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National Food Institute
Technical University of Denmark

Validation Report 24

Quantitative determination of pesticide residues in cereals

by GC-QTOF (HRMS)

(QuEChERS method)

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CONTENT:

1. Introduction

This report describes the validation of the QuEChERS method combined with GC- and LC-QTOF accurate mass determination. Accurate mass determination using the GC- and LC-QTOF was initially intended only for screening purposes, i.e. qualitative analysis. However with increasing resolution, increasing sensitivity as well as wider dynamic ranges of the high resolution MS systems it has become more realistic to also perform quantitative analysis on these instrument. The aim of the present validation study was therefore to evaluate whether the HRMS systems available (Agilent model 7200 GC/Q-TOF) can provide data which allow for, not only qualitative analysis, but also quantitative analysis.

The method was validated for 79 pesticides and metabolites in barley, oat, rice and wheat. The QuEChERS method is an extraction method which has been developed to be Quick, Easy, Cheap, Efficient, Rugged and Safe. The method is most commonly used on fruit, vegetables and cereals[1].

2. Principle of analysis

Sample processing:
The samples were milled with a sieve at 1 mm.

Extraction:
The sample is shaken and a salt and buffer mixture is added and the sample is shaken again.

Clean-up:
After centrifugation the supernatant is transferred to a clean tube and put in -80 degree freezer. When the extract is almost thawed it is centrifuged and the supernatant is transferred to a tube containing PSA and $MgSO_4$. An aliquot was withdrawn prior to this clean-up step and analysed by LC-MS/MS. After shaking and an additional centrifugation step the final extract is diluted 1:1 with acetonitrile to obtain the same matrix concentration as in the matrix matched calibration standards.

Quantification and qualification:
The final extracts are analysed by GC-QTOF.

GC-QTOF analysis:
The GC-system was a 7890B, Agilent Technologies equipped with PAL auto sampler system and Gerstel PTV injector. The injection volume used was 5 µl and the injector was programmed from initially 30°C for 0.8 min to 290°C at a rate of 480°C per min-1 and held for 2 min before further incensement to 310 °C at a rate of 720°C per min-1. Purge time was 3.05 minutes and aliquot of 5 µl extract was injected. Two columns, HP5-5MS UI, 15 m, 0.25 mm ID, 0.25 µm df with backflush was used. The oven was programmed from initially 60°C for 3 minutes increasing to 180°C at a rate

of 30°C min-1 and then further, after 0.8 min increased to 290°C at a rate of 5°C min-1. This temperature was maintained for 16 min. Runtime: 38 min. Backflush at 300°C for 2.7 min.

TOF instrument instrument was a 7200 GC/Q-TOF, Agilent Technologies which were managed in EI positive ionisation mode, with an acquisition rate of 4GHz and acquisition mode centroid. The source temperature was 230 °C and the software was MassHunter B 07.03.2129,
Processing of data was performed by the MassHunter Quantitave Analysis, B.08.00. This software gives a good overview of the data. The masses used to identify the compounds were imported from the Agilent Pesticide Spectrum Library. Masses for up to five ions (could include the molecular ion) were imported and the software choose the most intense ions. The method was then used to process a data set and the three best masses were kept and the other deleted. The ions were chosen based on mass accuracy, signal/noise and ion ratios. The masses of the ions included in the methods are listed in appendix 1.

3. Validation design

The method was sought validated for 108 pesticides or metabolites barley, oat, rice and wheat for the quantitative analysis and determination of LOQ and for barley, oat, rice and wheat for the qualitative analysis and determination of SDL. The quantitative results are reported separately.

Validation design quantitative analysis:
The validation was performed on 5 replicates of barley, oat, rice and wheat (20 samples) at three spiking levels; 0.01, 0.02 and 0.1 mg/kg (total of 60 samples). The calibration standard were matrix matched with rye matrix.

4. Linarity

The calibration curve is determined by the analysis of each of the analysts at least 4 calibration levels, i.e. 1, 3.33, 10, 33.3, 100, and 333 ng/ml. The quantification was performed from the mean of two bracketing calibration curves.
The calibration curves were in general fitted well with a linear curve, weiged 1/x and ignoring origin. The majority (76%) of the correlation coefficients (R) were higher or equal to 0.98. The poorest fit (R2=0.945) obtained for terbufos.

5. Validation parameters and criteria for quantitative analysis

Precision – repeatability

Repeatability was calculated for all pesticides and degradation products on both spiking levels (0.01 mg/kg and 0.1 mg/kg). Repeatability (RSD_r) in this validation calculated from the 20 replicate determinations. Repeatability were calculated as given in ISO 5725-2[2].

Accuracy – Recovery

The accuracy was determined from recovery studies in which samples were spiked at three concentration levels (0.01, 0.02 mg/kg and 0.1 mg/kg) with the relevant pesticides, isomers and degradation products.

Robustness

The QuEChERS method has, in connection with the development of the method, been shown to be robust by Anastassiades et al. 2003[1].

Limit of quantification, LOQ

The quantification limits (LOQ) was determined as the lowest spike level for which the acceptance criteria (se Section 6) was meet.

The obtained results including recovery, RSD_r, Combined Uncertainty (U_c) and limit of quantification (LOQ) are presented in appendix 2.

Criteria for the acceptance of validation results

For the pesticides to be accepted as validated the following criteria for precision and trueness must to be fulfilled:

1. The relative standard deviation of the repeatability should be ≤20%[3].
2. The average relative recovery must be between 70 and 120%[3].

If the above mentioned criteria have been meet, the quantification limits, LOQs is stated.

The combined uncertainty given by:

$$U_c = \sqrt{Bias^2 + RSD^2}$$

Where Bias is the measurement uncertainty of the method (100 - Recovery (%)) and RSD is the standard deviation and the bias or recovery. If Uc is found to be >20 the analytical result for the compound in question obtained with the method needs to be corrected for recovery.

6. Validation parameters and criteria for qualitative analysis

Selectivity: An Un-spiked sample of each of the five cereal matrices were analysed and screened for false detects.

Screening detection limit (SDL) is set to the lowest tested level, i.e. 0.01 or 0.1 mg/kg, at which the compound is detected in minimum 95% of the spiked samples, not necessarily meeting the MS-identification criteria defined in the SANTE/11813/2017, 01/01/2018.

7. Results and conclusion

The validation results obtained for the 90 pesticides or metabolites hereof are presented in appendix 1. LOQ at 0.01 was achieved for 60 compounds, LOQ at 0.02 mg/kg for 17 compounds and LOQ at 0.1 for 13 compounds.

9. References

1 EN 15662:2008. Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method

2 ISO 5725-2:1994. Accuracy (trueness and precision) of measurement methods and results – Part2. Basic method for the determination of repeatability and reproducibility of standard measurement method. First edition. December 1994.

3 Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed, Document No SANTE/11813/2017, 01/01/2018.

Appendix 1. Retention times, and quantifier ion and qualifier ions.

Pesticide name	RT	Quantifier ion	Qualifier ion1	Qualifier ion2
2,4,5-T-methylester	11.43	232.9768	267.9455	208.9322
2,4-DB-methylester	12.24	101.0594	161.9634	230.9974
2,4-DButylester	12.65	184.9997	174.9712	161.9634
2,4-Dmethylester	9.81	199.0155	233.9845	174.9712
2,6-Dichlorobenzamide	10.21	172.9555	174.9540	188.9743
4,4'-Dichlorobenzophenone	14.25	138.9942	110.9996	140.9916
Acetochlor	12.75	146.0961	162.0913	174.0913
Acibenzolar-S-methyl(BTH)	13.01	181.9852	134.9899	106.9950
Allidochlor	7.34	138.0908	132.0215	96.0808
Ancymidol	17.84	228.0897	121.0648	107.0240
Aspon(NDP)	14.00	210.9049	209.8970	114.9613
Atraton	11.78	196.1193	169.0958	154.0723
Atrazine-desethyl	10.09	172.0383	187.0619	145.0150
Azaconazole	17.46	216.9819	172.9556	218.9788
Aziprotryne	11.76	182.0492	139.0978	196.0651
Benfluralin	10.18	292.0542	264.0227	276.0591
Benodanil	18.67	230.9303	202.9352	322.9802
Benoxacor	12.26	120.0440	134.0600	259.0161
Benzoylprop-ethyl	20.91	105.0333	77.0386	292.0291
Butachlor	16.34	160.1119	176.1070	188.1070
Butafenacil	25.49	331.0102	179.9847	333.0062
Butamifos	16.62	286.1031	200.0107	231.9828
Butylate	8.16	146.0996	156.1383	174.0947
Carbetamide	14.27	119.0366	93.0573	120.0399
Chlorbenside	15.70	125.0148	89.0386	267.9875
Chloridazon	19.19	218.0163	124.9821	93.0100
Chloroneb	8.75	190.9661	205.9896	140.9738
Chlorthion	14.46	124.9817	296.9622	109.0049
Crimidine	8.86	142.0289	156.0323	171.0558
Cyfluthrin	25.79	206.0611	199.0554	163.0076
DDD,-op	18.44	235.0079	165.0699	212.0387
DDM/Dichlorophen	8.35	128.0018	141.0102	129.9994
Diallate	10.41	234.0714	86.0600	108.9606
Dibutylchlorendate	22.61	387.8211	370.8178	316.8750
Dicapthon	14.31	261.9936	124.9821	296.9622
Dichlobenil	7.71	170.9634	100.0182	172.9606
Dichlone	9.61	190.9840	162.9945	192.9865
Dichlormid	7.73	172.0522	124.0757	136.0757
Dichlorprop-methyl	9.61	161.9632	188.9868	248.0002
Diclobutrazol	17.41	270.0198	158.9763	216.9823
Diclofop-methyl	20.15	252.9831	340.0264	281.0131
Dimethenamid	12.63	154.0683	230.0401	203.0166

Pesticide name	RT	Quantifier ion	Qualifier ion1	Qualifier ion2
Dimethipin	11.05	75.9974	123.9647	118.0083
Diphenamid	14.70	167.0853	72.0444	152.0621
Dithiopyr	13.42	354.0587	286.0486	306.0548
Dodemorph	14.58	154.1224	238.2165	281.2713
Etaconazole	18.29	172.9556	245.0131	190.9661
Ethofumesate	13.62	161.0596	207.1016	137.0597
Famphur	19.19	218.0163	124.9822	93.0100
Fenfuram	11.81	109.0280	110.0317	201.0784
Fenobucarb	9.56	121.0642	150.1039	207.1254
Fenoprop-methyl	11.10	195.9244	222.9479	281.9612
Fensulfothion	18.23	293.0073	156.0062	141.0005
Fensulfothionsulfone	18.75	187.9957	324.0250	156.9954
Fluchloralin	11.65	306.0698	326.0150	264.0227
Fluoroglycofen-ethyl	23.81	343.9945	447.0327	345.0010
Fluridone	27.60	328.0951	329.1022	154.5477
Flurprimidol	12.60	269.0535	107.0240	189.0158
Hexazinone	19.96	171.0878	71.0604	128.0818
Iprobenfos	12.15	91.0540	204.0005	288.0944
Isazofos	11.91	118.9877	161.0350	162.0429
Isocarbamide	11.24	142.0608	130.0611	185.1159
Isocarbophos	14.37	135.9975	120.0206	121.0284
Isopropalin	14.85	280.1295	238.0822	264.1343
Mefenpyr-diethyl	20.81	252.9943	299.0349	227.0137
Metazachlor	15.08	132.0804	133.0886	209.0602
Methabenzthiazuron	10.14	136.0211	164.0403	135.0137
Metobromuron	12.29	169.9595	258.0000	196.9471
Mexacarbate	11.87	165.1145	164.1070	150.0913
mgk264	14.95	164.0706	111.0315	110.0237
Nitrothal-isopropyl	14.37	236.0555	194.0084	212.0190
Pentanochlor	13.64	141.0337	239.1071	197.0602
Pethoxamid	15.59	260.1649	131.0855	280.1099
Phenothrin	22.32	123.1168	183.0804	81.0699
Phoratesulfoxide	15.15	96.9505	199.0011	153.0134
Plifenate(Penfenate)	12.80	216.9818	169.9685	171.9655
Profluralin	11.33	318.0699	330.1060	347.1087
Prometryn	13.13	184.0660	241.1356	226.1121
Propachlor	9.64	120.0802	176.1070	120.0444
Propaphos	15.94	219.9956	140.0290	304.0893
Quinomethionate	15.83	233.9918	205.9967	116.0495
Quizalofop-ethyl	26.61	372.0885	243.0320	299.0582
Rabenzazole	14.26	212.1056	170.0713	195.0791
Secbumeton	11.78	196.1193	169.0958	210.1349
Tebupirimfos	12.07	152.0941	234.0222	261.0457
Terbufos	11.35	230.9734	96.9508	153.0134
Thenylchlor	20.05	127.0209	288.1053	141.0369

Pesticide name	RT	Quantifier ion	Qualifier ion1	Qualifier ion2
Tiocarbazill	14.39	91.0539	100.0757	156.1383
Tribufos	17.03	168.9905	146.9156	112.9279
Vernolate	8.27	128.1066	86.0600	146.0998

Appendix 2. Recoveries, repeatability (RSD_r) and Limit of Quantification (LOQ) for pesticides validated on three cereal commodities, wheat, oat and rice using QuEChERS method and LC-ESI-QTOF and GC-QTOF.

	Spike level 0.005 mg/kg				Spike level 0.01 mg/kg				Spike level 0.1 mg/kg				
Compound	**Recovery %**	**RSD_r, %**	**RSDR, %**	**Comb. Uncer-tainty (%)**	**Recovery %**	**RSD_r, %**	**RSDR, %**	**Comb. Uncer-tainty (%)**	**Recovery %**	**RSDr, %**	**RSDR, %**	**Comb. Uncer-tainty (%)**	**LOQ**
2,4,5-T-methylester	109	10	11	11	106	8	8	8	86	12	12	12	0.01
2,4-D Butyl ester	107	16	15	15	113	8	8	8	85	12	12	12	0.01
2,4-D methyl ester	111	10	12	12	106	8	12	12	91	12	12	12	0.01
2,4-DB-methylester	96	17	18	18	115	9	9	9	87	15	14	14	0.01
2,6-Dichlorobenzamide	107	20	20	20	98	10	9	9	*62*	12	18	18	0.01
4,4'-Dichlorobenzophenone	100	18	18	18	104	7	9	9	79	12	11	11	0.01
Acetochlor	89	17	*23*	*23*	91	10	14	14	79	19	17	17	0.01
Acibenzolar-S-methyl (BTH)	112	*33*	*31*	*31*	*124*	11	11	11	91	10	10	10	0.02
Allidochlor					93	19	20	20	85	17	17	17	0.02
Ancymidol	94	15	14	14	105	7	6	6	*65*	15	20	20	0.01
Aspon (NDP)	108	14	16	16	98	8	7	7	83	14	16	16	0.01
Atraton	102	12	14	14	99	7	10	10	75	14	15	15	0.01
Atrazine-desethyl (Desethyl-atrazine)	99	14	18	18	91	7	10	10	74	14	13	13	0.01
Azaconazole	101	14	16	17	102	7	8	8	71	15	19	19	0.01
Aziprotryne									85	12	11	11	0.10
Benfluralin	112	15	19	19	92	6	9	9	*61*	11	11	11	0.01
Benodanil	92	16	16	16	94	9	9	9	77	11	10	10	0.01
Benoxacor	103	15	19	19	113	10	10	10	84	18	17	17	0.01
Benzoylprop-ethyl	120	19	*39*	*39*	*37*	6	*67*	*67*	101	17	15	15	0.10
Butachlor					120	10	16	16	74	16	17	17	0.02
Butafenacil	*57*	*35*	*33*	*33*	*31*	*30*	*73*	*73*	80	13	12	12	0.10
Butamifos	103	*22*	*32*	*32*	109	14	13	13	76	13	14	14	0.02

	Spike level 0.005 mg/kg				Spike level 0.01 mg/kg				Spike level 0.1 mg/kg				
Compound	**Recovery %**	**RSD_r, %**	**RSDR, %**	**Comb. Uncer-tainty (%)**	**Recovery %**	**RSD_r, %**	**RSDR, %**	**Comb. Uncer-tainty (%)**	**Recovery %**	**RSDr, %**	**RSDR, %**	**Comb. Uncer-tainty (%)**	**LOQ**
Butylate	83	*32*	*33*	*33*	*62*	15	20	20	75	*21*	20	20	0.02
Chlorbenside	104	15	18	18	97	9	11	11	*69*	14	14	14	0.01
Chloridazon	97	14	19	19	105	8	17	17	79	17	19	19	0.01
Chloroneb	88	15	18	19	93	6	6	6	82	14	13	13	0.01
Chlorthion	101	18	20	20	*122*	11	12	12	79	15	14	14	0.01
Crimidine	95	17	16	16	95	7	7	7	83	15	14	14	0.01
Cyfluthrin									96	14	*22*	*22*	0.10
DDD,- op	84	*35*	*41*	*41*	*151*	*29*	*29*	*29*	83	17	18	18	0.10
DDM / Dichlorophen	85	11	11	11	92	7	8	8	73	15	15	15	0.01
Diallate	112	19	20	20	102	6	9	9	80	14	13	13	0.01
Dibutylchlorendate	106	12	18	18	*60*	12	13	13	75	19	19	19	0.01
Dicapthon	98	14	16	16	111	9	11	11	*69*	14	14	14	0.01
Dichlobenil	95	10	10	10	98	5	6	6	89	14	14	14	0.01
Dichlone	106	*34*	*34*	*34*	103	12	12	12	84	18	17	17	0.02
Dichlormid					96	8	8	8	76	16	16	16	0.02
Dichlorprop-methyl	101	11	14	14	96	8	8	8	83	15	14	14	0.01
Diclobutrazol	106	13	14	14	113	6	7	7	75	19	20	20	0.01
Diclofop-methyl	*42*	*29*	*31*	*31*	*31*	9	11	11	74	11	10	10	0.10
Dimethenamid (SAN 582H)	99	14	13	13	100	10	9	9	81	16	14	14	0.01
Dimethipin	120	*33*	*40*	*40*	*132*	12	12	12	85	15	14	14	0.02
Diphenamid	112	13	20	20	111	7	6	7	81	15	13	13	0.01
Dithiopyr	104	12	14	14	106	7	7	7	83	16	14	14	0.01
Dodemorph	78	*40*	*41*	*41*	95	19	20	20	77	14	13	13	0.02
Etaconazole	98	*44*	*41*	*41*	112	6	7	7	77	15	15	15	0.01
Ethofumesate	99	17	15	16	108	9	8	8	83	14	13	13	0.01
Famphur	97	14	19	19	105	8	17	17	79	17	19	19	0.01
Fenfuram	114	16	15	15	108	11	16	17	88	18	*26*	*26*	0.01

	Spike level 0.005 mg/kg				Spike level 0.01 mg/kg				Spike level 0.1 mg/kg				
Compound	**Recovery %**	**RSD_r, %**	**RSDR, %**	**Comb. Uncer-tainty (%)**	**Recovery %**	**RSD_r, %**	**RSDR, %**	**Comb. Uncer-tainty (%)**	**Recovery %**	**RSDr, %**	**RSDR, %**	**Comb. Uncer-tainty (%)**	**LOQ**
Fenobucarb (Baycarb)									109	20	19	19	0.10
Fenoprop-methyl	105	13	12	12	98	9	8	8	83	14	13	13	0.01
Fensulfothion	96	16	18	18	101	7	13	13	76	13	13	13	0.01
Fensulfothion sulfone	105	18	16	16	110	10	9	9	84	14	12	12	0.01
Fluchloralin	102	13	14	14	108	9	9	9	73	11	11	11	0.01
Fluoroglycofen-ethyl									*68*	10	14	14	0.10
Fluridone									78	11	10	10	0.10
Flurprimidol	103	12	12	12	100	7	7	7	79	15	14	14	0.01
Hexazinone	*67*	*30*	*43*	*43*	84	8	13	13	77	14	13	13	0.02
Iprobenfos					105	7	8	8	99	19	18	18	0.02
Isazofos (Miral)	89	19	18	18	98	9	9	9	81	17	20	20	0.01
Isocarbamide	103	15	15	15	106	8	9	9	79	13	13	13	0.01
Isocarbophos	105	13	15	15	*122*	8	8	8	80	15	14	14	0.01
Isopropalin	95	13	12	13	94	6	6	6	74	13	11	11	0.01
Mefenpyr-diethyl									84	12	12	12	0.10
Metazachlor	93	16	15	15	107	10	9	9	77	16	15	15	0.01
Methabenzthiazuron					97	11	11	11	*61*	*21*	18	18	0.02
Metobromuron	94	*53*	*50*	*50*	114	*32*	*30*	*30*	88	11	10	10	0.10
Mexacarbate	111	9	12	12	116	8	8	8	82	15	15	15	0.01
mgk 264	108	17	16	16	112	11	10	10	84	12	11	11	0.01
Nitrothal-isopropyl	102	15	17	17	105	8	8	8	76	12	11	11	0.01
Pentanochlor (Solan)	102	13	12	12	108	9	8	8	80	15	13	13	0.01
Pethoxamid	101	16	16	16	104	8	9	9	78	15	15	15	0.01
Phenothrin									77	15	15	15	0.10
Phorate sulfoxide	106	15	15	15	111	8	8	8	80	16	15	15	0.01
Plifenate (Penfenate)	107	19	17	17	108	9	9	9	84	13	13	13	0.01
Profluralin	102	16	16	16	94	10	9	9	71	12	12	12	0.01

	Spike level 0.005 mg/kg				Spike level 0.01 mg/kg				Spike level 0.1 mg/kg				
Compound	**Recovery %**	**RSD_r, %**	**RSDR, %**	**Comb. Uncer-tainty (%)**	**Recovery %**	**RSD_r, %**	**RSDR, %**	**Comb. Uncer-tainty (%)**	**Recovery %**	**RSDr, %**	**RSDR, %**	**Comb. Uncer-tainty (%)**	**LOQ**
Prometryn	98	14	13	13	106	7	8	8	78	14	14	14	0.01
Propachlor	99	11	13	13	101	10	12	12	79	16	15	15	0.01
Propaphos	100	15	13	13	92	11	20	20	72	17	17	17	0.02
Quinomethionate (MQD)					*61*	8	14	14	74	12	13	13	0.02
Quizalofop-ethyl	103	19	20	*21*	*54*	9	8	8	83	13	12	12	0.01
Rabenzazole	106	18	16	16	108	8	10	10	79	12	11	11	0.01
Secbumeton	102	12	14	14	95	7	11	11	75	14	15	15	0.01
Tebupirimfos	99	18	16	16	100	8	8	8	83	17	16	17	0.01
Terbufos	78	19	*84*	*84*	76	7	*37*	*37*	*59*	17	*55*	*55*	0.02
Thenylchlor	88	15	19	19	101	8	16	16	77	16	17	17	0.01
Tiocarbazil I (Drepamon)									75	19	17	17	0.10
Tribufos	108	16	17	17	109	6	6	7	77	13	12	12	0.01
Vernolate	85	*23*	*24*	*24*	91	7	7	7	78	18	19	19	0.02

Appendix 3: Principles of the QuEChERS method for cereal extraction

QuEChERS for cereals (FP417)

Weigh 5 g (±0.05 g) of flour into a 50 ml single use centrifuge tube (red cap). Add internal standard and/or spike standard (maximum 25 µl)

Add a ceramic homogenizer and 10 g of cold water and shake briefly

Add 10 ml acetonitrile and shake vigorously by hand for 1 min. (1. extraction)

Add the prepared mixture of 4 g $MgSO_4$, 1 g NaCl, 1 g Na_3 citrate dihydrate and 0.5 g Na_2H cirate sesquihydrate. Shake for a few seconds after each addition to prevent lumps.

Shake vigorously for 1 min. (2. Extraction with phase separation)

Centrifuge for 10 min at 4500 rpm

Transfer at least 8 ml of the extract to a 15 ml single use centrifuge tube and store in the freezer (-80˚C for 1 hour or over night). When the extract are almost thawed (i.e. About -40 ˚C) centrifugate (should be cold 5 C) for 5 min. at 4500 rpm.

Transfer 6 ml of the cold extract to a 15 ml single use centrifuge tube containing 150 mg PSA and 900 mg $MgSO_4$. Close the tube and shake vigorously for 30 seconds.

Centrifuge for 5 min. at 4500 rpm

Transfer 4 ml of the extract to a 15 ml single use centrifuge tube. Add 40 µl of 5% formic acid solution in acetonitrile (10 µl/ml extract). Dilute the extract 1:1 with acetonitrile

Transfer the final extract into auto sampler vials and analyse by GC and LC.