

Towards safer food supplements: importance and challenges of multi-analyte measurement

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1. Background

Plants such as *Aloe*, *Rheum*, *Rhamnus* and *Cassia senna* are often used in food supplements to improve bowel function. The downside of these preparations is the presence of hydroxyanthracene derivatives (HADs), naturally occurring plant substances. Although HADs are considered important biologically active components with their recognized laxative effect, recently, the European Commission prohibited a number of HADs due to toxicological concerns [1].

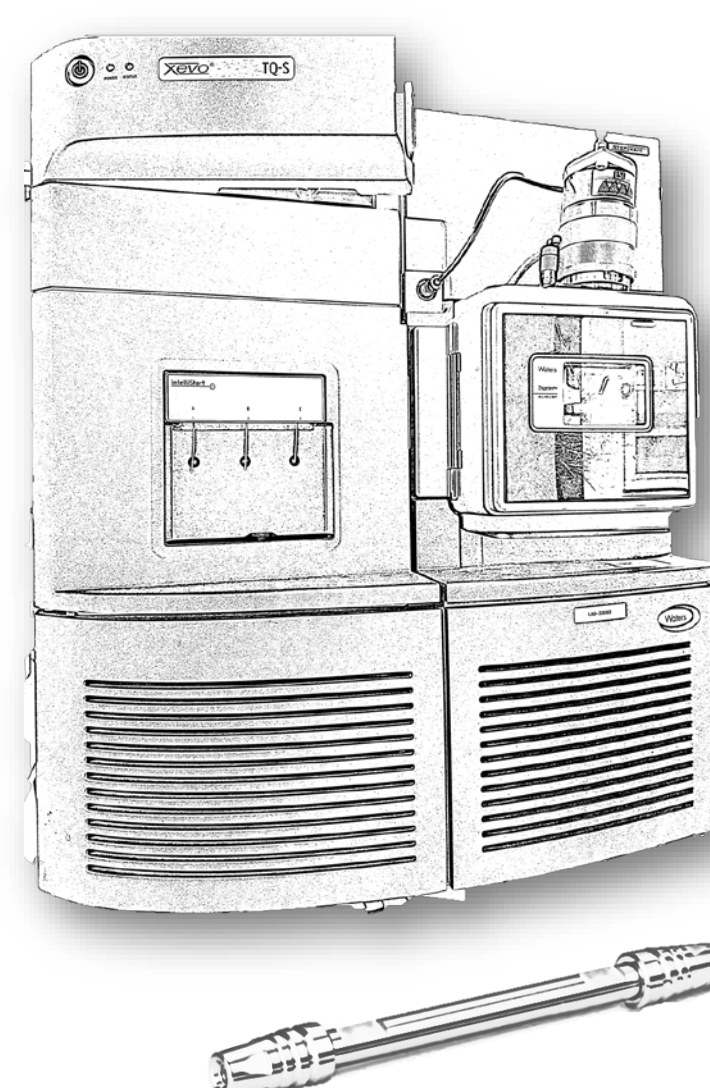
2. Research aim

In view of the recent update of the regulation, validated analytical methods for the determination of multiple HADs in food are in high demand for regulatory analysis and dietary intake studies. The aim of this study was the development and validation of a sensitive and quantitative method for the simultaneous analysis of 16 HADs in food supplements.

3. Methods

3.1. Analytical technique

Ultra high performance liquid chromatography – tandem mass spectrometry (UHPLC-MS)



ACQUITY UPLC H-class system (Waters) coupled to XEVO TQ-S triple quadrupole MS (Waters)

ACQUITY UPLC BEH C18 column (1.7µm, 2.1×100mm), 60°C

Mobile phase A: H₂O + 0.1% HCOOH

Mobile phase B: CH₃OH + 0.1% HCOOH

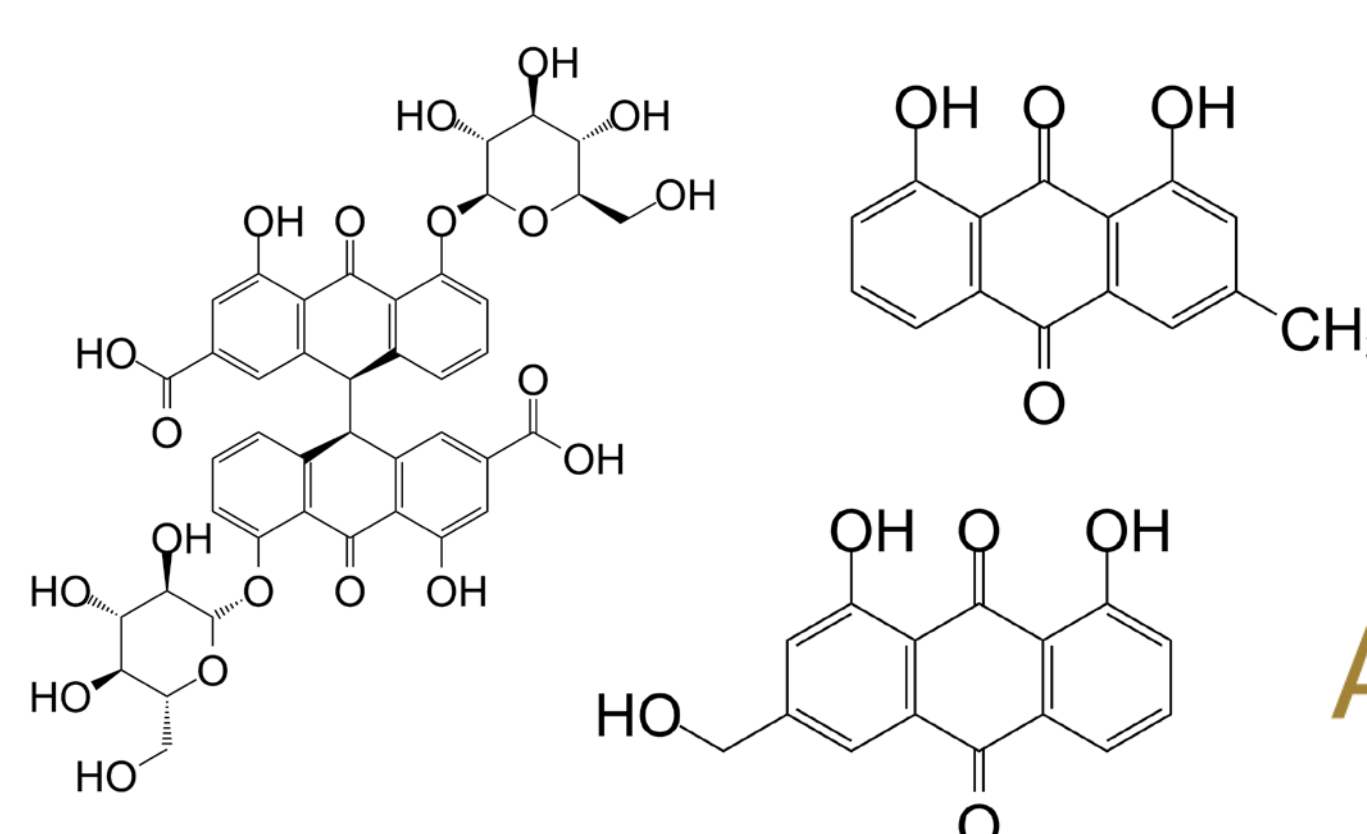
Flow 0.4 mL/min

Ionisation mode MS: ESI pos, neg

Acquisition mode MS: Multiple Reaction Monitoring

More details on the analytical conditions can be found in [2].

3.2. Target compounds

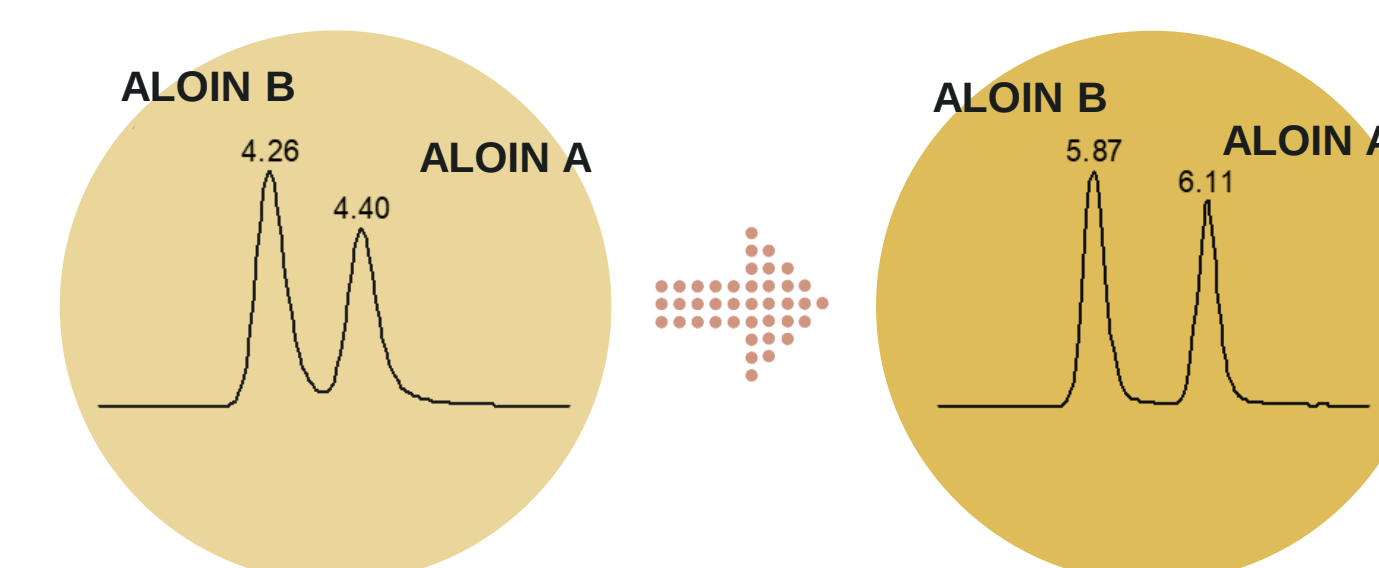
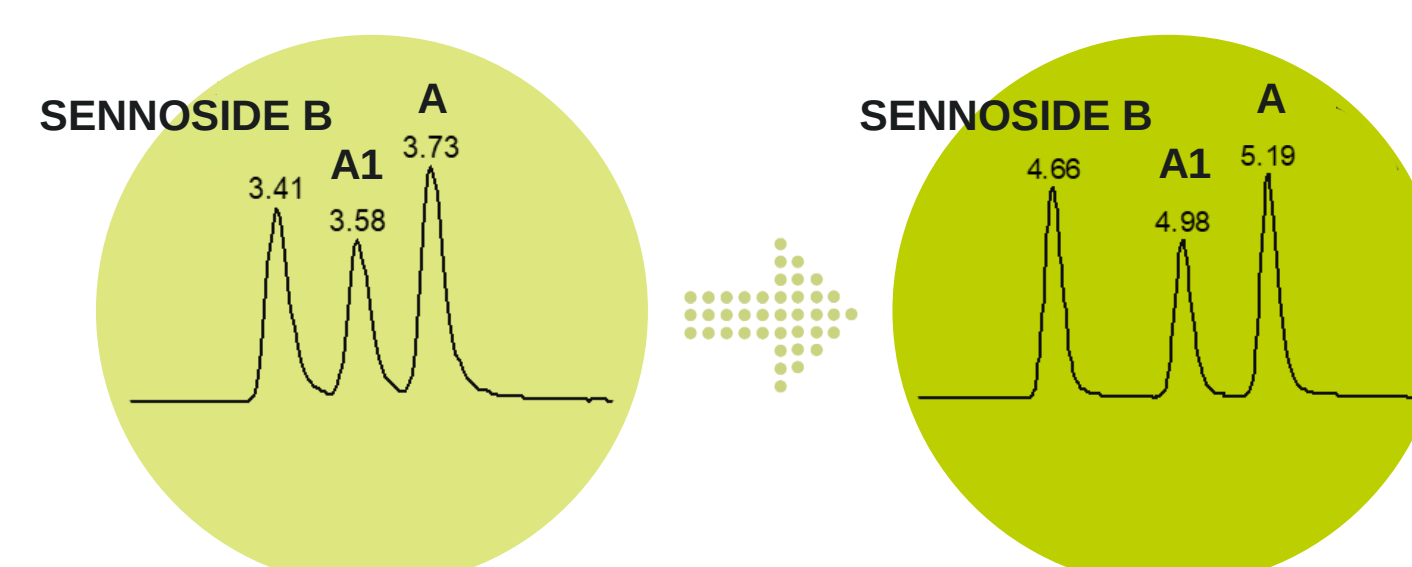


4. Results

4.1. Measurement challenges

Isomers

Aloins and some Sennosides are stereoisomers. They produced the same precursor and product ions in the mass spectrometer making them indistinguishable.



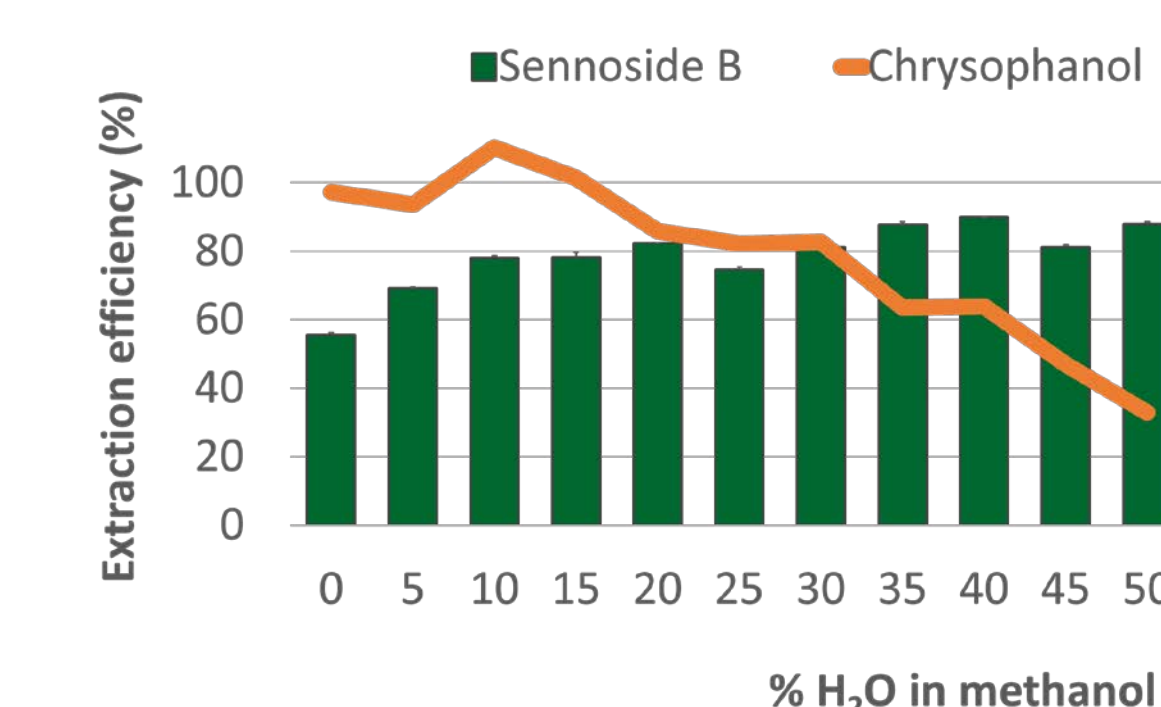
Here, the separating power of the liquid chromatography was relied on. And in view of individual quantification and analysis of highly contaminated samples, a better baseline separation was required.

This was successfully achieved by applying a shallower gradient slope, i.e. increasing phase B from 5% to 85% in 9 min instead of 5 min.

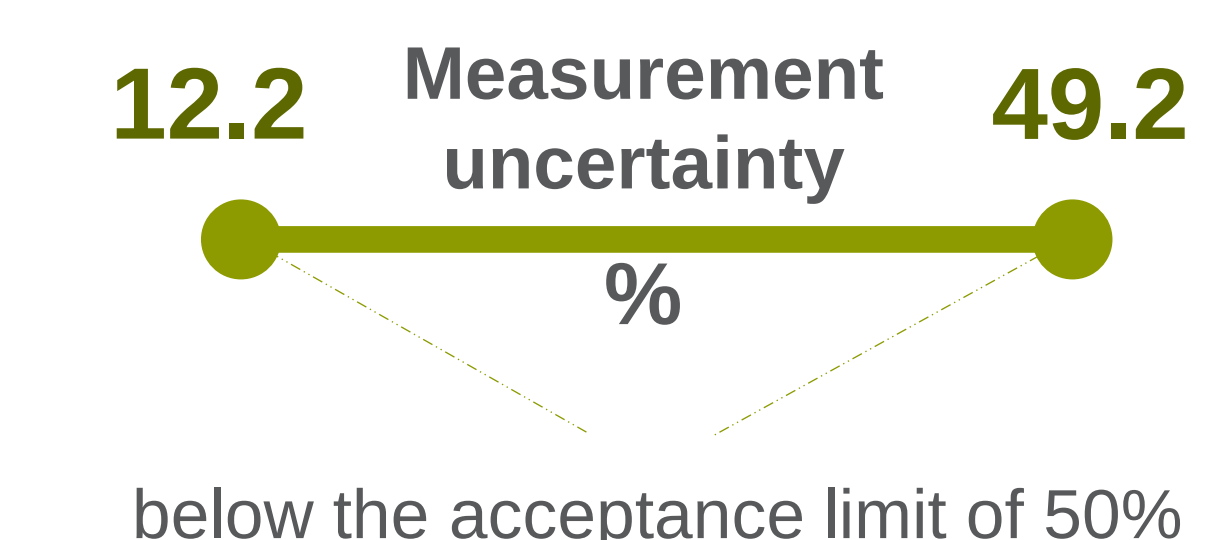
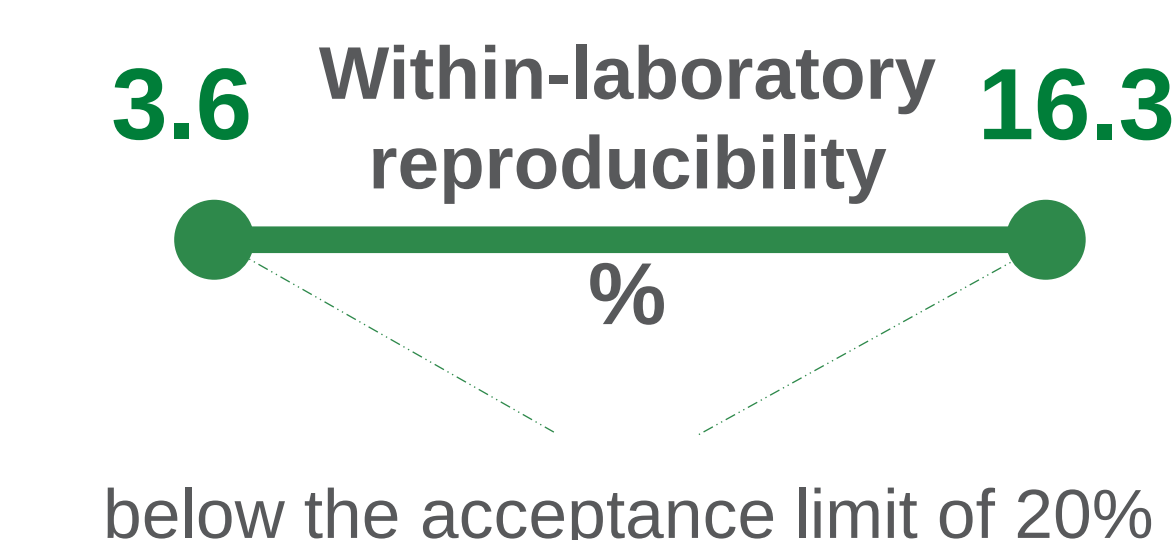
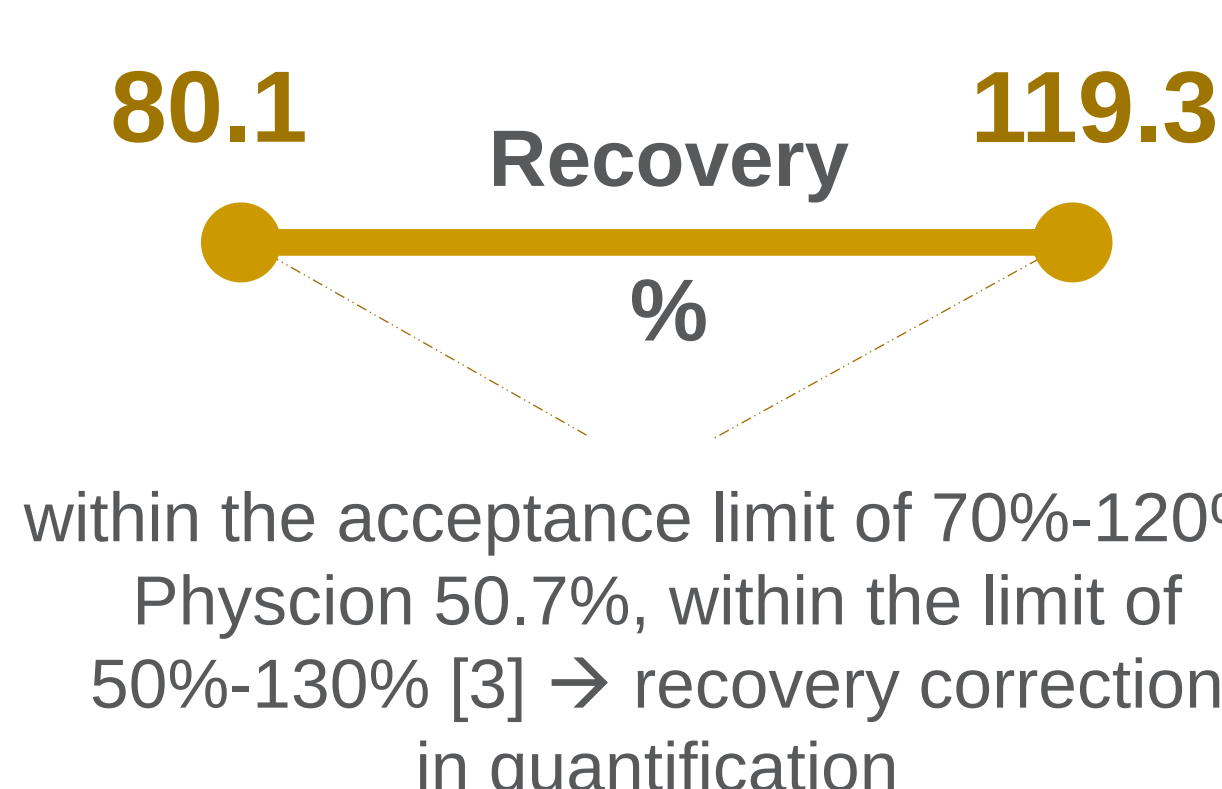
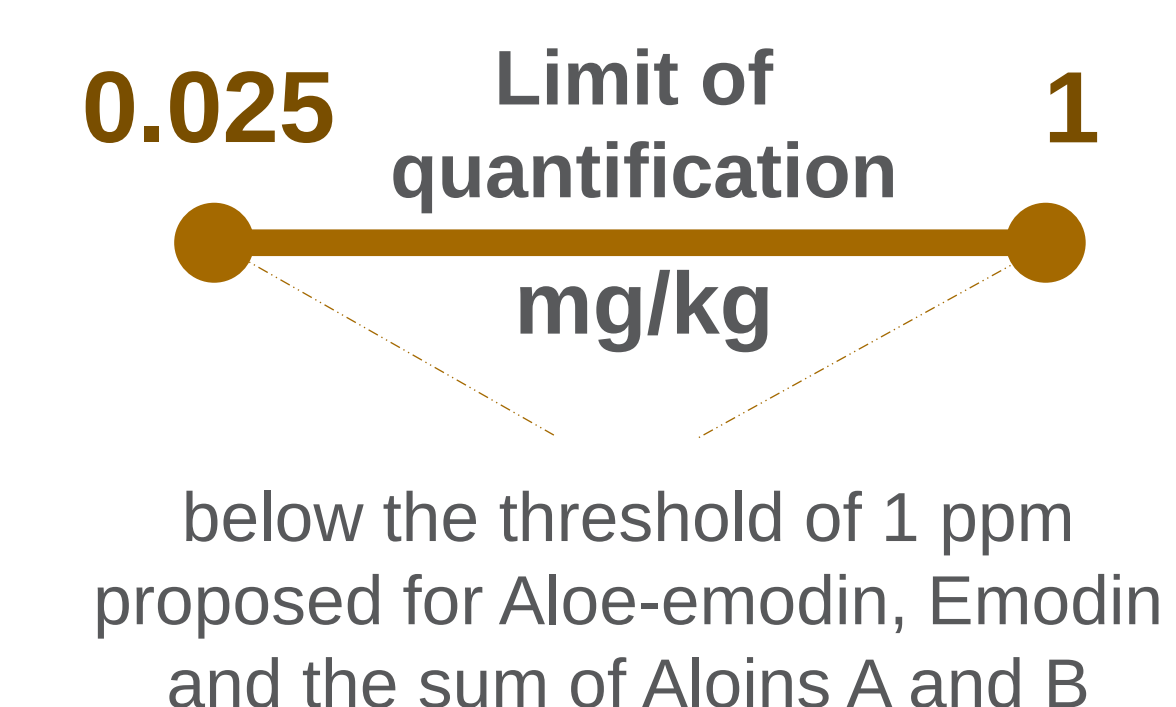
Different physicochemical properties

The diverse nature of HADs became pronounced in the extraction optimisation. With the increasing polarity of the extraction solvent, the extraction efficiency of a less polar Chrysophanol was considerably decreasing, while the results for more polar Sennosides improved.

A compromise was found at 20% of water in the extraction solvent.



4.2. Method validation



4.3. Method application

Analysis of 24 food supplements and dry herbal infusions purchased on the Belgian market revealed the following:

17 out of 24 samples: HADs >LOQ

Highest level: **19.81 g/kg** Glucofrangulin A

Lowest level: **0.13 mg/kg** Rhein

Up to **13** HADs present simultaneously in the same product

Highest sum of HADs: **41.05 g/kg** in a product with senna pods and rhubarb extract

58% of products significantly exceeded the proposed threshold for the content of Aloe-emodin, Emodin, Aloins A and B.

A meticulous method optimisation helped to overcome the challenges related to the specific character of HADs. The results of the food supplement analysis stressed the importance of simultaneous quantification of multiple HADs. This method is critical for collecting more occurrence data and facilitating accurate dietary exposure assessment.