EURL-SRM

EU Reference Laboratories for Residues of Pesticides
Single Residue Methods

EURL-SRM - Analytical Observations Report

concerning the following…

- **Compound(s)**: Fluoride (from sulfuryl fluoride applications and from natural sources)
- **Commodities**: Fruit and vegetables (fresh and dried), dry commodities
- **Extraction Method(s)**: a) Direct measurement; b) QuPPe; c) Microdiffusion Cell Approach
- **Instrumental analysis**: Ion-Selective Electrode (ISE)

Determination of fluoride ions in food

Version 1 (last update: 02.03.2022)

Background information / Initial Observations:

Fluorine is a ubiquitous element and thus naturally present in all living organisms. From the pesticide residue point of few, fluoride anion is a useful marker compound indicating fumigations with sulfuryl fluoride, which is applied for disinfestation of dry food commodities, e.g. before transportation. Sulfuryl fluoride is an approved active substance within the EU with applications being authorized in various member states[1].

Residues of both sulfuryl fluoride and fluoride ion, are regulated in Regulation (EC) No 396/2005 and MRLs have recently been revised[2]. Fluoride MRLs were lowered for several commodities, e.g. for coconuts from 30 to 15 mg/kg, for cocoa beans from 10 to 5 mg/kg, for animal tissues from 1 to 0.3 mg/kg, and for fruits, vegetables and fresh herbs from 2 mg/kg to 0.2 mg/kg. MRLs remained the same in the case of e.g. herbal infusions at 10 mg/kg, spices and coffee beans at 5 mg/kg, pulses, oilseeds, cereals and sugar plants at 2 mg/kg, and milk and eggs at 0.2 mg/kg. The MRLs were raised for tea from 350 to 400 mg/kg and for tree nuts from 25 to 30 mg/kg. In the case of honey, an MRL of 0.5* mg/kg was established.

A regular and sufficient intake of fluoride is recommended by the World Health Organization (WHO) to prevent caries. In addition, fluoride is reported to improve resistance and density of bones. On the other hand, excess fluoride intake can cause fluorosis, which affects the teeth and bones and has to be avoided. With fluoride concentrations being low in food commodities, adults usually reach their daily fluoride intake by fluoridated table salt. Excess intake possibly occurs in regions with unusual fluoride-rich drinking water. Collections of fluoride contents in food are available, e g. from the USDA [1] with focus on ready-to-eat food, but there are also various publications with short compilations of fluoride contents in miscellaneous products [2],[3], teas and other beverages [4] and infant food [5], [6], [7]. Fluoride content data are of particular interest within the public health context as the fluoride intake by the public has an influence on the occurrence and prevention of tooth decay and/or dental fluorosis.

[1] According to the EU Pesticides Database it is authorized in AT,BE,DE,ES,FR,IE,NL,PL,PT,SI (accessed 20 February 2023)
[2] Regulation (EU) 2022/1321

EU Reference Laboratory for Single Residue Methods
CVUA Stuttgart, Schaflandstr. 3/2, 70736 Fellbach, Germany
EURL@cvuas.bwl.de

Fluoride is naturally present in all food commodities and this aspects needs to be considered in MRL-setting. From the analytical point of view, the background levels may complicate validation at low levels, as the SANTE guidelines require that in recovery experiments the background levels in the blank matrix do not exceed 30% of the spiked amount.
For the determination of fluoride in food and water, usually, methods using ion-selective electrodes (ISE) or ion chromatography with conductivity detection (IC-ICD) are used. Due to the low concentrations of fluoride ion in food samples, an enrichment / isolation process via microdiffusion or steam-distillation may need to be conducted, which take advantage of fluoride tendency to evaporate in form of hydrofluoric acid under strongly acidic conditions. For microdiffusion, dry (or dried) commodities, are placed onto a cavity and mixed with a highly acidic solution. The released hydrofluoride is trapped into an alkaline trapping solution. Approaches involving mineralization (ashing) aim to analyze the total fluoride content in a sample, incl. organofluorine compounds (e.g. PFAS), which are not entailed in the residue definition stated in Regulation (EC) No 396/2005, which only refers to the free fluoride ions.

Compound details:

Fluoride ion (CAS: 7782-41-4), IUPAC: Fluoride	
Parameter	**Value**
Molecular Mass	18.998 g/mol
Exact mass	18.998403 Da
Pka	3.17 (refers to the corresponding acid, HF)
Residue definition EU	Sulfuryl fluoride and fluoride ion separately
Sulfuryl fluoride is approved in…	AT, BE, DE, ES, FR, IE, NL, PL, PT, SI
ADI / ARfD	0.004 mg/kg bw per day / 0.004 mg/kg bw (BfR 2004)

Chemicals[3]:

Substances/Chemicals	Concentration/Purity	CAS	Source
Fluoride standard solutions	1000 mg/L; 100 mg/L; 10 mg/L; suitable dilutions	7782-41-4	Mettler Toledo
TISAB II (with CDTA)**	-	-	Mettler Toledo
Perchloric acid $HClO_4$	70 – 72%	7601-90-3	Supelco Merck
Hexamethyldisiloxane (HMDS)	p.a.	107-46-0	Sigma Aldrich
NaOH	0.1 N	1310-73-2	Supelco Merck
Ultrapure water	-	7732-18-5	Water purification system in lab
* TISAB stands for Total Ionic Strength Adjustment Buffer. TISAB is used to level-out (mask) minor differences in the ionic strength and pH of sample solutions. Its addition increases the accuracy of potentiometric measurements by ion-selective electrodes (e.g. FSE). TISAB solutions contain water, NaCl, acetic acid and a complexing agent such as CDTA or citrate, that helps to break up complexes between F and metals such as Ca and Mg. ** CDTA stands for 1,2-Cyclohexanedinitrilotetraacetic acid which is multidentate ligand that is used for chelating metals ions.			

<u>HMDS-saturated $HClO_4$:</u>
118 ml ultrapure water are placed in a 200 mL flask and 82 mL 70% perchloric acid are added. Thereafter, 10 ml HMDS (hexamethyldisiloxane) are added [7].

All other materials and chemicals used as listed in the current QuPPe document [1].

[3] **Disclaimer**: Names of companies and brand names are given for the convenience of the reader and do not indicate any preference by the EURL-SRM towards these companies and their products

Materials and Apparatus[3]

- SevenCompact pH/Ion meter by Mettler Toledo
- PerfectION™ comb F (Flouride) Combination Electrode containing a reference electrode by Mettler Toledo, Model #: 51344715
- Bel-Art® Conway Diffusion Cell, 83 mm O.D.; white molded polypropylene with natural, clear polypropylene cover, by SP BelArt (USA), purchased from Merck, product #: BAF409410000
- Centrifuge suitable for the centrifuge tubes employed in the procedure and capable of achieving > 3,000 g, e.g. Rotanta 460 by Hettich, Tuttlingen/Germany.
- Disposable 50 mL PP "Falcon" tubes (e.g. Sarstedt / Nümbrecht, Germany, 114x28 mm, Art-No. 62.548.004), used for QuPPe and potentially also for ISE measurements
- Simport T550-10AT self-standing PP-tubes, ca. 15 mm inner diameter thus accommodating the electrode which has a dimeter of 12 mm. Can be used for ISE measurements

Sample preparations prior to ISE Measurements

QuPPe extraction

For "direct measurement", sample extracts were prepared following the QuPPe extraction method for fresh fruit and vegetables and dry commodities, see Figure 1 [1]. Contrary to the usual QuPPe procedure, for dry commodities EDTA was not used during extraction. The raw QuPPe-extracts (without further clean-up for the removal of proteins or lipids) were filtered after cold centrifugation and a 1 mL aliquot was transferred into a PP vessel, mixed with 1 mL TISAB II solution and measured using ISE.
The concentration was quantified against a fluoride calibration curve prepared in a reagent blank at a concentration range e.g. between 0.05−0.5 µg/mL.

Microdiffusion approach (for dry or dried samples)

Microdiffusion was conducted largely following the method by Tomori et al. [7]. The method uses a microdiffusion cell, that consists of an inner and an outer sector and a suitable lid, see Figure 3.
0.5 g of dry commodities are weighed into the outer sector of the cell. The inner sector, is filled with 1 mL of 0.1 N NaOH, which functions as a trapping solution. After adding 4 mL of a HMDS-saturated 5 M $HClO_4$ solution to the outer sector, where the sample material is located, the cell is covered by the lid and sealed with vaseline. Microdiffusion is conducted either at room temperature for 48 h or at 50 °C for 5 h (different conditions were tested, see below). During this step, fluoride evaporates in form of HF and is trapped in the alkaline trapping solution (diffusate) within the inner sector of the cell. After the diffusion time, the cell is re-opened and 1 mL TISAB II solution is added to the diffusate. The merged solution is then transferred into a suitable plastic vessel (see materials) for ISE measurement.

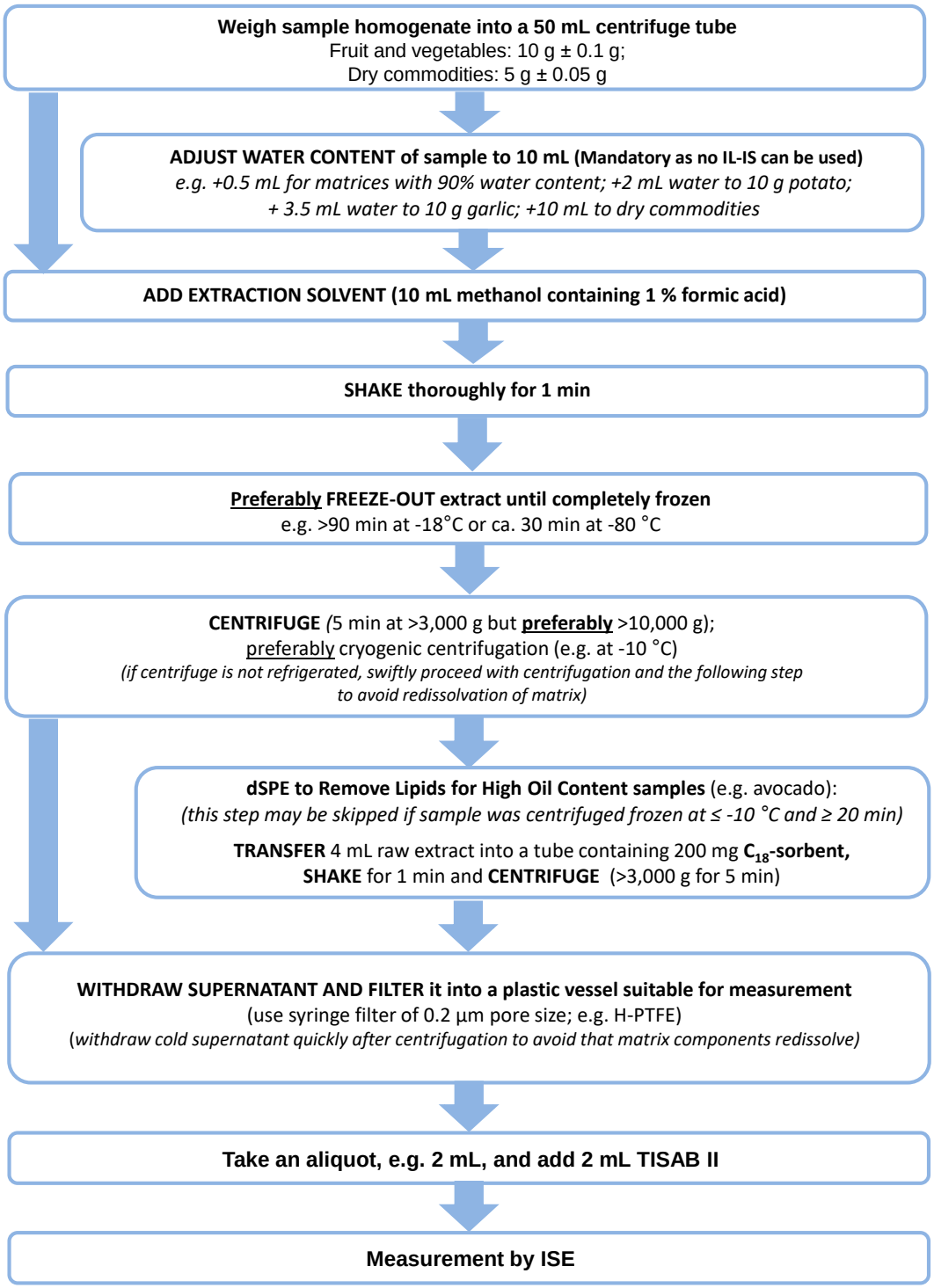

Figure 1: *Method at a glance – QuPPe extraction followed by direct measurement with ISE [1].*

Weigh sample homogenate into the outer sector of the microdiffusion cell
Dry commodities: 0.5 g ± 0.005 g

↓

Fill the inner sector of the microdiffusion cell with 1 mL 0.1 M NaOH (Fluoride trapping solution)

↓

Add 4 mL HMDS-saturated 5 M $HClO_4$ solution to the sample homogenate in the outer sector of the microdiffusion cell (Diffusion solution)

↓

Close the cell gas tight by using Vaseline on the lid

↓

Fluoride diffusion:
a) 48 h at room temperature
OR b) 5 h at 50 °C

↓

Add 1 mL TISAB II solution to the trap solution in the inner sector

↓

Transfer the solution into a vessel suitable for ISE measurement, e.g. tapered 10 mL or 50 mL-centrifugal tube

↓

Measurement by ISE

Figure 2: *Method at a glance – Microdiffusion procedure for dry commodities and measurement by ISE.*

Figure 3: *Pictures of the microdiffusion cells showing the procedure for microdiffusion for dry commodities.*

ISE-Measurement and Calibration Approach

Ion-selective electrodes are electrochemical sensors that convert the concentration (more precisely the activity) of a specific ion dissolved in a solution (in this case fluoride) into an electrical potential that is displayed by the ion-meter. The voltage of such electrode is proportional to the logarithm of the ionic activity (Nernst equation). This is why calibration curves are plotted in a semi-logarithmic scale with the concentration being in logarithmic scale, see Figure 4. The higher the concentration of the ion, the lower the potential measured by the ISE, therefore the calibration curves have a negative slope. At very low concentrations (< 0.1 µg/mL[4]), measurement becomes increasingly difficult because of the ubiquitous presence of fluoride. Absolutely 'residue' free matrix and/or reagents are usually not available. Choosing appropriate calibration ranges is therefore paramount for accurate quantification.

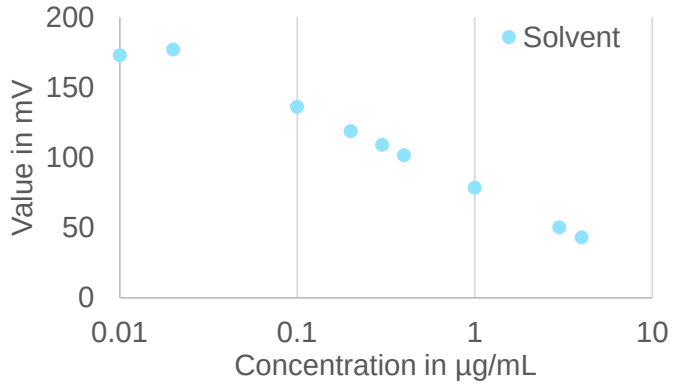

Figure 4: *Exemplary calibration curve in solvent (methanol containing 1% formic acid/H2O 1/1).*

As the electrode is also sensitive to OH^- ions and as ISE measurement is also affected by the overall ion strength, it is of foremost importance to mix all solutions to be measured with Total Ion Strength Buffer (TISAB) solution, in order to roughly equalize the ion strength and the pH of the measurement solution at an acidic pH of ca. 4.5-5.
In the experiments presented here TISAB II solution was used, which contains cyclohexylenedinitrilotetraacetate (CDTA), glacial acetic acid, NaCl and Na_3-Citrate. TISAB II was added to the measurement solutions at a 1 to 1 ratio.

[4] This corresponds to 0.2 mg/kg. In the case of QuPPe extracts (10 g analytical portions) the final conc. of the matrix is 0.5g/mL. In the case of dry products (e.g. spices) with an analytical portion size of 0.5 g and a diffusate volume of 1 mL, the final conc. of the matrix is also 0.5g/mL.

Experiments conducted and observations:

The ISE-measurement setup available for the measurements presented here was not automated. The ISE potentials displayed by the ion meter were therefore manually tracked into an excel sheet for calculations and the calibration diagrams were plotted there.
The ISE measurements were conducted either in 10 mL or 50 mL PP centrifuge tubes. Calibration solution were prepared using reagent blanks. Prior to ISE measurements, the solutions to be measured (i.e. calibration solutions, QuPPe extract aliquots, fluoride diffusates) were mixed with TISAB II solution at a 1+1 ratio in order to adjust and largely equalize the pH and ionic strength with those of the respective calibration solutions. In the present study, only TISAB II was used.

Matrix effects with ISE

Several calibration curves were prepared in matrix extracts following the QuPPe extraction procedure (cucumber and red currants) and solvent (methanol containing 1% formic acid/H_2O 1/1) and recorded. As shown in Figure 5, the presence of matrix did not significantly alter the slopes of the calibration curves. The lower graph shows a quasi-complete overlap of the calibration curves. The cucumber and the red currants extracts originally contained both ca. 0.03 µg/mL fluoride respectively. Therefore, the curves in Figure 5 start at 0.1 µg/mL (the concentration refers to the solutions prior to mixing with TISAB II). At lower levels, measurement becomes more error-probe.

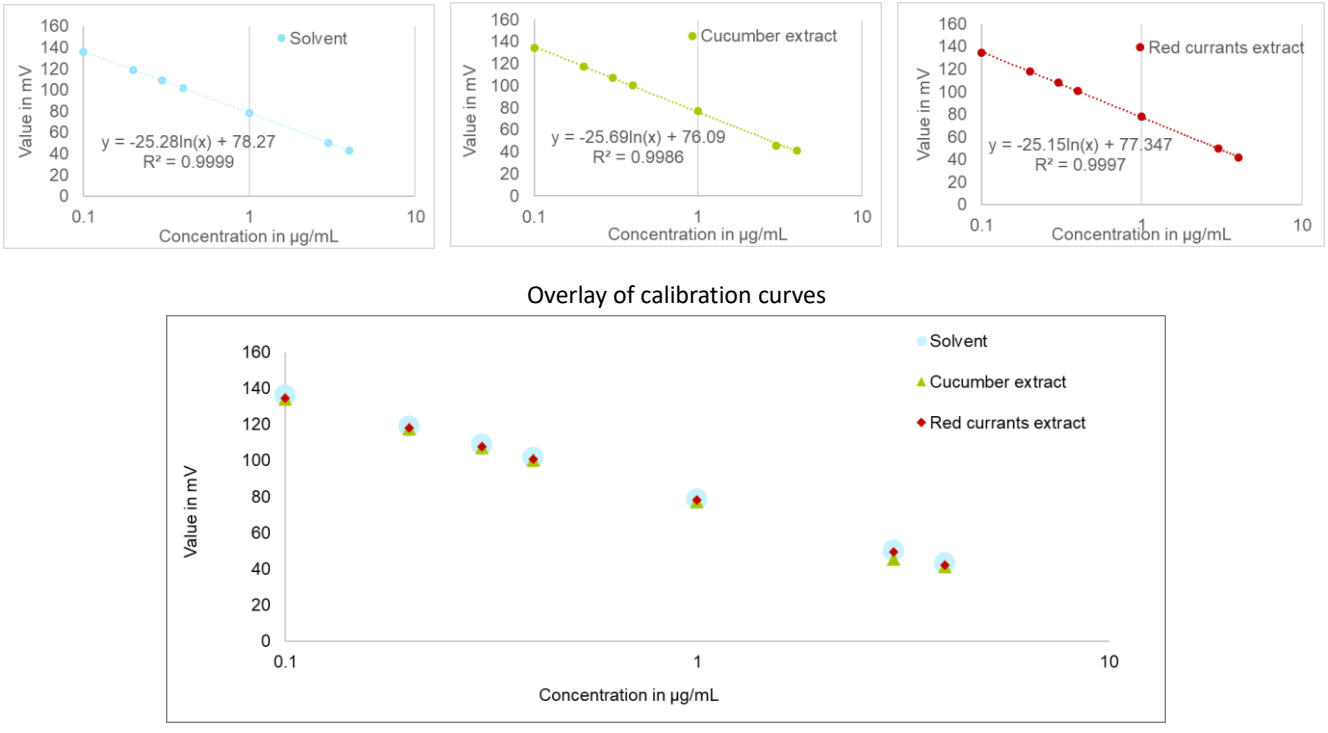

Figure 5: *Comparison of calibration curves showing no/negligible matrix effects.*

Microdiffusion conditions

In pre-experiments (results not shown here), a HMDS-saturated 1.5 M sulphuric acid (H_2SO_4) solution was tested. But it was observed that microdiffusion using this approach was less efficient than with perchloric acid, which provided double the yield. For the following experiments, it was decided to use HMDS-saturated perchloric acid.
In a further pre-experiment (results not shown here), the heated microdiffusion was tested at 60°C, but an extensive evaporation of the trap solution was observed. It was therefore decided to reduce the temperature to max. 50°C.
To further optimize the microdiffusion conditions, infant formula and wheat flour were analyzed under different conditions.

a) ISE measurement following microdiffusion at RT for 24 h;
b) ISE measurement following microdiffusion at RT for 48 h;
c) ISE measurement following microdiffusion at 50°C for 5 h.

The fluoride yields obtained are shown in Figure 6. As the overall kinetics slow-down with decreasing temperature, microdiffusion at room temperature was expectedly much slower, and it took 48 h to reach similar yields as in 5h at 50 °C.

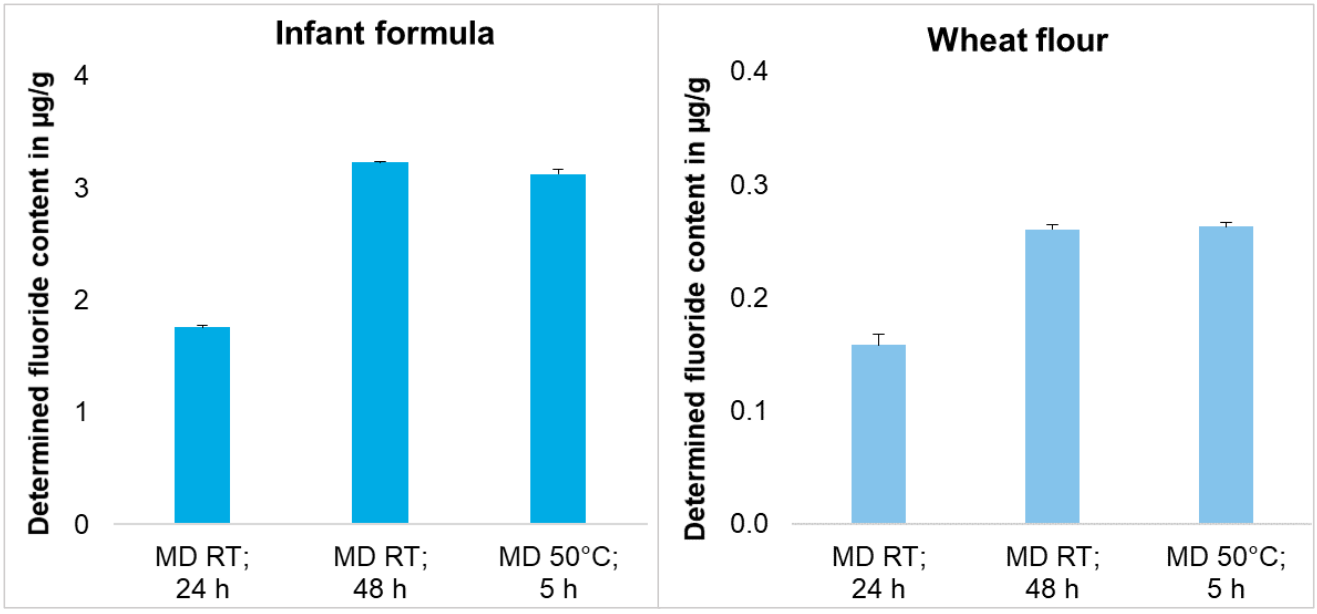

Figure 6: *Comparison of measurement and microdiffusion conditions for infant formula powder and wheat flour. MD = microdiffusion; MeOH-FA = methanol containing 1% formic acid.*

To determine the microdiffusion yield, oats were spiked with fluoride at 2 mg/kg (n = 5). The mean recovery based on an external calibration in purified H_2O/methanol containing 1% formic acid 1/1, was calculated at 96 % with a narrow variation (RSD: 2.1 %).

Microdiffusion of fresh commodities after drying

In an additional experiment, fresh commodities with high water content (e.g. cucumber) were first dried and then subjected to microdiffusion and ISE measurement. For this. 4 g of sample homogenate were weighed onto the outer sector of the microdiffusion cell and dried using a moisture analyzer (DBS-60-3 by Kern, Balingen/Germany). A drying cycle lasted approximately 20-25 min. The cells with the dried material were then directly employed for microdiffusion as described above. Mean recovery rates in cucumber spiked at 2 mg/kg prior to drying were determined to 109 % on average (108 and 110%).
This approach may be useful in cases where direct measurements are not possible due to sensitivity or interference problems.

Method Validation

The direct ISE-measurement approach (in QuPPe extracts) was validated in cucumber spiked with fluoride at 1 mg/kg and 5 mg/kg (n = 5). The microdiffusion approach (at 50°C) was validated in lentils spiked with fluoride at 1 mg/kg and 5 mg/kg (n = 5).

With background levels of fluoride being between 0.1 and 0.2 mg/kg in both matrices, validation at lower spiking levels than 1 mg/kg would not fulfill the AQC performance criteria.

Table 1: *Recovery data for fluoride from cucumber and lentils.*

Matrix Type	Matrix	Sample Weight + water addition	Spiking Level in mg/kg	Direct measurement			Following microdiffusion		
				n	Mean Rec.%	RSD %	n	Mean Rec.%	RSD %
High water	**Cucumber**	10 g	1	5	97	1.3			
			5	5	119	2.2			
Dry	**Lentils**	0.5 g (for microdiffusion)	1				5	113	0.25
			5				5	117	0.31

In a second experiment, the repeatability was checked by repeated measurements (n = 10) of cucumber extracts. For this, 10 analytical portions á 10 g of cucumber homogenate were spiked at 1 mg/kg prior to extraction. The RSD in this experiment was at 1.4%.

Fluoride contents in food

Fluoride contents in some samples were determined and are listed in Table 2.

Table 2: *Overview on fluoride contents in food determined by ISE in QuPPe extracts (high water content commodities) or in microdiffusates (dry and dried commodities).*

Matrix	Fresh or dry?	ISE measurement following QuPPe extraction (direct) or following microdiffusion (MD)	Fluoride in mg/kg[1)]	MRL
Cucumber	Fresh	Direct	~0.05	0.2 mg/kg
Red Currants			~0.06	
Lettuce 1			~0.06	
Lettuce 2			~0.05	
Lettuce 3			~0.06	
Spinach			~0.07	
Mushroom 1			~0.10	
Mushroom 2			~0.12	
Dried Plum (prunes)	Dry	MD	0.28	0.2 mg/kg for the fresh product (~1 mg/kg for the dried product)
Infant food formula 1			3.12	Roughly correlating with fluoride content labelled on the products
Infant food formula 2			0.39	
Infant food formula 3			0.88	
Caraway			0.58	5 mg/kg
Paprika powder			1.66	0.2 mg/kg for the fresh product (~1.4 mg/kg for the dried product). This would be an MRL exceedance.
Pepper spice			0.75	5 mg/kg
Wheat flour 1			0.26	2 mg/kg
Wheat flour 2			0.34	
Macadamia nuts			0.86	30 mg/kg

[1)] For fresh products values below 0.2 mg/kg are to be considered as semi-quantitative as the concentration in the extract is only 0.1 µg/mL

Discussion and conclusions:

The content of fluoride anions in food was measured by a fluoride ion-selective electrode (ISE) following three different sample preparation techniques:

1) In dry commodities, fluoride was isolated from the samples via microdiffusion prior and measured in the diffusate via ISE.
2) Commodities with high water content were either…
   a) directly extracted by the QuPPe method followed by ISE measurement, or
   b) first dried and then subjected to microdiffusion and ISE measurement.

Heating at 50 °C helped to reduce microdiffusion duration from 48 h to 5 h achieving nearly quantitative recoveries. Irrespective of whether microdiffusion was conducted on diffusates or QuPPe extracts, matrix effects were negligible, which opens the way for external calibration on solvents.
The procedures using direct measurement and microdiffusion were successfully validated at 1 mg/kg and 5 mg/kg in cucumber and lentils respectively. Natural background levels in food, limited the ability of validating at lower levels as the AQC criteria (SANTE/11312/2021) require that the background concentration does not exceed 30% of the spiked level. Validations at the low MRLs set for fruits and vegetables (0.2 mg/kg) are thus often difficult.

Literature:

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[8] Quick Method for the Analysis of Highly Polar Pesticides in Food Involving Extraction with Acidified Methanol and LC- or IC-MS/MS Measurement - I. Food of Plant Origin (QuPPe-PO-Method) – Version 12 (published on EURL-SRM website on July 23, 2021);
M. Anastassades; A.-K. Wachtler; D. I. Kolberg; E. Eichhorn; H. Marks; A. Benkenstein; S. Zechmann; D. Mack; C. Wildgrube; A. Barth; I. Sigalov; S. Görlich; D. Dörk and G. Cerchia.
URL: https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN

[9] Mettler Toledo: perfectION™ Guidebook for fluoride combined electrode

History

Action	When	Document Version
Experiments	April - September 2022	
Document placed online	March 2023	V1