A REVIEW ON ANALYTICAL METHOD FOR PARACETAMOL, CAFFEINE, IBUPROFEN IN MARKETED FORMULATION

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ABSTRACT

Purpose of this study was to develop a indicating UV- Spectrophotometrics &RP-HPLC method for Routine analysis of Paracetamol (PARA), Caffeine (CAF) and Ibuprofen (IBU) in their combined Marketed Dosage Formulation. Paracetamol, Caffeine and Ibuprofen in their combined Marketed Dosage Formulation is used for the treatment of analgesic and antipyretic (Anti-inflammatory). Specificity was shown by the separation of drugs with high degree of resolution between them and absence of any interference from the excipient or degradation products. This method was successfully applied to assay the drugs in tablets and capsules. Hence this newly developed method can be considered suitable and reliable for the routine analysis of PARA, CAF and IBU in their solid dosage forms. The Method was validated as per ICH, FDA and USP guidelines.

Key Words: Paracetamol; Caffeine; Ibuprofen; HPLC; UV- Spectrophotometrics.

INTRODUCTION[1,3,4]

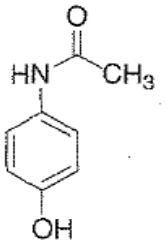
Paracetamol (Acetaminophen) is chemically N-(4-hydroxyphenyl) acetamide is a crystalline solid is a sparingly soluble compound which is classified under antipyretic analgesics. Drugs Classified under this class possess analgesic and antipyretic activity but lacks anti-inflammatory effects indicated for 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 pyrogens. Acetaminophen possesses analgesic activity in arthritis and musculoskeletal disorders.

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.

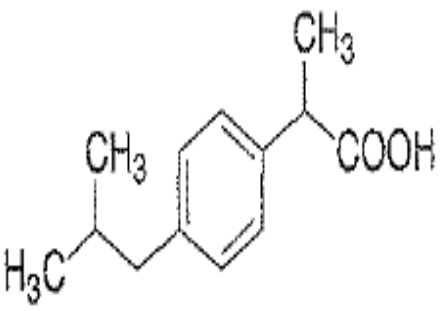
It is marketed as racemic mixture even its biological response owed almost evidently with S-(+)isomer. Ibuprofen is more potent than aspirin but less effective than indomethacin.

Ibuprofen produces moderate levels of gastric irritation. ibuprofen is indicated in patients suffering with rheumatoid arthritis, osteoarthritis, fever and dysmenorrhoea. Caffeine is chemically 1,3,7-trimethyl-1H-purine-2,6(3H,7H)dione acetamide is a crystalline solid is a sparingly soluble.

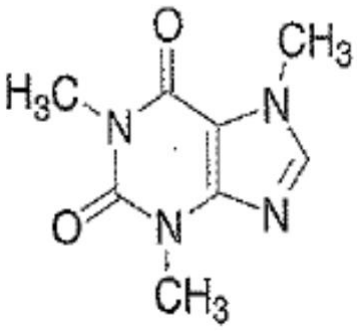
Compound which is chemically methyl xanthines naturally occur in coffee (coffee Arabica) which is generally termed as stimulant and as a bronchodilator. Caffeine is generally added to other over the counter(OTC) analgesic and stimulants.



Paracetamol



Ibufrophe



caffeine

3. METHOD[5,6,7,8]

The method was validated according to the ICH**(Q2A)** guidelines for the below following parameters.

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.

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.

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.

Precision

The inter day 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.

Linearity

The ability of the method to obtain test results proportional to the concentration of the analyte within a given range. It was evaluated by linear regression analysis, which was calculatedby
the least square regression method.

Limit of Detection (LOD)

The limit of detection (LOD) is the lowest concentration of analyte in a sample that can be detected but not necessary quantified. The obtained LOD values of specified impurities and API is discussed.

$$\text{Limit of Detection (LOD)} = 3.3 \times \sigma / S$$

Where σ = standard deviation of the response

S= slope of the calibration curve

Limit of Quantification (LOQ)

The limit of quantisation is the lowest concentration or amount of analyte that can be determined quantitatively within an acceptable level of repeatability precision and trueness. Limit of quantisation
(LOQ) = $10.0 \times \sigma / S$

Where, σ = the standard deviation of the response

S = Slop of the calibration curve

Selectivity and Specificity

The specificity was studied by injecting the unstressed and stressed standard solution, excipient and pharmaceutical preparation several times on several days. It was revealed that there was no interference of peak from excipient or any impurities in the region of all three drugs in the chromatogram. Chromatogram of blank and chromatograms of drugs from tablet sample.

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.

Repeatability

Six standard solutions of Ibuprofen, Caffeine and Paracetamol (100% level concentrations) were prepared using different weights. Six determinations were performed

Method: 1

Simultaneous determination of caffeine, Paracetamol and ibuprofen in pharmaceutical formulations By High-performance liquid chromatography with UV detection and by capillary electrophoresis with conductivity detection.

Method: 2

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

4. RESULTS: [9,10,11,12]

Method: 1

Simultaneous determination of caffeine, Paracetamol and ibuprofen in pharmaceutical formulations By High-performance liquid chromatography with UV detection and by capillary electrophoresis with conductivity detection.

Accuracy:

A sample was constituted (a total weight of276.46 mg of the placebo weighed was spiked with known quantity of standard samples of Ibuprofen, Caffeine and Paracetamol) at 80%, 100%, 120% concentration levels and assayed as per the method stated under analytical methods respectively. Three determinations were performed under each concentration levels respectively.

Table:1 Accuracy of Ibuprofen

% Level	Weight use(mg)	Weight obtained(mg)	% recovery	% Mean recovery± SEM
80	160.01	160.08	100.04	99.94±0.0681
	160.00	159.69	99.81	
	160.02	159.97	99.97	

120	200.00	199.78	99089	99.90±0.0636
	200.00	200.02	100.01	
	200.01	199.59	99.79	
180	240.01	239.77	99.92	99.97±0.0322
	240.03	239.93	99.96	
	240.00	240.06	100.03	

N=3 for each % level, SEM= Standard Error of The Mean

Table:2 Accuracy of Caffeine

% Level	Weight use(mg)	Weight obtained(mg)	% recovery	% Mean recovery± SEM
80	24.03	23.94	99.71	99.87±0.1090
	24.00	23.99	99.83	
	24.01	24.01	100.08	
120	30.00	30.04	100.13	99.86±0.1764
	30.00	29.86	99.53	
	30.01	30.00	99.93	
180	36.01	35.81	99.44	99.70±0.1405
	36.01	35.98	99.92	
	36.00	35.91	99.75	

N=3 for each % level, SEM= Standard Error of The Mean

Table:3 Accuracy of Paracetamol

% Level	Weight use(mg)	Weight obtained(mg)	% recovery	% Mean recovery± SEM
80	80.01	79.92	99.89	100.00±0.0702
	80.00	80.07	100.99	
	80.02	80.11	100.11	
120	100.01	99.89	99.88	99.87±0.0348
	100.00	99.93	99.93	
	100.01	99.82	99.81	
180	120.01	120.03	100.02	99.97±0.0321
	120.03	119.93	99.91	
	120.00	119.97	99.78	

N=3 for each % level, SEM= Standard Error of The Mean

Repeatability:

Six standard solutions of Ibuprofen, Caffeine and Paracetamol (100% level concentrations) were prepared using different weights. Six determinations were performed.

Table:4 Repeatability of Ibuprofen

Weight used(mg)	Weight obtained(mg)	% content	Mean± SEM	SD	%RSD
200.02	198.23	99.11	99.75±0.3472	0.8504	0.850

200.00	201.05	100.53			
200.01	197.97	98.98			
200.00	201.90	100.95			
200.02	198.98	99.02			
200.03	199.89	99.93			

N=6, SEM= Standard Error of the Mean, SD=Standard Deviation, RSD=Relative Standard Deviation

Table:5 Repeatability of Caffeine

Weight used(mg)	Weight obtained(mg)	% content	Mean± SEM	SD	%RSD
30.00	30.78	101.17	99.56±0.4286	1.0490	1.0150
30.03	39.86	99.53			
30.03	30.02	100.03			
30.01	29.94	100.95			
30.01	29.94	99.70			
30.00	29.24	98.13			

N=6, SEM= Standard Error of the Mean, SD=Standard Deviation, RSD=Relative Standard Deviation

Table:6 Repeatability of Paracetamol

Weight used(mg)	Weight obtained(mg)	% content	Mean± SEM	SD	%RSD
100.00	99.49	99.89	99.49±0.3982	0.9754	0.980
100.00	100.11	100.11			
100.01	98.12	98.12			
100.01	100.62	100.64			
100.03	98.59	98.56			
100.00	100.02	100.02			

N=6, SEM= Standard Error of the Mean, SD=Standard Deviation, RSD=Relative Standard Deviation

Intermediate precision:

Three samples were constituted (a total weight of276.46 mg of the placebo weighed was spiked with 200 mg of Ibuprofen, 30 mg of Caffeine and 500 mg of Paracetamol) and assayed as per the method stated under analytical methods respectively by two different analysts on the same day.

For Caffeine, specificity was determined by accurately weighing 30 mg of Caffeine, 200 mg of Ibuprofen, 500 mg of Paracetamol and 276.46 mg of the placebo and assaying as per the method for Caffeine. In order to establish specificity for Paracetamol, 500 mg of Paracetamol, 30 mg of Caffeine, 200 mg of Ibuprofen and 276.46 mg of the Placebo were accurately weighed and assayed as per the method for Paracetamol.

Table:7 Intermediate precision of Ibuprofen

Sample	Anylast 1(% content)	Anylast 2(% content)
1	101.38	100.35
2	99.31	102.41
3	99.31	100.35
Mean ±SEM	100.00 ± 0.6900	101.00 ± 0.6867
SD	1.195	1.189
%RSD	1.20	1.18

Table:8 Intermediate precision of Caffeine

Sample	Anylast 1(% content)	Anylast 2(% content)
1	99.41	101.70
2	101.17	99.63
3	99.89	101.04
Mean ±SEM	100.30 ± 0.7005	100.80 ± 0.6105
SD	1.213	1.057
%RSD	1.21	1.05

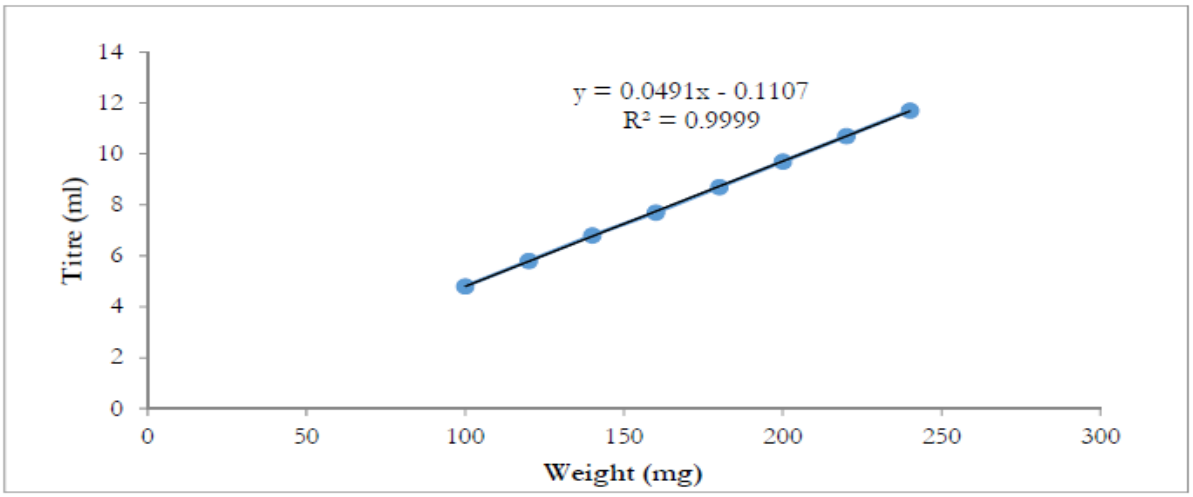
Table:8 Intermediate precision of Paracetamol

Sample	Anylast 1(% content)	Anylast 2(% content)
1	101.59	99.97
2	99.69	100.46
3	101.32	99.21
Mean ±SEM	100.90 ± 0.5935	99.88 ± 0.3636
SD	1.0280	1.6289
%RSD	1.028	1.63

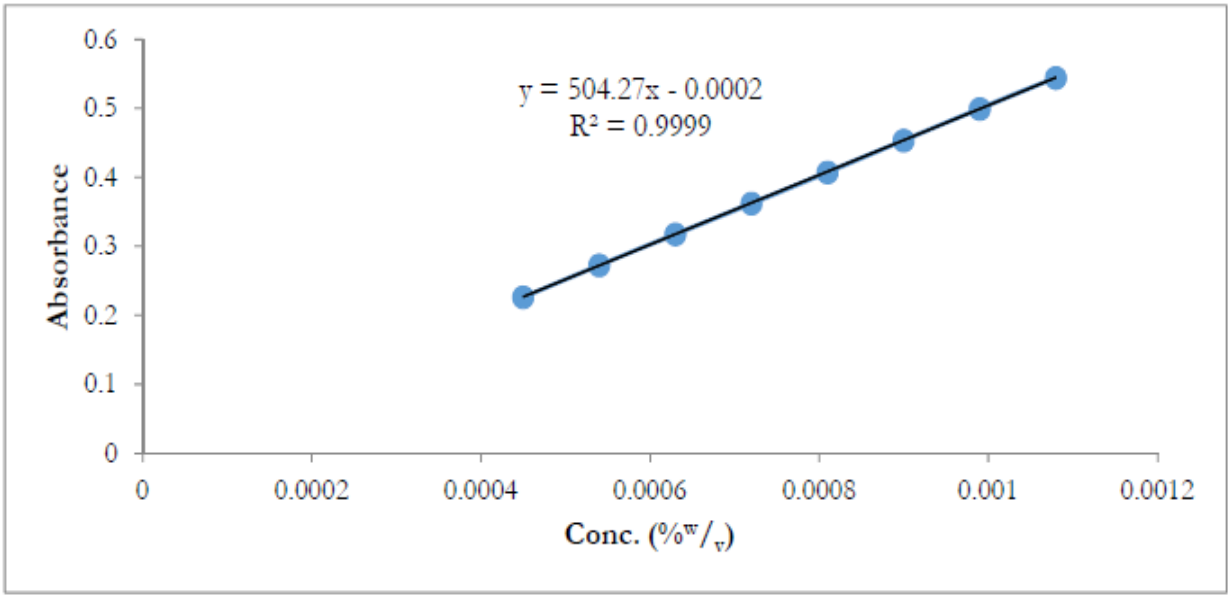
Linearity and range:

Standard solutions of Ibuprofen, Caffeine and Paracetamol over a range of 50% - 120% concentration levels were prepared using different weights. The titre values, absorbance at 257 nm and 273 nm were determined for Ibuprofen, Caffeine and Paracetamol respectively. Results are shown in Graphs 1, 2, and 3 and Table 10 for Ibuprofen, Caffeine and Paracetamol respectively.

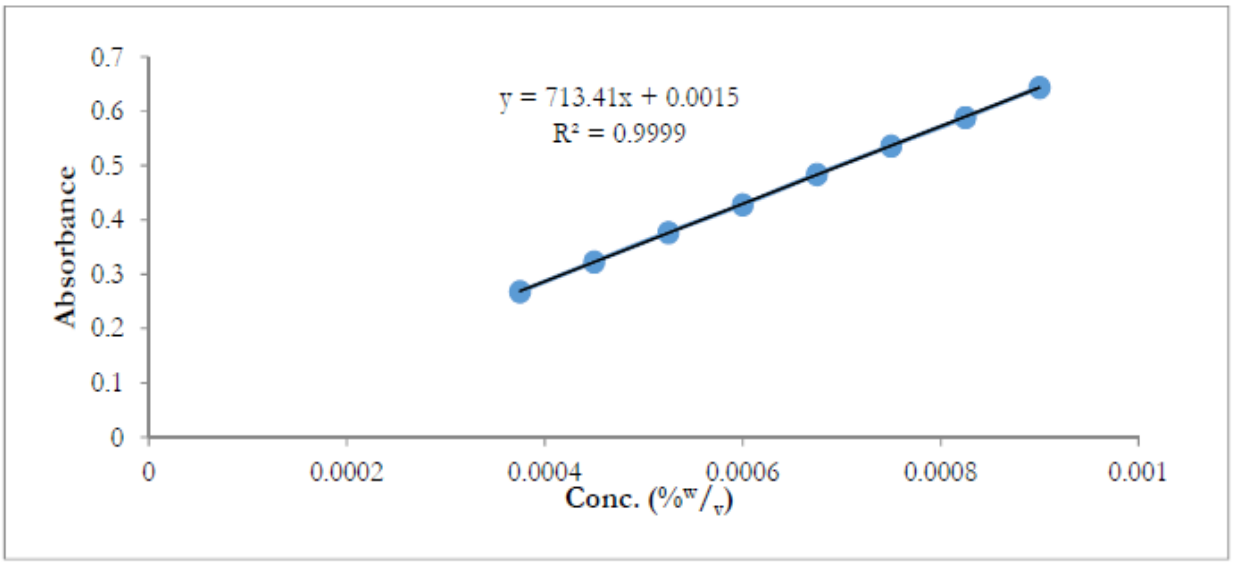
Parameters	Ibuprofen	Caffeine	Paracetamol
Slope± SE	0.04911±0.0002305	504.3±0.8878	713.4±2.1058
Intercept± SE	0.1107±0.04059	-0.000206±0.0007034	0.001487±0.001392
R^2	0.9999	0.9999	0.9999
$S_{Y/X}$ (SE)	0.02988	0.0005178	0.001024
F	45370	322700	114600



Graph. 1. Linearity curve for ibuprofen



Graph 2. Linearity curve for caffeine



Graph 3. Linearity curve for paracetamol

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Table :11 Result for LOD and LOQ

Parametrrs	Ibuprofen(mg)	Caffeine(µg/ml)	Paracetamol(µg/ml)
LOD	2.7275	0.0460	0.0644
LOQ	8.2651	0.1395	0.1951

Specificity:

In determining the specificity of Ibuprofen, 200 mg of Ibuprofen, 30 mg of Caffeine, 500 mg of Paracetamol and 276.46 mg of the placebo were accurately weighed and assayed as per the method for Ibuprofen.

Table:12 Specificity(Ibuprofen)

Sample	Titre(ml)
Ibuprofen	9.70
Ceffeine	(-)
Paracetamol	(-)
Placebo	(-)

(-): No Detection

Table:12 Specificity(Caffeine)

Sample	Absorbance @ 273 nm
Ibuprofen	(-)
Ceffeine	0.4603
Paracetamol	(-)
Placebo	(-)

(-): No Detection

Table:12 Specificity(Paracetamol)

Sample	Absorbance@257 nm
Ibuprofen	0.0404
Ceffeine	0.0404
Paracetamol	0.5423
Placebo	0.0455

(-): No Detection

Robustness:

A sample was constituted (a total weight of276.46 mg of the placebo weighed was spiked with 200 mg of Ibuprofen, 30 mg of Caffeine and 500 mg of Paracetamol) and assayed as per the methods stated for the ingredients respectively and variations were made to the shaking time and number of extractions

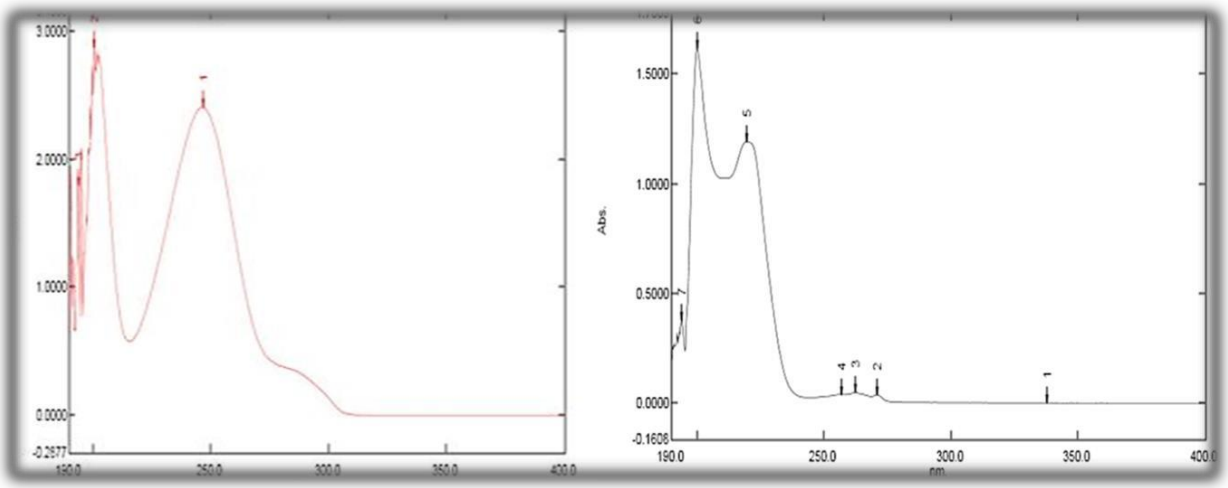
Sample	Original condition(Extraction with 5x20 ml petroleum ether)% content	Varied condition(Extraction with 3x20 ml petroleum ether)% content
1	99.31	99.02
2	98.61	98.12
3	100.35	100.09
Mean±SEM	99.42±0.5055	99.10±0.5499
SD	0.8755	0.9525
%RSD	0.88	0.96

Method-2[14,15,16,17]

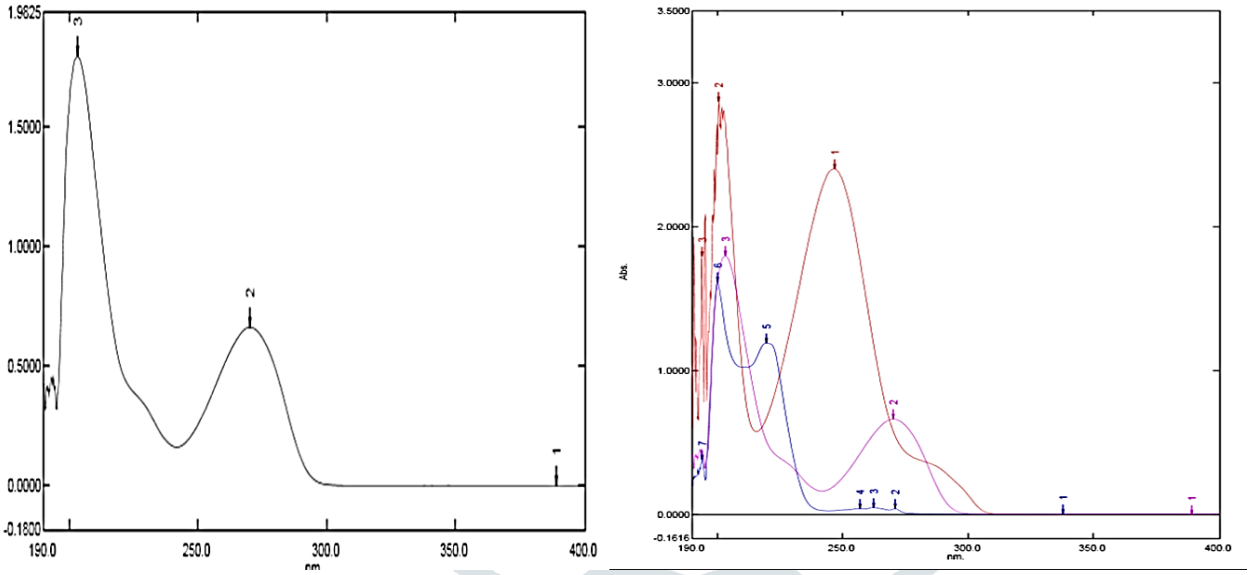
Validated Isocratic/Gradient RP-HPLC for Simultaneous Estimation of Paracetamol, Ibuprofen and Caffeine in Marketed Formulations Using Diclofenac as Internal Standard.

UV profiling of API's

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) isobestic point was found to be 203 nm. the same wavelength.



Absorption Maxima of Paracetamol **Absorption Maxima of Ibuprofen**



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. 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.

Assay

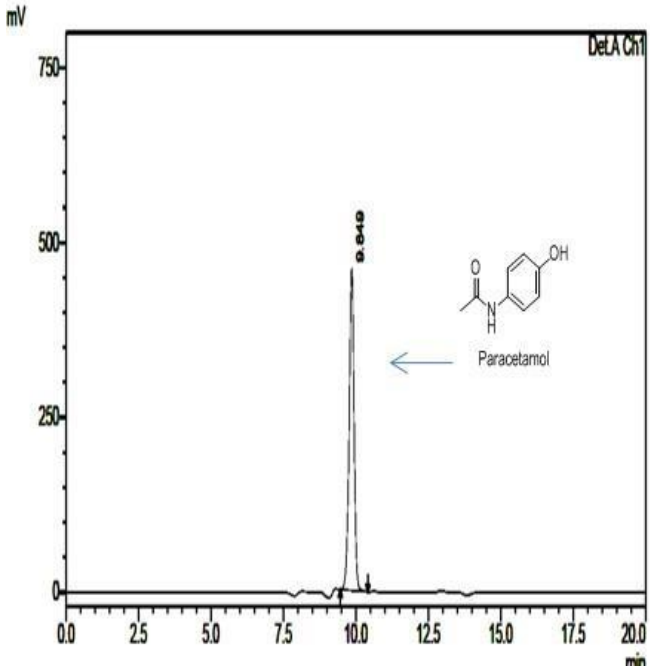
The amount of Paracetamol, Ibuprofen and Caffeine present per tablet was calculated by comparing the peak area, with that of the internal standard.

Selection and role of internal standard

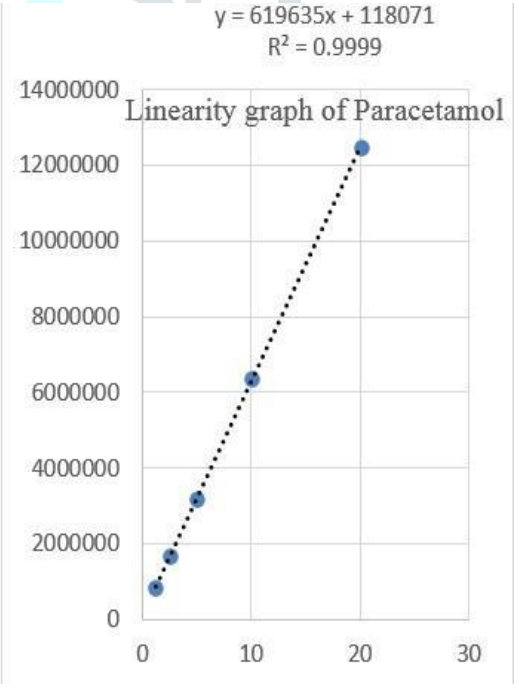
Conventional quantification techniques carry an error, to minimize the error in analysis, recovery and quantification of API was done with respect to internal standard method. criteria for selection of internal standard; structurally similar compounds have similar retention times, diclofenac is used as internal standard. 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.

Recovery experiment

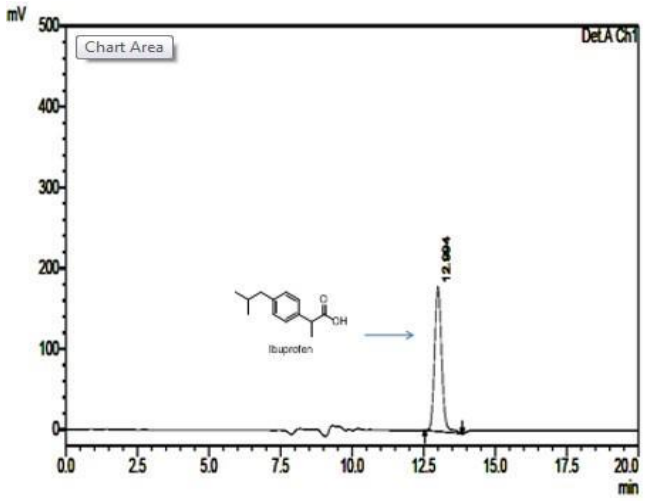
To study the accuracy, reproducibility and the precision of the proposed method recovery experiments were carried out. A fixed amount of pre-analyzed sample was taken and standard drug was added at three different concentrations and each level was repeated for 3 times. The recovery was estimated to be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

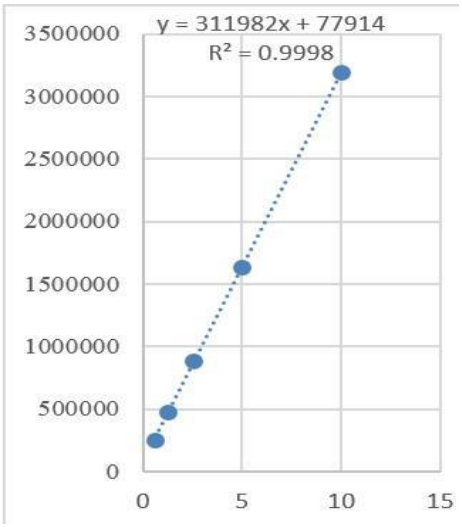


Chromatogram of paracetamol

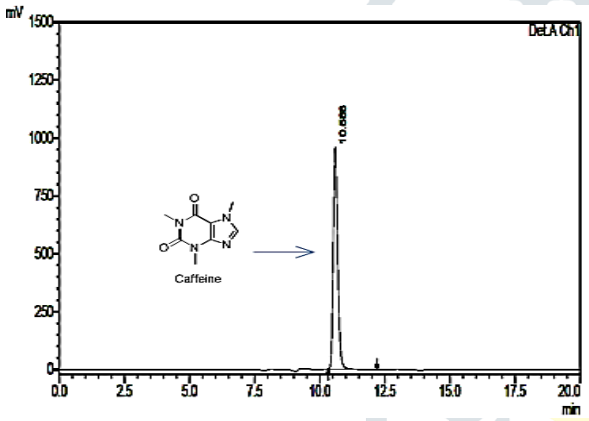


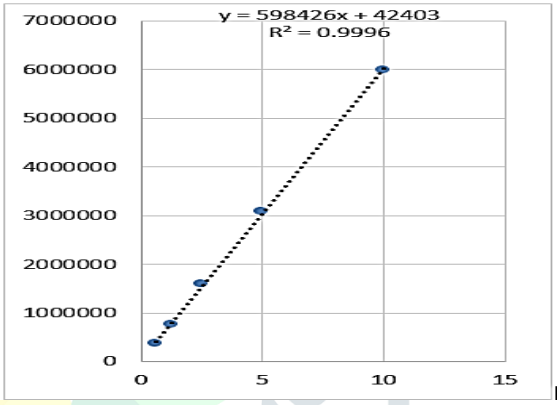
linearity graph of paracetamol



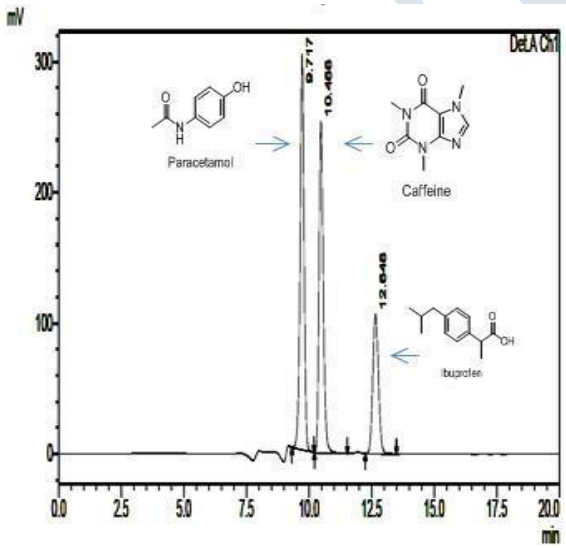


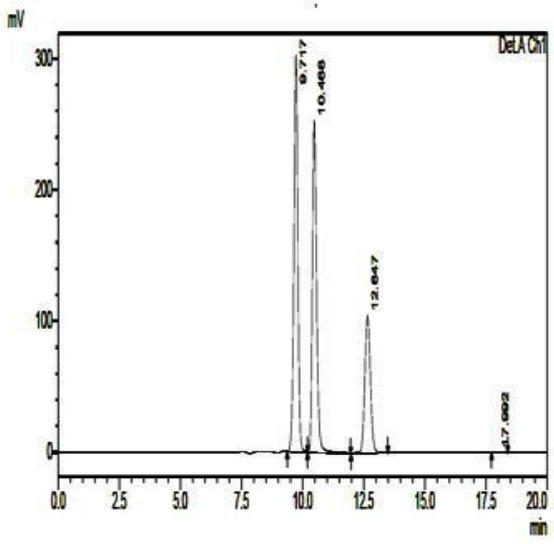
Chromatogram of Ibufropen **linearity graph of Ibufropen**



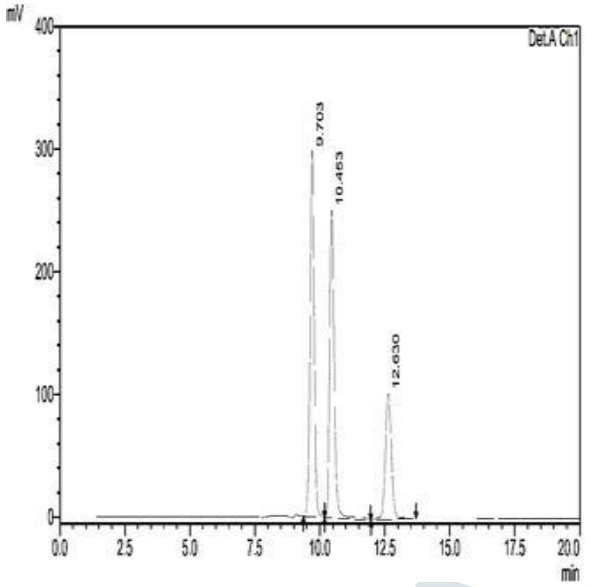


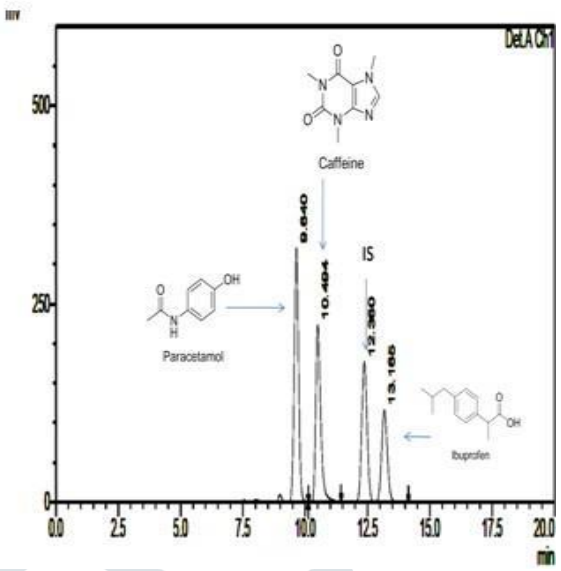
Chromatogram of Caffeine **linearity graph of Caffeine**



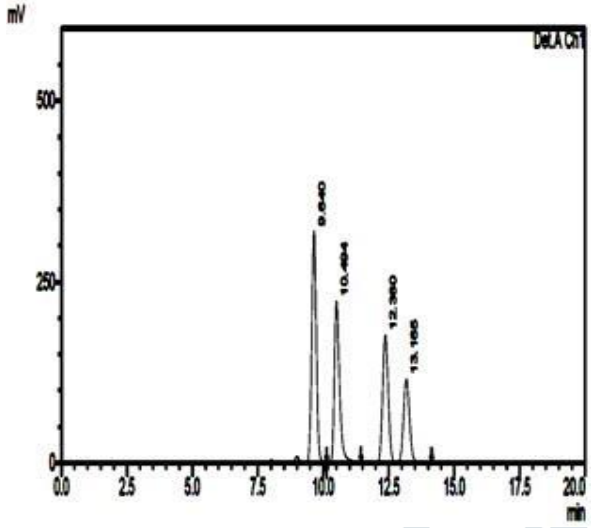


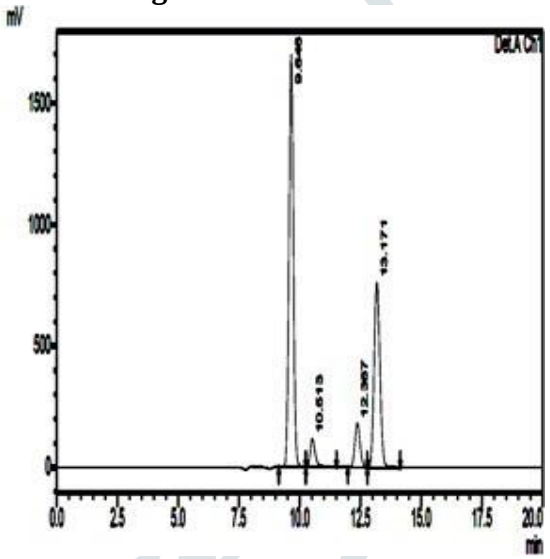
Chromatogram without Baseline **Correction with Baseline Correction.**





Chromatogram without internal standard Chromatogram with internal standard.





Chromatogram of Standard Assay Chromatogram of Assay of Formulation.

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 (µg/ml)	0.28481225	0.187937934	0.264438458
Limit of quantification (µg/ml)	0.94937417	0.626459779	0.881461526
Regression coefficient (r2)	0.999	0.999	0.999

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								

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						

Robustness study of Parecetamol

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	0.32523603
90 : 10	0.7	9067.419667	78.84922682	0.869588369	0.888	0.00953939	1.07425585
88 : 12	0.5	15478.77167	135.7386882	0.876934495	0.99166666	0.00709459	0.71542173
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

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

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

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:

C_{18} 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).

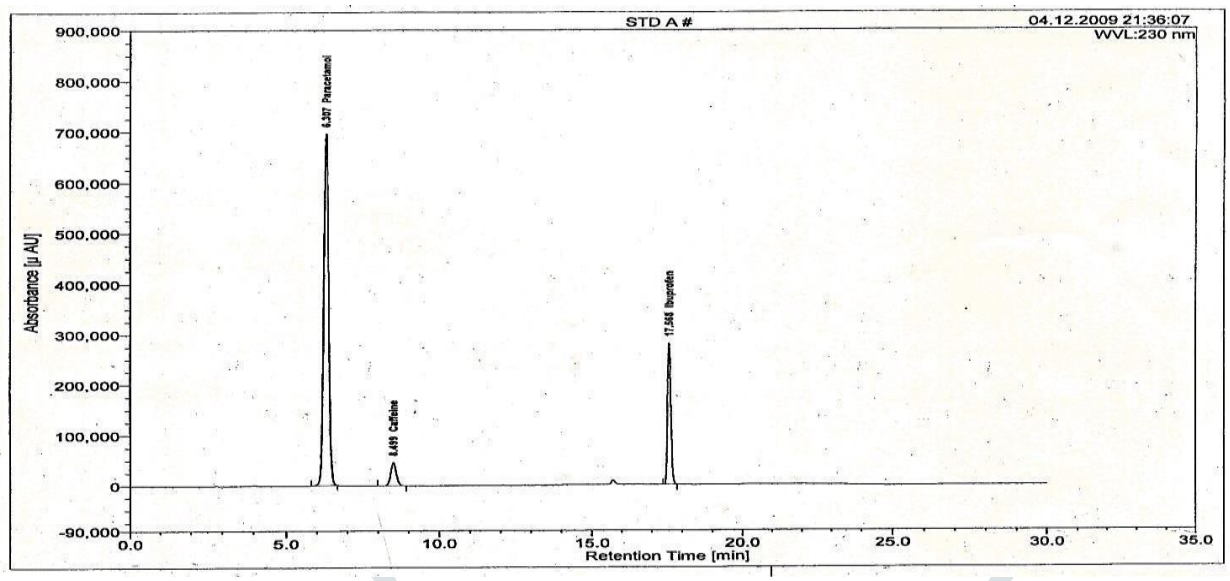
Detector: UV at wavelength 230 nm.

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.



HPLC (Gradient) Chromatogram of paracetamol, Ibuprofen andCaffeine

Gradient programming.

Time	KH_2PO_4 Buffer *p*H 3.0	Acetonitrile
0.01	85	15
5	85	15
12	25	75
20	25	75
22	85	15
30	85	15

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 %	

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.				

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	550272	39.4	98.6	2019414	200.1	100.1
2	7424325	319.8	98.4	552223	39.6	99.0	2026164	200.8	100.4
3	7437356	320.5	98.6	553173	39.7	99.2	2028301	201.1	100.6
4	7485967	322.8	99.3	554184	39.8	99.4	2033877	201.8	100.9
5	7550710	325.6	100.2	558013	40.1	100.1	2042587	202.7	101.4
6	7552770	325.2	100.1	557624	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

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 withmethanol.

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 . The above stock solution was further diluted 2 ml to 100 ml with methanol.

Linearity of API's at various spiking levels

sample Name	Level	Paracetamol			Caffeine			Ibuprofen		
		Peak Area	Conc.	Response Factor	Peak Area	Conc.	Respons Factor	e Peak Area	Conc.	Respon se 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%

5. DISCUSSION:

Method-1 is superior for Method-2

Method-2 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.

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