Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Data

Liquid chromatography–tandem mass spectrometry method for determination of apixaban in human plasma and its application to pharmacokinetic study in Indian population

1. Optimization of extraction method

Different sample preparation techniques have been tested, such as protein precipitation extraction (PPE) using methanol, acetonitrile and mixtures of both; liquid-liquid extraction (LLE) using diethyl ether, t-butyl methyl ether and dichloromethane and solid phase extraction (SPE) using Strata X, Oasis HLB, Hypurit HLB and Plexa bond elute using water, methanol, formic acid and ammonia in various washing trials. In order to eliminate interference during analyte and IS at the retention periods, optimization was performed. Trials have been performed to achieve consistent recovery. The samples prepared using Strata-X 33 µm, SPE (Phenomenex) polymer sorbent (30 mg/mL) and 10% methanol as washing solution and methanol as eluent for solid-phase extraction provided excellent and consistent recovery and cleanliness without compromise. For a stable, accurate approach that is essentially consistent, matrix effects may thus be removed using Strata-X. The results of each extraction trials are mentioned below:

Table 1: Protein Precipitation extraction (PPE)

Trial	Solvent used	Plasma sample volume	Results	Revised plasma sample volume	Result/ Inference
1	Methanol	100 µL	Low response	250 µL	Sufficient response, Poor chromatography, ion enhancement.
2	Acetonitrile	100 µL	Low response	250 µL	Sufficient response, ion enhancement.
3	Methanol : Acetonitrile (1:1, v/v)	100 µL	Low response	250 µL	Matrix effect due to ion enhancement not reduced with various
4	Methanol : Acetonitrile (2:1, v/v)	100 µL	Low response	250 µL	proportions trials; hence ruled out.
5	Methanol : Acetonitrile (1:2, v/v)	100 µL	Low response	250 µL	

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Table 2: Liquid-liquid extraction (LLE)

Trial	Solvent used	Solvent volume	Plasma sample volume	Results	Inference
1	Diethyl Ether	2 mL	100 µL	Low response	Not further used.
2	TBME	2 mL	100 µL	Good and consistent response but poor chromatography	Checked response with different proportions of organic solvents and with different make of available C18 columns[1]. Similar results with different column check were observed.
3	Dichloromethane	2 mL	100 µL	Response is satisfactory, good chromatography	
4	Dichloromethane : Diethyl Ether (1:1, v/v)	2 mL	100 µL	Response is satisfactory, good chromatography	Overall response using Gemini C18 column, 50 mm x 4.6 mm, 3µm was found as per requirements but ion enhancement still persists; hence ruled out.
5	Dichloromethane : TBME (1:1, v/v)	2 mL	100 µL	Response is satisfactory, good chromatography	

[1]Kromasil 100-5 C18, 50 mm x 4.6 mm, 5µm; Gemini C18 column, 50 mm x 4.6 mm, 3µm; and ACE super C18, 50 mm x 4.6 mm, 5µm
TBME t-butyl methyl ether

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Table 3: Solid phase extraction (SPE)

Trial	Plasma sample volume	Cartridges	Make	Sample pre-treatment condition	Elution	Further treatment after elution	Result/ Inference
1	100 µL	Strata X, (30mg/1mL)	Phenomenex	Neutral	Mobile phase	--	Slight ion enhancement observed; good response and chromatography.
2	100 µL	Strata X, (30mg/1mL)	Phenomenex	Neutral	Methanol	Evaporated to dryness in nitrogen evaporator at 50°C and reconstituted with 1000 µL of mobile phase.	Precise, accurate and reliable for further analysis under method validation.
3	100 µL	Oasis HLB, (1 cc/30 mg)	Waters	Neutral	Mobile phase	--	Slight ion enhancement observed; good response and chromatography.
4	100 µL	Oasis HLB, (1 cc/30 mg)	Waters	Neutral	Methanol	Evaporated to dryness in nitrogen evaporator at 50°C and reconstituted with 1000 µL of mobile phase.	Precise, accurate and reliable for further analysis under method validation.
5	100 µL	Hypurit HLB (1 cc/30 mg)	National Chromatography	Neutral	Mobile phase	--	Slight ion enhancement observed; good response and chromatography.
6	100 µL	Hypurit HLB (1 cc/30 mg)	National Chromatography	Neutral	Methanol	Evaporated to dryness in nitrogen evaporator at 50°C and reconstituted with 1000 µL of mobile phase.	Precise, accurate and reliable for further analysis under method validation.
7	100 µL	Plexa bond elute (50mg/1mL)	Agilent	Neutral	Mobile phase	--	Slight ion enhancement observed; good response and chromatography.
8	100 µL	Plexa bond elute (50mg/1mL)	Agilent	Neutral	Methanol	Evaporated to dryness in nitrogen evaporator at 50°C and reconstituted with 1000 µL of mobile phase.	Precise, accurate and reliable for further analysis under method validation.

All the SPE analysis was performed with Gemini C18 column, 50 mm x 4.6 mm, 3µm and a mixture of acetonitrile: 2 mM ammonium format buffer (50:50 v/v) as mobile phase.
All the results using above mentioned SPE cartridges were found to be similar. Further method of analysis was selected on the grounds that it would be the least expensive of all.

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2. Comparison with reported methods

Table 4: Comparison of salient features of the present techniques with reported techniques for quantitative determination of apixaban

Technique	LLOQ (ng/mL)	Linear range (ng/mL)	Extraction method	Sample volume (µL)	Run time (min)	Application	References No.
LC-MS/MS	1.01	1.01 - 280	SPE	100	1.8	PK study	Present method
LC-MS/MS	5	5 - 500	PPE	100	1.3	PK study	11
LC-MS/MS	1	1.0 - 1000	SPE	100	3	Clinical study	12
LC-MS/MS	9.7	9.7 - 970	PPE	50	1	PK study	13
LC-MS/MS	1	1 - 500	PPE	100	6	None	14
LC-MS/MS	23	23 - 750	PPE	100	4.75	Coagulation assay	15
LC-MS/MS	1	1 - 500	PPE	50	2.5	Clinical study	16
LC-MS/MS	1	1 - 500	SPE	200	10	Anticoagulant therapy	17
LC-MS/MS	1	1 - 100	PPE	100	3.5	PK study on rat	18
LC-MS/MS	2	2 - 500	PPE[1]	50	2	Clinical study	19
LC-MS/MS	1	1 - 1000	SPE[2]	100	4	Clinical study	20
LC-UV/VIS	16	20 - 200	PPE	100	7	TDM	21

[1]PPE followed by magnetic separation. [2]SPE 96 well plate method.
TDM therapeutic drug monitoring