Dear participants,

Firstly, we would like to thank each one of you for analysing the samples and submitting your results. In total, we received 35 sets of results from 32 laboratories, and we have analysed the samples ourselves on two different instruments. We have been evaluating some of the results, from first ten randomly selected laboratories so far, and thought that we would give you an update of how it is going.

When evaluating the performance of each semi-quantification approach across these ten laboratories, we have seen a general agreement of the errors, even though instruments of different types and from different vendors were used, see Figure 1A below. We could see that ionisation efficiency based approaches generally yielded lower prediction errors, while the close eluting approach yielded higher errors. We could also see more outliers when using the close eluting and *IE* 2 approaches compared to the other. We performed a Friedman test to see whether the mean errors for each approach was statistically different between the laboratories at 95% confidence level and could determine that at least one laboratory had a statistically different mean error in all matrices and dilutions.
Therefore, we compared the results from laboratories using orbitrap instrument and time of flight instrument, respectively, see Figures 1B and 1C. Visually, the results were more coherent using orbitrap instrumentation. This was also confirmed by Friedman test showing that for laboratories using orbitrap instrument, the mean errors for each approach were not statistically different as opposed to laboratories using ToF instrument. Generally, orbitrap yielded slightly lower prediction error than ToF instruments. On the other hand, orbitraps also yielded more outliers in total across the approaches, especially for the close eluting and *IE* 2 approaches. The outliers for orbitrap instruments were also shifted towards higher error than the outliers for ToF instruments.
In total, across all selected laboratories, approaches, and samples, 491 outliers were observed, with errors ranging over orders of magnitude, from approximately 11× to 2 000 000. All outliers corresponded to 37 unique compounds, out of total 41 possible suspects, however, some outliers were more striking than other. For example, those with extremely high prediction error or those that were labelled as outliers in multiple samples and/or approaches, and by multiple laboratories. One example is clotrimazole, which had prediction errors up to 70 000×. However, we have not yet seen any trend to this compound or other outliers as to whether they are always over- or underpredicted, but this is something we will investigate further.
Finally, a summary of errors for the investigated laboratories are shown in Table 1 below. As seen, the median errors are below 10× for all approaches, while the mean error looks not as good. Especially the structural similarity, close eluting, and *IE* 2 approaches have such high maximum errors which increases the mean error. All approaches have the majority of estimated concentrations within 10× error, with the ionisation efficiency based approaches seemingly performing better for more compounds, with over 80% estimations within 10×.

Table 1. Error summary for each approach, averaged over the ten evaluated laboratories.

Semi-quantification approach	Mean error	Median error	Maximum error	Percentage of error below 10×
Structural similarity	54×	4.6×	4 000×	70%
Parent – TP	25×	4.5×	470×	72%
Close eluting	4 700×	6.4×	150 000×	60%
***IE* 1**	15×	3.3×	990×	84%
***IE* 2**	76×	3.2×	7 900×	82%

There is still a lot of work left to do, including to compare the results from all laboratories, look at the raw data for peak verification, and maybe look for additional adducts in the raw data.
We would like to end this update with a reminder to answer the survey for those who have not done so yet. The survey can be found here and would provide us with valuable insights from all you participants. Finally, we would like to ask all of you to provide us with information on which processing software you used when integrating the peaks.

Once again, thank you for your participation.
We wish you all a nice summer.
On behalf of the organising team,
Louise Malm and Anneli Kruve

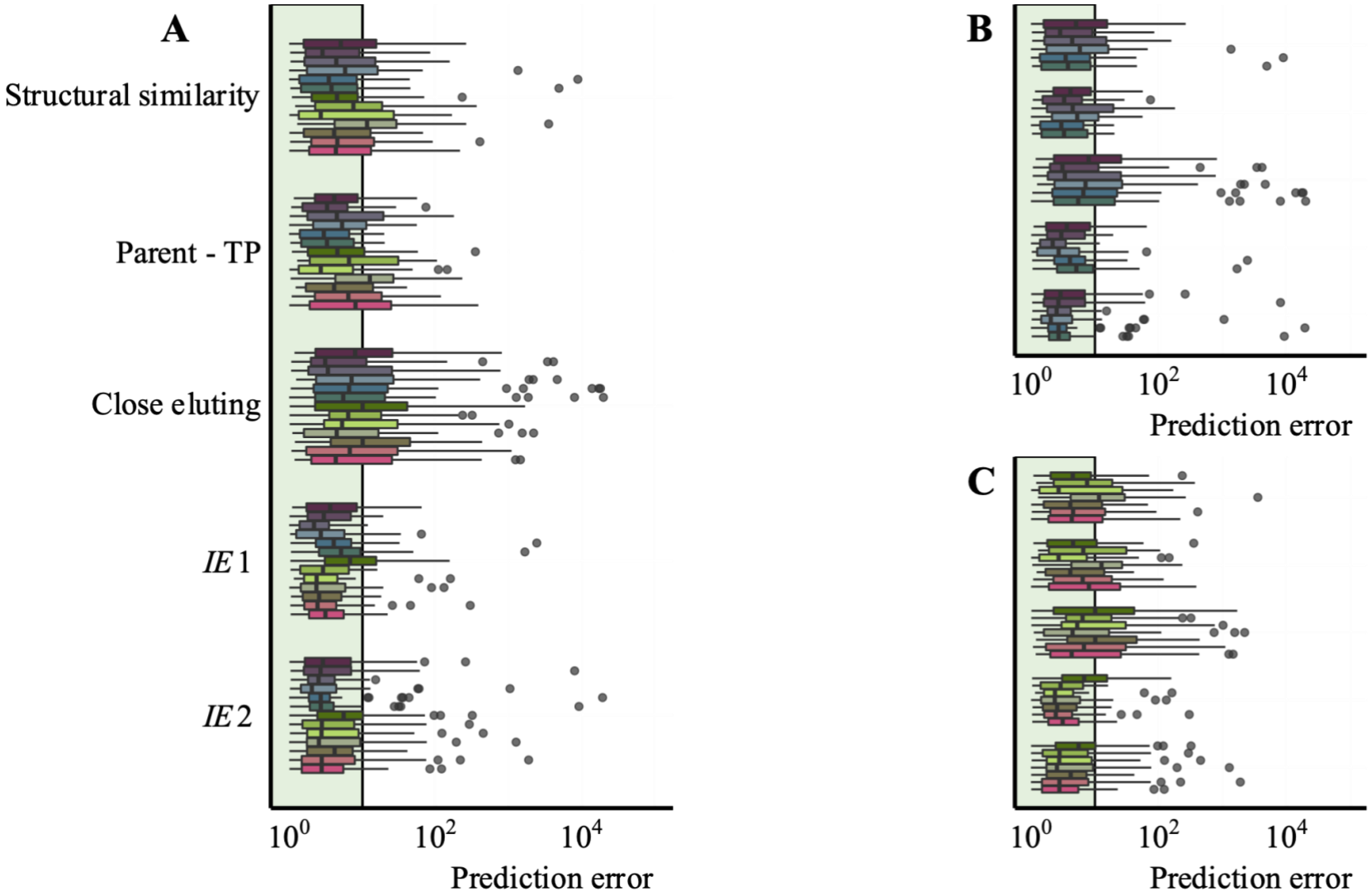

Figure 1. The results from ten investigated laboratories, presented as boxplots displaying the errors for each semi-quantification approach and laboratory, for one sample: the high concentrated HPLC water sample (S1a). **A** is showing the results for all ten laboratories (in total 13 results since three laboratories have two results). **B** is showing the comparison of laboratories using an orbitrap instrument (four versions of orbitrap instruments were used), and **C** is showing the comparison of ToF instruments (from four different vendors).