EUCHINASAFE 中欧食品安全

Delivering an Effective, Resilient and Sustainable
EU-China Food Safety Partnership

Measurement Using Internal Standardisation

Frankie Smith
Fera Science Ltd

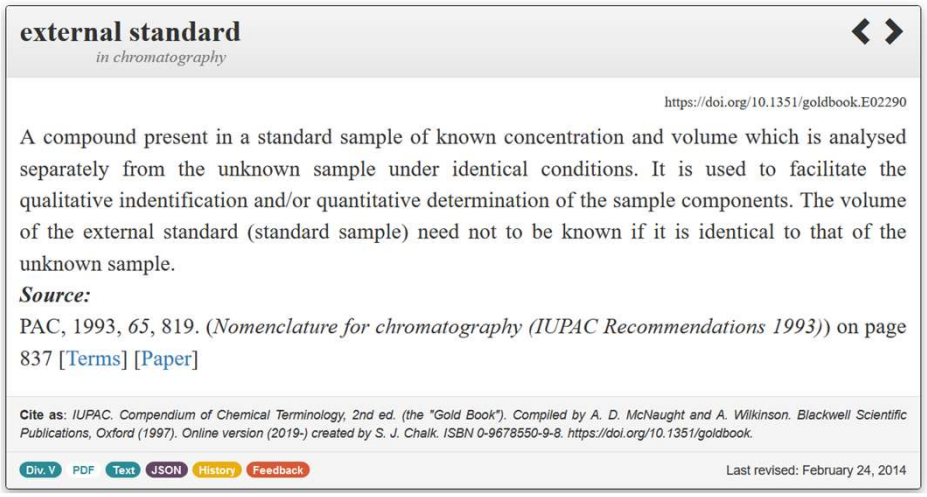
fera
Original thinking... applied

Overview

- External standardisation
  - What is it?
  - How is it applied?
  - What are the limitations?
- Internal standardisation
  - What is it?
  - How is it applied?
  - Calculations
  - Advantages/benefits

Just to cover what we'll be looking at in this session, first we'll look at why we use Internal Standardisation, and to do so it's worth looking at why we don't use the simpler process of External Standardisation.

External Standardisation – what is it?



external standard
in chromatography

https://doi.org/10.1351/goldbook.E02290

A compound present in a standard sample of known concentration and volume which is analysed separately from the unknown sample under identical conditions. It is used to facilitate the qualitative indentification and/or quantitative determination of the sample components. The volume of the external standard (standard sample) need not to be known if it is identical to that of the unknown sample.

Source:

PAC, 1993, *65*, 819. (*Nomenclature for chromatography (IUPAC Recommendations 1993)*) on page 837 [Terms] [Paper]

Cite as: *IUPAC. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997). Online version (2019-) created by S. J. Chalk. ISBN 0-9678550-9-8. https://doi.org/10.1351/goldbook.*

Div. V PDF Text JSON History Feedback Last revised: February 24, 2014

External standard is separate from the unknown analyte in the sample, so doesn't undergo the same effects as the unknown analyte in the sample – apologies for the typo (mis-spelling of identification on line 3 of description; that is present from the IUPAC website).

External Standardisation - process

- Prepare calibrant solutions of analyte at known concentrations
- Inject known volume of calibrant solutions into instrument/detector
- Measure response of analyte
- Plot calibration curve of analyte concentration vs response
- Inject same volume of unknown sample solution/extract
- Measure response of analyte
- Plot response on calibration curve to determine analyte concentration in unknown sample solution

Simple… but External Standardisation has its failings

Maybe split this into 2 slides – 1st the calibration and include a curve, 2nd the unknown measurement and application on curve

Also, calibrant solutions can be made in matrix matched extracts to reduce difference in matrix effects within the measurement.

External Standardisation - issues

What if…:

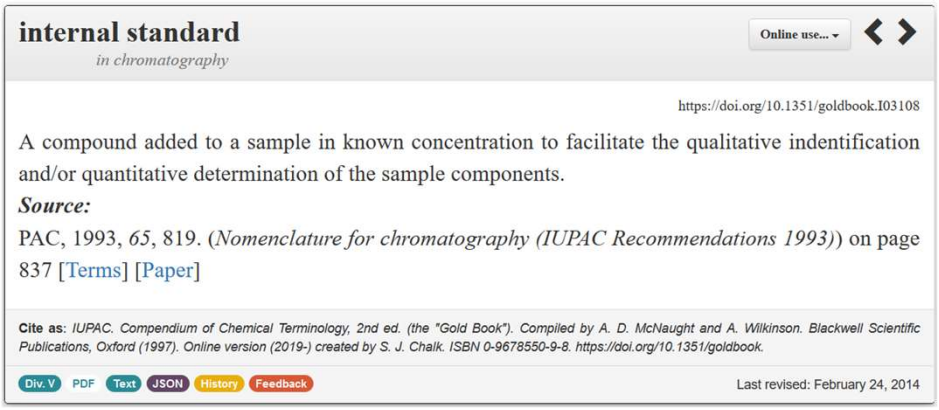
- the injector operates inconsistently?
- the analyte solutions have been diluted or concentrated since being prepared?
- the unknown sample has to undergo a series of extraction and purification procedures in order to attain the cleanliness to be adequately chromatographed, and concentration steps to yield sufficient concentration to allow detection and to be accurately measurable on the instrument?

So we need an alternative approach…

Analysis of POPs in Foods, Jan 2022

1. Modern injector systems are usually very reliable and consistent, but historically this was a major issue
2. You would hope the analyte solutions are correct – this can be avoided by preparing solutions at time of use
3. Unavoidable problems – complex extraction processes will deliver inconsistent recoveries even if carried out with great care. You could run the calibrant solutions through the same processes, but with inconsistent recoveries, the resulting calibration curve would be compromised and accuracy would be lost
4. Use of external standards is simple and relatively cheap, but only effective if the preparatory procedures prior to measurement on the instrument are simple and highly reproducible

Internal Standardisation – what is it?



internal standard
in chromatography

Online use...

https://doi.org/10.1351/goldbook.I03108

A compound added to a sample in known concentration to facilitate the qualitative indentification and/or quantitative determination of the sample components.

Source:

PAC, 1993, *65*, 819. (*Nomenclature for chromatography (IUPAC Recommendations 1993)*) on page 837 [Terms] [Paper]

Cite as: *IUPAC. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997). Online version (2019-) created by S. J. Chalk. ISBN 0-9678550-9-8. https://doi.org/10.1351/goldbook.*

Div. V PDF Text JSON History Feedback

Last revised: February 24, 2014

Internal Standardisation – process (1)

Selection of internal standard

- Internal standard should behave similarly to analyte of interest
- Should not be expected to be naturally present in samples
- Ideal – labelled isotope e.g. ^{13}C – elutes at same retention time but is separable by MS.

Addition of internal standard

- Ideally, before any sample handling - e.g. to an aliquot of provided sample (liquid or powder)
- Practical – after sample preparation (e.g. removal of skin/bones for fish) and sub-sampling (as whole sample would require a larger amount of internal standard to be added)

Analysis of POPs in Foods, York UK, Jan 2022

1. Selection – labelled isotope analogues are almost identical to analyte of interest. 13C preferable as natural abundance is very low (~1.1%, chances of all 12 carbon atoms in skeleton of dioxin, furan or PCB naturally being 13C is around 1 in 1.67 quadrillion). 2H can be used but can be less stable (and also wouldn't have much shift in mass if used for hexa- or hepta- chlorinated dioxins and can't be used for OCDD/F as there are no H in OCDD/F. Cl isotopes could be used, but as 37C is quite abundant, it wouldn't make a good choice.

2. Using labelled isotopes enables easily identifiable retention times as 13C m/z traces are expected to be much clearer of co-extractives than the native traces

3. Addition – ideally as early as possible in the analytical procedure, but practically after sample preparation (removal of bones/skin etc.), after homogenisation and sub-sampling.

Internal Standardisation – process (2)

- Sample must be homogenised
- Add known amount of internal standard to an accurately measured aliquot of the sample
- Homogenise the internal standard into the aliquot
- Extract from the aliquot, purify and concentrate the extract
- Prepare calibrant solutions containing both analyte and internal standard at known concentrations
- Inject calibrant solutions into instrument/detector and calculate the relative response factor (RRF) of analyte/internal standard for the calibrant solutions
- Inject sample extract into instrument/detector and measure the responses for the analyte and internal standard for the extract
- Calculate the amount of analyte in the original aliquot of sample using the RRF from the calibrant and the responses of the analyte and internal standard from the extract solution and from the known amount of internal standard added to the aliquot

Analysis of POPs in Foods, Jan 2022

1. Important that the sample is homogenised – doesn't matter how accurate internal standard addition is if aliquot is not truly representative of whole sample

6-8. Appreciate this isn't as straightforward as for external standard calibrations, so go into more detail on following slides.

Internal Standardisation – process (3)

Relative Response Factor (RRF) standard

Composition of RRF:

- Primary (native, certified) standards for all required analytes
- Internal standards (preferably ^{13}C labelled, certified)
- Sensitivity standards (preferably ^{13}C labelled)

1. Haven't mentioned sensitivity standards yet – sometimes referred to as syringe standards. These are also be added to the final extract prior to introduction to instrument and allow the recovery for each Internal standard to be determined (more of that later).

Internal Standardisation – calculations

For the calibrant:

$$RRF_{cal} = \frac{\text{Response native/Amount native}}{\text{Response Int. Std./Amount Int. Std.}}$$

For the extract:

$$Amount_{unknown} = \frac{\text{Response native x Amount Int. Std.}}{RRF_{cal} \text{ x Response Int. Std.}}$$

For the sample:

$$\text{Concentration} = \frac{\text{Amount of analyte calculated in extract}}{\text{Weight of extracted aliquot}}$$

Analysis of POPs in Foods, York UK, Jan 2022

1. Important to note that the Amounts for native and internal standards refer to amounts, not concentrations.
2. To quantify how much of the analyte is in the extract, need the Relative Response Factor from the Calibrant
3. If a sensitivity standard has been added at the end of extraction, the same equations can be used to calculate the recovery, but the where native is used above, it should be the Internal Std, and where the internal std is used above, it should be the sensitivity std.

Internal Standardisation – advantages

Multi-point calibration

- Most accurate but increases run time

Single point calibration

- A linear range must be established/validated prior to use and at regular intervals
- Reduces instrument run times
- Used when expected range is narrow

Recovery correction is not required

Accurate volumes of final extracts not essential

Processing losses can be quantified (QA)

Analysis of POPs in Foods, York UK, Jan 2022

1. Multi-point calibration is best – relative responses may not be linear across a broad range
2. Single point calibration saves time – one injection for a dioxin run can take around an hour on a GCMS system including oven cooling time etc. If a batch is analysed in two brackets, single point calibration could save about 12 hours compared to a 5 point calibration. Linearity of a range must be established.
3. No recovery correction required – the calculations inherently provide the amount of unknown in the original aliquot due to the use of relative ratios and relative responses
4. Accurate volumes of final extracts are not essential – the method measures the amount of analyte in the extract, not the concentration.
5. Use of a sensitivity standard can allow processing losses to be quantified, in other words, the extraction recovery can be calculated

www.euchinasafe.eu

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 727864 and from the Chinese Ministry of Science and Technology (MOST).