Analysis of dithiocarbamates in berries and leafy vegetables by ultra-high performance liquid chromatography coupled with tandem mass spectrometry

Supplementary material

Cécile Dionne Edoa, Justine Fontaine, Ken Goeury, Sébastien Sauvé

Department of Chemistry, Université de Montréal, Montreal, Quebec H3C 3J7, Canada

Additional details on the validation procedure

Accuracy and precision were evaluated using samples enriched to 20 µg/kg to calculate the percentage recovery, which also represents the yield of the methylation reaction (equation 1). This was done in triplicate and repeated over three days. Thus, samples were enriched on the same day and used to determine intra-day precision by calculating the standard deviation of their recovery percentages (replicability, $RSD_{(r)}$) (equation S1). The inter-day precision was evaluated by calculating the relative standard deviation (repeatability, $RSD_{(R)}$) (equation S2) of all the results of the samples enriched on three different days. To determine whether the averages of the 3-sample series are equivalent, an analysis of variance (ANOVA) was also performed.

Formula for calculating replicability

$$RSD_r = \frac{t(0{,}975, n-1) * S_1}{\sqrt{n}}$$ (n= 3 and $t_{(0.975)}$ = 4.303 (EQ S1) [1]

Where S_1: standard deviation of a series of measurements referring to repeatability

Formula for calculating repeatability

$$RSD_R = \frac{t(0{,}975, n-1) * S_2}{\sqrt{n}}$$ (n= 9 and $t_{(0.975)}$ = 2.306) (EQ S2) [1]

Where S_2: standard deviation of a series of measurements referring to repeatability

$$Recovery\ (\%) = \left(\frac{C_{sb} - C_{ns}}{C_{sa} - C_{ns}}\right) * 100$$ (EQ 1) [1]

Where C_{sb} : Concentration of the enriched sample before extraction using DTC standards

C_{sa} : Concentration of the enriched sample after extraction and clean-up using DTCs-Me standards

C_{ns} : Concentration of the unenriched sample

Calibration curves

Formula for calculating LOD

$LOD = 3 * S$ (n = 6) (EQ S3)

Formula for calculating LOQ

$LOQ = 10 * S$ (n = 6) (EQ S4)

Where S: standard deviation and n: number of samples

To do this, the areas of the peaks obtained were plotted against the concentrations of DTC-Me. In addition, the calibration curves in the matrix were produced on two different days, which enabled the concentrations of the analytes in our samples (each sample was extracted in triplicate) to be calculated. Based on the results obtained for each data series, an analysis of variance (ANOVA) was performed to determine whether the averages obtained from these curves were equivalent. In addition, given that the matrix effect can strongly influence analytical performance, it is important to evaluate both the identification and the quantification of an analyte [2]. To this end, analytical performance was evaluated by comparing the slope of the calibration curve prepared in the matrix ($a_{(M)}$) with the slope of the calibration curve prepared in the solvent ($a_{(S)}$) using equation 2 below [3].

$$Matrix\ effect\ (\%) = \left(\frac{a_M}{a_S} - 1\right) * 100 \quad \text{(EQ 2)}$$

Table SI-1a: Leafy vegetables samples information

Sample number	Leafy vegetables	Brands	Origin	Supermarket	Code
1	Romaine lettuce hearts	Dole	USA	IGA	A
2	Iceberg lettuce	Oak Canyon Farms	USA	IGA	B
3	Chopped spinach (frozen)	Artic Gardens	France	IGA	C
4	Fresh spinach	Harvest Fresh	USA	IGA	D
5	Curly lettuce	Folia (GreenHouses)	Canada	IGA	E
6	Rocket	Attitude	USA	IGA	F
7	Romaine lettuce hearts	Ocean Mist Farms	USA	Super-C	G
8	Cress	B&W (non-OGM)	USA	Super-C	H
9	Iceberg lettuce	Naturels Rewards	USA	Super-C	I
10	Fresh spinach	Les marques Métro	USA	Super-C	J
11	Curly lettuce	Good Leaf (no pesticides)	Quebec	Super-C	K
12	Frozen chopped spinach	Selection	France	Super-C	L
13	Romaine lettuce hearts	Happy Green	Mexico	Maxi	M
14	Iceberg lettuce	Happy Green	Mexico	Maxi	N
15	Fresh spinach	Queen Victoria	Canada	Maxi	O
16	Chopped kale	Arte*	Mexico	Maxi	p
17	Young spinach	Organics	USA	Maxi	Q
18	Chopped spinach (precooked)	President's choice	Belgium	Maxi	R
19	Romaine lettuce hearts	Tanimura & Antle	USA	Marché C&T	S
20	Fresh spinach	Frisco's	Canada	Marché C&T	T
21	Chinese spinach (frozen)	J.L Trading	China	Marché C&T	U
22	A-choy lettuce	Marché C&T	China	Marché C&T	V
23	Taiwanese bok choy lettuce	Marché C&T	Mexico	Marché C&T	W
24	Curly green lettuce	Marché C&T		Marché C&T	X

Table SI-1b: Berries samples information

Sample number	Berries	Brands	Origin	Supermarket	Code
25	Fresh blackberries	North Bay Produce	Mexico	Maxi	AA
26	Fresh blackberries	Wish Farms	Mexico	Maxi	BB
27	Fresh strawberries	Gem-Pack Berries	Mexico	Maxi	CC
28	Fresh blackberries	President's choice	Mexico	Maxi	DD
29	Fresh raspberries	Berry Valley	Mexico	Maxi	EE
30	Fresh blueberries	Local farm	Morocco	Maxi	FF
31	Fresh blueberries	President's choice	USA	Maxi	GG
32	Fresh blueberries	Naturip Farms	Canada	Maxi	HH
33	Frozen blueberries	No name	Canada	Maxi	II
34	Frozen blueberries	President's choice	Canada	Maxi	JJ
35	Frozen strawberries	No name	Chili	Maxi	KK
36	Frozen blackberries	President's choice	Mexico	Maxi	LL
37	Frozen strawberries	President's choice	Mexico	IGA	MM
38	Fresh raspberries	Driscoll's	USA	IGA	NN
39	Fresh strawberries	Naturipe Farms	Mexico	IGA	PP
40	Fresh blackberries	Driscoll's	Morocco	IGA	OO
41	Fresh blueberries	Shajara	Canada	IGA	QQ
42	Mixed berries	Snowcrest	Quebec	IGA	RR
43	Frozen blueberries	Bleu et Bon (no pesticides)	Canada	IGA	SS
44	Frozen blueberries	Compliments	Mexico	Super-C	TT
45	Fresh blackberries	Berry-Fresh	USA	Super-C	UU
46	Fresh blueberries	Clear Springs	Mexico	Super-C	VV
47	Fresh blackberries	Mariland Farms	USA	Super-C	WW
48	Fresh blueberries	Camposol	Canada	Super-C	XX
49	Mixed berries	Selection	Canada	Super-C	YY
50	Frozen blueberries	Irrésistible	Morocco	Super-C	ZZ
51	Frozen strawberries	Naturalia	Quebec	Super-C	FL

Table SI-2 : Ion Source properties

Parameters	Values
Ion source Type	H-ESI
Spray voltage	Static
Positive Ion (V)	3800
Negative Ion (V)	2500
Sheath Gas (Arb)	60
Aux Gas (Arb)	25
Sweep Gas (Arb)	1
Ion Transfer Tube Temp (ºC)	350
Vaporizer Temp (ºC)	400
Current LC Flow (µL/min)	0

Table SI-3: Optimized MS/MS parameters for methylated derivatives of dithiocarbamates (DMD-Me, EBD-Me, and PBD-Me).

Compounds	Molecular formula	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)	RF Lens (V)
DMD-Me	$C_4H_9NS_2$	136.04	88.00	13	40
		136.04	72.92	32	
EBD-Me	$C_6H_{12}N_2S_4$	241.70	193.00	11	54
		241.71	134.00	26	
PBD-Me	$C_7H_{14}N_2S_4$	255.00	206.92	14	54
		255.01	147.75	22	

[a] This transition was used for quantification

[b] This transition was used for confirmation

Table SI-4 : ANOVA results for averages recoveries (Fc = 5.14 and p = 0.05)

Matrix	Compound	Average recovery for each day (%)	F	Probability
Spinach	DMD-Me	76.5 69.5 69.4	1.46	0.30
	EBD-Me	35.8 31.7 23.6	5.35	0.05
	PBD-Me	7.7 5.9 5.3	3.94	0.08
Blueberries	DMD-Me	103 74.1 99.2	7.53	0.02
	EBD-Me	74.1 57.3 55.3	4.08	0.08
	PBD-Me	8.3 7.8 8.5	0.30	0.75

Table SI-5: Summary table of the main validation parameters (LOD, LOQ, recovery and RSD_R)

Matrix	Compounds	LOD (µg/kg of CS_2)	LOQ (µg/kg of CS_2)	Recovery (%)	RSD_R (%)
Leafy vegetables	DMD	0.08	0.27	72	4.8
	EBD	0.38	1.27	31	4.5
	PBD	0.01	0.03	6.3	0.84
Berries	DMD	0.04	0.14	92	12
	EBD	0.26	0.87	62	8.9
	PBD	0.01	0.03	8.2	1.1

Table SI-6: ANOVA results for calculation of DMD-Me concentrations using the 2 calibration curves in blueberries. Fc = 7.71 and Pc = 0.05

DMD-Me				
Sample	**Average.conc (2) (µg/kg)**	**Average.conc (3) (µg/kg)**	**F**	**Probability**
AA	0.52	0.54	1.65	0.27
BB	0.50	0.52	0.03	0.88
CC	1.11	1.17	0.05	0.84
DD	0.37	0.39	0.07	0.81
EE	0.38	0.39	0.07	0.81
FF	0.49	0.52	0.22	0.66
FL	0.69	0.72	0.08	0.79
GG	0.52	0.55	0.08	0.79
HH	0.63	0.66	3.26	0.15
II	0.78	0.82	0.13	0.74
JJ	1.15	1.21	0.11	0.75
KK	0.50	0.53	0.02	0.90
LL	1.26	1.32	0.05	0.84
MM	0.60	0.63	0.03	0.87
NN	1.23	1.29	0.01	0.92
OO	0.59	0.62	0.05	0.83
PP	0.34	0.36	0.25	0.65
QQ	0.74	0.78	0.03	0.88
RR	0.47	0.49	0.49	0.52
SS	0.51	0.53	0.15	0.72
TT	0.33	0.35	0.21	0.67
UU	0.52	0.54	0.03	0.86
VV	0.33	0.34	0.17	0.70
WW	0.42	0.44	0.02	0.89
XX	0.32	0.34	0.16	0.71
YY	0.55	0.58	0.16	0.71
ZZ	0.62	0.65	0.16	0.71

Table SI-7: ANOVA results for the calculation of EBD-Me concentrations using the 2 calibration curves in blueberries. Fc = 7.71 and Pc = 0.05

EBD-Me				
Sample	**Average.conc (2) (µg/kg)**	**Average.conc (3) (µg/kg)**	**F**	**Probability**
AA	2.32	2.80	4.34	0.11
BB	2.67	3.21	0.64	0.47
CC	1.24	1.49	2.72	0.17
DD	2.32	2.79	3.51	0.13
EE	1.48	1.78	9.36	0.04
FF	21.1	25.4	6.45	0.06
FL	99.5	120	0.42	0.55
GG	3.25	3.91	13.8	0.02
HH	2.81	3.38	2.58	0.18
II	1.63	1.96	1.14	0.35
JJ	0.95	1.15	0.19	0.69
KK	N/A	N/A	N/A	N/A
LL	1.75	2.11	0.22	0.66
MM	1.21	1.45	61.3	0.00
NN	43.8	52.7	0.14	0.73
OO	2.87	3.46	57.0	0.00
PP	1.34	1.61	0.23	0.65
QQ	2.37	2.85	6.91	0.06
RR	2.30	2.76	2.11	0.22
SS	2.48	2.98	9.81	0.04
TT	1.76	2.12	98.6	0.00
UU	11.4	13.7	0.79	0.42
VV	1.87	2.25	0.44	0.55
WW	0.99	1.19	0.78	0.43
XX	6.16	7.41	0.45	0.54
YY	2.98	3.58	483	0.00
ZZ	2.30	2.77	0.91	0.39

Table SI-8: ANOVA results for the calculation of PBD-Me concentrations using the 2 calibration curves in blueberries. Fc = 7.71 and Pc = 0.05

PBD-Me				
Sample	**Average.conc (2) (µg/kg)**	**Average.conc (3) (µg/kg)**	**F**	**Probability**
AA	0.04	0.05	0.07	0.81
BB	0.06	0.06	0.03	0.87
CC	0.05	0.06	0.15	0.72
DD	0.03	0.03	0.37	0.58
EE	0.10	0.10	0.10	0.77
FF	0.05	0.05	0.08	0.79
FL	0.20	0.22	0.03	0.87
GG	0.04	0.05	0.07	0.80
HH	0.06	0.06	0.01	0.91
II	0.08	0.08	0.17	0.71
JJ	0.06	0.06	0.01	0.91
KK	0.03	0.03	0.05	0.83
LL	0.11	0.12	0.00	0.95
MM	0.37	0.40	0.03	0.87
NN	0.07	0.07	0.02	0.91
OO	0.04	0.04	0.07	0.80
PP	0.05	0.06	0.15	0.71
QQ	0.07	0.07	0.40	0.56
RR	0.05	0.06	0.92	0.39
SS	0.07	0.07	0.03	0.88
TT	0.07	0.07	0.03	0.88
UU	0.04	0.05	0.30	0.61
VV	0.04	0.04	0.11	0.75
WW	0.04	0.04	0.02	0.90
XX	0.05	0.06	0.03	0.88
YY	0.06	0.06	0.13	0.73
ZZ	0.06	0.06	0.03	0.88

Table SI-9: ANOVA results for the calculation of DMD-Me concentrations using the 2 calibration curves in spinach. Fc = 7.71 and Pc = 0.05

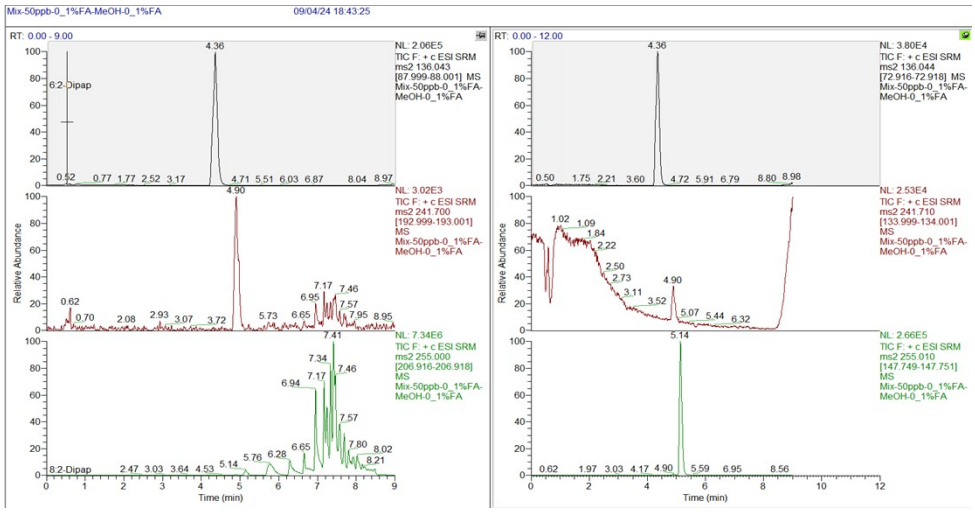
DMD-Me				
Sample	**Average.conc (2) (µg/kg)**	**Average.conc (3) (µg/kg)**	**F**	**Probability**
A	0.84	0.89	1.28	0.32
B	0.57	0.61	0.08	0.79
C	1.01	1.08	0.25	0.64
D	0.83	0.88	0.84	0.41
E	0.71	0.75	0.20	0.68
F	2.01	2.14	2.02	0.23
G	0.69	0.73	0.13	0.74
H	0.58	0.62	0.47	0.53
I	0.49	0.52	2.15	0.22
J	1.59	1.69	0.10	0.77
K	1.25	1.33	0.08	0.80
L	1.48	1.57	0.05	0.84
M	0.81	0.86	0.17	0.70
N	0.54	0.57	0.59	0.48
O	1.65	1.75	0.28	0.62
P	5.23	5.55	0.06	0.82
Q	1.89	2.01	0.15	0.71
R	0.56	0.59	0.15	0.71
S	1.16	1.23	0.38	0.57
T	0.50	0.53	0.65	0.47
U	0.85	0.90	2.18	0.21
V	1.23	1.30	0.06	0.82
W	2.03	2.15	0.30	0.61
X	1.07	1.14	0.77	0.43

Table SI-10: ANOVA results for the calculation of EBD-Me concentrations using the 2 calibration curves in spinach. Fc = 7.71 and Pc = 0.05

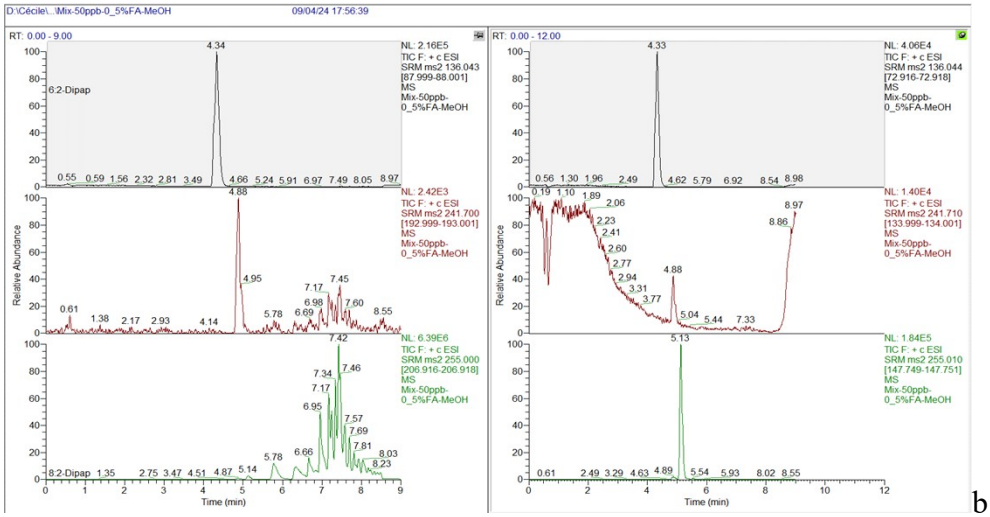
EBD-Me				
Sample	**Average.conc (2) (µg/kg)**	**Average.conc (3) (µg/kg)**	**F**	**Probability**
A	N/A	N/A	N/A	N/A
B	0.20	0.24	0.77	0.43
C	N/A	N/A	N/A	N/A
D	N/A	N/A	N/A	N/A
E	0.59	0.73	3.58	0.13
F	3.14	3.86	2.27	0.21
G	N/A	N/A	N/A	N/A
H	2.12	2.61	1.16	0.34
I	N/A	N/A	N/A	N/A
J	0.84	1.04	3.92	0.12
K	N/A	N/A	N/A	N/A
L	1.16	1.42	1.83	0.25
M	0.60	0.74	1.00	0.37
N	N/A	N/A	N/A	N/A
O	N/A	N/A	N/A	N/A
P	5.29	6.51	0.59	0.48
Q	1.90	2.34	4.06	0.11
R	1.19	1.47	4.06	0.11
S	1.55	1.91	73.9	0.00
T	N/A	N/A	0.34	0.59
U	2.74	3.38	0.34	0.59
V	2.11	2.59	1.29	0.32
W	4.39	5.41	12.0	0.03
X	8.04	9.90	0.58	0.49

Table SI-11: ANOVA results for the calculation of PBD-Me concentrations using the 2 calibration curves in spinach. Fc = 7.71 and Pc = 0.05

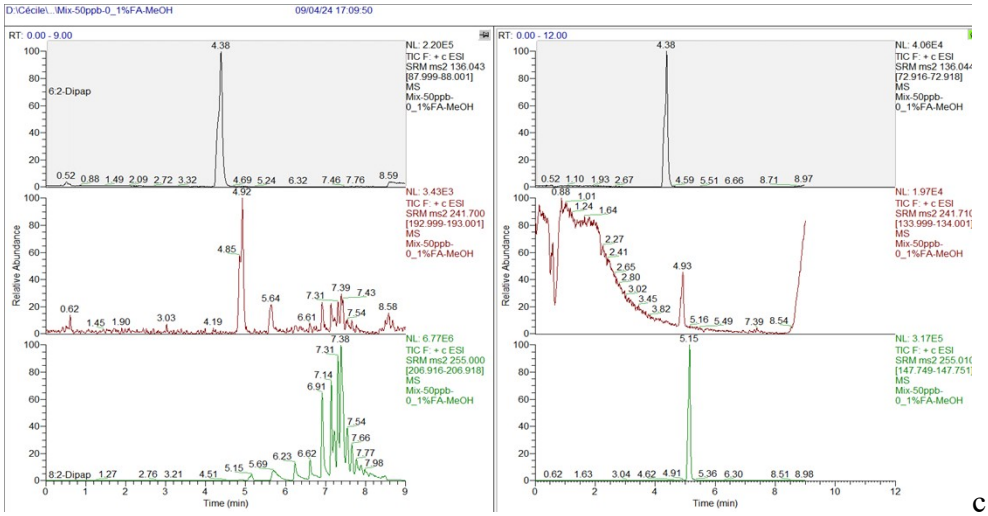
PBD-Me				
Sample	**Average.conc (2) (µg/kg)**	**Average.conc (3) (µg/kg)**	**F**	**Probability**
A	0.03	0.04	0.08	0.79
B	0.05	0.05	0.04	0.85
C	0.05	0.05	0.50	0.52
D	0.06	0.07	0.02	0.89
E	0.04	0.04	0.03	0.88
F	N/A	N/A	N/A	N/A
G	0.05	0.06	0.04	0.85
H	0.13	0.14	0.66	0.46
I	0.04	0.04	0.14	0.73
J	0.04	0.04	0.10	0.77
K	0.03	0.03	0.15	0.72
L	0.04	0.04	0.15	0.72
M	0.03	0.04	1.40	0.30
N	0.03	0.03	0.15	0.72
O	0.04	0.04	0.09	0.78
P	0.09	0.10	0.09	0.78
Q	0.07	0.07	0.09	0.78
R	0.12	0.13	0.01	0.92
S	0.04	0.04	0.13	0.74
T	0.12	0.13	0.01	0.91
U	0.05	0.06	0.14	0.73
V	0.03	0.03	0.14	0.73
W	0.07	0.08	0.14	0.72
X	0.08	0.09	0.07	0.81


Mix-50ppb-0_1%FA-MeOH-0_1%FA 09/04/24 18:43:25
RT: 0.00 - 9.00
RT: 0.00 - 12.00
6.2-Dipap
8.2-Dipap
Relative Abundance
Time (min)

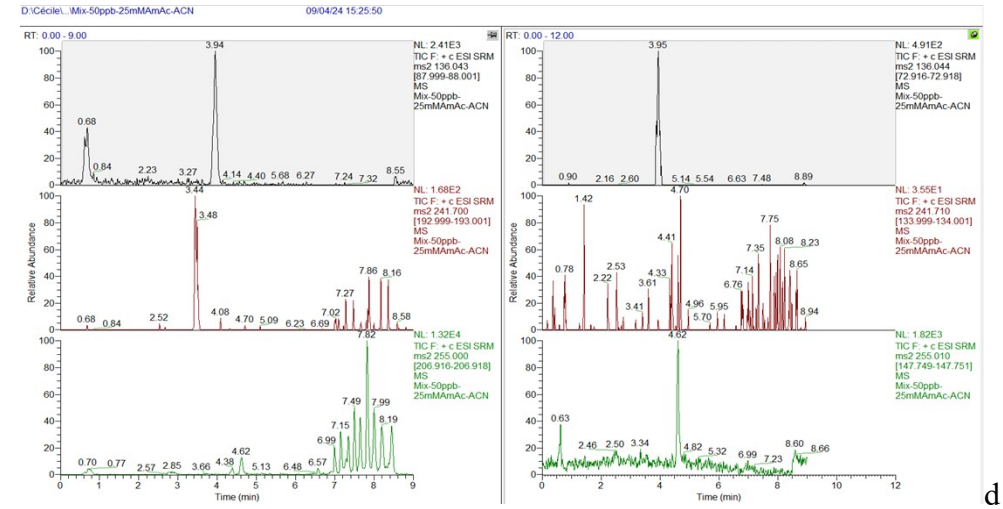
a


D:\Cécile\...\Mix-50ppb-0_5%FA-MeOH 09/04/24 17:56:39
RT: 0.00 - 9.00
RT: 0.00 - 12.00
6.2-Dipap
8.2-Dipap
Relative Abundance
Time (min)

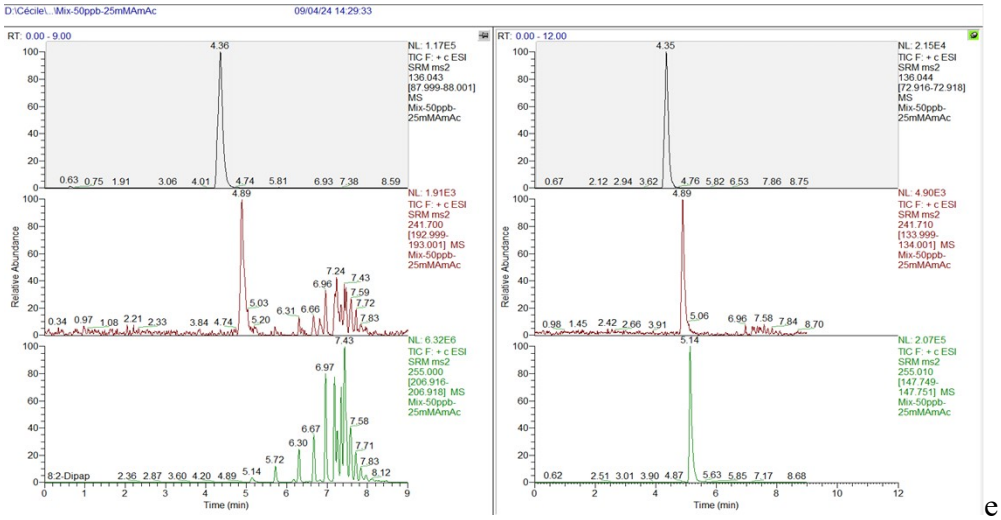
b


D:\Cécile\...\Mix-50ppb-0_1%FA-MeOH 09/04/24 17:09:50
RT: 0.00 - 9.00
RT: 0.00 - 12.00
6.2-Dipap
8.2-Dipap
Relative Abundance
Time (min)

c



d



e

Figure SI-1 : Chromatogram of mobile phases: (a) 0.1% formic acid methanol + 0.1% formic acid, (b) 0.5% formic acid methanol, (c) 0.1% formic acid-methanol, (d) 25 mM ammonium acetate acetonitrile, (e) 25 mM ammonium acetate methanol. Injection concentration: 50 µg/kg.

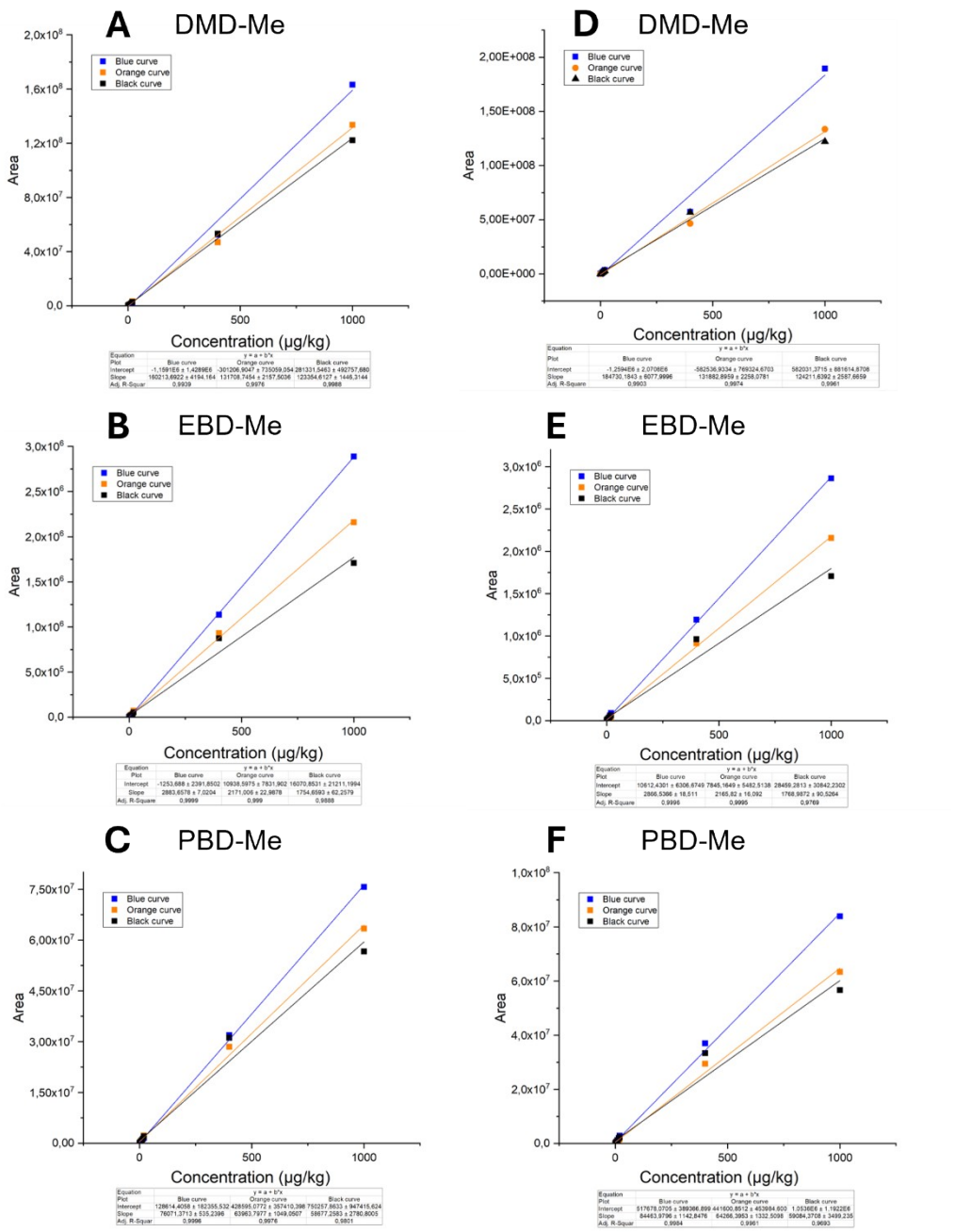


Figure SI-2: Differences between the three calibration curves prepared in the spinach (A, B, and C) and blueberry (D, E, and F) matrices. The curves are shown first using old methoxy-functionalized standards (blue) and second using new methoxy-functionalized standards (orange and black). Each curve is based on 10 calibration levels: 0.02 µg/kg, 1.0 µg/kg, 1.5 µg/kg, 2.0 µg/kg, 5.0 µg/kg, 10.0 µg/kg, 15.0 µg/kg, 20.0 µg/kg, 400 µg/kg and 1000 µg/kg.

References

1 Ministère de l'environnement et de la lutte contre les changements climatiques, 2002, 1–27.

2 W. Zhou, S. Yang and P. G. Wang, *Taylor & Francis*, 2017.

3 B. K. Matuszewski, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.*, 2006, **830**, 293–300.