Pesticide residue analysis in Hops: Four different procedures of sample preparation are currently used. Is one of them more useful than the others?

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INTRODUCTION

The aim of this study is to compare four different sample preparation procedures that are commonly used for pesticide residues analysis in hops. Our attention was then focused on ability of each method effectively minimize co-extraction of matrix components (chlorophyll, resins, bitter acids) which can most often cause a massive signal suppression, elevated background, and the other negative matrix effects. The recovery of fifty five spiked pesticides was also evaluated for each method.

The methods are based on a completely different approach for a sample preparation using various solvents for an extraction of pesticide residues from matrix. The clean-up step is also unique for each method. One of those is recently published the QuEChERS method in combination with a specially optimized mixture for the dispersive solid phase extraction. The results of the **AHA *(Arbeitsgruppe Hopfenanalyse)* collaborative trial** shows that all methods are more or less comparable. Therefore, the critical evaluation of each method for the purpose to choose the most effective one and easiest handling are more than desirable.

METHODS and EXPERIMENTS

Sample Preparation Protocol of Hengel-method [1]

- Transfer 0.5 g sample to 50 mL tube
- Add internal standard
  Add 15 mL of MeCN
  Then homogenize on Ultra-Turrax
- Precondition *SPE Strata X* column with 5 mL MeCN
  Apply unfiltered sample to the SPE column
  Collect eluent in a 45-mL conical tube
  Rinse the filtration cake with 10 mL MeCN
  Concentrate the extract to approx. 0.5 mL
- **LC-MS/MS analysis**
  Dissolve the extract in 25 mL of 10 mM ammonium acetate/MeOH (10:90, v/v)

HPLC – HR-MS/MS
LC System: Dionex UltiMate 3000 UHPLC system
Column: C18 Atlantis T3 column, 2.1×100 mm, 3 µm
Column Temp: 35 ˚C
Injection volume: 5 µL or 2 µL (QuEChERS)
Mobile phase A: 2 mM ammonium formate in water + 0.1 % formic acid
Mobile phase B: Methanol

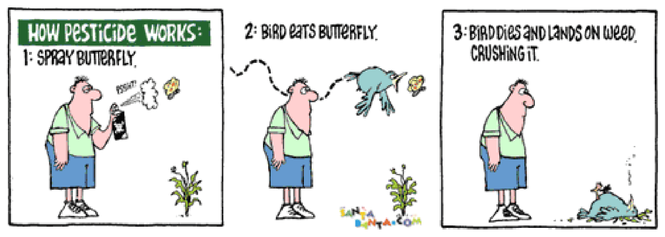
MS System: hybrid quadrupole-orbitrap Q-Exactive
Acquisition: Parallel reaction monitoring (PRM)
Isolation window: ± 0.7 amu
Resolution: 17,500

Sample Preparation Protocol of Biendl-method [2]

- Transfer 5 g sample into a 250 mL screw cap bottle
- Add 50 mL of water
  Add 100 mL of acetone
- Shake using a shaking device (225 rpm) for 30 min
- Transfer 30 mL of supernatant to a tube
  Add 6 mL of saturated NaCl solution
  Add 10 mL of dichloromethane
  Shake, then centrifuge (2 min/2000 rpm)
- Evaporate 7.5 mL of organic phase to dryness
  Dissolve in 10 mL of EtAc, then centrifuge
- Precondition SPE ***Mega Bond Elut-PSA*** cartridge with 10 mL MeCN
  Apply 5 mL of sample and collect eluate
  Elute with 10 mL of MeCN
  Then evaporate eluate to dryness
- **LC-MS/MS analysis**
  Dissolve the extract in 1 mL of 5 mM ammonium formate in MeOH/water
  Add internal standard

Sample Preparation Protocol of Alder-method with Chem Elut [3]

- Transfer 1 g sample to 50 mL tube
- Add internal standard
  Add 10 mL of water
  Add 20 mL of MeOH
  Disperse with Ultra-Turrax for 2 min
  Then centrifuge 7 min/5000 rpm
- Add 5 mL of 20% solution NaCl in water to 15 mL of supernatant, then mix
- Soak a ***ChemElut*** cartridge with the mixture
  Elute with 4 x 6 ml of dichloromethane
  Then evaporate eluate to dryness
- **LC-MS/MS analysis**
  Dissolve the extract in 5 mL of MeOH



Sample Preparation Protocol of QuEChERS [4]

- Transfer 1 g sample to 50 mL tube
- Add internal standard
  Add 10 mL of water
  Then shake
- Add 10 mL of MeCN
  Add salt mixture: 4g $MgSO_4$, 1g NaCl, 1 g trisodium citrate dehydrate, and 0.5 g disodium hydrogen citrate sesquihydrate
- Shake vigorously, then centrifuge (7 min/4500 rpm)
- Transfer 1 mL to tube with 150 mg $MgSO_4$ + 100 mg PSA + 25 mg C18 + 25 mg Z-Sep
- Shake vigorously, then centrifuge (5 min/10 000 rpm)
- **LC-MS/MS analysis**
  Analyze the MeCN extract directly

FINDINGS and RESULTS

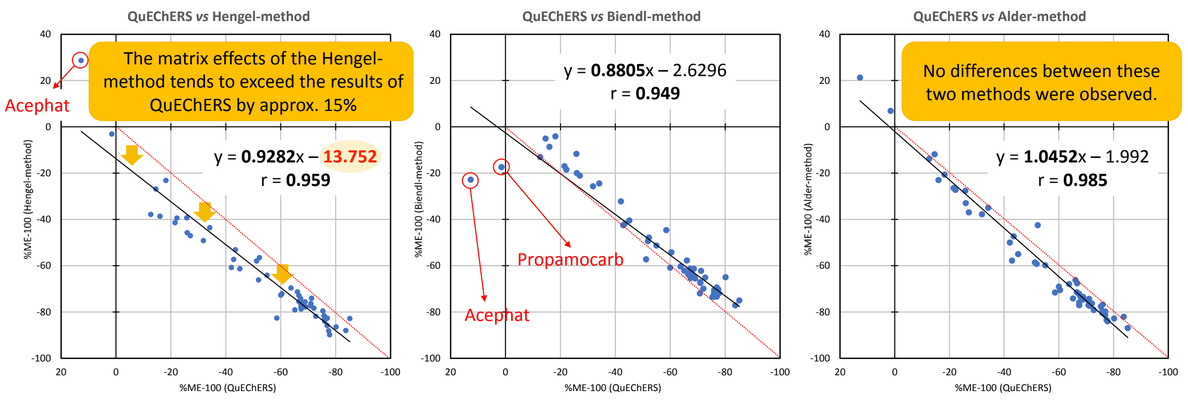
1. Evaluation of Sample Preparation Procedures

The study evaluates a solvents consumption, numbers of different steps involved in the sample handling protocol of the different sample preparation methods, and concentration of matrix in one milliliter of the final extract.

Method	Sample Size	Solvent(s)	LC and GC together	Evaporation	Centrifugation	Matrix concentration
Hengel-method	0.5 g	30 mL acetonitrile 5 mL acetone	?	Yes (1x)	No	0.02 g per mL
Biendl-method	5 g	100 mL acetone 10 mL dichloromethane 20 mL acetonitrile	No	Yes (2x)	Yes (2x)	0.125 g per mL
Alder-method with Chem Elut	1 g	20 mL methanol 40 mL dichloromethane 5 mL acetonitrile	Yes	Yes (1x)	Yes (1x)	0.1 g per mL
QuEChERS method	1 g	10 mL acetonitrile	Yes	No	Yes (2x)	0.1 g per mL

2. Matrix Effects Evaluation

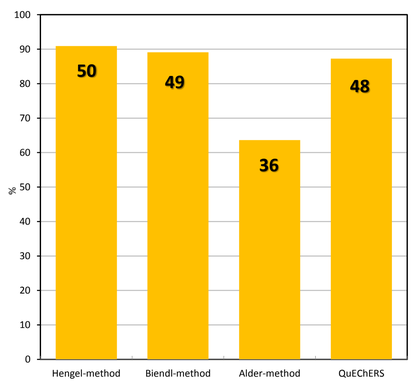
The ability of methods to effectively remove the co-extracted matrix components is illustrated by plotting the calculated *percent matrix effect minus 100* for Hengel, Biendl and Alder-methods versus QuEChERS. The evaluation uses a correlation coefficient and a regression line to estimate the differences between the pair of methods.



3. Recovery Evaluation

This study evaluated the performance of four sample processing strategies to determine the best method for hops analysis. Percent recovery values for 59 analytes were calculated. This percent recovery values for each pesticide for each sample preparation procedure was done at fortification level of 100 ppb. The percent of compounds that fell within the 80-120% recovery range was used to compare the sample preparation method.

% Pesticides with 80-120% Recovery



Some of pesticides with recovery out of the acceptable recovery range

	Analyte	Hengel-method	Biendl-method	Alder-method	QuEChERS
1.	Acephate	87.4	**12.6**	**53.1**	**64.7**
2.	Bifenthrin	85.4	90.0	**1.0**	95.3
3.	Chlorpyrifos	91.3	100.1	**59.6**	96.4
4.	Etoxazole	**122.8**	93.4	**78.2**	92.6
5.	Hexythiazox	85.4	97.5	**67.3**	100.1
6.	Pendimethalin	93.6	95.3	**26.0**	93.0
7.	Propamocarb	100.6	**7.9**	**49.5**	**8.1**
8.	Pymetrozin	**77.4**	**36.4**	81.6	**20.9**
9.	Spirodiclofen	108.9	**74.7**	**54.4**	91.0
10.	Thiabendazole	**73.8**	95.5	**121.9**	**77.3**

CONCLUSION

- **The Hengel-method seemed to be the most easy-handling among the tested methods.**
- **The QuEChERS method use the lowest solvent volume per one sample.**
- **All sample preparation procedures minimize matrix effects more or with the same effectivity.**
- **The most complex sample preparation procedure of the Biendl-method does not necessarily ensure the best suppression of the matrix effects.**
- **Application of PSA sorbent (the Biendl-method and the QuEChERS) negatively affect recovery of some compounds.**
- **Except the Alder-method, the methods have acceptable recoveries for 90% of tested pesticides.**

LITERATURE
[1] Hengel, J.H.: *J. Am. Soc. Brew. Chem.* 69(3): 121-26, **2011**.
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ACKNOWLEDGEMENT
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