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Stability Indicating HPLC Method Development and Validation for the Simultaneous Estimation of Propyphenazone, Caffeine and Paracetamol by Gradient Elution Technique

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ABSTRACT

The present work is a method development and validation for the simultaneous estimation as well as stability studies for the combined tablet formulation of Paracetamol, Caffeine and Propyphenazone by using reverse phase liquid chromatography with gradient elution where the stationary phase used was ODS column (150 mm,4.6 mm,5 µ) , mobile phases were buffer (Ortho phosphoric acid 0.1%):Acetonitrile(85:15) at the initial stage and buffer : Acetonitrile (15:85) at the later stage, pH of the system was maintained 3.0, flow rate 1.5 ml/min, eluent was monitored at 280 nm., retention time was found to be in minutes 2.268 , 3.059 and 8.5 for Paracetamol, Caffeine and Propyphenazone respectively. Linearity range was 62.5 µg/ml to 375 µg/ml, 12.5 µg/ml to 75 µg/ml and 37.5 µg/ml to 225 µg/ml respectively. The developed method was validated as per ICH guideline and found to be an ideal one for regular analysis in the laboratory.

Keywords: HPLC, Gradient elution, Propyphenazone, Paracetamol, and Caffeine.

1. INTRODUCTION

Paracetamol[1,2] is considered as one of the safest antipyretic and analgesics as it does not cause significant side effects except hepato toxicity a little in some cases when it is used for a long period of time or overdose. The main mechanism proposed is the inhibition of cyclo-oxegenase (COX), and recent finding suggests that it is highly selective for COX-2. Paracetamol is metabolized primarily in the liver, into toxic and non toxic products. Figure1 represents the chemical structure of Paracetamol. Chemically the compound is known as N-(4-hydroxyphenyl) ethanamide.

Caffeine [1,3] is a centrally acing drug used mostly along with pain relievers, cough formulation etc. Even though it is a drug but is found in many soft drinks, chocolate and some other OTC products. Figure 2 represents chemical structure of Caffeine. Chemically the compound is known as 1,3,7-Trimethylpurine-2,6-dione.

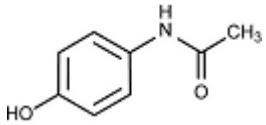

Figure 1: Chemical Structure of Paracetamol

Propyphenazone [1,4] is an analgesics, mostly used as a combination along with other antipyretic or anti inflammatory or analgesics for faster onset of action. It is one of the most popular choices indicated for headache. Figure 3 represents chemical structure of Propyphenazone.Chemically the compound is known as

4-Isopropyl-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one.

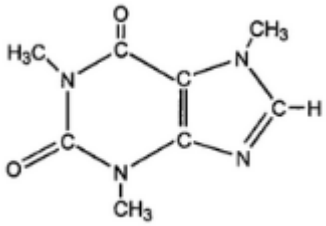

Figure 2: Chemical Structure of Caffeine

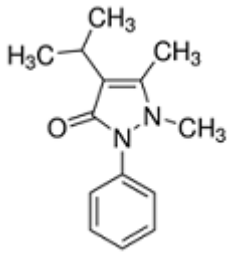

Figure 3: Chemical Structure of Propyphenazone

According to literature Survey [5-9] it was noted that there were very less number of work done for the combined formulation(tablet) of Propyphenazone, Paracetamol and Caffeine. Certain methods are available like JULIA AVRAMOVA[5] developed a method for determination of this drugs from blood sample by High Performance Liquid Chromatography, Deniz Emre, Nuran Ozaltin have innovated a method by capillary chromatography, but no good method was suggested for the above said three compounds in bulk as well as combined formulation like tablet by High Performance Liquid Chromatography. In fact the tablet form of these three combined drugs is extensively used in pain management. Therefore a good analytical method was required to develop for determination of the same. Hence we made a sincere attempt to find out a new, simple, rapid and accurate HPLC method for the assay of Propyphenazone, Caffeine and Paracetamol simultaneously.

2. MATERIALS AND METHODS

2.1 Instruments:

HPLC Waters 2695 Separations Module equipped with Quaternary pump with Auto sampler and Auto injector, PDA Detector 2996, Sonicator (Sartorius), Digital balance (Sartorius-M500P), PH meter (Thermo scientific).

2.2 Chemicals:

All the chemicals and reagent [10] were of analytical grade. Various chemicals used were Ortho phosphoric Acid (RFCL), Acetonitrile (Rankem), Methanol (Merck) and Milli Q water (Rankem).

2.3 Chromatographic Condition:

Mobile Phase- (A)Buffer : Acetonitrile(85:15)- zero to four minutes, (B)Buffer : Acetonitrile (15:85)- four to twelve minutes, Stationary Phase- ODS column(150mm,4.6mm,5µ), Flow rate 1.5 ml/min, PH 3.0, Temperature 30º C, Detecting wave length 280 nm.

2.4 Preparation of Mobile Phase:

Buffer: (0.1%OPA) 1 ml of Ortho phosphoric acid solution was taken in a 1000 ml of Volumetric flask, added about 100 ml of milli-Q water and final volume was made up to 1000 ml with milli-Q water. Buffer and Acetonitrile were taken in the ratio 85:15 “A” (initial). Buffer and Acetonitrile were taken in the ratio 15:85 “B” (final).

2.5 Diluent:

First dissolved in Methanol and made up the volume with the mixture of water and Acetonitrile (50:50).

2.6 Preparation of standard:

Accurately Weighed and transferred 15 mg of Propyphenazone, 25 mg of Paracetamol and 5 mg of caffeine working Standards into a 10 ml clean and dry volumetric flask, added 3/4th volume of diluent, sonicated for 5 minutes and made up to the final volume with diluents. 1 ml from the above stock solutions was taken into a 10 ml volumetric flask and made up to 10 ml.

2.7 Preparation of sample:

Ten tablets were weighed, powdered and required calculated quantity was transferred into a 100 ml volumetric flask, 30 ml of diluent was added and sonicated for 25 min, further the volume was made up with diluent and filtered. From the filtered solution 0.4 ml was pipetted out into a 10 ml volumetric flask and made up to 10 ml with diluent. The new method was developed and analytical/ chromatographic parameters were established after several trials.

2.8 Validation Parameters:

The method was validated as per ICH Guideline [11]. The validation parameters considered were accuracy, precision, intermediate precision, linearity, and limit of detection, limit of quantification and robustness studies.

2.9 System suitability:

The method was developed after considering many chromatographic parameters[12]. Initially the separation of compounds were done by Isocratic elution using C_{12} column, retention time was optimum for Paracetamol and Caffeine but for Propyphenazone it was more than 20 minutes. Tried with up to 90% organic part in mobile phase, Propyphenazone eluted in reasonable time, but again plate count was much less. With C_{18} column plate count was acceptable but retention time was again long which was not acceptable for regular analytical work. With gradient elusion all these obstacles were removed the Retention Time for Propyphenazone was reasonable for regular analysis. All other system suitability parameters were satisfactory.

2.10 Assay of marketed formulation:
The formulation (Tablet- Saridon) was purchased from local medical store and was assayed. Figure-4 is a representing typical chromatogram of paracetamol, Caffeine and Propyphenazone. The result in terms of percentage purity was found to be 99.89 %, 100.69% and 100.21% for paracetamol, Caffeine and Propyphenazone respectively.

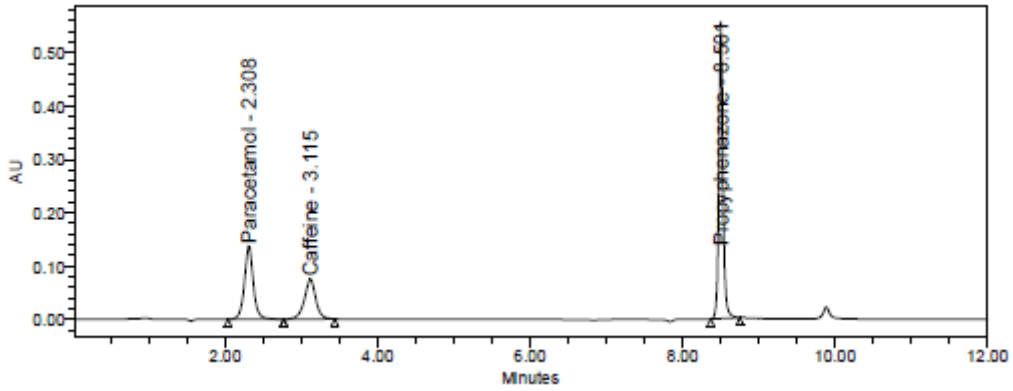

Figure 4: A typical chromatogram of Paracetamol, Caffeine and Propyphenazone

2.11 Stability Studies:
Force degradation or stability studies [13] were conducted by providing different physic-chemical environment. Figure 5 to Figure 10 represents the chromatograms due to stress degradation of the compounds.

2.12 Oxidation:
To 1 ml of stock solution of Paracetamol, caffeine and Propyphenazone, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 150 µg/ml, 250 µg/ml and 50 µg/ml solution and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

2.13 Acid Degradation Studies:
To 1ml of stock s solution Paracetamol, caffeine and Propyphenazone, 1 ml of 2N Hydrochloric acid was added and refluxed for 30 minutes at 60 ^{0}C.The resultant solution was diluted to obtain 150 µg/ml, 250 µg/ml and 50 µg/ml solution with mobile phase and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

2.14 Alkali Degradation Studies:
To 1 ml of stock solution Paracetamol, caffeine and Propyphenazone, 1 ml of 2N sodium hydroxide was added and refluxed for 30 minutes at 60 ^{0}C. The resultant solution was diluted to obtain 150 µg/ml, 250 µg/ml and 50 µg/ml solution with mobile phase and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

2.15 Dry Heat Degradation Studies:
The standard drug solution was placed in oven at 105 °C for 6 hours to study dry heat degradation. For HPLC study, the resultant solution was diluted to get 150 µg/ml, 250 µg/ml and 50 µg/ml solution with mobile phase and10 µl was injected into the system and the chromatograms were recorded to assess the stability of the sample.

2.16 Photo (UV) Stability Studies:
The photochemical stability of the drug was also studied by exposing the standard drug solution to UV Light by keeping the solution in a beaker inside the UV Chamber for 7 days. For HPLC study, the resultant solution was diluted to obtain 150 µg/ml, 250 µg/ml and 50 µg/ml solutions with mobile phase and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

2.17 Neutral (water) Degradation Studies:
Stress testing under neutral conditions was studied by refluxing the standard drug solution in water for 6 hrs at a temperature of 60 ºC. For HPLC study, the resultant solution was diluted to 150 µg/ml, 250 µg/ml and 50 µg/ml solution with mobile phase and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

3. RESULTS AND DISCUSSION

3.1 Method Validation:
For Linearity test gradually increased concentration of three drugs Paracetamol ranging from 62.5 µgm/ml to 375 µgm/ml, Caffeine 12.5 µgm/ml to 75 µgm/ml and Propyphenazone 37.5 µgm/ml to 225 µgm/ml in combined form was injected in the column. Concentration Vs area was used to build calibration curve. The Calibration curves for all the three drugs were linear at specified range and the value of 'r^2' was within the limit. Table-1 describes about regression analysis.

Table 1: Regression Analysis of Calibration Curve

Parameters	Paracetamol	Caffeine	Propyphenazone
Linearity (µgm/ml)	62.5-375	12.5-75	37.5-225
Correlation Coeff.(r)	0.999	0.999	0.999
Slope of Regression	4476	15833	14706
SD of Slope	19.86	16.26	4.12
Regression Intercept	858.5	510.5	444.1
SD of Intercept	154.58	384.07	330.84

Accuracy test was performed by spiking method. Table-2 describes the accuracy results. Each time (three replicates) with sample standard was added by 50%, 100% and 150%. The recovery was observed in terms of area and found to be within limit.

Table 2: Accuracy study

Amount of Sample			Amount of Standard			Amount recovered			% recovered		
Para	Caff	propy	Para	Caff	propy	Para	Caff	propy	Para	Caff	propy
250	50	150	125	25	75	124.15	25.04	74.70	99.32	100.01	99.60
250	50	150	250	50	150	251.13	49.80	150.49	100.45	99.61	100.33
250	50	150	375	75	225	374.68	74.09	223.26	99.91	98.78	99.22

Precision test was performed by injecting similar concentration for each compound six times into the column. Table-3 describes about precision observations, Limit of Detection, Limit of Quantification and other parameters. The result was analyzed in terms of standard deviation and %RSD. Limit of detection and quantification were calculated taking slope and standard deviation in consideration.

Table 3: Summary of Validation Parameters

Parameters	Paracetamol	Caffeine	Propyphenazone
Retention Time(min)	2.299	3.101	8.486
LOD (µgm/ml)	0.18	0.03	0.05
LOQ (µgm/ml)	0.55	0.09	0.14
Accuracy %	99.32-100.45	98.78-100.01	99.22-100.33
Intraday Precision RSD%	1.5	0.7	1.2
Inter day precision RSD%	0.8	1.0	0.6

Robustness study was done by changing chromatographic parameters. Table No.4 describes the Results of robustness studies. Chromatographic parameters like varying flow rate of mobile phase, temperature and mobile phase composition etc. were considered.

Table 4: Summary of Robustness Study

	Chromatographic Condition	Retention Time (minutes)	USP Theoretical Plates	Asymmetric Factor	% Assay
Paracetamol	Flowrate1.8ml/min	2.125	1137	0.96	99.22
	Flowrate1.2ml/min	2.310	637	1.01	100.04
	Buffer: ACN(93.5:6.5)	2.330	1141	1.04	99.03
	Buffer: ACN(76.5:23.5)	2.110	635	0.96	99.98
	Temperature(350c)	2.259	2564	1.05	99.99
	Temperature(250c)	2.299	2498	1.06	98.01
Caffeine	Flowrate1.8ml/min	2.774	1307	0.91	98.51
	Flowrate1.2ml/min	3.133	772	1.00	100.96
	Buffer: ACN(93.5:6.5)	3.150	1135	1.08	99.58
	Buffer: ACN(76.5:23.5)	2.781	766	0.92	100.55
	Temperature(350c)	3.020	2800	0.98	100.28
	Temperature(250c)	3.102	2748	1.02	98.97
Propyphenazone	Flowrate1.2ml/min	8.502	81438	0.98	99.00
	Flowrate0.8ml/min	8.505	65655	0.98	99.89
	ACN: Buffer (93.5:6.5)	8.501	73961	1.01	100.05
	ACN: Buffer (55:45)	8.785	65575	0.98	99.62
	Temperature(350c)	8.489	131352	1.14	98.88
	Temperature(250c)	8.491	152912	1.10	99.09

System suitability parameters are described in Table-5. Values of resolution indicate that the peaks were well separated. Values of asymmetric factor, theoretical plates were good enough.

Stability studies were conducted in different physicochemical condition. Tables 6 to Table 11 describe results of force degradation.

Table 5: System suitability parameters

Parameters	Paracetamol	Caffeine	Propyphenazone
Retention Time	2.299	3.101	8.486
Retention Time RSD	0.45	0.44	0.32
Theoretical plates	2610	2862	156648
Asymmetric Factor	1.04	0.98	1.12
Resolution	-	3.5	32.9
Capacity Factor	1.3	2.0	7.5

3.2 Acid environment

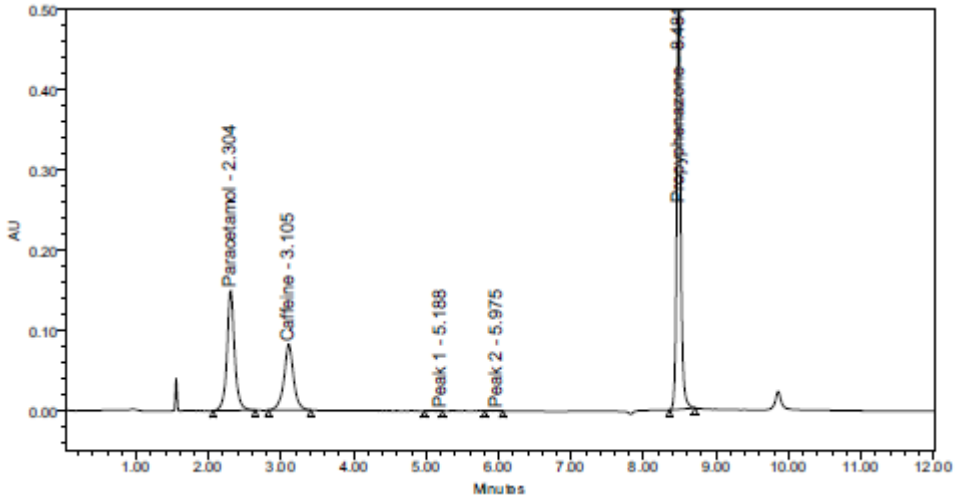

Figure 5: Chromatogram after acid degradation

Table 6: Acid Degradation Results

	Peak Name	RT	Area	%Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Paracetamol	2.304	1108250	28.04	0.072	0.282	2609	1.1
2	Caffeine	3.105	755919	19.30	0.296	0.427	2858	1.0
3	Peak1	5.188	557	0.01	82.410	90.000	7783	0.6
4	Peak2	5.975	875	0.02	50.922	90.000	23852	0.7
5	Propyphenazone	8.481	2161725	52.63	0.181	0.275	156781	1.2

3.3 Alkali environment

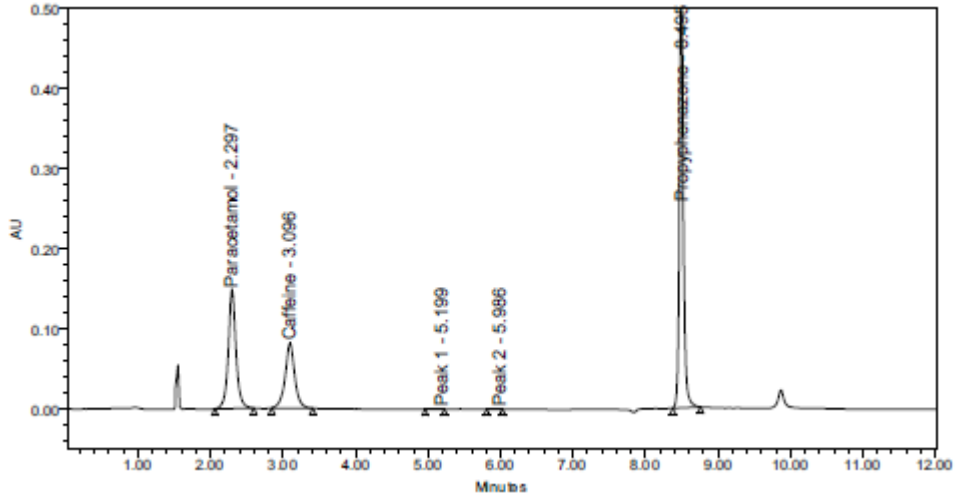

Figure 6: Chromatogram after alkali degradation

Table 7: Alkali Degradation Results

	Peak Name	RT	Area	%Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Paracetamol	2.297	1107212	27.86	0.076	0.277	2596	1.1
2	Caffeine	3.096	756631	19.11	0.235	0.389	2823	1.0
3	Peak1	5.199	716	0.02	67.675	90.000	6299	0.6

4	Peak2	5.986	608	0.02	66.057	90.000	13556	0.6
5	Propyphenazone	8.495	2168151	53.00	0.211	0.276	135881	1.1

3.4 Oxidation environment

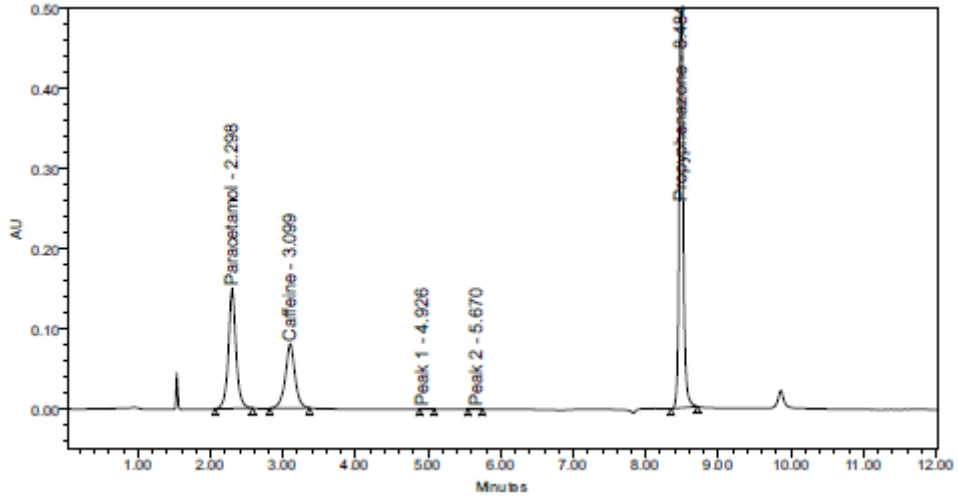

Figure 7: Chromatogram after Oxidation

Table 8: Results of Oxidation Degradation

	Peak Name	RT	Area	%Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Paracetamol	2.298	1129619	27.86	0.079	0.282	2561	1.0
2	Caffeine	3.099	770061	19.18	0.301	0.430	2748	1.0
3	Peak1	4.926	751	0.02			2631	2.2
4	Peak2	5.670	436	0.01			20065	0.8
5	Propyphenazone	8.484	2170375	52.93	0.221	0.280	144539	1.1

3.5 Elevated heat environment

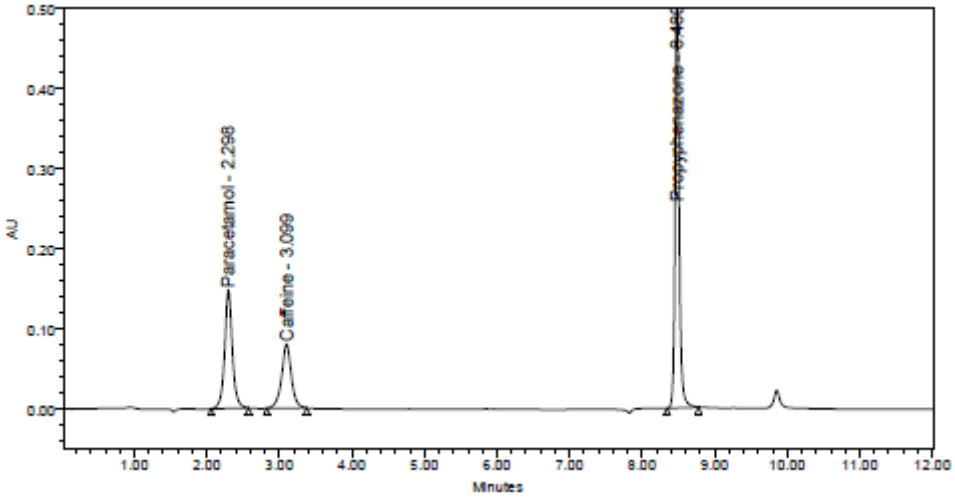

Figure 8: Chromatogram after dry-heat degradation

Table 9: Dry-heat Degradation Results

	Peak Name	RT	Area	%Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Paracetamol	2.298	1134765	27.71	0.076	0.284	2499	1.0
2	Caffeine	3.099	776853	19.08	0.265	0.441	2792	1.0
3	Propyphenazone	8.480	2183092	53.21	0.222	0.287	138016	1.1

3.6 Exposure to UV radiation

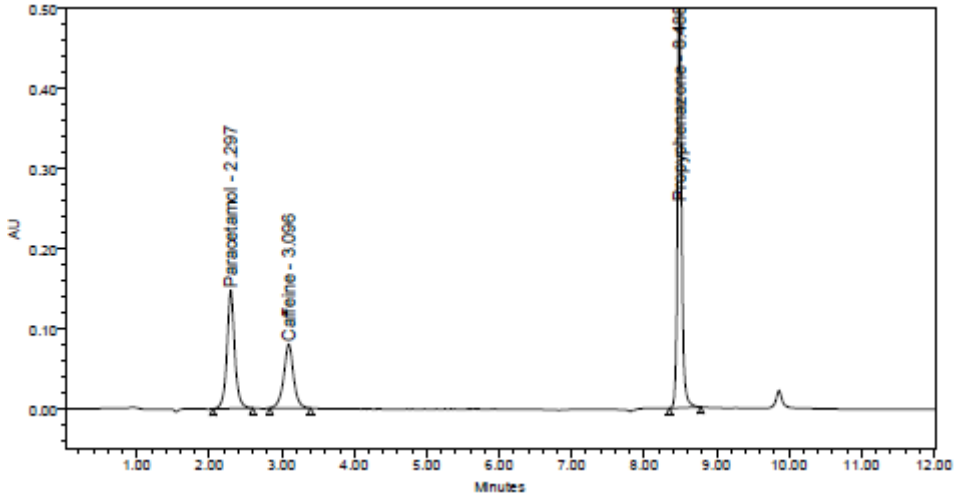

Figure 9: Chromatogram after UV degradation

Table 10: UV Degradation Results

	Peak Name	RT	Area	%Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Paracetamol	2.297	1149143	27.82	0.073	0.280	2455	1.0
2	Caffeine	3.095	774778	19.10	0.256	0.417	2709	1.0
3	Propyphenazone	8.488	2097161	53.08	0.219	0.280	136406	1.1

3.7 Neutral environment

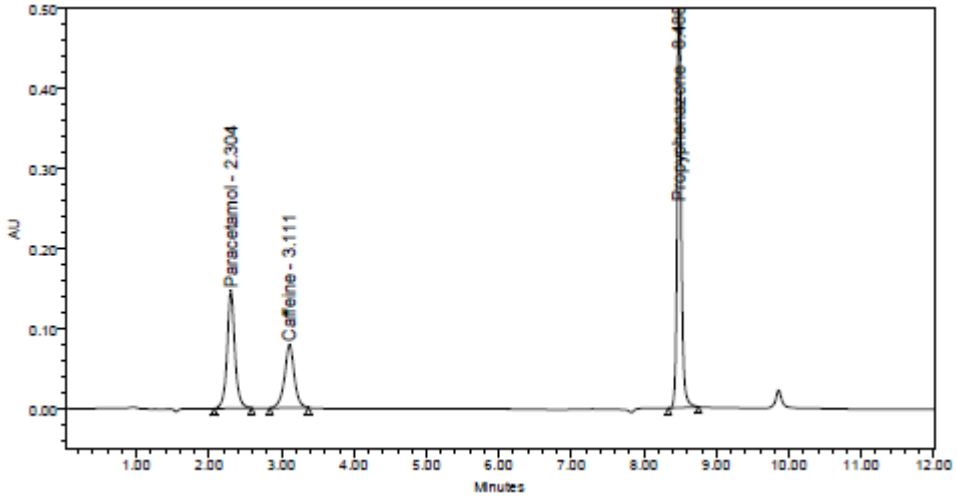

Figure 10: Chromatogram after Water degradation

Table 11: Water Degradation Results

	Peak Name	RT	Area	%Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Paracetamol	2.304	1137987	27.80	0.073	0.280	2528	1.0
2	Caffeine	3.111	784400	19.02	0.250	0.418	2729	1.0
3	Propyphenazone	8.488	2151606	53.18	0.202	0.283	131296	1.1

4. CONCLUSION

A rapid, simple accurate, precise and economic HPLC method was developed for the simultaneous estimation and stability studies of Propyphenazone, Caffeine and Paracetamol in bulk and their combined tablet dosage form. The results were found to be well within the limits as per ICH guideline. Hence the developed new

method can be used for the determination of the above mentioned drugs in the laboratory on regular basis.

5. ACKNOWLEDGEMENT

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