nanalysis

Benchtop qNMR Spectroscopy
A Journey Across Three Continents

SNUG PG Meeting
January 12th, 2023

Alexander Maier, PhD

Company Snapshot

Sell Direct in USA, Canada, Germany, and other parts of Europe;
Distributor Network of 45 dealers in the UK and the rest of world.

GPE SCIENTIFIC LIMITED

Nanalysis Scientific Corp.

TSXV : NSCI | OTC : NSCIF | FRA : 1N1

A global product development and manufacturing company designing a suite of leading-edge, high-performance magnetic resonance technologies to provide innovative solutions to customers in academia, government and industry.

Technology Leader

- Products in LF, HF and MRI
- Patent protected technology

MR Research Leader

- Exciting international research collaborations with government & industry

Growing Global Business

- > 200 employees
- 1000+ customers worldwide

nanalysis

Miniaturization: *accessible, affordable, automatable*

Magnet platform

- NdFeB magnets arranged in patented, highly homogenous compact Halbach based arrays
- Cryogen free

Electronics

- Condensed to a small circuit board stack
- Embedded computer for data acquisition and processing

100 MHz (2.35 T)
110 kg

60 MHz (1.4 T)
26 kg

nanalysis

Nanalysis Benchtop NMR Spectrometers




	60e	60PRO	100e	100PRO
Field strength	60 MHz (1.4 T)		100 MHz (2.35 T)	
Magnet	NdFeB		NdFeB	
Weight	26 kg		110 kg	
Cryogen Free	Y		Y	
Shielding	<2 Gauss outside enclosure		<2 Gauss outside enclosure	
Operating Temperature	18-26°C		18-26°C	
Touchscreen interface	Y		Y	
Nuclei	$^{1}H/^{19}F$	$^{1}H/^{19}F/X$ X: ^{11}B, ^{13}C, ^{31}P, …	^{1}H	$^{1}H/X$ X: ^{11}B, ^{13}C, ^{19}F, ^{31}P, …
Experiments	1D, T_1, T_2, COSY, JRES	1D, T_1, T_2, COSY, JRES DEPT, HSQC, HETCOR, HMBC, Decoupling	1D, T_1, T_2, COSY, JRES	1D, T_1, T_2, COSY, JRES DEPT, HSQC, HETCOR, HMBC, Decoupling

nanalysis

Geographic Distribution of NMR

	Country	%
1	United States	32
2	Germany	11
3	China	10
4	Japan	10
5	UK	6
6	France	4
7	India	4
8	Switzerland	3
	Total	***80***

SDI Global Assessment Report 2019

nanalysis

Quantitative NMR (qNMR)

NMR is inherently quantitative
Integration area of each signal is directly proportional to the number of chemically equivalent nuclei giving rise to that signal

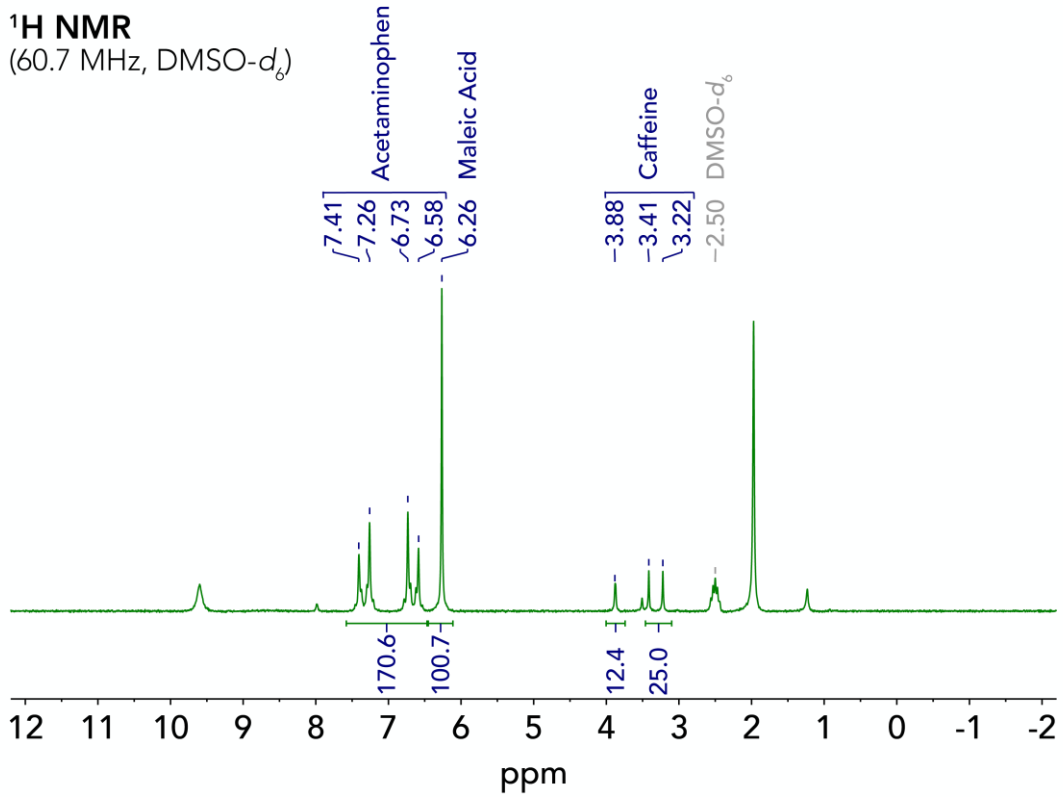
Components do not need to be separated
Provides a snapshot of everything in solution

A single product resonance can be used to quantify

The analysis is non-destructive
The analyte can be recovered

Calibrant methods:

1) **Internal calibrant**
2) External calibrant
3) **Calibration curve**
4) ERETIC (digital)



qNMR Experiments – Key Considerations

ACCURACY

Weighing

Volumes

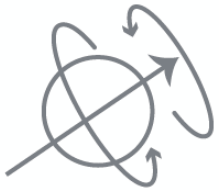
Internal calibrant purity

ACQUISITION PARAMETERS

Numbers of points

Acquisition time

Spectral width



RELAXATION

Scan delay must be chosen to ensure all spins of interest are fully relaxed between pulses

Typically, 5-7 times longest T_1

>> Please see our blog for more details!
Nanalysis website → Resources → Benchtop NMR blog
https://www.nanalysis.com/nmready-blog

nanalysis

Development of qNMR Methods

^{7}Li

Determination of lithium content in brine pools from Chile

^{31}P

Identification of lignin substructures in Canada

^{1}H

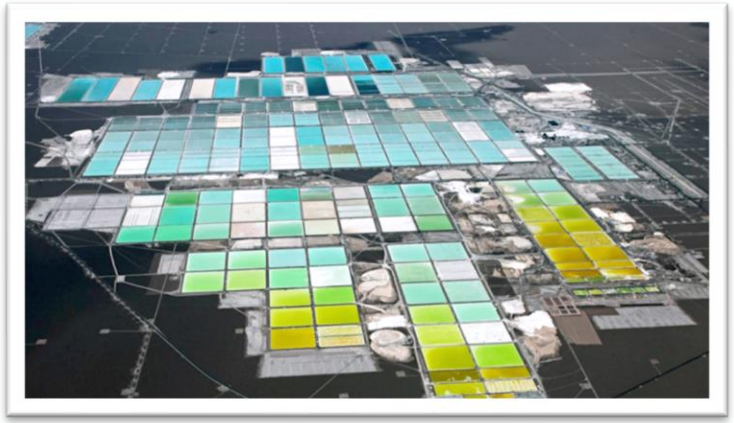
Quantification of MDMA in ecstasy tablets collected at music festivals in the UK

nanalysis

Quantification of Lithium Content in Brine Pools

Problem

- Lithium is becoming increasingly important in our society
- AA & ICP methods require cumbersome sample preps, are difficult to maintain, and are prone to matrix effects



NMR lithium analyzer

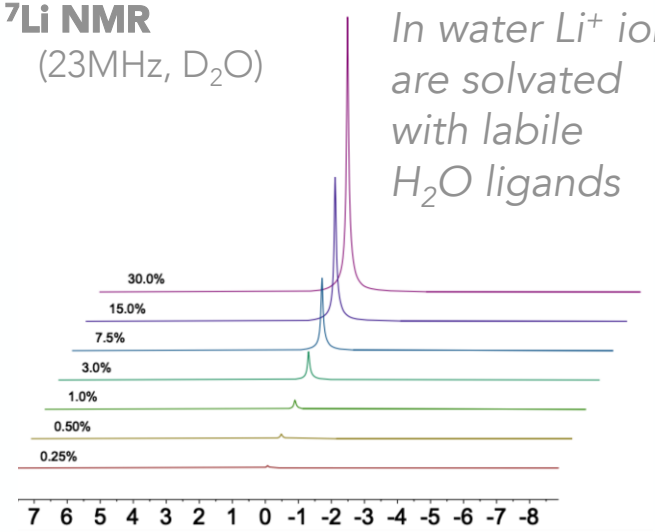
Benchtop qNMR for rapid, onsite, simple analysis of lithium content

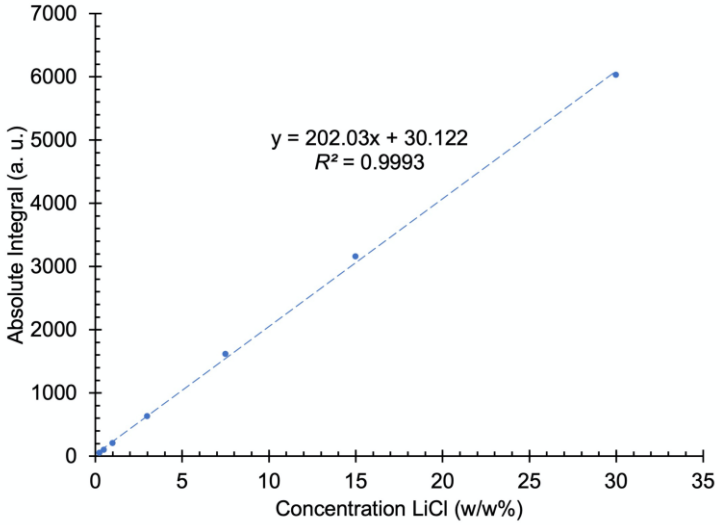
ROI

- Simple sample preparation
- Reduced operating expenditures
- Rapid & accurate results
- Automated procedure

^{7}Li NMR
(23MHz, D_2O)

In water Li^+ ions are solvated with labile H_2O ligands





nanalysis

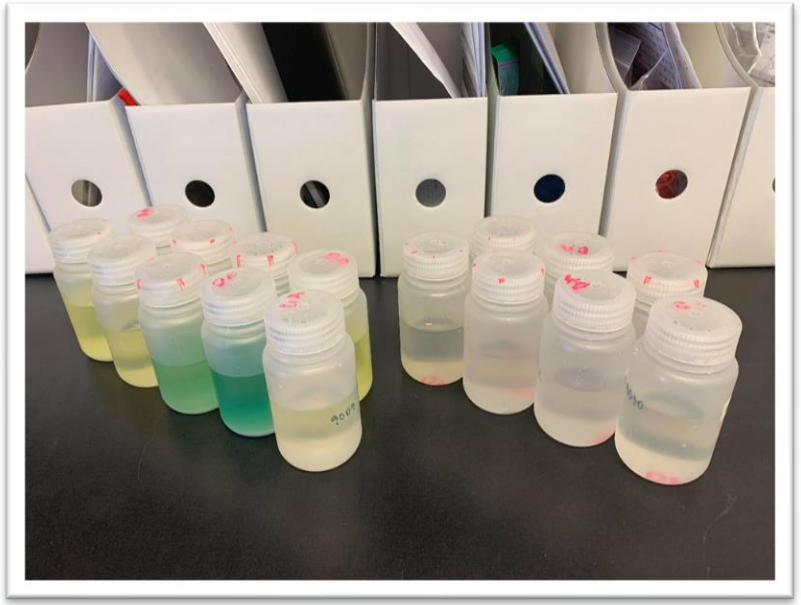
Araneda, J. F.; Hui, P.; Riegel, S. D.; Mercado, R.; Green, C. *Analyst* **2021**,*146*, 882-888
https://www.mining.com/tianqi-puts-worlds-biggest-lithium-plant-expansion-on-hold/
https://www.mining-journal.com/research/news/1177949/spodumene-influx-lifts-lt-lithium-price

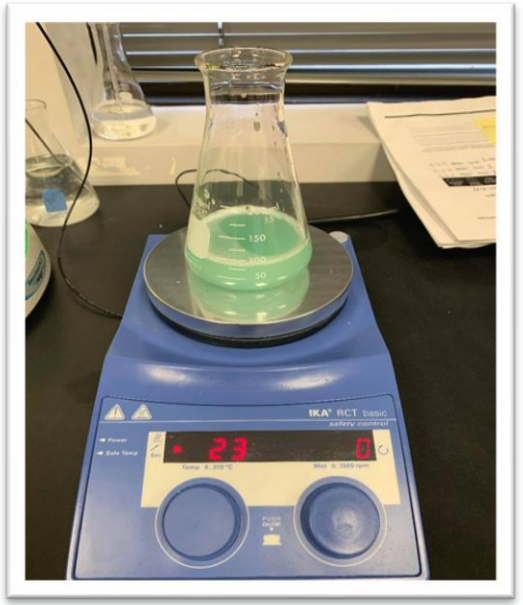
Quantification of Lithium Content in Brine Pools

Collaboration with Sociedad Chilena de Quimica's (SQM)

16 samples of underground brine pools were obtained

Two sets of these samples were prepared: one was sent to Nanalysis Corp. in Calgary, Canada, while the other remained at SQM in Chile

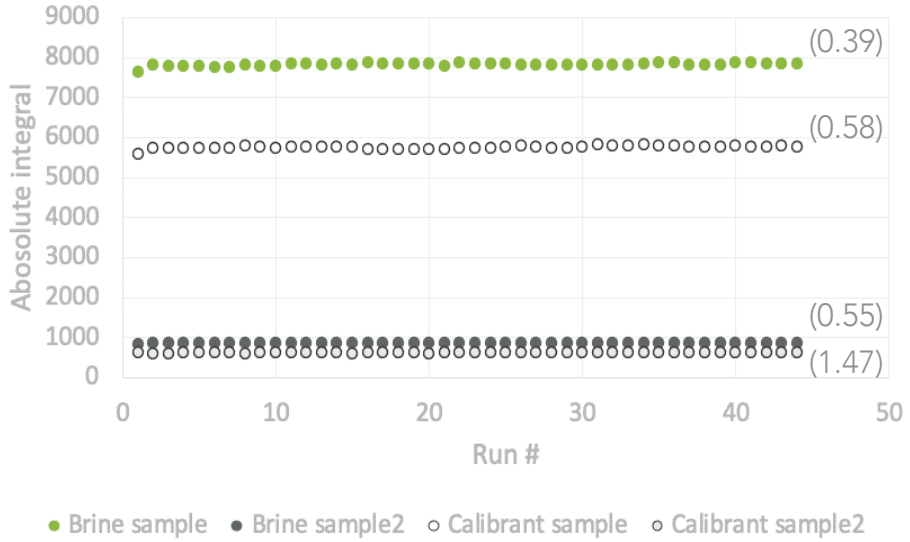




Araneda, J. F.; Hui, P.; Riegel, S. D.; Mercado, R.; Green, C. *Analyst* **2021**,*146*, 882-888
https://www.mining.com/tianqi-puts-worlds-biggest-lithium-plant-expansion-on-hold/
https://www.mining-journal.com/research/news/1177949/spodumene-influx-lifts-lt-lithium-price

Method Validation (excerpt)

22-day Long term Stability



44 absolute integrals plotted, (RSD)
(2 data points collected per day for 22 days)

Limit of detection (LOD)
Limit of quantification (LOQ)

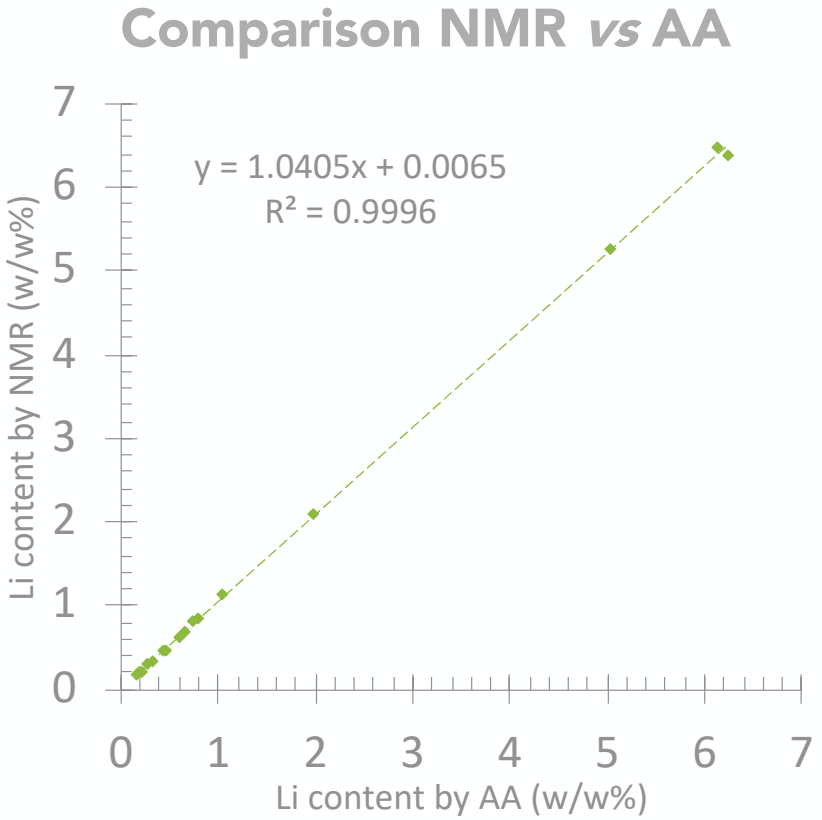
LOD (SNR = 3): 40 ppm Li^+
LOQ (SNR = 10): 100 ppm Li^+

Araneda, J. F.; Hui, P.; Riegel, S. D.; Mercado, R.; Green, C. *Analyst* **2021**,*146*, 882-888.

Sample Analysis Results

Sample	Li content by NMR	RSD[a]	Li content by AA	Diff (%)[b]
1	0.166	1.86	0.163	1.8
2	0.201	1.06	0.197	2.0
3	0.222	0.83	0.226	-1.8
4	0.292	1.56	0.289	1.0
5	0.349	0.70	0.339	2.9
6	0.467	0.68	0.439	6.4
7	0.478	0.34	0.471	1.5
8	0.617	0.23	0.599	3.0
9	0.684	0.46	0.654	4.6
10	0.811	0.51	0.755	7.4
11	0.850	0.55	0.802	6.0
12	1.136	0.27	1.060	7.2
13	2.110	0.13	1.969	7.2
14	5.271	0.06	5.032	4.7
15	6.380	0.11	6.243	2.2
16	6.480	0.04	6.144	5.5

- Values obtained from NMR are comparable to that of values from AA
- RSD values of replicates were under 2%
- Values differ up to a maximum of 7.2%



The intercept is 0.0065 (0.0366 and -0.0236)
The slope is 1.0405 (1.029–1.052)
(R^2) is equal to 0.9996

Araneda, J. F.; Hui, P.; Riegel, S. D.; Mercado, R.; Green, C. *Analyst* **2021**,*146*, 882-888.
Harvey, D. *Analytical Chemisty 2.1*, Open Education Resource (OER) LibreTexts Project Miller, J. N. *Analyst* **1991**, *116*, 3–14.

Development of qNMR Methods

^{7}Li

Determination of lithium content in brine pools from Chile

^{31}P

Identification of lignin substructures in Canada

^{1}H

Quantification of MDMA in ecstasy tablets collected at music festivals in the UK

nanalysis

Lignin Characterization

Lignin is a by-product of paper production

- Monomers are important building blocks
- Historically obtained from petroleum feedstocks
- Complex cross-linked phenolic polymers
- Structure needs to be determined

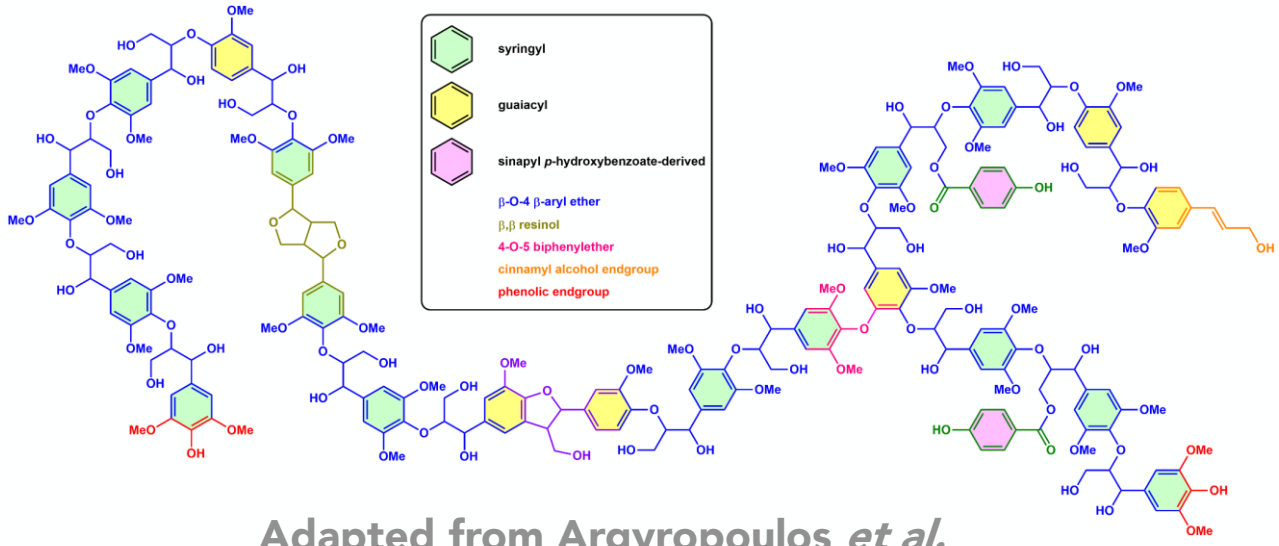
Challenge with lignin is structure determination

- Different for each lignin and very complex
- $^{1}H/^{13}C$ NMR analyses suffer from too much overlap

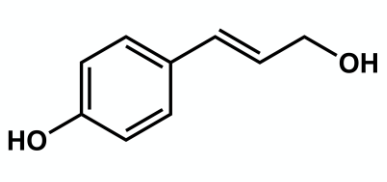
Lignin was successfully derivatized with many nuclei

^{31}P is an excellent nucleus for NMR analysis of lignin

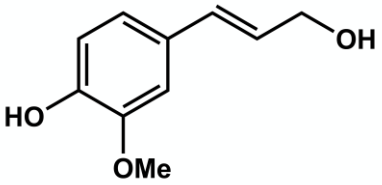
- 100% natural abundance
- Large chemical shift range
- Gives rise to sharp lines



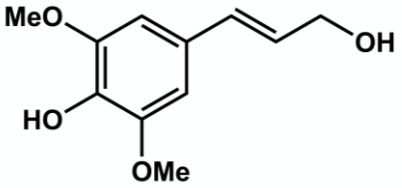
Adapted from Argyropoulos *et al.*



***p*-Coumaryl Alcohol**



Coniferyl Alcohol



Sinapyl Alcohol

nanalysis

Freudenberg, K. *Nature* **1959**, *183*, 1152–1155
Argyropoulos, D. S. in *Lignin and Lignans: Advances in Chemistry* **2010**, pp. 245–266

Phosphitylation Approach

This analysis was part of a round-robin study performed to assess a test method described in a future Canadian Standard Assoc. standard method

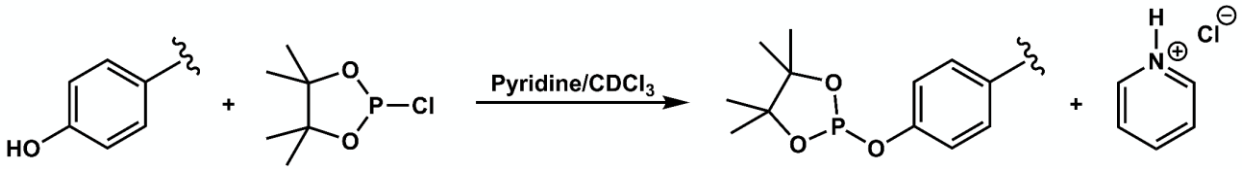
Phosphitylation of alcohol groups with TMDP

- Cyclohexanol used as internal calibrant

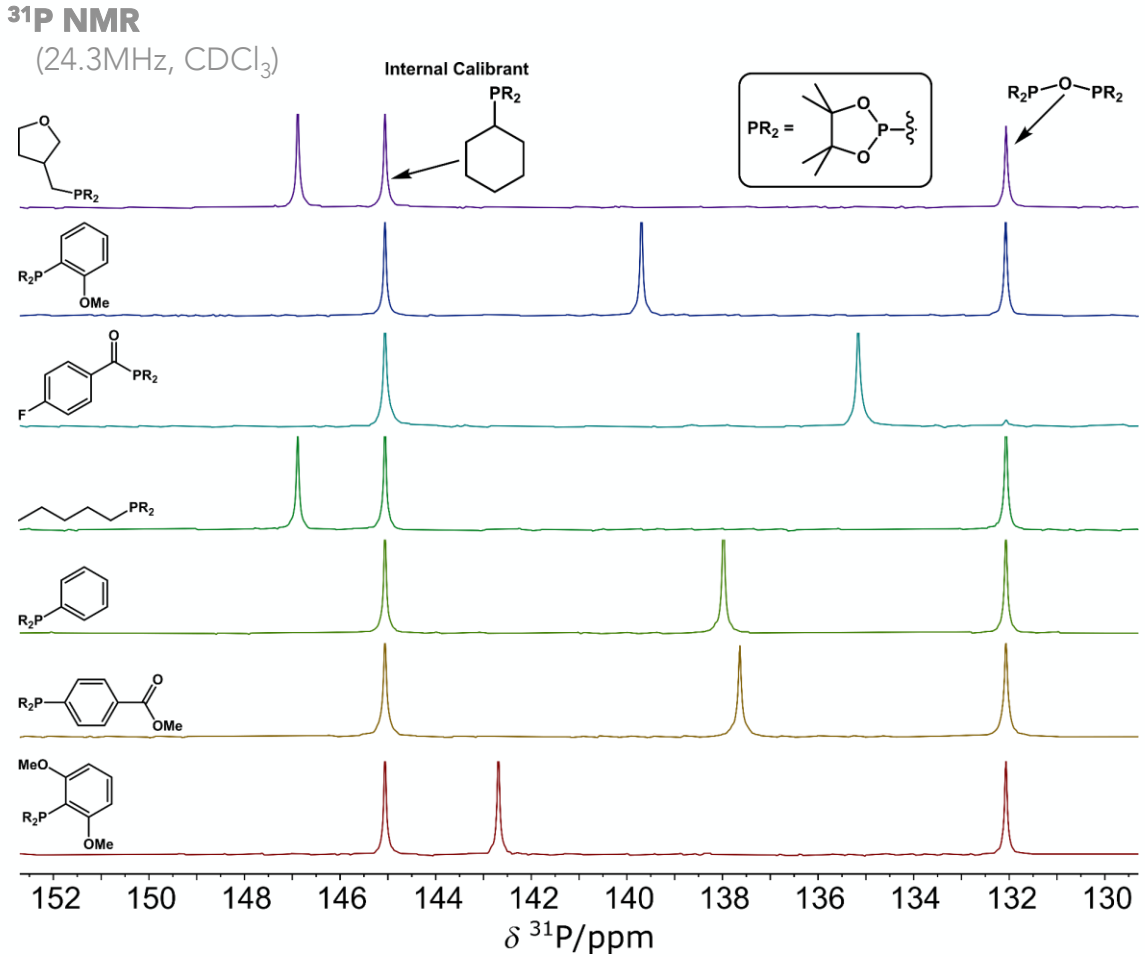
Reaction is immediate and quantitative

- ^{31}P signals in δ **= 130-150 ppm range**

Large potential applicability for this approach, but lack of access (for many) to a high-field instrument



TMDP



Meng, X.; Crestini, C.; Ben, H.; Hao, N.; Pu, Y.; Ragauskas, A. J.; Argyropoulos, D. S. *Nat. Protoc.* **2019**, *14*, 2627–2647
CSA W213 Lignin – Quantification of hydroxyl groups by ^{31}P NMR – publication early 2022

Identification of Lignin Substructures

$^{31}P\{^{1}H\}$ NMR
(*Kraft Softwood Lignin*, $CDCl_3$)

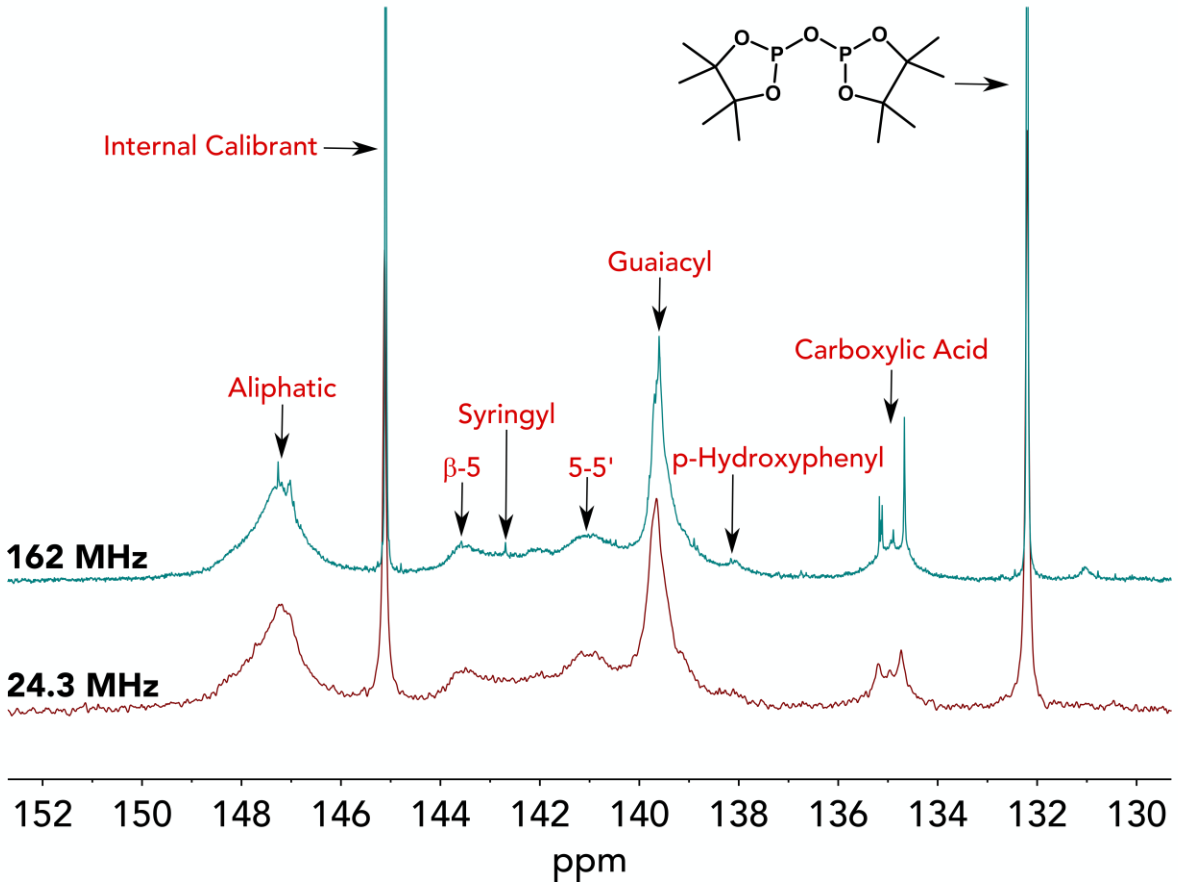


Table 1. Chemical shift ranges (^{31}P NMR) used for integration of various regions for the quantification of lignin substructures.

Hardwood and wheat straw	
Functional group	Chemical shift range (ppm)
Aliphatic	150.0–145.4
Internal calibrant	145.4–144.6
β-5	144.5–143.3
4-*O*-5'	143.3–141.6
5–5'	141.6–140.4
Guaiacyl	140.4–138.5
p-Hydroxyphenyl	138.5–137.0
Carboxylic acid	136.0–133.6

Softwood	
Functional group	Chemical shift range (ppm)
Aliphatic	150.0–145.4
Internal calibrant	145.4–144.6
β-5	144.5–143.0
Syringyl	143.0–141.8
5–5'	141.8–140.4
Guaiacyl	140.4–138.3
p-Hydroxyphenyl	138.3–137.0
Carboxylic acid	136.0–133.6

Meng, X.; Crestini, C.; Ben, H.; Hao, N.; Pu, Y.; Ragauskas, A. J.; Argyropoulos, D. S. *Nat. Protoc.* **2019**, *14*, 2627–2647
Ahmad, Z.; Mahmood, N.; Yuan, Z.; Paleologou, M.; Xu, C. C. *Molecules* **2018**, *23*, 2464
Araneda, J. F.; Burton, I. W.; Paleologou, M; Riegel, S. D.; Leclerc, *Can. J. Chem.* **2022**, doi: 10.1139/cjc-2022-0041.

nanalysis

Identification of Lignin Substructures - Analysis Results

Table 2. Comparison of concentrations (in mmol/g) obtained from 24.3 MHz (Bench) and 162 MHz (HF) ^{31}P NMR analysis.

	KSW			KSW			KHW			OHW			SOD		
Functional group	Bench[a]	HF[b]	Error	Bench[c]	HF[d]	Error	Bench	HF	Error	Bench	HF	Error	Bench	HF	Error
X (Aliphatic OH)	1.82 (0.3)	1.71	6%	1.88 (0.7)	1.71	10%	2.02 (1.4)	1.85	9%	2.44 (3.1)	2.37	3%	1.75 (1.1)	1.64	7%
X (5–5′)	0.77 (0.4)	0.65	18%	0.72 (1.4)	0.66	9%	0.34 (1.4)	0.33	3%	0.24 (6.1)	0.22	9%	0.34 (3.2)	0.33	3%
X (4-*O*-5′ or Syringyl OH)[e]	0.45 (0.9)	0.41	10%	0.56 (0.5)	0.41	37%	1.90 (2.0)	1.85	3%	1.50 (1.6)	1.51	1%	1.15 (0.5)	1.13	2%
X (*β*-5)	0.46 (1.8)	0.42	10%	0.50 (1.1)	0.42	19%	0.30 (4.2)	0.26	15%	0.28 (5.6)	0.26	8%	0.28 (1.9)	0.27	4%
X (Guaiacyl OH)	1.80 (1.5)	1.61	12%	1.82 (1.2)	1.63	12%	1.05 (2.2)	1.01	4%	0.80 (1.8)	0.82	2%	1.19 (1.4)	1.15	3%
X (*p*-Hydroxyphenyl OH)	0.13 (11.9)	0.16	19%	0.15 (3.6)	0.16	6%	0.12 (2.9)	0.19	37%	0.08 (16.1)	0.09	11%	0.46 (5.6)	0.47	2%
X (Carboxylic acid OH)	0.52 (0.7)	0.54	4%	0.56 (2.0)	0.54	4%	0.37 (8.7)	0.41	10%	0.17 (3.7)	0.18	6%	0.98 (0.3)	0.95	3%

Note: The benchtop NMR values are presented as the average of triplicate runs, and the relative standard deviations are provided in parentheses. Results for the individual triplicate analyses are included in the supplementary information (Table S1). [a]1024 scans.

[b]128 scans.

[c]2048 scans.

[d]256 scans.

[e]4-*O*-5′ for KHW/OHW and syringyl OH for KSW/SOD. Error (%) = |content by Bench − content by HF|/content by HF x 100.

Araneda, J. F.; Burton, I. W.; Paleologou, M; Riegel, S. D.; Leclerc, *Can. J. Chem.* 2022, doi: 10.1139/cjc-2022-0041.

Development of qNMR methods

^{7}Li

Determination of lithium content in brine pools from Chile

^{31}P

Identification of lignin substructures in Canada

^{1}H

Quantification of MDMA in ecstasy tablets collected at music festivals in the UK

nanalysis

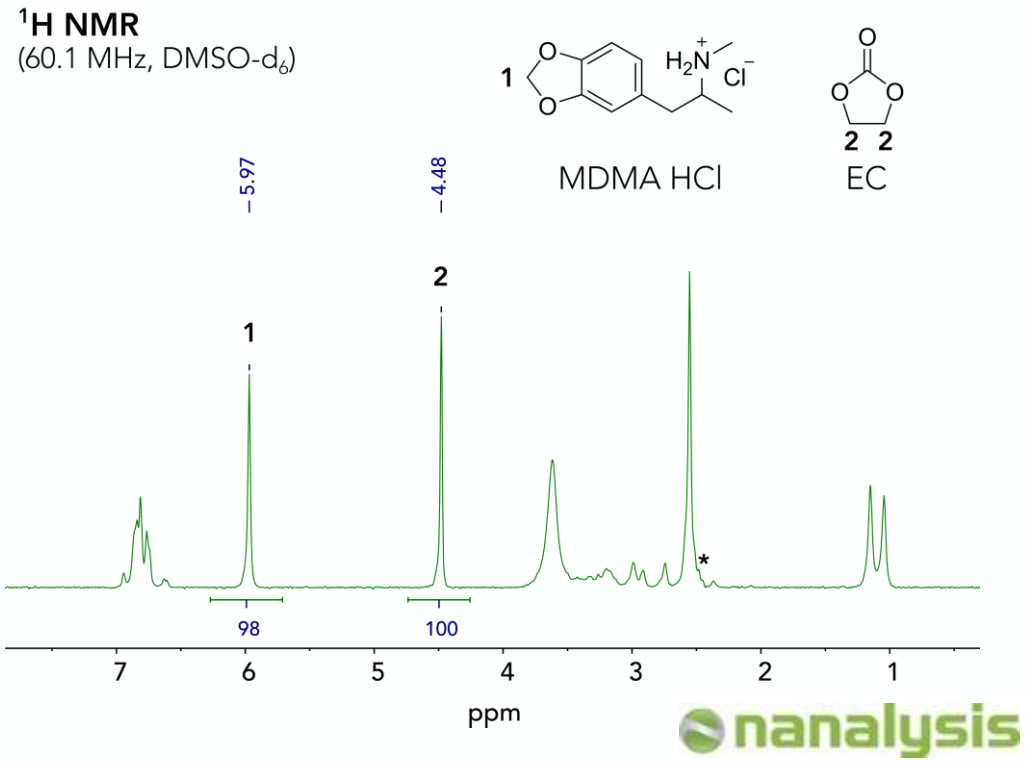
Quantification of MDMA in Ecstasy Tablets

Problem

- MDMA quantification important for harm reduction and law enforcement
- Issues of overdoing and deaths related to drug poisoning
- Public Health England: £ 10.7 billion illicit drug costs in 2010/2011 in the UK

Method validation and street sample analysis of 100 tablets collected at music festivals in the UK

- Benchtop qNMR with ethylene carbonate (EC) as internal calibrant



ROI

- Simple sample preparation
- Robust method
- Rapid & accurate results

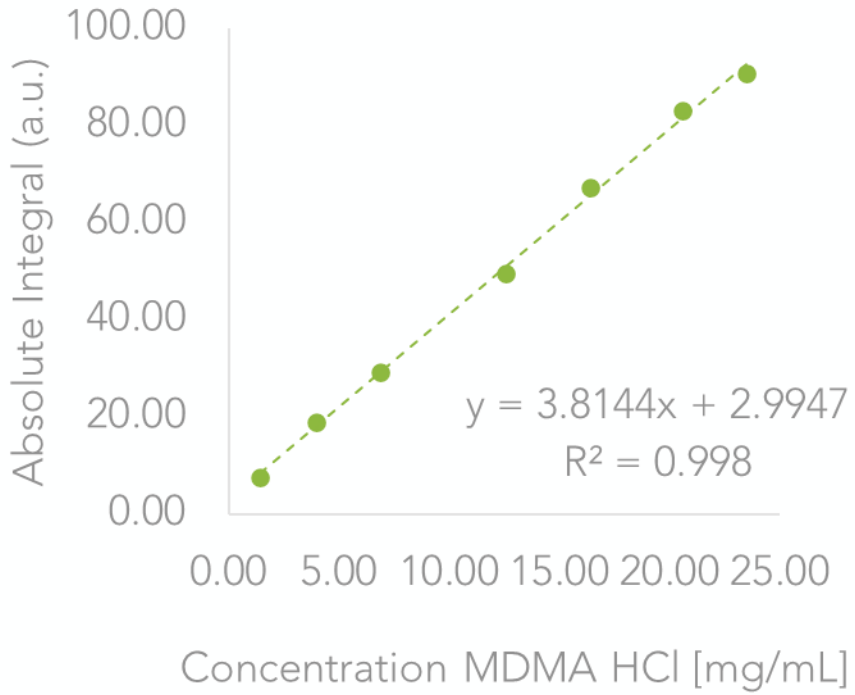
Frinculescu, A.; Maier, A. F. G.; Shine, T.; Ramsey, J.; Araneda, J. F.; Riegel, S. D.; Frascione, N.; Abbate, V. *J. Pharm. Biomed. Anal.* **2022**, 214. 114728.

Method Validation (excerpt)

External quality testing

Tablet ID	MDMA content in wt%		Deviation in %
	NMR results	LC-MS results	
16152	47.52	46.0	3.3
18861	31.45	33.3	-5.6
20252	33.95	33.2	2.3
20284	20.06	20.0	0.3
20307LA	30.79	32.7	-5.8
22192/4	38.84	37.1	4.7
24803	2.20	2.25	-2.2
27099	12.92	12.8	0.9
28042	41.53	40.6	2.3
29596	60.69	59.8	1.5

Linearity



Limit of detection and limit of quantification

- LOD: 0.10 mg/mL (SNR ≥ 3); LOQ: 0.33 mg/mL (SNR ≥ 10)

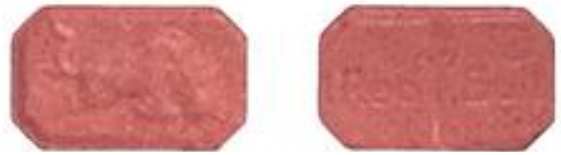
Frinculescu, A.; Maier, A. F. G.; Shine, T.; Ramsey, J.; Araneda, J. F.; Riegel, S. D.; Frascione, N.; Abbate, V. *J. Pharm. Biomed. Anal.* **2022**, 214. 114728.

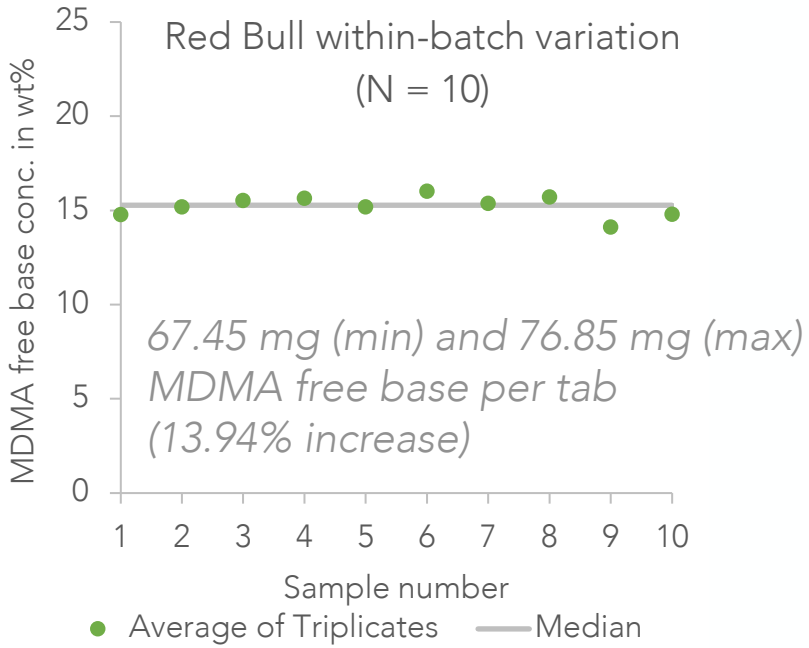
nanalysis

Street Sample Analysis (excerpt)

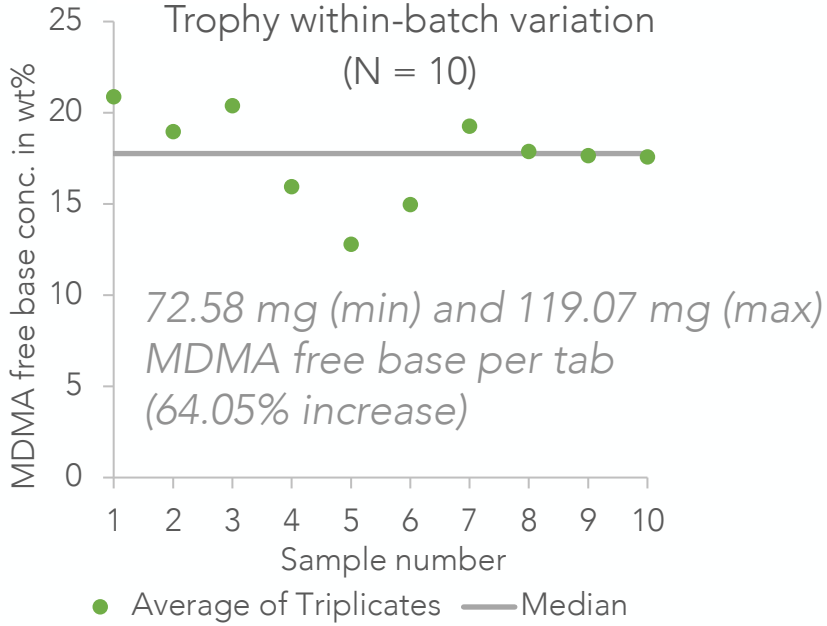
Within Batch Variation Testing

Small variation in MDMA content for the Red Bull tab





High variation in MDMA content for the Trophy tab



Frinculescu, A.; Maier, A. F. G.; Shine, T.; Ramsey, J.; Araneda, J. F.; Riegel, S. D.; Frascione, N.; Abbate, V. *J. Pharm. Biomed. Anal.* **2022**, 214. 114728.

Conclusions

Benchtop technology offers unparalleled NMR access to academic and industrial users by **addressing accessibility issues**

- Low upfront and recurring costs
- No maintenance
- Expert staff not required
- Low infrastructure requirements
- Compact all in one benchtop NMR systems

We have demonstrated highly effective methods using **^{7}Li**, **^{31}P**, and **^{1}H qNMR** (for others, please inquire)

- Determination of lithium content in brine pools in Chile
- Identification of substructures in lignin from Canada
- Quantification of MDMA in ecstasy tablets collected at music festivals in the UK

nanalysis

Acknowledgments

Susanne Riegel
Juan Araneda
Matthew Leclerc
Paul Hui
Jonathan Ma
Thais Barbosa
Kiana Nayebi

Simon Rainbow
James Lyons
Thomasine Curzon

Rodrigo Mercado
Christopher Green

Michael Paleologou

Ian Burton

Melanie Pinatton

Anca Frinculescu
Trevor Shine
John Ramsey

Vincenzo Abbate
Nunzianda Frascione

Thank you for your time.

Please come by our booth tomorrow and chat with us!