



Validated Isocratic/Gradient RP-HPLC for Simultaneous Estimation of Paracetamol Ibuprofen and Caffeine in Marketed Formulations Using Diclofenac as Internal Standard

Palled PJ, Dushyanth Reddy V*, Mannor VS and Bharat Chowdary

Department of Pharmaceutical Chemistry, KLE's University College of Pharmacy, KLE University Belagavi 590001, India

*Corresponding author: Dushyanth Reddy V, Department of Pharmaceutical Chemistry, KLE's University College of Pharmacy, KLE University, Belagavi 590001, India, Tel: 08312471399; E-mail: dushyanthreddy233@gmail.com

Received: October 16, 2016; Accepted: January 16, 2017; Published: January 22, 2017

Abstract

Objective: To develop a validated RP-HPLC method for simultaneous estimation of Paracetamol Ibuprofen and Caffeine.

Methods: The HPLC instrument used was Shimadzu LC-20AD with reverse phase ODS-Hypersil-C18 (250 mm × 4.6 mm, 5 μm) column using Acetonitrile:water (90:10) pH adjusted to 2.8 as mobile phase. The flow rate was maintained at 0.3 ml/min and UV detection was carried at 203 nm.

Results: The method was validated for linearity, accuracy, precision, specificity, robustness and ruggedness according to ICH guidelines. The retention time for Paracetamol, Ibuprofen and Caffeine was found to be 9.7, 12.66 and 10.48 respectively. The regression analysis showed good linearity over the concentration range of 1.25 μg/ml to 20 μg/ml for paracetamol, 0.625 μg/ml to 10 μg/ml for Ibuprofen and 0.625 μg/ml to 10 μg/ml for Caffeine. The recovery studies of the method gave good results in the range of 99.89% to 100.48% with less than 2% of RSD.

Conclusion: The method was found to be suitable for the analysis of marketed formulation in presence of other excipients.

Keywords: Paracetamol; HPLC; Chromatography; Ibuprofen; Anti-inflammatory

Introduction

Paracetamol (Acetaminophen) is chemically N-(4-hydroxyphenyl) acetamide is a crystalline solid is a sparingly soluble compound which is classified under antipyretic analgesics. Drugs Classified under this class possess analgesic and antipyretic activity but lacks anti-inflammatory effects indicated for use in patients who are sensitive to aspirin with usual adult dosage 325 mcg to 650 mcg dose greater than 2.6 g/day are not advisable for prolonged treatment owing to its hepatotoxicity. Antipyretic effect of acetaminophen affords to the inhibition of endogenous leukocytic pyrogens released from cells upon external stimuli or upon activation with exogenous pyrogen. acetaminophen possesses analgesic activity in arthritis and musculoskeletal disorders.

Citation: Palled PJ, Dushyanth Reddy V, Mannor VS, et al. Validated Isocratic/Gradient RP-HPLC for Simultaneous Estimation of Paracetamol Ibuprofen and Caffeine in Marketed Formulations Using Diclofenac as Internal Standard. Anal Chem Ind J. 2017;17(1):116.
© 2017 Trade Science Inc.

Acetaminophen is available in various formulations say suppositories, tablets, capsules, granules and solutions. Ibuprofen is chemically 2-(4-isobutylphenyl) propionic acid is a crystalline solid is a sparingly soluble compound. It is classified as Non-Steroidal anti-inflammatory drug it was the first NSAID approved after Indomethacin. First NSAID to become over the counter (OTC) drugs. It is marketed as racemic mixture even its biological response owed almost evidently with S-(+) isomer. Ibuprofen is more potent than aspirin but less effective than indomethacin. Ibuprofen produces moderate levels of gastric irritation [1]. ibuprofen is indicated in patients suffering with rheumatoid arthritis, osteoarthritis, fever and dysmenorrhea. Caffeine is chemically 1,3,7-trimethyl-1H-purine-2,6(3H,7H)-dione acetamide is a crystalline solid is a sparingly soluble compound which is chemically methyl xanthines naturally occur in coffee (coffee Arabica) which is generally termed as stimulant and as a bronchodilator. caffeine is generally added to other OTC analgesic and stimulants.

Methodology

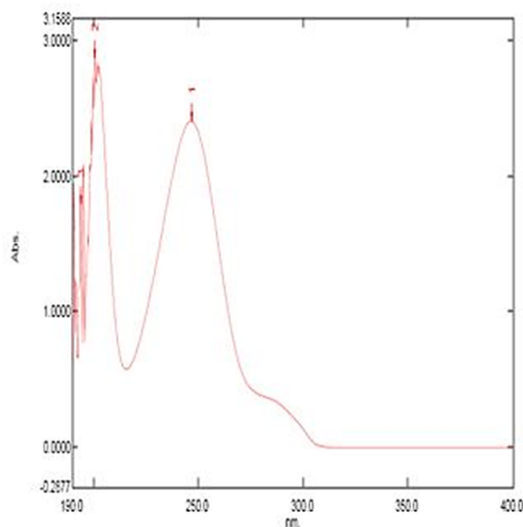
1. UV Profiling of API's
2. HPLC Method Development
 - a. Isocratic
 - b. Gradient
3. Selection of Internal Standard
4. Assay Methodology for Quantification of API's

Chromatography

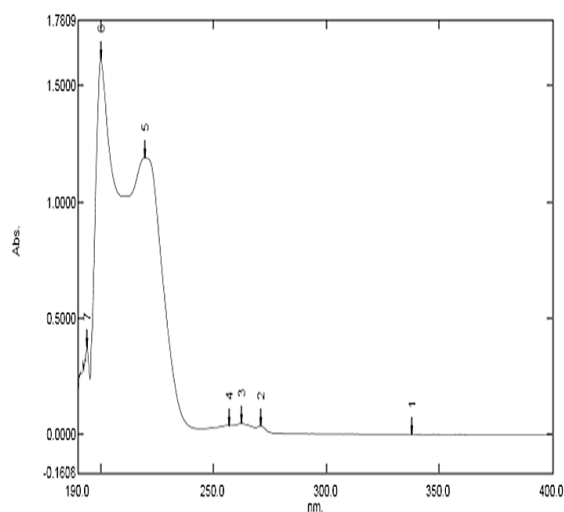
Ultraviolet spectroscopy (absorption spectroscopy) is one of the most widely used technique for quantification of organic and inorganic molecules. Chromatography is a technique for separation of organic molecules. Hyphenated instrumentation technique say (HPLC-UV/LC-MS/LC-NMR) is a technique to reconcile output abnormalities of one instrument with input abnormalities of other instruments. Lower edge or conventional instruments say UV/Fluorescence/IR serve as detectors in high end or Hyphenated instrumentation technique say (HPLC-UV/LC-MS/LC-NMR). High Performance Liquid Chromatography is a technique of separation of molecules (in $\mu\text{g-ng/mL}$) in shorter time. Various literature reported HPLC methods are available for simultaneous estimation of paracetamol and ibuprofen either alone or in combination with other API's but very few direct HPLC method for simultaneous estimation of paracetamol, ibuprofen and caffeine according our knowledge. We employ a criteria of standard addition method for inclusion of internal standard in quantification of API's.

UV profiling of API's

As the current research work is executed with HPLC with UV detector (Dual Wavelength detector), we had performed spectral scan for all the API's of interest both individually and simultaneously. Our aim was to identify an isobestic point (wavelength at which all API's have common absorbance). Identification of Isobestic point using UV spectroscopy is the criteria for wavelength selection in HPLC-UV. In UV scan of API's Paracetamol, Ibuprofen and Caffeine (absorption maxima as a function of wavelength) (FIG. 1 and 2) isobestic point was found to be 203 nm. The same wavelength was selected in HPLC-UV(LC-20-AD).



Absorption Maxima of Paracetamol



Absorption Maxima of Ibuprofen

FIG. 1. Absorbance maxima as a function of wavelength.

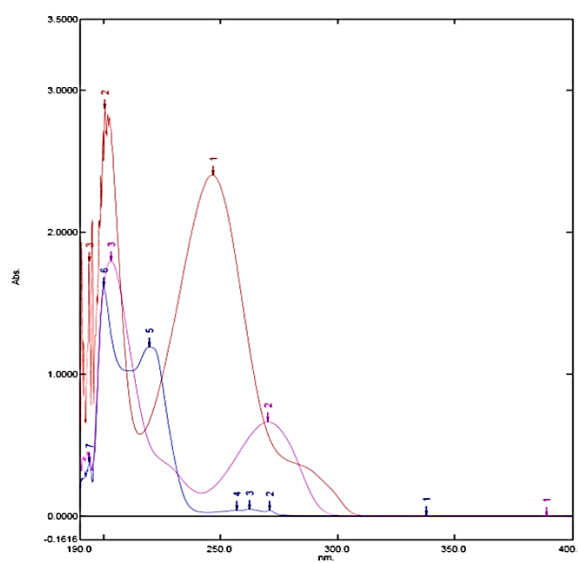
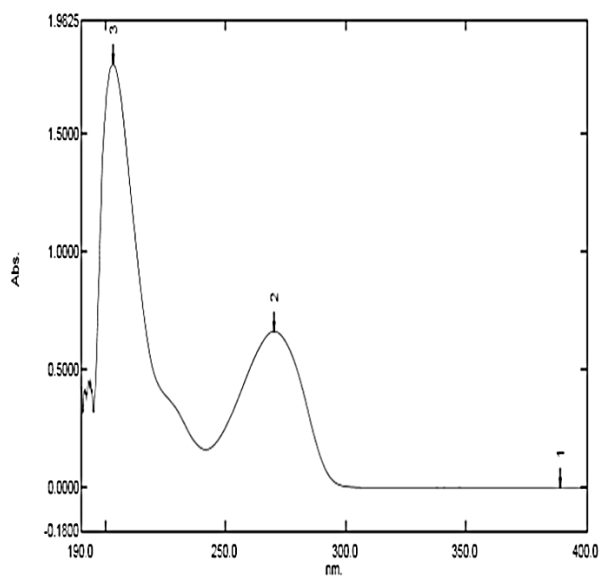


FIG. 2. Absorption maxima of Caffeine and isobestic point.

HPLC Method Development

Mobile phase selection

Various combinations of mobile phases with and without buffer at varied pH from 2-8, with more emphasis on final pH of mobile phase is adjusted to ± 2 units of pka value of API. Various combinations of mobile phase tried are highlighted in Table.

Column or stationary phase selection

Various stationary phases (columns) have been screened for current research, the best fit column with respect to tailing factors and theoretical plates, eventually ODS Hypersil C18 was selected for analysis.

Materials, reagents and chemicals

Paracetamol, Ibuprofen and Caffeine pure drug was obtained as gift sample from Sun Pharma Sparc Research Centre Vadodara. The other chemicals like methanol, acetonitrile and milli-Q waters were procured from Milli pore water system available at BSRC. The tablet dosage forms imol plus procured from local market [2].

Instrumentation

UV instrument consist of Shimadzu UV, PH meter, Milli pore water purification system. The HPLC system consist of Shimadzu LC 20AD, Hamilton injector of 20 µl capacity and detected by SPD-20A UV detector with LC-Solutions software. The stationary phase used is reverse phase ODS-Hypersil-C18.

Chromatographic conditions

The mobile phase consists of Acetonitrile:water (90:10) pH adjusted to 2.8, all the solvents used in analysis are filtered through 0.45 µ Sartorius filter paper, degassed by ultra-sonication for 10 min. The flow rate was maintained at 0.3 ml/min. Aliquots of the samples (20 µl) (injection volume) were injected and the total run was kept at 20 min. the chromatogram was monitored at 203 nm.

Standard solutions and Calibration curves

The standard stock solutions of the drug was prepared by dissolving 10 mg pure drug in 100 ml of mobile phase. Serial dilutions were made from stock solutions using acetonitrile.

Sample preparation

Ten tablets of Paracetamol, Ibuprofen and Caffeine were weighed and grinded into fine powder in mortar and pestle. A mass of powder equivalent to 100 mg of Paracetamol, Ibuprofen and Caffeine was accurately weighed and transferred to a volumetric flask containing acetonitrile. The resultant solution was sonicated for 5 min and filtered through nylon filter and the volume was made up to 100 ml using acetonitrile. serial dilutions were made from the stock solution of formulation, and were analyzed for their recovery studies [3].

Method validation

The method was validated according to the ICH guidelines for the following parameters.

Linearity and sensitivity

Standard solutions of Paracetamol, Ibuprofen and Caffeine was prepared in the concentration range of 5 µg/ml to 25 µg/ml. Then 20 µl of each solution were injected in triplicate on to the column and the chromatogram was developed using above mobile phase Acetonitrile:water (90:10) pH adjusted to 2.8 ratio. The RF values were plotted against the corresponding concentration to obtain the calibration graph. The LOD and LOQ were calculated based on the equation: $LOD=3.3 \times A/B$ and

$LOQ=10 \times A/B$. Where, A is SD of peak areas of the drugs taken as a measure of noise and B is the slope of corresponding calibration curve FIG. 3-5.

Precision

The interday and intraday precision studies were conducted by using three different concentrations of the standard (initial, medium and final concentrations) in triplicate in a day and on three consecutive days.

Accuracy

The accuracy of the method was examined by performing recovery studies in triplicate using standard addition method (50%, 100% and 150%). Accurately known amount of sample was added to a known amount of pre-analyzed tablet powder and was analyzed (TABLE 1).

Robustness

Robustness of the method was determined by introducing small changes in the mobile phase composition, change in flow rate and detection wavelength. During initial stages of development of method, the method was subjected to small changes and the effect of small changes in method on the detection of Paracetamol, Ibuprofen and Caffeine with respect to peak shape, RT values and stability were studied (TABLE 2-5).

Results and Discussion

Optimization of the RP-HPLC method

Various solvent systems were evaluated to obtain better chromatogram. Initially, methanol, HPLC grade water, acetate buffer, acetonitrile and phosphate buffer were tried in different ratios [4]. But the resolution was not satisfactory. Finally, the mobile phase consisting of Acetonitrile:water (90:10) pH adjusted to 2.8 found to be optimum.

Linearity and sensitivity

The linearity was evaluated by determining six working standard solutions containing 5 µg/ml to 25 µg/ml of Paracetamol, Ibuprofen and Caffeine in triplicate with good correlation coefficient in the concentration range. ($r=0.989$). The LOD and LOQ was found to be 0.0532 µg/ml and 2.344 µg/ml respectively (FIG. 5-8).

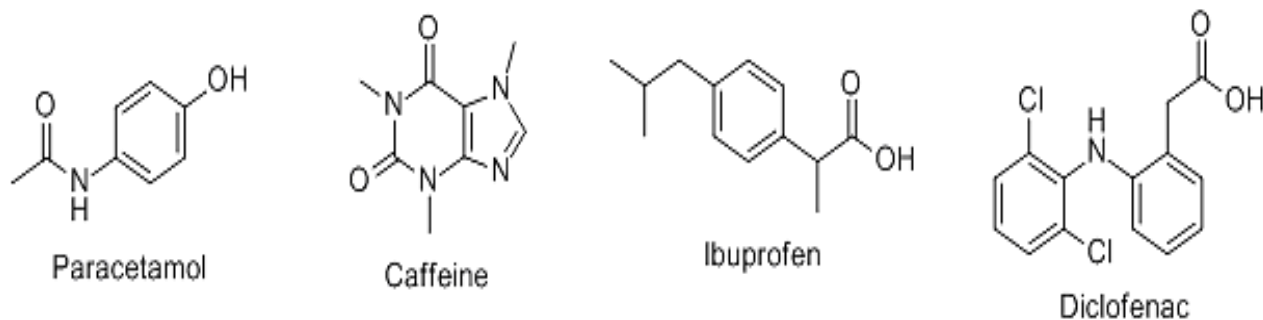
Assay

The amount of Paracetamol, Ibuprofen and Caffeine present per tablet was calculated by comparing the peak area, with that of the internal standard.

Selection and role of internal standard

Conventional quantification techniques carry an error, to minimize the error in analysis, recovery and quantification of API was done with respect to internal standard method. criteria for selection of internal standard; structurally similar compounds have similar retention times, diclofenac is used as internal standard [5]. The conventional quantification technique (using linearity and regression is obsolete and carry certain amount of random error. Internal standard analysis always carry standard amount of determinate error throughout the analysis.

The structures of API's were drawn using Chem. Draw Ultra-10



Assay Methodology

$$\frac{\text{Peak Area of Internal Standard}}{\text{Peak Area of API (or) Analyte}} = F \frac{\text{Conc of Internal Standard}}{\text{conc of API (or) Analyte}}$$

Recovery experiment

To study the accuracy, reproducibility and the precision of the proposed method recovery experiments were carried out. A fixed amount of pre-analyzed sample was taken and standard drug was added at three different concentrations and each level was repeated for 3 times. The recovery was estimated to be more than 100% [6].

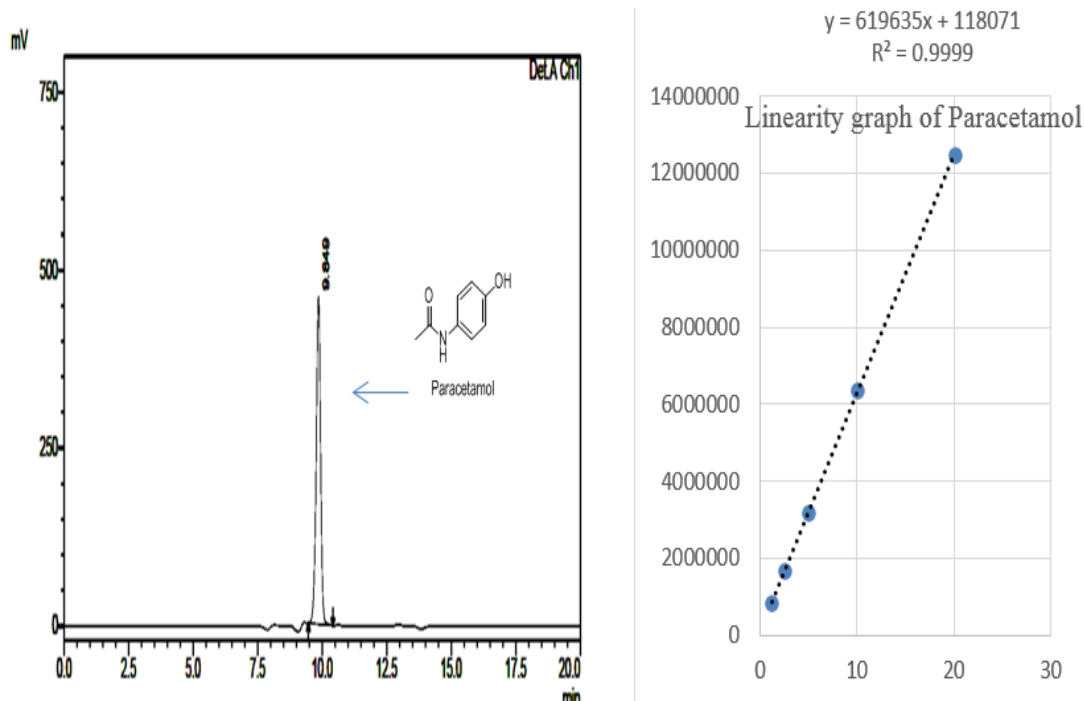


FIG. 3. Chromatogram of paracetamol linearity graph of paracetamol.

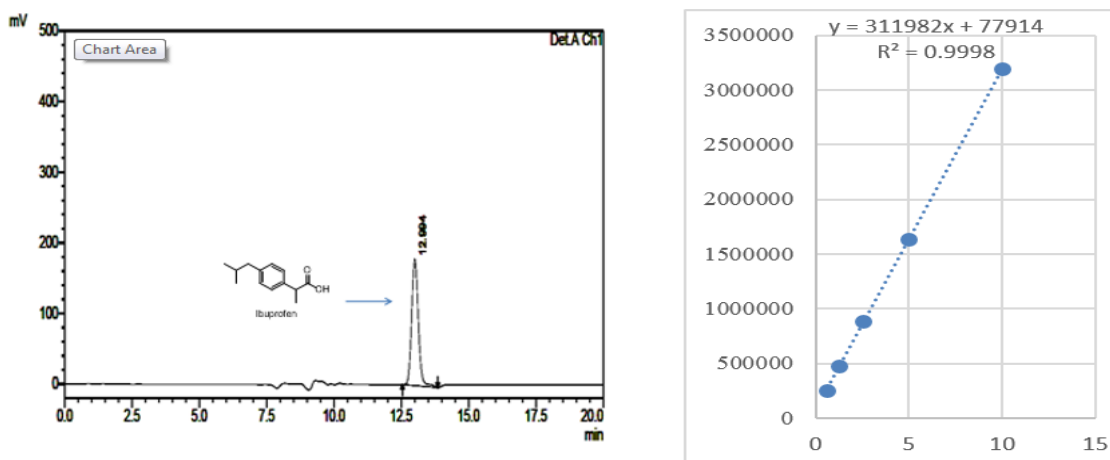


FIG. 4. Chromatogram of Ibuprofen linearity graph of Ibuprofen.

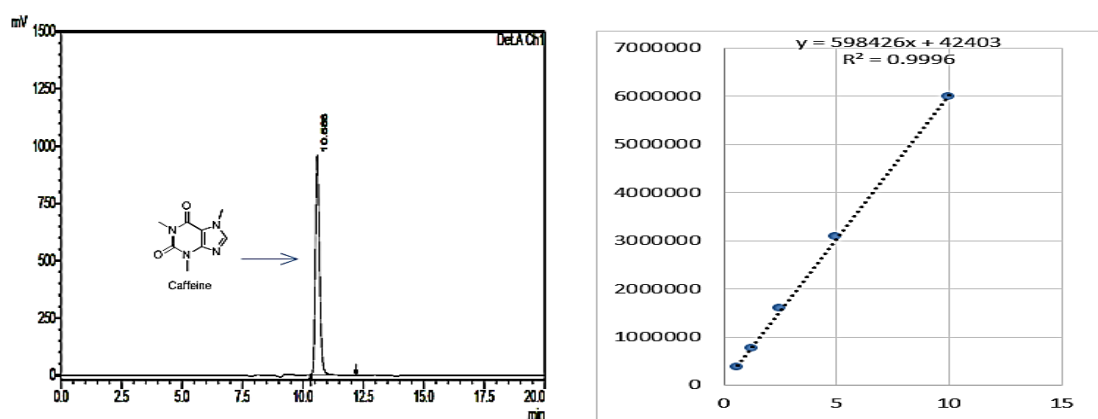


FIG. 5. Chromatogram of Caffeine linearity graph of Caffeine.

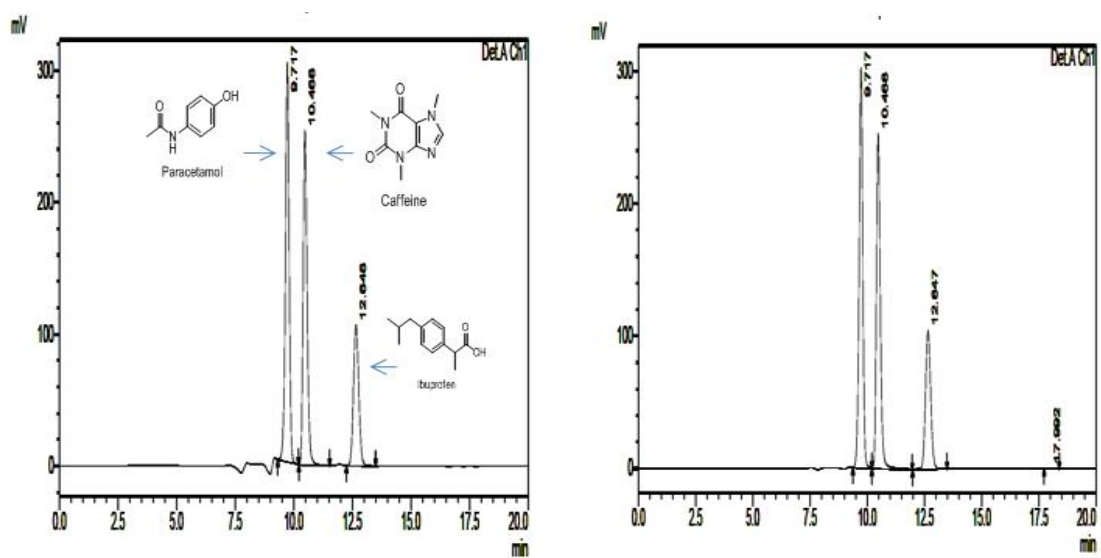


FIG. 6. Chromatogram without Baseline Correction with Baseline Correction.

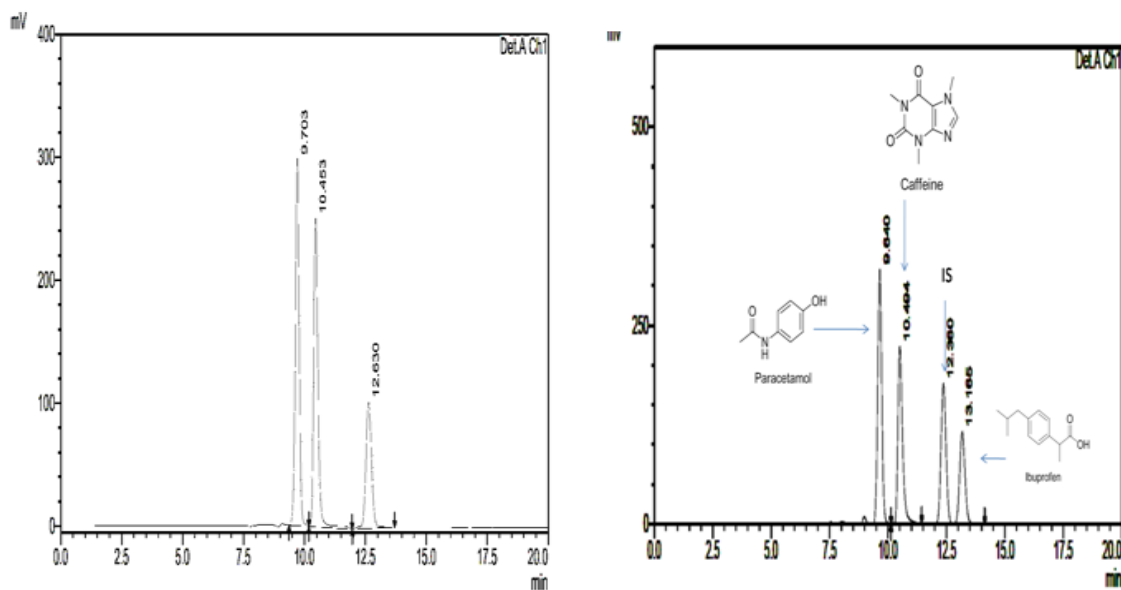


FIG. 7. Chromatogram without internal standard Chromatogram with internal standard.

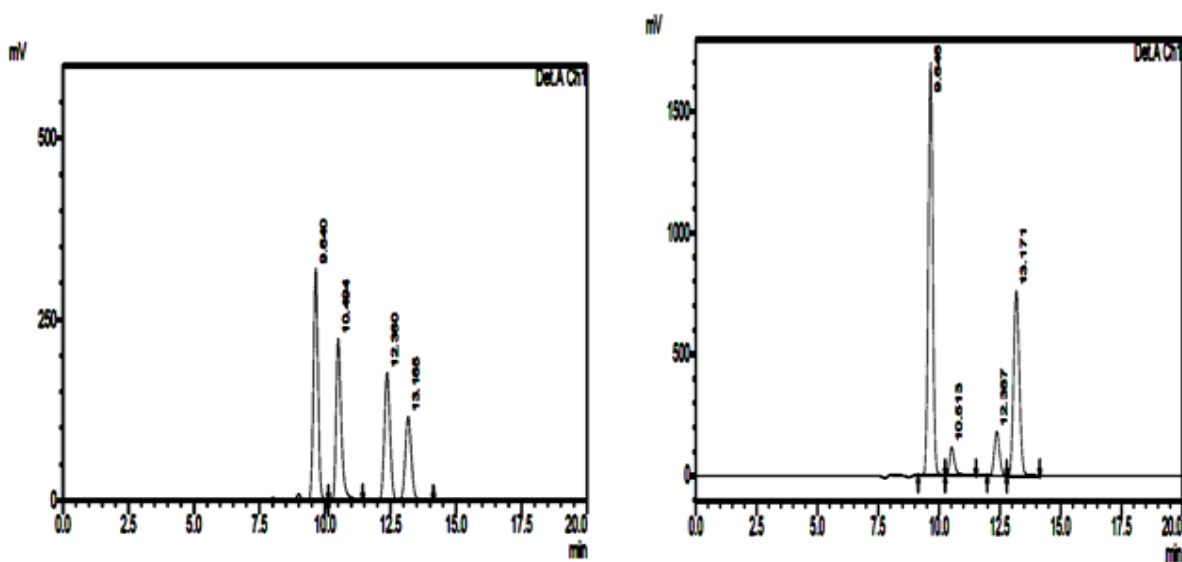


FIG. 8. Chromatogram of Standard Assay Chromatogram of Assay of Formulation.

TABLE 1. Linearity parameters for calibration curve.

Parameter	Paracetamol	Ibuprofen	Caffeine
Retention time (Rt)	9.7	12.66	10.48
Linearity range (µg/ml)	1.25 µg/ml to 20 µg/ml	0.625 µg/ml to 10 µg/ml	0.625 µg/ml to 10 µg/ml
Regression equation	$y=61963x + 11807$	$y=31198x + 77914$	$y=59842x + 42403$

Limit of detection ($\mu\text{g/ml}$)	0.28481225	0.187937934	0.264438458
Limit of quantification ($\mu\text{g/ml}$)	0.94937417	0.626459779	0.881461526
Regression coefficient (r^2)	0.999	0.999	0.999

TABLE 1a. Accuracy.

Paracetamol			Caffeine			Ibuprofen		
Peak Area	Conc.	RSD	Peak Area	Conc.	RSD	Peak Area	Conc.	RSD
888147	1.25	0.69258	783034	1.25	0.442707	470798	1.25	0.692588
1659642	2.5	0.53228	1527003	2.5	0.69224431	869036.667	2.5	0.53228782
3197167	5	0.36195	3035632	5	0.709403	1645744	5	0.361959
n=3								

TABLE 1b. Precision.

S.no	Intra Day Precession			Inter day precession		
	Paracetamol	Ibuprofen	Caffeine	Paracetamol	Ibuprofen	Caffeine
1	6385180	3189497	5990397	6385180	3189497	5990397
2	6394967	3179829	6011626	6394967	3179829	6011626
3	6498615	3218876	6057253	6498615	3218876	6057253
4	6401950	3184222	6014400	6400632	3186556	5960127
5	6342337	3204056	5944931	6366615	3256463	5917302
6	6400594	3223011	5978122	6394706	3199781	5987988
AVG	6403941	3199915.167	5999455	6406786	3205167	5987449
SD	51392.4	18266.60881	38005.54	46554.44	28573.27	47187.29
RSD	0.802512	0.570846659	0.633483	0.726643	0.891475	0.788103
n=3						

TABLE 2. Robustness study for Paracetamol.

Mobile Phase	Flow	Theoretical plates			Tailing factor		
		AVG	STDEV	RSD	AVG	STDEV	RSD
90 : 10	0.5	12323.978	56.20974539	0.456100663	0.93933333	0.00305505	0.325236032
90 : 10	0.7	9067.419667	78.84922682	0.869588369	0.888	0.00953939	1.074255857
88 : 12	0.5	15478.77167	135.7386882	0.876934495	0.99166666	0.00709459	0.715421736
88 : 12	0.7	11609.59933	154.6095267	1.331738695	1.021	0.00754983	0.739454891
92 : 08	0.5	5950.82	40.23684315	0.67615628	0	0	0

92 : 08	0.7	4445.731	49.73684297	1.118755115	0	0	0
---------	-----	----------	-------------	-------------	---	---	---

TABLE 3. Robustness parameters of Ibuprofen.

Mobile Phase	Flow	Theoretical plates			Tailing factor		
		AVG	STDEV		AVG	STDEV	
90 : 10	0.5	12864.37067	116.9338918	90 : 10	0.5	12864.37067	116.9338918
90 : 10	0.7	11470.76967	130.9244533	90 : 10	0.7	11470.76967	130.9244533
88 : 12	0.5	14257.88433	209.0422122	88 : 12	0.5	14257.88433	209.0422122
88 : 12	0.7	12586.42633	104.8210764	88 : 12	0.7	12586.42633	104.8210764
92 : 08	0.5	12718.54433	71.83204801	92 : 08	0.5	12718.54433	71.83204801
92 : 08	0.7	11747.39667	135.0314692	92 : 08	0.7	11747.39667	135.0314692

TABLE 4. Robustness parameters of Caffeine.

Mobile Phase	Flow	Theoretical plates			Tailing factor		
		AVG	STDEV	RSD	AVG	STDEV	RSD
90 : 10	0.5	15841.41867	104.1076266	0.657186258	1.31	0.007549834	0.576323239
90 : 10	0.7	12461.14467	201.2005414	1.614623269	1.361	0.02433105	1.787733293
88 : 12	0.5	16618.58767	209.3578273	1.259781105	1.2573	0.011590226	0.921810109
88 : 12	0.7	13637.39533	201.6091045	1.478354918	1.324	0.012529964	0.94637191
92 : 08	0.5	14483.07133	84.91817942	0.58632715	0	0	0
92 : 08	0.7	12089.129	67.05188783	0.554646144	0	0	0

TABLE 5. Ruggedness.

Ruggedness study for Paracetamol			Ruggedness study for Ibuprofen		Ruggedness study for Caffeine		
S.NO	Analysit-1		Analyst-2	Analyst-1	Analyst-2	Analyst-1	Analyst-2
1	Peak area	3208599	3240878	1641232	1686064	3051201	3020092
2	Peak area	3193454	3148553	1652496	1699056	3044639	3116484
3	Peak area	3189449	3238949	1643503	1681409	3011056	3097548
	AVG	3197167	3209460	1645744	1688843	3035632	3078041
	STDEV	10100.61	52755.83	5956.917	9145.834	21534.85	51070.9
	RSD	0.315924	1.64376	0.361959	0.541544	0.709403	1.659201

Validated gradient RP-HPLC method for estimation of Paracetamol, Caffeine and Ibuprofen

Stationary phase: C₁₈ 250 mm × 4.6 mm, 5 μ, Inertsil ODS 3V.

Mobile phase A: 6.8 gm of KH₂PO₄ in 1L Milli-Q water pH adjusted to 3 with Orthophosphoric acid

Mobile phase B: Filtered and degassed Acetonitrile (HPLC grade).

Detector: UV at wavelength 230 nm.

Flow rate: 1 mL/min (Run Time 30mins)

Injection volume: 20 μl

Mode: Gradient

Elution order: Paracetamol, Caffeine and Ibuprofen.

Retention time: Paracetamol, Caffeine and Ibuprofen is 6, 8 and 17 min (TABLE 6), FIG. 9 and 10.

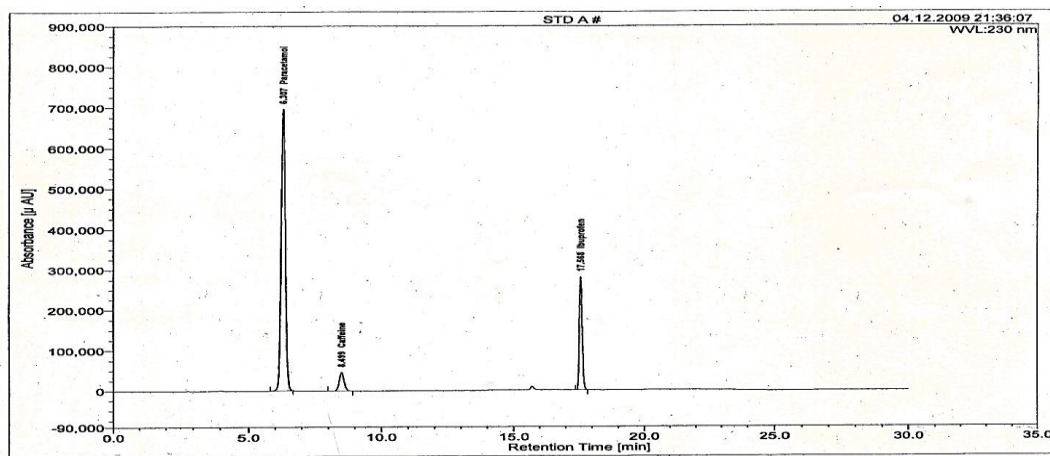


FIG. 9. HPLC (Gradient) Chromatogram of paracetamol, Ibuprofen and Caffeine.

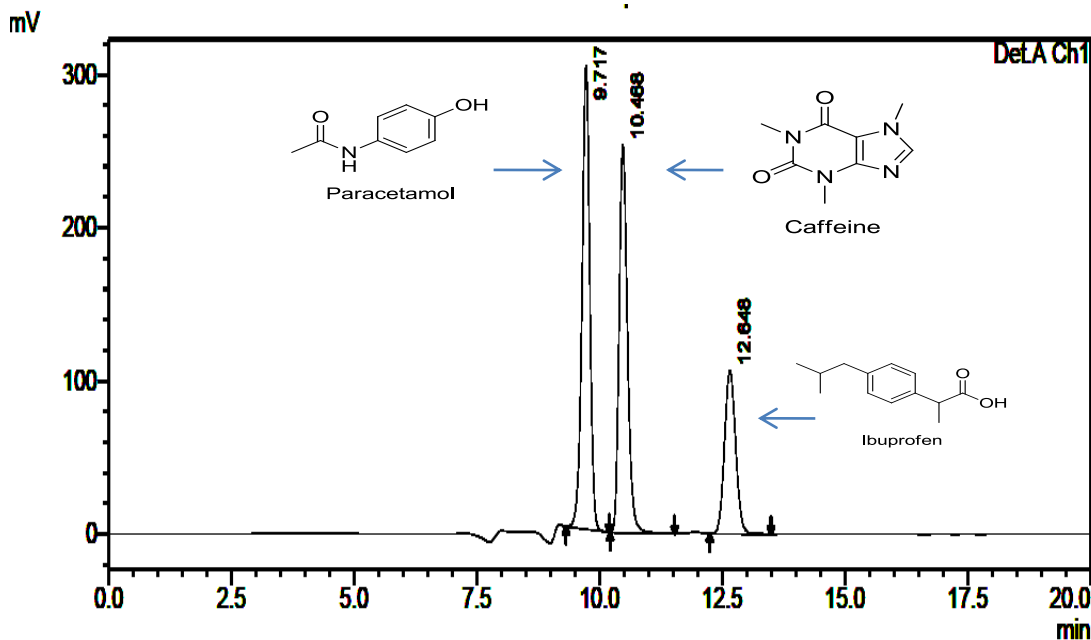


FIG. 10. HPLC (Isocratic) chromatogram of Paracetamol, Ibuprofen and Caffeine.

TABLE 6. Gradient programming.

Time	KH ₂ PO ₄ Buffer pH 3.0	Acetonitrile
0.01	85	15
5	85	15
12	25	75
20	25	75
22	85	15
30	85	15

Preparation of samples and standard stock solution A: Weigh accurately 130 mg of Paracetamol, 16 mg Caffeine, and 80 mg Ibuprofen into a 50 ml volumetric flask. Add sufficient amount of methanol, sonicate to dissolve, cool and dilute up to the mark with methanol. The above stock solution was further diluted 5 ml to 100 ml with methanol [6].

Standard stock solution B: Weigh accurately 136.5 mg of Paracetamol, 16.8 mg Caffeine, and 84 mg Ibuprofen into a 50 ml volumetric flask. Add sufficient amount of methanol, sonicate to dissolve, cool and dilute up to the mark with methanol. The above stock solution was further diluted 5 ml to 100 ml with methanol.

Sample stock solution: Twenty tablets were weighed accurately and finely powdered. The powder equivalent to 1 tablet was taken in a 50 ml volumetric flask, methanol was added and sonicate for 20 minutes, cool and further diluted up to the mark with methanol. The above stock solution was further diluted 2 ml to 100 ml with methanol. Then filtered through 0.45 μ membrane filter.

Placebo for Paracetamol: Weigh accurately 0.135 gm of placebo taken into 50 ml volumetric flask. Add 16 mg Caffeine, 80 mg Ibuprofen and sufficient amount of methanol, sonicate for 20 minutes, cool and dilute up to the mark with methanol. The above solution further diluted 5 ml to 100 ml with methanol. Then filtered through 0.45 μ membrane filter.

Placebo for Caffeine: Weigh accurately 0.135 gm of placebo taken into 50 ml volumetric flask. Add 130 mg Paracetamol, 80 mg Ibuprofen and sufficient amount of methanol, sonicate for 20 minutes, cool and dilute up to the mark with methanol. The above solution further diluted 5 ml to 100 ml with methanol. Then filtered through 0.45 μ membrane filter.

Placebo for Ibuprofen: Weigh accurately 0.135 g of placebo taken into 50 ml volumetric flask. Add 130 mg Paracetamol, 16 mg Caffeine and sufficient amount of methanol, sonicate for 20 min, cool and dilute up to the mark with methanol. The above solution further diluted 5 ml to 100 ml with methanol. Then filtered through 0.45 μ membrane filter (TABLES 7-10).

TABLE 7. Data of specificity test for Paracetamol.

Sample Name	Peak area Paracetamol	Retention time	Area Caffeine	Retention time	Peak area of Ibuprofen	Retention time	Similarity factor Paracetamol/Ibuprofen/Caffeine
STD A	7557233	6.32	564849	8.54	2016114	17.58	
STD A	7580906	6.31	566807	8.53	2020175	17.57	
STD A	7588646	6.31	567043	8.52	2022898	17.57	
STD A	7587502	6.31	567267	8.52	2021357	17.57	
STD A	7584143	6.30	566700	8.49	2019249	17.57	
STD A	75944237	6.30	567367	8.49	2021417	17.56	
STD B	7568881	6.31	565933	8.50	2016610	17.56	0.98
RSD	0.17%	0.11%	0.16%	0.22%	0.12%	0.03%	

TABLE 8. System Suitability.

Parameter	Acceptance	Paracetamol	Caffeine	Ibuprofen
Theoretical Plates	NLT 2000	7771	10581	135129
Tailing Factor	NMT 2	1.0	1.1	1.1
Capacity Factor	NLT 2	1.5	2.4	6.0
Similarity Factor	0.98 to 1.02	0.98	0.98	0.98
%RSD of STD A for Area	NMT 2	0.17%	0.16%	0.12%
%RSD of STD A for RT	NMT 2	0.11%	0.22%	0.03%
%RSD of STD for Area	NMT 2	0.17%	0.18%	0.20%
%RSD of STD for Retention time	NMT 2	0.11%	0.22%	0.04%
The given method is specific.				

TABLE 9. Repeatability of Paracetamol Ibuprofen and Caffeine.

Sample Name	Paracetamol			Caffeine			Ibuprofen		
	Area	Amount mg/tab	% Label Claim	Area	Amount mg/tab	% Label Claim	Area	Amount mg/tab	% Label Claim
1	7402044	318.8	98.1	5502 72	39.4	98.6	2019414	200.1	100.1
2	7424325	319.8	98.4	5522 23	39.6	99.0	2026164	200.8	100.4
3	7437356	320.5	98.6	5531 73	39.7	99.2	2028301	201.1	100.6

4	7485967	322.8	99.3	5541 84	39.8	99.4	2033877	201.8	100.9
5	7550710	325.6	100.2	5580 13	40.1	100.1	2042587	202.7	101.4
6	7552770	325.2	100.1	5576 24	40	99.9	2042212	202.3	101.2
SD		2.881	0.886		0.229	0.573		0.972	0.486
% RSD		0.89	0.89		0.58	0.58		0.48	0.48

TABLE 10. Summary of repeatability.

Parameter	Acceptance	Paracetamol	Caffeine	Ibuprofen
%RSD of Assay	NMT 2	0.89	0.58	0.48
Similarity Factor	0.98 to 1.02	0.98	0.98	0.98

Linearity

Standard solution (50% Level): Weigh accurately 32.50 mg of Paracetamol, 4.02 mg Caffeine, and 21.0 mg Ibuprofen into a 50 ml volumetric flask. Add sufficient amount of methanol, sonicate to dissolve, cool and dilute up to the mark with methanol. The above stock solution was further diluted 2 ml to 100 ml with methanol.

Standard solution (60% Level): Weigh accurately 39.3 mg of Paracetamol, 4.83 mg Caffeine, and 25.5 mg Ibuprofen into a 50 ml volumetric flask. Add sufficient amount of methanol, sonicate to dissolve, cool and dilute up to the mark with methanol. The above stock solution was further diluted 2 ml to 100 ml with methanol.

Standard solution (80% Level): Weigh accurately 52.80 mg of Paracetamol, 46.42 mg Caffeine, and 34.1 mg Ibuprofen into a 50 ml volumetric flask. Add sufficient amount of methanol, sonicate to dissolve, cool and dilute up to the mark with methanol. The above stock solution was further diluted 2 ml to 100 ml with methanol.

Standard solution (100% Level): Weigh accurately 64.90 mg of Paracetamol, 8.06 mg Caffeine, and 40.5 mg Ibuprofen into a 50 ml volumetric flask. Add sufficient amount of methanol, sonicate to dissolve, cool and dilute up to the mark with methanol. The above stock solution was further diluted 2 ml to 100 ml with methanol.

Standard solution (120% Level): Weigh accurately 77.30 mg of Paracetamol, 9.62 mg Caffeine, and 48.5 mg Ibuprofen into a 50 ml volumetric flask. Add sufficient amount of methanol, sonicate to dissolve, cool and dilute up to the mark with methanol. The above stock solution was further diluted 2 ml to 100 ml with methanol.

Standard solution (140% Level): Weigh accurately 90.0 mg of Paracetamol, 11.18 mg Caffeine, and 56.5 mg Ibuprofen into a 50 ml volumetric flask. Add sufficient amount of methanol, sonicate to dissolve, cool and dilute up to the mark with methanol. The above stock solution was further diluted 2 ml to 100 ml with methanol.

Standard solution (150% Level): Weigh accurately 32.50 mg of Paracetamol, 4.02 mg Caffeine, and 21.0 mg Ibuprofen into a 50 ml volumetric flask. Add sufficient amount of methanol, sonicate to dissolve, cool and dilute up to the mark with methanol [7]. The above stock solution was further diluted 2 ml to 100 ml with methanol (TABLE 11-20).

TABLE 11. Linearity of API's at various spiking levels.

Sample Name	Level	Paracetamol			Caffeine			Ibuprofen		
		Peak Area	Conc.	Response Factor	Peak Area	Conc.	Response Factor	Peak Area	Conc.	Response Factor
1	50%	64188	64.80	59372.5	4731	8.01	35378.8	17414	41.92	24958.2
2	60%	77398	78.36	59118.8	5712	9.63	35481.5	20893	50.90	24582.5
3	80%	103953	105.28	59075.1	7684	12.80	35888.8	27871	68.07	24504.7
4	100%	127640	129.41	59158.1	9468	16.07	35319.7	34086	80.84	25257.3
5	120%	152373	154.13	59279.0	11336	19.19	35405.6	40589	96.81	25130.7
6	140%	177636	179.46	59290.9	13263	22.30	35612.5	47167	112.78	25049.0
7	150%	189707	192.02	59194.8	14203	24.07	35291.7	50228	119.77	25123.2
SD				105.75			208.79			288.66
% RSD				0.18%			0.59%			1.16%

TABLE 12. Summary of linearity.

Name	%Y Intercept	Correlation coefficient	Response Factor (%RSD)
Paracetamol	-0.1	1.000	1.18%
Caffeine	0.4	1.000	0.59%
Ibuprofen	-1.6	1.000	1.16%
Acceptance	-2 to +2	NLT 0.99	NMT 5%

TABLE 13. Robustness (Low pH).

Sample Name	Paracetamol			Caffeine			Ibuprofen		
	Peak Area	Amount mg/tab	%Label Claim	Peak Area	Amount mg/tab	%Label Claim	Peak Area	Amount mg/tab	%Label Claim
1	7406357	326.6	100.5	552989	39.9	99.8	2073280	199.8	99.9
2	7338309	323.4	99.5	552303	39.9	99.7	2077918	200.2	100.1

3	7360016	324.4	99.8	553631	40	99.9	2084750	200.8	100.4
SD		1.61	0.49		0.04	0.12		0.51	0.25

TABLE 14. Summary of Robustness (low pH).

Parameter	Acceptance	Paracetamol	Caffeine	Ibuprofen
% Label Claim	95% to 105%	99.9	99.8	100.1
Similarity Factor	0.98 to 1.02	0.99	0.98	1.01

TABLE 15. Robustness (High pH).

Sample Name	Paracetamol			Caffeine			Ibuprofen		
	Peak Area	Amount mg/tab	%Label Claim	Peak Area	Amount mg/tab	%Label Claim	Peak Area	Amount mg/tab	%Label Claim
1	7364772	321.1	98.8	554575	40	100.0	2046088	200.8	100.4
2	7400597	322.8	99.3	555880	40.1	100.3	2055765	201.9	100.9
3	7369187	321.6	99.0	554293	40	100.1	2045168	201	100.5
SD		0.86	0.26		0.05	0.14		0.56	0.28
RSD		0.27	0.27		0.15	0.15		0.28	0.28

TABLE 16. Summary of robustness (high pH).

Parameter	Acceptance	Paracetamol	Caffeine	Ibuprofen
% Label Claim	95% to 105%	99.0	100.1	100.6
Similarity Factor	0.98 to 1.02	1.00	0.99	1.00

TABLE 17. Summary of robustness (low flow rate).

Parameter	Acceptance	Paracetamol	Caffeine	Ibuprofen
% Label Claim	95% to 105%	99.4	99.0	99.0
Similarity Factor	0.98 to 1.02	0.98	0.98	1.00

TABLE 18. Robustness (Low flow rate).

Sample Name	Paracetamol			Caffeine			Ibuprofen		
	Peak Area	Amount mg/tab	%Label Claim	Peak Area	Amount mg/tab	%Label Claim	Peak Area	Amount mg/tab	%Label Claim
1	8197386	323.5	99.5	617630	39.7	99.3	2282308	198.6	99.3

2	8172205	322.2	99.1	615123	39.5	98.8	2273102	197.6	98.8
3	8184580	323	99.4	615999	39.6	99.0	2275068	197.9	99.0
SD		0.66	0.20		0.10	0.25		0.50	0.25
RSD		0.20	0.20		0.25	0.25		0.26	0.26

TABLE 19. Robustness (High flow rate).

Sample Name	Paracetamol			Caffeine			Ibuprofen		
	Peak Area	Amount mg/tab	%Label Claim	Peak Area	Amount mg/tab	%Label Claim	Peak Area	Amount mg/tab	%Label Claim
1	6668504	322.6	99.3	498142	39.6	99.0	1837498	198.6	99.3
2	6673044	322.9	99.4	498715	39.7	99.2	1838632	198.8	99.4
3	6639102	321.3	98.9	496605	39.5	98.7	1831277	198	99.0
SD		0.84	0.26		0.08	0.20		0.40	0.20
RSD		0.26	0.26		0.21	0.21		0.20	0.20

TABLE 20. Summary of robustness (High flow rate).

Parameter	Acceptance	Paracetamol	Caffeine	Ibuprofen
% Label Claim	95% to 105%	99.2	99.0	99.2
Similarity Factor	0.98 to 1.02	0.98	0.98	0.98

Accuracy

Requirements

Standard solution (Recovery 50% level): Weigh accurately 162.5 mg of Paracetamol, 20 mg Caffeine, and 100 mg Ibuprofen into a 50 ml volumetric flask. Add sufficient amount of methanol, sonicate to dissolve, cool and dilute up to the mark with methanol. The above stock solution was further diluted 2 ml to 100 ml with methanol.

Standard solution (Recovery 100% level): Weigh accurately 325 mg of Paracetamol, 40 mg Caffeine, and 200 mg Ibuprofen into a 50 ml volumetric flask. Add sufficient amount of methanol, sonicate to dissolve, cool and dilute up to the mark with methanol. The above stock solution was further diluted 2 ml to 100 ml with methanol [8].

Standard solution (Recovery 150% level): Weigh accurately 487.5 mg of Paracetamol, 60 mg Caffeine, and 300 mg Ibuprofen into a 50 ml volumetric flask. Add sufficient amount of methanol, sonicate to dissolve, cool and dilute up to the mark with methanol. The above stock solution was further diluted 2 ml to 100 ml with methanol (TABLE 21-25).

TABLE 21. Recovery table of Paracetamol, Caffeine and Ibuprofen.

Level	Paracetamol			Caffeine			Ibuprofen		
	Peak Area	Amount mg/tab	%Label Claim	Peak Area	Amount mg/tab	%Label Claim	Peak Area	Amount mg/tab	%Label Claim
50%	3816748	101	0.22	288181	103	0.73	1050819	101	0.12
100%	7573648	99	0.14	562381	100	0.31	2035228	101	0.08
150%	11261934	99	0.03	839915	100	0.15	2991330	99	0.17

TABLE 22. Summary of accuracy.

Parameter	Acceptance Criteria	Paracetamol	Caffeine	Ibuprofen
Recovery 50%	98% to 102%	101	103	101
Recovery 100%	98% to 102%	99	100	101
Recovery 150%	98% to 102%	99	100	99
%RSD of Recovery	NMT 2	0.03	0.15	0.17
Similarity Factor	0.98 to 1.02	1.00	0.99	1.00

TABLE 23. Intermediate Precision Data for Paracetamol, Ibuprofen and caffeine.

Sample Name	Paracetamol			Caffeine			Ibuprofen		
	Peak Area	Conc.	Response Factor	Peak Area	Conc.	Response Factor	Peak Area	Conc.	Response Factor
1	6960251	320.8	98.7	544569	39.7	99.2	544569	39.7	99.2
2	7113910	323.7	99.6	550444	39.6	99.0	550444	39.6	99.0
3	6944705	319.3	98.2	543543	39.5	98.8	543543	39.5	98.8
4	7138815	324.8	99.9	552295	39.7	99.3	552295	39.7	99.3
5	7117815	323.8	99.6	550579	39.6	99.0	550579	39.6	99.0
6	6931597	319	98.2	543636	39.6	98.9	543636	39.6	98.9
SD		2.51	0.77		0.07	0.19		0.07	0.19
% RSD		0.78	0.78		0.20	0.20		0.20	0.20

TABLE 24. Summary of intermediate precision (Ruggedness).

Parameter	Acceptance	Paracetamol	Caffeine	Ibuprofen
-----------	------------	-------------	----------	-----------

%RSD of Assay	NMT 2	0.78	0.20	0.18
% Variation	NMT2	0.1	0.3	1.5
Similarity Factor	0.98 to 1.02	0.98	0.99	1.00

TABLE 25. Solution stability of Paracetamol Ibuprofen and Caffeine.

Paracetamol					Caffeine				Ibuprofen			
	Area	mg/tab	% Label Claim	% relative differ	Area	mg/tab	% Label Claim	% relative differ	Area	Amount mg/tab	%Lab el Claim	% relative differ
0	7411567	322.6	99.3	0.0	553845	39.4	98.6	0.0	2052065	201.2	100.6	0.0
2	7436506	323.7	99.6	0.3	555885	39.6	99.0	0.4	2069954	202.9	101.5	0.9
4	7434897	323.6	99.6	0.3	555679	39.6	98.9	0.3	2067476	202.7	101.3	0.8
8	7445787	324.1	99.7	0.5	556076	39.6	99.0	0.4	2067943	202.7	101.4	0.8
12	7446004	324.1	99.7	0.5	556104	39.6	99.0	0.4	2063880	202.3	101.2	0.6
16	7419306	322.9	99.4	0.1	554280	39.5	98.7	0.1	2058591	201.8	100.9	0.3
20	7386753	321.5	98.9	0.3	552865	39.4	98.4	0.2	2055739	201.5	100.8	0.2
24	7461670	324.7	99.9	0.7	556740	39.7	99.1	0.5	2063585	202.3	101.1	0.6

Orthogonal analysis

API's from other sources need to be characterized by conventional as well as orthogonal testing's as described in ICH Q6B [9]. Additional testing should include parameters or method to determine the suitability of the reference material not necessarily captured by the drug substance (e.g., more extensively in tracing related substance impurities).

Conclusion

The switch over of two different methods say Isocratic/gradient analysis for the same sample ensured greater resolution of analyte of interest in comparison with isocratic in gradient mode, and there is no relative substance impurities detected by variation in the method confirming the purity of the samples and the method is validated according to ICH 21 CFR guidelines and the method is robust in both isocratic and gradient mode.

References

1. Ali NW, Hegazy MA, Abdelkawy M, et al. Simultaneous determination of methocarbamol and its related substance (guaifenesin) in 2 ternary mixtures with ibuprofen and diclofenac potassium by RP-HPLC method. J Liq Chromatogr Relat Technol. 2012;2229-42.
2. Elkady EF, Fouad MA. Two liquid chromatographic methods for the simultaneous determination of ibuprofen and methocarbamol or chlorzoxazone in the presence of their degradation products. J Liq Chromatogr Relat Technol. 2012;882-95.

3. Gul S, Sultana N, Arayne MS, et al. New method for optimization and simultaneous determination of sparfloxacin and non-steroidal anti-inflammatory drugs: Its *in-vitro* application. Am J Analyt Chem. 2012;328-37.
4. Harde M, Wankhede S, Chaudhari P. Development of validated UV spectrophotometric method for simultaneous estimation of ibuprofen, paracetamol and caffeine in the bulk drug and marketed formulation. World J Pharm Res. 2015;1428-36.
5. Haritha P, Sreenivasa Rao B, Sunandamma Y. Method development and validation for simultaneous determination of Paracetamol and Ketoprofen by RPHPLC Technique. Int J Appl Biol Pharm. 2014;110-22.
6. Liu J. Determination of 4-isobutyl acetophenone in ibuprofen injections by HPLC. 2012;672-73.
7. N Bassanese D. Protocols for finding the most orthogonal dimensions for two dimensional high performance liquid chromatography. Talanta. 2015;402-8.
8. Rajavel P. Method develop. Asian J. 2013;8-17.
9. Tuani YT, Nartey VK, Akanji O, et al. Simultaneous quantification of Paracetamol and Ibuprofen in fixed dose combination using RP-HPLC with UV detection. IJPCR. 2014;7-15.