International Journal of Pharmacy and Pharmaceutical Sciences

Print ISSN: 2656-0097 | Online ISSN: 0975-1491 Vol 11, Issue 10, 2019

Original Article

QUANTITATIVE ANALYSIS OF PAMABROM AND IBUPROFEN IN SYNTHETIC MIXTURE USING 1ST ORDER DERIVATIVE SPECTROSCOPY

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Received: 06 Jul 2019, Revised and Accepted: 02 Sep 2019

ABSTRACT

Objective: The preliminary goal was to develop and validate 1st order derivative spectroscopic method for quantitative analysis of Pamabrom (PAMA) which is a xanthine diuretic and ibuprofen (IBU) which is a non-steroidal anti-inflammatory agent from its synthetic mixture.

Methods: Analytical method was developed on Shimadzu double beam spectrophotometer equipped with UV probe 2.42 as software using methanol as solvent. Quantification of PAMA was carried out at zero cross over point of IBU that is 291 nm and for IBU, it was achieved at 278 nm which is zero cross over point of PAMA. Method was validated according to ICH Q2 R1 guidelines.

Results: Method showed a linear response in the range of 2-12 µg/ml of PAMA and 20-120 µg/ml of IBU. Method was found to be accurate with recovery between 99.7–100.9 % for PAMA and 100.3–100.7 % for IBU. The method was found to be accurate and precise for quantitative analysis of PAMA and IBU.

Conclusion: The developed method was successfully validated as per ICH Q2 R1 guidelines and was successfully applied for quantitative analysis of a synthetic mixture of PAMA and IBU.

Keywords: Pamabrom, Ibuprofen, Derivative spectroscopy, Zero crossover point, Analytical method validation

DOI: http://dx.doi.org/10.22159/ijpps.2019v11i10.34797

INTRODUCTION

Pamabrom (PAMA) belongs to the class of xanthene diuretics, which basically contains purine moiety and is approved by US FDA for treatment of menstruation bloating, premenstrual dysphoric disorders, while ibuprofen (IBU) is non-steroidal anti-inflammatory (NSAID's) drug that is propionic acid derivative which is approved by Indian pharmacopoeia (IP), United states pharmacopoeia (USP) and Japanese pharmacopoeia (JP) for management of mild-to-moderate pain associated with dysmenorrhea and arthritis [1-7]. 40–90 % of women of childbearing age complained of primary dysmenorrhea, which is characterized in cyclic pelvic pain during the menstrual period, vomit and headache. NSAIDs were administered to patients because prostaglandins and leukotriene were reported as major factors for dysmenorrhea. Among the all NSAID's, IBU has shown better tolerability which forms the basis of combining PAMA and IBU for treatment of pain associated with renal calculi and dysmenorrhea. PAMA and IBU are officially quantified by HPLC [8]. Several analytical methods are available which can determine IBU and PAMA individually or in combination with another drug [9-26]. Literature review revealed one complex RP-HPLC method for quantitative analysis of PAMA and IBU.

Among different analytical methods, UV spectrophotometric method is perhaps the most quickest and robust method and no UV spectrophotometric method has been reported for estimation of IBU and PAMA in synthetic mixture. More specifically derivative spectroscopy has the advantage to be more specific in comparison to other multicomponent UV spectrophotometric methods.

So by considering the above facts, it was decided to develop and validate 1st order derivative spectrophotometric method for quantitative analysis of IBU and PAMA, as it has less complexity and offers a better economy.

MATERIALS AND METHODS

Materials

PAMA was obtained as gratis sample (99.95% pure) for research purpose from Amoli organics, Vadodara while IBU was obtained as gratis sample from OSAKA pharmaceuticals, Sakrda, Baroda. Methanol (LR grade) was purchase from SD fines.

Instrument and experimental conditions

Spectrophotometric analysis was performed on Shimadzu UV-1800 double beam spectrophotometer having a path length of 1 cm matched pair of quartz cell. Obtained spectra of PAMA and IBU were derivatized to 1st order using UV probe 2.42 as software at delta Λ of 10 nm.

Preparation of master stock solution

For the method development purpose, 10 mg of PAMA was weighed and diluted to 10 ml (1000 µg/ml) and was further diluted to give final concentration of 100 µg/ml. In a similar way, 50 mg of IBU was weighed and diluted to 50 ml (1000 µg/ml) and was further diluted to give the final concentration of 200 µg/ml.

Selection of analytical wavelength

The working standards of PAMA (2-12 µg/ml) and IBU (20-120 µg/ml) were prepared in 10 ml volumetric flask using methanol as a solvent. They were scanned in the UV range of 200–400 nm and D ° spectra is recorded by UV spectrophotometer. All the D ° spectra of PAMA and IBU were transformed into D^1 spectra with the help of UV probe 2.42 software. For confirmation of D^1 spectra of PAMA and IBU, D ° and D^1 spectra of the same were overlapped.

Preparation of solutions for analytical method validation

Preparation of solution for linearity and range

To check the linearity of the method, PAMA was prepared in the concentration range of 2-16 µg/ml and IBU was prepared in the range of 20-160 µg/ml from master stock solution in 10 ml volumetric flask. When D^1 Absorbance was plotted against concentration, non-linearity was observed above 12 µg/ml for PAMA and above 120 µg/ml for IBU so final range for validation was selected at mixture containing 2-12 µg/ml for PAMA and 20-120 µg/ml for IBU. All prepared solution was scanned between 200-400

nm and all spectra were derivatized to 1st order. D^1 absorbance was obtained at selected wavelength and mean D^1 absorbance was plotted against concentration.

Intermediate precision (Repeatability)

To adjudge the repeatability of the analytical method, the solution of linearity studies were analyzed for five-time with same conditions. Mean D^1 absorbance was recorded at all concentration for PAMA and IBU and were observed for relative standard deviation.

Method precision

Method precision was determined by performing intraday and interday precision. The mixture that represents overall range (2+20, 8+80 and 12+120µg/ml) were analyzed on the same day at different time interval for intraday precision. The mixture that represents overall range (2+20, 8+80 and 12+120 µg/ml) were analyzed on different days for interday precision.

Accuracy study

Accuracy of the analytical method was adjudged by spiking of placebo with standard solution. The mixture containing 100 mg of directly compressible lactose, 2 mg of talc and 2 mg of magnesium stearate was selected as placebo and was spiked at 50, 100 and 150% of target concentration (8+80 µg/ml) (table 1). Each spiked concentration was analyzed for three times and mean % recovery was observed at each spiked level.

Table 1: Preparation of solutions for accuracy study

Level of spiking	Quantity of placebo (mg)	The volume of stock solution (ml)	The volume of diluent taken (ml)	Final concentration (µg/ml)	
				PAMA	IBU
Unspiked	104	0	10	-	-
50 %	104	0.4	9.6	4	40
100 %	104	0.8	9.2	8	80
150 %	104	1.2	8.8	12	120

A stock solution was prepared by weighing 5 mg PAMA and 50 mg IBU and was dissolved in 50 ml methanol (100 µg/ml for PAMA and 1000 µg/ml for IBU respectively)

Solvent stability

Solvent stability was determined by scanning the same solution prepared in selected solvent (methanol) at 3 different time interval that is at 0 hour, 6 h and 24 h. The mixture of 12+120 µg/ml solution of PAMA and IBU in methanol were scanned at the selected time interval and characteristics of spectra were compared (λ_{max}).

Assay

As the proposed synthetic mixture is having dose of 20 mg of PAMA and 200 mg of IBU, 20 mg of PAMA and 200 mg of IBU was mixed with selected placebo, and diluted appropriately to give a mixture containing 8 µg/ml of PAMA and 80 µg/ml of IBU. This mixture was scanned between 200-400 nm and was derivatized to 1st order. D^1 absorbance was measured at selected wavelengths and was transformed to concentration with help of linear regression equation. This mixture was analyzed for three times and the mean % assay was drawn.

RESULTS AND DISCUSSION

Selection of analytical wavelength

Three different ZCP at 278 nm, 316 nm and 245 nm was observed in overlain D^1 spectra of PAMA (fig. 1). Three different ZCP at 250 nm, 261 nm and 291 nm were observed in overlain D^1 spectra of IBU (fig. 2). For determination of analytical wavelength D^1 spectra of PAMA and IBU were overlapped (fig. 3). While recording D^1 absorbance of PAMA at ZCP of IBU, nonlinearity was observed at 250 and 261 nm while at 291, the linear response was observed with concentration (fig. 4). In similar way at ZCP of PAMA, linearity was observed only at 278 nm for IBU (fig. 5). So 291 nm and 278 nm was selected as analytical wavelength for quantitative determination of PAMA and IBU respectively.

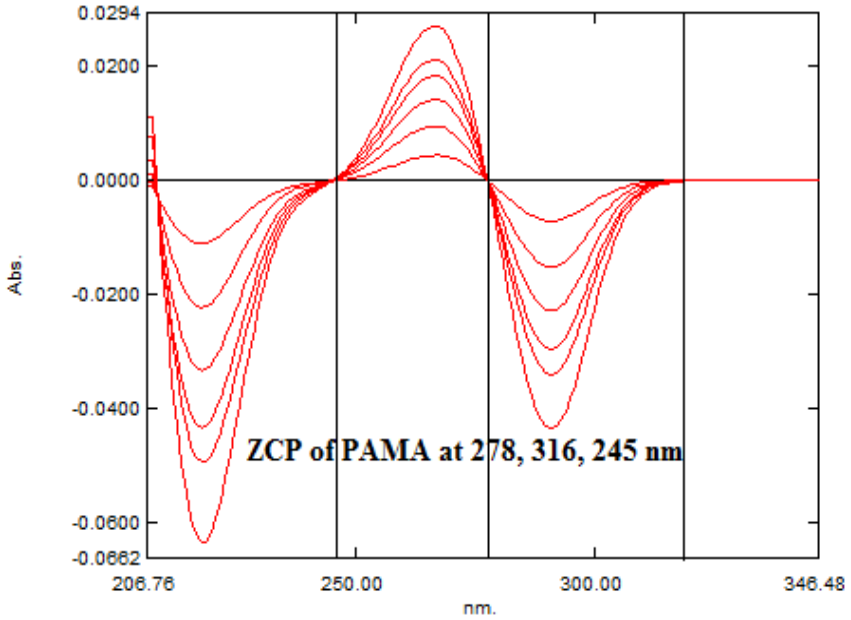

Fig. 1: Zero cross over point of PAMA

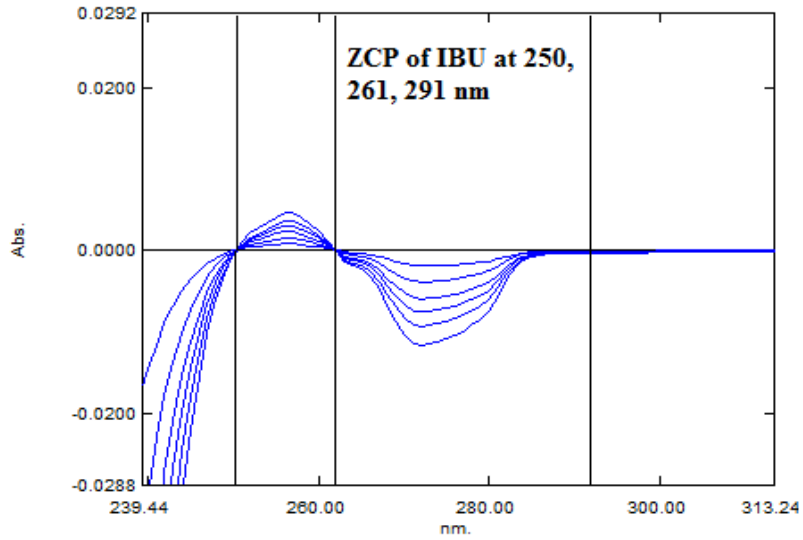

Fig. 2: Zero cross over point of IBU

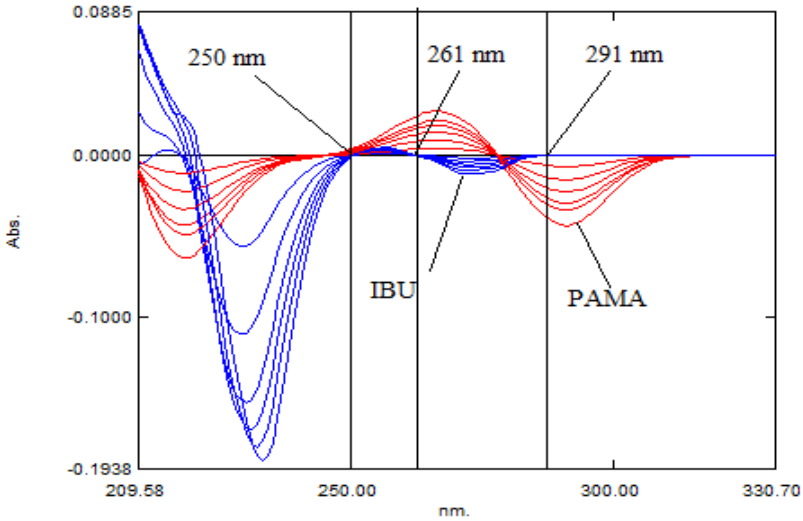

Fig. 3: Overlain D^1 spectra of IBU and PAMA

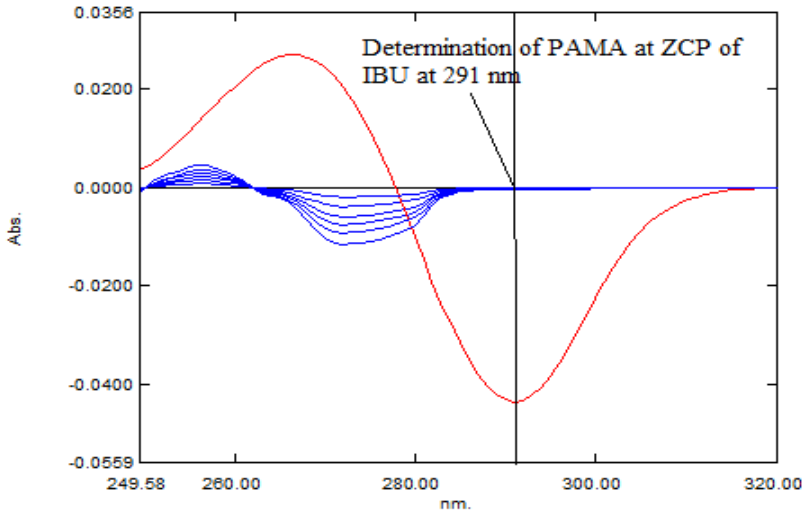

Fig. 4: Determination of PAMA at ZCP of IBU at 291 nm (12 µg/ml)

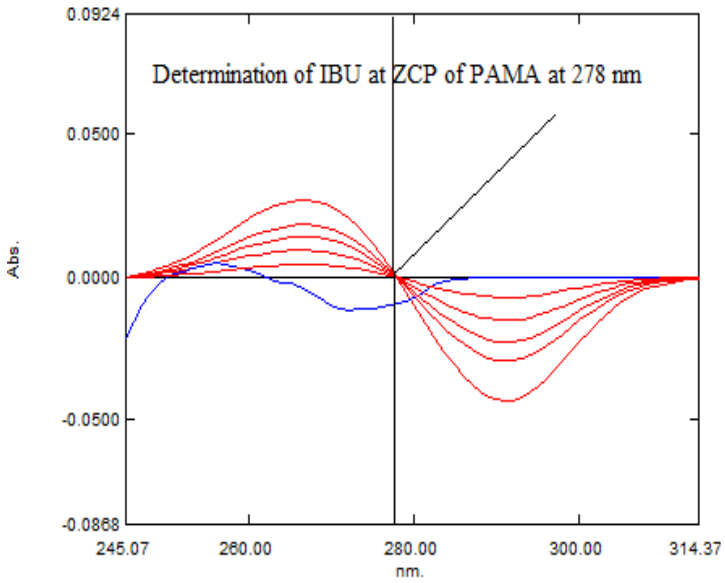

Fig. 5: Determination of IBU at ZCP of PAMA at 278 nm (120 µg/ml)

Analytical method validation

Linearity and range

When D^1 Absorbance was plotted against concentration, non-linearity was observed above 12 µg/ml for PAMA and above 120 µg/ml for IBU, so final range for validation was selected at mixture containing 2-12 µg/ml for PAMA and 20-120 µg/ml for IBU (fig. 6). When the calibration curve was plotted for given concentration range (fig. 7 and 8), value of linear regression coefficient was found to be 0.99898 for PAMA and 0.99876 for IBU. Regression equation was found to be y = 0.00361 X–0.00051 for PAMA and y = 0.00008 X–0.00011 for IBU. Linearity data for both drugs is shown in table 2.

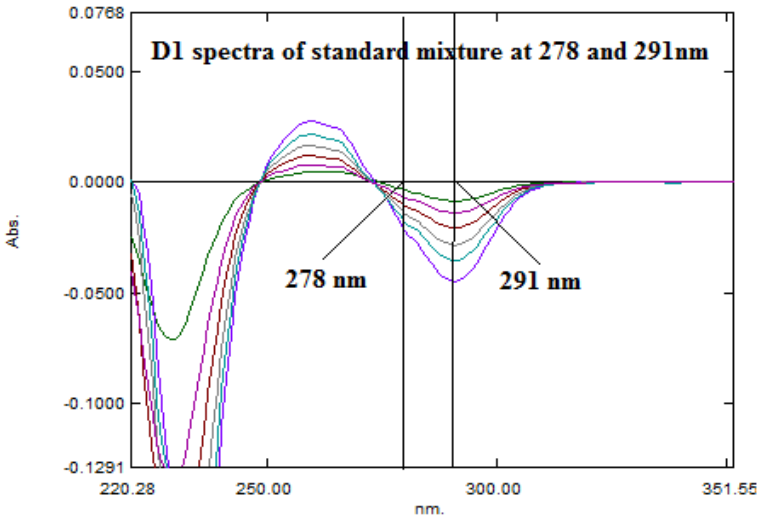

Fig. 6: D^1 Spectra of a standard mixture of IBU (20-120µg/ml) and PAMA (2-12 µg/ml) for linearity stud

Table 2: Linearity data for PAMA and IBU

S. No.	For PAMA			For IBU		
	Conc. (µg/ml)	mean±SD	RSD	Conc. (µg/ml)	mean±SD	RSD
1	2	-0.00716±0.00011	1.592	20	-0.00159±0.000013	0.815
2	4	-0.01516±0.00011	0.752	40	-0.00317±0.000042	1.329
3	6	-0.02276±0.00011	0.500	60	-0.00478±0.000045	0.947
4	8	-0.02944±0.00011	0.387	80	-0.00616±0.00011	1.850
5	10	-0.03654±0.00011	0.312	100	-0.00756±0.00011	1.508
6	12	-0.04358±0.00008	0.191	120	-0.00936±0.00011	1.218

(n= 5 determinations)

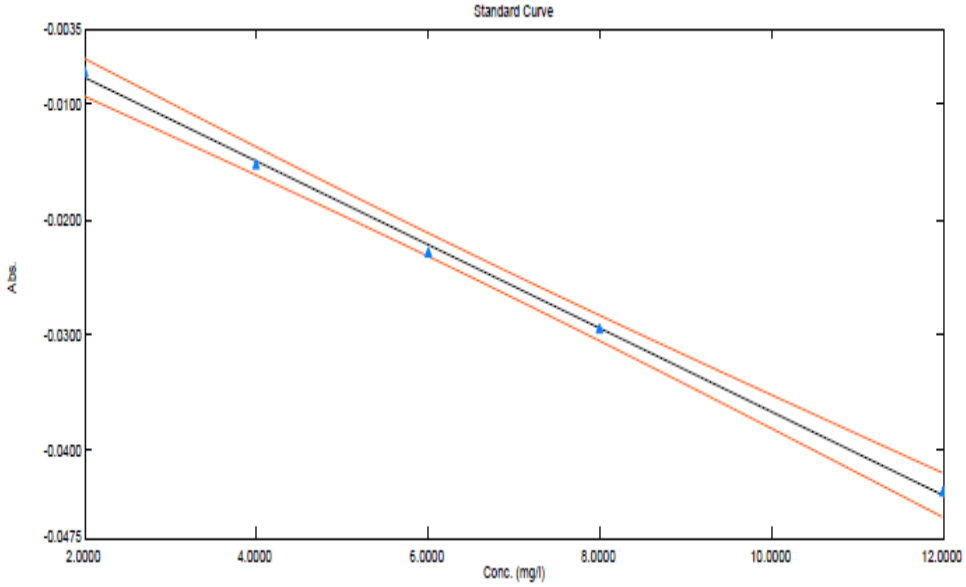

Fig. 7: Calibration curve of PAMA at 291 nm

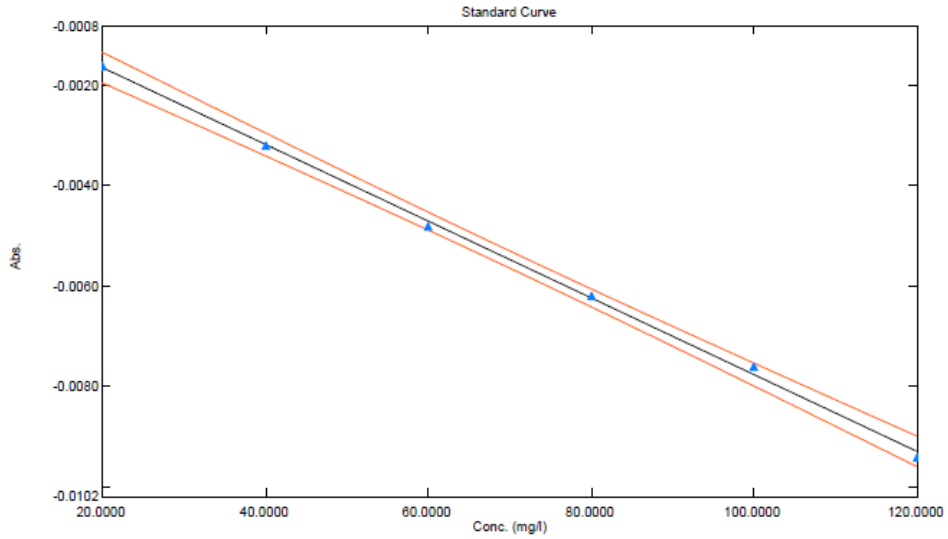

Fig. 8: Calibration curve of IBU at 278 nm

Repeatability

When all mixture were analyzed at all concentration, calculated relative standard deviation at each level was found to be less than 2 so that method was found to be repeatable over the range of 2-12 µg/ml for PAMA and 20-120 µg/ml for IBU. Repeatability data are shown in table 3 and 4 for PAMA and IBU, respectively.

Method precision

For determining interday and intraday precision, RSD was monitored at selected concentration level, which was found to be less than 2 so the method was found to be precise for estimation of PAMA and IBU. Data for intermediate precision are given in table 5 and 6 for PAMA and INU respectively.

Table 3: Repeatability data for PAMA at 291 nm

Conc. (µg/ml)	2	4	6	8	10	12
	-0.0072	-0.0152	-0.0228	-0.0294	-0.0365	-0.0436
D^1 Absorbance	-0.0071	-0.0153	-0.0227	-0.0295	-0.0368	-0.0435
	-0.007	-0.015	-0.0228	-0.0296	-0.0364	-0.0437
	-0.0072	-0.0151	-0.0226	-0.0293	-0.0365	-0.0436
	-0.0073	-0.0152	-0.0229	-0.0294	-0.0367	-0.0435
Mean	-0.00716	-0.01516	-0.02276	-0.02944	-0.03654	-0.04358
SD	0.000114	0.000114	0.000114	0.000114	0.000114	0.00008
R. SD	1.592	0.752	0.500	0.387	0.312	0.191

(n= 5 determinations)

Table 4: Repeatability data for IBU at 278 nm

Conc.(µg/ml)	20	40	60	80	100	120
	-0.00161	-0.0032	-0.0048	-0.0062	-0.0076	-0.0094
D^1 Absorbance	-0.00159	-0.00319	-0.0047	-0.0061	-0.0077	-0.0095
	-0.00161	-0.0031	-0.0048	-0.0063	-0.0075	-0.0093
	-0.00158	-0.00318	-0.00481	-0.0062	-0.0076	-0.0092
	-0.0016	-0.0032	-0.00479	-0.006	-0.0074	-0.0094
Mean	-0.00159	-0.00317	-0.00478	-0.00616	-0.00756	-0.00936
SD	0.00001	0.00004	0.00004	0.00011	0.00011	0.00011
R. SD	0.815	1.329	0.947	1.850	1.508	1.218

(n= 5 determinations)

Table 5: Intraday and interday precision for PAMA

Conc. (µg/ml)	Intraday (mean+SD)	RSD	Inter-day (mean+SD)	RSD
2	-0.007192+0.000013	0.18	-0.007228+0.000019	0.26
8	-0.0294+0.00015	0.53	-0.02942+0.00019	0.65
12	-0.04362+0.00025	0.59	-0.0446+0.00035	0.79

(n=3 determinations)

Table 6: Intraday and interday precision for IBU

Conc. (µg/ml)	Intraday (mean+SD)	RSD	Inter-day (mean+SD)	RSD
20	-0.00162+0.000019	1.19	-0.001736+0.000021	1.19
80	-0.0062+0.000042	0.67	-0.0066+0.000026	0.38
120	-0.00932+0.000113	1.20	-0.00966+0.000036	0.36

(n=3 determinations)

Table 7: Accuracy data of PAMA and IBU by derivative spectroscopy method

Level of spiking	Total placebo (mg)	Amount of std. drug added (µg/ml)		Amount of drug recovered (µg/ml)		% recovery	
		PAMA	IBU	PAMA	IBU	PAMA	IBU
Unspiked	104	-	-	-	-	-	-
50 %	104	4	40	4.03+0.025	40.2+0.32	100.9+0.6	100.6+0.6
100 %	104	8	80	8+0.03	80.5+0.64	99.9+0.3	100.7+0.8
150 %	104	12	120	11.95+0.04	120.4+0.65	99.7+0.2	100.3+0.5

(n=3 determinations)

Table 8: Assay of the synthetic mixture by validated 1st order derivative spectroscopic method

Drug	Amount taken (µg/ml)	Amount recovered (µg/ml)	% Assay
PAMA	8	7.99+0.6	100.0+0.6
IBU	80	80.14+0.7	100.1+0.7

(n=3 determinations)

Accuracy study

Spiked placebo with standard solution at 50, 100 and 150% level was analyzed for % recovery, which was found within 98 to 102, so the method was found to be accurate (table 7).

Solvent stability

As the λ_{max} was stable over a period of 24 h, the solvent was found to be suitable and the drug was found to be stable.

Assay

When prepared synthetic mixture was analyzed by the developed and validated method, % assay was found to be 100.0+0.6 for PAMA and for100.1+0.7 IBU (table 8).

CONCLUSION

The 1st order derivative spectroscopic method was developed and validated as per ICH Q2 R1 guidelines and was successfully applied for determination of PAMA and IBU from its synthetic mixture. The present method was found to be economical in terms of cost and time. Commonly used excipient didn't interfered in the estimation of PAMA and IBU so the method was found to be specific. Method was also found to be repeatable and precise.

ACKNOWLEDGMENT

Authors are extremely grateful to OSAKA pharmaceuticals limited and Amoli organics for providing a gratis sample of IBU and PAMA. The authors are also grateful to Smt. S. M. Shah Pharmacy College for providing excellent research facilities and promoting research activities.

AUTHORS CONTRIBUTIONS

All authors have contributed equally for the successful execution of research and completion with beneficial outcomes.

CONFLICT OF INTERESTS

Declared none

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