

Choice of anticoagulant and Comparison of counter ions

- Results from two EBF surveys

Presenter: *Carl Sennbro on behalf of EBF*

Presented at: *EBF open meeting , 02 December 2010, Barcelona*

Aim

- To present results from two EBF surveys
- Part I: Anticoagulant mini-survey
 - Overview of the policy on and choice of anticoagulant within EBF companies
- Part II: Counter ion data comparison
 - Collection of data in order to compare LC-MS assay performance using different counter ions

Part I. Anticoagulant mini-survey

Aim

- To map the company policy on usage of anticoagulants and counter ions
- To map the company choice of anticoagulant

Mini-survey design

- Part A: General questions on policy for usage of anticoagulants and counter ions within various bioanalytical issues
- Part B. Choice of anticoagulant in various applications

Outcome

- Survey distributed in September – 2009
- Responder rate 25/27 companies
- Results presented and discussed at EBF closed meetings 2010

Part I. Anticoagulant mini-survey

Conclusions I – anticoagulant policy

- Calibrator/QC samples in Method validation *versus* Method application
 - Most companies (21/25) **do not** allow different anticoagulants
 - Most companies (18/25) **do** allow different counter ions (same anticoagulant)

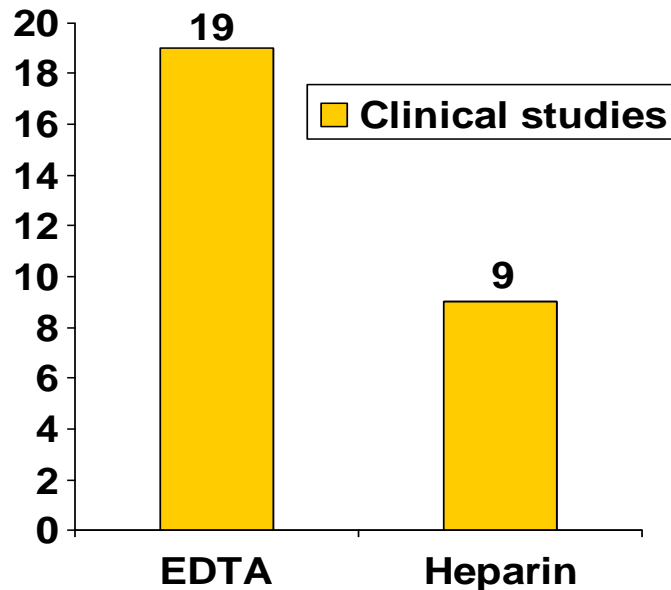
- Study samples *versus* Calibrator/QC samples
 - Most companies (21/25) **do not** allow different anticoagulants
 - Most companies (16/25) **do** allow different counter ions (same anticoagulant)

- 10 of 25 companies cover these issues in an SOP.

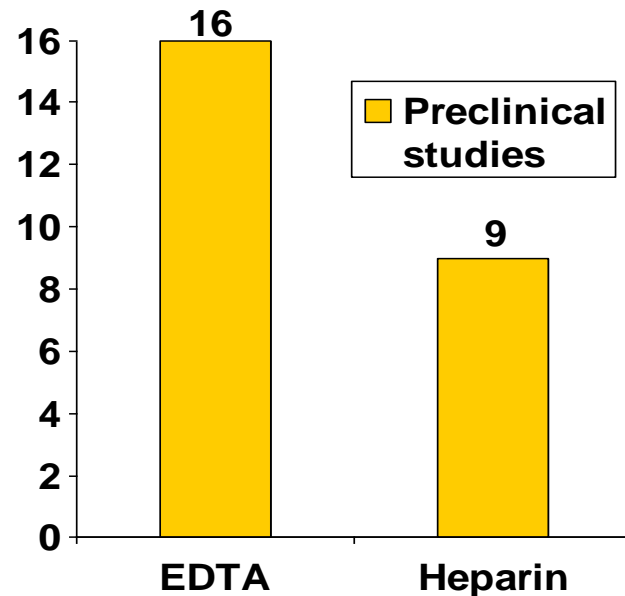
Part I. Anticoagulant mini-survey

Conclusions II – anticoagulant choice

- EDTA is the most commonly used anticoagulant



In clinical studies:
For one company – different preferences in Europe vs US
For two companies – the choice is project dependent

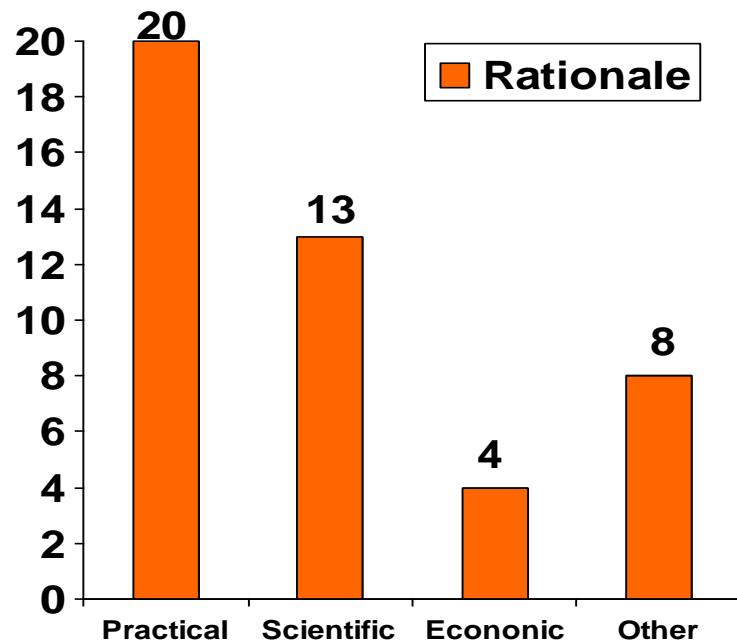


For all companies:
Same choice in non-regulated studies

Part I. Anticoagulant mini-survey

Conclusions III – anticoagulant choice

- The rationale for the choice of anticoagulant

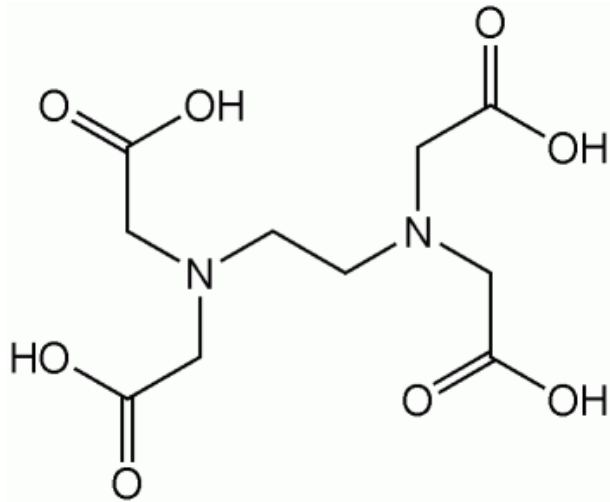


Main specified rationale:

Less problems with clotting using EDTA

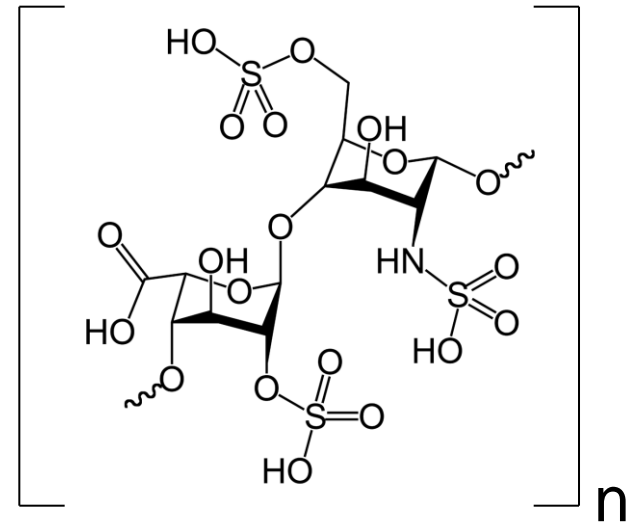
Sadagopan et al., Rapid Comm Mass Spectrom
2003;17;1065

EDTA



Ethylenediaminetetraacetic acid
Commercially synthesized
Chelating agent
Mw 292 g/mol
Various salts available

Heparin



Variably-sulfated glycosaminoglycan
Extracted from mammals (porcine)
Medical agent
Mw 12-15 kDa (average)
Various salts available

Part II. Counter ion comparison

Aim

- To compare experimental data on counter ion impact in LC-MS assays

Study design

- To analyze one batch of spiked Calibrator/QC plasma samples including
 - Calibration curve in plasma with validated anticoagulant and counter ion
 - One set of QCs in plasma with validated anticoagulant and counter ion
 - One set of QCs in plasma with validated anticoagulant but **different** counter ion
 - 2-3 levels of QCs, 3-6 replicates of each level
 - Results delivered as calculated Precision and Accuracy
- Each company to provide experimental data for at least one compound
 - Also, description of the assay and analyte(-s) delivered
- Pair-wise comparison of calculated precision and accuracy

Part II. Counter ion comparison

Conclusions

- Results within acceptance criteria regardless of counter ion and anticoagulant
- No significant differences (neither statistical nor practical) for Precision nor Accuracy
- No specific parameter (e.g. sample prep, internal standard, etc) affected the results

Part II. Counter ion comparison

Survey outcome

- Survey distributed in June – 2009
- Responder rate 15/27 companies
- Results presented and discussed at EBF closed meetings 2010

Summary of assay and analyte data

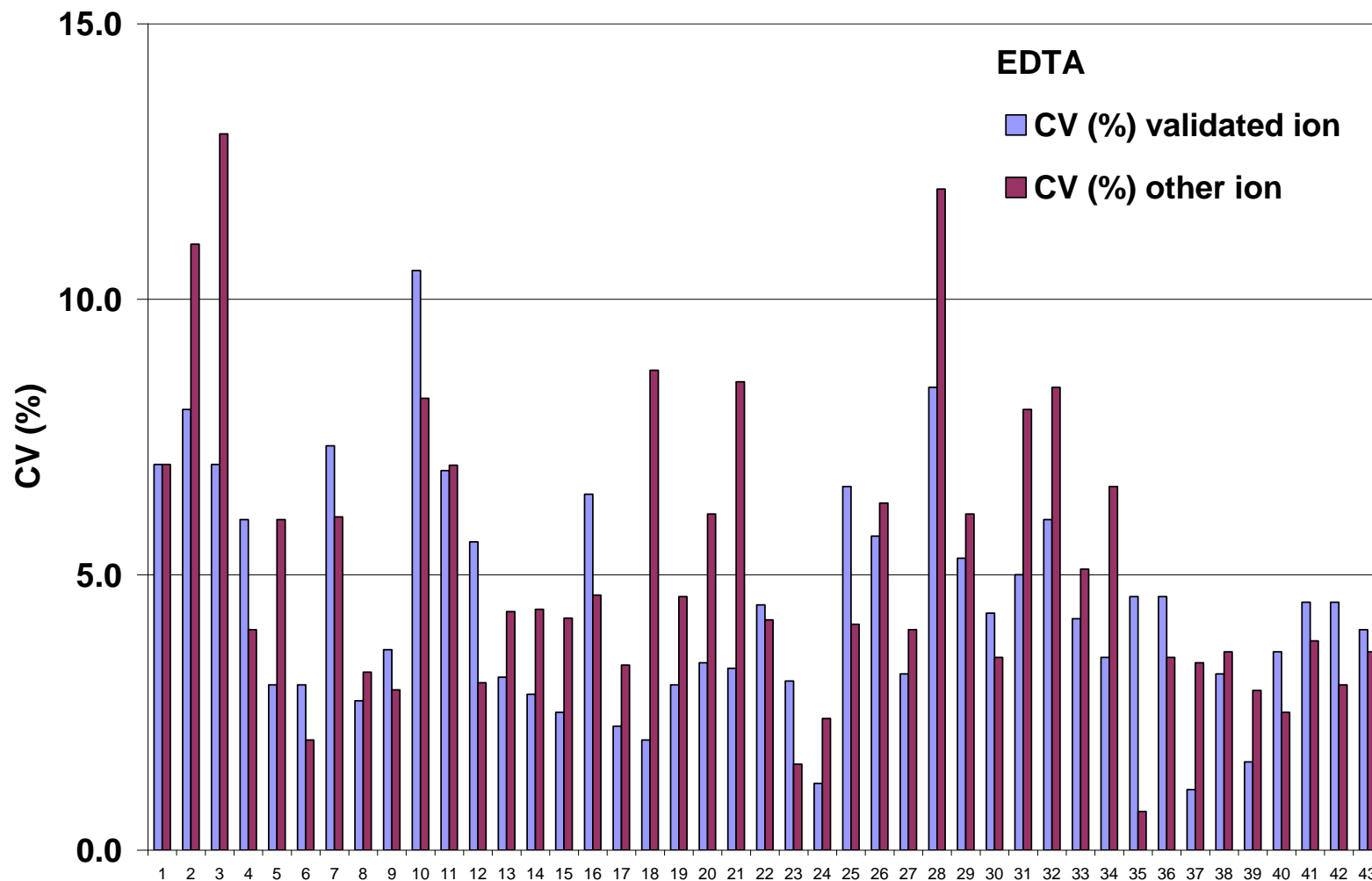
- Exclusively LC-MS/MS assays (n=42) for 34 compounds
- Stable isotope labelled IS for 32 of 42 assays
- Species: Human (n=32), rat (n=5), dog (n=4), primate (n=1)
- Sample preparation techniques
 - PP, SPE, LLE, DBS, Derivatization, Hydrolyzation
- Mw = 206 – 1069 Da (average 461 Da)
- Analyte type: acids (n=7), neutrals (n=6), bases (n=12), unspecified (n=9)
- Log P 1.1 – 7.1 (average 3.9)

Part II. Counter ion comparison

Precision & Accuracy data for comparison

- Data for EDTA counter ion comparison; 43 pairs of measurements for 15 compounds
 - For Heparin counter ion comparison; 51 pairs of measurements for 15 compounds
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- Data also included comparison of different anticoagulants: 21 pairs of measurements for 9 compounds – data **not shown** in this presentation

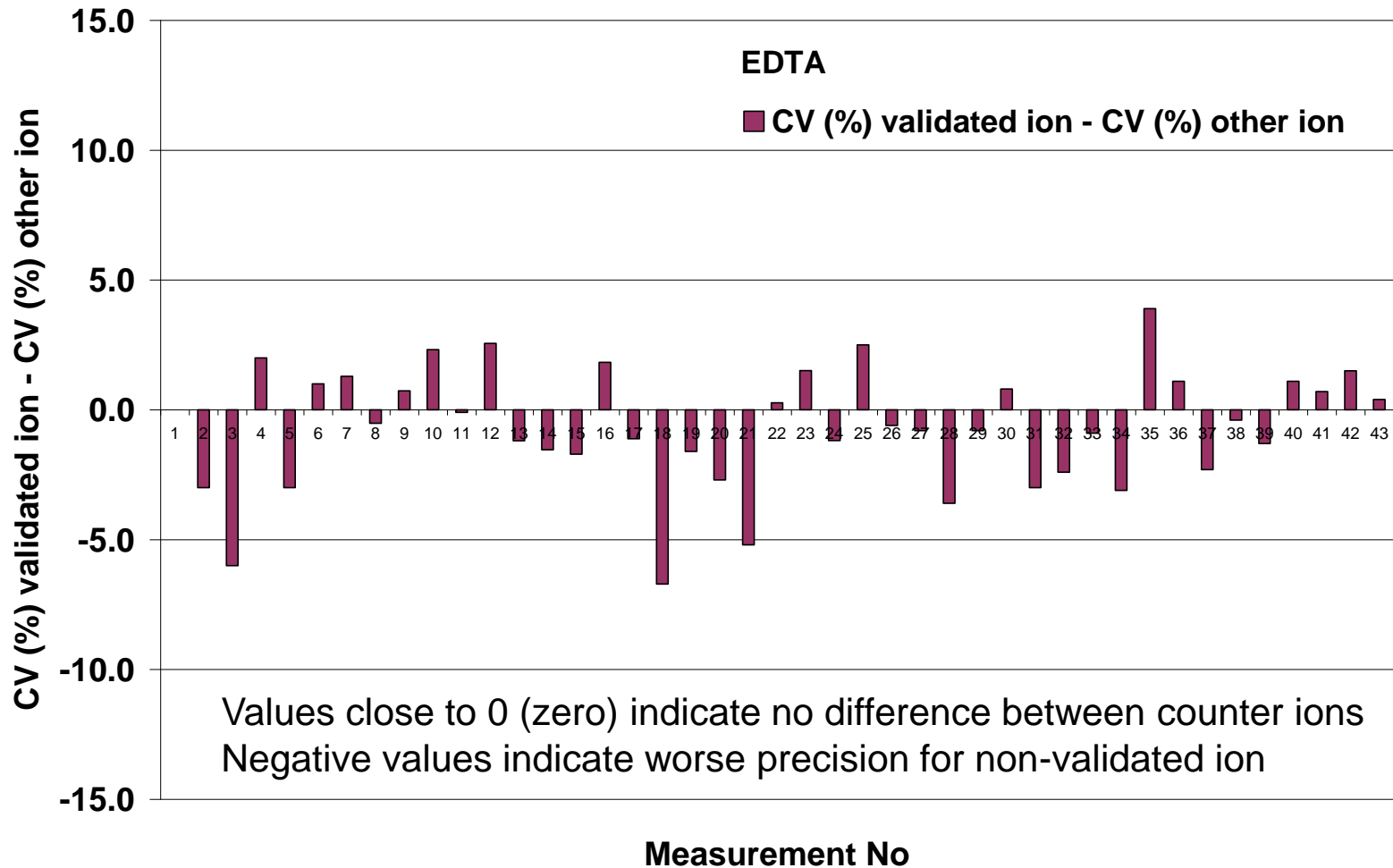
Results, Precision EDTA



Pair-wise comparison (n=43)

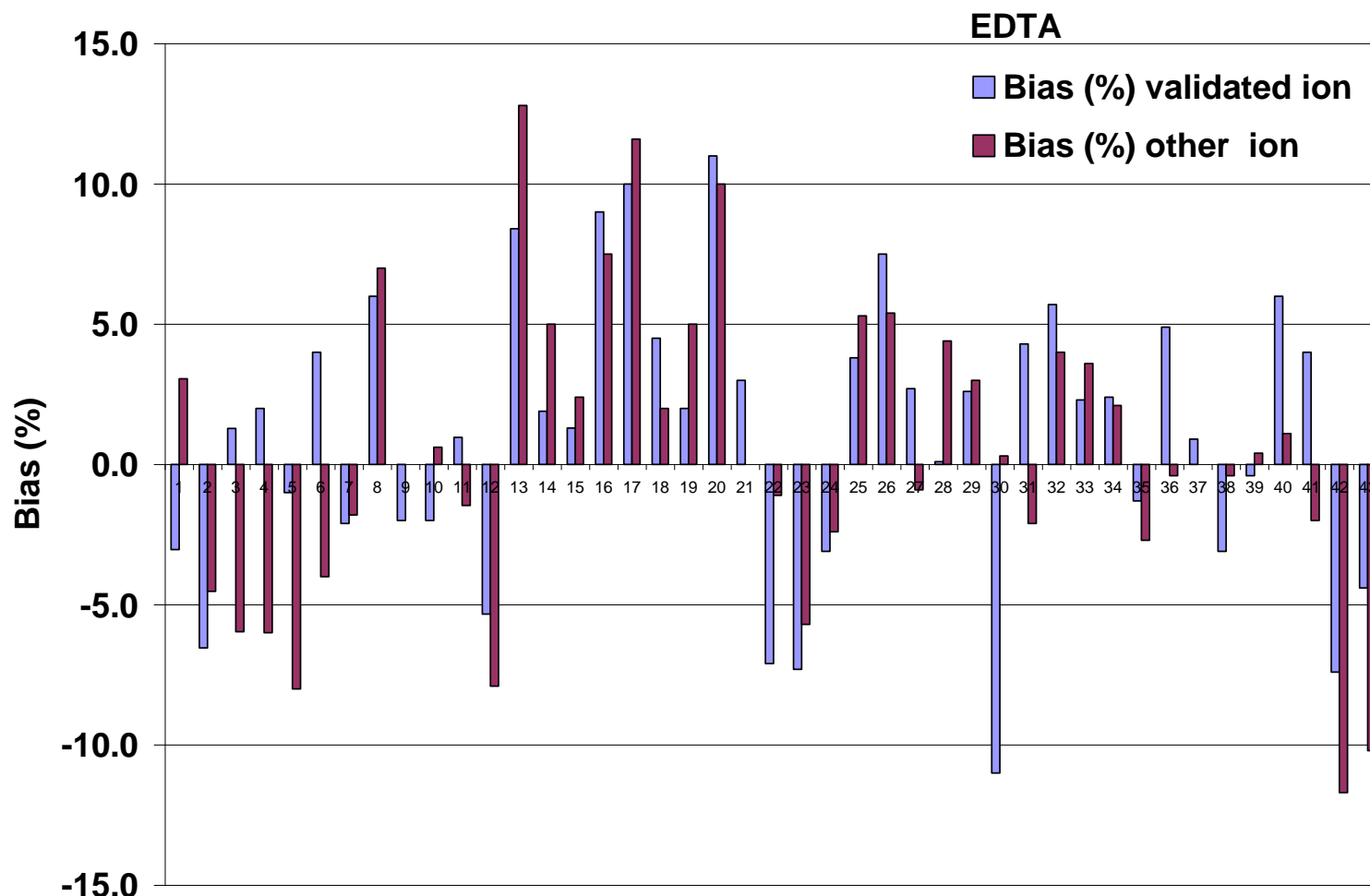
All measurements within acceptance criteria of 15%

Results, Precision difference EDTA



EDTA. Paired t-test: $p = 0.060$
Average diff: - 0.7 %

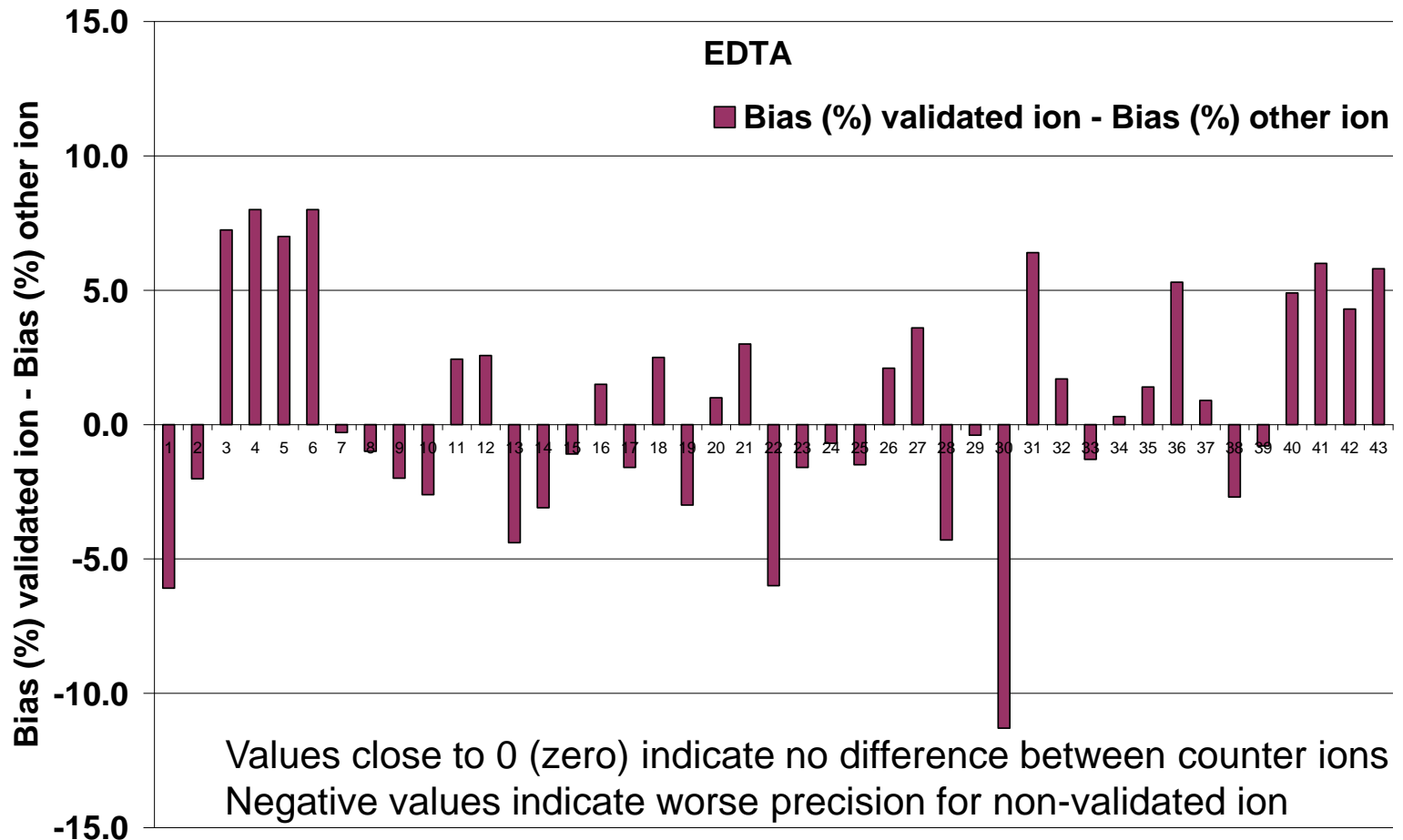
Results, Accuracy bias EDTA



Pairwise comparison (n=43)

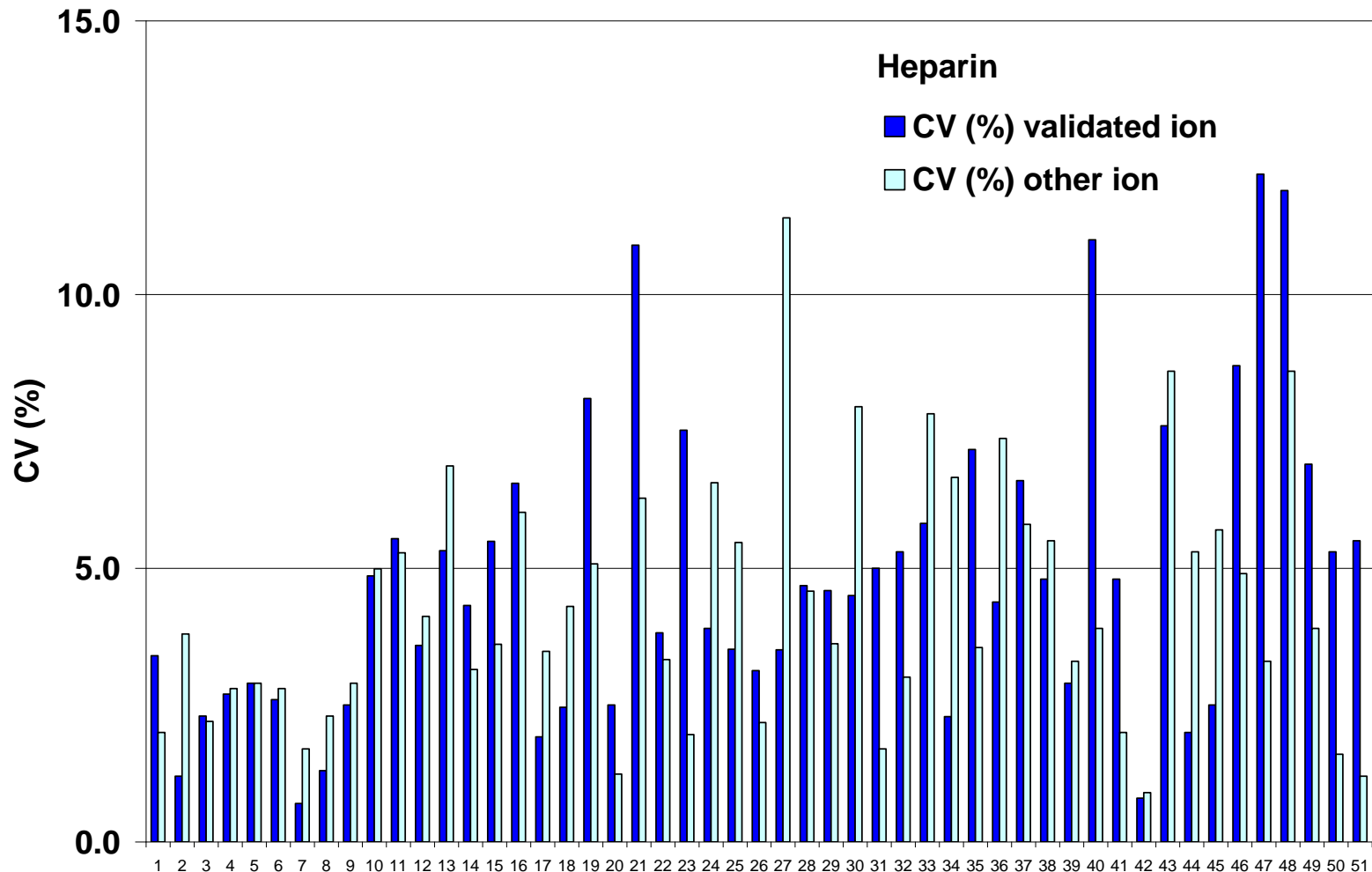
All measurements within acceptance criteria +/- 15%

Results, Accuracy bias difference EDTA



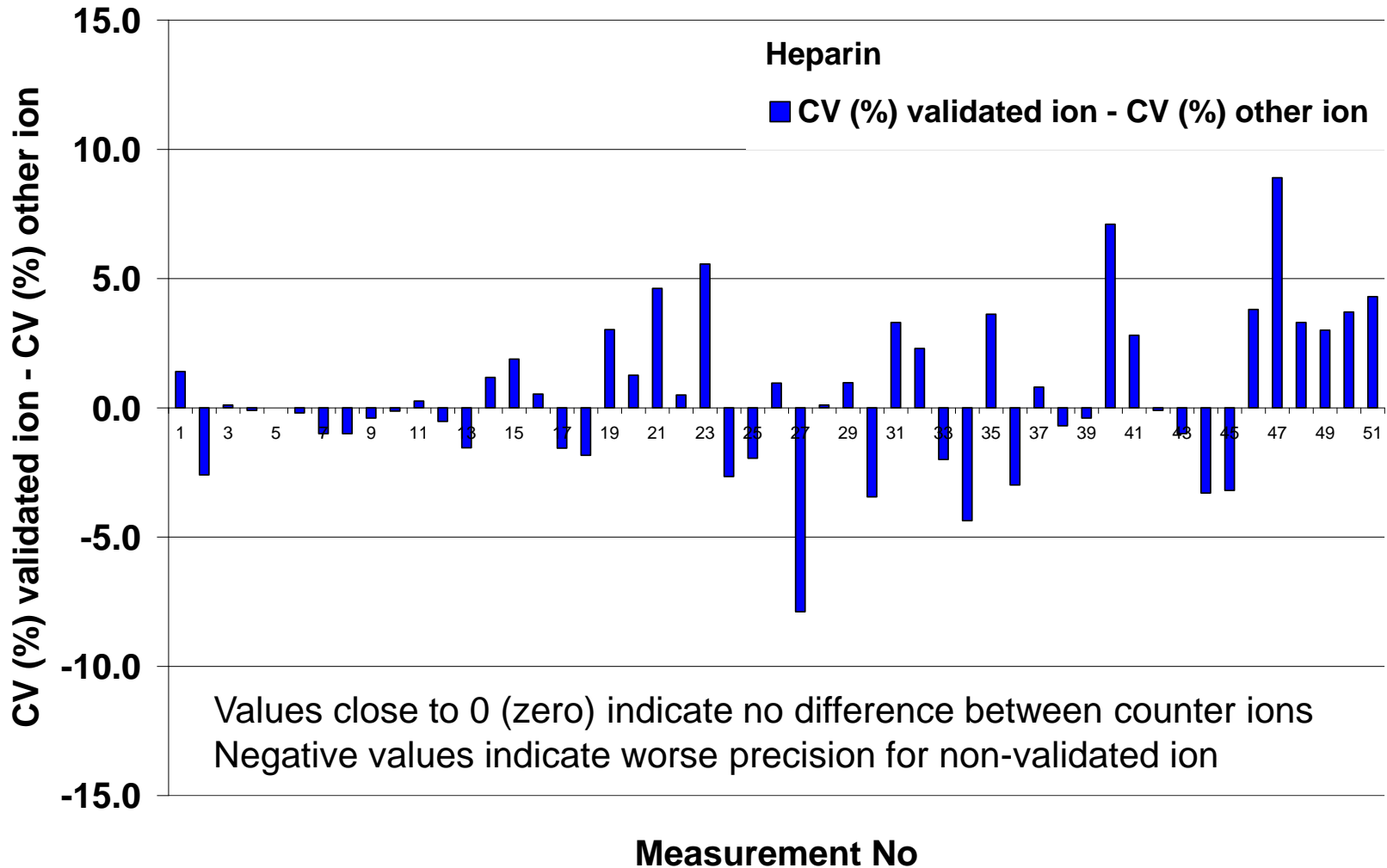
EDTA. Paired t-test: $p = 0.31$
Average diff: + 0.6 %

Results, Precision Heparin



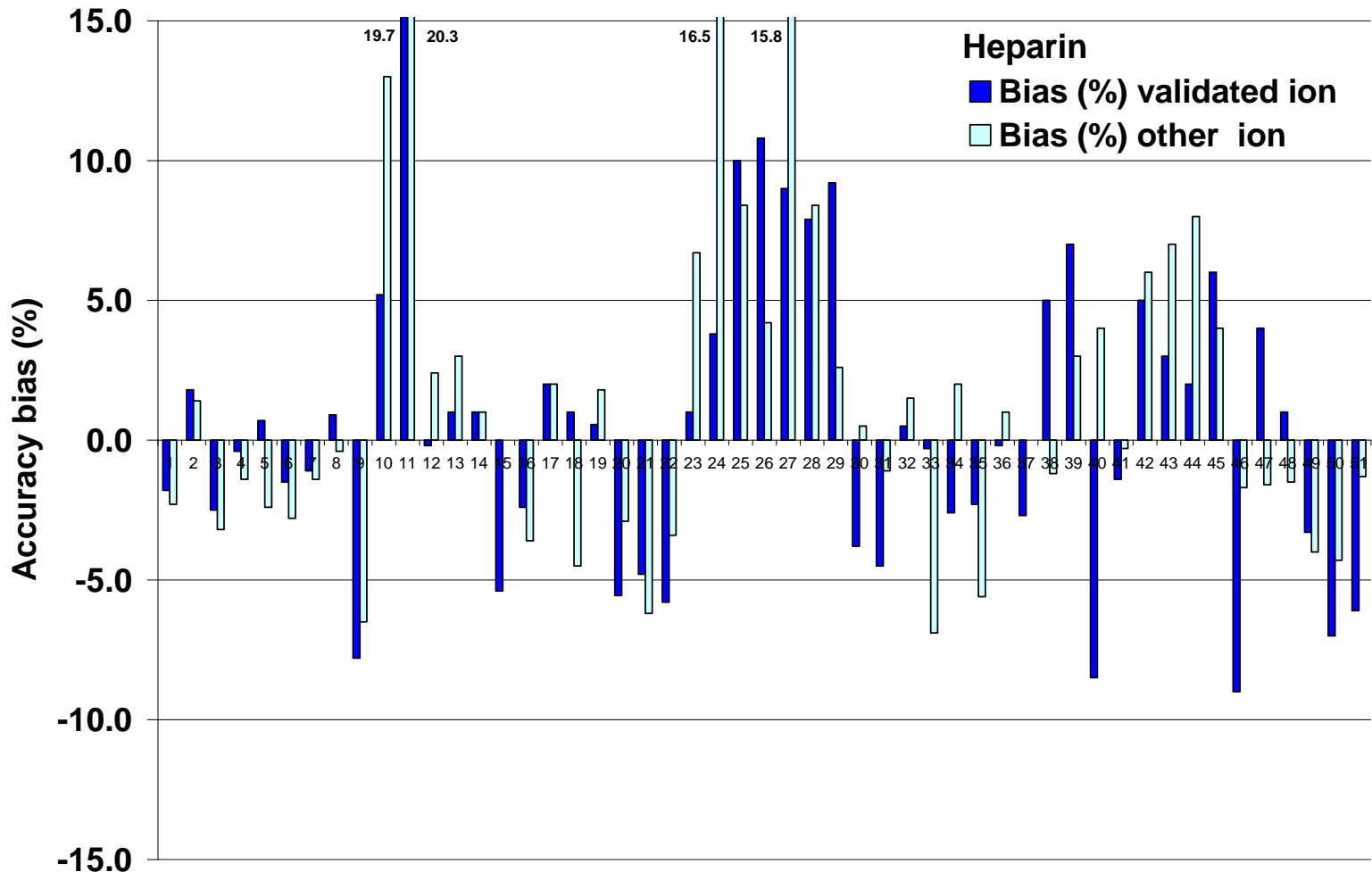
Pair-wise comparison (n=51)
All measurements within acceptance criteria of 15%

Results, Precision difference Heparin



Heparin. Paired t-test: $p = 0.26$
Average diff: + 0.5 %

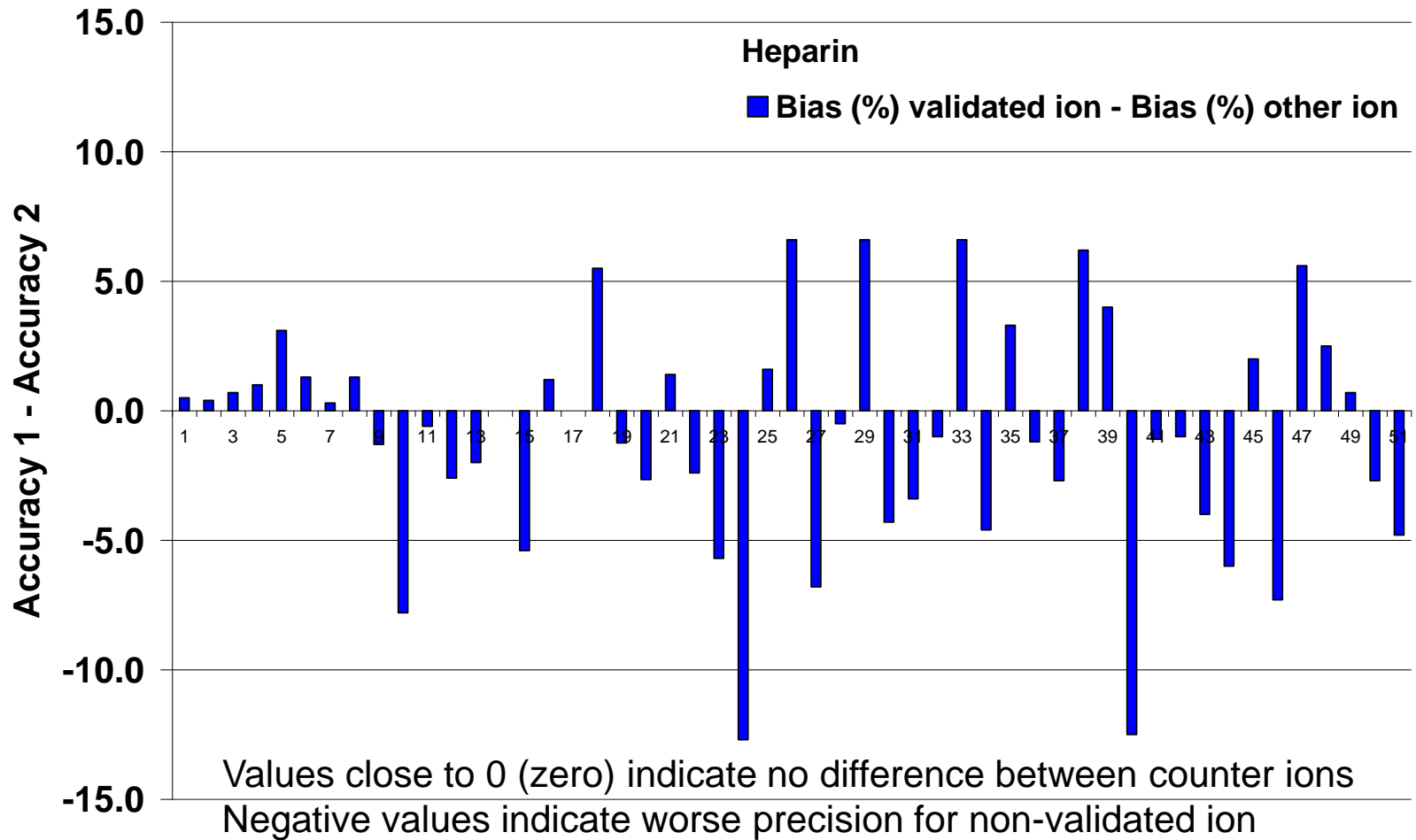
Results, Accuracy bias Heparin



Pair-wise comparison (n=51)

96 % of measurements within acceptance criteria +/- 15%

Results, Accuracy bias difference Heparin



Heparin. Paired t-test: $p = 0.15$
Average diff: - 0.9 %

Conclusions, counter ion comparisons

- Results within acceptance criteria regardless of counter ion and anticoagulant
- No significant differences (neither statistical nor practical) for Precision nor Accuracy
- No specific parameter (e.g. sample prep, internal standard, etc) affected the results
- Results in compliance with previous findings by Bergeron et al (2009); Bioanalysis 1(3); 537-48.
- These observations justify an EBF suggestion/recommendation

Main conclusion, counter ion comparisons

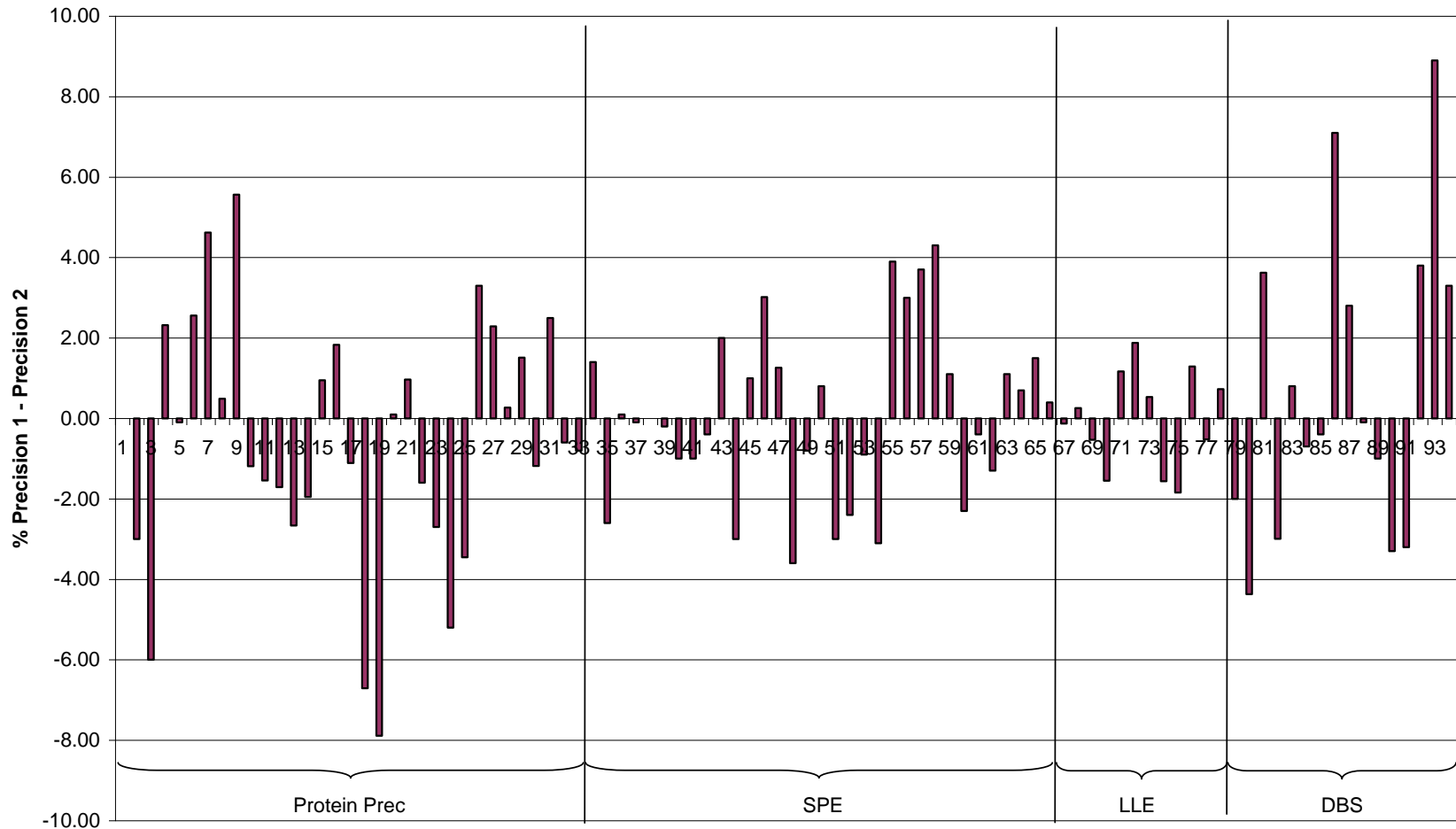
➤ EBF suggestion/recommendation

In regulated bioanalysis, collected plasma samples containing different counter ions but same anticoagulant may be regarded as **equal** matrices

Acknowledgement

- Magnus Knutsson, Ferring A/S
- Peter van Amsterdam, Abbott
- All contributing EBF companies

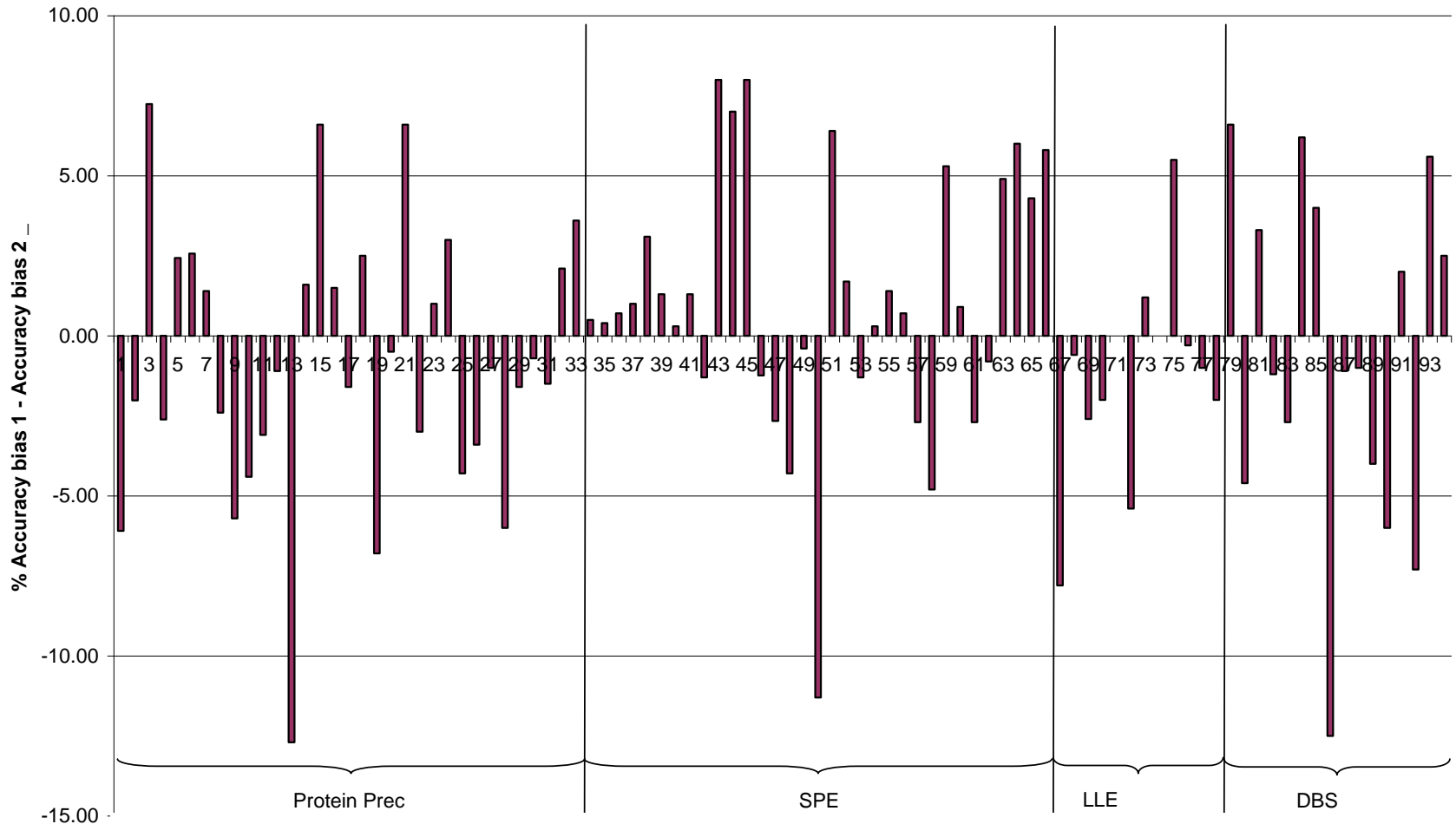
Back-up slide 1a: Different sample preps



Average CV Difference

PP: -0.6 % SPE: +0.1 % LLE: -0.0 % DBS: +0.8 %

Back-up slide 1b: Different sample preps



Average Bias Difference

PP: -0.9 % SPE: +1.1 % LLE: -1.3 % DBS: -0.6 %

Back-up slide 2: Internal standard

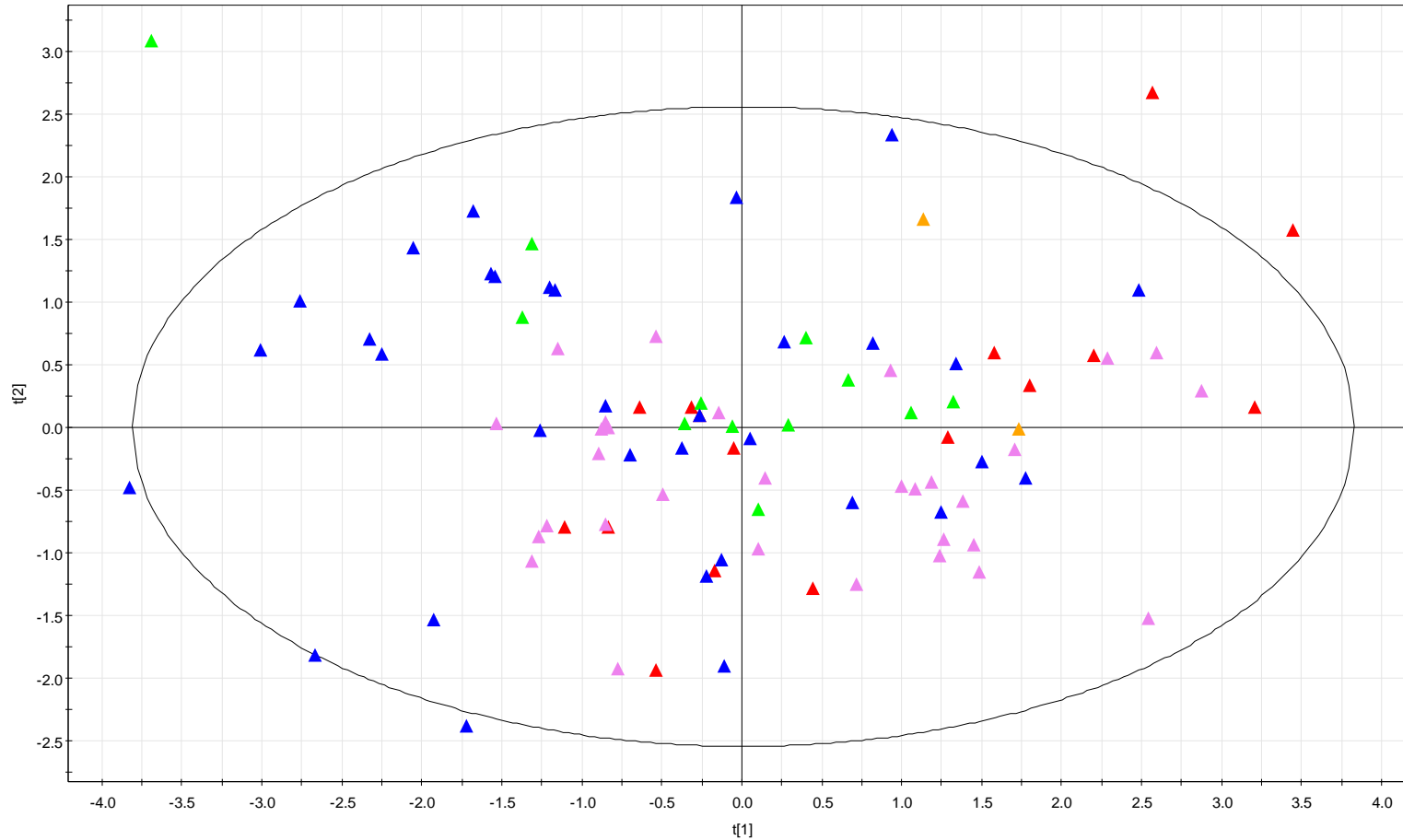
Compared assays with or without SIL IS.

CV difference	
With SIL IS	+0.7 %
Without SIL IS	-0.3 %
Bias difference	
With SIL IS	-0.4 %
Without SIL IS	-0.1 %

Back-up slide 3a: PLS model,

Evaluation_Heparin_data_survey_20101119.M5 (PLS), PLS prec diff
t[Comp. 1]/t[Comp. 2]
Colored according to Obs ID (Sample prep)

- ▲ DBS
- ▲ LLE
- ▲ PP
- ▲ PP + SPE
- ▲ SPE

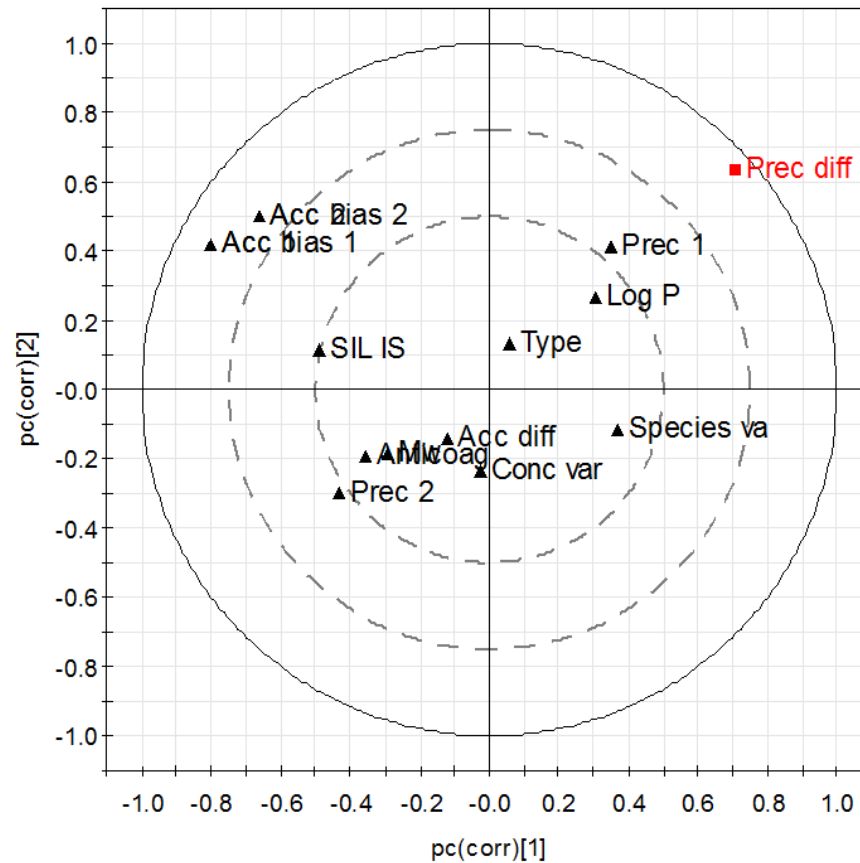


R2X[1] = 0.234553 R2X[2] = 0.103526
Ellipse: Hotelling T2 (0.95)

SIMCA-P+ 12.0.1 - 2010-11-19 10:35:21 (UTC+1)

Back-up slide 3b: PLS model

Evaluation_Heparin_data_survey_20101119.M5 (PLS), PLS prec diff
pc(corr)[Comp. 1]/pc(corr)[Comp. 2]
Colored according to model terms



R2X[1] = 0.234553 R2X[2] = 0.103526