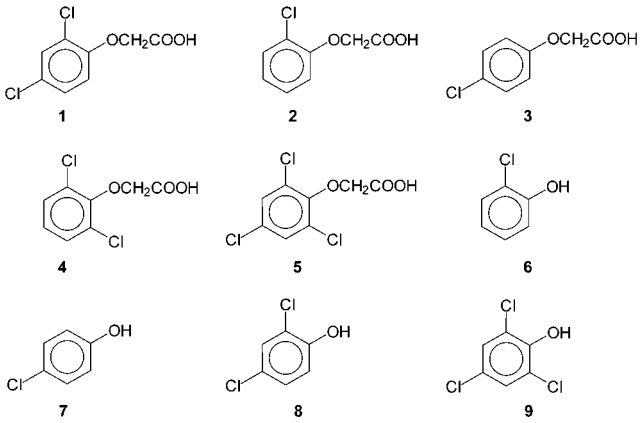

Figure 1. Chlorophenoxyacetic acids and chlorophenols that may be present as impurities in technical grade samples of 2,4-D, **1**.

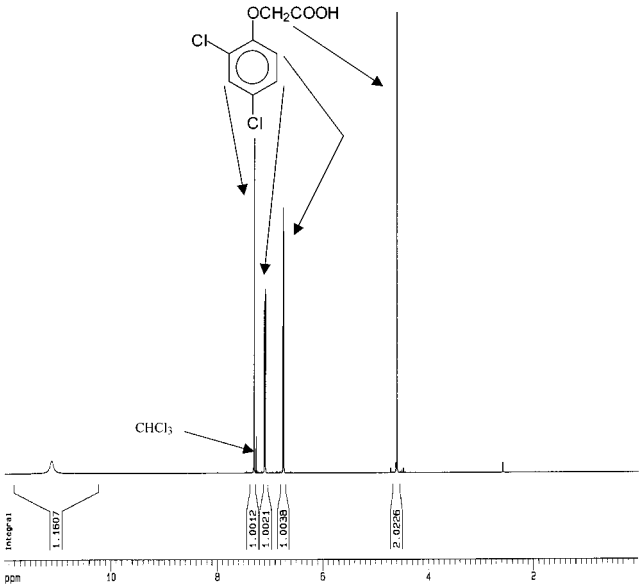

Figure 2. 600 MHz ^{1}H NMR spectrum of 2,4-D, **1**, in $CDCl_3$ + DMSO-d_6 demonstrating the uniformity of integration across the spectrum.

RESULTS AND DISCUSSION

Difference QNMR Analysis of Technical Grade 2,4-D, 1. The major impurities that may occur in 98% technical 2,4-D, **1**, are shown in **Figure 1** and are predominantly other chlorinated phenoxyacetic acids, **2**−**5**, together with a mixture of chlorophenols, **6**−**9**, of which 2,4-dichlorophenol, **8**, is the major constituent. However, the quantity of the combined chlorophenols was <0.3% according to the manufacturer.

The manufacturer claimed >99% purity for **1** using HPLC, with the major impurities identified as 2-chlorophenoxyacetic acid, **2**, and 2,4,6-dichlorophenoxyacetic acid, **5**, but because these results were at odds with those from HPLC of the acids and GC-MS analysis of the derived methyl esters as shown in **Table 1**, we investigated the purity determination of **1** using the QNMR difference method.

The use of 600 MHz ^{1}H NMR spectroscopy was crucial for allowing all impurities to be rapidly identified and quantified from a single spectrum, because there was insufficient dispersion at the lower field frequency of 300 MHz. Two important points

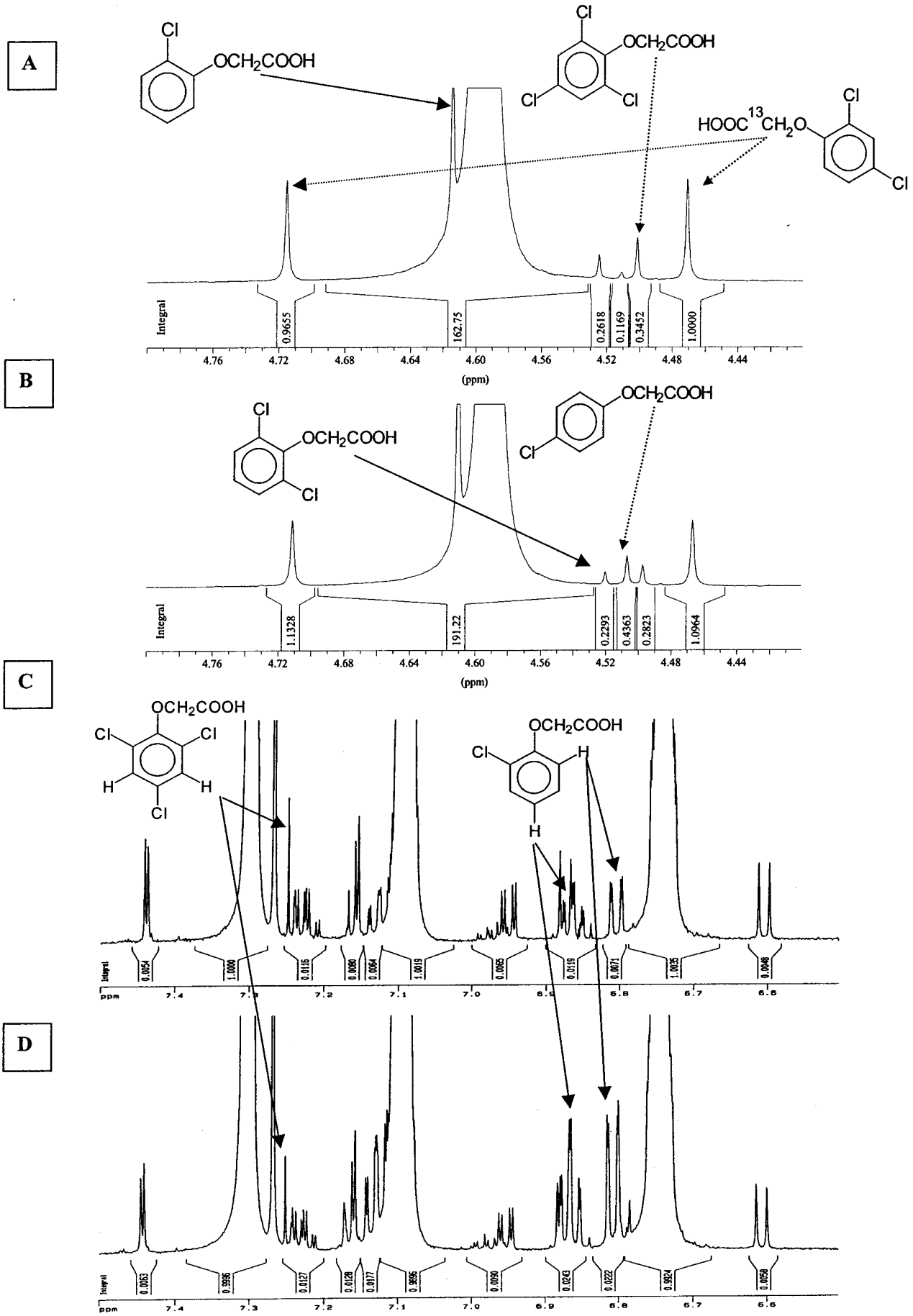

Figure 3. Expansions of the 600 MHz ^{1}H NMR spectrum of two batches of 2,4-D, **1**, in $CDCl_3$ + DMSO-d_6: (A) sample 19; (B) sample 126, methylene region; (C) sample 19; (D) sample 126, aromatic region.

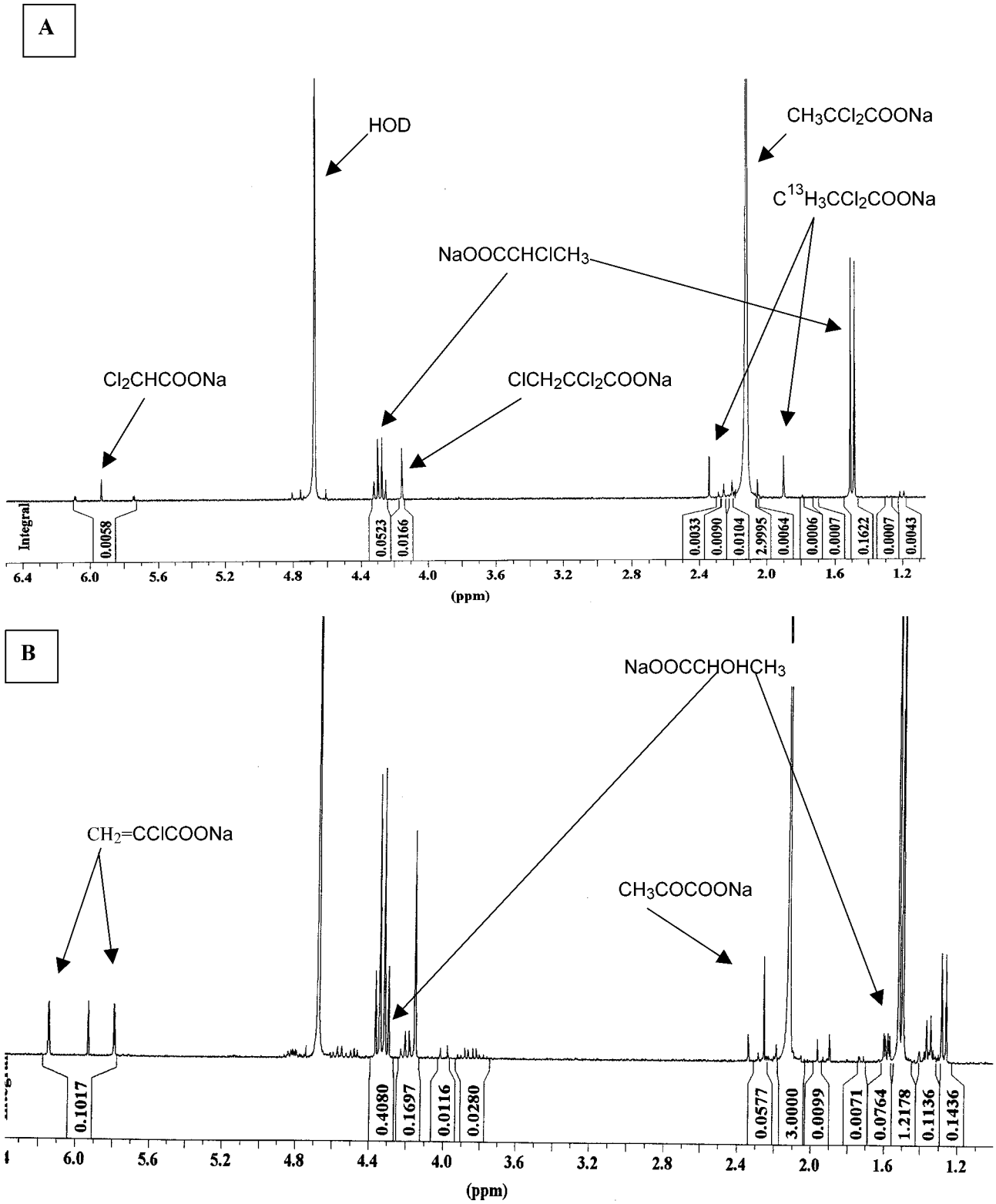

Figure 4. 300 MHz ^{1}H NMR spectra of Dalapon sodium, **10**, in D_2O showing (A) sample acceptable for registration and (B) sample unacceptable for registration.

are noteworthy: The ^{1}H NMR spectrum of technical grade **1** shown in **Figure 2** reveals high uniformity of integration values across the spectrum, and the peaks of the methylene groups of all the chlorophenoxyacetic acids present in the technical grade material are sufficiently resolved at δ_H ~4.5 and occur as singlets, simplifying analysis for assignment and quantitation (see **Figure 3A**,**B**). Interpretation of the NMR spectra was simplified by the presence of considerably more 2-chlorophenoxyacetic acid, **2**, in one sample (126, 1.5%) than was found in the other four (<0.8%). Details of the aromatic region (δ_H ~7.5−6.5) of two technical samples (19 and 126) are presented in **Figure 3**,**D** as evidence supporting the assignments.

As would be expected on mechanistic grounds, the percentage of 4-chlorophenoxyacetic acid, **3**, detected in all samples relative

Table 2. Comparison of Percent Purity of Dalapon Sodium, **10**, by ^{1}H QNMR at 300 MHz and by HPLC

	^{1}H QNMR					
	difference method[a]	internal standard method[b]		HPLC[c]		
sample	**10** (%)	**10**	SD (%)	**10** (%)	SD (%, $n = 6$)	moisture[d]
A1	94.0	91.1	2.08 (n= 7)	90.2	3.48	0.35
A2	93.7	90.7	0.41 (n = 6)	91.7	0.25	0.40
A3	65.8	61.6	0.70 (n = 7)	69.0	0.83	1.00
A4	65.8	60.4	0.26 (n = 6)	69.5	0.23	0.50
A5	91.7	88.2	0.39 (n= 6)	90.7	0.40	1.00
C1	93.8	93.5	1.77 (n = 6)	98.2	0.95	1.06
C2	95.6	96.4	1.18 (n = 6)	97.9	0.52	1.04
C3	94.7	97.4	0.88 (n = 7)	99.2	0.68	1.02
C4	95.5	96.1	0.51 (n =7)	98.3	0.97	1.02
C5	95.0	97.5	0.61 (n =7)	98.5	0.23	0.85

[a] Not corrected for moisture and inorganic salts. [b] Measured with respect to DMSO2 (see Materials and Methods). [c] According to CIPAC method (*19*), calibrated with respect to 2,2-dichloropropionic acid certified at 99%. [d] Determined by Karl Fischer method.

to that of **2** remained constant, enabling its assignment (**Figure 3**). Similarly, the methylene and aromatic singlet signals of 2,4,6-trichlorochlorophenoxyacetic acid, **5**, (singlet at δ_H 7.25) may be easily assigned by comparison of the expanded ^{1}H NMR spectra of the two samples.

The QNMR results in **Table 1**, derived from the spectra using eq 2 based on the area of the ^{13}C satellite peak of **1** (*2*), reveal that the manufacturer's claims of >99% purity could not be substantiated for three of the five samples of **1** and that only ^{1}H QNMR afforded a comprehensive profile of the impurities present. ^{1}H QNMR therefore provided a superior alternative to the chromatographic methods for both determining the purity of **1** and simultaneously allowing identification and quantification of all major organic impurities.

Analysis of Purity of and Impurities in Technical Grade Dalapon Sodium, 10. The ^{1}H NMR spectrum, run in D_2O at 300 MHz, of a typical technical grade sample of Dalapon sodium, **10**, is shown in **Figure 4A**, together with the assignment of the peaks due to the major impurities. **Figure 4B** shows a ^{1}H 300 MHz spectrum of a distinctly inferior technical sample of **10**.

The percentage purity of **10** was first determined by using the difference method as described above for **1**, and the results for 10 different samples of **10** are presented in **Table 2**.

The quantification of **10** using DMSO2 as an internal standard was also performed. DMSO2 was chosen because it is readily available in high purity, is inexpensive, is readily soluble and inert, and has a very simple NMR spectrum consisting of a single six-proton singlet. Commercially available DMSO2 showed no signals from impurities in the 600 MHz ^{1}H spectrum between 0 and 11 ppm. The purity of this material was also tested by DSC, an independent primary method of analysis, and found to be >99.9% pure. Moisture content, determined by the Karl Fischer method was <0.05%.

The mean purities of six or seven separate determinations for each of the 10 samples of **10**, with DMSO2 as an internal standard, are presented in **Table 2**. The results demonstrate satisfactory relative standard deviations and quantitative values consistently 3−4% lower than those obtained by the difference method for the samples A from one supplier (A1−A5), but not lower than those from the other supplier (C1−C5) (**Table 2**) for reasons that are unclear. Values for the moisture content, determined separately, are also shown in **Table 2**. Discrepancies between percent purity values obtained by the difference method and those obtained by the internal standard method may be in part explained by the presence of variable amounts of moisture and inorganic salts that were not included in the calculations. Total inorganic salts present in samples of **10** were not determined in this work.

Comparison of ^{1}H QNMR and HPLC Methods for Analysis of Dalapon Sodium, 10. The purity of **10** is usually established by the official CIPAC HPLC method (*19*), which also gives methods for the quantitation of the likely impurities, which are listed in **Tables 3** and **4**, namely, the sodium salts of 2-chloropropionic acid, **11**, 2,2,3-trichloropropionic acid, **12**, chloroacetic acid, **13**, dichloroacetic acid, **14**, and trichloroacetic acid, **18**. There are several fundamental weaknesses of the official HPLC method from CIPAC. First, it is necessary to quantitate **10** and all impurities by UV detection at 214 nm, a measurement that can be unduly influenced by small amounts of highly absorbing impurities such as acrylic acids, **16**. Second, major impurities can be incorrectly identified by HPLC, when it is based only on comparison with the retention time of a standard reference material. Third, certified standards for many of the anticipated impurities are not commercially available. Finally, minor impurities in excess of 0.1%, such as lactic acid, **15**, 2-chloroacrylic acid, **16**, and pyruvic acid, **17**, can be readily identified by NMR (see **Table 3**) but are not included in the CIPAC HPLC method (*19*).

Whether HPLC or GC was used for analysis, it was impossible to accurately quantify **10** and all associated impurities in technical grade material without having access to standard reference materials for all substances present. Comparison of results obtained by QNMR and HPLC are shown in **Tables 2** and **3** and demonstrate the shortcomings of the official CIPAC HPLC method for performing purity analysis of **10**. In **Table 2** the purity of **10** was determined by HPLC by comparing the peak due to **10** with an external standard of 2,2-dichloropropionic acid certified at 99% purity. The HPLC results are in reasonable agreement with those obtained by the QNMR method, using DMSO2 as an internal standard, for one set of samples of uneven quality, A1−A5, but for the second set, C1−C5, of a more consistent quality, the HPLC method gives significantly higher values that the QNMR method.

In **Table 3**, on the other hand, the relative amounts of **10** and impurities were calculated from the ratio of a particular peak area to the total area of all peaks from the HPLCl that is, no standard reference materials were used to calibrate the detector response. Inspection of **Table 3** reveals a significant difference in results obtained by QNMR and HPLC, and therefore this assumption that there is a uniform response factor for each component is clearly incorrect. Furthermore, analysis of the certified reference material, 2,2-dichloropropionic acid, gave a purity of only 75% based on the detection of all peaks at 214 nm. In addition, HPLC quantitation of **10** by peak ratios, shown in **Table 3**, is clearly not consistent with the impurity profile obtained by QNMR. For example, one major impurity, **12**, in samples A, is clearly different from that present in samples C, that is, **13**, and the HPLC method was unable to recognize this difference.

A GC-MS impurity profile was also obtained for samples C and is shown in **Table 4**. Because **10** is highly water soluble, as are all the most probable impurities, the conversion of the carboxylate salts to esters for GC or GC-MS analysis is not routine, with the underlying assumption that the mixture of esters prepared from technical grade carboxylate salts contains the same relative ratio that was present in the original sample.

Table 3. Comparison of ^{1}H QNMR Results Obtained at 300 MHz by the Difference Method[a] and HPLC[b] Results Showing Purity of and Impurities in Technical Grade Dalapon Sodium, **10**

	substance[c] (%)												
	10		**11**		**12**		**13**		**14**		**15**	**16**	**17**
sample	NMR	HPLC	NMR	HPLC	NMR	HPLC	NMR	HPLC	NMR	HPLC	NMR	NMR	NMR
A1	94.0	82.3	4.0	2.7	0.9	3.1	ND	3.6	0.5	1.2	0.1	ND	0.1
A2	93.7	85.4	2.2	2.4	3.4	3.2	ND	2.3	0.2	0.9	ND	2.2	0.3
A3	65.8	49.6	20.8	24.6	5.7	3.0	ND	9.7	0.3	2.2	2.3	2.3	1.3
A4	65.8	47.6	21.0	27.7	5.6	3.0	ND	9.4	0.3	2.0	2.1	0.7	1.8
A5	91.7	78.4	4.6	6.0	0.2	3.0	ND	3.2	0.9	2.0	0.5	ND	0.7
C1	93.8	84.0	4.2	2.6	0.1	2.0	0.7	2.4	1.3	1.6	ND	ND	0.1
C2	95.6	82.4	3.1	2.4	0.1	3.5	0.8	2.4	0.8	1.6	ND	ND	0.1
C3	94.7	83.3	3.3	2.8	0.1	2.4	0.1	2.4	0.9	1.7	ND	ND	0.1
C4	95.5	83.1	3.2	2.9	<0.1	2.4	0.5	2.4	0.7	1.7	ND	ND	0.1
C5	95.0	82.3	3.5	3.0	<0.1	2.4	0.6	2.5	0.9	1.9	ND	ND	0.1
standard[d]	NM	75.7	NM	2.0	NM	2.2	NM	2.6	NM	2.2	NM	NM	NM

[a] Quantitation in the QNMR difference method (see text) used in this table represents the ratio of total signal, corrected for differences in molecular weight. [b] Using the ratio of a peak area to the total area for all peaks in the HPLC trace assuming a response factor of 1 for each component. [c] **10** = 2,2-dichloropropionic acid, sodium salt; **11** = 2-chloropropionic acid; **12** = 2,2,3-trichloropropionic acid; **13** = chloroacetic acid; **14** = dichloroacetic acid; **15** = lactic acid; **16** = 2-chloroacrylic acid; **17** = pyruvic acid. ND = not detected; NM = not measured. [d] Certified reference standard of 2,2-dichloropropionic acid, 99% purity.

Table 4. Comparison of ^{1}H QNMR[a] Results Obtained at 300 MHz by the Difference Method and GC-MS[b] Results Showing Purity of and Impurities in Technical Grade Dalapon Sodium, **10**

	10[c]		**11**[c]		**12**[c]		**13**[c]		**14**[c]		**17**[c]	**18**[c]
sample	NMR	GC-MS	NMR	GC-MS	NMR	GC-MS	NMR	GC-MS	NMR	GC-MS	NMR	GC-MS
C1	93.8	94.8	4.2	4.2	0.1	0.22	0.7	ND	1.3	0.41	0. 1	0.1
C2	95.6	95.6	3.1	3.9	0.1	0.30	0.8	ND	0.8	ND	0.1	0.1
C3	94.7	95.3	3.3	3.9	0.1	0.30	0.1	ND	0.9	ND	0.1	0.1
C4	95.5	94.6	3.2	4.1	<0.1	0.34	0.5	ND	0.7	ND	0.1	0.1
C5	95.0	95.3	3.5	3.1	<0.1	0.32	0.6	ND	0.9	ND	0.1	0.1

[a] Results of QNMR difference method (see text) used in this table represent the ratio of impurity peak area to total peak area, corrected for differences in molecular weight. [b] GC-MS method, using the ratio of a peak area to the total area for all peaks in the GC trace, assuming a response factor of 1 for each component. [c] **10** = 2,2-dichloropropionic acid, sodium salt; **11** = 2-chloropropionic acid; **12** = 2,2,3-trichloropropionic acid; **13** = chloroacetic acid; **14** = dichloroacetic acid; **17** = pyruvic acid; **18** = trichloroacetic acid. ND = not detected.

Although the profile generated with this set was not standardized with reference materials, the results compare moderately well with those from QNMR analysis, suggesting that this underlying assumption may have some merit. Note, however, that GC-MS analysis failed to detect amounts of **13** and **14**, readily detected and quantified by QNMR as shown in **Table 4**.

Uncertainty Budgets for QNMR. The recently introduced ISO Guide 17025 requires the establishment of an uncertainty estimate for results reported (*21*). This has led to a discussion of the relative merits of uncertainties established by so-called "bottom up" and "top down" methods (*22*, *23*). "Bottom up" refers to a theoretical approach in which the relationship between individual measurement steps and the final result is established and the uncertainties of the individual steps propagated to that of the final result. In "top down" methods, precision estimates are obtained from repeated measurements conducted in such a way that the method is exposed to the full gamut of possible sources of uncertainty. Practical applications combine aspects of both approaches.

The uncertainty associated with the measurement of relative peak area for the calculation of purity based on the difference method concerns the linearity of peak heights across the NMR spectrum and the sufficiency of the establishment of the baseline. The completeness of the impurities observed is not part of the uncertainty budget but a source of systematic error in the method as a whole.

Difference Method. The purity of an analyte as deteremined by the difference method relies on the relative peak areas of different compounds calculated according to the equation

$$\%\ \text{purity} = \frac{w_{\text{anl}}}{w_{\text{anl}} + \Sigma_i\, w_i} \tag{3}$$

where w_i is the weight of impurity i expressed as $w_i = (\text{MW})_i I_i / \rho_i$ and w_{anl} as $w_{\text{anl}} = (\text{MW})_{\text{anl}} I_{\text{anl}} / \rho_{\text{anl}} \alpha$. ρ and I are defined as before, and MW is the molecular weight of the compound. For the analyte, the area of the ^{13}C satellite peak is chosen to provide an area commensurate with the impurity peaks, and so the equation is divided by $\alpha = 0.0055$. The uncertainty in w therefore essentially resides in the measurement of peak area (I). The uncertainties in molecular weights are negligible; ρ is a known integer, and the parameter α is the peak area of the ^{13}C satellite conventionally set at 0.55%. As all peak area measurements are made from the same spectrum, we need only be concerned about the peak to peak repeatability and the linearity of the spectrum. The latter includes a number of instrumental parameters and the choice of baseline, but can be estimated. The uncertainty, u, in the purity is therefore expressed in terms of the uncertainties of each peak area:

$$\frac{u_{\text{purity}}}{\text{purity}} = \sqrt{\left(\frac{u_{I_{\text{analyte}}}}{I_{\text{analyte}}}\right)^2 + \sum_i \left(\frac{u_{I_i}}{I_i}\right)^2} \tag{4}$$

If the simplifying assumption may be made that there is a

common uncertainty, u_{peak}, in the measurement of a peak area, the overall uncertainty in the purity is $u_{purity} = u_{peak}\sqrt{n}$, where n is the total number of peaks measured (analyte plus impurities). Any constant, relative systematic error in the measurement of peak area will cancel in the determination of purity. We estimate u_{peak}/I_{peak} to be 0.2%, and so for the compounds listed in **Table 1**, $n = 6$ and the expected relative uncertainty in the purity of **1** by the difference method is 0.5%.

Internal Standard Method. The uncertainty of a measurement that takes the ratio of the area of an analyte peak to that of an internal standard depends on the purity of the standard, the uncertainties in the weights of standard and sample used in making the measured solution, and the uncertainties in the peak areas. The greatest uncertainty, ~0.5%, resides in the uncertainty of the purity of the standard, and this translates to a similar uncertainty in the purity of the analyte. Because the purity of such chemicals is typically near 100%, whether the uncertainty is expressed as a relative uncertainty or an absolute percentage does not affect the final result significantly. We have repeated the experimental design of Maniara et al. (*3*) with **10** as the analyte and DMSO2 as the internal standard, in which three weighings, two independent acquisitions of the spectra, and two independent processings of the data files were nested within two analysts using two spectrometers, giving 48 experiments performed in total. In a fixed effects model the categories of "analyst" and "spectrometer" were significant, and the overall standard deviation of the data was 0.36%. The design and methodology will be described in detail elsewhere.

For some samples the standard deviation for replicate measurements is greater than the estimates here (sample A1; **Table 2**). We infer that the intrabatch variability of the samples of **10** contributed the majority of the observed variance.

Conclusion. QNMR analysis of agrochemicals in this paper is both more accurate and more precise than standard HPLC methods. The analysis of percent active ingredient and impurities may be carried out from a single data set by the difference method or by using an internal standard reference material. In principle, these two methods should give the same result, provided that all organic impurities can be separately identified and quantified in the NMR spectrum and that all inorganic impurities are quantified separately. In practice, these conditions were satisfied with 2,4-D, **1**, whereas for Dalapon sodium, **10**, the inorganic impurities were not quantified.

QNMR also allows identification and quantification of the major components and their organic impurities unobtainable by the chromatography methods, particularly with the many representatives that are unsuitable for direct HPLC or GC determination because they lack a suitable UV chromophore or are highly polar. Sample preparation for QNMR requires fewer steps, and there is no need for derivatization as with GC-MS, nor for standard reference materials for each substance determined, thereby avoiding assumptions inherent in derivatization regimes and detector response. Indeed, standard reference materials were not available for many of the impurities present in technical grade **1** and **10**, and this lack of availability would preclude accurate determination of an impurity profile for **10** by HPLC, GC-FID, or GC-MS because the relative detector response factors of each impurity are mandatory for determining the amount present.

Finally, DMSO2, used here as an internal standard for the quantification of Dalapon sodium, **10**, fulfills the conditions of being an excellent internal standard for many QNMR measurements in that it is readily available in a highly pure commercial form that is inexpensive, stable, and soluble in both organic solvents and water and has a simple NMR spectrum at a convenient chemical shift (*5*). Because NMR is a primary ratio method (*16*), DMSO2 also represents a standard reference material against which the purity of all other standards might be conveniently assessed.

ACKNOWLEDGMENT

We thank Dr. G. Ball for fruitful discussions, H. Stender for expert assistance obtaining NMR spectra, and Dr. L. Besley of the Australian National Measurement Laboratory for the measurements of DMSO2 by DSC.

LITERATURE CITED

(1) Rackham, D. M. Recent Applications of Quantitative NMR in Pharmaceutical Research. *Talanta* **1976**, *23*, 269−274.

(2) Holzgrabe, U.; Diehl, B. W. K.; Wawer. I. NMR Spectroscopy in Pharmacy. *J. Pharm. Biomed. Anal.* **1998**, *17*, 557−616.

(3) Maniara, G.; Rajamoorthi, K.; Rajanand, S.; Stockton, G. W. Method Performance and Validation for Quantitative Analysis by ^{1}H and ^{31}P NMR Spectroscopy. Applications to Analytical Standards and Agricultural Chemicals. *Anal. Chem.* **1998**, *70*, 4921−4928, and references cited therein.

(4) Wells, R. J.; Saed Al-Deen, T.; Hook, J. M.; Hibbert, D. B. *Proceedings of the 3rd Biennial Conference*; Metrology Society of Australia: Melbourne, 1999; pp 261−265. Wells, R. J.; Hook, J. M.; MacKay, J. *Proceedings of the 3rd Biennial Conference*; Metrology Society of Australia: Melbourne, 1999; pp 267−271.

(5) Wells, R. J.; Cheung, J. Quantitative Analysis of Technical Grade Agrochemicals by High Field ^{1}H NMR Spectroscopy. *The Chemistry Preprint Server CPS*:analchem/0103002, 2001.

(6) Pauli, G. F. qNMR−A Versatile Concept for the Validation of Natural Product Reference Compounds. *Phytochem. Anal.* **2001**, *12*, 28−42, and references cited therein.

(7) Brasch, N. E.; Finke, R. G. A Simple, Convenient and Direct Method for Assessing the Purity of Cobalamins. *J. Inorg. Biochem.* **1999**, *73*, 215.

(8) Larive, C. K.; Jayawickrama, D.; Orfi, L. Quantitative Analysis of Peptides with NMR Spectroscopy. *Appl. Spectrosc.* **1997**, *51*, 1531.

(9) Ahvazi, B. C.; Crestini, C.; Argyropoulos, D. S. ^{19}F Nuclear Magnetic Resonance Spectroscopy for the Quantitative Detection and Classification of Carbonyl Groups in Lignans. *J. Agric. Food Chem.* **1999**, *47*, 190−201.

(10) Vlahov, G.; Schiavone, C.; Simone, N. Quantitative ^{13}C NMR Method Using the DEPT Pulse Sequence for the Determination of the Geographical Origin (DOP) of Olive Oils. *Magn. Reson. Chem.* **2001**, *39*, 689−695.

(11) Spyros, A.; Dais, P. Application of P-31 NMR Spectroscopy in Food Analysis. 1. Quantitative Determination of the Mono- and Diglyceride Composition of Olive Oils. *J. Agric. Food Chem.* **2000**, *48*, 802−805.

(12) Meusinger, R. Qualitative and Quantitative determination of Oxygenates in Gasolines using ^{1}H Nuclear Magnetic Resonance Spectroscopy. *Anal. Chim. Acta* **1999**, *391*, 277−288.

(13) Clarke, D. Acetone and Ethyl Acetate in Commercial Nail Polish Removers: A Quantitative NMR Experiment Using an Internal Standard. *J. Chem. Educ.* **1997**, *74*, 1464. Lefevre, J. W.; Silveira, A. Quantitative Determination of Methylcyclohexanone Mixtures Using ^{13}C NMR Spectroscopy. *J. Chem. Educ.* **2000**, *77*, 83−85, and references cited therein.

(14) Rabestein, D.; Keire, D. A. Quantitative Chemical Analysis by NMR. In *Modern NMR Techniques and Their Application in Chemistry*; Popov, A. I., Hallenga, Eds.; Dekker: New York, 1991; Vol. 11, pp 323−369.

(15) Derome, A. E. *Modern NMR Techniques for Chemistry Research*; Pergamon Press: Oxford, U.K., 1989; pp 168−172.

(16) Jancke, H. NMR Spectroscopy as a Primary Analytical Method. *CCQM Rep.* **1998**, *98* (2), 1−12.

(17) Griffiths, L.; Irvine, A. M. Assay by Nuclear Magnetic Resonance Spectroscopy: Quantification Limits. *Analyst* **1998**, *123*, 1061−1068.
(18) CIPAC (Collaborative International Pesticides Analytical Council). *Handbook*; Black Bear Press: Cambridge, U.K., 1995; Vol. 1C, p 2062.
(19) CIPAC (Collaborative International Pesticides Analytical Council). *Handbook*; Black Bear Press: Cambridge, U.K., 1988; Vol. D, p 54.
(20) Sanders, J. K. M.; Hunter, B. K. *Modern NMR Spectroscopy: A Guide for Chemists*; Oxford University Press: Oxford, U.K., 1990; Chapter 3, pp 61−65.
(21) ISO/IEC. *General Requirements for the Competence of Calibration and Testing Laboratories*; Guide 17025; ISO: Geneva, Switzerland, 2000.
(22) ISO. *Guide to the Expression of Uncertainty in Measurement*; ISO: Geneva, Switzerland. 1995.
(23) EURACHEM. *Quantifying Uncertainty in Analytical Measurements*; EURACHEM Secretariat: Teddington, U.K., 1995.

Received for review October 30, 2001. Revised manuscript received March 11, 2002. Accepted March 15, 2002.

JF0114379