System Suitability

María de Castro

GasIRMS Field Applications Specialist EMEA/Global

London, 22-23 September 2023

The world leader in serving science

System Suitabilty Test

- *Used to verify that an analytical instrument is suitable for its intended purpose the day the analysis is done.*
- *To prove that system is working perfectly before the analysis.*
- *System Suitability Test (SST) is generally performed to evaluate the suitability and effectiveness of the entire chromatographic system not only prior to use but also during the time of analysis (due to several reasons the performance and the capacity of the entire chromatographic system may abruptly or mildly change during their regular uses).*

➢ *There are several instruments involved in the GC-C-IRMS analysis, not just the GC-IRMS:*

  - *IRMS*
  - *GC-C-IRMS*
  - *Sample Preparation (SPE, HPLC,…)*

System Suitability GC-IRMS

- Autofocus (IRMS, not required only daily basis, only for troubleshooting purposes)
- Backgrounds (GC-IRMS)
- Standard onoff (IRMS)
- Peak Shape (IRMS, in Thermo Instruments only for troubleshooting purposes)
- Linearity (IRMS)
- Solvent Injection for system conditioning and backgrounds check (GC-IRMS)
- Internal Standard (GC-IRMS) - RT
- Certified Reference Materials (GC-IRMS)
  - Control / Allows to perform normalization (reference gas correction)
- GC QCs / Reference Mixes (GC-IRMS)
  - Control
- Urine Quality Controls (GC-*IRMS + Sample Preparation*)

❖ Linearity for target compounds?

Backgrounds Check and Record

GC - IRMS

Instrument Logbook

Date	H_2O		N_2		O_2		Ar		CO_2		REMARKS / Maintenances	Injections #	Total Injections #	Onoff CO2 (‰)	Linearity Reference Gas (‰/V)
	Backflush mode	Straight mode	Backflush mode	Straight mode	Backflush mode	Straight mode	Backflush mode	Straight mode	Backflush mode	Straight mode					
10/19/2022	4398	4309	378	760	1038	1478	11	26	168	196	Oxidación 15hs antes, PTV				
25/10/2022	2122		127		815		6		147					0.021	
26/10/2022		3900		800		1000		26		200					
27/10/2022	1772	496	58	731	650	1384	4	10	136	250					
2/11/2022	1791	1521	307	112	345	834	26	9	133	118					
4/11/2022	2400	2387	349	239	49650	49720	20	15	565	540	Despues de 45 min de oxidacion + 45 min BF				
7/11/2022	4225	3928	68	135	1175	1250	4	8	245	236	Cambio a columna TG-624SilMS/SSL/new septum/Liner HS				
11/11/2022							12	25			BTEX Supelco	12	12	0.025	0.004
14/11/2022												6	18		
23/11/2022												24	42		
24/11/2022												11	53		
25/11/2022					270	590	10	45			BTEX Schimmelmann	11	64		

Linearity Reference Gas (‰/V)	TransferTest (3ul air, Split 1:20, 2mlmin column flow)	P He (bar)	P He+O2 (bar)	P O2 (bar)	Flows (ml/min)-Disconnected				
					GC oven Set	GC column Set	BF CN	BF HTC	HTC+O2
		1.5		0.55	80	1.5	3.50/3.10		
0.004	10.5				50	2	3.70/3.45		

LINEARITY CALCULATION		
	Ampl. 44 (V)	d13C(‰)
	0.48	-0.05
	0.48	-0.07
	0.80	0.00
	1.98	-0.04
	2.15	-0.07
	2.65	-0.12
	4.56	-0.08
	6.10	-0.09
	6.10	-0.10
	8.74	-0.04
Linearity (‰/V)	0.003	≤ 0.06

Stability: Standard onoff test

IRMS

- Specification for ^{13}C reference gas pulses precision: 0.06‰
- Necessary to check the IRMS stability as well as to condition the instrument (must be performed just before running the samples sequence)
- First run is generally drifting, some instruments require more than one run to get stable
- Minimum requirement n=3 stable runs (n=4/5, first one or two runs for conditioning)
- ➢ Can also be used to control sensitivIty

Sample List

	Label	Status	Comment	Evaluate	Sample Type
1	co2 onoff		Conditioning	☑	Unknown
2	co2 onoff		Conditioning	☑	Unknown
3	co2 onoff		co2 onoff	☑	Unknown
4	co2 onoff		co2 onoff	☑	Unknown
5	co2 onoff		co2 onoff	☑	Unknown

Peak Finder Results Statistical View 1

Columns

Statistic	d 13C/12C
Mean	0.023
SqrSum	0.009
Std Dev	0.020
Max	0.046
Min	-0.014

Peak	Dilution	Retention Time (s)	Width (s)	Total Area (Vs)	44.00 m/z Amplitude (V)	44.00 m/z Background (V)	45.00 m/z Background (V)	46.00 m/z Background (V)	d 13C/12C
Peak 1	0.00	48.9	24.9	51.755	2.667	0.005	0.005	0.006	0.046
Peak 2	0.00	98.6	24.9	51.737	2.665	0.005	0.005	0.006	0.022
Peak 3		148.4	24.9	51.670	2.663	0.005	0.005	0.006	0.000
Peak 4	0.00	198.3	24.7	51.768	2.664	0.005	0.006	0.006	-0.014
Peak 5	0.00	248.1	24.7	51.705	2.664	0.005	0.006	0.006	0.022
Peak 6	0.00	297.8	24.9	51.747	2.667	0.005	0.006	0.006	0.018
Peak 7	0.00	347.6	24.9	51.732	2.668	0.005	0.006	0.006	0.044
Peak 8	0.00	397.3	24.9	51.836	2.667	0.005	0.006	0.006	0.016
Peak 9	0.00	447.3	24.9	51.700	2.663	0.005	0.006	0.006	0.043
Peak 10	0.00	497.0	24.7	51.768	2.667	0.005	0.005	0.006	0.036

Linearity Reference Gas (IRMS)

IRMS

- Slope must be ≤ 0.066 ‰/V (≤ 0.02 ‰/nA)
- Must be performed regularly (once a month)?
  - ❖ WADA -TD2022IRMS: within the specifications of the instrument manufacturer

CO2

Columns

Peak	Total Area (Vs)	44.00 m/z Amplitude (V)	d 13C/12C
Peak 1	9.382	0.484	-0.088
Peak 2	9.376	0.484	-0.042
Peak 3	15.602	0.804	0.000
Peak 4	38.629	1.993	-0.092
Peak 5	41.821	2.160	-0.120
Peak 6	51.483	2.659	-0.134
Peak 7	88.919	4.598	-0.135
Peak 8	118.226	6.137	-0.147
Peak 9	118.997	6.141	-0.170
Peak 10	167.984	8.796	-0.153

Peak Finder Results Statistical View 1

Columns

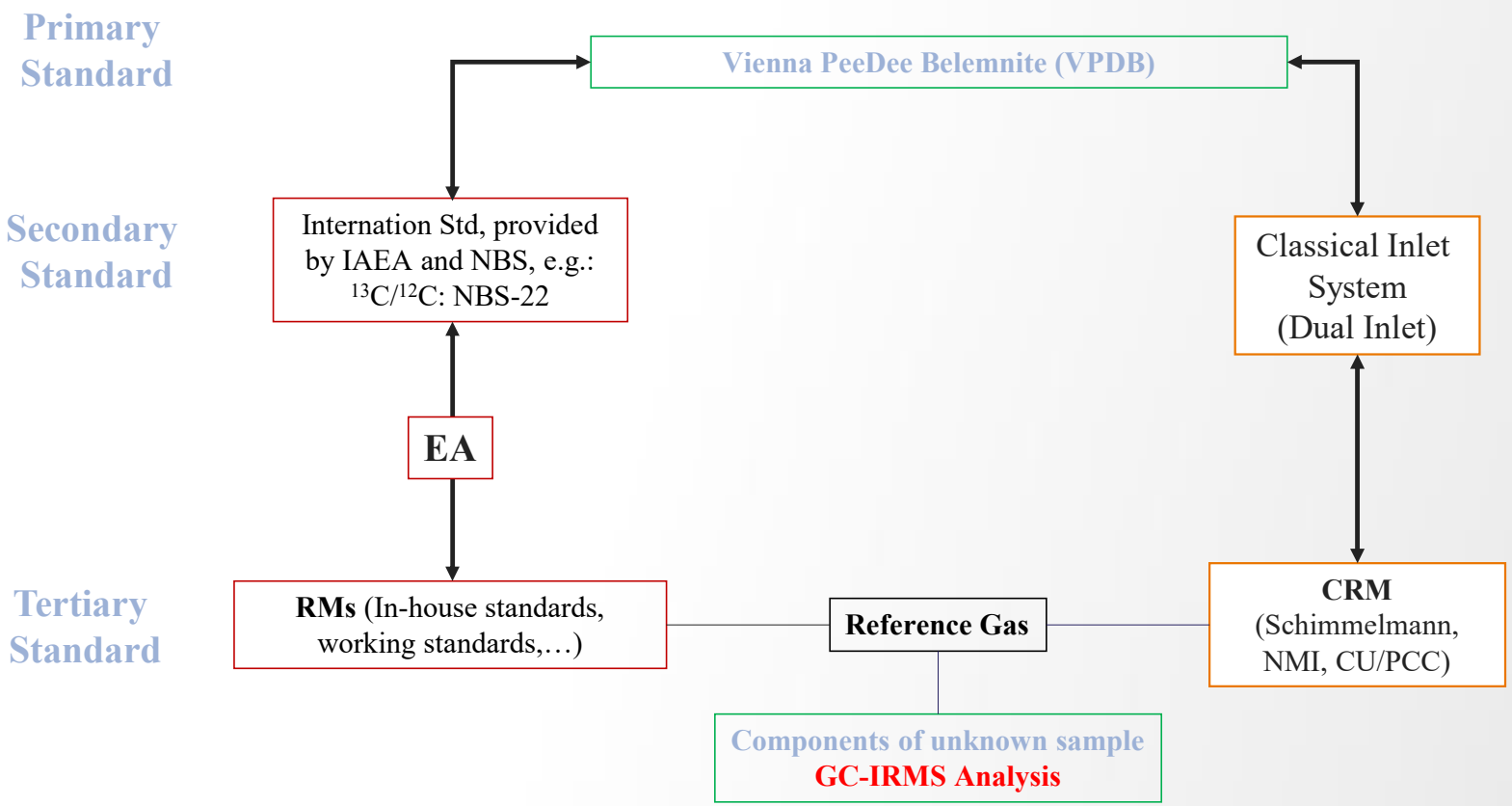
Statistic	d 13C/12C
Mean	-0.108
SqrSum	0.142
Std Dev	0.053
Max	0.000
Min	-0.170
Regression Slope (vs Peak ID)	-0.014
Regression Offset (vs Peak ID)	-0.029
Regression Coefficient (vs Peak ID)	0.812
Regression Slope (vs Main Mass Amplitude [/V])	-0.015
Regression Offset (vs Main Mass Amplitude [/V])	-0.058
Regression Coefficient (vs Main Mass Amplitude [/V])	0.782

Additional Results

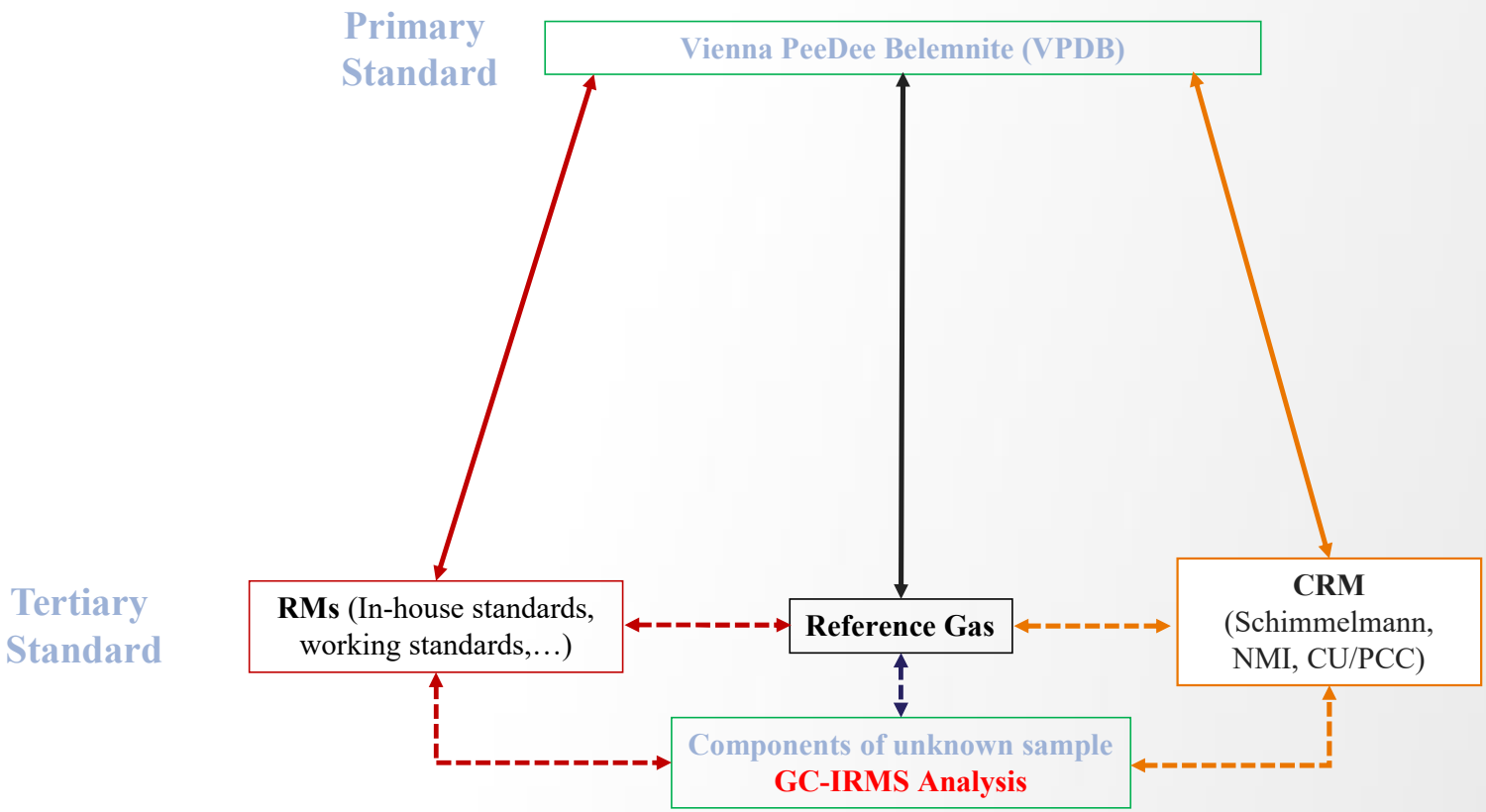
LINEARITY CALCULATION

	Ampl. 44 (V)	d13C(‰)
	0.48	-0.09
	0.48	-0.04
	0.80	0.00
	1.99	-0.09
	2.16	-0.12
	2.66	-0.13
	4.60	-0.14
	6.14	-0.15
	6.14	-0.17
	8.80	-0.15
Linearity (‰/V)	-0.008	≤ 0.06

Isotopic Standarization



Isotopic Standarization



Precision and Accuracy

Certified / In-house RMs (GC – IRMS) & Urine Quality Controls (GC-*IRMS + Sample Preparation)*

- Certified Reference Materials: Control / Allows to normalize the data (= reference gas correction)

Forensic Isotope Ratio Mass Spectrometry (FIRMS)

For results to be comparable between laboratories, measurements must be traceable to the international δ-scales and, because isotope ratio measurements are reported relative to standards, a key aspect is the correct selection, calibration, and use of international and in-house RMs.

Four principles:

The principle of identical treatment by which samples and RMs are processed in an identical manner and which incorporates three further principles;

- The principle of identical correction (by which necessary corrections are identified and evenly applied),
- The principle of identical scaling (by which data are shifted and stretched to the international δ-scales),
- The principle of error detection by which quality control (QC) and quality assurance (QA) results are monitored and acted upon.

To achieve both good repeatability and good reproducibility it is essential to obtain RMs with internationally agreed δ-values. These RMs will act as the basis for QC and can be used to calibrate further in-house QC RMs tailored to the activities of specific laboratories.

The δ-values assigned to RMs must be recorded and reported with all data.

Reference materials must be used to determine what corrections are necessary for measured data.

Each analytical sequence of samples must include both QC and QA materials which are subject to identical treatment during measurement and data processing. Results for these materials must be plotted, monitored, and acted upon.

Periodically international RMs should be analysed as an in-house proficiency test to demonstrate results are accurate.

Isotopic Data Corrections

- Blank Correction (EA-IRMS)
- Drift Correction: A linear regression line is fitted through the $\delta^{13}C$ values vs. measurement time t_{sample} (sample start time). The slope *m* of that line is then used to correct the other samples:

$$\delta\,^{13}C_{corr} = \delta^{13}C_{measured} - m \times t_{sample}$$

- Linearity Correction

$$\delta^{13}C_{corr} = \delta^{13}C_{meas} - m \times Ampl.$$

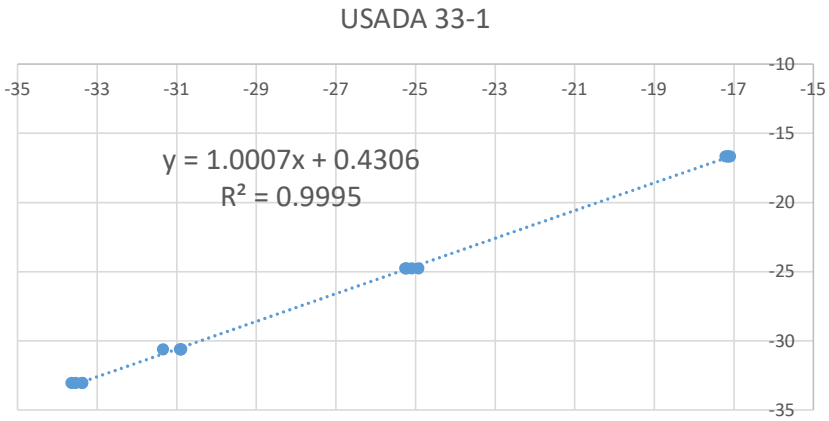
- Normalization: data is shifted and stretched to the international δ-scales. A linear regression line is fitted through the measured $\delta^{13}C$ values vs. the certified $\delta^{13}C$

$$\delta^{13}C_{corr} = Slope \times \delta^{13}C_{meas} + Intercept$$

Normalization

	Raw Data	Certified	Offset	Corrected	Offset	Ref. Gas Correction 0.41	Offset
5a-ol_ac	-31.338	-30.61	-0.73	-30.93	-0.32	-30.93	-0.32
5a-ol_ac	-30.887	-30.61	-0.28	-30.48	0.13	-30.47	0.14
5a-ol_ac	-30.898	-30.61	-0.29	-30.49	0.12	-30.49	0.12
5a-ol_ac	-30.913	-30.61	-0.30	-30.50	0.11	-30.50	0.11
Choles_ac	-25.221	-24.77	-0.45	-24.81	-0.04	-24.81	-0.04
Choles_ac	-24.922	-24.77	-0.15	-24.51	0.26	-24.51	0.26
Choles_ac	-25.095	-24.77	-0.32	-24.68	0.09	-24.68	0.09
Choles_ac	-25.252	-24.77	-0.48	-24.84	-0.07	-24.84	-0.07
andro_ac	-33.645	-33.04	-0.61	-33.24	-0.20	-33.23	-0.19
andro_ac	-33.375	-33.04	-0.34	-32.97	0.07	-32.96	0.08
andro_ac	-33.538	-33.04	-0.50	-33.13	-0.09	-33.13	-0.09
andro_ac	-33.368	-33.04	-0.33	-32.96	0.08	-32.96	0.08
11ketoE_ac	-17.202	-16.69	-0.51	-16.78	-0.09	-16.79	-0.10
11ketoE_ac	-17.147	-16.69	-0.46	-16.73	-0.04	-16.73	-0.04
11ketoE_ac	-17.089	-16.69	-0.40	-16.67	0.02	-16.68	0.01
11ketoE_ac	-17.144	-16.69	-0.45	-16.73	-0.04	-16.73	-0.04
		Average	-0.41				

Slope	1.001
Intercept	0.431
Correlation	1.000



Precision and Accuracy

Sample List

	Label	Status	Comment	Evaluate	Sample Type	Reference
1	Solvent			☑	Unknown	
2	ISTD			☑	Drift Correction	
3	MX018-1			☑	Delta Standard (CSIA)	MX018-1
4	MX018-2			☑	Delta Standard (CSIA)	MX018-2
5	MX018-1			☑	Unknown	
6	MX018-2			☑	Unknown	
7	RM_QC			☑	Drift Correction	
8	RM_QC			☑	Unknown	
9	F1_QCN_11-Keto			☑	Unknown	
10	F2_QCN_Testo			☑	Unknown	
11	F3_QCN_11-EpiT			☑	Unknown	
12	F4_QCN_5b-diol			☑	Unknown	
13	F5_QCN_5a-diol			☑	Unknown	
14	F6_QCN_PD			☑	Unknown	
15	MX018-1			☑	Unknown	
16	MX018-2			☑	Unknown	
17	RM_QC			☑	Drift Correction	
18	F1_QCP_11-Keto			☑	Unknown	
19	F2_QCP_Testo			☑	Unknown	
20	F3_QCP_11-EpiT			☑	Unknown	
21	F4_QCP_5b-diol			☑	Unknown	
22	F5_QCP_5a-diol			☑	Unknown	
23	F6_QCP_PD			☑	Unknown	
24	RM_QC			☑	Unknown	
25	RM_QC			☑	Unknown	
26	MX018-1			☑	Unknown	
27	MX018-2			☑	Unknown	
28	ISTD			☑	Drift Correction	

Sample List

	Label	Status	Comment	Evaluate	Sample Type	Reference
1	Solvent			☑	Unknown	
2	ISTD			☑	Unknown	
3	MX018-1			☑	Delta Standard (CSIA)	MX018-1
4	MX018-2			☑	Delta Standard (CSIA)	MX018-2
5	MX018-1			☑	Unknown	
6	MX018-2			☑	Unknown	
7	RM_QC			☑	Unknown	
8	RM_QC			☑	Unknown	
9	F1_QCN_11-Keto			☑	Unknown	
10	F2_QCN_Testo			☑	Unknown	
11	F3_QCN_11-EpiT			☑	Unknown	
12	F4_QCN_5b-diol			☑	Unknown	
13	F5_QCN_5a-diol			☑	Unknown	
14	F6_QCN_PD			☑	Unknown	
15	MX018-1			☑	Delta Standard (CSIA)	MX018-1
16	MX018-2			☑	Delta Standard (CSIA)	MX018-2
17	RM_QC			☑	Unknown	
18	F1_QCP_11-Keto			☑	Unknown	
19	F2_QCP_Testo			☑	Unknown	
20	F3_QCP_11-EpiT			☑	Unknown	
21	F4_QCP_5b-diol			☑	Unknown	
22	F5_QCP_5a-diol			☑	Unknown	
23	F6_QCP_PD			☑	Unknown	
24	RM_QC			☑	Unknown	
25	RM_QC			☑	Unknown	
26	MX018-1			☑	Delta Standard (CSIA)	MX018-1
27	MX018-2			☑	Delta Standard (CSIA)	MX018-2
28	ISTD			☑	Unknown	

Precision and Accuracy

Sample List

	Label	Status	Comment	Evaluate	Sample Type	Reference
1	Solvent			☑	Unknown	
2	ISTD			☑	Unknown	
3	MX018-1			☑	Unknown	
4	MX018-2			☑	Unknown	
5	MX018-1			☑	Unknown	
6	MX018-2			☑	Unknown	
7	RM_QC			☑	Unknown	
8	RM_QC			☑	Unknown	
9	F1_QCN_11-Keto			☑	Unknown	
10	F2_QCN_Testo			☑	Unknown	
11	F3_QCN_11-EpiT			☑	Unknown	
12	F4_QCN_5b-diol			☑	Unknown	
13	F5_QCN_5a-diol			☑	Unknown	
14	F6_QCN_PD			☑	Unknown	
15	MX018-1			☑	Unknown	
16	MX018-2			☑	Unknown	
17	RM_QC			☑	Unknown	
18	F1_QCP_11-Keto			☑	Unknown	
19	F2_QCP_Testo			☑	Unknown	
20	F3_QCP_11-EpiT			☑	Unknown	
21	F4_QCP_5b-diol			☑	Unknown	
22	F5_QCP_5a-diol			☑	Unknown	
23	F6_QCP_PD			☑	Unknown	
24	RM_QC			☑	Unknown	
25	RM_QC			☑	Unknown	
26	MX018-1			☑	Unknown	
27	MX018-2			☑	Unknown	
28	ISTD			☑	Unknown	

Australian Government
Department of Industry, Science and Resources

National Measurement Institute

Steroid CRM for GC-C-IRMS applications

Fong Liu
National Measurement Institute, Australia (NMIA)

Current CRMs for GC-C-IRMS application

CRM	Steroid/Matrix type	$\delta^{13}C_{VPDB}$ ± U(‰)	Traceability	Intended application
MX016	19-Norandrosterone in 20% methanol/water	-29.7 ± 0.8	CU-USADA 34-1	Calibration, Validation, QC
MX017	11 steroid metabolites in freeze dried urine	-30 to -20 (0.4 to 0.9)	Multipoint normalisation using MX018	Validation, matrix matched QC
MX018	13 pure steroid metabolites	-32 to -13 (0.1 to 0.6)	IAEA-CH-6, IAEA-CH-7	Multi-point calibration
MX020	Boldenone BM 1 (pure steroids)	-30.38 ± 0.29 -29.71 ± 0.28	USGS4-40, IAEA-CH-7	Calibration, Validation, QC
MX021	Formestane (pure steroid)	-30.71 ± 0.48	USGS4-40, IAEA-CH-7	Calibration, Validation, QC

New CRMs to be released –April 2024

- MX023 to MX028 – Six steroid CRM certified for $\delta^{13}C$
- For validation of GC-C-IRMS confirmatory analysis by linear mixing model (LMM)
- Funded by Sport Integrity Australia (SIA)

CRM	Steroid	Dried steroid (mg)
MX023	Androsterone	1
MX024	Etiocholanolone	1
MX025	Testosterone	0.5
MX026	5α-androstane-3α-17β-diol	0.5
MX027	5β-androstane-3α-17β-diol	0.5
MX028	Pregnanediol	0.5

Australian Government
Sport Integrity Australia

SPORT INTEGRITY AUSTRALIA

Preparation and handling of ampoule CRM

CRITICAL NOTE: Thorough rinsing of all glassware and tips with methanol is critical to minimise contamination for the stock and working solutions

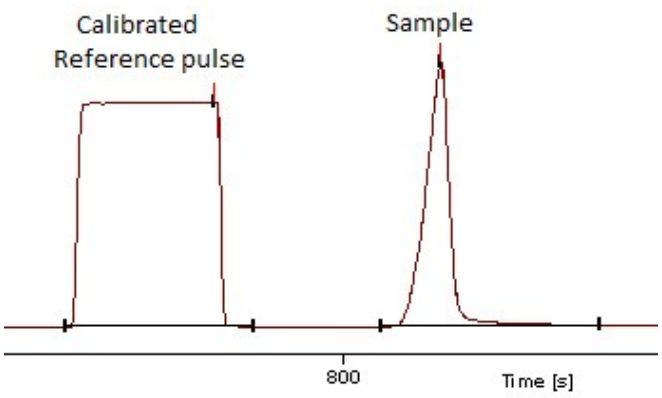
- **Ampoule reconstitution for stock solution ie. MX018**
  - 2 mL of 2-propanol, equilibrate 3 hours
  - Transfer to a clean vial

- **Storage of stock solution**
  - +4^{o}C protected from light when not in use

- **Working standard preparation**
  - In GC vial fitted with 200 µL insert (furnaced 500^{o}C for 7 hours)
  - 10 µL stock solution, internal standard, make up to 100 µL with cyclohexane
  - Use only rinsed pipette tips for both stock solution and solvent

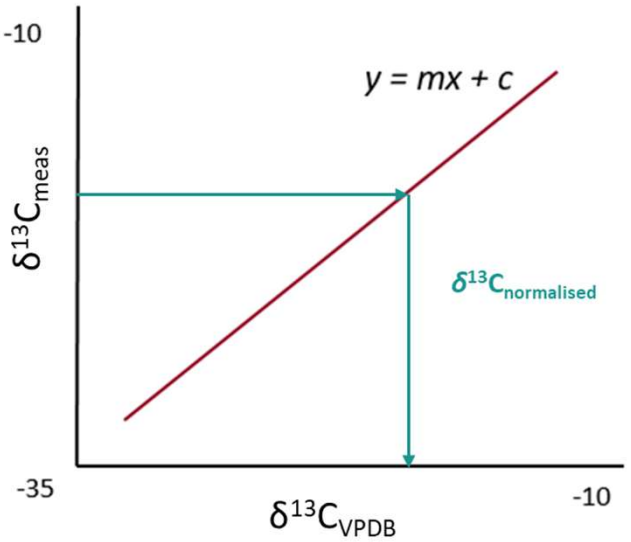
Two approaches to $\delta^{13}C$ measurement

Reference gas approach (MX005)



- $\delta^{13}C$ of CO_2 reference gas calibrated with steroid CRM
- Sample $\delta^{13}C$ is measured against an average batch $\delta^{13}C$ for reference gas
- Essentially a single point calibration!

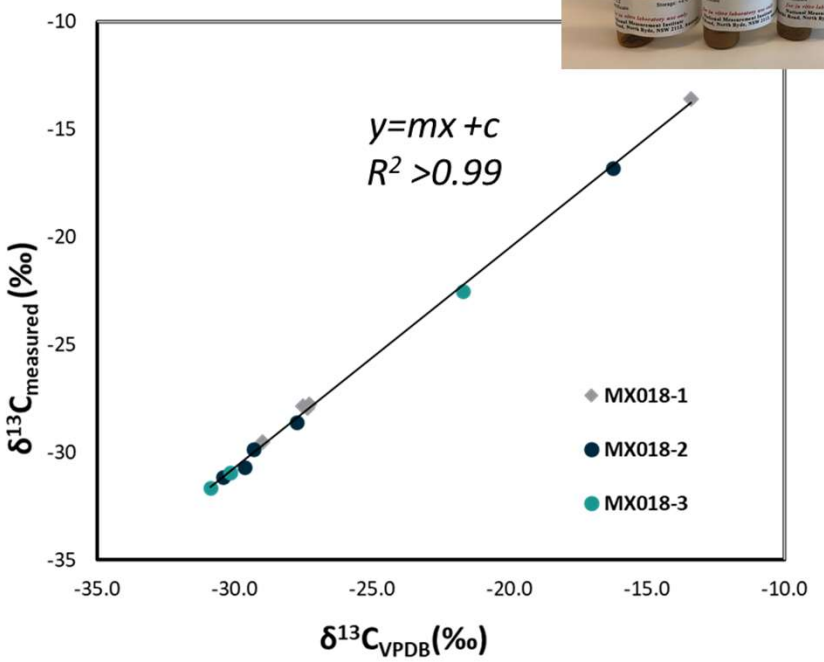
Multi-isotopic calibration (MX017)



- δ-values for samples and standards expressed as relative to an approximate $\delta^{13}C$ value of the reference gas
- Sample normalised using linear calibration function, $y = mx + c$

MX018 CRM for Multi-point calibration

- System suitability-zero enrichment, linearity
- $\delta^{13}C$ -13 to -32‰ using 3 mixes
  - 15 calibration points, *y=mx+c*
  - $\delta^{13}C_{norm} = (\delta^{13}C_{meas} - c)/m$
- Bracketing approach
- Set acceptance criteria for batch ie.
  - R^2 >0.99
  - In-house QC Spike (water/matrix)
  - QC standard to check calibration bias using CRM



Set criteria for passing ie. Deviation of normalised QC within ± 0.5 ‰

NMI Contact

Technical information

Email: fong.liu@measurement.gov.au

Sale of material:

Email: chemref@measurement.gov.au

Department of Industry, Science and Resources | **National Measurement Institute**

105 Delhi Rd
North Ryde, NSW 2113
Australia