

VALIDATION ASSAYS IN WHOLE BLOOD, WHICH IS THE TARGET?

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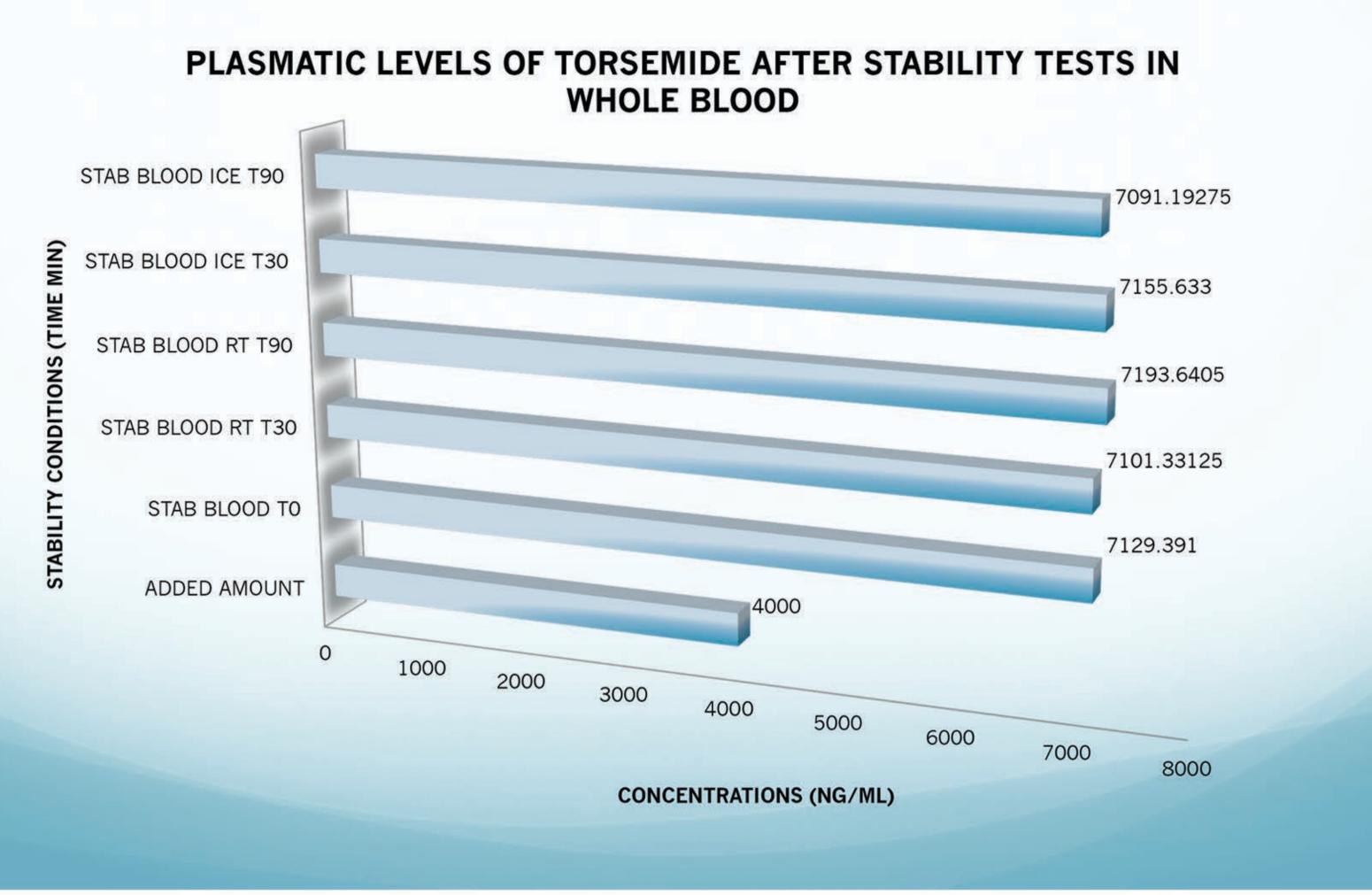
INTRODUCTION

- Validation experiments with analyte/s in whole blood are required by regulatory authorities but the aim can be difficult to understand. First of all after a compound is added in whole blood 3 important events took place:
- 1) Distribution between plasma and cellular compartment (red blood cells, white blood cells and platelets)
- 2) Metabolization/degradation, if exist, in plasma
- 3) Intracellular metabolization/degradation (if present).
- While points 2 and 3 are clear and an evaluation of these aspects is normally considered the target on whole blood assay validation, point 1 remains, in our opinion, neglected and the impact on final results underestimated.

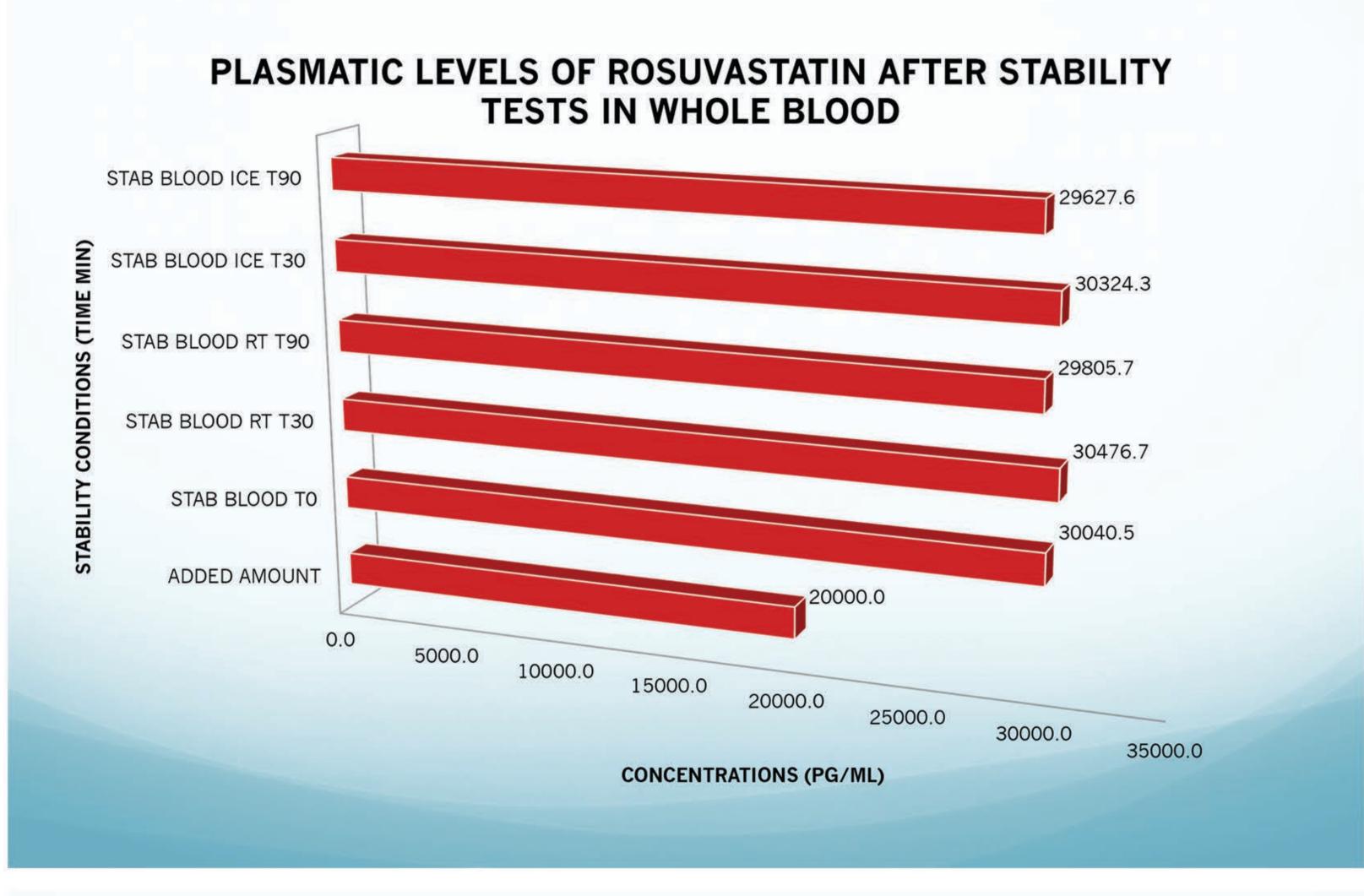
MATERIALS AND METHODS

- These validation experiments were carried-out as follows:
- 1) Aliquots of whole human blood freshly collected (within 6 hours) were incubated at 37C for 30 min with different drugs at concentrations close to Cmax
- 2) Samples were then splitted in 2 tubes and separately kept at room temperature or on ice
- 3) At 30 and 90 min (at room temperature or on ice) plasma was separated by centrifugation and plasmatic concentrations were determined by validated LC-MS/MS methods
- 4) Stability results were calculated comparing the concentrations in plasma after storage to the ones of the samples at the end of the 37C incubations
- 5) Experiments carried out on 6 drugs, 2 non penetrating in cells (NP compounds), 2 passively diffusing in cells (PD compounds) and 2 probably actively transported intracellular (AT compounds) are presented; it is important to note that all of them are not metabolized/degraded in whole blood.

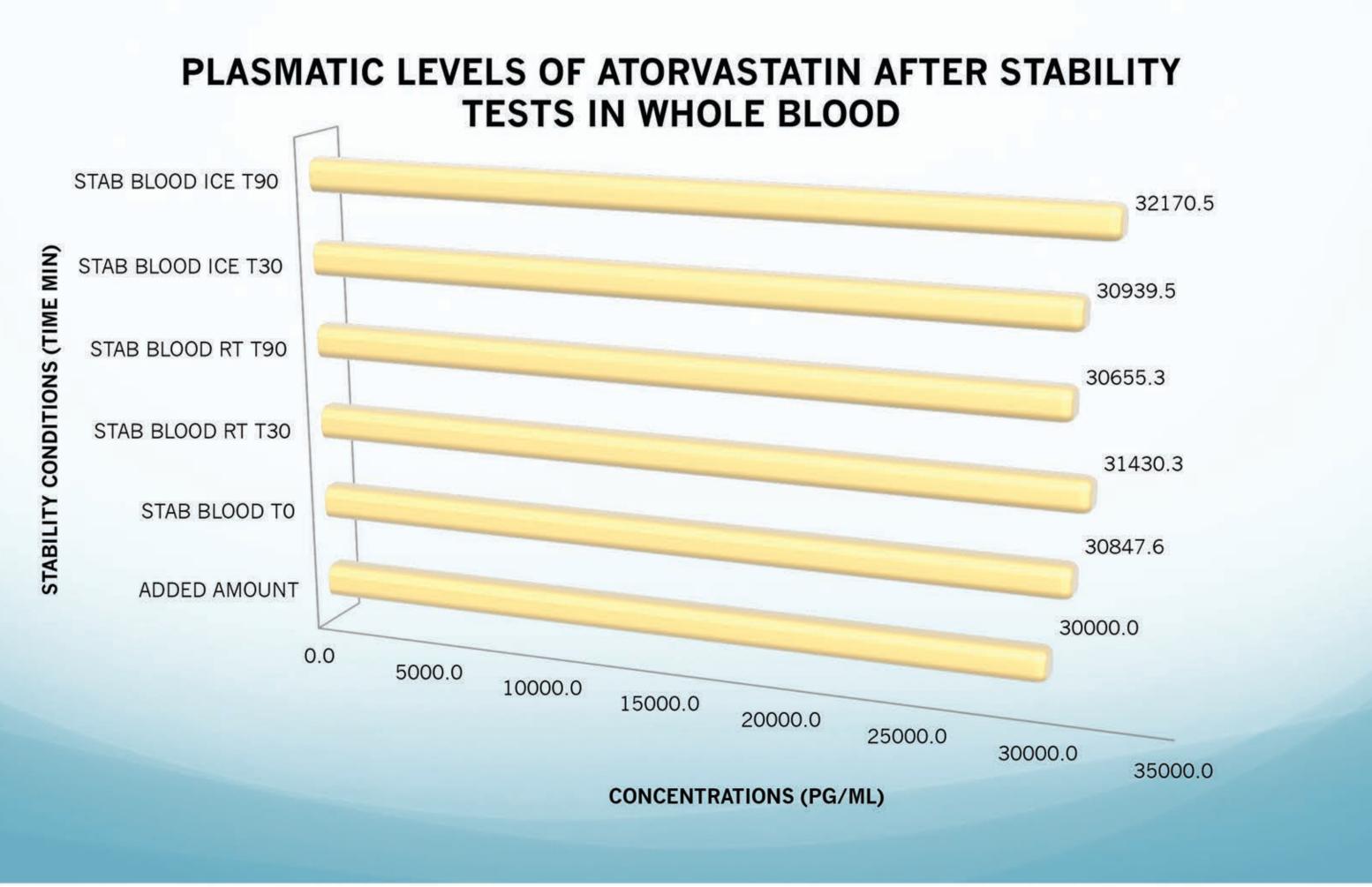
WHOLE BLOOD STABILITY OF A DRUG W/O INTRACELLULAR PENETRATION (NP) - TORSEMIDE



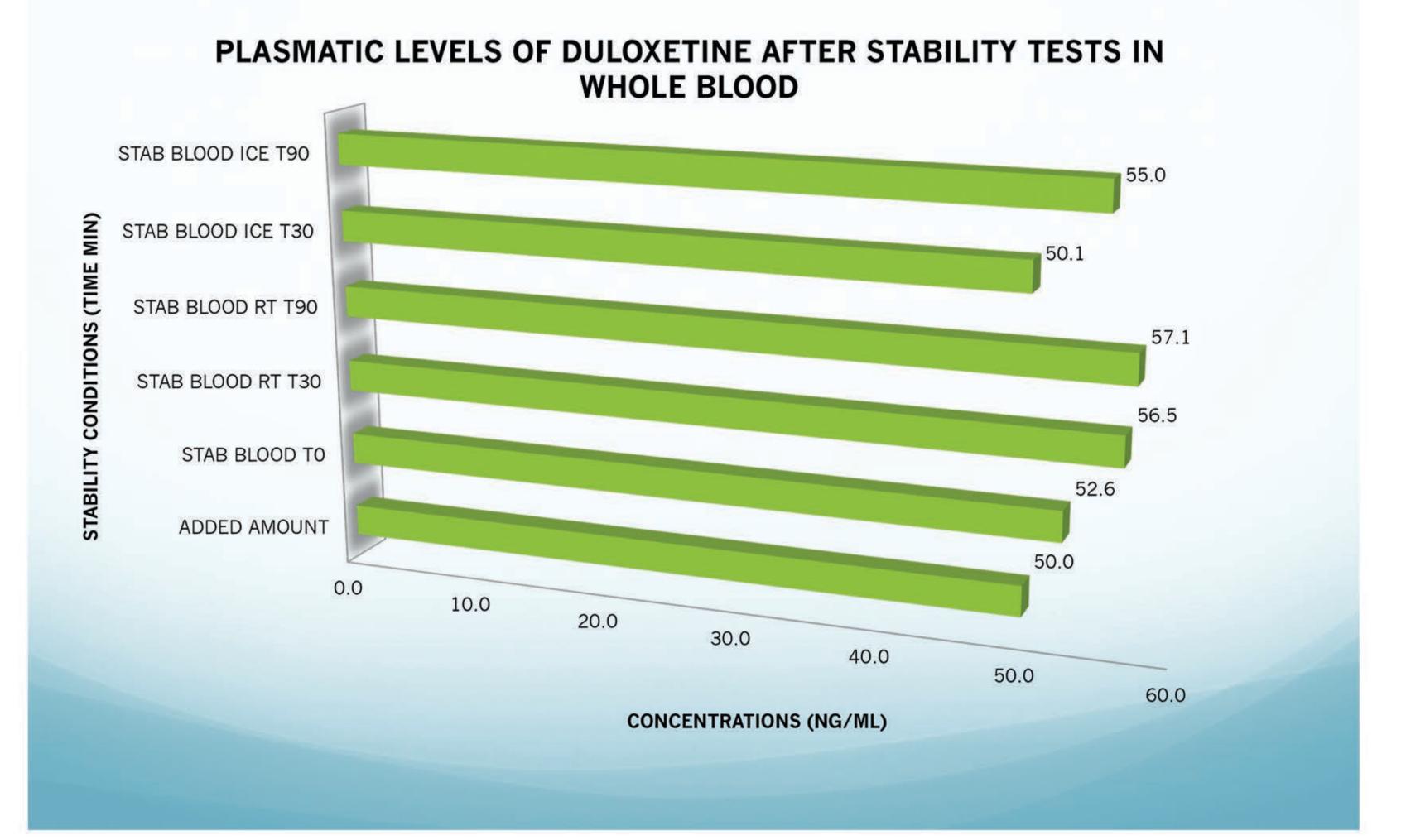
WHOLE BLOOD STABILITY OF A DRUGS W/O INTRACELLULAR PENETRATION (NP) - ROSUVASTATIN



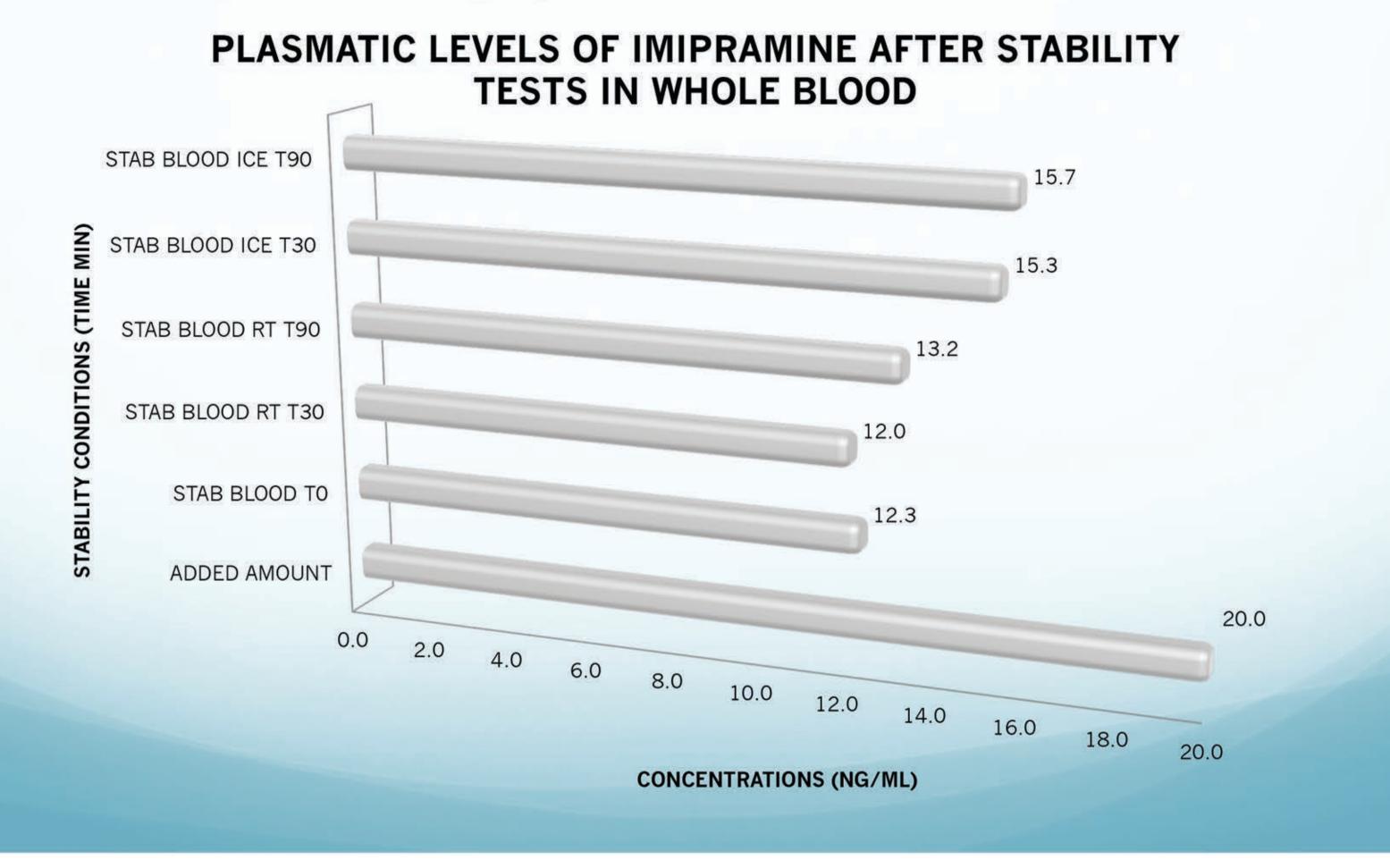
WHOLE BLOOD STABILITY OF A DRUG WITH INTRACELLULAR PASSIVE DIFFUSION (PD) - ATORVASTATIN



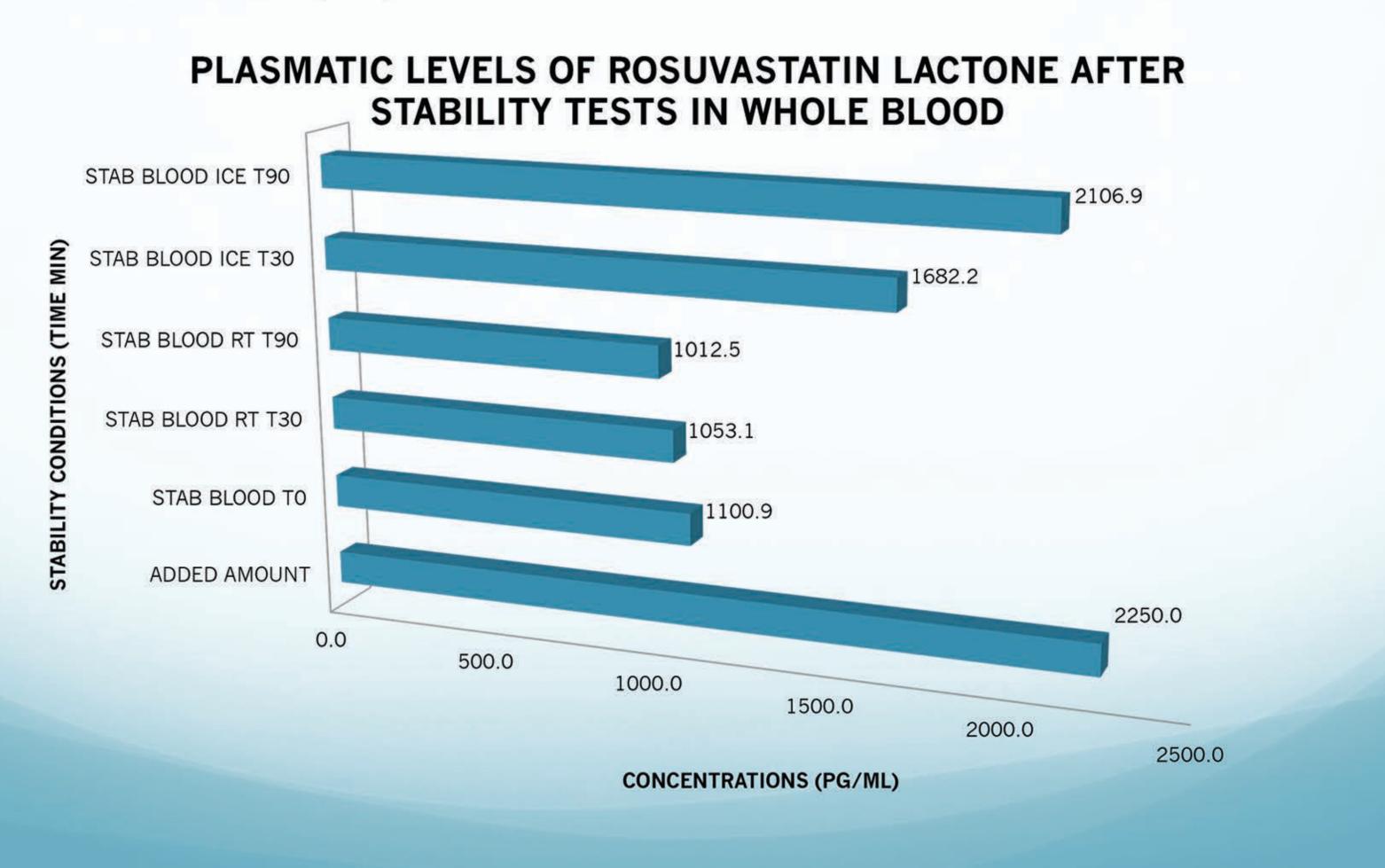
WHOLE BLOOD STABILITY OF A DRUG WITH INTRACELLULAR PASSIVE DIFFUSION (PD) - DULOXETINE



WHOLE BLOOD STABILITY OF A DRUG WITH POSSIBLE ACTIVE INTRACELLULAR TRANSPORT (AT) - IMIPRAMINE



WHOLE BLOOD STABILITY OF A DRUG WITH POSSIBLE ACTIVE INTRACELLULAR TRANSPORT (AT) – ROSUVASTATIN LACTONE



RESULTS

- The results with the 2 first drugs (NP compounds) are predictable and show plasmatic concentrations higher then blood spiking that can be estimated excluding the cellular volume. Sample handling conditions (time and temperature) didn't influence the results.
- With the 2 drugs of the PD group the plasmatic concentration are almost identical to the ones spiked in blood. Sample handling conditions (time and temperature) didn't influence the results.
- In the 3rd group (AT) relevant effects are observed with cold temperature storage, probably due to a reduction in the active transport mechanisms, bringing to a decrease in the intracellular levels of these drugs.

CONCLUSIONS

- In the previously reported experiments it can be observed that the commonly used approach to rapidly cool blood samples can significantly alter the real plasmatic concentrations of such compounds changing the blood partition between plasma and cells
- This fact, altering the plasmatic concentrations, can introduce an unexpected variable when performing PK and bioequivalence studies.
- It seems therefore important to draw attention on this phenomenon and to consider it in future validation to really understand the best conditions of blood sample handling until freezing.

