



# PFAS analysis at low ppt-level in fruits and vegetables

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## Introduction

Per and polyfluorinated compounds (PFAS) are a group of man-made substances that recently received a lot of attention. Because many PFAS are already harmful at low concentrations (several ng/week), the European Food Safety Authority (EFSA) set a safety threshold for the most relevant PFAS that accumulate in the body. The threshold: a group tolerable weekly intake for PFOA, PFNA, PFHxS, and PFOS (also called the EFSA 4) of 4.4 ng/kg body weight per week, has large implications on the required detection limits of PFAS in food matrices. Also, EFSA reports a lack of data on PFAS in fruits and vegetables, which are an important part of the human diet. Current methodologies often lack sensitivity. In this current research, we attempted to push the boundaries of our clean-up method and LC-MS/MS. This study aimed to develop a method for the analysis of 16 PFAS in fruit and vegetables, down to the low-ppt level, especially the EFSA 4 PFAS. The developed method was applied in a large-scale study on fruits and vegetables (n=866) from kitchen gardens in the vicinity of two large fluorochemical production plants (FPP) in the Netherlands.

## Method

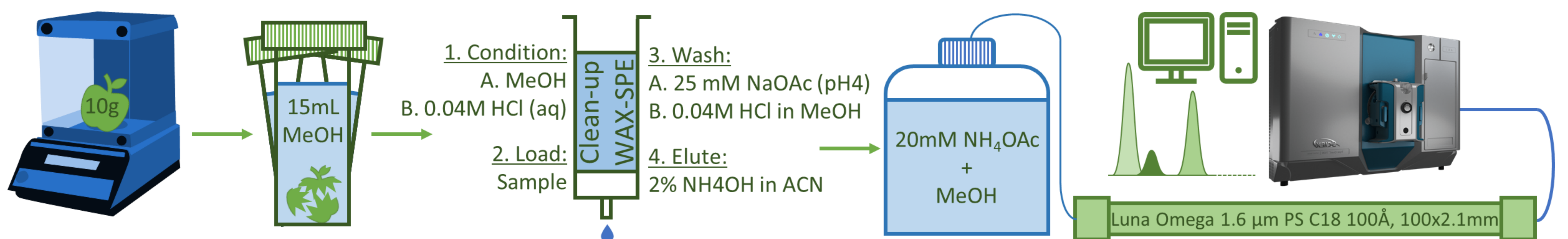
The developed method is based on an existing method that is used in our lab. The method is altered in order to lower the detection limit. The method consists of a solid-phase extraction (SPE; weak anion exchange), followed by detection by UHPLC (SCIEX Triple Quad™ 7500 LC-MS/MS). The alternations to the existing method are shown in Table 1. An overview of the method is depicted in Figure 1. The PFAS that were included are ten perfluorinated carboxylic acids (PFCAs; C5-C14), five perfluorinated sulfonates (PFSAs; C4,6-8,10), and GenX (HFPO-DA); Table 2. The method was validated on five categories of fruits and vegetables: leaf crops, fruits, tuber crops, garlic/onions, and 'other' vegetables. In each category, six different fruits/vegetables were included. PFAS were quantified based on matrix fortified calibration curves, prepared in fruits or vegetables of the same category as the actual sample.

**Table 1.** Efforts to reduce the chemical blank signal and increase sensitivity.

Old method	New method
- 1 g sample	- 10 g sample
- 1 mL reconstitution	- 0.4 mL reconstitution
- Old generation LC-MS/MS	- New generation LC-MS/MS
- Contaminated solvents	- Higher quality solvents
- Slow elution on a small-particle column	- Fast elution on an ultra small-particle column
- 2.5 ppb internal standard	- 5 ppt internal standard
<b>Challenges</b>	<b>Outcome</b>
- Lowering LOQs	- Lower chemical blank signal
- Background contamination (mainly PFOA)	- Higher signal
- Contamination from isotopically labeled internal standards	

**Table 2.** Overview of PFAS and MS/MS transitions used in the method.

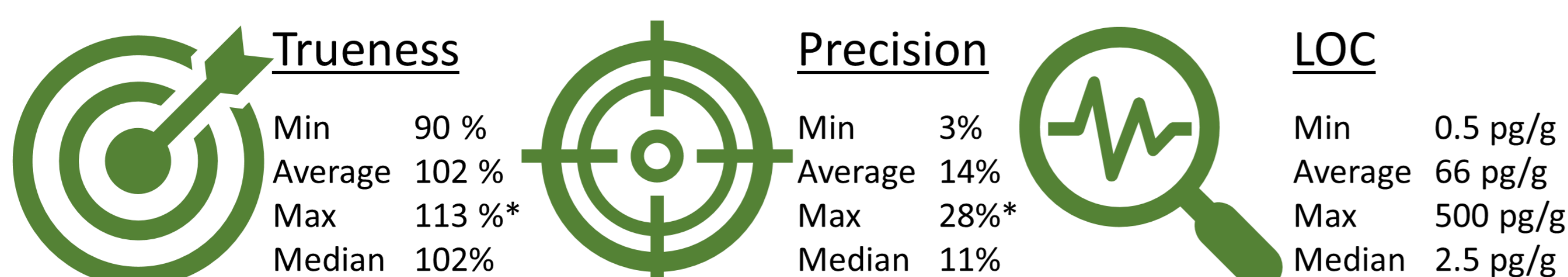
PFCAs			PFSAs		
Quantification-pair	Internal standard		Quantification-pair	Internal standard	
PFFPA	263-219	13C-PFFPA	PFBS	299-99	13C-PFBS
PFHxA	313-119	13C-PFHxA	PFHxS	399-99	180-PFHxS
PFHpA	363-169	13C-PFHpA	PFHpS	449-99	180-PFHpS
PFOA	413-169	13C-PFOA	PFOS	499-99	13C-PFOS
PFNA	463-219	13C-PFNA	PFDS	615-570	13C-PFDoDA
PFDA	513-219	13C-PFDA			
PFUnDA	563-269	13C-PFUnDA			
PFDoDA	613-319	13C-PFDoDA			
PFTTrDA	663-269	13C-PFTTrDA			
PFTeDA	713-319	13C-PFTeDA			
			Others		
			GenX	285-169	13C-GenX



**Figure 2.** Schematic overview of the clean-up and detection methodology. All samples were grinded and homogenized before they were weighed.

## Validation results and conclusion

The trueness, precision, and limit of confirmation (LOC) were calculated for all matrix categories. For most PFAS, an LOC of 2.5 pg/g was achieved with excellent trueness and precision. An overview of the performance characteristics is given in Figure 2. The method was demonstrated to be quantitative for the perfluorinated carboxylic acids, with chain lengths C5 – C14 and C16 (excluding C13, due to the lack of isotopically labeled internal standard), and the perfluorinated sulfonates with chain length C4 – C10, as well as for GenX.



**Figure 2.** Overall trueness, precision, and limit of confirmation of the method. The trueness and precision of PFTTrDA did not meet the quality criteria. The trueness was 134%-146% and the precision 44%-64%. Therefore, PFTTrDA can only be reported qualitatively. The LOC is defined as the lowest concentration at which both MS-transitions are visible, and their ion-ratio deviates less than 30% of the mean.

## Survey

The samples in the vicinity of the FPPs showed elevated concentrations of PFHxA, PFHpA, PFOA, and GenX. Especially concentrations of PFOA and GenX were significantly elevated; up to 5.000 pg/g. Fruits and garlic/onions were, in general, less contaminated than leaf crops and tuber crops. The Dutch Institute for Public Health and the Environment (RIVM) performed a risk assessment on the results and has issued general advice regarding the consumption of vegetables from these gardens. The advisory report is published on the RIVM website.

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