

# Suitability of a Well-Established Chromogenic Anti-Factor Xa Assay for Measuring the Pharmacodynamics of Rivaroxaban

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## Introduction

◆ Rivaroxaban is an oral, direct Factor Xa inhibitor that concentration-dependently inhibits Factor Xa activity<sup>1</sup>

– Phase III studies of rivaroxaban have recently been completed for the prevention of venous thromboembolism in adults after total hip and knee replacement;<sup>2-5</sup> rivaroxaban is also in advanced clinical development for other thromboembolic disorders

◆ Rivaroxaban was found to have predictable pharmacokinetics and pharmacodynamics, and a low propensity for food–drug and drug–drug interactions, and therefore can be used at a fixed dose without the need for routine coagulation monitoring<sup>6</sup>

◆ A quantitative determination of rivaroxaban might be valuable in some cases, such as severe overdose or to measure compliance

## Objective

◆ To evaluate the suitability of the commercially available Coamatic<sup>®</sup> Heparin (Chromogenix Instrumentation Laboratory, Milan, Italy) assay to detect rivaroxaban across a broad range of plasma concentrations

## Methods

◆ The Coamatic Heparin assay used in this study was designed following the principle of one-stage heparin detection for use on automated clinical laboratory instruments<sup>7</sup>

◆ Standard plasma (containing rivaroxaban 500 ng/mL) was obtained by mixing pooled normal plasma with an appropriate solution of rivaroxaban dissolved in dimethyl sulfoxide (DMSO)

- ◆ Calibration plasma was produced for a concentration range of rivaroxaban of 0–500 ng/mL through further dilutions of the standard plasma with pooled normal plasma
- ◆ After 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 bovine 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 using a microtiter plate (manual method) showed a good correlation between rivaroxaban concentration (0–500 ng/mL) and optical density (Figure 1)

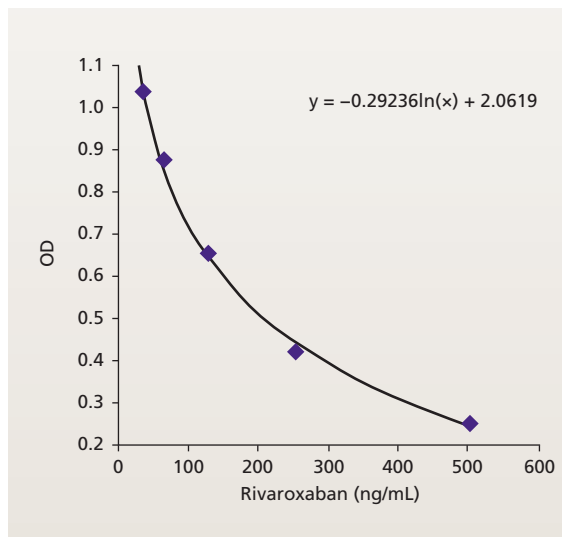


Figure 1. Manual method: correlation between rivaroxaban concentration (0–500 ng/mL) and optical density. OD, optical density.

- ◆ The results obtained with the manual endpoint method were then applied to the automated coagulation analyzer ACL TOP<sup>®</sup> (Instrumentation Laboratory, Lexington, MA, 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  $r^2$ -value of the calibration curve was 0.9915 (Figure 2A)
- In the high range (31–500 ng/mL), calibrators and samples had to be prediluted 1:5 with pooled normal plasma (to avoid consumption of reagents during the reaction), and the  $r^2$ -value of the calibration curve was 0.9954 (Figure 2B)

- ◆ 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. The concentration of rivaroxaban in unknown spiked samples 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 ( $r^2=0.9997$ ; Figure 3A)

- In the high range (31–500 ng/mL, with predilution), there was also a linear correlation between the obtained values and the expected values ( $r^2=0.9967$ ; Figure 3B)

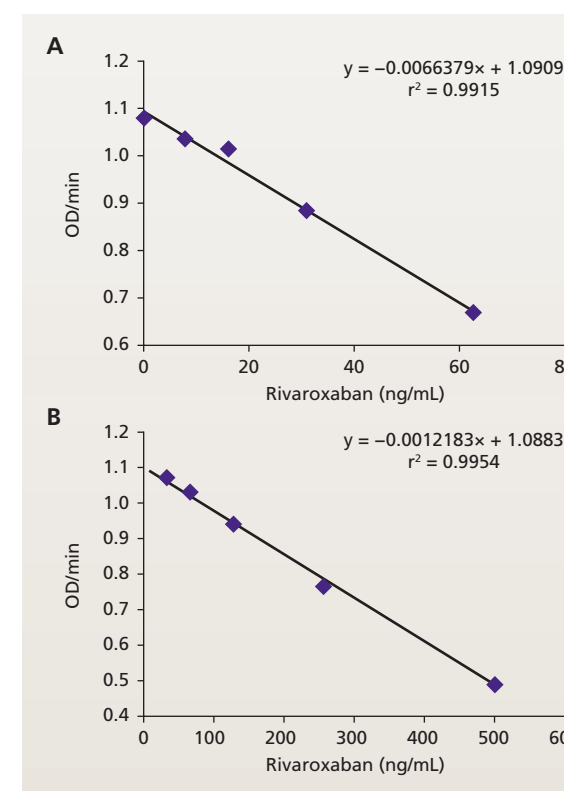


Figure 2. Automated method: calibrations for the (A) low (0–63 ng/mL) and (B) high (31–500 ng/mL) ranges of rivaroxaban concentrations. OD, optical density.

Table 1. Precision and accuracy of the automated method

Rivaroxaban concentrations in spiked plasma		30 ng/mL	100 ng/mL	300 ng/mL
Precision between series	Mean	28.06	79.80	310.39
	Standard deviation	0.80	7.15	5.52
	Coefficient of variation (%)	2.84	8.96	1.78
Precision over all series	Mean	28.06	79.80	310.32
	Standard deviation	1.15	8.57	13.45
	Coefficient of variation (%)	4.11	10.73	4.33
Values obtained by high-performance liquid chromatography–mass spectrometry		31.06	101.46	314.44

Table 2. Influence of DMSO on optical density

		OD/min				Mean
DMSO 0%		1.114	1.116	1.113	1.117	1.116
DMSO 1%		1.111	1.111	1.116	1.112	1.111
DMSO 2%		1.109	1.110	1.111	1.108	1.108

DMSO, dimethyl sulfoxide; OD, optical density.

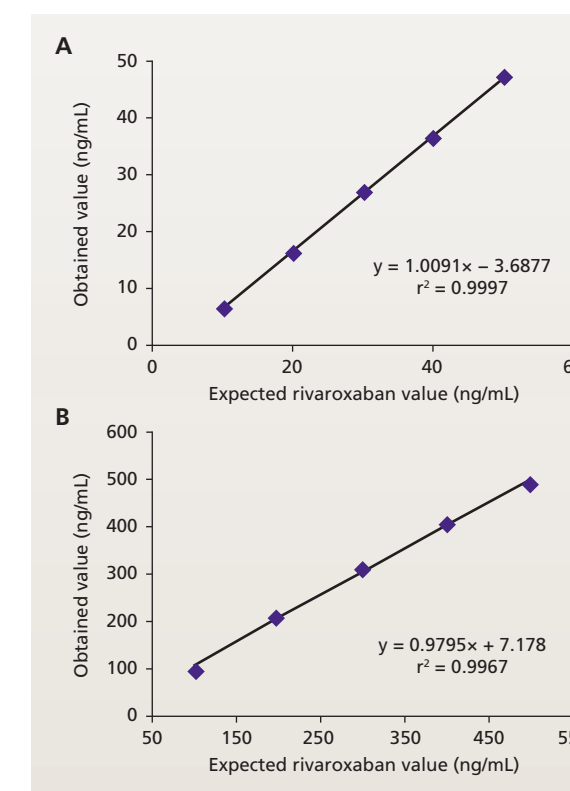


Figure 3. Linearity of the automated method for the (A) low (0–63 ng/mL) and (B) high (31–500 ng/mL) ranges of rivaroxaban concentrations.

## Conclusion

◆ The well-established commercially available Coamatic Heparin assay is suitable for measuring plasma levels of rivaroxaban in the range of 0–500 ng/mL

- ◆ Because rivaroxaban was dissolved in 100% DMSO and further diluted with pooled normal plasma (0.16% DMSO for rivaroxaban standard 500 ng/mL; less for lower concentrations of rivaroxaban, due to dilution with plasma), the influence of DMSO on the Coamatic Heparin method was also tested

- Pooled normal plasma was compared with pooled normal plasma containing 1% and 2% DMSO

- There was a small change of 0.003–0.004 optical density units with DMSO, corresponding to an overestimation of approximately 4 ng/mL in plasma with 1% DMSO (Table 2)

- Because the highest concentration of DMSO used was 0.16%, the influence of DMSO on the Coamatic Heparin assay is not considered to be significant

## References

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## Disclosure of conflict of interest

This study was supported by Bayer Schering Pharma AG and Johnson & Johnson Pharmaceutical Research & Development, L.L.C. The data contained within this poster do not support or recommend the use of rivaroxaban in indications or countries in which it is not licensed.

Presented at the International Society on Thrombosis and Haemostasis (ISTH) XXII Congress, Boston, MA, USA; July 11–16, 2009