Synthesis of a Novel Isotopically Labelled Standard for Quantification of γ-Nonalactone in New Zealand Pinot noir via SIDA-SPE-GC-MS

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Supplementary Information

Wine Technical Data

Table S1: Measured RS, TA, free and total SO_2 of wine samples in this work

Sample	Alcohol (% v/v)	RS (g L^{-1})	TA (g L^{-1})	pH	Free SO_2 (ppm)	Total SO_2 (ppm)
PN_1	12.3	0.244	4.85	3.49	3.70	31.4
PN_2	12.7	-0.146	4.72	3.60	5.78	16.1
PN_3	13.2	0.491	5.13	3.71	12.6	52.0
PN_4	13.0	0.679	5.35	3.67	11.3	45.5
PN_5	12.7	-0.0894	4.94	3.72	7.90	22.8
PN_6	13.9	5.48	4.90	3.61	9.40	41.1
PN_7	12.7	0.514	4.94	3.64	9.20	29.6
PN_8	12.5	0.461	4.35	3.60	7.52	35.7
PN_9	14.1	1.78	5.42	3.71	8.61	30.9
PN_10	13.2	0.439	5.12	3.60	8.59	32.7
PN_11	13.5	2.48	5.03	3.61	8.81	56.1
PN_12	13.6	2.03	4.89	3.94	8.46	45.8
Dry red cask wine	11.9	5.48	6.13	3.46	24.2	106

$^{2}H_{6}$-γ-Nonalactone Synthesis

Potassium 4-hydroxynonanoate **2***.* To a stirred solution of γ-nonalactone **1** (2.49 g, 15.43 mmol) in methanol (30 mL) was added potassium hydroxide (<85 % purity, 1.12 g, 15.43 mmol). This mixture was stirred at room temperature for 3 days. The solvent was removed *in vacuo* to yield the *title compound* **2** (3.22 g, 98 %) as a white solid which was used in the following reaction without further purification.

^{1}H NMR (400 MHz, $^{2}H_{2}O$) 0.89 (3H, t, *J* = 6.6 Hz, H9), 1.24-1.58 (8H, m, H-5, H-6, H-7, H-8), 1.62-1.84 (2H, m, H-3), 2.17-2.36 (2H, m, H-2), 3.61-3.71 (1H, m, H-4). ^{1}H NMR was in agreement with literature values.[3]

Isopropyl 4-hydroxynonanoate **3***.* Potassium 4-hydroxynonanoate **2** (1.00 g, 4.71 mmol) was dissolved in DMSO (30 mL) with heating. The resulting solution was cooled to room temperature, then isopropyl bromide (3.70 mL, 23.00 mmol) was added. This mixture was stirred at room temperature for 2 days, then diluted with water (20 mL) and extracted with diethyl ether (3 x 15 mL portions), dried with anhydrous magnesium sulfate and concentrated *in vacuo*. The resulting oil was purified by column chromatography (5 % v/v Et_2O/CH_2Cl_2) to give the *title compound* **3** (0.484 g, 47 %) as a pale-yellow oil.

^{1}H NMR (400 MHz, $C^{2}HCl_3$) 0.87 (3H, t, *J* = 6.8, H-9), 1.21 (6H, d, *J* = 6.3 Hz, H-2'), 1.22-1.47 (8H, m, H-5, H-6, H-7 and H-8), 1.60-1.85 (2H, m, H-2), 2.33-2.46 (2H, m, H-3), 3.42-3.74 (1H, m, H-4), 5.00 (1H, sept, *J* = 6.3 Hz, H-1'). ^{1}H NMR was in agreement with literature values.[3]

Isopropyl 4-oxononanoate **4***.* Oxalyl chloride (0.25 mL, 2.88 mmol) was added to a mixture of DMSO (0.41 mL, 5.7 mmol) and CH_2Cl_2 (9 mL) at – 78 °C under an atmosphere of nitrogen, over 2 mins. This mixture was stirred at – 78 °C for 30 mins, then a solution of isopropyl 4-hydroxynonanoate **3** (0.40 g, 1.85 mmol) in CH_2Cl_2 (1.5 mL) was added and the resulting mixture was stirred at – 78 °C for 45 mins. Triethylamine (1.6 mL, 11.6 mmol) was added slowly, and this mixture was stirred at – 78 °C for 30 mins, then 0 °C for 30 mins, then room temperature for 30 mins. The reaction mixture was poured into a rapidly stirred solution of $NaHSO_4$ (1.0 M, 12 mL), the layers separated, and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL portions). The combined organic extracts were concentrated under reduced pressure, and the resulting residue was taken up in diethyl ether (10 mL) and washed with a 1.0 M solution of $NaHSO_4$ (3 x 5 mL portions), water (5 mL), saturated $NaHCO_3$ (5 mL) and brine (5 mL). The organic phase was dried with anhydrous magnesium sulfate, filtered, and concentrated *in vacuo* to

yield the crude product as a pale-yellow oil. This oil was purified by column chromatography (1:1 ethyl acetate: petroleum ether) to give the *title compound* **4** (0.328 g, 83 %) as a pale-yellow oil.

^{1}H NMR (400 MHz, C^{2}HCl$_3$) 0.88 (3H, t, *J* = 7.0 Hz, H-9), 1.22 (6H, d, *J* = 6.3 Hz, H-2'), 1.25-1.32 (4 H, m, H-7, H-8), 1.58 (2H, qn, *J* = 7.2 Hz, H-6), 2.43 (2H, t, *J* = 7.5 Hz, H-5), 2.53 (2H, t, *J* = 6.5 Hz, H-2), 2.71 (2H, t, *J* = 6.7 Hz, H-3), 4.98 (1H, sept, *J* = 7.4 Hz, H-1'). ^{1}H NMR was in agreement with literature values.[3]

$^{2}H_6$-Oxononanoic acid **5***.* Isopropyl 4-oxononanoate **4** was heated under reflux in 20 % (w/v) ^{2}HCl/$^{2}H_2O$ solution (2.3 mL) and $^{2}H_2O$ (4 mL) under nitrogen for 7 days. This reaction mixture was allowed to cool and was then extracted with diethyl ether (3 x 6 mL portions). The organic extracts were combined, then dried with anhydrous magnesium sulfate, filtered, and concentrated *in vacuo*. The resulting residue was again heated under reflux in 20 % (w/v) ^{2}HCl/$^{2}H_2O$ solution (2.3 mL) and $^{2}H_2O$ (4 mL) under nitrogen for 7 days. This reaction mixture was allowed to cool and was then extracted with diethyl ether (3 x 6 mL portions). The organic extracts were combined, then dried with anhydrous magnesium sulfate, filtered, and concentrated *in vacuo* to give the *title compound* **5** (0.234 g, 93 %) as a yellow oil.

^{1}H NMR (400 MHz, C^{2}HCl$_3$) 0.86 (3H, t, *J* = 7.1 Hz, H-9), 1.23-1.32 (4H, m, H-7, H-8), 1.55 (2H, *t*, J = 7.0 Hz, H-6), 2.35-2.44 (0.29H*, m, H-5), 2.46-2.60 (1.83H*, m, H-2), 2.62-2.71 (0.30H*, m, H-3). ^{1}H NMR integrals denoted * represent partially deuterated positions.

^{13}C NMR (100 MHz, C^{2}HCl$_3$) 13.9 (CH3, C-9), 22.4 (CH2, C-8), 23.4 (CH2, C-6), 27.5 (CH2, C-2), 31.3 (CH2, C-7), 36.6 (CH2, C-3), 58.2 (CH2, C-3), 175.9 (COOH, C-1)

***m/z* (ESI^{+})** 173 (MH+, 100 %), 174 (10), 175 (2), 131 (1)

HRMS Found (MH^{+}): 173.1185, $C_9H_{17}O_3^{+}$ requires 173.1178†

†The most abundant molecular ion of a mixture of analogues.

$^{2}H_6$-γ-Nonalactone **6***.* $^{2}H_6$-oxononanoic acid **5** (0.230 g, 1.28 mmol) was added to a solution of sodium borohydride (0.129 g, mmol) in $^{2}H_2O$ (6 mL), and this mixture was stirred under nitrogen at room temperature for 24 h. This reaction was quenched by the careful addition of 20 % (w/v) ^{2}HCl/$^{2}H_2O$ solution (pH 2), then stirred under nitrogen at room temperature for an additional 24 h. The mixture was extracted with diethyl ether (3 x 15 mL portions), then the combined organic extracts were dried with anhydrous magnesium sulfate and

concentrated *in vacuo* to give the crude product. The crude product was purified by column chromatography (1:1 diethyl ether: petroleum ether) to give the *title compound* **6** (73.5 mg, 35 %) as a colourless oil.

Rf (1:1 diethyl ether: petroleum ether) 0.36

IR: υ_{max}(film)/cm^{-1} 2927 and 2861, 1767, 1634, 1522, 1467 and 1424, 1336, 1264 and 1193, 1132, 1024 and 902

^{1}H NMR (400 MHz, C^{2}HCl$_3$) 0.89 (3H, t, *J* = 6.7 Hz, H-9), 1.25- 1.50 (6H, m, H-6, H-7 and H-8), 1.52-1.77 (0.99H*, m, H-5) 1.78-1.88 (0.18H*, m, H-3a) 2.24-2.34 (0.20H*, m, H-3b), 2.47-2.56 (1.74H*, m, H-2), 4.46 (1H, s, H-4). ^{1}H NMR integrals denoted * represent partially deuterated positions.

^{13}C NMR (100 MHz, C^{2}HCl$_3$) 14.0 (CH3, C-9), 22.5 (CH2, C-6, C-7, or C-8), 24.8 (CH2, C-6, C-7, or C-8), 28.8 (CH2, C-2), 31.5 (CH2, C-6, C-7 or C-8), 80.9 (CH, C-4), 177.3 (COO, C-1)

***m/z* (ESI^{+})** 239 (100 %), 223 (20), 181 (2), 157 (MH+, 3), 144 (5), 133 (3)

HRMS Found (MH^{+}): 157.1159, $C_9H_{17}O_2^{+}$ requires 157.1229[ŧ]

[ŧ]The most abundant molecular ion of a mixture of analogues.

Retention Times

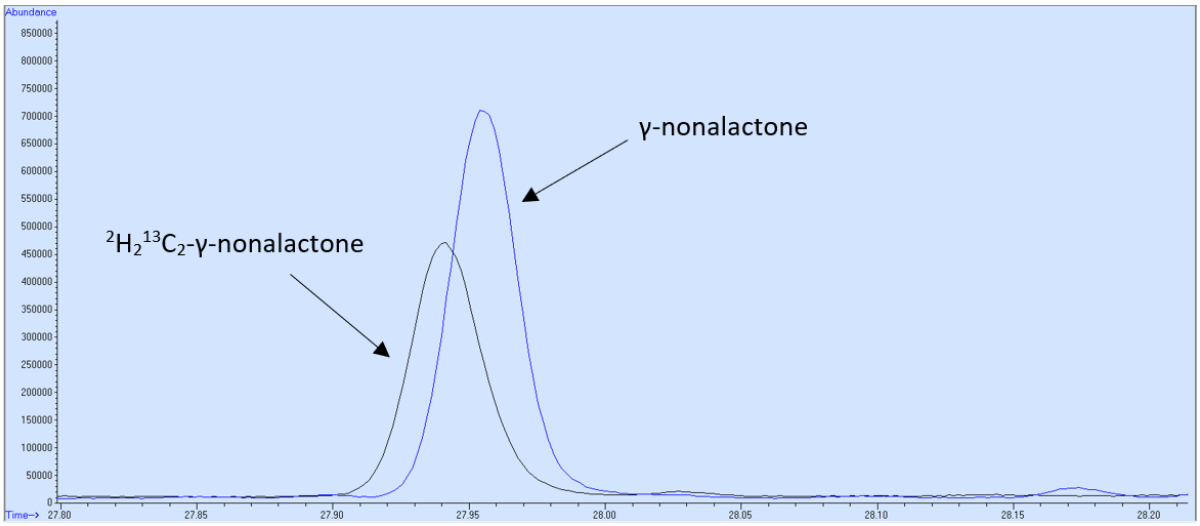
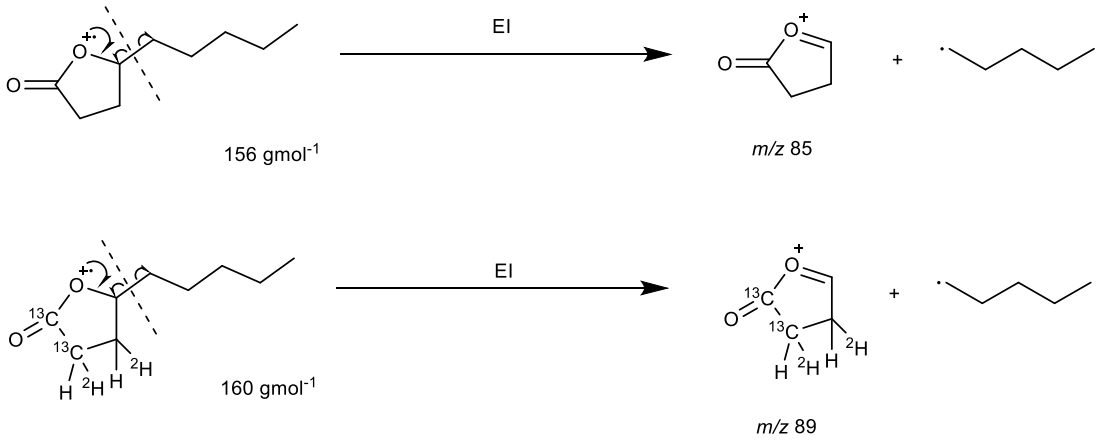


Fig. S1: Excerpt of chromatogram showing base peaks corresponding to γ-nonalactone 1 and $^{2}H_{2}{}^{13}C_{2}$-γ-nonalactone 1

Ions used For Quantification



Scheme S1: Hypothesised fragmentation pathway of (a) γ-nonalactone and (b) $^{2}H_{2}{}^{13}C_{2}$-γ-nonalactone to produce their respective base peaks during EI mass spectrometry

Characterisation of $^{2}H_{6}$-Oxononanoic acid 5

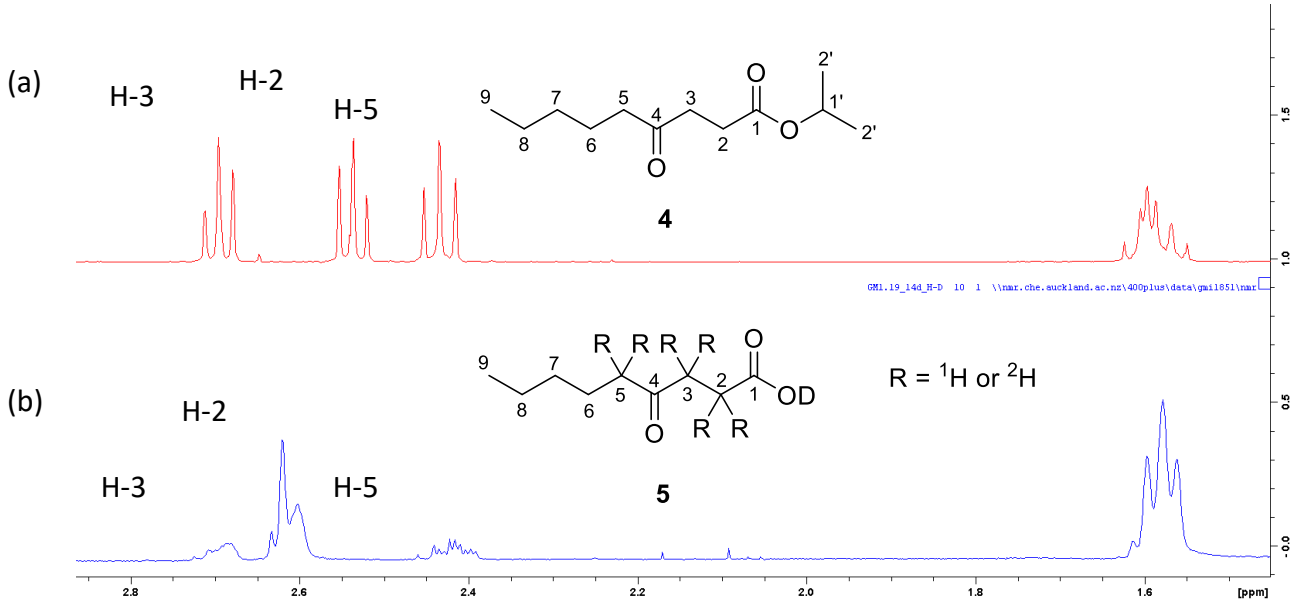


Fig. S2: Excerpts from ^{1}H NMR spectra of (a) isopropyl 4-oxononanoate 4 prior to deuterium exchange and (b) 4-oxononanoic acid 5 after 14 days deuterium exchange reaction conditions. Peaks corresponding to exchangeable protons are shown (H-2, H-3, and H-5). Incomplete deuterium exchange is shown by remaining peaks corresponding to these protons

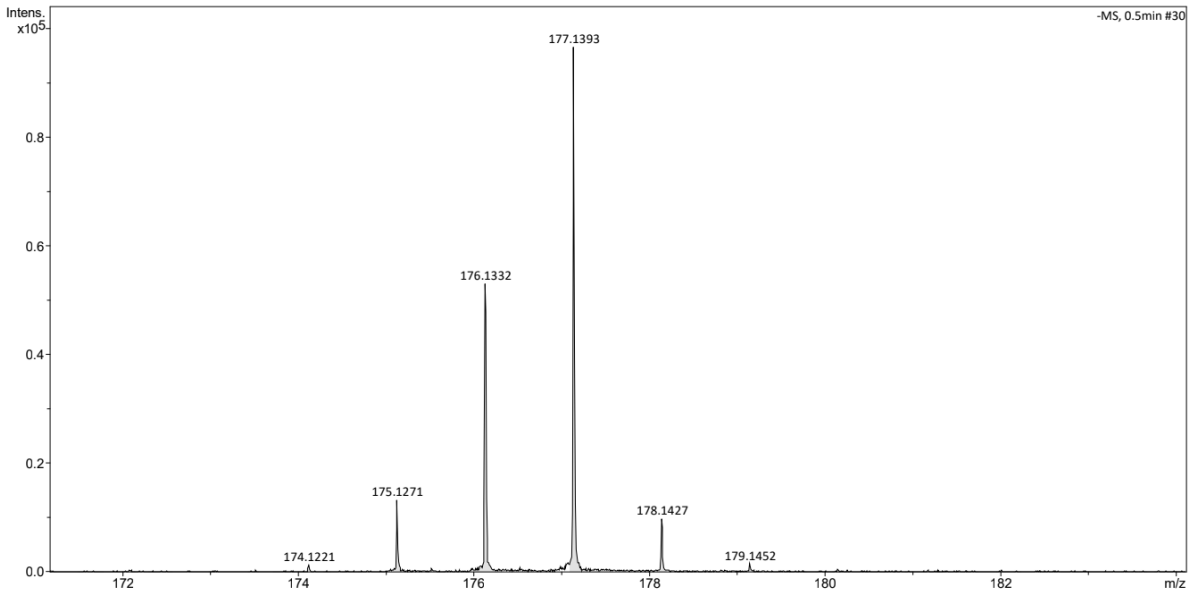


Fig. S3: Excerpt from HRMS analysis (negative ion mode) of 4-oxononanoic acid 5 after 14 days of deuterium exchange conditions. *m/z* 177 corresponds to full deuterium exchange ($C_9H_9{}^2H_6O_3^-$), whilst the presence of *m/z* 176 and *m/z* 175 likely indicate incomplete exchange ($C_9H_{10}{}^2H_5O_3^-$ and $C_9H_{11}{}^2H_4O_3^-$, respectively)

Verification of suitable SIDA internal standard

Table S2: Analysis of isotopologue distribution of $^{2}H_{2}{}^{13}C_{2}$-γ-nonalactone after different model wine spiking conditions. "M" refers to a *m/z* ratio of 85, corresponding to the natural analogue of γ-nonalactone. The relative integrals of M+2, M+3 and M+4 (corresponding to *m/z* 87, 88 and 89, respectively) were monitored and are shown here

	Peak areas corresponding to specified *m/z* ratio					Relative peak area of specified *m/z* ratios		
Sample	**M+0**	**M+1**	**M+2**	**M+3**	**M+4**			
	85	**86**	**87**	**88**	**89**	**M+4/M+3**	**M+4/M+2**	**M+3/M+2**
30min_RT_a	1417	1221	8053	27753	35838	1.29	4.45	3.45
30min_RT_b	809	867	7882	27653	35237	1.27	4.47	3.51
30min_RT_c	689	932	8950	30254	39822	1.32	4.45	3.38
30min_HEAT_a	481	915	7301	25221	32520	1.29	4.45	3.45
30min_HEAT_b	564	767	6037	20009	26790	1.34	4.44	3.31
30min_HEAT_c	492	1151	7285	26139	35354	1.35	4.85	3.59
180min_RT_a	721	898	7371	26458	34704	1.31	4.71	3.59
180min_RT_b	568	1197	9198	31786	41949	1.32	4.56	3.46
180min_RT_c	196	1614	8981	32240	43309	1.34	4.82	3.59
180min_HEAT_a	533	813	6023	21328	27790	1.30	4.61	3.54
180min_HEAT_b	301	1017	6720	22613	28806	1.27	4.29	3.367
180min_HEAT_c	521	541	4941	17694	22741	1.29	4.60	3.58

Calibration Comparison and Verification

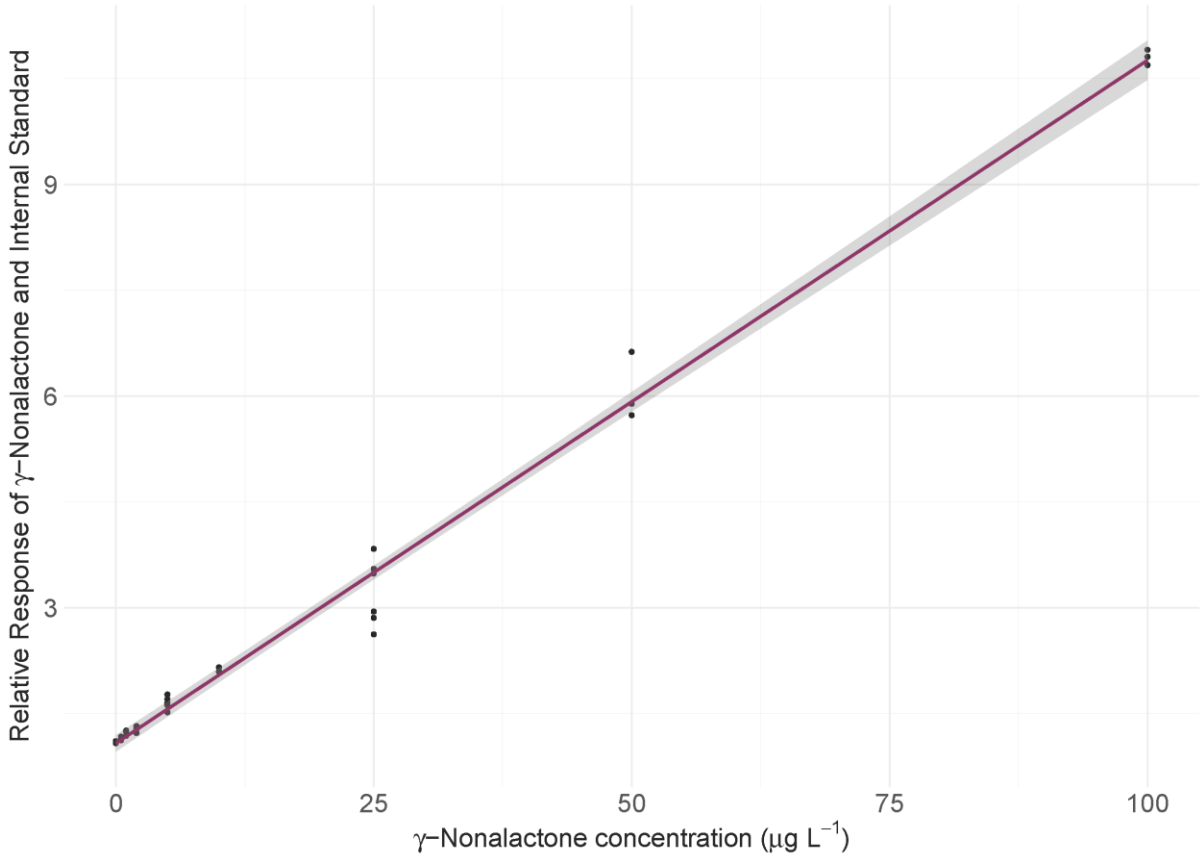


Fig. S4: Calibration curve constructed using a dry red wine matrix, showing relative response of γ-nonalactone 1 and $^{2}H_{2}{}^{13}C_{2}$-γ-nonalactone 15 with varying concentrations of γ-nonalactone

Table S3: Dry red wine calibration data

	Dry Red Wine Calibration Data
slope	0.09684
slope standard error	0.00159
degrees of freedom	32
95 % t-statistic for 99 df	2.037
95 % confidence interval (+/-)	0.00323883
Upper	0.10007883
lower	0.09360117
R^2	0.9914

Table S4: Model Wine calibration data, with associated LOD and LOQ

	Model Wine Calibration Data
slope	0.0958
slope standard error	0.000374
degrees of freedom	99
95 % t-statistic for 99 df	1.984
95 % confidence interval (+/-)	0.000742
upper	0.0966
lower	0.0951
R^2	0.999
a = estimate of intercept	0.0624
s = standard error of line	0.104
LOD = a+3.29s	0.406
LOQ = a+10s	1.11

Table S5: Relative response of γ-nonalactone and $^{2}H_{2}{}^{13}C_{2}$-γ-nonalactone in instrumental repeats. This data was used to find the repeatability of this analytical method

Sample	Relative Response of γ-nonalactone and $^{2}H_{2}{}^{13}C_{2}$-γ-nonalactone
MWI_5a_1	0.543
MWI_5a_2	0.515
MWI_5a_3	0.527
MWI_5a_4	0.523
MWI_5a_5	0.539
MWI_5a_6	0.537
MWI_5a_7	0.535
n	7
Standard deviation	0.0101
Reproducibility	0.00380
% Reproducibility	0.380

Table S6: Relative response of γ-nonalactone and $^{2}H_{2}{}^{13}C_{2}$-γ-nonalactone in experimental repeats. Repeats were conducted on different days (indicated by letters a, b, c, d, and e) and in triplicate (indicated by numbers 1, 2 and 3). * Represents outliers, detected by Grubbs' test and these were excluded from averages

Sample	**Relative Response of γ-nonalactone and $^{2}H_{2}{}^{13}C_{2}$-γ-nonalactone**	Average
MWI_5r_1a	0.577	0.577
MWI_5r_2a	0.586	
MWI_5r_3a	0.568	
MWI_5r_1b	0.619	0.588
MWI_5r_2b	0.554	
MWI_5r_3b	0.591	
MWI_5r_1c	0.0104*	0.569
MWI_5r_2c	0.576	
MWI_5r_3c	0.562	
MWI_5r_1d	0.545	0.537
MWI_5r_2d	0.523	
MWI_5r_3d	0.538	
MWI_5r_1e	0.547	0.542
MWI_5r_2e	0.516	
MWI_5r_3e	0.564	
n	14	
Standard deviation	0.0269	
Repeatability	0.00720	
% Repeatability	0.720	

Wine Data

Table S7: Relative response of γ-nonalactone and $^{2}H_{2}^{13}C_{2}$-γ-nonalactone for each NZ Pinot noir sample, measured in triplicate and averaged. The calculated γ-nonalactone concentration, vintage, region, and price ($NZ) are also provided for each wine. * Represents outliers, detected by Grubbs' test and these were excluded from averages

	Relative response γ-Nonalactone/Internal Standard Area								
Sample	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Calculated γ-Nonalactone Concentration (μg L^{-1})**	**Standard deviation (±)**	**Vintage**	**Region**	**Price ($NZ)**
PN_1	1.13	1.15	1.12	1.13	11.2	0.0183	2019	Marlborough	32.99
PN_2	0.864	0.875	0.961	0.900	8.74	0.0532	2020	Waipara Valley	18.99
PN_3	1.06	2.31*	1.06	1.01	9.93	0.00359	2021	Central Otago	22.99
PN_4	2.23	2.22	2.21	2.22	22.5	0.0100	2021	Marlborough	24.99
PN_5	0.964	0.923	0.915	0.934	9.10	0.0265	2020	Central Otago	28.99
PN_6	1.27	1.26	1.24	1.26	12.5	0.0132	2019	Central Otago	54.99
PN_7	1.31	1.29	1.30	1.30	12.9	0.00728	2019	Central Otago	42.99
PN_8	0.848	0.847	0.878	0.858	8.30	0.0178	2019	Marlborough	52.99
PN_9	0.957	1.03	0.497*	0.994	9.72	0.0530	2019	Waipara Valley	61.99
PN_10	1.25	1.27	1.29	1.27	12.6	0.0208	2019	Central Otago	49.99
PN_11	1.01	1.02	1.01	1.01	9.90	0.00622	2018	Central Otago	99.99
PN_12	0.932	0.870	0.902	0.902	8.76	0.0309	2019	Central Otago	52.99