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Research Article

METHOD DEVELOPMENT AND VALIDATION OF RAPID ISOCRATIC RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL, CAFFEINE, AND PROPYPHENAZONE IN PHARMACEUTICAL FORMULATION

SAYALI JADHAV, GAYATRI BHOIR, VANDANA JAIN

Department of Quality Assurance, Oriental College of Pharmacy, Sanpada, Navi Mumbai, Maharashtra, India
***Corresponding author: vandana.jain@ocp.edu.in**

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ABSTRACT

Objective: The objective is to develop a novel, rapid, simple, precise, accurate, and reproducible RP-HPLC method for simultaneous estimation of paracetamol, caffeine, and propyphenazone in bulk and a pharmaceutical dosage form.

Method: Optimized chromatographic conditions were used for isocratic elution with Shimadzu C18 (4.6×250 mm, 5 µm), methanol, and 20mM phosphate buffer (60:40, v/v, pH 2.5) as mobile phase, flow rate 1.2 mL/min, and UV detector at λ max 272 nm. The method was validated for specificity, precision, linearity, accuracy, robustness, and solution stability as per the International Council for Harmonization (ICH) guidelines.

Result: It was discovered that the retention time for paracetamol, caffeine, and propyphenazone was found to be 2.6 min, 3.0 min, and 7.5 min. The method proved to be rapid, simple, linear (R^2>0.999), precise {relative standard deviation (RSD<2.0%)}, accurate (recovery 98–102%), sensitive, and robust.

Conclusion: The proposed novel isocratic RP-HPLC method was found to be rapid (short run time of 10 min), highly selective, accurate, and sensitive. The method has been successfully applied to the simultaneous analysis of paracetamol, caffeine, and propyphenazone in a pharmaceutical formulation.

Keywords: Paracetamol, Caffeine, Propyphenazone, RP-HPLC, Validation.

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INTRODUCTION

The present work is a method development and validation for the simultaneous estimation for quantitative analysis of the widely prescribed combination of paracetamol, caffeine, and propyphenazone in a tablet dosage form used as an analgesic for treating headaches, common cold, and menstrual cramps. Paracetamol is a good and promptly acting antipyretic since it does not contain any significant side effects except hepatotoxicity in some cases when used for a long period or overdose [1]. Paracetamol is a COX-2 inhibitor [2]. Paracetamol is adequately absorbed when taken by oral route and evenly distributed throughout the body approximately $1/4^{th}$ of which is plasma protein bounded. Metabolic process is predominately carried out by conjugating with glucuronic acid and sulfate. The conjugate is excreted rapidly through urine systemic bioavailability is dose-dependent and generally ranges from 70 to 90% [3]. Chemically paracetamol is N-(4-hydroxyphenyl) acetamide. Caffeine is 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione [4]. Caffeine is a xanthine alkaloid substance used as a psychotropic stimulant drug. Caffeine is a CNS stimulant that inhibits the phosphodiesterase enzyme and also shows an antagonistic effect at central adenosine receptors [5]. It is mostly used along with pain relievers, and cough formulations. Propyphenazone is 1,2-dihydro-1,5-dimethyl-4-(1-methylethyl)-2-phenyl-3H-pyrazol-3-one. It has the same analgesic and antipyretic properties as paracetamol. Propyphenazone is a non-steroidal anti-inflammatory (NSAID) and non-acidic drug derived from pyrazolone. Propyphenazone is a selective COX-2 inhibitor [6]. However, the HPLC method for simultaneous estimation of a formulation containing paracetamol, caffeine, and propyphenazone has not been reported. Combinations of these APIs relieve headaches. Paracetamol and propyphenazone block the release of certain chemical messengers that cause fever, pain, and inflammation (redness and swelling) [7,8]. Caffeine helps narrow the blood vessels in the brain to reduce headaches [9]. The developed method was validated as per the International Council for Harmonization (ICH) guidelines [10].

METHODS

Instrument

HPLC chromatographic separation was performed on the Shimadzu LC 2030 model using Lab Solution software. For obtaining the maximum wavelength of the compounds, UV visible spectrometer was used.

Chemicals

HPLC-grade API and chemicals were purchased. Thermo Fisher Scientific India Pvt. Ltd Supplied methanol. High Purity Laboratory Chemicals Pvt. Ltd. supplied potassium dihydrogen phosphate. Mobile phase was prepared using double-distilled water.

Chromatographic conditions

The method was developed using RP, prontosil C18 column (250×4.6 mm, 5 µ). Total runtime for the process was 10 min. Methanol was used as a mobile phase: Orthophosphoric acid of pH- 2.5 in the ratio of 60:40 v/v was employed at a flow rate of 1.2 mL/min and the column's temperature was maintained at 30°C. A UV–visible detector was used to find a 272nm wavelength.

Selection of wavelength

Suitable wavelength for HPLC analysis was determined by ultraviolet (UV) spectrum in the range of 200-400 nm for individual drug solutions of paracetamol, caffeine, and propyphenazone then overlapped. Hence 272 nm, λ max was selected as a suitable wavelength (Fig. 1).

Preparation of phosphate buffer pH 2.5

Potassium phosphate 1.36 gm was accurately weighed and dissolved in 500 ml of distilled water and the pH was adjusted up to 2.5 using ortho-phosphoric acid.

Preparation of standard stock solutions

Paracetamol, caffeine, and propyphenazone were weighed at 250 mg, 50 mg, and 150 mg, and transferred to three separate 100 mL volumetric flasks. About 50 ml of methanol was added and sonicated for 15–20 min and volumes were made up to the mark with methanol, respectively. Final concentrations of paracetamol, caffeine, and propyphenazone of 25 µg/mL, 5µg/mL, and 15µg/mL were made by suitable dilution.

Preparation of sample solution

Ten tablets were weighed and crushed finely. Tablet powder equivalent to 250 mg of paracetamol, 50 mg of caffeine, and 150 mg of propyphenazone was transferred to a 100 ml volumetric flask and centrifuged for 20 min at 10,000 rpm. The solution was further diluted to obtain paracetamol 25 µg/mL, caffeine 50 µg/mL, and propyphenazone 15 µg/mL.

RESULT AND DISCUSSIONS

Method Development

The C18 column was selected for the RP-HPLC method to estimate paracetamol, caffeine, and propyphenazone. Method optimization was performed by the trial-and-error method. Changing the mobile phase ratio, flow rate, and pH optimized conditions to obtain a good resolution, retention time, acceptable number of theoretical plates, and tailing factor. Initially, trials were taken using the 20 mM phosphate buffer pH adjusted with ortho-phosphoric acid at various pH values and different mobile phase proportions. The trial obtained optimal separation using an isocratic combination of mobile phase consisting of methanol: 20 mM potassium phosphate buffer pH 2.5 adjusted with ortho-phosphoric acid (60:40 v/v), and the flow rate selected was 1.2 mL/min the retention time observed was 2.6 min for paracetamol, 3.0 min caffeine, and 7.5 min propyphenazone (Figs. 2-7 and Table 1).

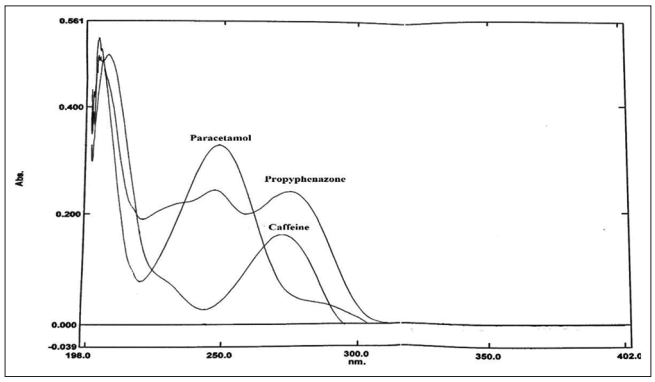

Fig. 1: UV overlap spectrum of paracetamol 25 ppm, caffeine 50 ppm, and propyphenazone 15 ppm using UV detector and lab solution software generated within the range of 400–200 nm

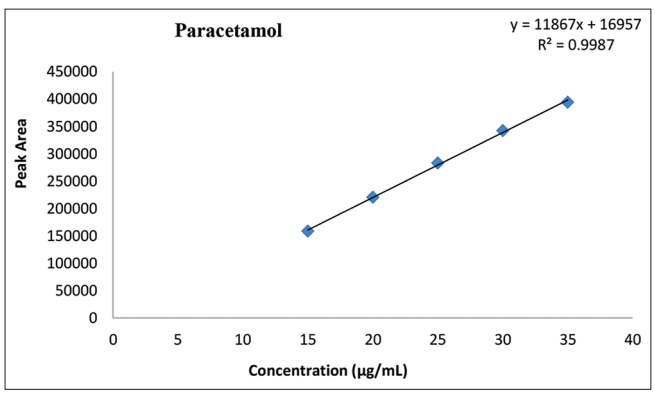

Fig. 2: Calibration curve of paracetamol 25 ppm; x is concentration, y is absorbance, R^2 is correlation coefficient

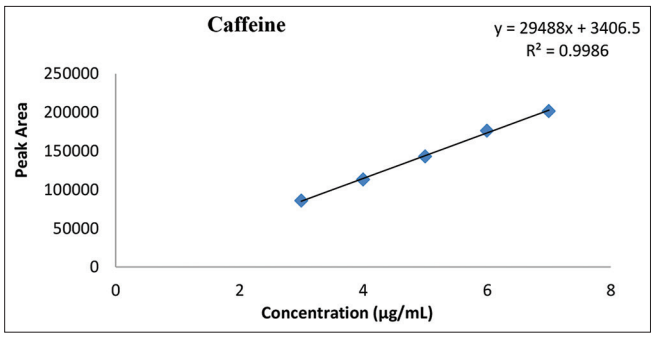

Fig. 3: Calibration curve of Caffeine 50 ppm

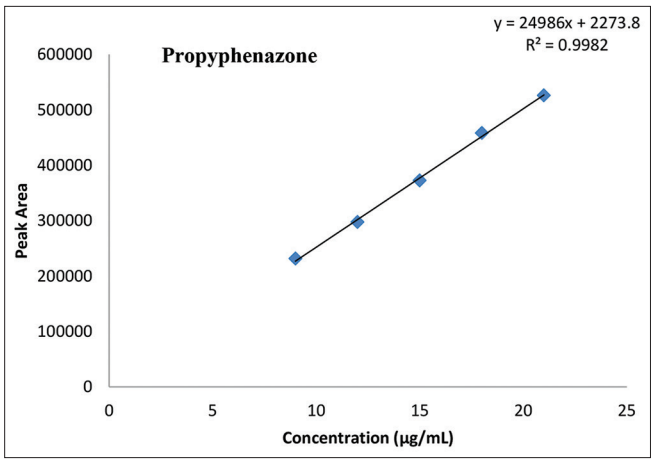

Fig. 4: Calibration curve of Propyphenazone 15 ppm

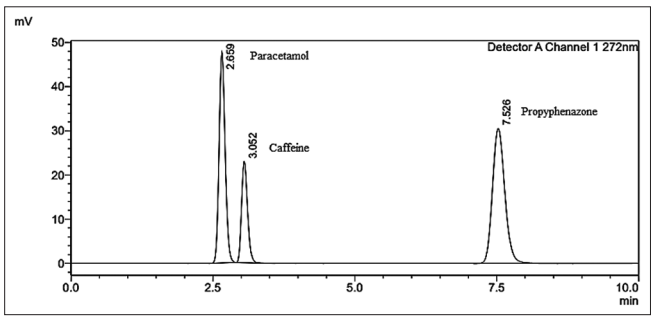

Fig. 5: Chromatogram of sample solution of paracetamol 250 mg, caffeine 50 mg, and propyphenazone 150 mg

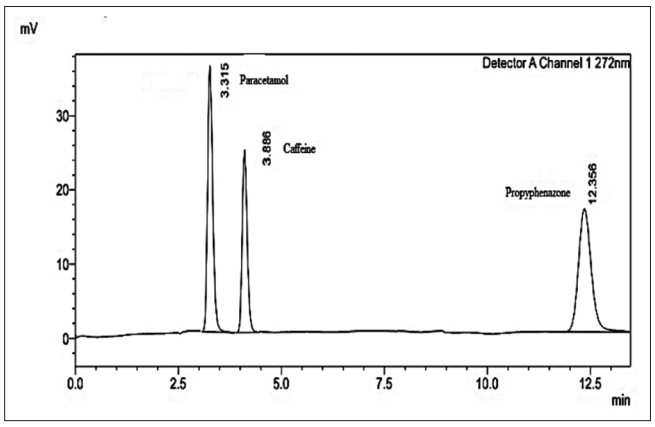

Fig. 6: Chromatogram of paracetamol, caffeine, and propyphenazone using mobile phase as methanol and phosphate buffer (55:45v/v), pH= 3

METHOD VALIDATION

Specificity

Specificity of the developed RP-HPLC method was determined by injecting blank, working standard, and sample solutions. The method was found to be specific since the complete separation was achieved in chromatograms of paracetamol, caffeine, and propyphenazone. The retention time of standard drugs and drugs in the sample solution was the same.

System precision

Six replicates (n=6) of standard solution at the working concentration of four compounds were used to test system precision. Because the %RSD of the peak areas obtained was <2.0%, indicating good reproducibility, the repeatability was quantified in terms of % RSD, and the devised technique was determined to be precise (Table 2).

Method precision

Method precision was determined by injecting six duplicates of the sample under the repeatability test at working concentration. Because the %RSD of the obtained peak regions was <2.0% indicating acceptable reproducibility. As a result, the developed procedure was determined to be precise (Table 3).

Linearity

Linearity was determined in the range of 10–35 µg/mL, 2–7 µg/mL, and 6–21 µg/mL for paracetamol, caffeine, and propyphenazone, respectively, with an acceptable coefficient of correlation (R^2). A calibration curve was constructed by plotting concentration (µg/mL) on the X-axis against the peak area on the Y-axis. R^2 was found to be 0.9989, 0.9987, and 0.9985 (Fig. 8-10 and Table 4).

Accuracy

Method's accuracy was determined by calculating recovery studies on the test sample at three distinct concentration levels (80%, 100%,

Table 1: Optimized chromatographic conditions

Parameters	Optimized conditions
Column	Shimadzu C18 (4.6×250 mm, 5 µm)
Mobile phase	Methanol: 20 mm Phosphate buffer (60:40 v/v)
Flow rate	1.2 mL/min
Run time	10 min
Column temperature	30°C
Injection volume	10 µL
Detection wavelength	272 nm
Retention time of paracetamol	2.6 min
Retention time of caffeine	3.0 min
Retention time of propyphenazone	7.5 min
Run time	10 min

Table 2: Result of system precision study for paracetamol, caffeine, and propyphenazone

S. No.	Paracetamol 25 µg/mL	Caffeine 5 µg/mL	Propyphenazone 15 µg/mL
	Peak area		
1	282143	142842	379120
2	283798	145031	375951
3	284979	144887	379091
4	281845	146556	379454
5	282255	143101	376036
6	283670	143711	379484
Average	283115	144354.66	378189.33
SD	1228.51	1404.33	1708.89
% RSD	0.43	0.97	0.45

Table 3: Result of method precision study for paracetamol, caffeine, and propyphenazone

	% Assay of peak area		
	Paracetamol 25 µg/mL	Caffeine 5 µg/mL	Propyphenazone 15 µg/mL
1	101.20	100.61	98.51
2	100.03	99.96	98.63
3	101.57	99.66	99.98
4	102.17	100.58	98.94
5	101.58	98.72	100.08
6	101.43	99.69	98.34
Average	101.33	99.87	99.08
SD	0.71	0.69	0.76
% RSD	0.70	0.70	0.76

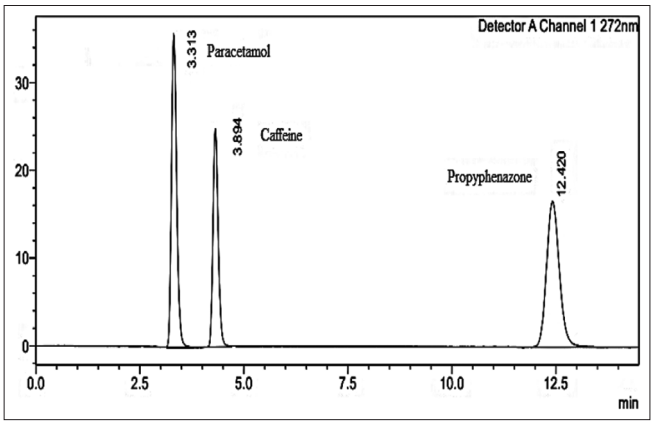

Fig. 7: Chromatogram of paracetamol, caffeine, and propyphenazone using mobile phase as methanol: Phosphate buffer (55:45 v/v), pH= 2.5, Flow rate= 1ml/min

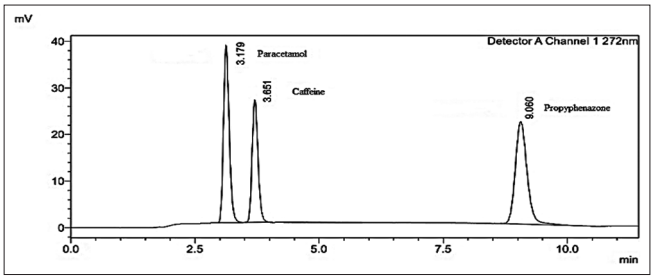

Fig. 8: Chromatogram of paracetamol, caffeine, and propyphenazone using mobile phase as methanol: Phosphate buffer (60:40v/v), pH= 2.5, Flow rate= 1ml/min

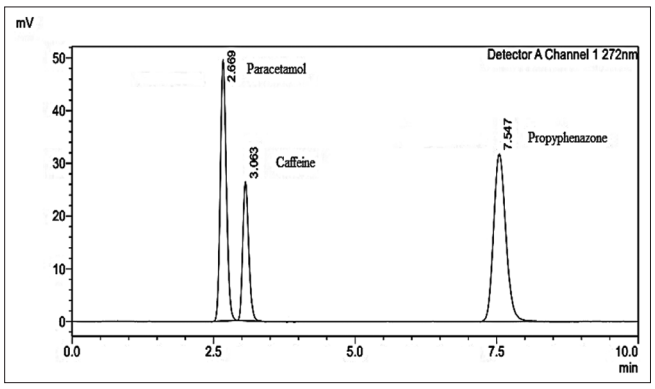

Fig. 9: Chromatogram of paracetamol, caffeine, and propyphenazone using mobile phase as methanol: Phosphate buffer (60:40v/), pH=2.5, Flow rate= 1.2ml/min

and 120%) using the standard addition method. At each level, three replicates were injected into a chromatographic system. The mean percent recovery was within a range of 98–102% and the established method was proven to be accurate based on the percent recovery result (Fig. 4). The results of recovery studies are shown in Table 5.

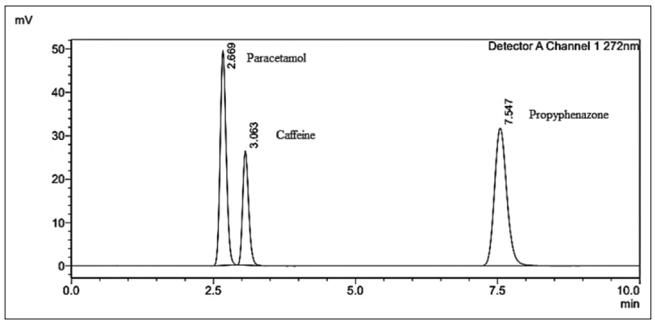

Fig. 10: Chromatogram of a standard mixture of paracetamol, caffeine, and propyphenazone

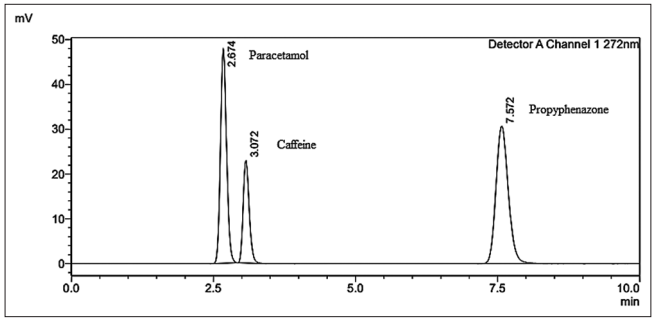

Fig. 11: Chromatogram of a sample mixture of paracetamol, caffeine, and propyphenazone

Table 4: Result of linearity study for paracetamol, caffeine, and propyphenazone

Parameters	Paracetamol	Caffeine	Propyphenazone
Linearity range (µg/ml)	15–35 µg/ml	3–7 µg/ml	9–21 µg/ml
Slope	11867	29488	24986
Intercept	16957	3406.5	2273.8
Correlation coefficient (R2)	0.998	0.998	0.998

Robustness

Robustness was performed by a small variation in the chromatographic conditions such as flow rate (±0.1 ml), wavelength (±1 nm), and temperature (±1°C). It was found that none of the above parameters caused an alteration in the peak area and retention time. % RSD was within limits and the method was found to be robust (Table 6).

ASSAY OF MARKETED FORMULATION

Assay of marketed formulation (Saridon)

The % assay of the marketed formulation containing Paracetamol (250 mg), Caffeine (50 mg) and Propyphenazone (150 mg) was found to be 101.25% for Paracetamol, 99.94% for Caffeine, and 99.69% for Propyphenazone (Fig. 11 and Table 7).

Sensitivity

Limit of detection (LOD) = 3.3 × σ/S

σ It is the standard deviation of the peak areas of the drug and S is the slope of the calibration curve.

Paracetamol: 1.08 µg/mL
Caffeine: 0.22 µg/mL
Propyphenazone: 0.75 µg/mL
Limit of Quantification (LOQ) = 10×σ/S

σ is the standard deviation of the peak areas of the drug, and S is the slope of the corresponding calibration curve.

Paracetamol: 3.27 µg/mL
Caffeine: 0.68 µg/mL
Propyphenazone: 2.29 µg/mL

The aim of developing the chromatographic method was to develop a method that is reliable and accurate for the quantification of paracetamol, caffeine, and propyphenazone in a pharmaceutical dosage form. For analysis optimized chromatographic conditions were used and the findings were within acceptable limits and ranges. A suitable wavelength of 272 nm was selected. The system precision, method

Table 5: Result of recovery study for paracetamol, caffeine, and propyphenazone

Pre-analyzed sample solution	Level of recovery (%)	Amount added (µg/mL)	Amount recovered (µg/mL)	% recovery
Paracetamol	80	19.82	19.70	99.39
	100	24.81	24.81	100.01
	120	29.75	29.74	99.95
Caffeine	80	3.93	3.92	99.82
	100	4.92	4.89	99.39
	120	5.92	5.91	99.94
Propyphenazone	80	11.87	11.84	99.76
	100	14.83	14.82	99.88
	120	17.83	17.75	99.58

Table 6: Result of robustness study for paracetamol, caffeine, and propyphenazone

Parameter	Deviation	%RSD (Peak Area)		
		Paracetamol	Caffeine	Propyphenazone
Flow rate (mL/min)	0.9 mL/min	0.39	1.06	0.37
	1.1 mL/min	0.11	1.05	0.11
Wavelength (nm)	271 nm	0.25	0.64	0.37
	273 nm	0.25	0.82	0.42
Column temperature (°C)	29°C	0.19	0.33	0.49
	31°C	0.64	1.37	0.33

Table 7: % assay of marketed formulation

Tablet	Drug	% Assay
Saridon	Paracetamol	101.25
	Caffeine	99.94
	Propyphenazone	99.69

precision, and accuracy were given by % RSD and found to be <2% indicating the method is precise and accurate. The calibration curves of paracetamol, caffeine, and propyphenazone were found to be linear over concentration ranges of 10–35 µg/ml, 2–7 µg/ml, and 6–2 µg/ml, respectively. Retention times for samples were 2.6 min for paracetamol, 3.0 min for caffeine, and 7.5 min for propyphenazone was found for the developed analytical method. The % assay was found to be 101.25% for paracetamol, 99.94% for caffeine, and 99.69% for propyphenazone determining the purity of the sample.

CONCLUSION

The study describes a novel rapid RP-HPLC systemic technique for the simultaneous estimation of paracetamol, caffeine, and propyphenazone in a pharmaceutical oral dosage form. All three drugs have good resolution with a short analysis time of below 10 minutes, hence making it an economic method. The developed isocratic HPLC technique was found to be uncomplicated, linear, accurate, precise, robust, specific; and sensitive which made the technique versatile and valuable for the simultaneous estimation of three drugs in the pharmaceutical dosage form. Acceptable values of precision and accuracy were obtained at all levels by this method as per guidelines for assay validation. The developed method can be used in routine chemical analysis of paracetamol, caffeine, and propyphenazone in the pharmaceutical dosage form. The proposed technique was validated, and proven for adequate selectivity and lower limits of detection and quantification.

LIST OF ABBREVIATIONS

RP-HPLC: Reverse phase high-performance liquid chromatography; UV: ultraviolet; ICH: International Conference on Harmonization; RSD: Relative standard deviation; LOD: Limit of detection; LOQ**:** Limit of quantitation

CONFLICTS OF INTEREST

The authors state that they have no conflicts of interest.

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AUTHOR'S CONTRIBUTION

Dr. Vandana Jain supervised experiments, and analyzed data as well. The data regarding the topic was collected, validated, analyzed, and drafted by Sayali Jadhav and Gayatri Bhoir. The final manuscript was read and approved by all writers.

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