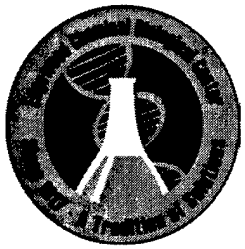

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ECBC-TR-416

NMR METHOD FOR THE QUANTITATIVE ANALYSIS OF LIQUID SAMPLES

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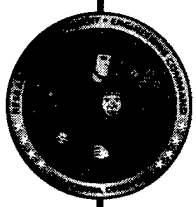
January 2005



ABERDEEN PROVING GROUND, MD 21010-5424

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
XX-01-2005	Final	Oct 2001 - Sep 2002

4. TITLE AND SUBTITLE
NMR Method for the Quantitative Analysis of Liquid Samples

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
O'Connor, Richard J.; Brickhouse, Mark D.; Rice, Jeffrey S.; Durst, H. Dupont (ECBC); McGarvey, David J.; Creasy, William R.; Pence, John; and Montgomery, Jennifer L. (EAI Corporation)

5d. PROJECT NUMBER
62262255200

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES)
DIR, ECBC, ATTN: AMSRD-ECB-RT-PD/AMSRD-ECB-RT-PC, APG, MD 21010-5424
EAI Corporation, 1308 Continental Drive, Suite J, Abingdon, MD 21009

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-416

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
This procedure is based on previously published ERDEC-TR-449, Nuclear Magnetic Resonance (NMR) Analysis of Chemical Agents and Reaction Masses Produced by their Chemical Neutralization, and ECBC-TR-253, NMR Method for the Quantitative Purity Analysis of Feedstock Samples. The procedure replaces the method ACT-021. This procedure uses an internal standard to establish an absolute weight percent for the analytes in question. Identifying the structures of other components in the mixture is not required to determine the weight percent of the analyte. The weight percent calculations are not negatively affected by the presence of undetectable components (e.g., elemental sulfur, inorganic salts, etc.) in the sample.

15. SUBJECT TERMS
NMR
Nuclear Magnetic Resonance
Quantitative

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Sandra J. Johnson
U	U	U	UL	18	19b. TELEPHONE NUMBER *(include area code)* (410) 436-2914

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PREFACE

This work described in this report was authorized under Project No. 62262255200. The work was started in October 2001 and completed in September 2002.

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NMR METHOD FOR THE QUANTITATIVE ANALYSIS OF LIQUID SAMPLES

1. INTRODUCTION

1.1 Purpose.

This procedure is based on the previously published ERDEC-TR-449[1] and ECBC-TR-253.[2] The procedure replaces the method ACT-021. This procedure uses an internal standard to establish an absolute weight percent for the analytes in question. Identifying the structures of other components in the mixture is not required to determine the weight percent of the analyte. The weight percent calculations are not negatively affected by the presence of undetectable components (e.g., elemental sulfur, inorganic salts, etc.) in the sample.

1.2 Analyte Concentration Range.

Analytes can be quantified at any concentration level from the detection limit to neat material. The following detection limit study (Tables 1 and 2) was performed using proton NMR on the Bruker AC-300. Detection limits for other nuclei and other instruments were estimated using the knowledge of the operator. Detection limits will depend on the matrix and interferences. If detection limits are required for a particular measurement, they should be determined for the particular matrix, analyte, and instrument and given in the analysis report.

1.3 Sample Matrices and Interferences.

This method can be performed on liquid matrices that solubilize the internal standard. Generally, either TEP (triethylphosphate) or HMPA (hexamethylphosphoramide) have been soluble in each AWCA matrix. Other internal standards may be used, as long as they are stable and the signals from the standard do not interfere with other peaks in the spectra. For basic matrices, TEP is the preferred internal standard. The internal standards must also be commercially available with a high purity.

Paramagnetic metals such as iron and chromium at fairly low concentrations will broaden the peaks and either increase the error of the analysis, or invalidate it entirely. Ongoing research on the use of Tiron as an iron chelator and spin-relaxation agent may partially solve this problem in the future.

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[1] Brickhouse, M.D.; Rees, M.S.; O'Connor, R.J.; Durst, H.D. *Nuclear Magnetic Resonance (NMR) Analysis of Chemical Agents and Reaction Masses Produced by Their Chemical Neutralization*; ERDEC-TR-449; U.S. Army Edgewood Research, Development and Engineering Center: Aberdeen Proving Ground, MD, 1997; UNCLASSIFIED Report (AD-A339 308).

[2] O'Connor, R.J.; Brickhouse, M.D.; McGarvey, D.; Durst, H.D.; Creasy, W.R.; Ruth, J.L. *NMR Method for the Quantitative Purity Analysis of Feedstock Samples*; ECBC-TR-253; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2002; UNCLASSIFIED Report (AD-A406 815).

Table 1. ACWA P&A Results for 50 ppm TDG Standard in D_2O on AC-300 Using Proton NMR Results for H_2O Solvent Suppression, Standard in DI Water, 8 Runs on Each of Two Days

Sample No. NB130P22C
Integration from NUTS, IS=HMPA, compound is TDG

File	Int. IS	Int. cmp.	µg/g sample (ppm)
AR090F.105	1031556	169.03	45.07
AR091F.105	1033091	170.46	45.39
AR092F.105	1030570	164.89	44.01
AR093F.105	1030391	140.83	37.60
AR094F.105	1027744	148.59	39.77
AR095F.105	1027010	135.38	36.26
AR096F.105	1030428	175.91	46.96
AR097F.105	1028647	159.97	42.78
AR090F.107	1059223	178.18	46.27
AR091F.107	1056418	182.84	47.61
AR092F.107	1057849	184.3	47.92
AR093F.107	1059150	180.48	46.87
AR094F.107	1057912	179.9	46.78
AR095F.107	1058795	189.81	49.31
AR096F.107	1056339	156	40.62
AR097F.107	1056515	184.06	47.92
Average Concentration (ppm)			44.45
Standard deviation			3.95
MDL (ppm)			10.27
Sample concentration			45.81
Recovery %			97.02%

Table 2. ACWA P&A Results for 200 ppm TDG Standard in ACWA Sample on AC-300 Using Proton NMR Results for no H_2O Solvent Suppression, 8 Runs on Each of Two Days

ACWA Sample CAT 04302-1, FBSC03HA01KX

Sample No. NB130P23C
Integration from NUTS, IS=HMPA, compound is TDG

File	Int. IS	Int. cmp.	µg/g sample (ppm)
AR090F.110	100000	98.72	247.99
AR091F.110	99809	65.79	165.59
AR092F.110	98407	92.07	235.03
AR093F.110	99030	65.59	166.38
AR094F.110	97825	39.24	100.77
AR095F.110	98619	57.3	145.96
AR096F.110	98380	75.06	191.66
AR097F.110	97904	43.57	111.80
AR090F.112	98588	55.45	141.29
AR091F.112	98575	69.54	177.22
AR092F.112	99136	34.16	86.56
AR093F.112	98622	45.76	116.56
AR094F.112	98222	58.74	150.23
AR095F.112	98559	48.33	123.18
AR096F.112	98294	53.45	136.60
AR097F.112	98301	65.26	166.77
Average Concentration (ppm)			153.97
Standard deviation			44.62
MDL (ppm)			116.11
Sample concentration			202.3
Recovery %			76.11%

1.4 Throughput.

Sample throughput depends on the nucleus that is being used for the analysis, and the sensitivity required. A typical experiment may allow three samples to be analyzed in an 8-hr day. The best throughput for proton NMR runs is a sample per hour, or 8 samples in an 8-hr day. With an autosampler, samples can be run 24 hr a day.

2. RISK AND SAFETY ASSESSMENT

All analyses should be performed in accordance with all appropriate Federal, State and local laws, as well as Army Regulations. Samples containing chemical agent should be handled in accordance with AR 50-6 and all other applicable regulations. Exposure to chemical agents or other super toxic materials may result in injury or death.

3. SCIENTIFIC BASIS

NMR spectroscopy has been a proven method for the identification and quantification of chemical materials for many years.[3]

4. TRAINING

Operators should have a Master's degree in chemistry, or the equivalent in work experience. Specific training in the use of the instrument, as well as in the handling of hazardous materials, should be obtained.

5. APPARATUS

5.1 Instrumentation.

Any make or model of NMR spectrometer may be used. Sensitivity and selectivity will depend on the field strength, type of probe, and processing software.

5.2 Glassware, Miscellaneous Equipment, and Supplies.

NMR sample tubes such as the 5 mm Wilmad 507-PP or the 535-PP may be used.

[3] Silverstein, R.M.; Bassler, G.C.; Morrill, T.C. *Spectrometric Identification of Organic Compounds*; 5th ed., John Wiley and Sons, Inc.: New York, 1991.

5.3 Chemicals.

Deuterated solvents and internal standards may be purchased from commercial vendors, such as the Aldrich Chemical Corporation. Deuterated solvents may be kept for years if properly stored in a sealed container. Internal standards should be kept in sealed containers and kept dry. Hydrolysis of the internal standards would create additional peaks in the spectra and affect the quantification of the other analytes. A certificate of analysis should be obtained with each bottle of standard material. The standard should be checked within a month of the analysis to be sure that hydrolysis or absorption of water has not taken place.

6. PROCEDURE

6.1 Sample Preparation.

This procedure should be performed under appropriate engineering controls, in accordance with all appropriate surety and safety regulations.

a. If the sample contains two layers, each should be analyzed separately.

b. Tare a screw-cap vial and cap. Dispense ~0.1mL of neat internal standard (usually TEP for acidic samples, TEP or HMPA for neutral or basic samples) of known purity into the vial and replace the cap. The exact weight is determined using an analytical balance capable of measuring to the hundredth of a milligram. Record the weight, and tare the balance.

c. Add 0.1-1.0mL of the neat sample into the vial. Replace the cap and record the weight. Add 0.1mL D_2O as a lock solvent for aqueous samples. Non-aqueous samples could be prepared using an organic solvent such as $CDCl_3$. Samples that are reactive or insoluble in common solvents may be analyzed without the use of a lock signal, or by the use of a coaxial insert.

d. Mix the sample vial on a vortexing apparatus to assure homogeneity.

6.2 Obtaining NMR Spectra.

Operating parameters vary according to the sample. The sample spectra (Figures 1 and 2) show example parameters that may be used. The important parameters to consider include the following:

- *delay time* should be 5 to 10 times the T1 value for maximum quantitation accuracy, but shorter delay times can be used to improve signal to noise ratios and sensitivity with less accurate quantitation;

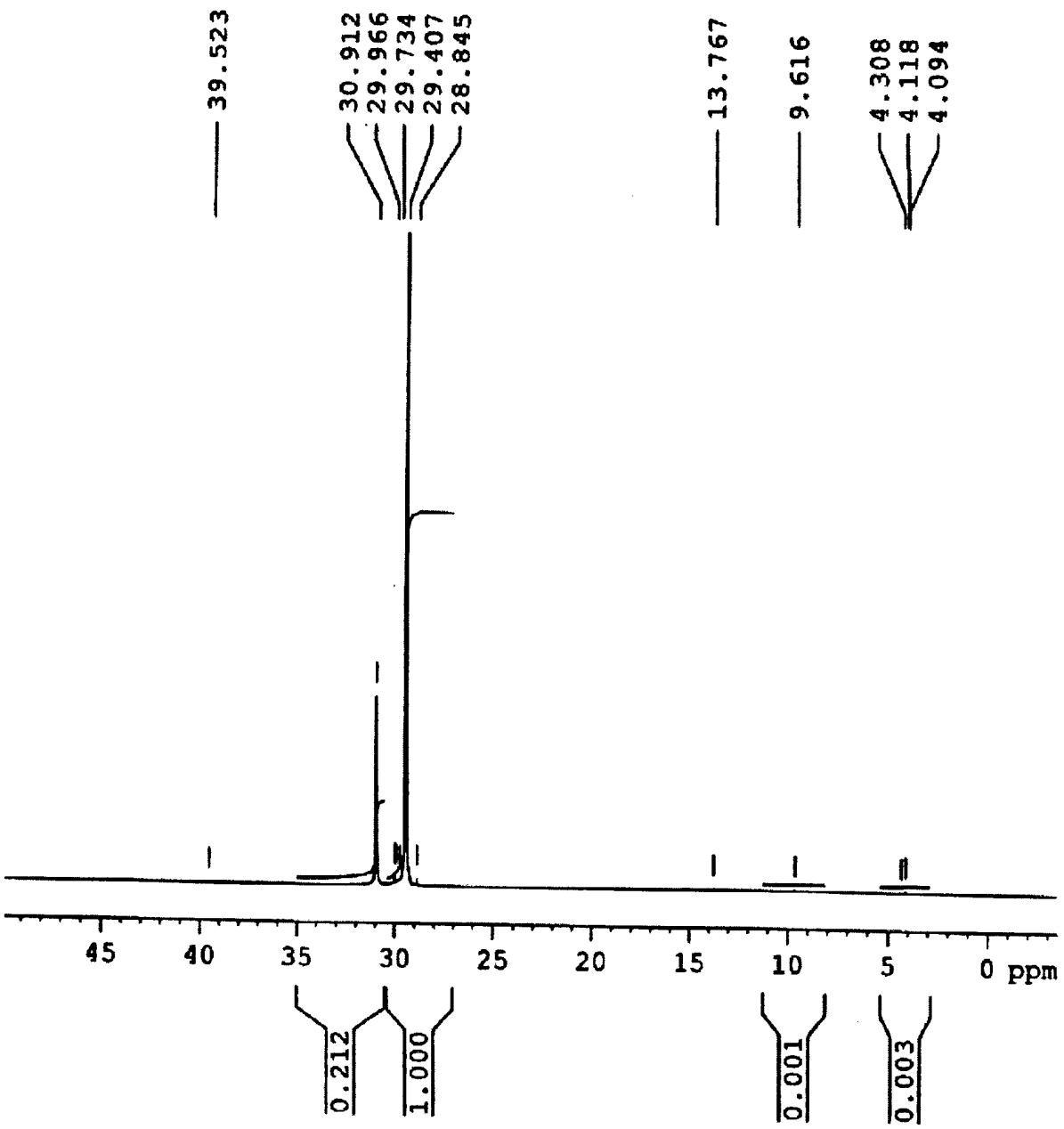

Figure 1. ^{31}P Spectrum of a Sample Containing HMPA Internal Standard

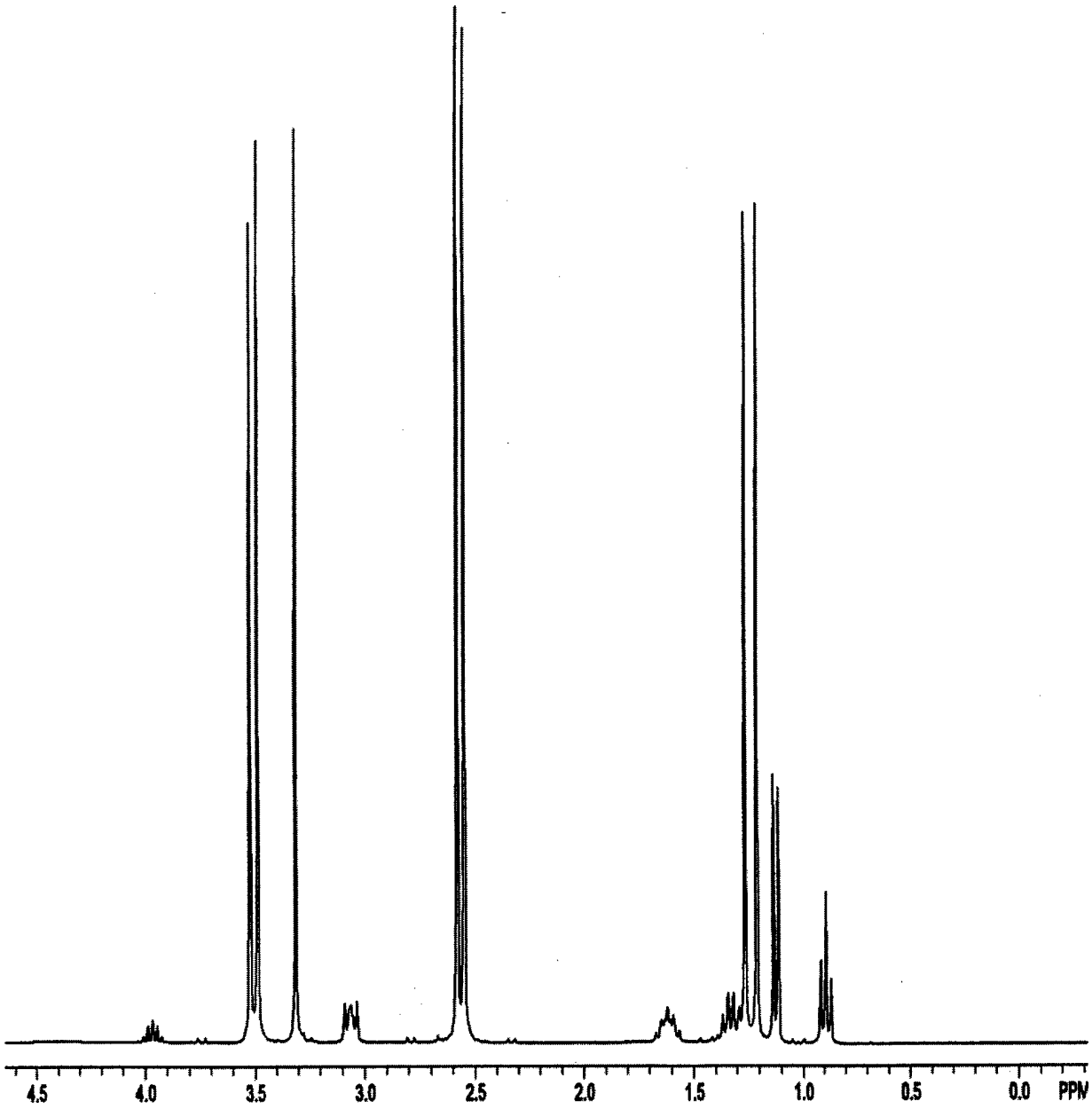

Figure 2. ^{1}H Spectrum of a Sample Containing HMPA Internal Standard

- *line broadening* should be large enough to give acceptable line shapes and baseline flatness;

- *number of scans* is determined by the signal to noise ratio that is required and limits on analysis time;

- *number of data points* is determined by the required spectral resolution to resolve interferences;

- *pulse width PW90 and amplifier gain* are adjusted to give the largest signal without saturating the detector electronics;

- *spectral width* must be wide enough so that peaks of interest are not near the edge of the spectrum.

a. Place the NMR tube into the spinner, using vendor supplied depth gauge to orient sample at maximum sensitivity position to the coils inside the probe. Lower the sample into the magnet, lock onto the deuterium signal and shim to maximize lock signal.

b. Tune probe for optimal frequency and impedance match for the sample.

c. To correctly set up the instrument parameters, it is necessary determine the PW90 for each nucleus, and the T1 values, unless those values are known from similar samples run previously.

d. Enter sample name and information into NMR operating system and acquire data. If necessary, setup multiple samples on the autosampler with careful attention to placing samples in the correct positions. Acquire data for all samples by ^{1}H (detection limit 20-150 ppm). To save instrument time, if no analytes are detected, do not run ^{13}C. At the discretion of the analyst, ^{13}C NMR may be used as an additional tool for the identification and quantification of some analytes. It is particularly useful when iron contamination or other factors broad the proton peaks to the point where the ^{1}H spectrum is not interpretable. ^{31}P NMR spectra should be obtained for all samples derived from phosphorus-containing agents.

6.3 Spectral Data Analysis.

a. Apply appropriate windows functions such as exponential multiplication to enhance signal-to-noise (line broadening for ^{31}P and ^{13}C should generally be 1-5 Hz, and 0.5 – 1 for ^{1}H).

b. Fourier transforms the resulting data to convert from time to frequency and produces the NMR spectrum.

c. Correct the baseline and phase all peaks in the spectrum.

d. Reference the chemical shift against the internal standard, if needed. (TEP should have a shift of 0.01 ppm for ^{31}P NMR).

e. Integrate all peaks in the spectrum to obtain areas.

7. CALCULATION, CALIBRATION, AND DOCUMENTATION

The NMR quality control standard (ethyl benzene or triphenylphosphate standards from Bruker Instruments) is analyzed to assure that the spectrometer meets signal to noise specifications and chemical shift criteria defined for the instrument.

The weight percent of each analyte for the sample can be calculated with the following equation when an internal standard (IS) is present:

$$\frac{\text{Area under analyte Peak}}{\text{Area under IS Peak}} \times \frac{\text{Molecular Weight of analyte}}{\text{Molecular Weight of IS}} \times \frac{\text{Weight of IS}}{\text{Weight of analyte}} \times 100\% = \text{Wt. \% Analyte}$$

If analytical precision is to be reported, from at least seven replicates calculate the mean and standard deviation. The mean value ± 2 standard deviations will provide a 95% confidence range. Generally, time does not allow for the acquisition of 7 spectra, and a single spectrum is used.

The following precision and accuracy study shows that the preparation of seven separate samples does not significantly affect the results of the analysis. The variability between the seven analyses of 0.8 ppm is lower than the average variability within each sample (1.0 ppm).

Sample No. 1, proton run with presat
Integration from Varian, IS=TEP, compound is TDG, (same integral ranges for all)

File	Detection	Wt. IS (mg)	Wt. Sample (mg)	Int. IS	Int. cmp.	Conc. Cmp. (ppm)
ACWA 1-1	H-1 at 2.67	0.04925	1018.3	63.63	36.37	41.723145
ACWA 1-2	H-1 at 2.67	0.04925	1018.3	62.42	37.58	43.946943
ACWA 1-3	H-1 at 2.67	0.04925	1018.3	63.84	36.16	41.345781
ACWA 1-4	H-1 at 2.67	0.04925	1018.3	62.81	37.19	43.220824
ACWA 1-5	H-1 at 2.67	0.04925	1018.3	62.85	37.15	43.146860
ACWA 1-6	H-1 at 2.67	0.04925	1018.3	63.59	36.41	41.795306
ACWA 1-7	H-1 at 2.67	0.04925	1018.3	62.61	37.39	43.592063

Average concentration of compound by NMR (ppm)	42.681560
Standard deviation (ppm)	1.034878
Expected concentration of compound in sample (ppm)	44.32
Recovery % of compound	96.30%
Calculated MDL (ppm)	3.09

Sample No. 2, proton run with presat
Integration from Varian, IS=TEP, compound is TDG, (same integral ranges for all)

File	Detection	Wt. IS (mg)	Wt. Sample (mg)	Int. IS	Int. cmp.	Conc. Cmp. (ppm)
ACWA 2-1	H-1 at 2.67	0.05809	1183.7	63.71	36.29	42.189488
ACWA 2-2	H-1 at 2.67	0.05809	1183.7	63.92	36.08	41.807544
ACWA 2-3	H-1 at 2.67	0.05809	1183.7	63.81	36.19	42.007297
ACWA 2-4	H-1 at 2.67	0.05809	1183.7	63.35	36.65	42.850141
ACWA 2-5	H-1 at 2.67	0.05809	1183.7	63.31	36.69	42.924010
ACWA 2-6	H-1 at 2.67	0.05809	1183.7	64.75	35.25	40.322202
ACWA 2-7	H-1 at 2.67	0.05809	1183.7	63.88	36.12	41.880102

Average concentration of compound by NMR (ppm) 41.997255
Standard deviation (ppm) 0.863989

Expected concentration of compound in sample (ppm) 43.68
Recovery % of compound 96.15%

Calculated MDL (ppm) 2.58

Sample No. 3, proton run with presat
Integration from Varian, IS=TEP, compound is TDG, (same integral ranges for all)

File	Detection	Wt. IS (mg)	Wt. Sample (mg)	Int. IS	Int. cmp.	Conc. Cmp. (ppm)
ACWA 3-1	H-1 at 2.67	0.05129	1046.7	63.99	36.01	41.618522
ACWA 3-2	H-1 at 2.67	0.05129	1046.7	63.51	36.49	42.492021
ACWA 3-3	H-1 at 2.67	0.05129	1046.7	63.62	36.38	42.290680
ACWA 3-4	H-1 at 2.67	0.05129	1046.7	64.28	35.72	41.097104
ACWA 3-5	H-1 at 2.67	0.05129	1046.7	63.74	36.26	42.071828
ACWA 3-6	H-1 at 2.67	0.05129	1046.7	63.65	36.35	42.235890
ACWA 3-7	H-1 at 2.67	0.05129	1046.7	63.7	36.3	42.144687

Average concentration of compound by NMR (ppm) 41.992962
Standard deviation (ppm) 0.477619

Expected concentration of compound in sample (ppm) 43.75
Recovery % of compound 95.98%

Calculated MDL (ppm) 1.43

Sample No. 4, proton run with presat
Integration from Varian, IS=TEP, compound is TDG, (same integral ranges for all)

File	Detection	Wt. IS (mg)	Wt. Sample (mg)	Int. IS	Int. cmp.	Conc. Cmp. (ppm)
ACWA 4-1	H-1 at 2.67	0.05016	1062	62.22	37.78	43.284273
ACWA 4-2	H-1 at 2.67	0.05016	1062	63.49	36.51	40.992526
ACWA 4-3	H-1 at 2.67	0.05016	1062	63.15	36.85	41.597029
ACWA 4-4	H-1 at 2.67	0.05016	1062	62.86	37.14	42.117802
ACWA 4-5	H-1 at 2.67	0.05016	1062	62.92	37.08	42.009662
ACWA 4-6	H-1 at 2.67	0.05016	1062	61.96	38.04	43.765035
ACWA 4-7	H-1 at 2.67	0.05016	1062	63.24	36.76	41.436381

Average concentration of compound by NMR (ppm)	42.171815
Standard deviation (ppm)	1.005575
Expected concentration of compound in sample (ppm)	45.36
Recovery % of compound	92.97%
Calculated MDL (ppm)	3.01

Sample No. 5, proton run with presat
Integration from Varian, IS=TEP, compound is TDG, (same integral ranges for all)

File	Detection	Wt. IS (mg)	Wt. Sample (mg)	Int. IS	Int. cmp.	Conc. Cmp. (ppm)
ACWA 5-1	H-1 at 2.67	0.05377	1098.8	64.78	35.22	40.154568
ACWA 5-2	H-1 at 2.67	0.05377	1098.8	63.06	36.94	43.264280
ACWA 5-3	H-1 at 2.67	0.05377	1098.8	64.15	35.85	41.274237
ACWA 5-4	H-1 at 2.67	0.05377	1098.8	64.59	35.41	40.489946
ACWA 5-5	H-1 at 2.67	0.05377	1098.8	64.89	35.11	39.961300
ACWA 5-6	H-1 at 2.67	0.05377	1098.8	65.42	34.58	39.039208
ACWA 5-7	H-1 at 2.67	0.05377	1098.8	63.87	36.13	41.778957

Average concentration of compound by NMR (ppm)	40.851785
Standard deviation (ppm)	1.387665
Expected concentration of compound in sample (ppm)	43.80
Recovery % of compound	93.27%
Calculated MDL (ppm)	4.15

Sample No. 6, proton run with presat
Integration from Varian, IS=TEP, compound is TDG, (same integral ranges for all)

File	Detection	Wt. IS (mg)	Wt. Sample (mg)	Int. IS	Int. cmp.	Conc. Cmp. (ppm)
ACWA 6-1	H-1 at 2.67	0.05016	1037.8	62.98	37.02	42.878816
ACWA 6-2	H-1 at 2.67	0.05016	1037.8	63.85	36.15	41.300606
ACWA 6-3	H-1 at 2.67	0.05016	1037.8	64.29	35.71	40.518695
ACWA 6-4	H-1 at 2.67	0.05016	1037.8	64.3	35.7	40.501049
ACWA 6-5	H-1 at 2.67	0.05016	1037.8	63.23	36.77	42.420861
ACWA 6-6	H-1 at 2.67	0.05016	1037.8	64.59	35.41	39.991682
ACWA 6-7	H-1 at 2.67	0.05016	1037.8	63.47	36.53	41.984617

Average concentration of compound by NMR (ppm) 41.370904
Standard deviation (ppm) 1.091097

Expected concentration of compound in sample (ppm) 44.36
Recovery % of compound 93.26%

Calculated MDL (ppm) 3.26

Sample No. 7, proton run with presat
Integration from Varian, IS=TEP, compound is TDG, (same integral ranges for all)

File	Detection	Wt. IS (mg)	Wt. Sample (mg)	Int. IS	Int. cmp.	Conc. Cmp. (ppm)
ACWA 7-1	H-1 at 2.67	0.05207	1053.5	64.75	35.25	40.610427
ACWA 7-2	H-1 at 2.67	0.05207	1053.5	64.94	35.06	40.273357
ACWA 7-3	H-1 at 2.67	0.05207	1053.5	65.71	34.29	38.927295
ACWA 7-4	H-1 at 2.67	0.05207	1053.5	64.42	35.58	41.200589
ACWA 7-5	H-1 at 2.67	0.05207	1053.5	64.9	35.1	40.344155
ACWA 7-6	H-1 at 2.67	0.05207	1053.5	63.85	36.15	42.234329
ACWA 7-7	H-1 at 2.67	0.05207	1053.5	65.33	34.67	39.587619

Average concentration of compound by NMR (ppm) 40.453967
Standard deviation (ppm) 1.070637

Expected concentration of compound in sample (ppm) 43.36
Recovery % of compound 93.30%

Calculated MDL (ppm) 3.20