Analysis of 2-Phenylphenol (sum) using QuEChERS involving Acidic Hydrolysis

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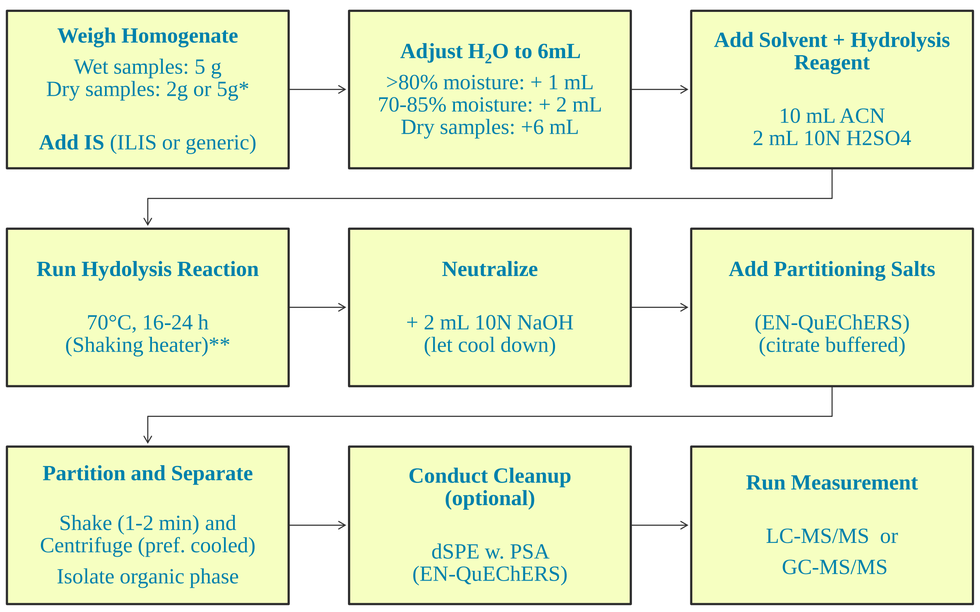
1. Introduction

2-Phenylphenol (OPP) has a long history of food related uses. Originally classified as a food additive (E231/E232) it was eventually declared as a PPP active substance. Currently EU-registrations concern post harvest treatment for mold-control in citrus. Elsewhere other types of fruits are also treated. Being effective against fungi, bacteria and viruses, OPP is also contained in biocidal products for e.g. households, farms and food processing sites. It is also reported as a preservative of paper, polymers and dyes. Contamination of food products following biocidal uses, and migration of OPP into food are thus also an issue.

Since 2018, the residue definition for food of plant origin entails OPP conjugates. The types of conjugates are not specified, but based on metabolism studies, sugar conjugates are known to be prominent in fruit. As the glycosidic linkage of OPP to sugars is chemically quite resistant to alkaline hydrolysis, a new QuEChERS-based method entailing a strong acidic hydrolysis step was introduced (AcH-QuEChERS).

2. Sample Preparation

OPP and OPP glucoside (OPP-Glc) show high recoveries using CEN-QuEChERS, but for the analysis of OPP (sum) a hydrolysis step is needed (➔ **AcH-QuEChERS):**



* 2 g or 5 g considering clumping behavior and measurement sensitivity
** Make sure the material is moving and well dispersed during reaction

3. Measurement

Screening for free OPP in routine QuEChERS extracts (w/o hydrolysis) prior to conducting AcH-QuEChERS is reasonable. **Screening via GC-MS/MS** is **possible but tricky**. Analyte protectants (APs) reduce tailing but low-level measurements are compromised by background contamination, as OPP-artefacts seem to be formed during the hot GC-injection, increasing the risk of false positives. Partial decomposition of OPP-Glc to OPP in the hot injector also compromises measurement accuracy of free OPP in samples. **LC-MS/MS circumvents these problems but is less sensitive** in measuring OPP than GC-MS/MS. OPP-Glc can also be sensitively measured via LC-MS/MS using the formate adduct as parent ion. Still, OPP (sum) cannot be covered by simply measuring free OPP and OPP-Glc in QuEChERS extracts, as OPP-Glc constitutes only a fraction of the conjugated OPP (see details to the right).

Table 1: LC-Settings for free OPP and OPP-Glc

Parameter		Settings	
Column / Pre-column		Acquity UPLC BEH C18,1.7µm; 2.1x100mm / Van Guard BEH C18, 1.7µm	
LC-Settings		Temp.: 40°C / inj. Vol.: 2µL / Flow rate: 0.4 mL/min	
Eluent A/B	**OPP**	A: 0,01% acetic acid in Water/ACN 95/5 / B: 0,01% acetic acid in ACN	
	OPP-Glc	A: 0,1% formic acid in Water/MeOH 95/5 / B: 0,1% formic acid in MeOH	
Gradient		80 % A ➔ 70 % A	0 min ➔ 4 min
		70 % A ➔ 10 % A	4 min ➔ 7, hold till 8.5 min
		10 % A ➔ 80 % A	8.5 min ➔ 14.5 min

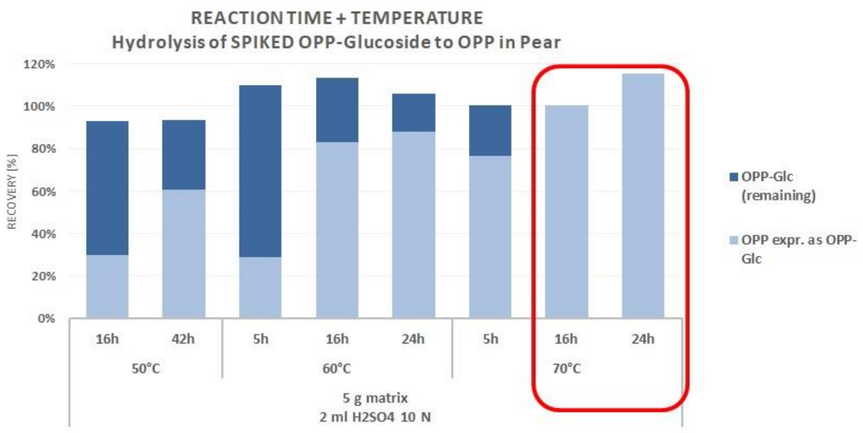
Table 2: Mass-Transitions for OPP (for GC- & LC-MS/MS) and for OPP-Glc (for LC-MS/MS)

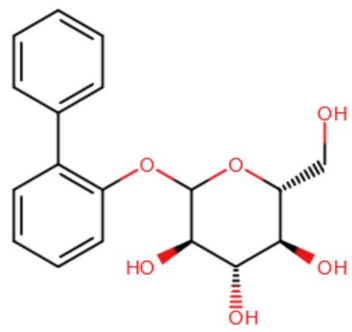
Analyte	LC Mass Transitions (m/z)	GC Mass Transitions (m/z)
OPP	169/115 ; 169/141 ; 169/93 (Parent: $[M-H]^+$)	170/115, 170/141, 169/115
OPP-Glc	377/45; 378/45; 377/169; 378/170 (Parent: $[M+HCOO]^-$)	

4. Hydrolysis of OPP-Glc

The glycosidic bond between OPP and glucose proved very resistant to hydrolysis. We have tested the hydrolytic behavior of OPP-Glc and the stability of OPP both via alkaline and acidic hydrolysis, with the latter proving more effective. A high reaction temp. (70°C) and acid-to-sample ratio (5 g sample + 2 mL 10 N H_2SO_4) as well as an extended reaction time (16-24h) were needed for achieving good hydrolysis yields for OPP-Glc. The reaction can be conducted unattended over-night in a shaking water bath.

Interestingly, AcH-QuEChERS was also shown to be effective in breaking up esters and glucosides of phenoxyalkanoic acids (like 2,4-D), so this approach might also be useful for covering the full RD of such pesticides.

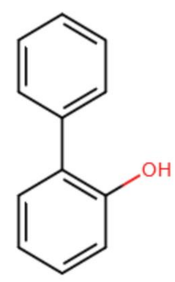


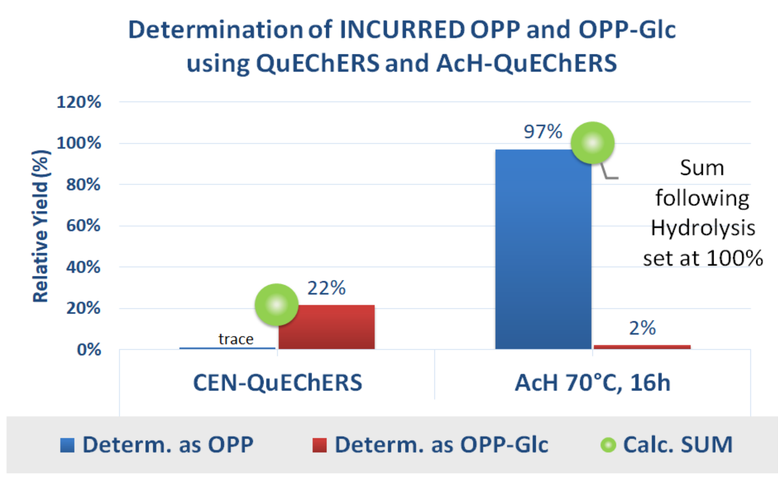


4. Hydrolysis of Incurred OPP-Conjugates

To test the behavior of conjugated OPP in real samples, organic pears were superficially treated with a Na-OPP solution and left standing for several days to built up conjugated residues. The samples were analyzed both via QuEChERS (for OPP & OPP-Glc) and via AcH-QuEChERS (for OPP-sum). In one sample >97% of total OPP was present in conjugated form (22% as OPP-Glc), while free OPP was only found in traces. In a second sample 75% of OPP was present in conjugated form (42% as OPP-Glc). The result achieved by AcH-QuEChERS (OPP-sum) was set at 100%. These experiments show, that OPP-Glc forms only a fraction of the conjugated OPP in pears and that OPP (sum) cannot be simply calculated by adding up free OPP and Glc-bound OPP. Other types of acid-hydrolysable OPP-conjugates form a considerable share of the residue. The hydrolysis conditions of AcH-QuEChERS were shown to be also effective for incurred OPP-conjugates in superficially treated pear samples (plateau reached).

AcH-QuEChERS





Analysis of citrus fruits showed that the share conjugated OPP to total OPP is typically well below 50%, with varieties with sweeter peel (e.g. mandarins) showing a higher share.

5. Conclusion and Outlook

AcH-QuEChERS allows the determination of OPP (sum) and involves only little additional effort compared to normal QuEChERS. A large portion of OPP is present in conjugated form (at least in pears). Analysis of OPP-Glc via QuEChERS and LC-MS/MS is possible with satisfactory recoveries and sensitivity, but it does not suffice for determining OPP (sum).

The share of conjugated OPP in other types of commodities as well as the source of the frequent OPP-findings in GC-analyses need to be elucidated.

Ref: Document SRM-53 on EURL Website

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