

# Strategies for the prevention of non-specific binding in the quantitative determination of drugs in cerebrospinal fluid

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## 1- ABSTRACT

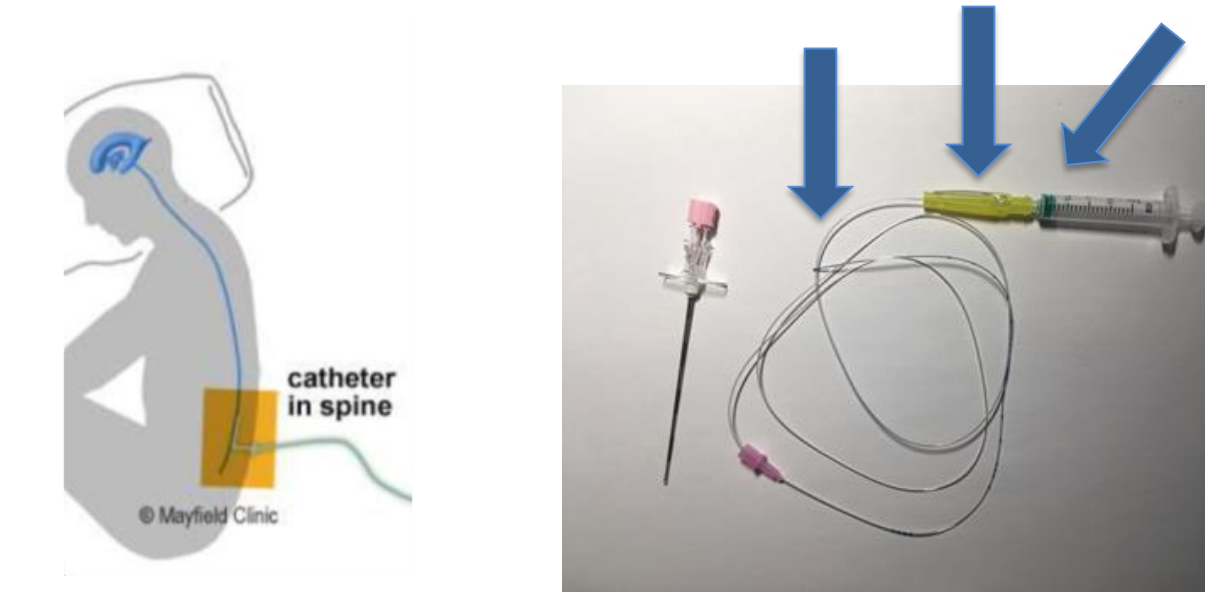
The last years there is an increasing interest in the analysis of drug candidates in cerebrospinal fluid (CSF). Sampling of CSF is done by means of a lumbar puncture or by serial sampling using a catheter. One of the main challenges with quantitative CSF analysis is the occurrence of non specific binding to the collection materials, as this leads to an underestimation of the results. The options for addition of stabilizers are limited as reagents are usually not allowed for invasive medical actions. In this poster we demonstrate strategies to prevent adsorption for the key CSF collection methods.

## 2- TYPES OF CSF COLLECTION

Lumbar puncture sampling, needle



Serial sampling, catheter



Blue arrows indicate places in which adsorption can occur.

Adsorption to tubing and needle can not be avoided: first drops will saturate the system and will be discarded.

## 3- NON SPECIFIC BINDING

Adsorption to the collection vial and pipette tip may lead to underestimation of the original concentration.

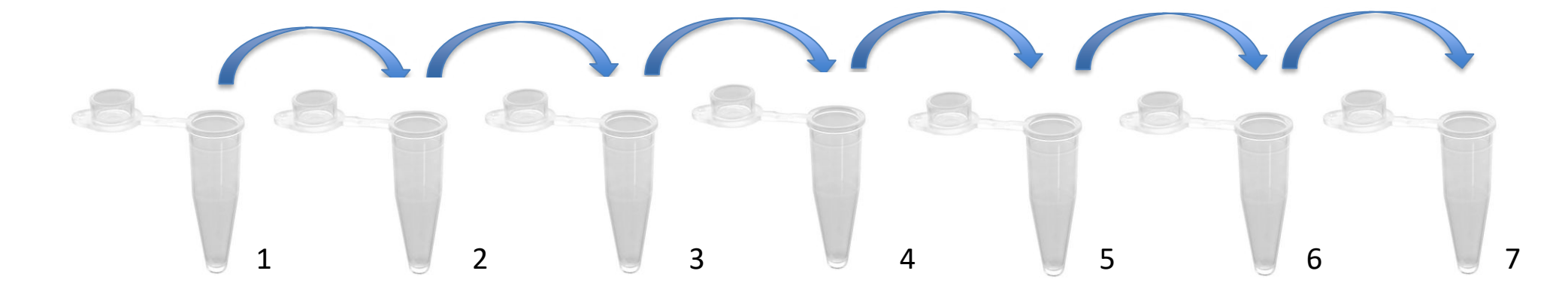


Adsorption occurs from matrix with low levels of suspended protein to bind to, such as CSF.

Matrix	Protein content
Plasma	70 g/L
CSF	0.5 g/L
Urine	<0.3 g/L

## 4-TESTING STRATEGY

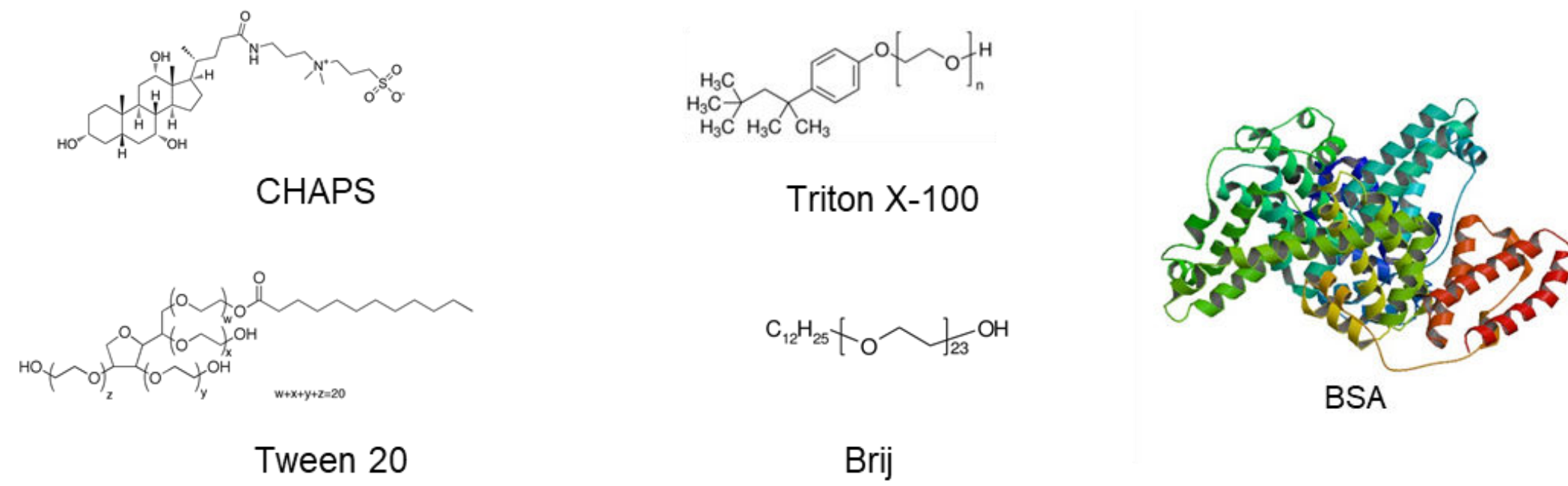
Transfer test: compound is spiked in matrix and transferred several times. All fractions are analyzed to study adsorption.



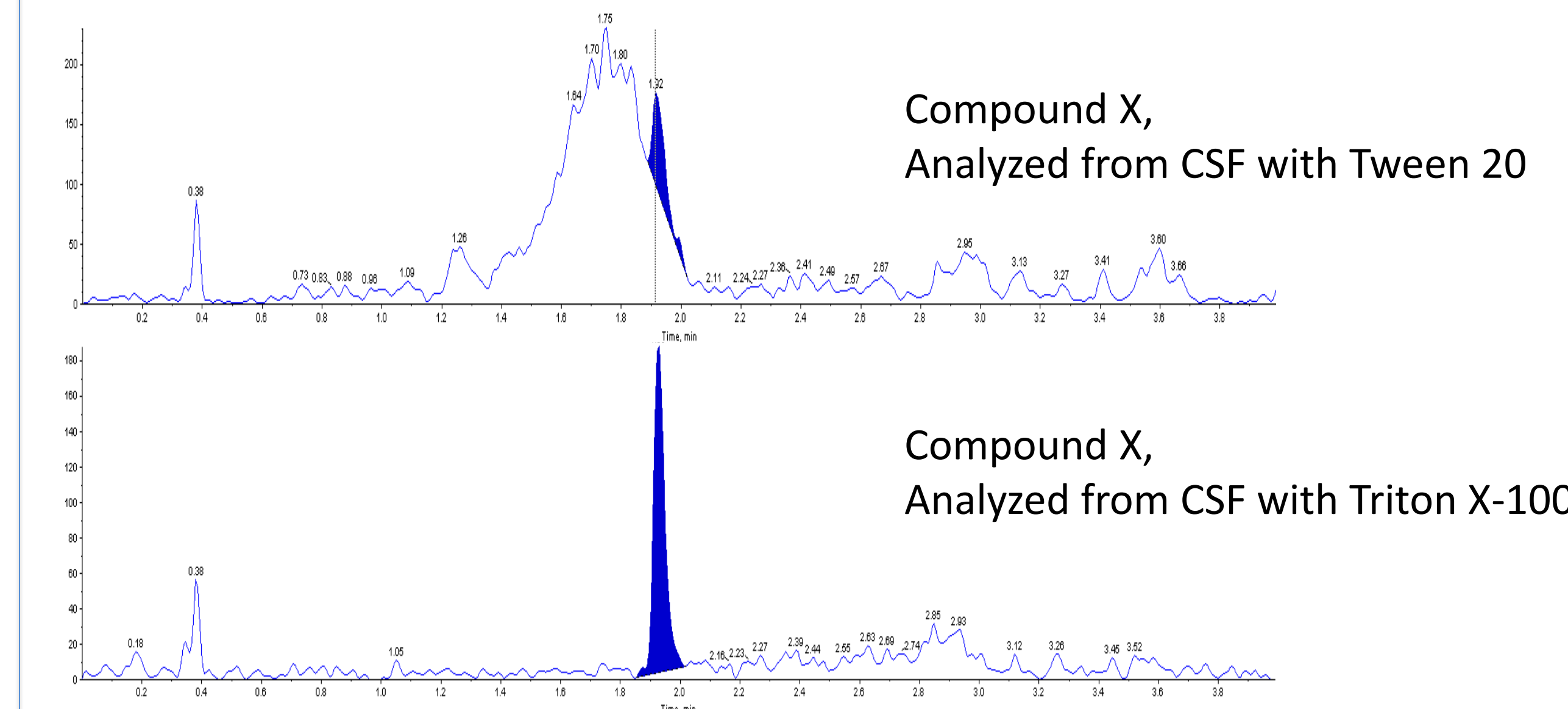
- Surface/volume ratio should mimic the real sample.
- Each tube needs to stand at room temperature >15 min.
- Adsorption might also occur in the pipet tip.
- Freezing can cause additional adsorption issues.

## 5- PREVENTION OF ADSORPTION

Below are the most often used stabilizers added to CSF for prevention of adsorption. Selection criteria are: ability to prevent adsorption, effect on the LC-MS method in terms of selectivity and sensitivity, price.



## 6- EXAMPLE INTERFERENCE



## 7- EXAMPLE

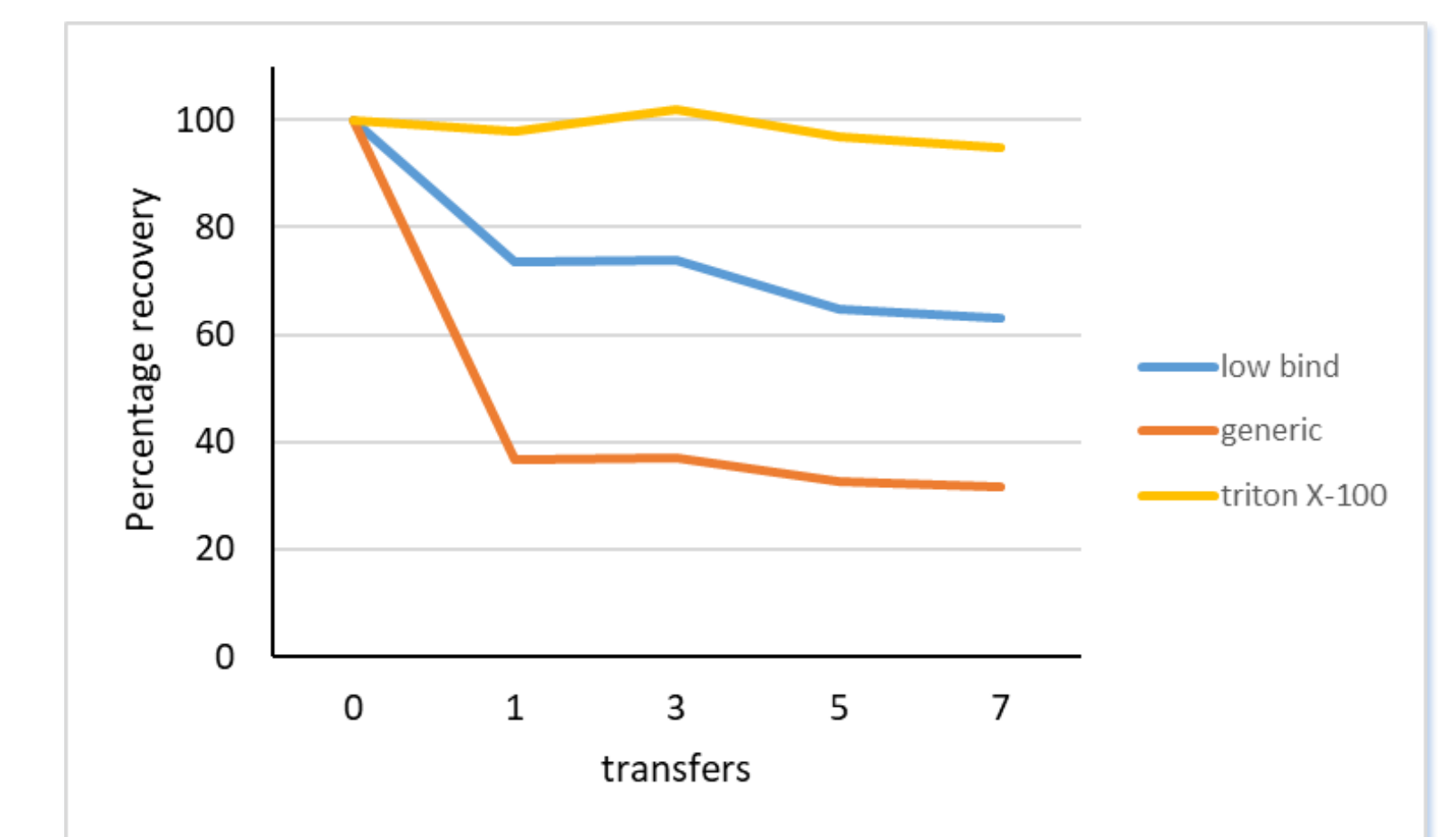
An LC-MS/MS method was developed for compound X in CSF to support a Phase 1 clinical study in which CSF was collected by both lumbar puncture and serial sampling.

Adsorption was tested using the strategy described in section 4 by spiking the compound at low QC level in CSF in normal tubes and low binding tubes and in CSF containing 0.2 v-% Triton-X100.

In addition, it was tested if adding the surfactant to spiked CSF would still completely redissolve the compound after 48 hours.

## 8- RESULTS

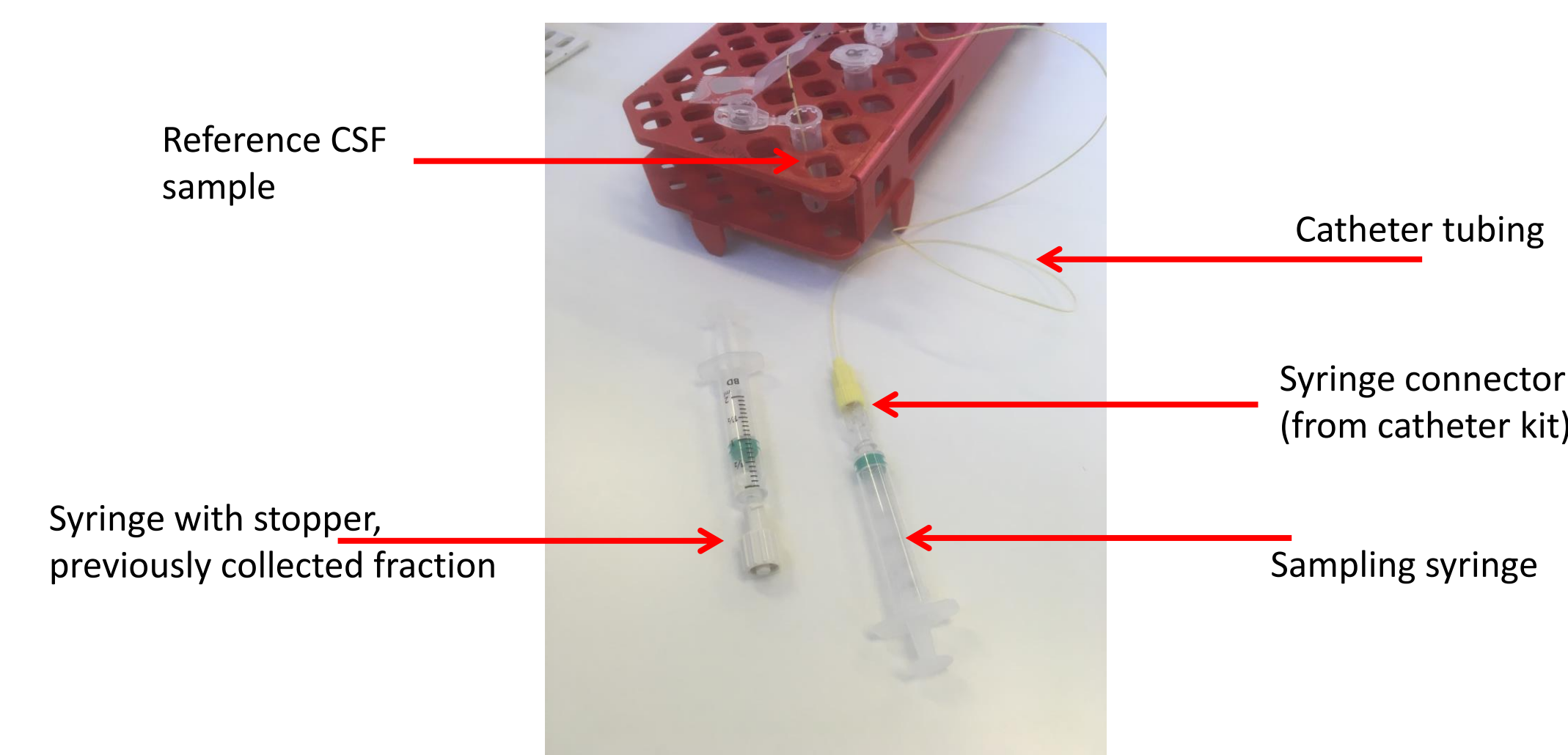
Transfer test for compound X



## 9- TESTING OF CATHETER AND NEEDLE

In order to test the adsorption of compound X in the catheter or needle, CSF spiked at the low QC level was transferred through the catheter to the collection syringe. The response was determined over subsequent fractions to determine the required waste volume before saturation of the catheter tubing. See section 10 for setup.

## 10- TESTING SETUP



## 11- STABILIZATION STRATEGY

- ▶ Discard a first CSF volume if catheter adsorption occurs; adsorption is assessed in validation.
- ▶ Collect sample volume.
- ▶ Transport vials (lumbar puncture) or syringes (serial) to the lab within 48 hours; period is assessed in validation.
- ▶ Weigh syringes or vials to determine CSF volume.
- ▶ Calculate and add volume stabilizer solution to the vial or the syringe.
- ▶ Correct for dilution caused by the addition of stabilizer to the CSF.

## 12- CONCLUSIONS

- ▶ Adsorption in sample container can result in underestimation of the actual concentration. This is typically encountered for CSF.
- ▶ Assessment strategy involves adsorption testing by transfers, selection of stabilizers, re-dissolving test and adsorption test of (transient) sample containers.
- ▶ Testing of the degree of adsorption and of contact time dependency needs to be done as early as possible, to allow strategies to be developed and shared with the clinical staff.
- ▶ Preparation of a clear clinical lab manual instructions and training of personnel are of key importance.

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