

BACKGROUND

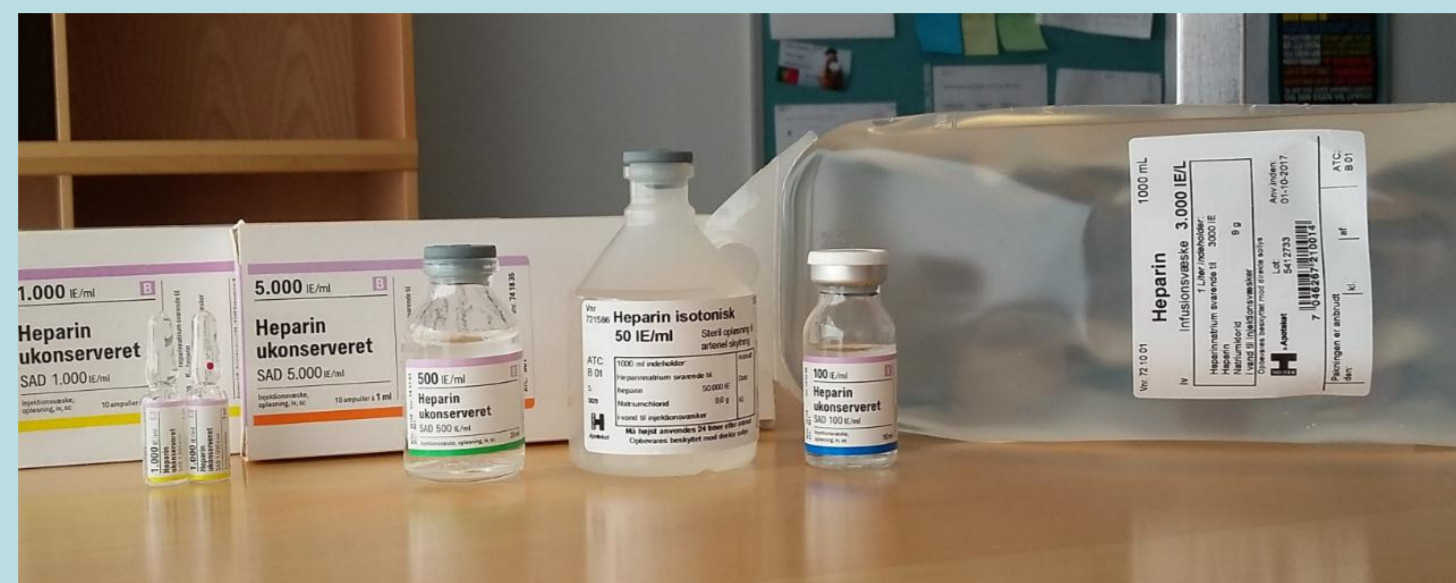


Fig. 1. Assortment of ready to use heparin IV solutions from the Capital Region Pharmacy. Licensed products with a colored label, magistral black and white.

Danish hospital pharmacies have by tradition produce and deliver a broad range of magistral and licensed preparations for hospital use. Among these products are unfractionated Heparin formulations as injectables presented as ready to use preparations reducing the manipulations by nurses in clinical area for patient safety reasons. By January first 2015 a new Assay of Heparin was adopted in the European Pharmacopoeia. The method was implemented as general tool in the Quality Control department, being the only biological assay used in the laboratory. The assay, carried out by a laboratory robot, is far more accurate than the old Ph. Eur.. This enabled us to study the decay of heparin activity due to autoclaving at 120°C for 30 minutes, our favorite method of sterilization.

MATERIALS AND METHODS

The assay is carried out according to the description in the monograph. For the sample/reagents manipulations and final absorption measurements a lab robot ACL TOP 300 from ILS was used. Tolls for the statistical evaluation were Combistat a program mentioned and presented in Ph. Eur. describing the Parallel Line Assay group of evaluations. Reagents are bought from Provision Kinetics, Arlington, Wisconsin, USA. Standard: Heparin Sodium BRP 1000 IU/ml, Ph. Eur. from EDQM. Heparin IV solutions are formulated without preservations only added WFI and sodium chloride to give isotonic solutions. A standard production autoclave from Geringe Sweden performed the sterilization process.



Fig. 2. The ACL TOP 300 robot for used for the heparin assay, Assay of Heparin Ph. Eur. 2.7.5..

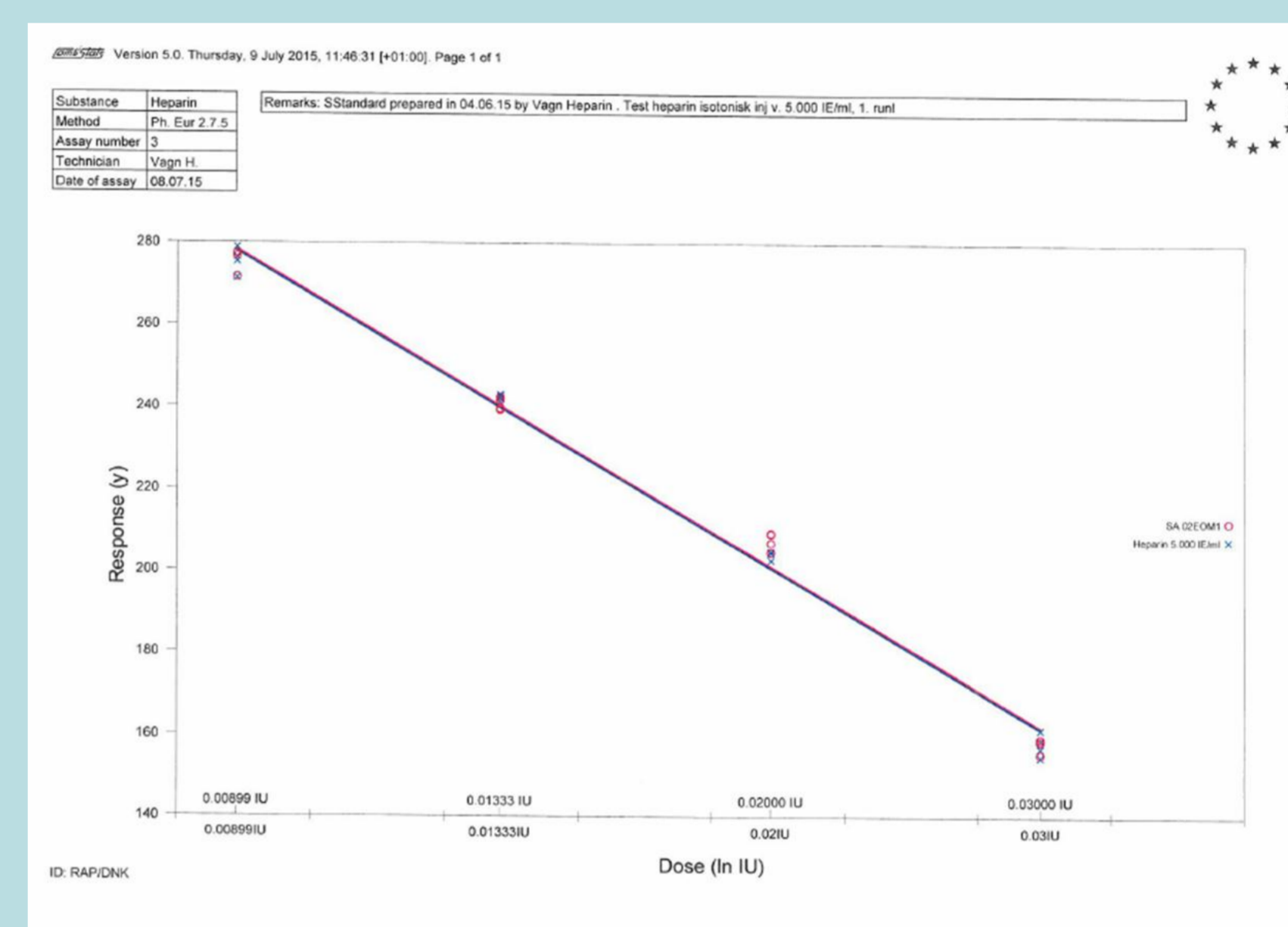


Fig 3. Assay of a 5.000 IU/ml heparin IV solution.

RESULTS

The assay.

An example to illustrate the accuracy of the assay is given in the table of fig. 4. The potency was 100,1%, standard deviation: 0,9%. Ph. Eur. Ph. Eur. limit for the assay: 90 – 110%! By dilution of the sample, the core of the assay is always carried out at the same concentration range 0,03 – 0,009 International Units/ml. Evaluation of the statistical validity of the assay according to the Ph. Eur. must be done using a.o. the probability for “Non parallelism” and “Non linearity” both factors being > 0,05. These factors has a strong tendency not to fulfill this demand. A close evaluation has shown that the deviation from the limit is due to the high accuracy of the method being far more precise than the old Ph. Eur. method based on the coagulation of sheep blood. The use of a robot has further increased the reproducibility of the many manipulations. Ph. Eur. Is informed about the problem.

The influence of sterilization.

Potency of a IV “Heparin 100 IU/ml “ nominal potency before autoclaving 108 IU/ml had a potency of 95,6 IU/ml (n=5) after sterilization. The decay in potency was 11% allowing us to add a surplus of 11 % to the product in the preparation process. The lethality of the autoclaving process, F_0 , was 34 at 120°C.

Results Overview for Heparin BRP 1000 IU					
Potency (IU/ml)			Potency (%) relative to assay		
Lower limit	Estimate	Upper limit	Lower limit	Estimate	Upper limit
991,5	1002,6	1013,7	99,1	100,3	101,4
983,0	997,6	1012,3	98,3	99,8	101,2
988,3	1010,9	1033,9	98,8	101,1	103,4
984,3	1001,2	1018,4	98,4	100,1	101,8
988,2	1006,1	1024,3	98,8	100,6	102,4
963,7	985,2	1007,2	96,4	98,5	100,7
983,2	1000,6	1018,3	98,3	100,1	101,8
10,03	8,79	9,58	1,00	0,88	0,96
1,02	0,88	0,94	1,02	0,88	0,94

Fig. 4. Result of 6 samples of Heparin BRP 1000 IU/ml

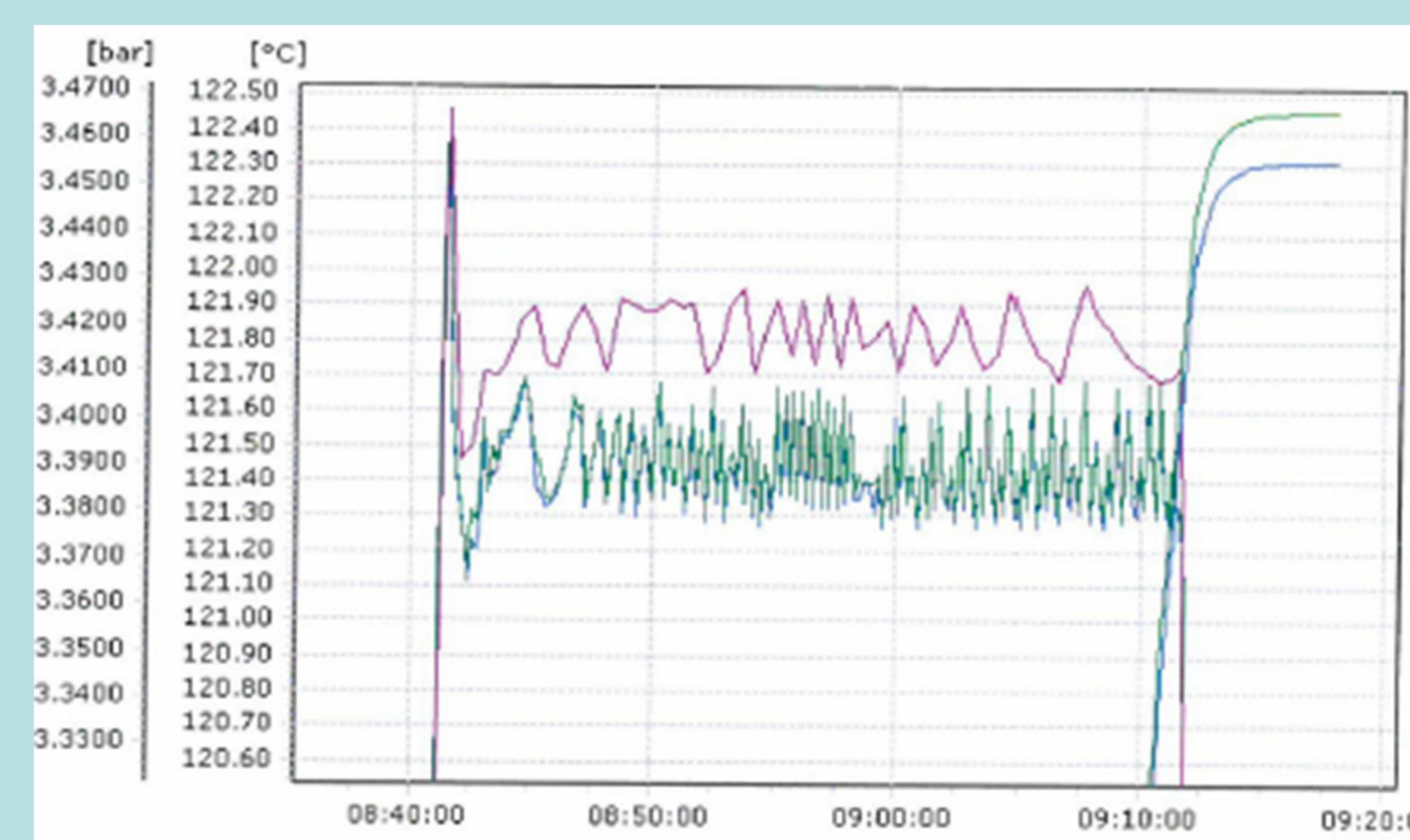


Fig. 5. Steam sterilization graph of temperatures. Temperature highest point in the load (violet) and lowest (green). F_0 34.

CONCLUSION

The project has shown the successful implementation of the recent Ph. Eur. 2.7.5 assay of unfractionated heparin done by the staff of a European Hospital Pharmacy. The implemented assay has the same robustness and precision as the classic physico- chemical methods used in a quality control of the hospital pharmacy. It has further been shown, that heparin IV solutions can be sterilized by a standard steam sterilization method, if a surplus of 11% heparin as the active ingredient is added to the product during manufacturing.

The outcome of the project has also shown the successful collaboration between individuals from Portugal and Denmark.