Original Article

Biodistribution Study of Pantoprazole Sodium in Rodent Tissues: A Tool for Pharmacokinetic Study

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Abstract

Background: Pantoprazole sodium is one of the most widely used drugs for treating gastric acid–related disorders as well as is the most popular drug among various cancer therapy protocols for treating gastric disturbances pertained to the chemotherapy. The present study aims to validate high-performance liquid chromatographic (HPLC) method for the quantification of pantoprazole sodium in mice plasma and various tissue homogenates including kidney, heart, prostate, lung, pancreas, liver, and brain. Pantoprazole sodium estimation was done using 100 µL aliquot, which was injected into HPLC system, and the separation was achieved using Shimadzu C18 column at 40°C. Mobile phase composed of acetonitrile/dibasic phosphate buffer (40:60, v/v), pH = 7.4 was isocratically pumped at 1.0 mL min^{-1}, and detection was performed at wavelength of 290 nm. **Material and Methods:** All the samples including tissues and plasma were collected after 4 h of oral administration of pantoprazole sodium to Swiss albino mice (10 mg/kg, p.o.). **Results:** Bioanalytical method was further validated according to the standard guidelines and portrays to be selective as well as linear ($r^2 \geq 0.999$) over the concentration range of 10–50 ng/injection. The intraday (% relative standard deviation [RSD] = 0.29%–1.21%) and inter-day precision (%RSD = 0.52%–2.88%) was found to be within the layout standards by International Council for Harmonization. Pantoprazole sodium extraction recovery was achieved between 64.15% and 78.17% demonstrating the suitability of the method. **Conclusion:** Bio-distribution study so carried out by bioanalytical technique can be used as an aiding tool for the quantification of pantoprazole sodium in all the studies involving the pharmacokinetic profiling of drug in various tissues of rodents.

Keywords: *Bioanalytical method, liquid chromatography, pantoprazole sodium, pharmacokinetic, tissue distribution*

Introduction

Pantoprazole [Figure 1], 5-(difluoro methoxy)-2-[(3, 4-dimethoxy-2-pyridyl) methylsulfinyl]-1-H benzimidazole sodium sesquihydrate, a hydrophilic molecule has been used for more than three decades for acid-related disorders. Being a member of the class proton-pump inhibitor (PPI), pantoprazole works by inhibiting the H^+/K^+ adenosine triphosphatase enzyme system (the proton pump) of the gastric parietal cell. Because of its high susceptibility to degradation in an acidic gastric environment, it is formulated as a gastroresistant pharmaceutical dosage form.[1-4]

Pantoprazole, compared with other members of the class, has shown a better pharmacokinetic profile as it is most stable in neutral as well moderately acidic conditions and also has not much interaction with cytochrome P_{450} system and thus being one of the PPIs with lesser known drug–drug interactions.[5,6]

Pantoprazole being a highly protein bound drug, much less has been known about the distribution of pantoprazole into different tissues. Also, keeping in mind of the fact that pantoprazole has shown to increase the antitumor activity with various chemotherapeutic agents such as doxorubicin in experimental tumors. The quantification of pantoprazole in human urges to understand its distribution in various tissues for a better therapeutic approach with optimal dose regimes.[2]

Various analytical techniques have been reported in the literature for the estimation of pantoprazole in various dosage forms alone or in combinations.[7] Many modified methods for the estimation of

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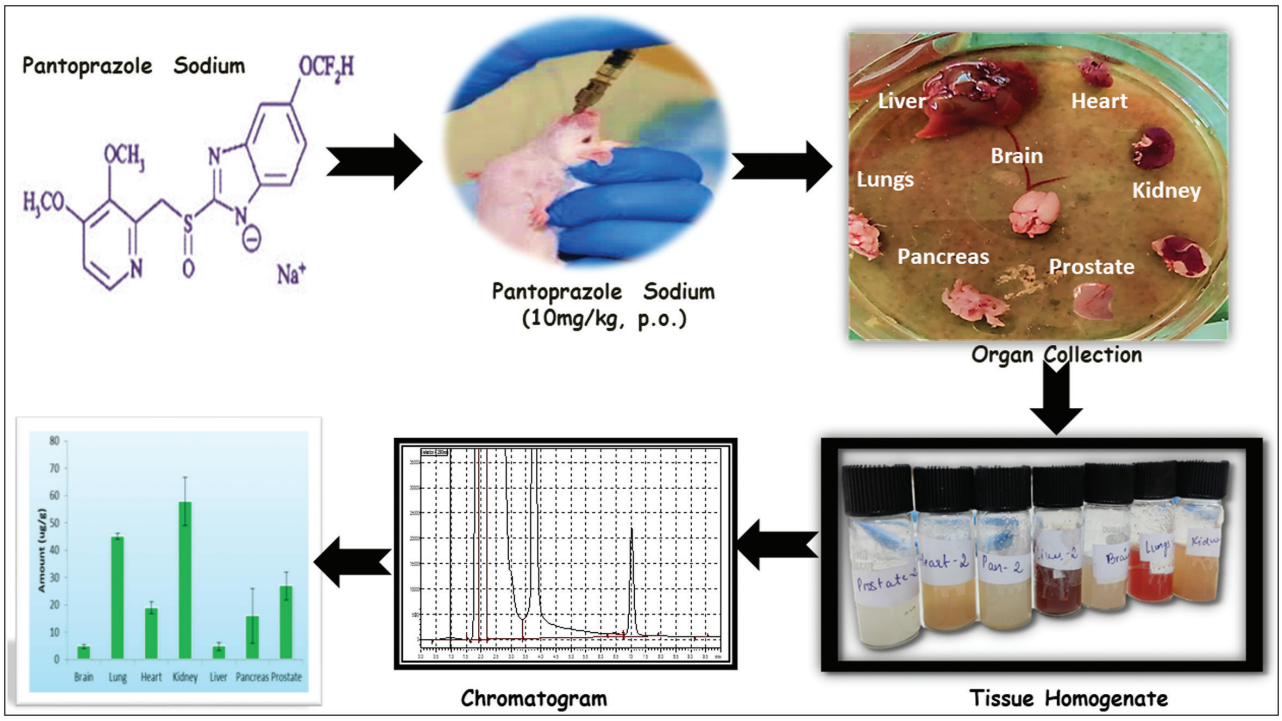
Graphical Abstract



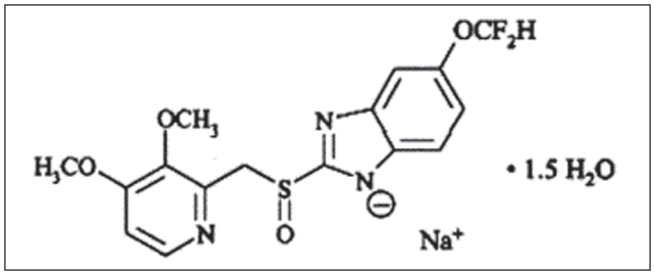


Figure 1: Pantoprazole sodium

the pantoprazole within biological fluids, i.e., either serum or plasma in rodents and humans, have been established including spectrophotometry,[8,9] capillary electrophoresis,[10] voltammetry,[11] polarography, and high-performance liquid chromatography (HPLC).[6,9]

Bhaskara *et al.* (2011) established a method with liquid chromatography with tandem mass spectrometry for the estimation of pantoprazole sodium in urine samples. The method however can be claimed as a sensitive one but at the same time renders the feasibility on account of the cost.[12] Considering the existing established high performance thin layer chromatography methods for the quantification of pantoprazole sodium in various samples including human plasma[2,3] or urine samples, scarcity has been observed in data available for the quantification of pantoprazole sodium in various tissues of rodents.[13] However, *in vivo* studies have been reported that of quantification in neonatal calves and rats but the biodistribution data lack that of in rodents. Available studies include for pantoprazole estimation in plasma, gastric fluid, or in stomach tissue homogenates.[13-16]

Therefore, the present study aims to attempt for the estimation of pantoprazole sodium in various tissues of rodents with a minor modification in the extraction procedure, which will lay a pathway for estimation of pantoprazole sodium in various tissues and further could be used as a model to evaluate the amount of pantoprazole in various tissues.

Materials and Methods

Chemicals and reagents

Pantoprazole (purity > 99%) was obtained as a gift sample from Intas Pharmaceuticals Pvt. Ltd., (Ahmedabad, Gujarat). All solvents, i.e., methanol, acetonitrile and phosphate buffer, orthophosphoric acid, of analytical grade were purchased from S.D. Fine Chem Ltd. (Vadodara, Gujarat). Ultra-pure water was prepared by Milli-Q System from Millipore (Milford, USA). Water for injection was used for the preparation of dosing solutions for animals.

Animals

Swiss albino mice of 10–12 weeks and weighing around 25–30 g were used for the tissue distribution study of pantoprazole sodium. The animals were housed in Maliba Pharmacy College animal house. Animals were kept within temperature range of 21°C–25°C and humidity of 45%–65%, with a 12-h light/dark cycle, and were given full access to food and water. All experimental methods were carried out in compliance

with CPCSEA guidelines, and the study was only initiated after receiving approval from the Institutional Animal Ethics Committee (protocol number: MPC/IAEC/06/2020).

Stock solution and calibration standards

Pantoprazole sodium 1 mg/mL standard stock was prepared in deionized water, and subsequent dilutions were made using a mobile phase. In order to attain a standard concentration within the range of 10–50 ng/injection, a serial dilution technique was employed.

Sample preparation and extraction

Sample preparation was done after oral administration of drug. Pantoprazole sodium (10 mg/kg) was administered orally to animals, and after 4 h, the animals were sacrificed using an overdose of urethane. Blood collection was performed through the retro-orbital plexus. Collected blood samples were allowed to clot at the room temperature, and the so-obtained serum after centrifugation was stored at −21°C till further analysis. Followed by blood collection, the animals were dissected, and all the organs, i.e., kidney, heart, prostate, lung, pancreas, liver, and brain, were isolated. The organs were dried, weighed, and stored at −21°C till further analysis. Organs were homogenized in the solvent composed of phosphate buffer (pH = 7.4) and methanol (80:20, %v/v) for 25 min.

After homogenization, the samples were centrifuged for 20 min at 10,000 rpm, and the supernatant so obtained was collected. For the quantification of pantoprazole sodium in collected tissues, 1 mL of tissue supernatant so obtained was taken in eppendorf, and 30 µL of 1 µg/mL of the standard solution was spiked to each eppendorf and was vortexed for at least 20 s. Further, the sample passed through a syringe filter and 100 µL aliquot from each sample vial was injected to the liquid chromatography system.

Instrument and chromatographic conditions

The system consisted of a Shimadzu Prominence LC-20AD (Kyoto, Japan) equipped with an SPD-20A UV-visible detector and SIL-20AC HT autosampler. A Shimadzu C18 reversed-phase column was used with 5.0 µm particle size, 250 mm length, and 0.46 mm internal diameter. High-speed homogenizer (Kinematica, Switzerland) was used for obtaining tissue homogenate. Chromatography was performed at 40°C at a flow rate of 1 mL/min using an isocratic condition with a detection wavelength 290 nm and injection volume 10 µL. The mobile phase consisted of a phosphate buffer: acetonitrile (60:40 %v/v), adjusted to pH = 7.4 with 5% orthophosphoric acid.

Method validation

The validation of the present study method was performed using parameters such as selectivity, linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, and recovery, and the stability study for the solution was considered using the recent version of Food and Drug Administration (FDA) guidance for industry[17] and the European Medicines Agency (EMA)[18] on the bioanalytical method validation.

To assess selectivity in each of the examined matrixes, blank plasma samples, and supernatant of kidney, heart, prostate, lung, pancreas, liver, and brain, homogenates from three different mice were analyzed to detect possible endogenous substances that could have the same retention time as that of drug.

Linearity was assessed through calibration curves constructed in five different days (n = 5) using nine calibration standards covering the concentration range of 10–50 ng/injection. Curves were obtained by plotting mean peak area against the corresponding nominal concentrations, being data subjected to a weighted linear regression analysis.

The precision of the methods was ascertained by intraday and interday repeatability precisions. The method's accuracy was assessed by comparing the area of drug extracted from the plasma to the area of drug standard. The LOD and LOQ of the developed method calculated using the standard deviation (SD) of the intercepts and the mean slope of the calibration curves of pantoprazole sodium using the formula LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ is the SD of the intercepts of five calibration curves and S is the mean slope of five calibration curve.

Method application

The method developed was applied to estimate the drug in the plasma and tissue samples collected in a triplicate manner.

Results and Discussion

Optimization of sample extraction

Chromatograms of all the tissues did not reveal any significant interference at the retention time of pantoprazole sodium. Hence, protein precipitation with methanol was a crucial step for the optimization of all samples in order to obtain cleaner chromatograms with no significant interferences at pantoprazole sodium retention time. The blank tissue homogenates and plasma samples extracted using the solvent resulted in lesser interferences, although tissue samples had endogenous interferences, but the extraction procedure with methanol and phosphate buffer reduced it as well as the interferences occurred were much before the retention time of pantoprazole sodium. Liver, kidney, and lungs samples showed much protein content, owing to the size and consideration of tissue type, which needed further treatment with methanol to precipitate out the protein remaining.

Method validation

Selectivity

Selectivity regarding the endogenous interferences in retention time was achieved after evaluating blank plasma and supernatants of tissue homogenates from three different animals. Blank chromatograms were overlayed with actual mouse plasma samples to produce representative chromatograms.

Linearity

Nine calibration standards covering over the range of 10–50 ng/injection were used to obtain a calibration curve, which showed to be linear for all tested biological matrices (i.e., plasma and seven tissue samples homogenates). Data are represented in Tables 1-3 and in Figures 2 and 3.

Precision

As per the standards laid by both FDA and EMA guidelines for acceptance criteria, the present method showed to be precise for the estimation of pantoprazole sodium in various tissues of mouse. Data depicted in Table 4 showed the inter- and intraday % relative standard deviation [%RSD] values that were within the limits as reported by the standard guidelines.

Table 1: Calibration curve data

Amount of drug (ng/injection)	Peak area (mean ± SD)	%RSD
10	38348.00 ± 809.56	2.11
15	57564.00 ± 808.00	1.40
20	76938.00 ± 1061.01	1.38
25	96261.99 ± 1105.42	1.15
30	115732.02 ± 1920.34	1.66
35	134807.00 ± 2125.13	1.58
40	153939.00 ± 3317.29	2.15
45	172453.99 ± 3261.83	1.89
50	192428.60 ± 896.40	0.46

%RSD = % relative standard deviation

Table 2: Summary of data of the linearity for pantoprazole sodium

Parameter	Values
Linearity range	10–50 ng
Regression line equation	y = 3827.8x + 407.35
Slope ± SD	3827.80 ± 31.77
Intercept ± SD	407.35 ± 81.92

x = independent variable

Table 3: Data for LOQ and LOD

Parameters	Results
Standard deviation of the Y-intercepts of five calibration curves	81.92
Mean	3827.80
LOD = 3.3× (SD/slope)	0.071
LOQ = 10× (SD/slope)	0.21

Extraction recovery

Recovery from the mouse plasma was performed with the protein precipitation followed by centrifugation to achieve clear plasma without protein interference. Data as represented in Table 5 show the recovery from mouse plasma ranged from 64.15% to 78.17%. The present study data fall in accordance to the international validation guidelines, giving a concrete base for the utilization of the developed method for the quantification of pantoprazole sodium in various rodent tissues.

Solution stability

Post analysis of pantoprazole sodium in mouse plasma and all the tissue homogenates, for 72 h, no significant loss of pantoprazole sodium was observed on all the different working conditions. All the samples were analyzed at the room temperature for a minimum of 5 h with two freeze thraw cycles for homogenates stored at −21°C, which were without significant degradation.

Method application

The modified method was validated by the estimation of pantoprazole sodium in various samples including plasma, kidney, heart, prostate, lung, pancreas, liver, and brain upon a single oral administration of pantoprazole sodium (10 mg kg^{-1}) to all the mice. The results for the quantification of pantoprazole sodium in various tissues as shown in Table 6 as well as the study chromatograms of the analysis of all the samples collected upon the completion of 4 h of pantoprazole sodium administration are shown below [Figures 4–13], which evidenced estimated concentration falling within calibration range and the method to be having utmost suitability for the analysis of pantoprazole sodium in various tissues of rodents. Moreover, several studies have also demonstrated that pantoprazole sodium

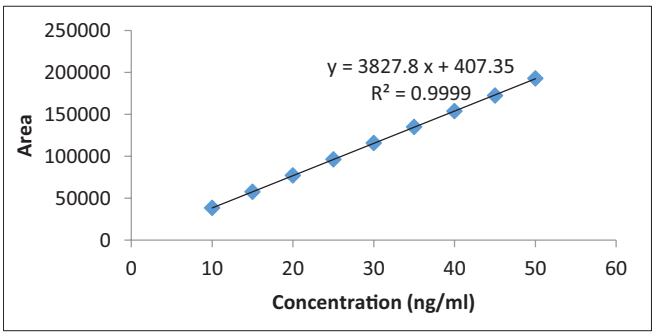


Figure 2: Calibration curve of pantoprazole sodium

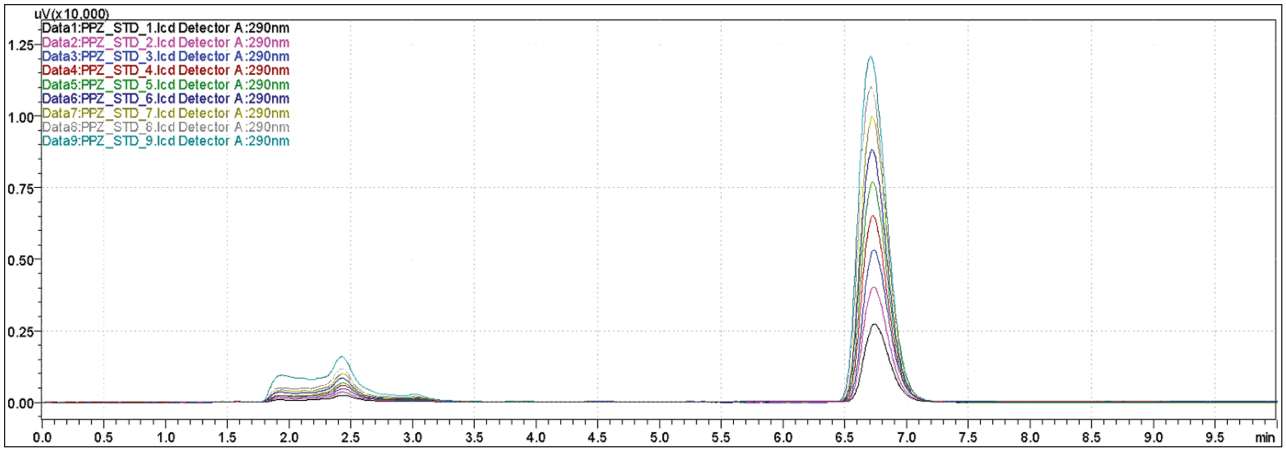


Figure 3: Overlay chromatogram for the linearity of reference standard pantoprazole sodium (10–50 ng/injection) by HPLC

Table 4: Intermediate precision data for pantoprazole sodium

S. no.	Amount of drug (ng/injection)	Intraday precision		Interday precision	
		Peak area (mean ± SD)	%RSD	Peak area (mean ± SD)	%RSD
1	10	38322.23 ± 652.02	0.29	38946.66 ± 112.78	1.70
2	15	57554.48 ± 1140.72	0.19	57627.38 ± 106.73	1.98
3	20	77104.93 ± 1454.67	0.44	76855.12 ± 339.29	1.89
4	25	97026.83 ± 500.19	0.92	95623.33 ± 880.93	0.52
5	30	116194.10 ± 15841.07	1.21	114547.19 ± 1380.94	1.36
6	35	135703.86 ± 2446.81	0.48	134091.40 ± 637.58	1.80
7	40	154451.75 ± 4443.89	1.05	156101.37 ± 1632.23	2.88
8	45	173757.30 ± 3658.25	0.74	170196.93 ± 1265.74	2.12
9	50	191249.57 ± 3149.57	0.52	193514.72 ± 1013.88	1.65

%RSD = % relative standard deviation

Table 5: Pantoprazole sodium recovery (%) from mouse plasma

Amount of drug (ng/injection)	Standard drug Peak area (mean ± SD)	Drug recovered from plasma Peak area (mean ± SD)	%Recovery
10	38548.00 ± 883.13	28247.97 ± 350.27	73.28
15	57564.00 ± 722.70	38683.01 ± 603.45	67.20
20	76938.00 ± 949.00	54202.82 ± 682.95	70.45
25	96262.00 ± 929.71	74121.74 ± 837.57	77.00
30	113746.67 ± 1717.62	85780.58 ± 1475.42	75.41
35	139629.00 ± 5505.68	89579.25 ± 1316.81	64.15
40	157268.33 ± 2967.07	109789.29 ± 2437.32	69.81
45	169878.33 ± 4573.79	132806.82 ± 2310.84	78.17
50	193295.67 ± 2164.79	146844.88 ± 1703.40	75.96

and its metabolites build up in the tissues. Additionally, the results revealed inconsistencies between research conducted on veterinary species including calves, dogs, and goats and those conducted on humans.[19] The current work thus paves the way for future research into pantoprazole metabolism as well as the identification and measurement of different pantoprazole metabolites in various tissues where parent drug accumulation occurs.

Conclusion

The quantification of pantoprazole sodium in rodent plasma and various tissues including kidney, heart, prostate, lung, pancreas, liver, and brain was successfully performed using a sensitive and simple approach. All the data fall in accordance to international validation guidelines, giving a concrete base for the utilization of developed method for the quantification of pantoprazole sodium in various rodent

Table 6: Biodistribution of pantoprazole sodium

Organ	Organ weight (g) (n = 3)	Amount (ng/g of tissue)	Amount (µg/g of tissue) (mean ± SD)
Brain	0.653	4148.80	4.88 ± 0.72
	0.434	5595.36	
	0.233	4889.66	
Lung	0.22	44079.57	45.13 ± 1.10
	0.222	45154.88	
	0.21	46278.60	
Heart	0.141	20295.74	18.96 ± 2.86
	0.133	20137.52	
	0.164	16449.39	
Kidney	0.200	66234.88	57.93 ± 8.82
	0.168	58884.89	
	0.343	48668.40	
Liver	1.276	4670.23	4.78 ± 5.12
	1.638	6234.75	
	1.728	3450.41	
Pancreas	0.218	20486.53	16.05 ± 10.06
	0.224	15574.42	
	0.223	12090.99	
Prostate	0.077	27074.91	26.97 ± 5.06
	0.08	31981.95	
	0.096	21865.21	

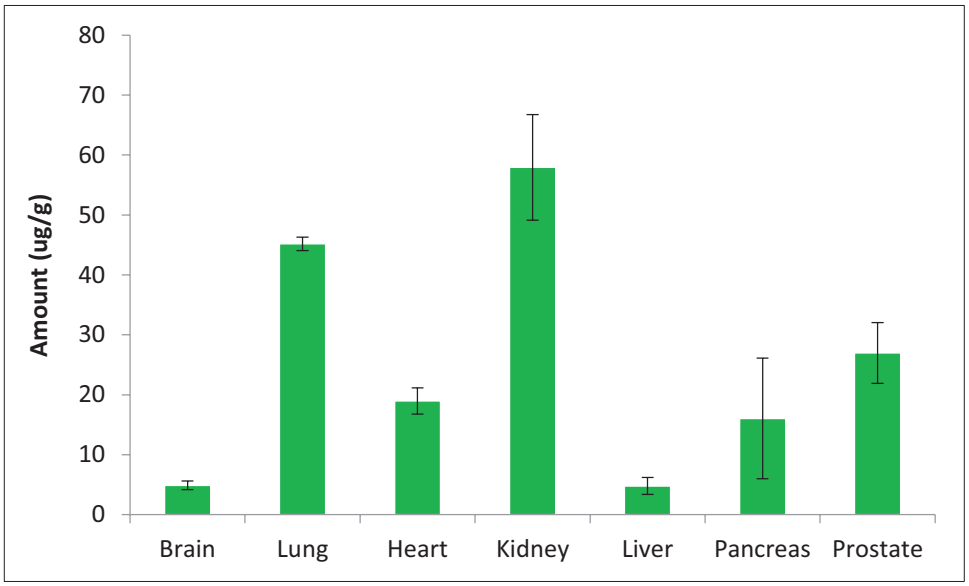


Figure 4: Biodistribution of pantoprazole sodium in rodent tissues

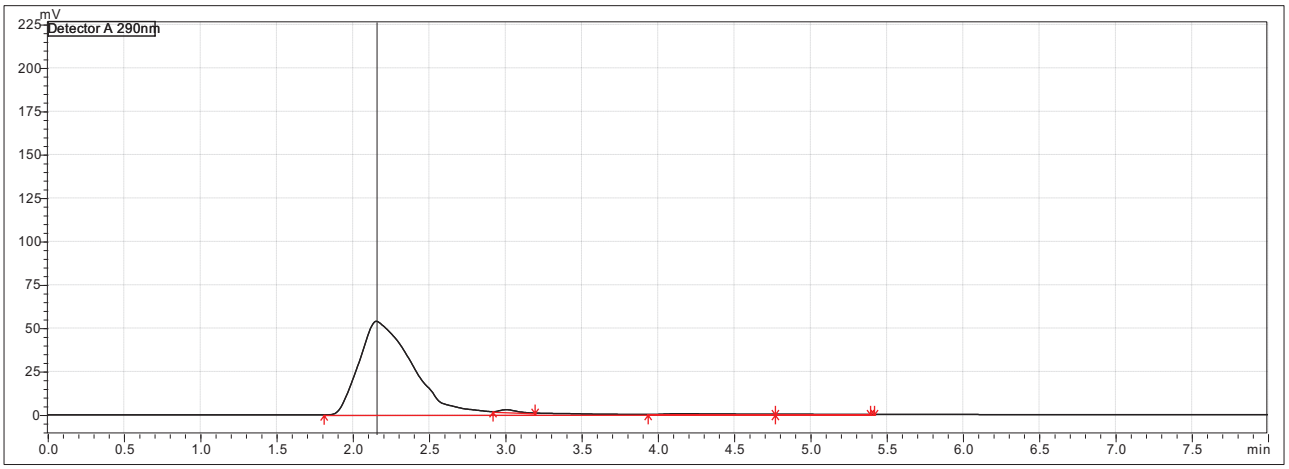


Figure 5: Estimation of pantoprazole sodium in blank tissue homogenate of mouse

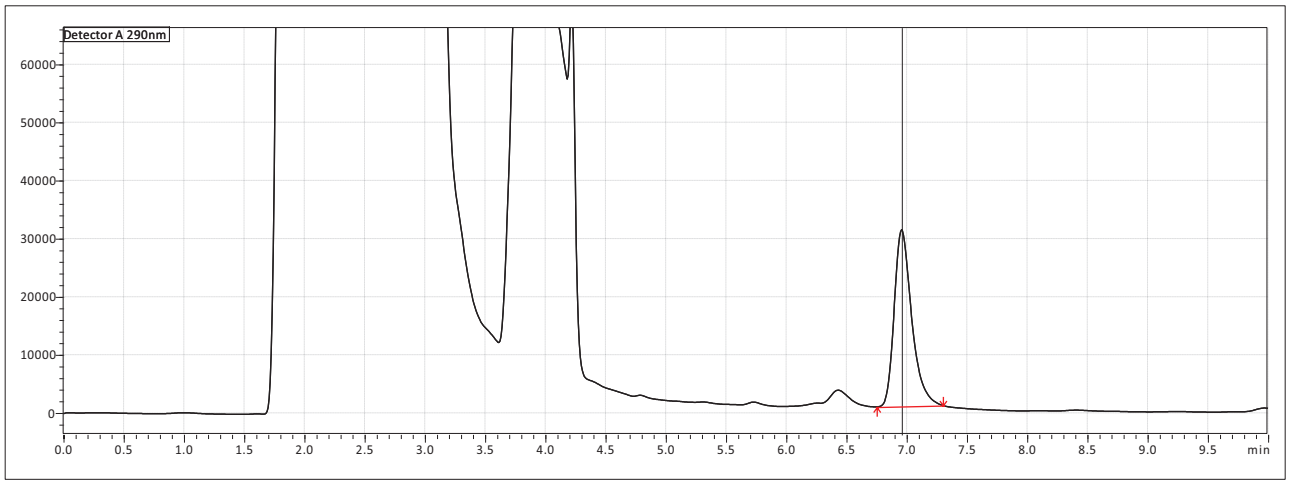


Figure 6: Estimation of pantoprazole sodium in liver tissue homogenate of mouse

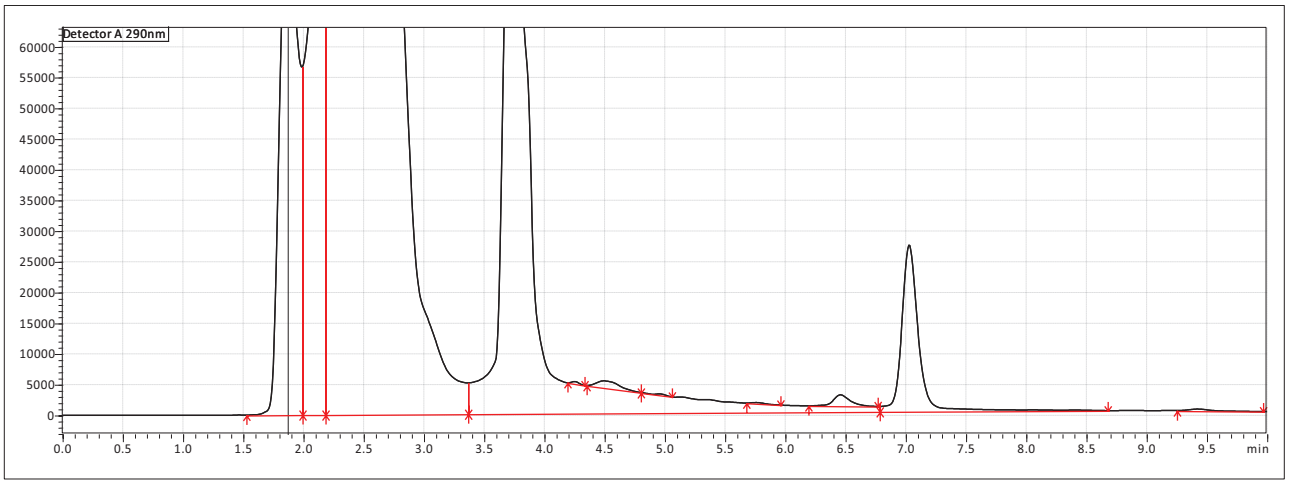


Figure 7: Estimation of pantoprazole sodium in brain homogenate of mouse

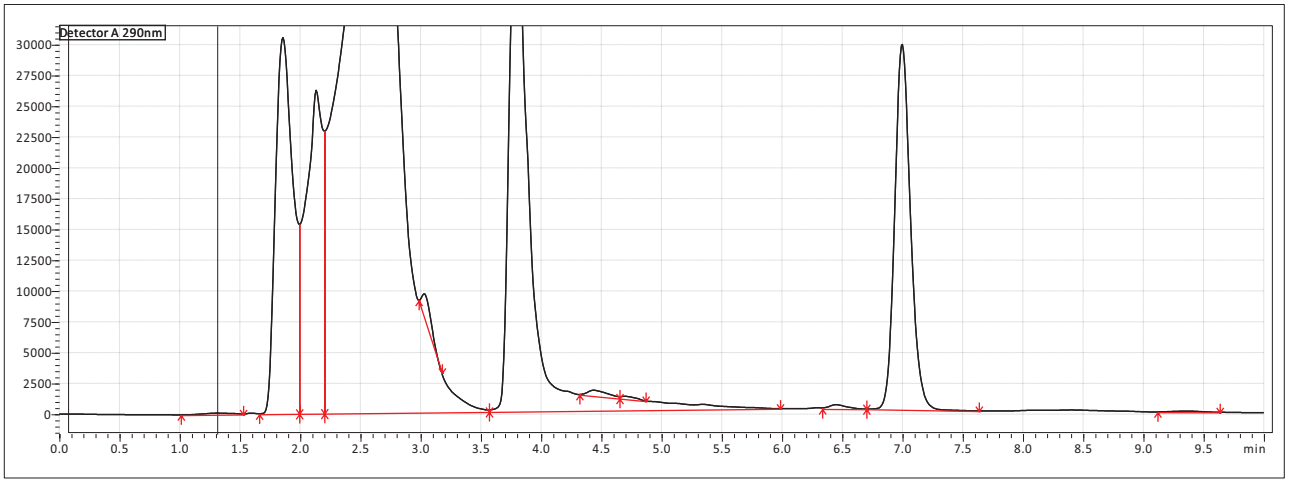


Figure 8: Estimation of pantoprazole sodium in heart homogenate of mouse

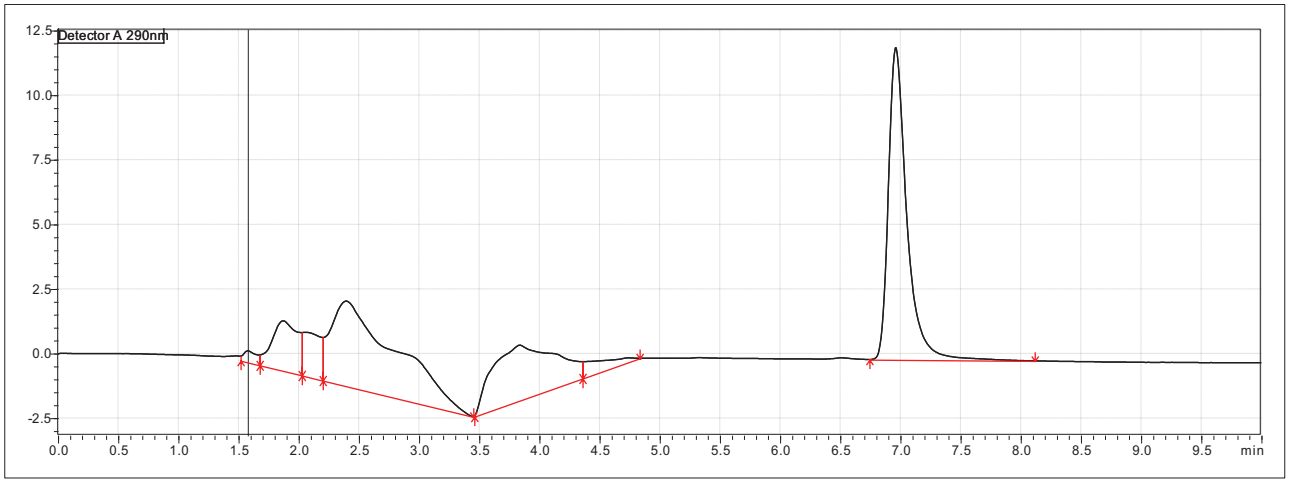


Figure 9: Chromatogram of standard pantoprazole sodium

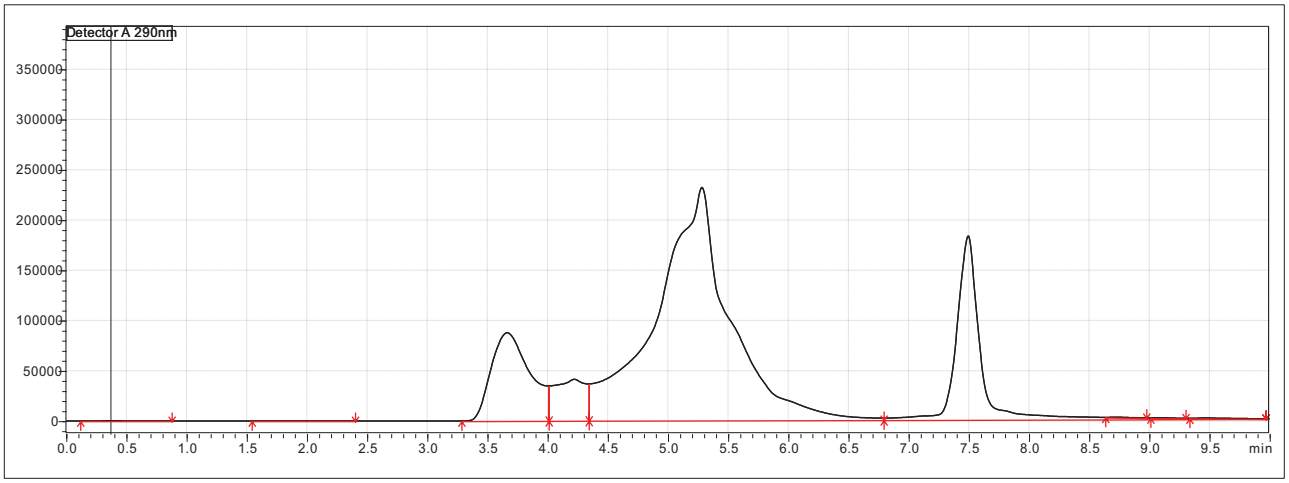


Figure 10: Estimation of pantoprazole sodium in kidney tissue homogenate of mouse

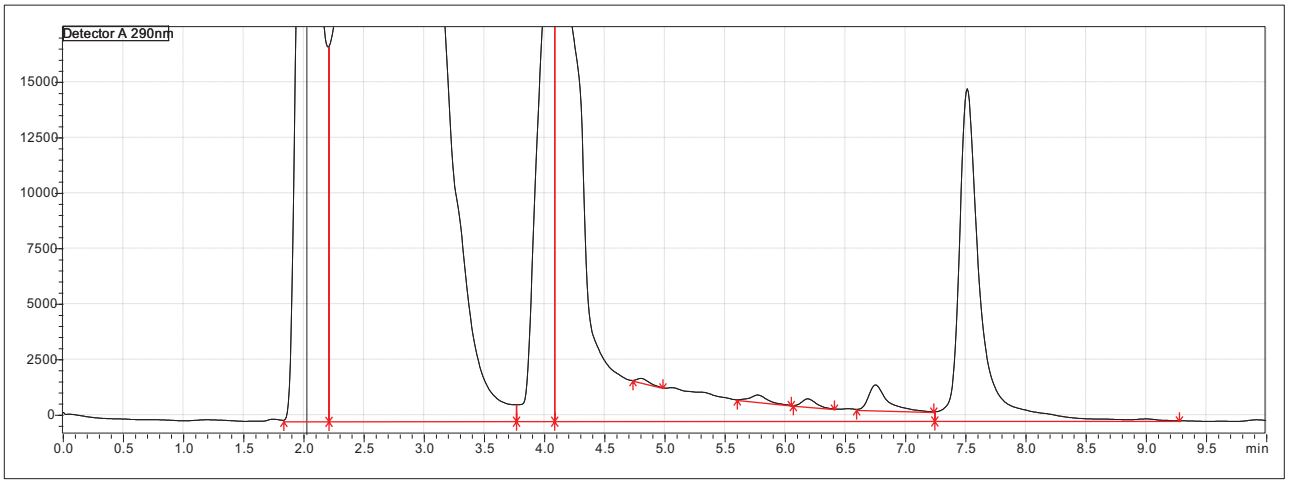


Figure 11: Estimation of pantoprazole sodium in lung tissue homogenate of mouse

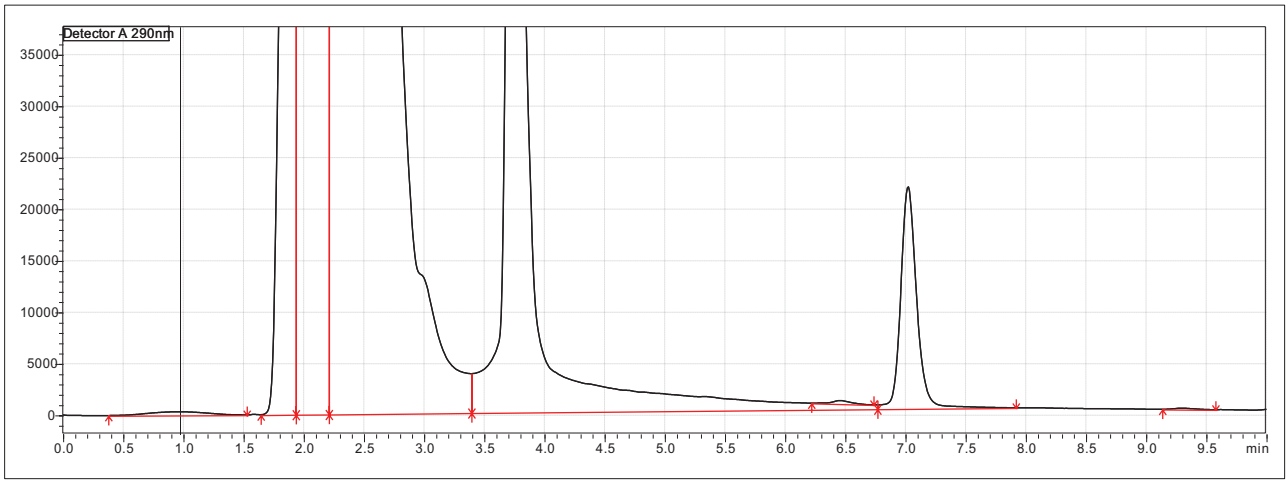


Figure 12: Estimation of pantoprazole sodium in pancreas tissue homogenate of mouse

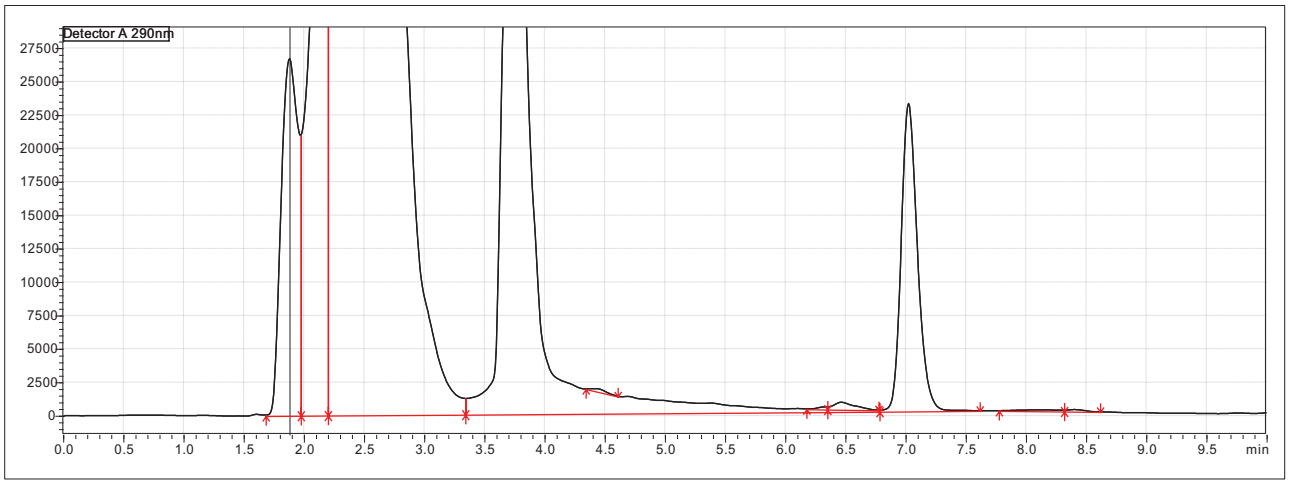


Figure 13: Estimation of pantoprazole sodium in prostate tissue homogenate of mouse

tissues and can be used for kinetics as well as to quantify the drug at the active targeted site.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Ethical approval

All procedures were performed in the study only after receiving the ethical approval from Animal Ethics Committee of Maliba Pharmacy College (protocol no.: MPC/IAEC/06/2020).

Authors' contributions

All authors contributed to the study conception and design. Material preparation and data collection were done by BVD. Data analysis and method development were carried out by PD and DD. Conceptualization and supervision of the work were done by BV. The first draft of the article was written by BVD and PD, and the review of the article was done by RV. All authors commented on previous versions of the article. All authors read and approved the final article.

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