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**Dissertations-Kurzfassung**

**Determination of the non-vitamin K antagonist oral anticoagulants apixaban and dabigatran in plasma, serum and urine samples**

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The non-vitamin K antagonist oral anticoagulants (NOACs) dabigatran and apixaban were developed to overcome drawbacks of vitamin-K antagonists (VKAs) which require routine coagulation monitoring. Clinical studies were performed for the development of these NOACs for various indications without generating laboratory values on coagulation parameters or without developing new methods for their determination.

The following topics were investigated in the project: 1. Determination concentrations of the NOACs dabigatran and apixaban in plasma samples by chromometric and chromogenic methods. 2. Concentrations of dabigatran and apixaban in serum and urine samples in vitro and ex vivo were analysed by chromogenic methods. 3. Comparison of results obtained from serum samples with those obtained from plasma samples. 4. Dabigatran and apixaban in urine samples were assessed by a point of care method (POC) which qualitatively determined the presence or absence of the NOACs by identification of a colour by the naked eyes.

Methods for dabigatran in plasma samples were: chromogenic direct thrombin inhibitor assay (DTI), S2238 chromogenic assay, prothrombin time (PT), activated partial thromboplastin time (aPTT), prothrombinase induced clotting time assay (PiCT), Hemoclot (HTI), Ecarin clotting time (ECT) and Heptest. Methods for apixaban were: chromogenic tests Coamatic, HemosIL, S2222, and clotting coagulation assays PT, aPTT, and Heptest. In serum and urine samples the chromogenic assays were adopted for both NOACs.

The mean concentrations of dabigatran in plasma and serum samples from patients were  $113.5 \pm 84.3$  ng/mL and  $71.1 \pm 67.7$  ng/mL using the S2238 method and  $143.1 \pm 102.0$  ng/mL and  $23.6 \pm 21.0$  ng/mL with the DTI chromogenic method, respectively. Concentrations in serum were significantly lower compared to plasma samples ( $p < 0.0001$ ). HTI and ECT showed the best correlation ( $r = 0.9061$ ) among those assays. Correlations for PT, aPTT or PiCT with HTI or with ECT were low ( $r$  between 0.4798 and 0.6755). Concentrations in urine were  $5641.6 \pm 4319.7$  ng/mL and  $4730.0 \pm 3770.2$  ng/mL by using the S2238 and DTI chromogenic assays, respectively.

During treatment with apixaban concentration in serum was  $282.1 \pm 144.5$  ng/mL and in plasma  $199.1 \pm 66$  by using the Coamatic assay which were similar to the results obtained by HemosIL and S2222 chromogenic assays (all  $p < 0.0001$ ). Variances of the three assays were lower using plasma compared to serum samples (all  $p < 0.0001$ ). Correlations of plasma and serum samples were high and ranged between  $r = 0.7883$  and  $r = 0.93778$  for the three chromogenic assays.

Patients identified the colour of prepared urine samples from patients on treatment with the NOACs. The sensitivity, specificity, accuracy, positive predictive value, and negative predictive values of the POC tests for dabigatran or apixaban were all above 95% (mean values). Additional studies showed no interaction of dabigatran and apixaban on the qualitative POC methods in the presence of heparin, low-molecular weight heparin and fondaparinux if antithrombin was not added to urine samples. Hirudin and argatroban may interfere with the POC dabigatran method.

The results indicate that apixaban concentrations can be determined specifically from serum samples. Dabigatran determinations in serum samples demand improvements because of the high variability and less sensitivity. The high precisions, sensitivities and accuracies of POC methods proved that POC methods can accurately assess urine samples from patients by qualitative determination of the presence of the NOAC dabigatran and apixaban.