Matrix effects impact using different chromatographic column sizes

Table of contents

1. Aim and scope

This document reports the matrix effects evaluation data in two chromatographic columns for 209 pesticides included in the European Union Multi Annual Control Program (EU-MACP) and the Working Document [1,2] using a multiresidue method by LC-MS/MS. These columns, which differ in their particle size and length, were two of the most popular column sizes used in routine laboratories.

2. Short description

The retention times for 209 pesticides were experimentally determined for both chromatographic columns tested. Then, homogenous tomato, orange, and onion samples were extracted using QuEChERS without a clean-up step. The obtained extracts were used to prepare matrix-matched calibration curves with concentrations ranging between 5 µg/L and 500 µg/L. The four calibration curves (including solvent) were injected in the LC-MSMS instrument using each chromatographic column and matrix effects were evaluated.

3. Apparatus and consumables

- Automatic pipettes, suitable for handling volumes from 10 µL to 5000 µL and from 1 mL to 5 mL.
- Graduated 10 mL pipette.
- 50 mL and 15 mL PTFE centrifuge tubes.
- Vortex Shaker IKATM 4 Basic.
- Axial shaker Agytax SR1 CP57.
- Centrifuge Orto Alresa Consul 21, suitable for the centrifuge tubes employed in the procedure and capable of achieving at least 4000 rpm.
- Concentration workstation.
- Injection vials, 2 mL, suitable for LC and GC auto-sampler.

4. Chemicals

- Acetonitrile ultra-gradient grade
- Trisodium citrate dihydrate
- Disodium hydrogenocitrate sesquihydrate
- Sodium chloride
- Anhydrous magnesium sulphate
- Anhydrous calcium chloride
- Ammonium formate

- Ultra-pure water
- Methanol HPLC grade
- Formic acid
- Pesticide standards

5. Procedure

5.1. Sample preparation

Tomato,orange and onion samples were extracted following the QuEChERS extraction procedure without a clean-up step. Removing the clean-up step the amount of matrix components in the extract increase.

5.2. Pesticide stock solutions and working mix solutions

Individual pesticide stock solutions (1000–2000 mg/L) were prepared in acetonitrile or ethyl acetate and were stored in screw-capped glass vials in the dark at -20 °C. Working mixes were prepared in 10 mL volumetric flasks by pipetting the appropriate volume of each stock solution.

5.3. Instrumentation and analytical conditions for the LC- MS/MS system

5.3.1. Nexera UC (Shimadzu)

- Mobile phase A: Water (0.1 % formic acid, 5 mM ammonium formate, 2 % MeOH)
- Mobile phase B: Methanol (0.1 % formic acid, 5 mM ammonium formate, 2 % water)
- Column temperature: 40 °C
- Flow rate: Column A: 0.3 mL/min
  Column B: 0.5 mL/min
- Injection volume: 2 µL

These parameters were used in combination with the two different chromatographic columns evaluated in this technical report:

- Column A: fully porous C8 2.1x100 mm and 1.8 µm particle size
- Column B: fully porous C8 2.1x150 mm and 3.0 µm particle size

Same run time was applied in both columns evaluation (15min)
Mobile phase gradient:

min	Mobile phase A	Mobile phase B
0	100 %	0 %
1.5	75 %	25 %
10	0 %	100 %
12	0 %	100 %

Re-equilibration time with initial mobile phase set for 3 minutes.

5.3.2. 8060 triple quadrupole system (Shimadzu)

- Ionisation mode ESI: Positive mode and negative mode
- Capillary (positive and negative): 4 kV
- Nebulizing gas flow: 3 L/min
- Heating gas flow: 10 L/min
- Drying gas flow: 10 L/min
- Interface temperature: 350°C
- DL temperature: 250°C
- Heat block temperature: 400°C

6. Results

6.1. Matrix effects evaluation

Matrix effects were calculated comparing the slopes of the matrix-matched calibration curves (5-500 µg/Kg range) with the slope of the calibration curve prepared in solvent. The equation used to calculate this was as follows:

$$ME\ (\%) = \left(\left(\frac{\text{matrix} - \text{matched slope}}{\text{solvent slope}}\right) - 1\right) x100$$

Coeluting compounds from the matrix can produce enhancement or suppression of the analyte signal. When the analytes enter the ion source at the same time as the matrix compounds, usually signal suppression is the predominant phenomenon in liquid chromatography. Matrix effects modify the reproducibility and accuracy of the method leading to an erroneous quantification. Matrix effects can differ

depending on the column size used. The elution of matrix compounds could not be the same if different particle size/length columns are used. Between 0 and 20% is considered a low or non-existent matrix effect; however, modifications of the signal between 20 and 50% and >50% are considered medium and strong matrix effects, respectively.

Table 1. Matrix effects in three different matrices using both columns

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Matrix Effects		Tomato	Orange	Onion
Column A	0-20	90%	70%	52%
	20-50	9%	20%	43%
	>50	1%	10%	5%
Column B	0-20	88%	61%	55%
	20-50	5%	24%	34%
	>50	7%	15%	11%

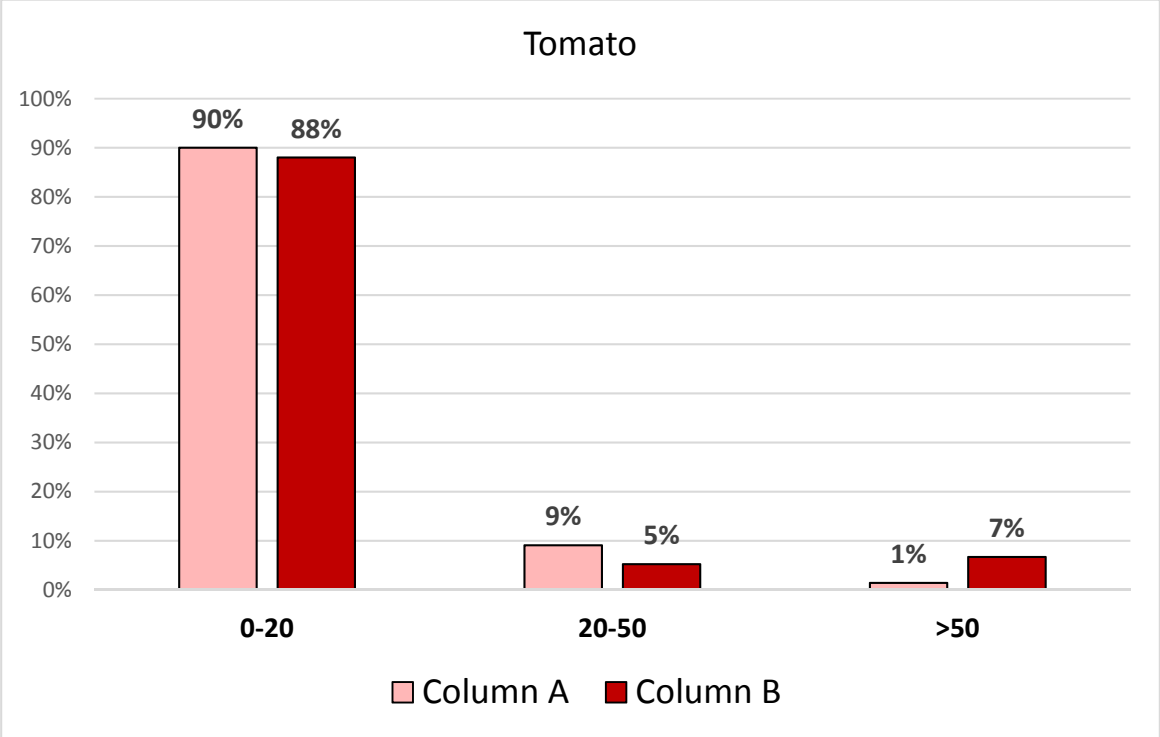

Figure 1. Matrix effects in tomato matrix.

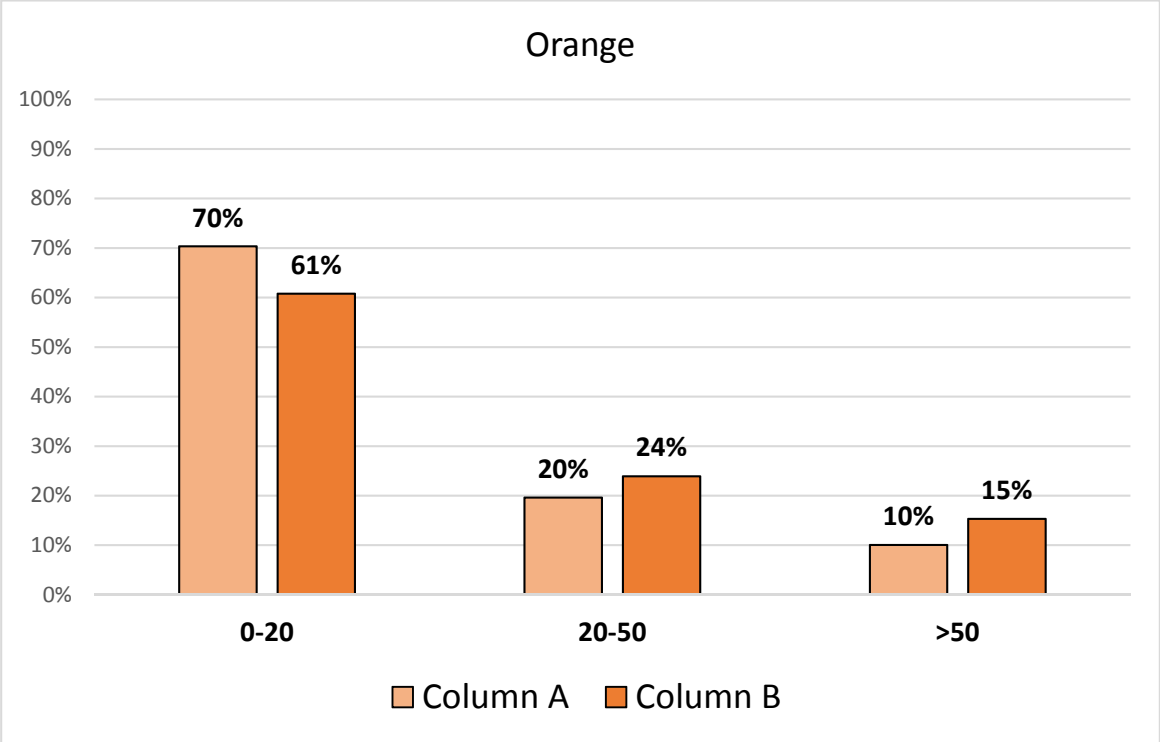

Figure 2. Matrix effects in orange matrix.

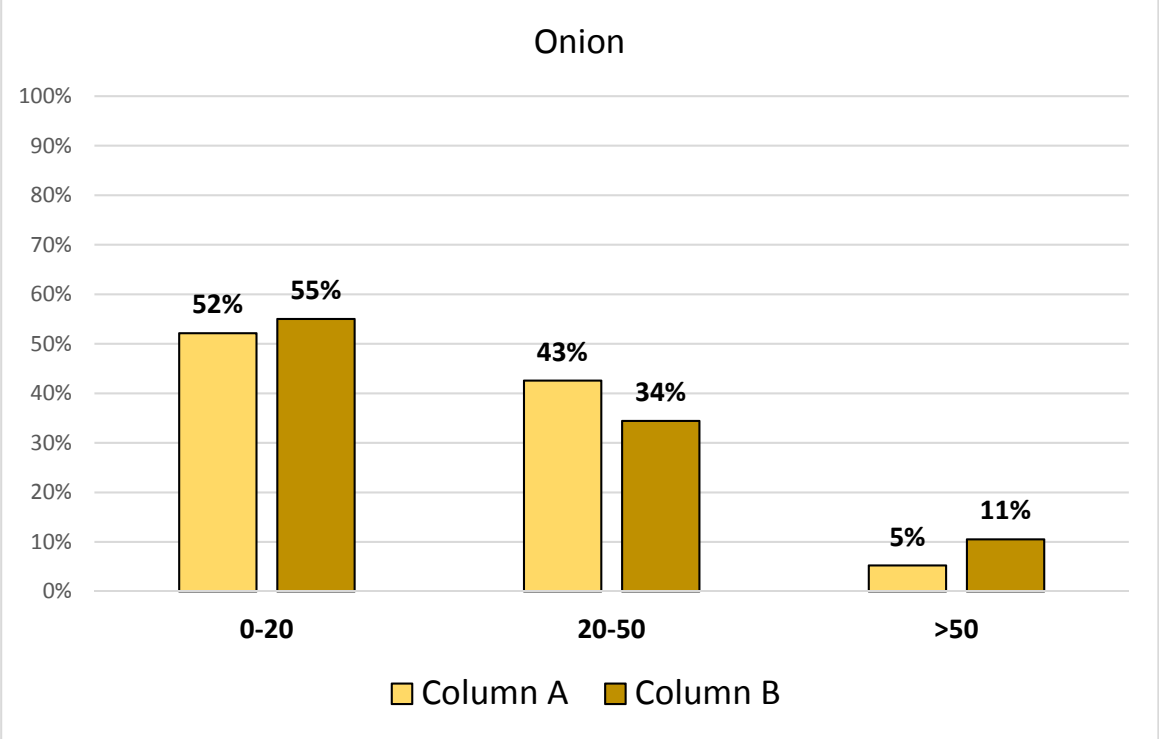

Figure 3. Matrix effects in onion matrix.

6.2. Conclusions

The objective of this study was to compare two of the typical columns employed in routine laboratories and evaluate the matrix effects by analysing three different matrices (tomato, orange, and onion). These matrix effects can be affected by the distribution of the coextracted compounds along with the chromatogram and the number of plates provided by the column. The column dimensions were 2.1x100mm,1.8 µm particle size (Column A) and 2.1x150mm, 3.5 µm particle size (Column B). Both columns used fully porous particles and employed C8 as stationary phase. Matrix effects results were similar in both columns. However, results were slightly better using column A, specially considering the percentage of compounds with strong matrix effects (above 50%). This fact makes sense taking into account chromatography resolution. Narrower peaks might be able to avoid the coelution with matrix components reducing ion suppression in the source (Figure 4). Other concerns should take into account like solvent waste which increase by 60% using column B as higher mobile phase flow was necessary. Nonetheless, despite in general terms matrix effects were similar, sometimes one compound can produce a different response in a specific matrix depending on the column, as can be observed in figures 5-8 with chlorantraniliprole and bromacil in orange matrix.

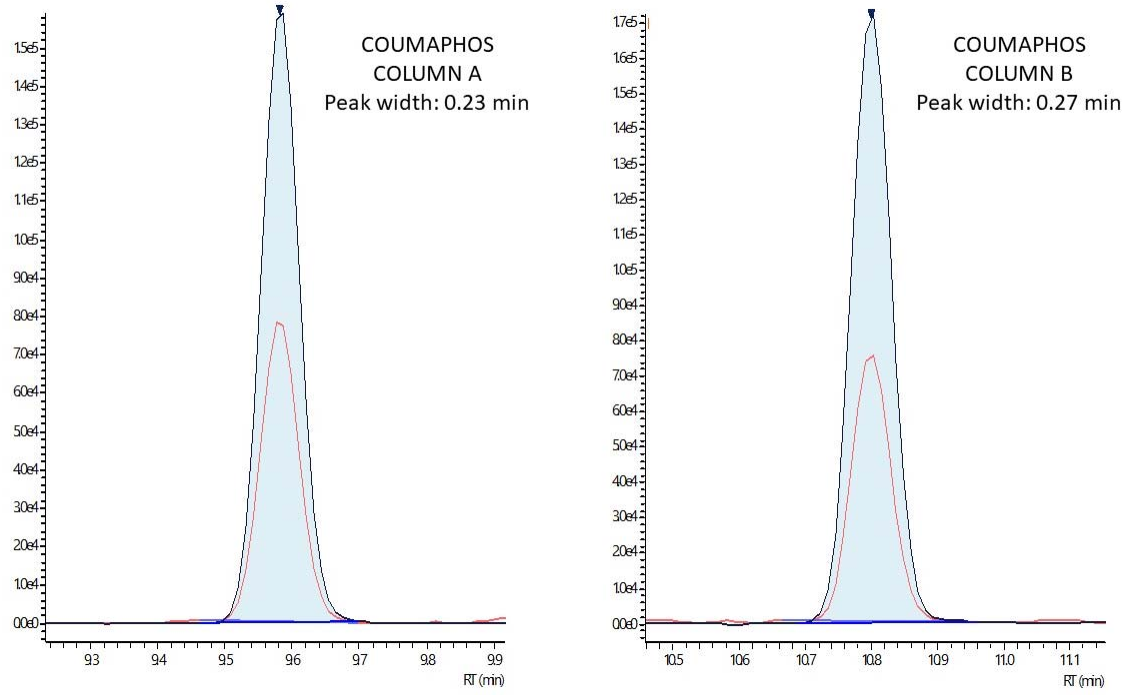

Figure 4. Chromatograms of coumaphos at the concentration level of 10 µg/Kg in tomato matrix.

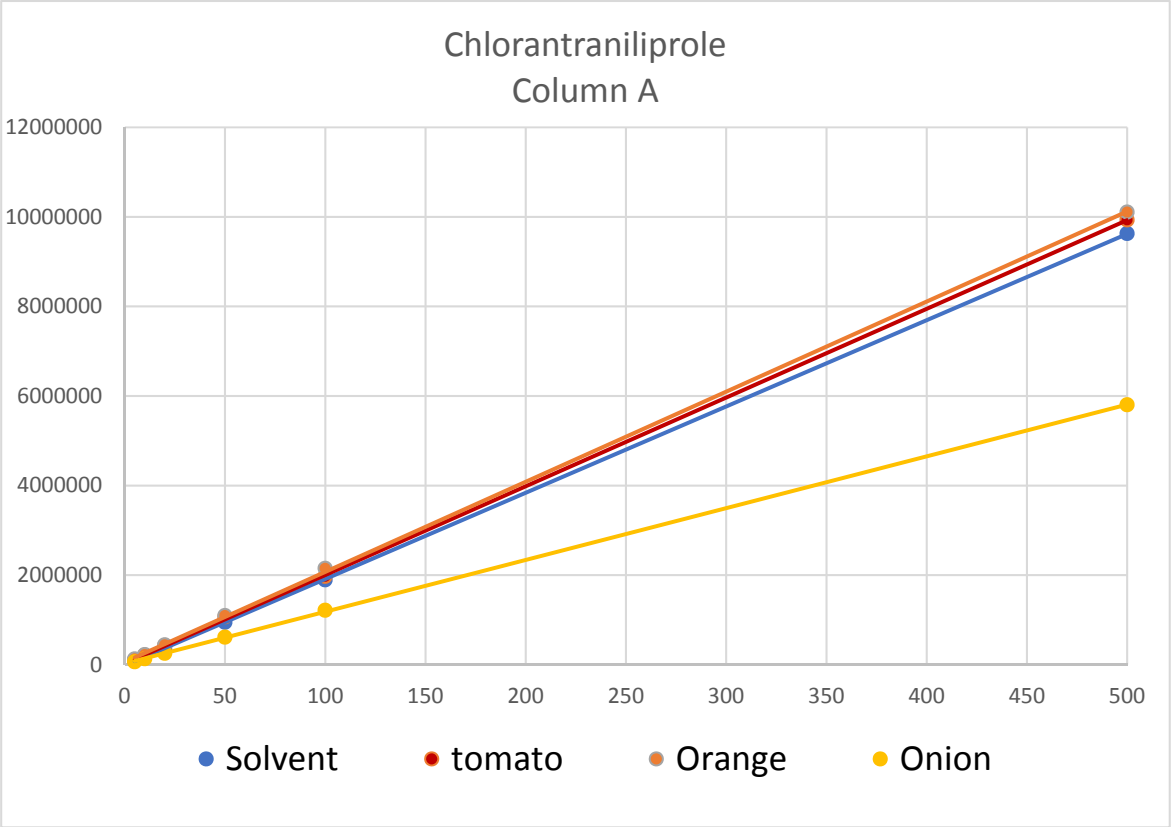

Figure 5. Solvent and matrix-matched slopes for chlorantraniliprole using column A.

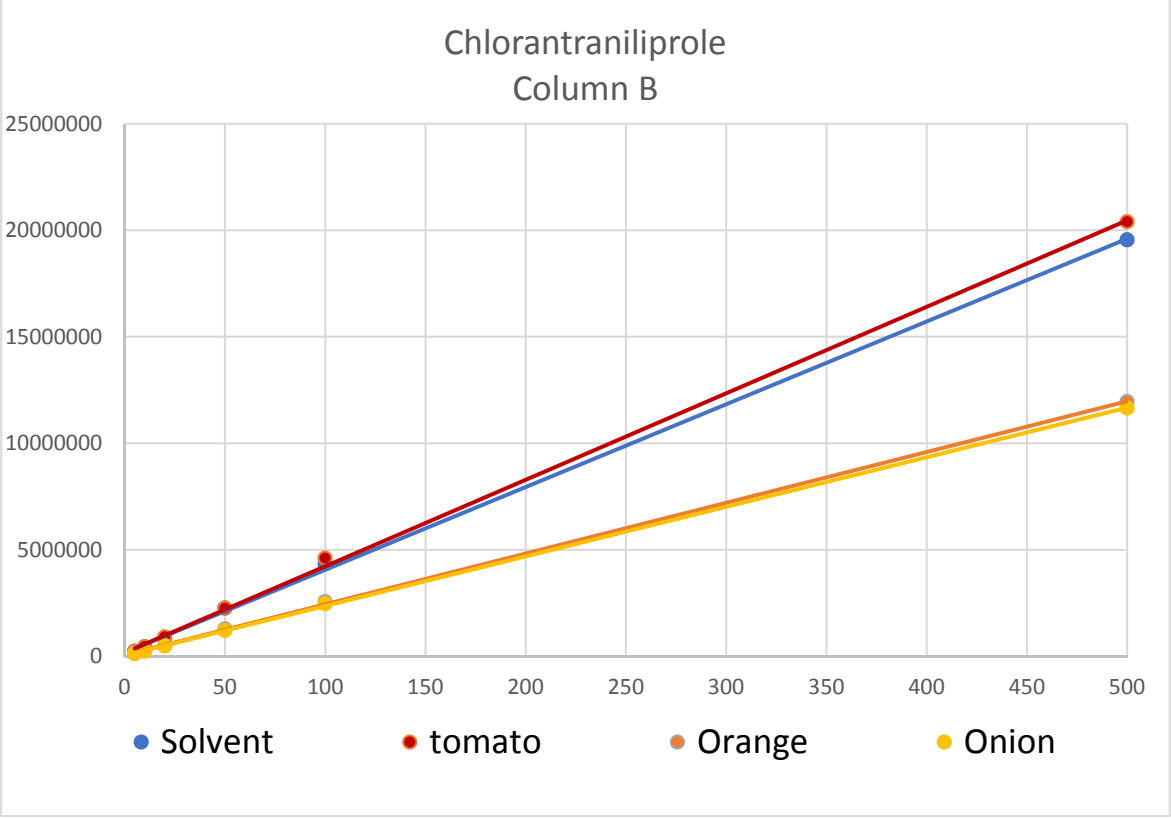

Figure 6. Solvent and matrix-matched slopes for chlorantraniliprole using column B.

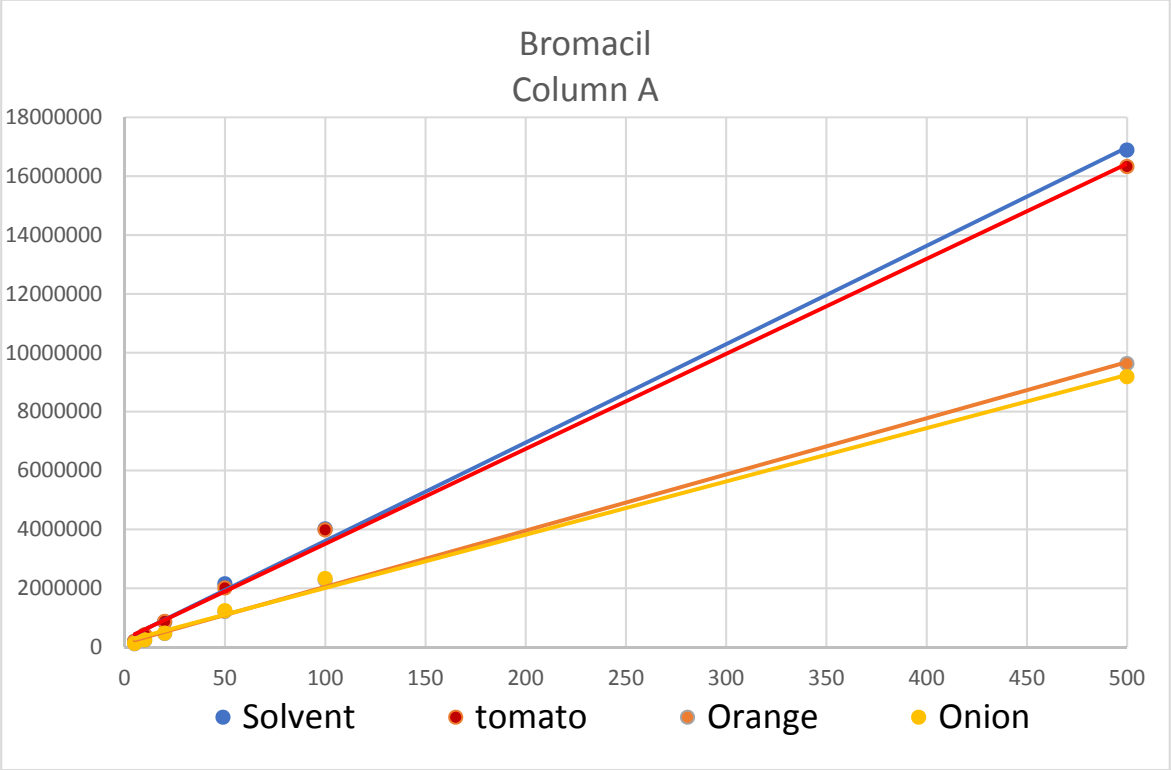

Figure 7. Solvent and matrix-matched slopes for Bromacil using column A.

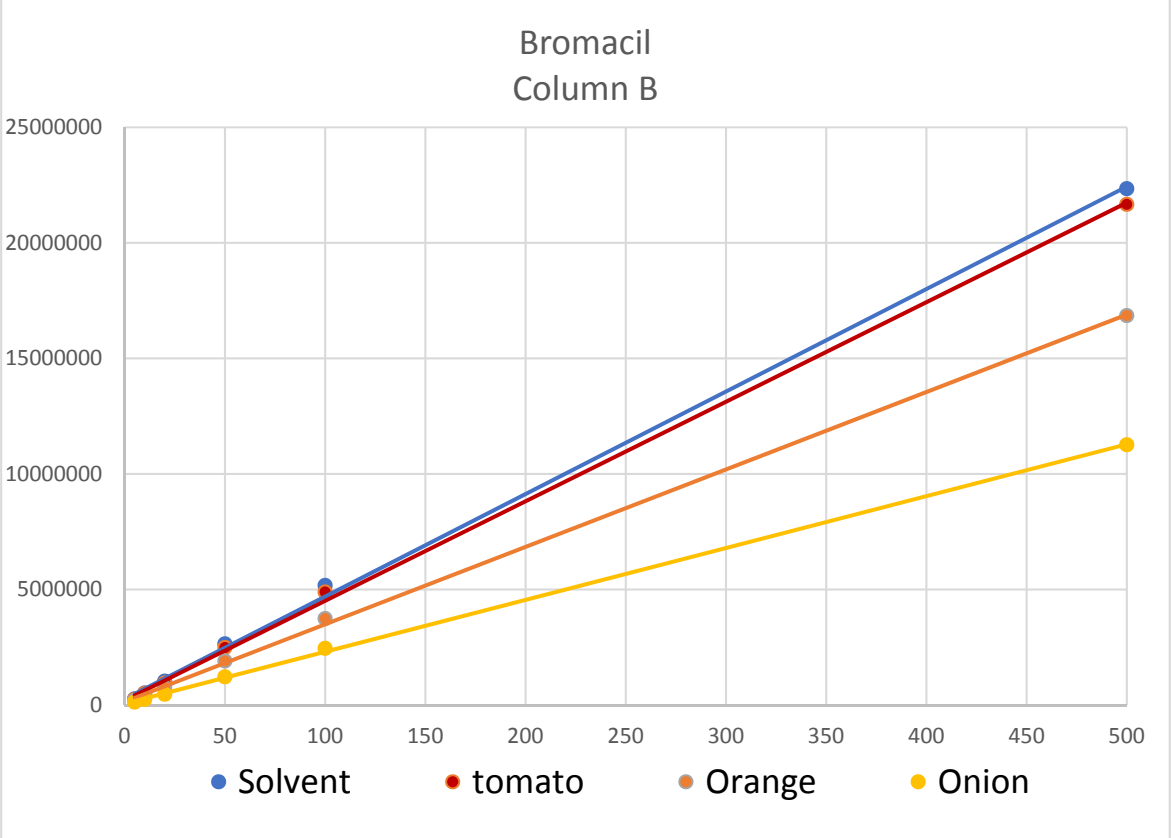

Figure 8. Solvent and matrix-matched slopes for Bromacil using column B.

7. References

[1] COMMISSION IMPLEMENTING REGULATION (EU) 2019/533 of 28 March 2019 concerning a coordinated multiannual control programme of the Union for 2020, 2021 and 2022 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin.

[2] Working document on pesticides to be considered for inclusion in the national control programmes to ensure compliance with maximum residue levels of pesticides residues in and on food of plant and animal origin (SANCO/12745/2013).

APPENDIX I: MASS TRANSITIONS

Table 1. Detection parameters for the selected compounds analysed by LC-MS/MS.

Compound	Precursor Ion (1)	Product ion (1)	Precursor Ion (2)	Product ion (2)	Ret. Time Column A (min)	Ret. Time Column B (min)
2,4-D	220.9	163.05	218.9	161.15	7.762	8.815
Acephate	184	143	184	125	3.341	4.265
Acetamiprid	223.1	126.1	223.1	99.05	5.524	6.749
Alachlor	270.1	162.15	270.1	147.1	9.359	10.423
Ametoctradin	276.1	176	276.1	149.15	10.028	10.89
Anilofos	368	125	368	199.05	9.71	10.645
Atrazine	216.1	174.1	216.1	104.05	7.968	9.388
Azinphos-ethyl	346	97	346	132.1	9.101	10.429
Azinphos-methyl	318	76.95	318	132.05	8.266	9.744
Azoxystrobin	404	371.95	404	328.95	8.491	9.795
Bendiocarb	224.1	109	224.1	81.05	7.248	8.559
Bifenazate	301.1	198.1	301.1	170	9.063	10.175
Bifenazate_diazene	299	213.05	299	184	9.916	11.23
Bitertanol	338	99.15	338	269.05	9.807	10.743
Boscalid	343	306.95	343	271.95	8.718	9.791
Bromacil	261	204.9	261	188	7.212	8.443
Bromuconazole	375.9	159	379.9	161.05	9.004	9.995
Bupirimate	317.2	166	317.2	108	9.283	10.443
Buprofezin	306.2	201.05	306.2	116.15	10.539	11.405
Carbaryl	202.1	127	202.1	145.1	7.436	8.821
Carbendazim	192.1	160.15	192.1	132.15	4.359	5.858
Carbofuran	222.1	123.15	222.1	165	7.274	8.51
Chlorantraniliprole	483.9	452.9	483.9	285.9	8.292	9.411
Chlorbromuron	295	205.9	295	182.1	8.703	10
Chlorfenvinphos	359	169.95	359	155.15	9.812	10.692
Chlorfluazuron	539.9	382.85	539.9	158	10.91	11.721
Chloridazon	222.1	104.1	222.1	92.15	5.511	7.009
Chlorotoluron	213.1	72.15	213.1	139.95	7.758	9.076
Chloroxuron	291.1	72.15	291.1	218.05	8.979	10.108
Chlorpyrifos	350	197.95	352	199.85	10.578	11.651
Chromafenozide	394.9	175.15	394.9	91.1	9.268	10.26
Clofentezine	303	138.15	303	102.1	9.659	11.135
Clomazone	239.9	125	241.9	127	8.448	9.723
Coumaphos	363	227	363	306.8	9.576	10.805
Cyazofamid	325	108.1	325	261	9.282	10.377
Cyflufenamid	413.1	295.05	413.1	241	9.974	10.934
Cymoxanil	199.1	111.15	199.1	128.15	5.867	7.129
Cyproconazole	292.1	125.05	292.1	89	9.163	10.061

Cyprodinil	226.1	93	226.1	108	9.265	10.73
Cyromazine	167.2	85.1	167.2	125.15	2.101	3.789
Deet	192.1	91	192.1	119	8.15	9.307
Demeton-S-methyl	231.1	89	231.1	61.1	7.303	8.533
Demeton-S-methyl-sulfone	263	169.05	263	109.05	4.462	5.532
Diazinon	305.1	169.1	305.1	96.95	9.792	10.9
Dichlorvos	238	220.9	238	109.1	7.184	8.473
Dicrotophos	237.9	127	237.9	112.15	4.951	6.06
Diethofencarb	268.2	226.05	268.2	124.15	8.517	9.69
Difenoconazole	406.1	250.9	406.1	188	9.948	10.87
Difenoxuron	287.1	72.05	287.1	123.15	7.956	9.36
Diflubenzuron	311	158.1	311	141.05	9.282	10.392
Dimethoate	230	125	230	198.9	5.595	6.909
Dimethomorph	388.1	301	388.1	165.1	8.815	9.84
Diniconazole	326.1	70.05	328	70	10.07	10.902
Diuron	233	72.1	233	46.15	7.762	9.043
Desethyl_terbuthylazine	202	146.05	202	104	7.482	8.73
Edifenphos	311	109	311	111.05	9.631	10.765
Emamectin B1a	886.4	158.2	886.4	82.05	10.231	10.789
EPN	324.1	295.85	324.1	156.95	9.996	11.174
Epoxiconazole	330	121.1	330	101.1	9.209	10.257
Ethiofencarb	226.1	107	226.1	77	7.688	8.934
Ethion	385	143	385	198.9	10.579	11.562
Ethiprole	397	350.9	397	254.85	8.719	9.587
Ethirimol	210.2	140.2	210.2	98.15	6.564	7.692
Ethoprophos	243.1	172.9	243.1	131	9.353	10.365
Etofenprox	394.2	177.05	394.2	107.05	11.443	12.331
Etoxazole	360.1	141.1	360.1	113.05	10.809	11.725
Fenamidone	312.1	92.1	312.1	236	8.756	9.919
Fenamiphos	304.1	216.95	304.1	201.95	9.445	10.375
Fenamiphos-sulfone	336.1	266	336.1	188	7.385	8.35
Fenamiphos-sulfoxide	320.1	233	320.1	108	7.257	8.185
Fenarimol	331	268	331	189	9.223	10.286
Fenazaquin	307.2	161.1	307.2	131.2	11.031	11.964
Fenbuconazole	337.1	70.1	337.1	125.05	9.299	10.218
Fenhexamid	302.1	97.1	302.1	143	9.162	10.087
Fenobucarb	208.1	95	208.1	152.1	8.66	9.744
Fenoxycarb	302.1	116.15	302.1	256.05	9.393	10.518
Fenpropimorph	304.2	117	304.2	147.1	8.496	9.35
Fenpyroximate	422.1	366	422.1	138.1	10.876	11.798
Fenthion-sulfone	311	125.1	311	109	7.544	8.898

Fenthion-sulfoxide	295	279.9	295	109	7.373	8.692
Fenuron	165	72.15	165	77	5.378	6.855
Fipronil	435	330	435	250.05	9.522	10.336
Flazasulfuron	408	182.05	408	139	8.26	9.441
Fluacrypyrim	427.2	145.15	427.2	205.05	10.121	11.105
Fluazifop	327.7	282	327.7	254	8.63	9.593
Fludioxonil	247	180.15	247	169.15	8.747	10.063
Flufenacet	364.1	152.05	364.1	194	9.325	10.262
Flufenoxuron	489	140.9	489	158.1	10.729	11.52
Fluopicolide	383	172.85	383	144.95	8.939	10.021
Fluopyram	397	145	397	208	9.217	10.161
Fluquinconazole	376	307	376	349	9.041	10.132
Flusilazole	316.1	247	316.1	165.1	9.451	10.356
Flutriafol	302.1	70.05	302.1	123	7.97	9.06
Fluxapyroxad	382	342	382	313.9	8.885	9.917
Fosthiazate	284.1	104.1	284.1	227.85	7.772	8.896
Haloxyfop	362.1	315.95	364.1	318.15	9.468	10.321
Hexaconazole	314.1	70	314.1	159	9.896	10.7
Hexythiazox	353.1	228	353.1	168	10.718	11.554
Imazalil	297.1	159.05	297.1	200.85	7.698	8.618
Imidacloprid	256.1	174.95	256.1	209	5.02	6.261
Indoxacarb	527.7	203	527.7	218	10.098	10.966
Ioxynil	369.8	127.05	369.8	215	8.08	9.31
Iprodione	330.1	245	332.1	247	9.351	10.262
Iprovalicarb	321.2	119.15	321.2	203	9.314	10.242
Isoprocarb	194.1	95	194.1	137	8.041	9.225
Isoprothiolane	290.8	188.9	290.8	231	8.875	10.168
Isoproturon	207.2	72.15	207.2	165	8.069	9.257
Isoxaflutole	360.1	251	360.1	144	8.158	9.337
Kresoxim-methyl	314.1	235	314.1	267	9.55	10.758
Lenacil	234.9	153.15	234.9	136	8.042	9.234
Linuron	248.8	182.05	248.8	160	8.57	9.881
Malathion	348.1	127.05	348.1	330.9	8.939	10.126
Malathion D10	340.9	132.1	340.9	290.05	8.902	10.111
Mandipropamid	412.1	327.9	412.1	125.1	8.763	9.841
Mepanipyrim	224.1	106.05	224.1	104.1	8.947	10.507
Metaflumizone	506.8	178.05	506.8	287	10.444	11
Metalaxyl	280.1	220	280.1	192.05	8.147	9.232
Metconazole	320.1	70.15	320.1	125.05	9.837	10.648
Methamidophos	142.2	93.95	142.2	124.9	2.662	3.92
Methidathion	320	145	320	302.8	8.239	9.683
Methiocarb	226.1	121.1	226.1	169.05	8.681	9.885
Methiocarb-sulfoxide	242.1	185.05	242.1	122.1	5.309	6.623
Methomyl	163	87.9	163	106.15	4.306	5.753

Methoxyfenozide	369.2	149.15	369.2	91.15	9.08	10.127
Metobromuron	259	170	259	148.1	7.802	9.197
Metolachlor	284.1	252.05	284.1	176.1	9.422	10.472
Metolcarb	166.1	109.1	166.1	94.05	6.807	8.203
Metrafenone	409	209.1	409	227	9.917	11.033
Monocrotophos	240.9	193	240.9	127.1	4.658	5.791
Monolinuron	215.1	99.1	215.1	148	7.558	8.989
Monuron	199.1	72.15	199.1	126	6.933	8.328
Myclobutanil	289.1	70.05	289.1	125	9.04	9.923
Neburon	274.8	87.95	274.8	114.15	9.548	10.576
Nitenpyram	271.1	225	271.1	237	4.112	5.009
Novaluron	493	141.05	493	158	10.18	11.074
Omethoate	214.1	125	214.1	183	3.641	4.574
Oxadiargyl	358.1	340.9	358.1	223	9.864	10.858
Oxadixyl	296.2	279.05	296.2	219.05	6.696	7.85
Oxamyl	237.1	72.1	237.1	90	4.109	5.2
Oxasulfuron	407.1	150.15	407.1	107	6.801	8.156
Paclobutrazol	294.1	70.1	294.1	125.05	8.924	9.86
Penconazole	284.1	158.95	284.1	123	9.67	10.549
Pencycuron	329.1	125	329.1	89	10.016	10.933
Pendimethalin	282.2	212	282.2	194	10.648	11.713
Phenthoate	321	79.05	321	246.85	9.548	10.78
Phosalone	368	182	368	111	9.841	10.848
Phosmet	335	160.1	335	317.9	8.325	9.773
Phoxim	299	77.1	299	129.1	9.799	11.069
Pirimicarb	239.2	182.05	239.2	85.05	6.863	8.427
Pirimicarb-desmethyl	225.1	168.05	225.1	180	5.188	6.5
Pirimiphos-methyl	306.1	164.1	306.1	108.15	9.852	11.069
Prochloraz	376	307.95	376	70	9.72	10.66
Profenofos	375	304.7	375	346.75	10.343	11.255
Promecarb	208.1	109.1	208.1	151.15	8.896	10.005
Prometryn	242.1	158	242.1	200.15	8.885	10.205
Propamocarb	189.2	102.15	189.2	74.15	3.724	4.598
Propaquizafop	443.8	100.15	443.8	299	10.325	11.241
Propargite	368.2	231.1	368.2	175.1	10.811	11.613
Propazine	230.2	146.15	230.2	188.1	8.677	9.942
Propiconazole	342	158.9	342	69.1	9.776	10.628
Propoxur	209.9	111.15	209.9	93.1	7.21	8.448
Propyzamide	256	190	256	173	8.937	10.096
Proquinazid	373.1	288.8	373.1	330.9	10.831	11.889
Prosulfocarb	252.1	91	252.1	128.1	10.278	11.273
Prothiophos	345	240.9	347	242.75	11.157	12.087
Pymetrozine	218.1	105	218.1	78.1	3.418	4.291
Pyraclostrobin	388	194.1	388	133	9.693	10.884

Pyridaben	365.2	147.1	365.2	308.9	11.116	11.876
Pyridaphenthion	341.1	188.95	341.1	204.9	9.013	10.161
Pyridate	379.1	206.95	379.1	104.15	11.283	12.022
Pyrimethanil	200.1	107	200.1	183	8.251	9.832
Pyriproxyfen	322.1	96.05	322.1	184.95	10.452	11.613
Quinalphos	299.1	163	299.1	147.05	9.453	10.805
Quinoclamine	208	88.95	208	105.05	6.673	8.362
Quinoxyfen	308	197	308	271.95	10.52	11.536
Quizalofop (free acid)	345	299	345	243.95	9.184	10.11
Quizalofop-ethyl	373.1	298.9	373.1	270.9	10.196	11.176
Rotenone	395.1	213	395.1	192	9.27	10.519
Simazine	202	104.05	202	96.05	7.13	8.713
SpinetoramA	748.5	142.15	748.5	98.05	10.012	10.55
SpinetoramB	760.6	142.1	760.6	98.15	10.227	10.793
Spinosad A	732.6	142.2	732.6	98.1	9.674	10.283
Spinosad D	746.6	142.1	746.6	98.1	9.934	10.538
Spirodiclofen	411.1	313.05	411.1	294.9	11.051	11.775
Spiromesifen	371.2	273.1	371.2	255.2	10.905	11.509
Spirotetramat	374.1	216	374.1	302	9.179	10.2
Tebuconazole	308.2	70.05	308.2	125.05	9.653	10.479
Tebufenozide	353.2	133.1	353.2	297	9.606	10.48
Tebufenpyrad	334.2	117	334.2	145.15	10.436	11.237
Teflubenzuron	378.8	339	378.8	358.9	10.393	11.357
Terbuthylazine	230.1	174.05	230.1	104	8.787	10.052
Terbuthylazine-desethyl	202.1	146.05	202.1	104.05	7.482	8.728
Terbutryn	242.1	157.95	242.1	90.9	8.887	10.205
Tetraconazole	372	159	372	70.1	9.293	10.181
Tetramethrin	332.2	164.1	332.2	135.1	10.47	11.374
Thiabendazole	201.8	175	201.8	131.15	4.804	6.658
Thiacloprid	252.8	126.05	252.8	90.1	5.964	7.244
Thiamethoxam	292	211.1	292	132	4.449	5.562
Thiobencarb	257.8	125.1	257.8	89	9.935	10.988
Triazophos	314.1	162.05	314.1	119.15	9.025	10.293
Trichlorfon	257	109	257	220.8	5.598	6.742
Tricyclazole	190.1	136	190.1	109	6.17	7.699
Trifloxystrobin	408.8	186.1	408.8	145.1	10.169	11.195
Triflumizole	346	278	348	280	10.267	11.099
Triflumuron	359	156.05	359	139.05	9.79	10.769
Triticonazole	318.1	70.15	318.1	125	9.213	10.07
Tritosulfuron	446	194.9	446	145.05	8.341	9.39
XMC (3,5-xylyl methylcarbamate)	180.1	123.1	180.1	108	7.671	8.795
Zoxamide	336	186.95	336	159	9.756	10.731