Analytical measurement uncertainty of food carotenoid determination

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Introduction

Carotenoid determination in food is a complex analytical process involving several mass transfer steps (extraction, evaporation, saponification, etc.). For consistent interpretation of an analytical method result it is necessary to evaluate the confidence that can be placed in it; this can be provided by the quantification of its accuracy (trueness and precision) in the form of a measurement uncertainty estimate. The Guide to the expression of Uncertainty in Measurement issued by the International Organization for Standardization[1] establishes rules for evaluating and expressing uncertainty. Although it is a very powerful tool[2], it is even more complex when analytical methods include mass transfer steps that lack descriptive models for the behaviour of the analyte in the analytical system. The guide was interpreted for analytical chemistry by EURACHEM, whose second edition[3] already includes the possibility of using interlaboratory information and also the use of information obtained from analytical methods in-house validation.

Aim

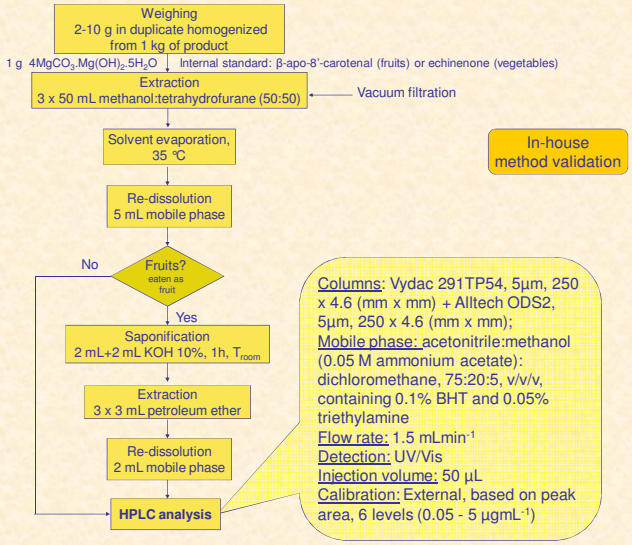
To estimate the analytical measurement uncertainty in the determination of the carotenoids, α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, zeaxanthin, in fruits and vegetables, by a HPLC method.
To contribute to the definition of the number of significant figures in the expression of carotenoid results obtained by HPLC methods, namely for Food Composition Databases.

Material and methods

Food Matrices

Pear	Apple	Cherry	Peach	Orange	
Kale	Cabbage	Turnip leafs	Leaf beet	Purslane	Tomato

Analytical method scheme



Measurement uncertainty evaluation

The measurement uncertainty was estimated based on in-house method development and validation studies, including precision and bias studies, using a methodology previously described[4]. Uncertainty contributions not adequately covered by these studies were quantified through the evaluation of the individual uncertainty components. All terms were joined according to the combination laws, taking into account the sensitivity coefficients and the variances for each influence. Thereafter, the expanded uncertainty was calculated for a confidence level of 0.05, using the coverage factor 2[3].

The measurement uncertainty was also evaluated based on laboratory participations in proficiency tests (BIPEA (France), NFA (Sweden)). International proficiency tests on carotenoids are scarce and only on β-carotene.

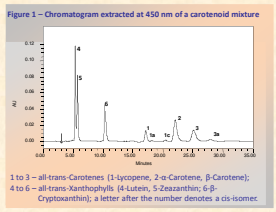
Bibliography

[1] ISO. Guide to the expression of uncertainty in measurement (GUM). International Organization for Standardization (1995), Geneva, Switzerland.
[2] Analytical Methods Committee. Uncertainty of measurement: implications of its use in analytical science. *The Analyst*, 120 (1995), 2303-2308.
[3] EURACHEM/CITAC Guide CG4: Quantifying Uncertainty in Analytical Measurement (2000), 2nd edition ; www.eurachem.ul.pt.
[4] Dias, M. Graça, Camões, M. Filomena, Oliveira, Luísa (2008). Uncertainty estimation and in-house method validation of HPLC analysis of carotenoids for food composition data production. *Food Chemistry* 109, 815-824.

Results

In-house method validation

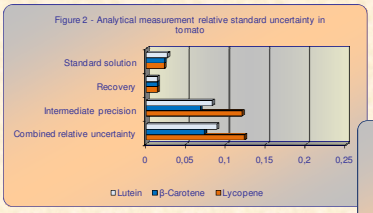
The analytical method was unbiased, based both on reference material analysis and on recovery studies, (*z*-score for the analysis of the standard reference material between −1.5 and +0.1; mean recovery 93.7%, not statistically different from 100%, confidence level 0.05).

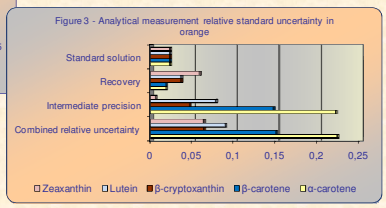


Analytical measurement uncertainty

•Based on in-house development and validation studies, and quantification of individual components

Analytical measurement relative standard uncertainties (components and combined) are presented in figures 2 and 3, for the matrices tomato and orange, respectively, illustrating examples of carotenoid determination without and with the saponification step in the analytical process.





Analytical measurement combined relative standard uncertainty

- 0.25-0.50 – Analytes in the quantification limit vicinity
- 0.15-0.22 – Xanthophylls in ester form or analytes with interferences in the vicinities
- 0.028-0.13 – Carotenes

•Based on proficiency tests

From the five rounds (BIPEA, NFA) on β-carotene (0.845-3.50 mg/100 g), only three gave reference values.
$|z\text{-score}|_{laboratory} \leq 2$ (acceptable).

Analytical measurement relative standard uncertainty

0.17 – Based on the relative differences between the laboratory and the reference values laboratory results.

0.19 – Based on all participant results.

Matrices
Baby food
Fruit juice
Fruit purée

Conclusions

- Certified **reference material** analysis and laboratory participation in **proficiency tests** showed **good laboratory performance**.
- Different approaches to quantify the analytical measurement uncertainty originated different values. The approach based on the **results of the method validation and individual components encompasses all uncertainty sources** and is more discriminative relative to matrix.
- The **precision** is the **largest contribution** to the **measurement uncertainty**. To reduce this term further experiments would be needed to show where improvements could be made.
- The **measurement uncertainty estimation at supralaboratory level** (proficiency tests results) **is easier to perform but could not identify all sources of uncertainty**. When based on all participants results might not reflect the laboratory performance.
- The great majority of food items showed results with **analytical measurement relative uncertainties between 0.050 and 0.15**, but higher relative uncertainties may occur, namely near quantification limits and when the analytical process involves a saponification step.
- Taking into account the results of the **measurement uncertainty evaluation**, the **maximum number of significant figures**, for the method and matrices studied, would be **2**, which should be considered in Food Composition Databases and subsequent studies with these data.