

Establishment and Clinical Evaluation of a Novel Method to Measure Apixaban in Patients with Atrial Fibrillation Using Dried Blood Spot Sample Collection



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Introduction

Apixaban is a non-vitamin K oral anticoagulant (NOAC) which specifically inhibits factor Xa. Apixaban concentration in patients' plasma can vary with disease status and concurrent medications, thus concentration monitoring should be considered to ensure its safety and efficacy. Dried blood spot (DBS) sampling strategy gained high attentions in recent years because of the growing importance of personalized medicine. We have established a novel method to measure apixaban concentration in DBS.

Material and Methods

The postcolumn infused-internal standard (PCI-IS) method was adapted for correction of matrix effect in liquid chromatography electrospray ionization mass spectrometry and estimation of blood volume on DBS cards. Paired plasma and DBS samples collected from patients with atrial fibrillation were used to establish the correlation between plasma and DBS concentrations.

Results

Under optimal conditions, apixaban could be quantified within 5min. Validation results indicated the accuracies of the method were within 93.3-97.0 % and intra-day and inter-day precisions were all below 10.0% for four concentration levels. The correlation analysis also showed good linear relationship between DBS and plasma concentrations with the Pearson correlation coefficient (R^2) being 0.970. The conversion factor was calculated from the ratios between plasma and DBS concentrations and over 90% of the calculated concentrations were within the 95% confidence interval (CI) of bias using the conversion factor for concentration estimation.

Conclusion

This novel DBS concentration measurement method represented as an efficient and effective strategy for monitoring apixaban concentration in patients which could benefit personalized medicine for apixaban therapy.

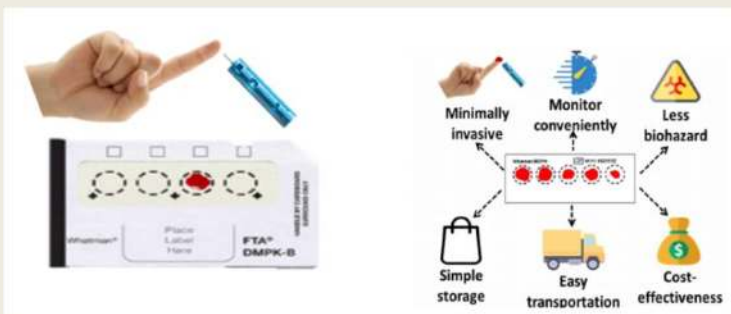


Figure 1. Concept and application of DBS in measuring drug concentration

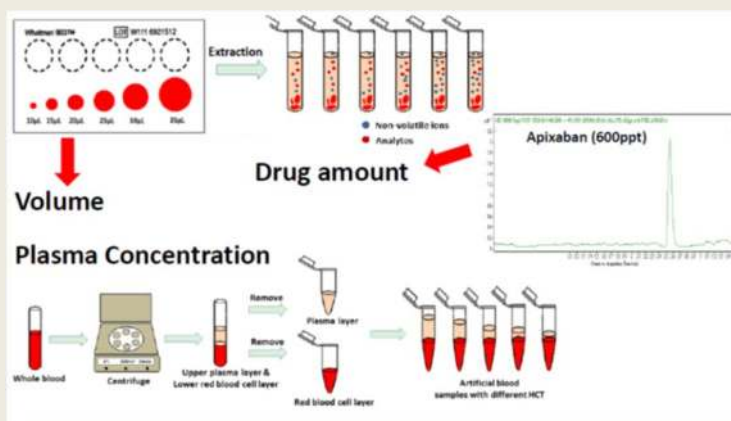


Figure 2. Comparison of methods in measuring apixaban concentration by DBS (upper) and standard plasma (lower panel).

Table 1. The blood volume estimation accuracy of the PCI-IS method. (n = 3)

Volume (μL)	Accuracy (%)						
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
10	107.2±14.7	110.5±8.2	119.5±19	119.8±10.2	116.3±2.7	97.5±7.8	98.2±11.7
15	102.6±8.1	108.3±3.5	102.3±7.0	100.7±5.3	114.1±4.3	96.9±3.4	103.2±6.5
20	108.3±24.4	118.7±8.5	119.4±3.4	116.7±5.9	107.3±4.1	100.6±3.4	118.0±5.5
25	95.9±11.6	108.4±6.0	113.4±3.6	101.1±11.0	103.4±0.9	87.2±7.3	95.1±0.8
30	87.1±10.1	89.0±2.8	90.7±4.1	90.7±4.4	100.0±8.4	91.7±9.0	94.5±2.3
35	87.8±6.4	93.9±11.8	91.0±6.7	91.5±2.2	88.0±9.4	86.1±5.8	90.2±2.0

Table 2. Quantification accuracy and precision of the LC-MS/MS method.

Concentration (ng mL ⁻¹)	Accuracy (%)	Precision	
		Intra-day (RSD %)	Inter-day (RSD %)
10	93.3±4.8	3.7	4.8
25	94.6±5.2	1.8	5.1
200	97.0±9.5	4.1	9.6
600	88.9±4.4	1.5	4.6

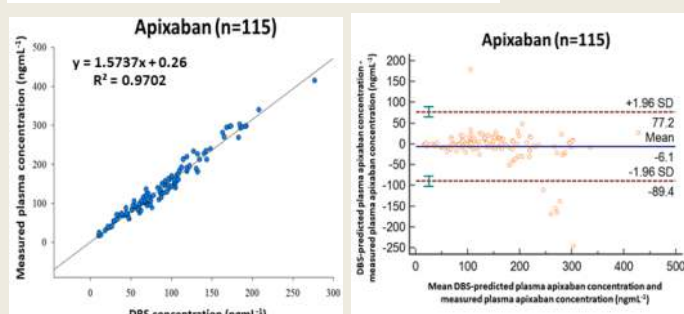


Figure 3. (A) Correlation between DBS and plasma concentrations. (B) Bland-Altman plots for the calculated and measured concentrations.