Agence nationale de sécurité du médicament et des produits de santé

Variations in internal standard response

Some thoughts and real-life cases

Olivier Le Blaye
Inspector

21 November 2013
EBF 6th Open Meeting

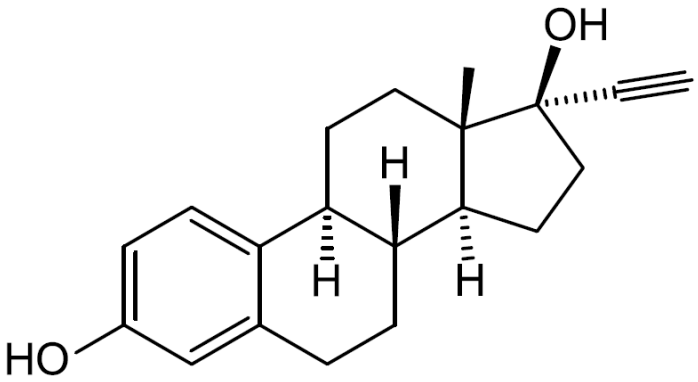
Disclaimer

- **The views expressed in this presentation are mine and may not represent those of either ANSM, EMA, the PKWP or the GCP IWG**

Preliminary observation: choice of IS

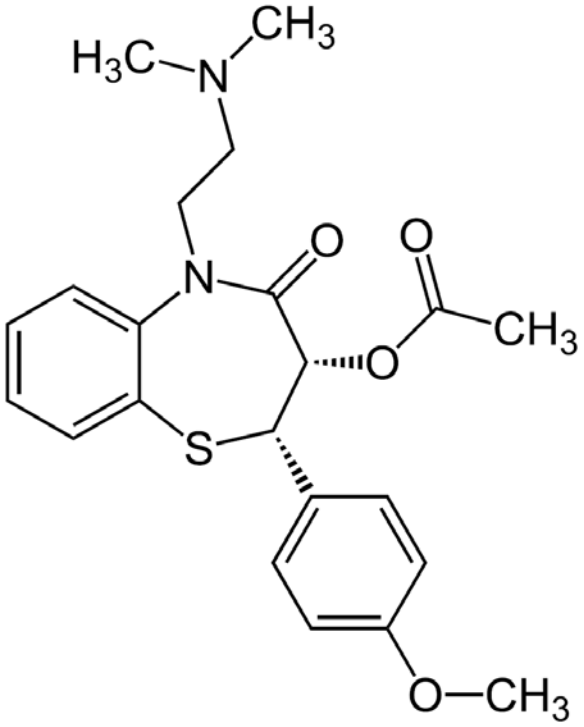
- **A minority of the studies I inspect use a stable-isotope labelled IS (SIL IS)**
  - Very few have a structural analogue
  - Most IS used are not structurally or chemically related to the analyte
    - Some have the same indication: so what ?

WTF ?



Analyte: ethinyl estradiol

Derivatisation with dansyl chloride



IS: diltiazem

No derivatisation

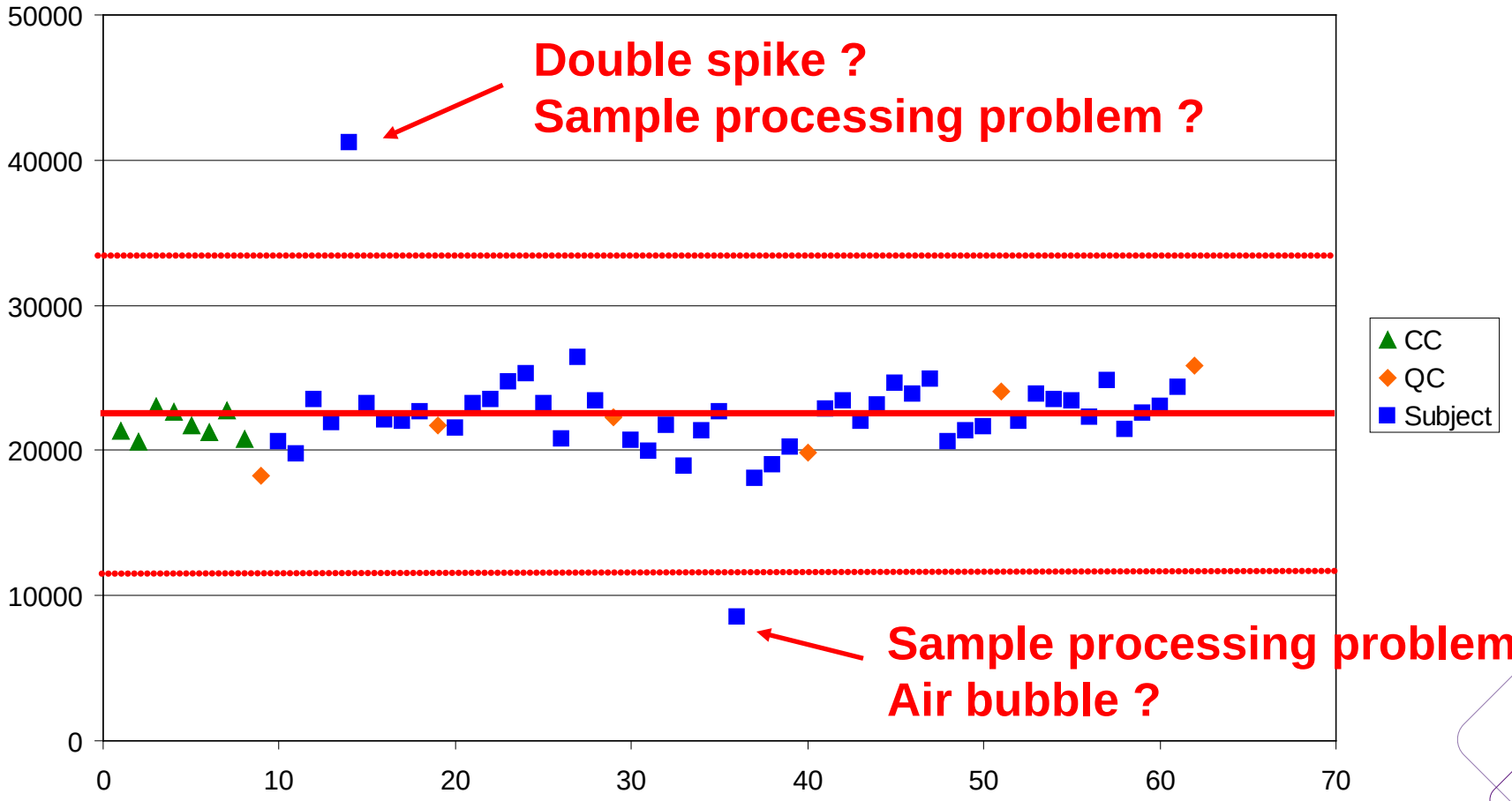
Variations in internal standard response

- **Different situations**
  - Isolated variation (limited number of isolated samples)
  - Systematic differences, trends…

Variations in internal standard response

- **Isolated variation**
  - Easy to detect and to handle
  - Often seen in SOPs: repeat if IS response deviates by more than x % from mean
    (of CC / QC ? of whole run ?)
  - Influence on overall study results limited, unless Cmax sample

IS variation: individual samples



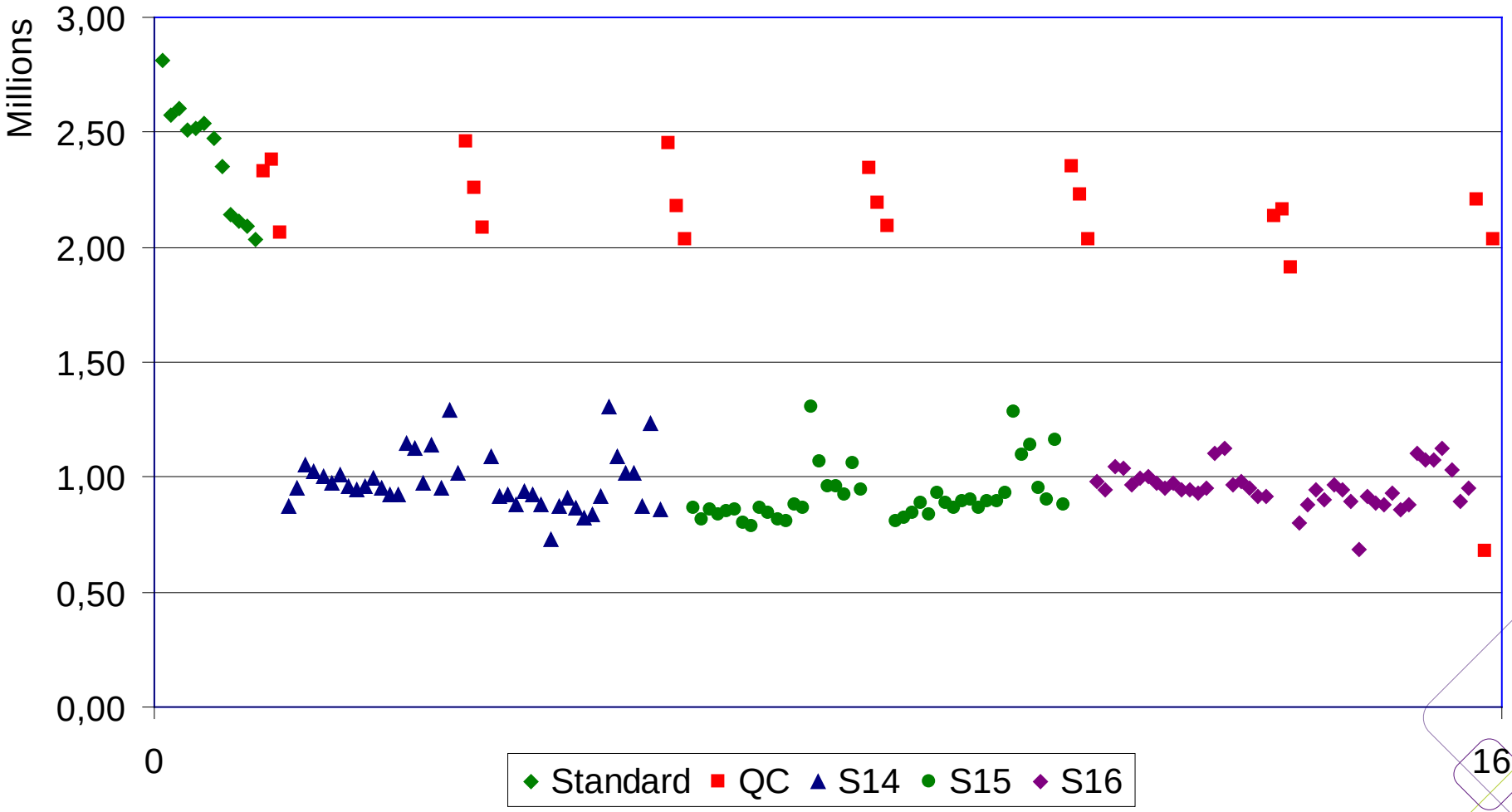
IS variation: individual samples

- Usually well described in SOPs, monitored by labs, few inspection findings
- If variation due to IS addition error: stable isotope labelled IS will not compensate
- If variation due to other reasons: SIL IS may compensate. But:
  - Low response: analyte S/N ? LLOQ ?
  - High response: linearity ?

Variations in internal standard response

- **Systematic differences, trends…**
  - Multiple possible reasons
  - May or may not affect the results
  - More difficult to capture in SOP, may require lab investigations, scientific approach

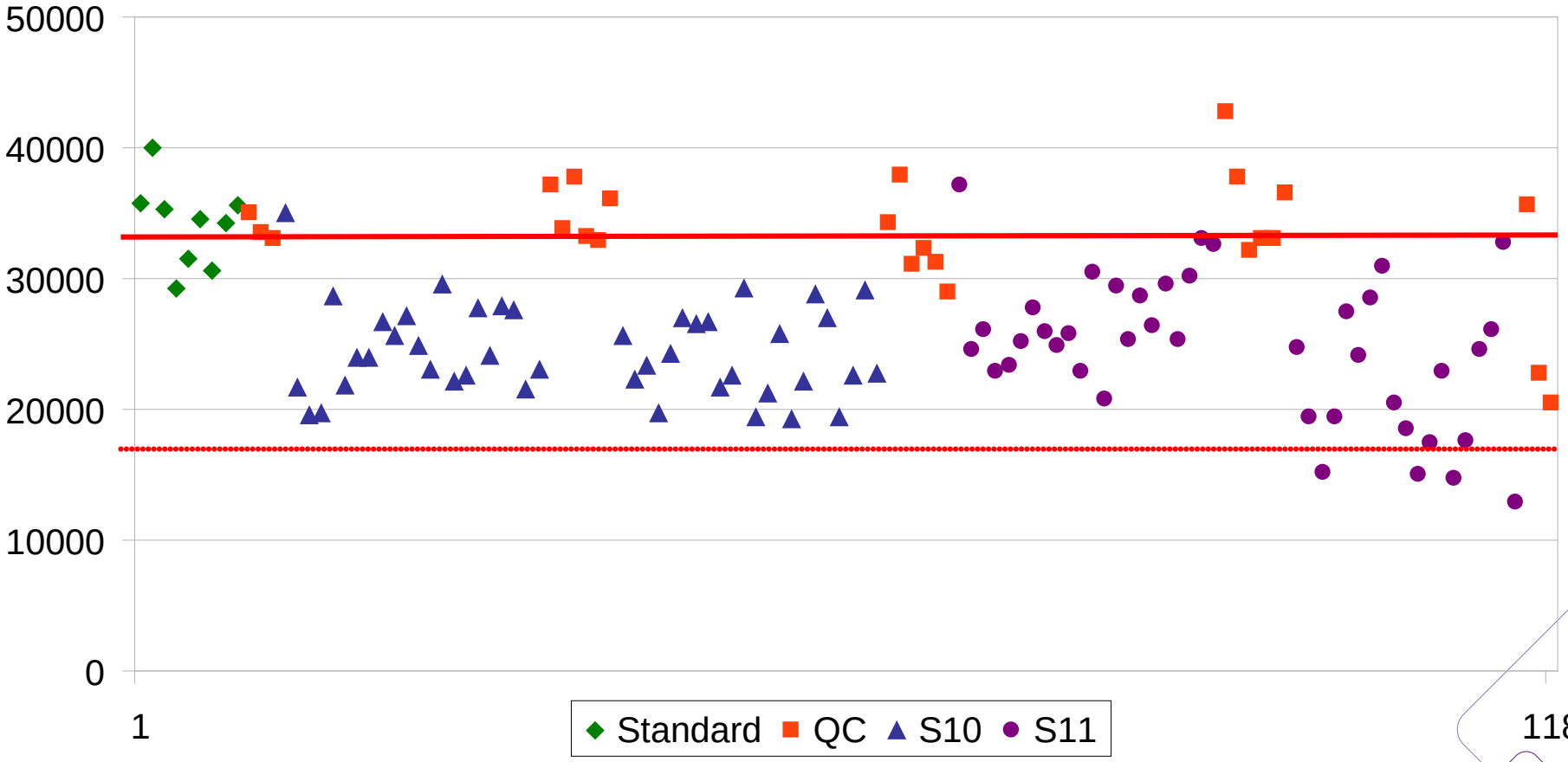
Variations in internal standard response
Systematic differences



Systematic differences

SOP with ± 50 % rule may not help !

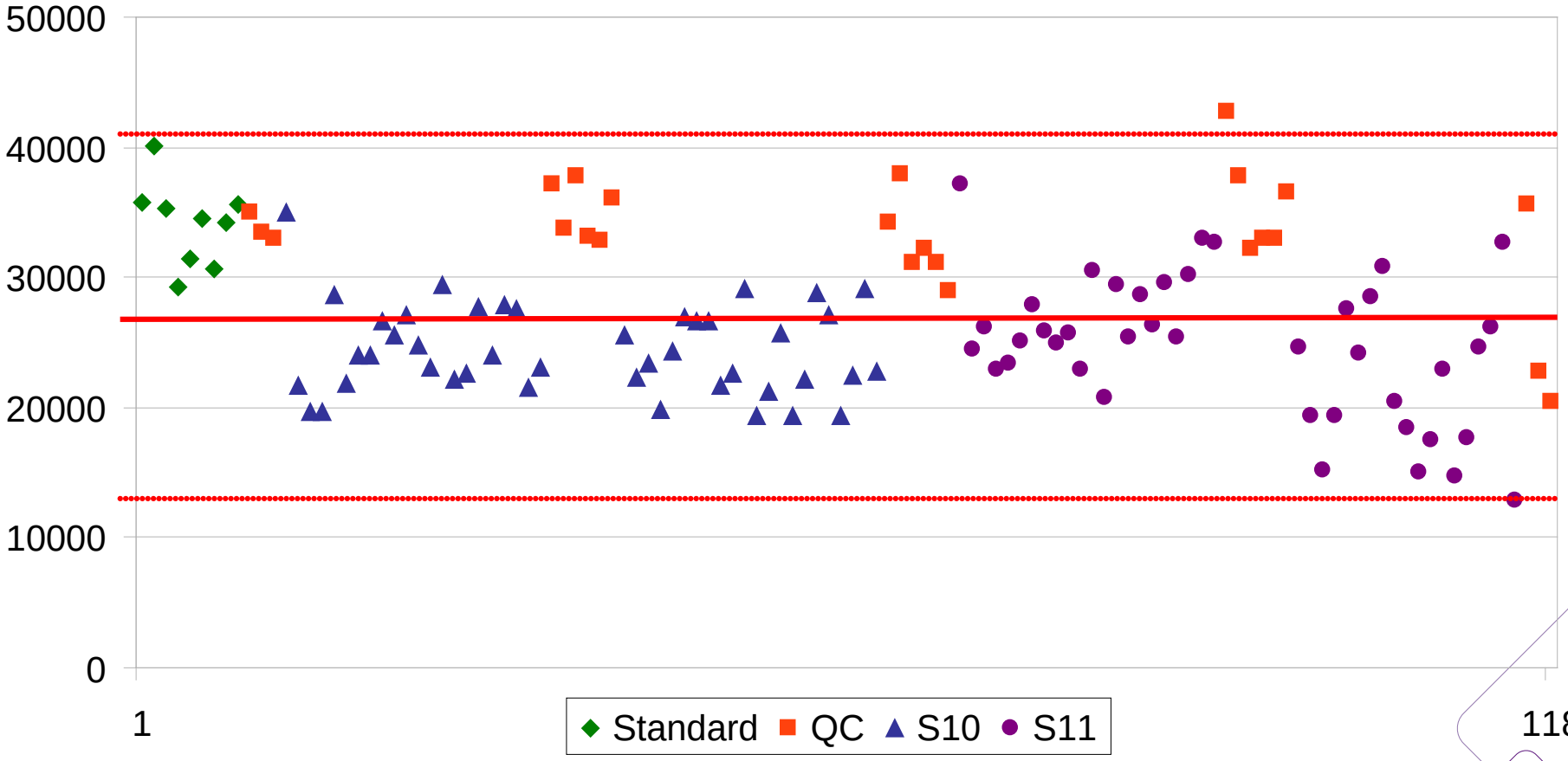
± 50 % of mean IS response of CC / QC samples



Systematic differences

SOP with ± 50 % rule may not help !

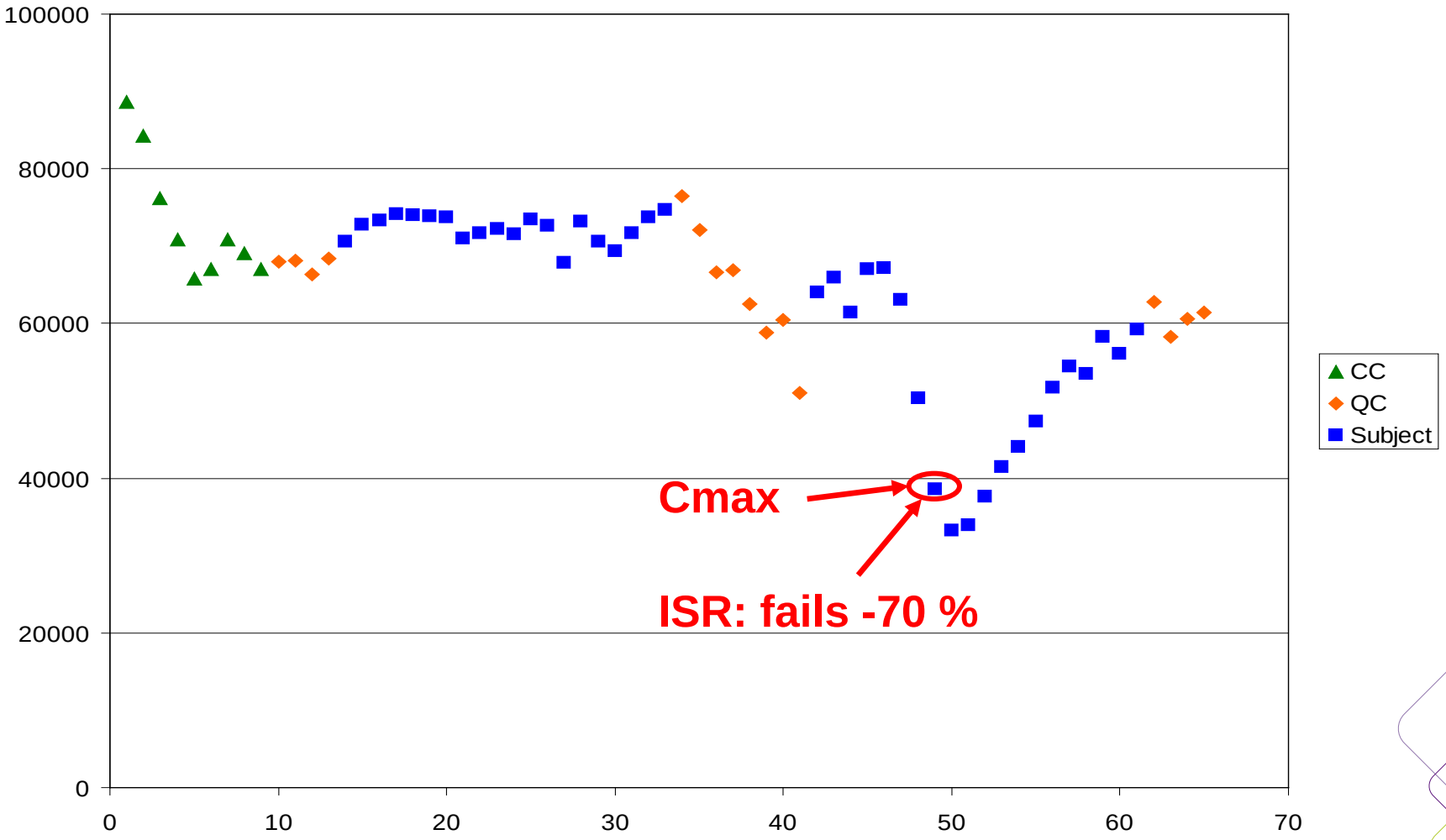
± 50 % of mean IS response of all samples



Variations in internal standard response
Systematic differences

- **Applying blindly an SOP with ± x % acceptance criteria may result in the blind acceptance of possibly inaccurate results !**

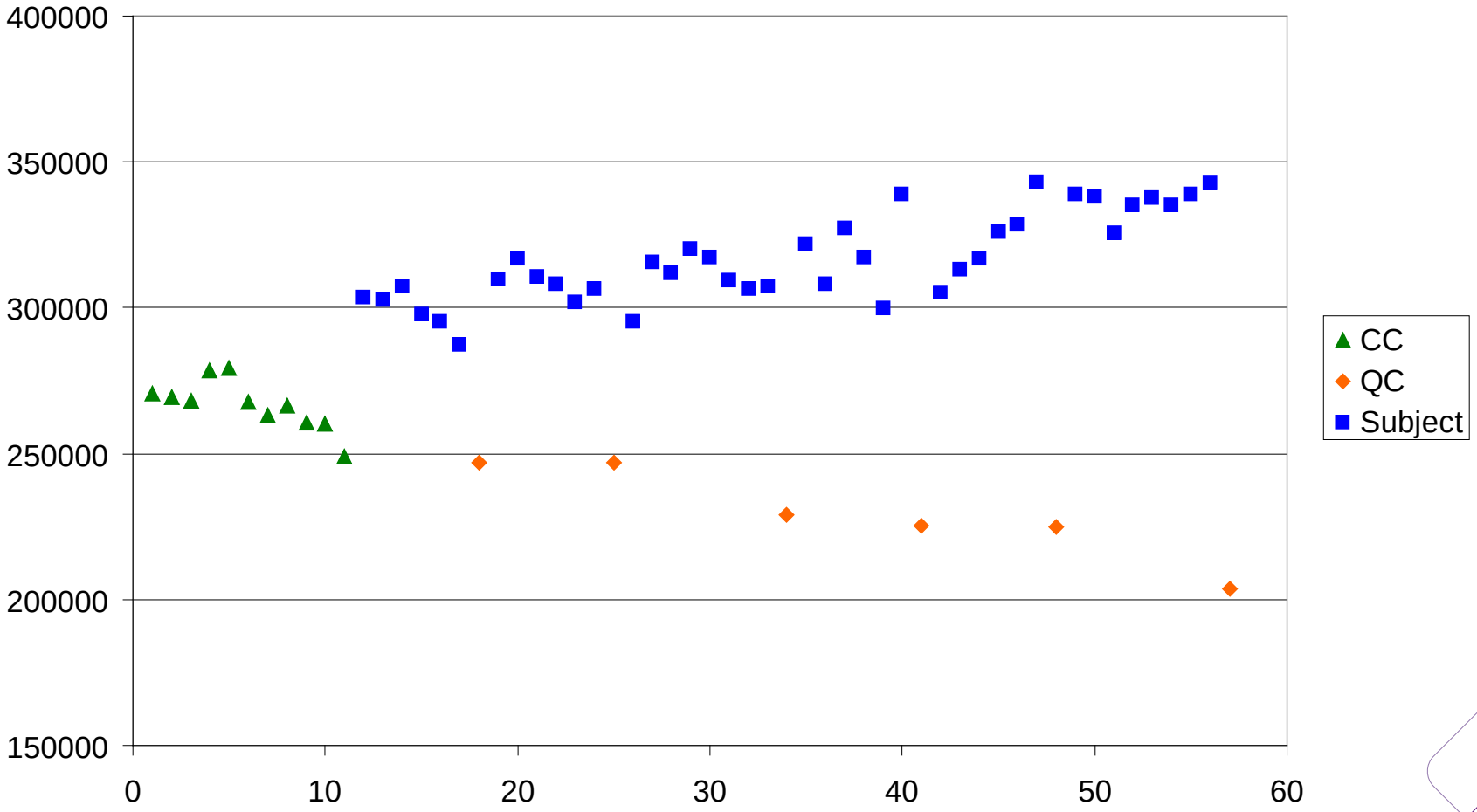
Variations in internal standard response
Trends – case 1



Variations in internal standard response
Trends – case 1

- **Lab did not monitor IS response**
- **SOP with ± x % rule may not help anyway**
  - IS response obviously results in inaccurate results
    - Tmax at 10’ with bolus IV administration; other datasets: 1-2’
    - ISR: 70 % difference
  - What % difference in IS would mean inaccurate results ?

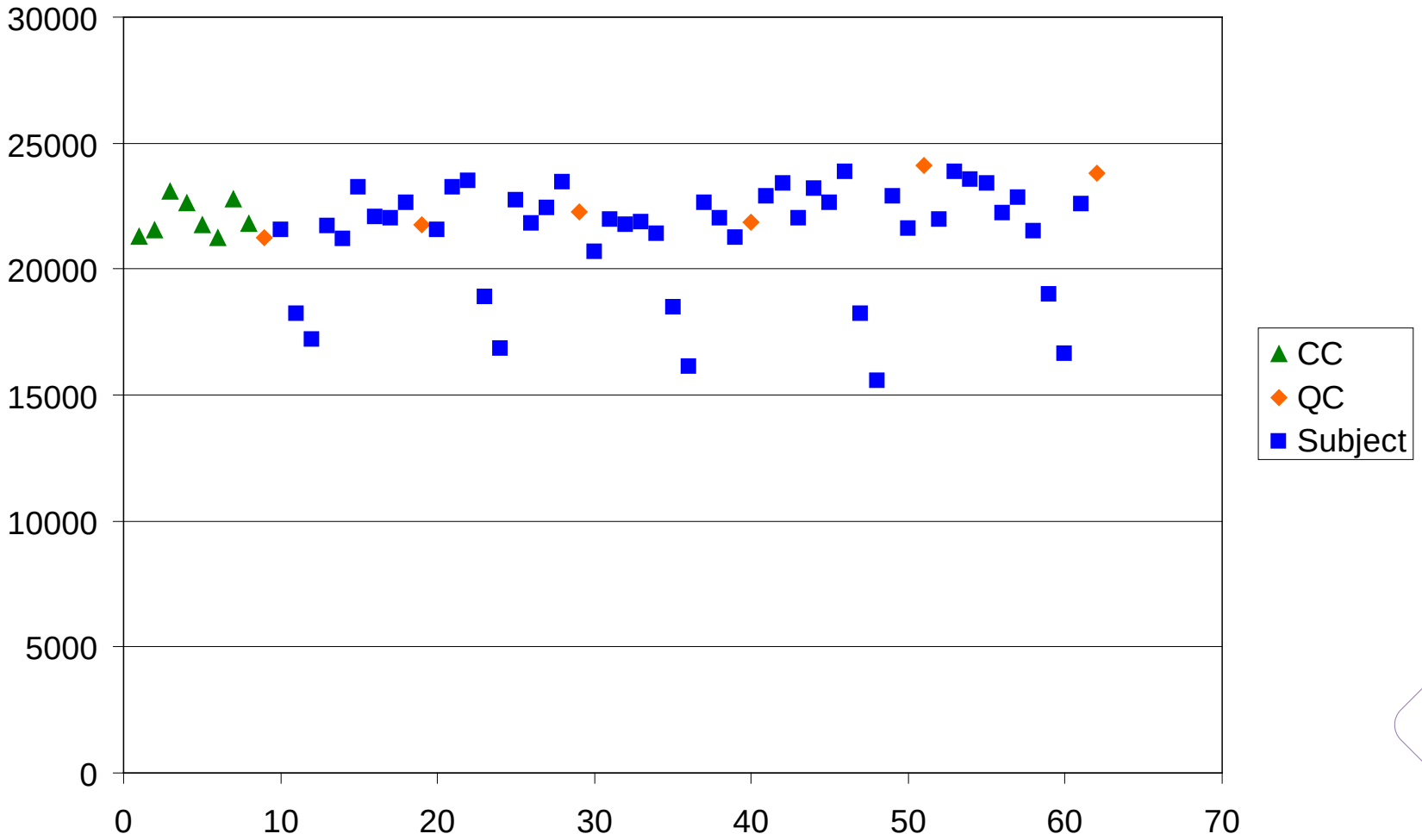
Variations in internal standard response
Trends – case 2



Variations in internal standard response
Trends – case 2

- **Gradual increase in subject IS, decrease in QC IS**
  - Are QCs representative of subject samples ? Accuracy of subject samples ?
  - Subject samples with highest IS result were re-analysed
    - No IS response trend in repeat run
    - Repeat result confirmed initial value, data accepted

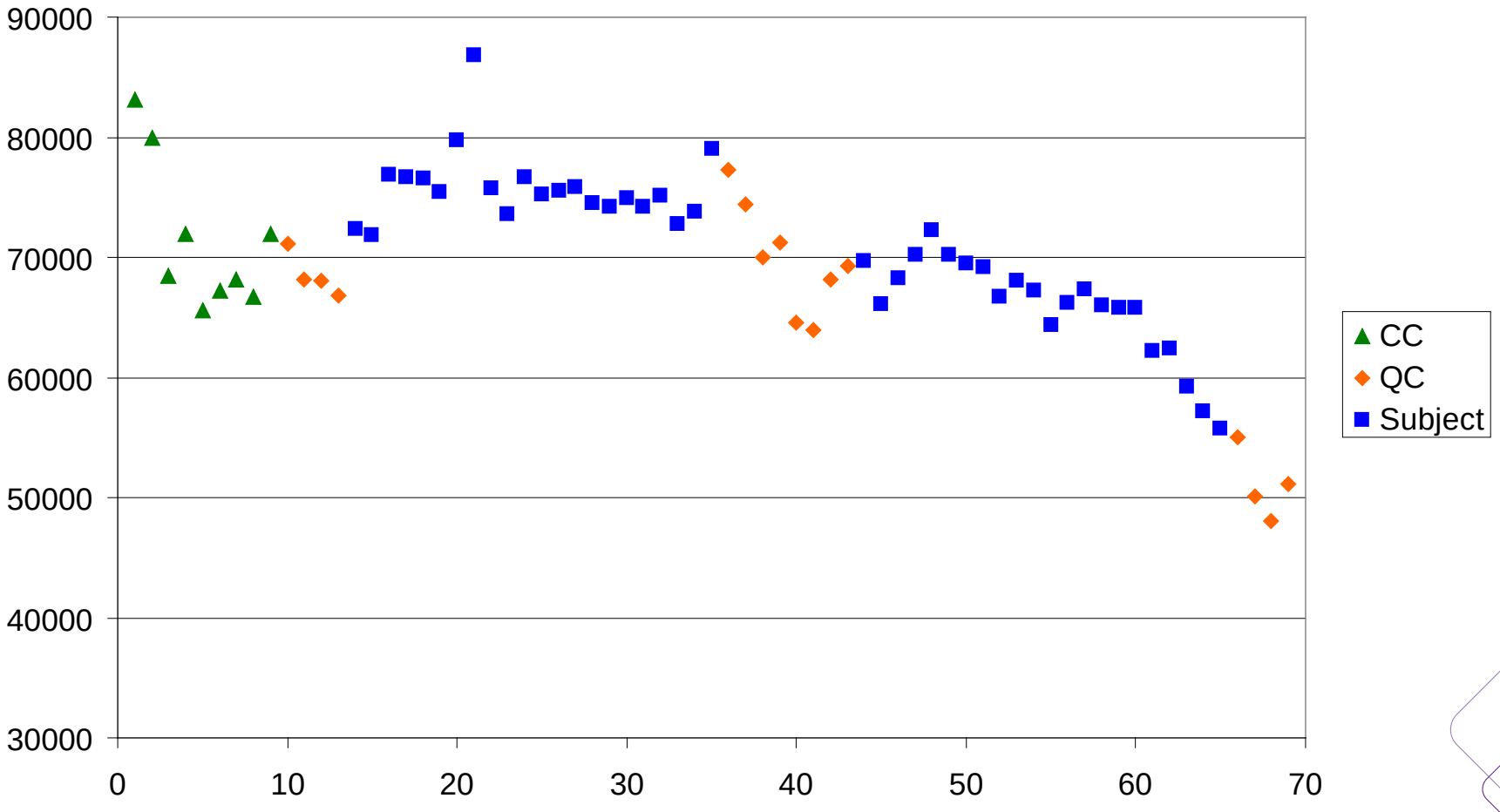
Variations in internal standard response
Trends – case 3



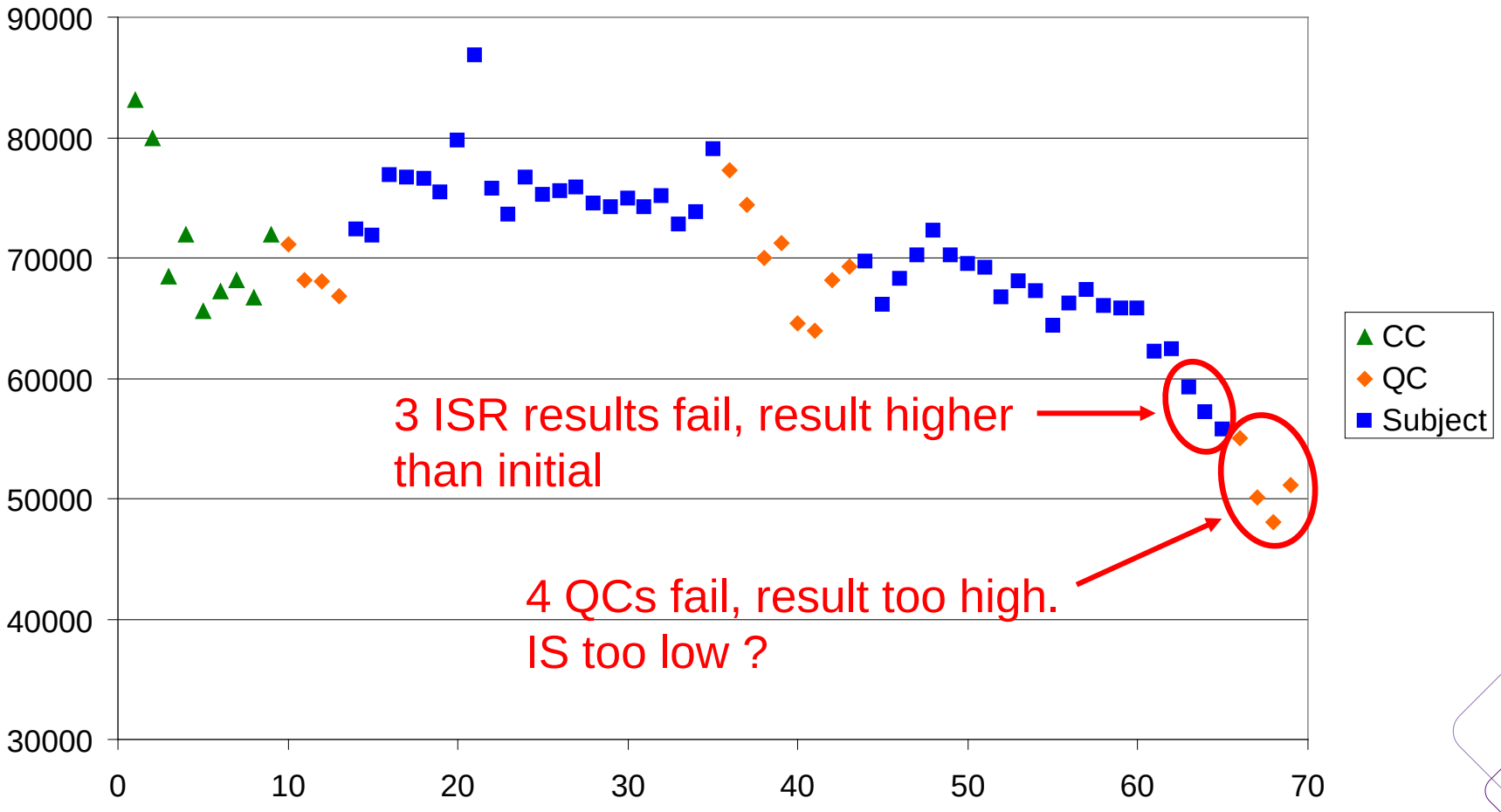
Variations in internal standard response
Trends – case 3

- **IS response systematically lower every 11 and 12 samples**
  - Post-inspection root-cause analysis
    - IS added with Gilson 401 dilutor, series of 12 fractions
    - Dilutor checked periodically, but only 10 fractions
    - Due to piston seal problem: fractions 11 and 12 too low
    - Incorrect IS addition resulted in inaccurate results
    - Affected all runs, possibly other studies

Variations in internal standard response
Trends – case 4, ISR run



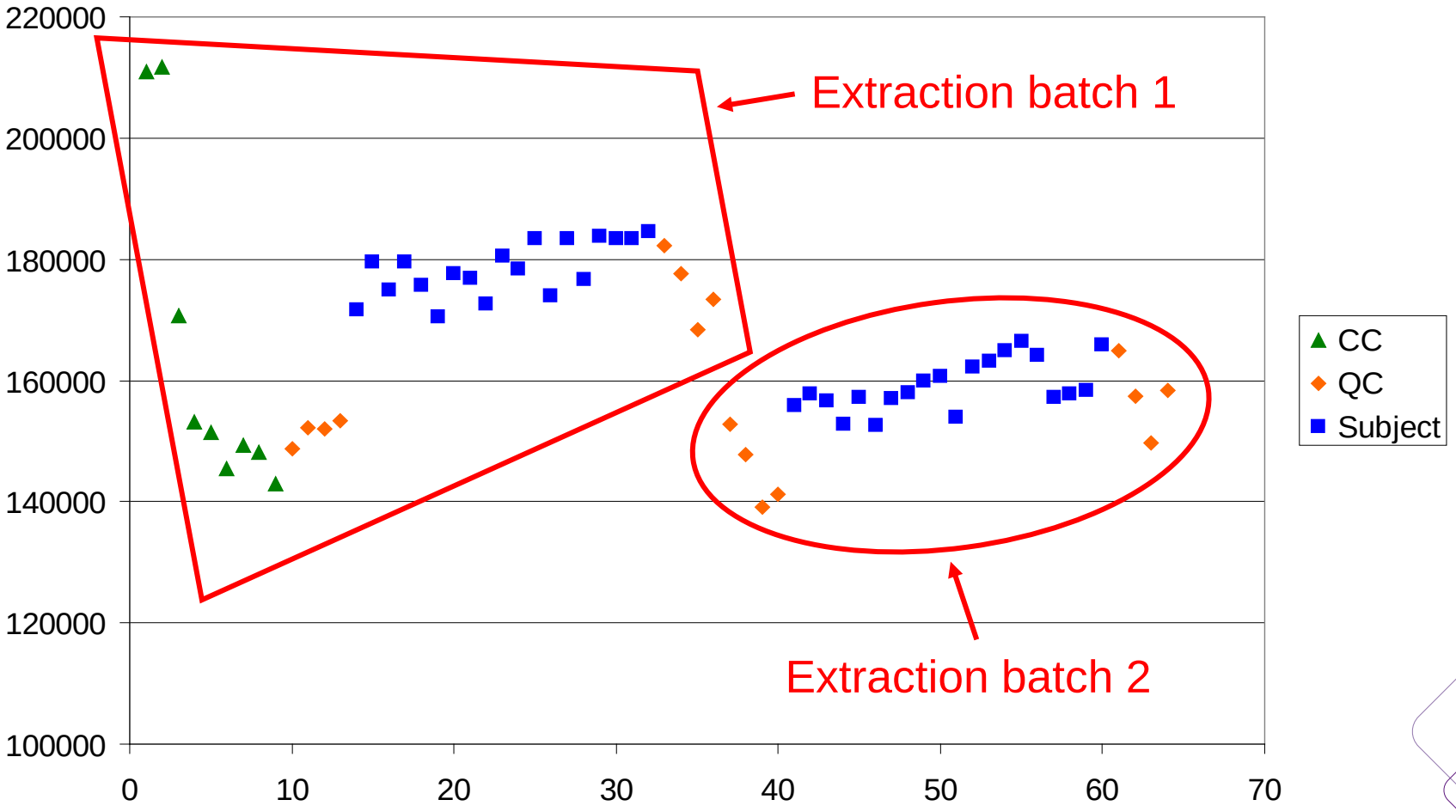
Variations in internal standard response
Trends – case 4, ISR run



Variations in internal standard response
Trends – case 4, ISR run

- Low IS response could be the reason for QC and ISR failure in this run
- Variations in IS response appeared to be the reason for failure of several run / extraction batches in this study – but not always: some runs with low or high IS response in QC sets, result OK
- IS not monitored during study, issue not identified, not investigated

Variations in internal standard response
Trends – case 5



Variations in internal standard response
Trends – case 5

- Samples processed in 2 batches per run, injected in the processing order
  - CC, 2 sets QC, period 1
  - 2 sets QC, period 2 from the same subject
- Difference in IS response between the two extraction batches
- EMA guideline: acceptance criteria for QC samples
  - First for the whole run
  - Then for each extraction batch

Variations in internal standard response
Systematic differences, trends

- **Multiple possible situations**
- **Multiple possible reasons**
- **Some may affect accuracy, others not**
- **Difficult to plan / describe in SOP**
- **Criteria such as ± x % may not be relevant**
  - May not be sensitive enough: ± x % inaccuracy ?
  - If limits too tight: may result in numerous unneeded repeats
- **ISR results can help in discussion (too late ?)**

Variations in internal standard response

Systematic differences, trends

- **“We are using an SIL IS anyway, gold standard, will compensate for any variation, no concern, no need to check IS response”**

Variations in internal standard response
Systematic differences, trends

- **“We are using an SIL IS anyway, gold standard, will compensate for any variation, no concern, no need to check IS response”**
  - Not an acceptable answer if the root cause for the variation has not been identified
  - SIL IS can compensate for some sources of variations, but not all !
  - If you don’t know why the IS varies, you don’t know whether the SIL IS will compensate

Variations in internal standard response
Systematic differences, trends

- **IS variations may trigger laboratory investigations to identify root cause**
  - Depending on the result: may require the re-analysis of study samples
  - We, inspectors, should be ready to accept decisions which are science-driven and not just based on SOPs, if:
    - Based on facts and solid scientific arguments
    - Well documented
  - Lack of SOP or inadequate criteria are not valid reasons to accept obviously inaccurate results

See Tan et al., Journal of Chromatography B, 877 (2009) 3201-3209 for 12 more cases !

Thank you for your attention !

Agence nationale de sécurité du médicament et des produits de santé

Avertissement

- Lien d'intérêt : personnel salarié de l'ANSM (opérateur de l'Etat).
- La présente intervention s'inscrit dans un strict respect d'indépendance et d'impartialité de l'ANSM vis-à-vis des autres intervenants.
- Toute utilisation du matériel présenté, doit être soumise à l'approbation préalable de l'ANSM.

Warning

- Link of interest: employee of ANSM (State operator).
- This speech is made under strict compliance with the independence and impartiality of ANSM as regards other speakers.
- Any further use of this material must be submitted to ANSM prior approval.