Measuring Rivaroxaban activity: Use of a well established chromogenic anti FXa method

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Introduction
Rivaroxaban is an oral, direct Factor Xa inhibitor that can be used at a fixed dose without monitoring, given its predictable pharmacokinetics and pharmacodynamics, and low propensity for food and drug interactions.

In order to measure compliance with rivaroxaban, or in the event of severe overdose, quantitative determination of rivaroxaban plasma concentrations would be helpful and the use of a well-established assay would be the preferred option.

The commercially available COAMATIC® Heparin (Chromogenix Instrumentation Laboratory, Milan, Italy) assay was developed for use with automated clinical laboratory instruments and is based on the principle of one-stage heparin detection. The assay contains a Factor Xa reagent and a chromogenic substrate.

Objective
To determine the suitability of the COAMATIC® Heparin assay as a method for monitoring the pharmacodynamic effects of rivaroxaban.

Methods

- Pooled normal plasma was mixed with an appropriate solution of rivaroxaban dissolved in 100% dimethyl sulphoxide (DMSO) to achieve standard plasma (containing rivaroxaban 500 ng/ml).
- Further dilutions of the standard plasma with pooled normal plasma produced calibration plasma for a concentration range of rivaroxaban of 0–500 ng/ml.
- Following incubation of the plasma samples or calibrators with the chromogenic substrate (S-2732; Chromogenix Instrumentation Laboratory) for 120 seconds, the reaction was initiated by the addition of an excess of the Factor Xa.
  - Hydrolysis of the substrate by Factor Xa resulted in the release of the yellow dye para-nitroaniline.
  - The change in optical density due to the release of para-nitroaniline was measured at 405 nm.

Results

- A preliminary evaluation on a microtiter plate (manual method) showed a good correlation between rivaroxaban concentrations (0–500 ng/ml) and the optical density (Figure 1).
- The results obtained with the manual endpoint method were then applied to the automated coagulation analyzer ACL TOP® (Instrumentation Laboratory, Lexington, Massachusetts, USA), using the settings of COAMATIC® Heparin for the measurement of low molecular weight heparins.

Two concentration ranges of rivaroxaban were studied in order to achieve linear calibration curves:
  - In the low range (0–63 ng/ml), calibrators and samples were used undiluted, and the r² value of the calibration curve was 0.9915 (Figure 2).
  - In the high range (31–500 ng/ml), calibrators and samples had to be prediluted 1:5 with normal pooled plasma (to avoid consumption of reagents during the reaction), and the r² value of the calibration curve was 0.9954 (Figure 2).

- To assess the precision of the automated method, rivaroxaban spiked in plasma was measured in six series on 3 consecutive days, and the coefficient of variation over all series was 4.11%, 10.73%, and 4.33% for plasma spiked with rivaroxaban 30, 100, and 300 ng/ml, respectively (Table 1).

- To test for accuracy, the actual concentration of rivaroxaban in spiked plasma was determined by high-performance liquid chromatography–mass spectrometry (HPLC–MS). The concentration of rivaroxaban in unknown spiked samples as measured by HPLC–MS was 31.1, 101.5, and 314.4 ng/ml for plasma spiked with rivaroxaban 30, 100, and 300 ng/ml, respectively (Table 1).

- The linearity of the automated method was tested in both the low and the high ranges by measuring dilutions of a spiked sample (Figure 3):
  - In the low range (0–63 ng/ml, without predilution), the measured rivaroxaban values in the spiked samples correlated highly with the expected values (r²=0.9997; Figure 3).
  - In the high range (31–500 ng/ml, with predilution), there was also a linear correlation between the obtained values and the expected values (r²=0.9967; Figure 3).

Conclusions

- The well established commercially available COAMATIC® Heparin assay is suitable for measuring plasma levels of factor Xa inhibitor rivaroxaban in the range of 0–500 ng/ml.
- The method needs to be transferred to other automated coagulation instruments to validate the suitability of this assay.

References
4. COAMATIC® Heparin. Product Monograph is available online at: www.chromogenix.com