COMPARISON OF DIFFERENT CLEAN-UP PROCEDURES FOR THE DETERMINATION OF PLANT PROTECTION PRODUCTS IN TOBACCO AND TOBACCO PRODUCTS BY LC-MS/MS

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Summary

Plant protection products (PPPs) are widely used to protect plant products including tobacco against a wide range of pest and plant diseases. During the last years methodologies for the analysis of PPP residues have been established at different industry and contract laboratories and a large variety of procedures for extraction, clean-up, separation and detection were set up.

Due to the trend of using polar PPP, the LC-MS/MS technique has been implemented by an increasing number of laboratories. The sample solutions for LC-MS/MS analysis are obtained by extraction, clean-up steps, *e.g.* gel permeation chromatography (GPC) and dissolving in LC eluent. As recommended in several publications, a matrix-matched standard calibration is used to reduce matrix effects for the calculation of the results. In this work, a systematic investigation was carried out in order to gain more knowledge about the relationship between selected tobacco matrices, different types of sample preparation, and matrix effects.

Different tobacco grades (air cured, flue cured and oriental), blended tobacco, and pipe tobacco were analysed and it was demonstrated that the sample preparation as well as the matrices of individual tobacco grades influence the recovery rates for certain PPPs. Matrix effects were particularly observed for the DFG S 19 method.

In this study, it was also demonstrated, that in case of azoxystrobin, linuron, oxamyl, imidacloprid, thiodicarb and aldicarb, the reduction of matrix interferences noticeably depends on the sample preparation applied. The range of the recovery rates of these PPPs determined by DFG S 19, DFG S 19 (ex GPC) and QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) were found to be hardly comparable.

1. Introduction

The analysis of PPP residues in food has been performed in laboratories for more than 40 years. Some methods still in used today were developed 30 years ago when analytical needs were less demanding. At that time, extensive solvent usage, extended analysis time and manual labour were only a minor issue. These methods applied for the analysis of common PPPs are far from ideal. [1]

In addition, in the last years a trend of using polar PPPs was observed. The well-established GC analysis is less suitable for these polar compounds. Consequently, the introduction of more effective and robust analytical methodologies and techniques are essential for laboratories to meet the demands of today.

The analysis of polar PPP residues requires the highly sensitive and selective LC-MS/MS-technique. Due to the complexity of the tobacco matrix and the different physicochemical properties of PPPs, the LC-MS/MS technique is not applicable for the direct determination of

PPPs without sample preparation. Therefore, clean-up steps are essential for tobacco samples.

In the present study, different published sample preparations, *e.g.* DFG S 19, simplified DFG S 19 and QuEChERS were examined systematically to assess the effect of different tobacco grades on matrix interferences.

2. Experimental

Studied material

Three different tobacco grades (air cured, flue cured, oriental), blended tobacco, and pipe tobacco low in PPP residues have been selected for this study.

Sample preparation - Extraction and clean up based on DFG S19 methodology

The tobacco samples were ground and homogenised. A representative proportion of 15 g was weighed and mixed with 100 mL water. To this solution, 200 mL of a *cyclo*-hexane/ethyl acetate (1:1) mixture and 20 g of sodium chloride were added. After filtration, an aliquot of 200 mL was extracted with 100 mL *cyclo*-hexane/ethyl acetate (1:1) mixture containing 20 g sodium chloride. The aqueous phase was removed and discarded. Sodium sulphate (25 g) was added to the organic layer and mixed well. Subsequently, the organic phase was filtered through a layer of sodium sulphate and the eluate was evaporated to 2 mL. This residue was dissolved in 15 mL of a *cyclo*-hexane/ethyl acetate (1:1) mixture and 2 g of sodium sulphate was added. An aliquot of 10 mL was subject to the clean up step by GPC. A 60 µL aliquot of the GPC extract was transferred into another tube and 2940 µL of ammonium formiate solution (10 mM) in methanol/water (20:80) was added. The resulting solution was filtered and the transferred into a LC vial.

Extraction and clean up based on simplified DFG S19 methodology (S 19 (ex GPC))

The tobacco samples were ground and homogenised. A representative proportion of 15 g was weighed and mixed with about 60 mL water. To this solution, 200 mL of ethyl acetate and 70 g of sodium sulphate were added. After filtration, an aliquot of 100 mL was evaporated to 1 mL. This solution was extracted with 1.5 mL ethyl acetate and 2.5 mL *cyclo*-hexane, centrifuged and filtered. 60 µL of the extract was transferred into another tube and 2940 µL of ammonium formiate solution (10 mM) in methanol/water (20:80) was added. An aliquot was transferred into a LC vial.

Extraction and clean up based on QuEChERS methodology

The tobacco samples were ground and homogenised. A representative proportion of 2 g was weighed and mixed with 10 mL water and 10 mL acetonitrile. To this solution, 6.5 g of buffer salt mixture (containing 4 g of magnesium sulphate, 1 g of sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogencitrate sesquihydrate) were added, mixed well and centrifuged. An aliquot of 1.5 mL of the acetonitrile extract was transferred in a cap lock tube containing 225 mg magnesium sulphate and 40 mg PSA sorbent and mixed well. After centrifugation, an aliquot of the acetonitrile extract was diluted 1:10 with ammonium formiate solution (10 mM) in methanol/water (20:80). The resulting solution was filtered and transferred into a LC vial.

Instrumentation - Liquid chromatography-mass spectrometry

The LC system consisted of an Agilent 1100 liquid chromatograph equipped with a vacuum degasser, a solvent delivery compartment with high pressure mixing chamber, an autosampler and a column oven. The injection volume was 20 µL. The separation of the agrochemicals was performed using a C 18 Synergi Fusion®-RP analytical column (50 x 2 mm (i.d.), 4 µm, Phenomenex). The separation column was protected by a guard column (C 18 Synergi Fusion®-RP, 4 x 2 mm (i.d.), Phenomenex). The total flow rate was adjusted to 0.2 mL/min.

The mobile phase consisted of a 10 mM ammonium formiate solution in methanol/water (20:80; pH: 4.1) (Eluent A) and methanol (Eluent B). Initial gradient was 100 % Eluent A, decreasing to 10 % A over 11 min. This proportion was held until 23 min and returned to the initial condition within 2 min. The column was then re-equilibrated for 10 min. The total run time was 35 min. The retention times for individual PPPs are given in Tab. 1. An integral switching valve on the mass spectrometer was used to divert the LC-flow to waste for the first 2.0 min.

Tab. 1: LC-MS/MS conditions: molar weight, precursor ion, primary and secondary traces, DP, CE for each agrochemical and retention time.

Agrochemical	Molar weight	Precursor ion	Primary trace			Secondary trace			Ret. time (min)
			Mass (m/z)	DP (V)	CE (V)	Mass (m/z)	DP (V)	CE (V)	
Azoxystrobin	403	[M+H]+	404 → 372	41	19	404 → 344	41	25	15.0
Oxamyl	219	[M+NH_4]+	237 → 72	26	21	237 → 90	26	13	3.6
Methomyl	162	[M+H]+	163 → 88	41	11	163 → 106	41	11	4.2
Carbaryl	201	[M+H]+	202 → 145	61	13	202 → 127	61	39	13.7
Metalaxyl	279	[M+H]+	280 → 192	51	19	280 → 220	51	15	14.4
Linuron	249	[M+H]+	249 → 160	66	21	249 → 133	66	45	15.2
Propoxur	209	[M+H]+	210 → 111	51	17	210 → 168	51	11	13.1
Carbendazim	191	[M+H]+	192 → 160	51	21	192 → 132	51	37	8.9
Thiodicarb	355	[M+H]+	355 → 88	56	21	355 → 108	56	19	14.0
Imidacloprid	256	[M+H]+	256 → 175	71	23	256 → 209	71	21	9.4
Aldicarb	190	[M+NH_4]+	208 → 116	26	13	208 → 89	26	19	12.1
Aldicarb-sulfoxid	206	[M+H]+	207 → 89	46	19	207 → 132	46	11	2.6
Aldicarb-sulfon	222	[M+NH_4]+	240 → 76	36	17	240 → 86	36	21	3.6
Ethiofencarb	225	[M+H]+	226 → 107	61	21	226 → 164	61	11	13.9
Ethiofencarb-sulfoxid	241	[M+H]+	242 → 107	41	21	242 → 185	41	13	8.7
Ethiofencarb-sulfon	257	[M+NH_4]+	275 → 107	26	29	275 → 201	26	13	8.9

The MS/MS detection was performed on a Q Trap 2000 instrument (Applied Biosystems). The mass spectrometer was operated with TurboIonspray® source in the positive mode (ESI +). The specific parameters for ionisation were as follows: curtain gas (CUR), 30 a.u.; ionspray voltage, 4500 V; temperature of the turbo heater gas, 400 °C; nebuliser gas (GS1), 60 a.u.; turbo gas (GS2), 60 a.u. Nitrogen was used as the curtain gas, nebuliser gas and turbo gas. The exhaust gas and curtain gas regulators were set at 3.5 bar each. The GS1/GS2 regulator was set at 6.5 bar. Unit mass resolution settings were used for Q1 and Q3. The analytical dependent parameters, declustering potential (DP) and collision energy (CE) were optimised for each compound. The data were acquired using Analyst® software, version 1.4.1 and are listed in Tab. 1.

3. Results and Discussion

LC-MS/MS analysis

The LC-MS/MS performance gave sharp peaks for all analytes irrespective of the applied sample preparation and tobacco matrix. (Fig. 1). The retention time ranged from 2.6 to 15.0 min. Some compounds were detected in their protonated form and others as adduct ions (Tab. 1). For each compound, the transition with the highest MRM response was chosen as quantifier and a second transition was used as qualifier to confirm the identity of the compound.

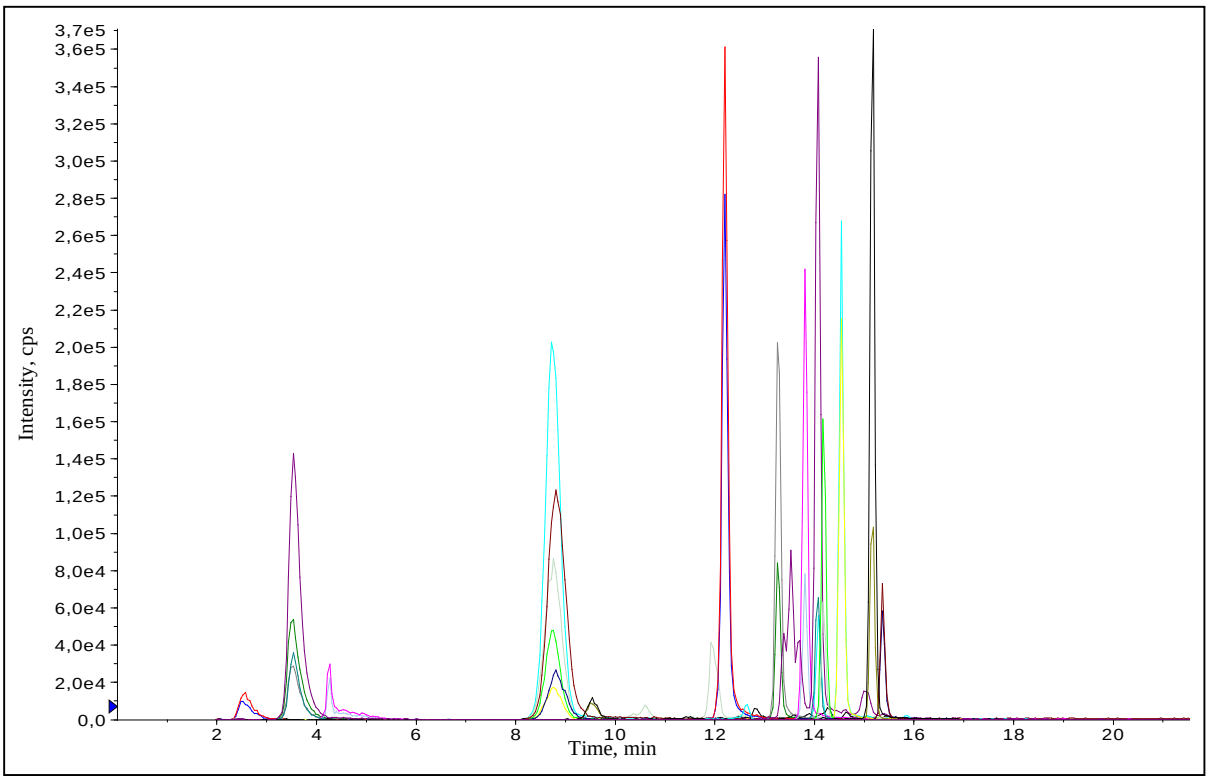

Fig 1: Chromatogram of a matrix matched standard solution (100 µg/L) including 16 agrochemicals and their metabolites (Matrix: pipe tobacco)

Sample preparation and matrix impact

It is well known that the MS-MS response depends on the eluent and matrix interferences entering the interface and the detector. Co-eluting substances could be the reason for quantification problems caused by compound specific suppression or enhancement. One way to compensate signal suppression is the use of matrix-matched standard which requires a blank matrix with the same matrix compounds as the analysed sample. Due to the complexity of the tobacco matrix, the selection of an appropriate matrix standard is very important since tobacco products consist of different tobacco types (oriental, air cured, flue cured). Another way to eliminate matrix interferences is the optimisation of sample preparation, dilution of sample extracts or variation of chromatographic conditions.

In the present study, a matrix-matched calibration was carried out for each sample preparation and matrix in order to evaluate the results of previous studies. Tobacco samples (see study material) were spiked with a PPP solution and a recovery rate study was conducted for selected PPPs to compare the sample preparations DFG S 19, DFG S19 (ex GPC), and QuEChERS with regard to the influence of the tobacco matrix. The recovery rates of the

studied PPPs are shown in Tab. 2 and 3. Recovery rates between 70 and 120 % are generally considered acceptable for the quantification of trace compounds.

Tab. 2: Recovery rates obtained by DFG S 19 and DFG S 19 (ex GPC).

Compounds	DFG S 19 - Recovery rates [%]					DFG S 19 (ex GPC) - Recovery rates [%]				
	Oriental	Air cured	Flue cured	Blended tobacco	Pipe tobacco	Oriental	Air cured	Flue cured	Blended tobacco	Pipe tobacco
Azoxystrobin	76.1	99.3	67.5	98.0	94.9	63.5	65.5	63.5	44.9	44.6
Oxamyl	68.4	76.3	58.8	67.5	74.9	89.7	90.5	78.5	77.2	75.2
Methomyl	62.5	78.3	70.0	80.3	87.8	85.0	129.4	87.5	86.2	82.5
Carbaryl	86.4	103.4	86.3	97.3	87.3	85.7	96.7	70.5	74.0	92.0
Metalaxyl	73.9	151.0	71.0	82.6	100.1	95.2	95.0	83.2	86.1	76.0
Linuron	64.8	80.1	61.8	86.0	75.2	75.7	61.0	44.0	50.0	39.7
Propoxur	77.5	85.6	77.0	90.0	102.6	97.7	100.2	85.2	90.2	73.5
Carbendazim	73.9	108.0	86.3	77.0	93.4	125.7	117.0	90.5	120.5	81.0
Thiodicarb	45.9	55.4	66.5	77.1	78.09	89.0	97.2	75.0	84.0	62.5
Imidacloprid	43.8	63.1	88.5	79.0	82.3	94.0	94.0	71.5	87.5	69.7
Σ Aldicarb	65.1	66.1	76.3	84.0	74.4	87.3	82.4	74.0	81.6	68.9
Σ Ethiofencarb	76.4	95.1	85.8	107.0	89.3	89.9	95.1	75.6	82.8	74.5

Tab. 3: Recovery rates obtained by QuEChERS.

Compounds	QuEChERS Recovery rates [%]				
	Oriental	Air cured	Flue cured	Blended tobacco	Pipe tobacco
Azoxystrobin	101.3	101.8	97.0	102.5	99.0
Oxamyl	103.3	104.3	100.3	101.0	103.5
Methomyl	86.5	95.8	114.5	136.0	99.5
Carbaryl	100.5	97.5	94.0	104.5	90.5
Metalaxyl	99.0	98.5	98.5	105.6	105.3
Linuron	90.5	94.8	99.0	101.8	98.8
Propoxur	101.5	100.0	93.8	100.0	95.3
Carbendazim	113.8	108.3	103.3	114.0	90.0
Thiodicarb	78.3	85.8	80.8	81.5	82.5
Imidacloprid	114.8	99.0	105.0	100.3	95.0
Σ Aldicarb	101.3	103.6	99.58	100.1	99.3
Σ Ethiofencarb	102.8	102.9	104.3	103.5	102.7

Recovery rates between 43 % and 108 % were found for the investigated PPPs in different tobacco matrices using the sample preparation described in DFG S 19.

For oxamyl, imidacloprid, and thiodicarb, recovery rates were found even lower than 60 %.

The results obtained for the DFG S 19 (ex GPC) sample preparation showed recovery rates ranging from 40 % to 129 %. For azoxystrobin and linuron, recovery rates below 70 % were detected.

Recovery rates of all PPPs tested with QuEChERS ranged between 86 % and 136 %.

Validation study

A validation study was performed for each sample preparation methodology. For this evaluation, tobacco samples (see study material) were treated with PPP solution. The spiked tobacco samples were analysed using the different sample preparation described above. Six replicates were carried out for the calculation of validation parameters which are listed in Tab. 4.

The results of the validation study can be summarised as followed: For most of the analysed PPPs, the limits of quantification (LOQ's) obtained by DFG S 19 method were found to be lower than the LOQ's of the DFG S 19 (ex GPC) and QuEChERS.

However, the LOQ's of all PPPs were lower than the respective Guidance Residue Levels (GRLs).

The repeatabilities (r) determined for DFG S 19, DFG S 19 (ex GPC) and QuEChERS were found to be comparable.

Tab. 4: Validation parameters and Guidance Residue Levels (GRLs)

Compounds	GRL	Limit of Quantification [mg/kg]			Repeatability [mg/kg]		
		DFG S19	DFG S 19 (ex GPC)	QuEChERS	DFG S19	DFG S 19 (ex GPC)	QuEChERS
Azoxystrobin	2.50	0.05	0.12	0.11	0.05	0.07	0.06
Oxamyl	0.50	0.10	0.20	0.09	0.01	0.03	0.02
Methomyl	-	0.35	0.58	0.61	0.05	0.04	0.05
Carbaryl	0.50	0.15	0.11	0.20	0.06	0.04	0.05
Metalaxyl	2.00	0.14	0.23	0.24	0.04	0.04	0.08
Linuron	NYA*	0.17	0.25	0.26	0.08	0.08	0.12
Propoxur	0.20	0.11	0.19	0.13	0.04	0.09	0.06
Carbendazim	2.00	0.12	0.17	0.26	0.05	0.05	0.08
Thiodicarb	-	0.07	0.16	0.12	0.05	0.05	0.08
Σ Methomyl + Thiodicarb	1.00	-	-	-	-	-	-
Imidacloprid	5.00	0.21	0.36	0.22	0.14	0.11	0.08
Aldicarb	-	0.05	0.19	0.10	0.08	0.07	0.05
Aldicarb-sulfoxid	-	0.07	0.20	0.19	0.06	0.06	0.10
Aldicarb-sulfon	-	0.07	0.19	0.11	0.07	0.06	0.06
Σ Aldicarb	0.50	-	-	-	-	-	-
Ethiofencarb	-	0.13	0.19	0.26	0.06	0.09	0.08
Ethiofencarb-sulfoxid	-	0.13	0.18	0.17	0.06	0.06	0.05
Ethiofencarb-sulfon	-	0.10	0.16	0.10	0.04	0.05	0.05
Σ Ethiofencarb	NYA*	-	-	-	-	-	-

NYA* = Not Yet Assessed

Effect of different sample preparation procedures

In the recovery rate study, it could be demonstrated that the sample preparation significantly influences the detected amounts of PPPs. The range of the recovery rates for azoxystrobin, linuron, oxamyl, imidacloprid, thiodicarb, and aldicarb determined by DFG S 19, DFG S 19 (ex GPC) and QuEChERS were found to be hardly comparable.

A reason for the different recovery rates could be the fact that systematic and random errors are more likely to occur for sample preparation procedures including many complicated analytical steps, *e.g*. DFG S19. The DFG S 19 is an effective, but complicated and time consuming method. Therefore, it is not surprising, that the best recovery rates for all PPPs investigated were found by using the QuEChERS sample preparation.

However, the validation study showed that the QuEChERS sample preparation resulted in the highest LOQ's of all PPPs. As expected, the DFG S 19 method featured the lowest LOQ's due to the effectiveness of its sample preparation. The LOQ's of all PPPs were found to be lower than the respective GRLs.

Effect of different tobacco matrices

Matrix interferences could be observed particularly for the DFG S 19 method. As it was shown for the example thiodicarb, differences between recovery rates for individual tobacco matrices obtained by DFG S 19 were found to be higher than for the other sample preparations applied. The lowest recovery rates were detected for different PPPs in the matrices of oriental and virginia tobacco grades using the DFG S 19 methodology.

Furthermore, the pipe tobacco matrix had an impact on the recovery rates using DFG S 19 (ex GPC) methodology. For this method, the lowest recovery rates were calculated for carbaryl, thiodicarb, azoxystrobin, linuron, and aldicarb.

An influence of different tobacco matrices could not be identified for the QuEChERS sample preparation.

4. Conclusion

In this study, the effects of different tobacco grades and sample preparations on the matrix interference were systematically examined. It was demonstrated that the sample preparation as well as the matrices of individual tobacco grades influence the results of recovery rates significantly. Matrix interferences could be observed particularly for the S 19 method.

Furthermore, it could be demonstrated, that in case of azoxystrobin, linuron, oxamyl, imidacloprid, thiodicarb and aldicarb the degree of matrix interferences noticeably depends on the applied sample preparation. The range of the recovery rates of these PPPs determined by S 19, S 19 (ex GPC) and QuEChERS were found to be hardly comparable. The validation study showed that the applied sample preparations result in different LOQ's. The highest LOQ's were calculated for the QuEChERS method.

Therefore, it can be concluded that all investigated sample preparations are suitable for the determination of PPPs in tobacco matrices. However, depending on the analytical needs, the most suitable sample preparation should be applied to meet the individual requirements.

QuEChERS seems to be the method of choice in case regulatory bodies set limits above the determined LOQ's. The advantages of QuEChERS are obvious: minor matrix interferences and a rapid sample preparation.

References

[1] Anastassisdes et al.: *Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and «Dispersive Solid-Phase Extraction» for the Determination of Pesticide Residues in Produce;* J. of ACOC Inter., 86 (2), 412-431, **2003**

[2] S. Otte, M. Intorp: *Analysis of Pesticides in Tobacco by LC-MS/MS: Specific Matrix Effects on Quantification for Different Tobacco Grades,* CORESTA Smoke Science & Product Technology Joint Meeting, Stratford-upon-Avon, **2005**

[3] T. Synnerdahl et al.: *A Program for multiresidue testing on tobacco*, CORESTA Sub-group on Pesticide Residues; 42nd Meeting, Bayreuth, **2003**

[4] T. Anspach: *QuEChERS Multi Residue Method- an Effective Tool for the Determination of Agrochemical Residues in Tobacco by LC-MS/MS;* CORESTA Congress, Paris, **2006**