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A NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF FLUPIRTINE AND PARACETAMOL IN BULK AND DOSAGE FORM

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Abstract:

A simple, rapid, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Paracetamol and Flupirtine in Tablet dosage form. A YMC C18 5mm column having 250 x 4.6mm id in Isocratic mode with mobile phase containing 0.1M Potassium dihydrogen phosphate: methanol (70:30) was used. The flow rate was 0.8ml/min and effluents were monitored at 271nm. The method was validated for Accuracy, Precision, Specificity, Linearity and Sensitivity. The retention times of Paracetamol and Flupirtine were 1.8min and 3.0min respectively. The calibration curves were linear over a concentration range from 81.25-243.75 µg/ml for Paracetamol and over a concentration range from 25-75 µg/ml for Flupirtine. Limit of detection (LOD) and Limit of quantitation (LOQ) were 3.83 and 12.77 for Paracetamol and 1.05 and 3.51 for Flupirtine respectively. The developed method was fast, accurate, precise and successfully applied to estimate the amount of Paracetamol and Flupirtine in bulk sample and tablet dosage form so it can be used for regular quality control of the drug.

KEYWORDS: Flupirtine, Paracetamol, RP-HPLC, YMC C18, Potassium dihydrogen phosphate, methanol.

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INTRODUCTION:

The importance of Chromatography[1][2] is increasing rapidly in pharmaceutical analysis in exact differentiation, selective identification and quantitative determination of structurally closely related compounds. Another important field of application of chromatographic methods is the purity testing of final products and intermediates (detection of decomposition products and by-products). As a consequence of the above points, chromatographic methods are occupying an ever-expanding position in the latest editions of the pharmacopoeias and other testing standards.

The modern form of column chromatography has been called high performance, high pressure, High resolution and high speed liquid chromatography. High Performance Liquid Chromatography (HPLC) is a special branch of column chromatography in which the mobile phase is forced through the column at high speed. As a result the analysis time is reduced by 1-2 orders of magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possible increasing the column efficiency substantially.

There are several reasons for developing new HPLC methods [3] of analysis, There may not be a suitable method for a particular analyte in the specific sample matrix, Existing method may be too expensive, time consuming, or energy intensive, or they may not be easily automated, Existing method may be too error, artifact, and/ or contamination prone, or they may be unreliable, Existing method may not provide adequate sensitivity or analyte selectivity in samples of interest,There may be a need for an alternative method to confirm, for legal, or scientific reasons analytical data originally obtained by existing methods, Newer instrumentation and techniques may have been evolved that provide opportunities for improved methods, including improved analyte identification or detection limits, greater accuracy or precision, or better return on investment.

Most of the drugs in multicomponent[4] dosage forms can be analyzed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation of this method. HPLC method eliminates tedious extraction and isolation procedures.

Reversed phase mode is the most popular mode for analytical and preparative separations of compound of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is nonpolar hydrophobic packing with octyl or octa decyl functional group bonded to silica gel and the mobile phase is polar solvent. An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization control, ion suppression, ion pairing and complexation) to control retention and selectivity. The polar compound gets eluted first in this mode and nonpolar compounds are retained for longer time. The different columns used are octa decyl silane (ODS) or C_{18}, C_8, etc., (in the order of increasing polarity of the stationary phase).

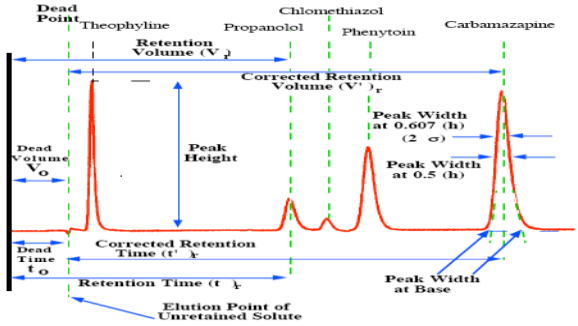

Fig. 1: Nomenclature of the Chromatogram

Flupirtine Maleate is a unique centrally acting, non-opioid analgesic with muscle relaxant and neuro protective properties. It is an amino pyridine derivative. It provides pain relief through interaction with non-opiate neural pathways in the nervous system[5]. Flupirtine Maleate is chemically designated as Ethyl 2-amino-6-[(4-fluorobenzyl) amino] pyridin-3-yl Carbamate Maleate. Its moelecular formula is $C_{15}H_{17}FN_4O_2.C_4H_4O_4$ and its molecular weight is 420.40. Flupirtine Maleate was formulated with Paracetamol, a weak inhibitor of PG synthesis of COX-1 and COX-2 in broken cell systems. The opening of these channels inhibits exaggerated neuronal action potential generation and controls neuronal excitability[6]. Some neuroprotective effects due to NMDA Receptor antagonistic properties of Flupirtine may also be used in the treatment of Creutzfeldt-Jakob disease, Alzheimer's disease, and multiple sclerosis[7].

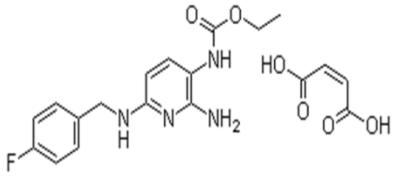

Fig. 2: Chemical Structure of Flupirtine

Paracetamol is a common analgesic and antipyretic drug, that is used for the relief of fever, headaches and other minor aches and pains. Their determination in pharmaceuticals is of paramount importance, since an overdose of Paracetamol can cause fulminating

hepatic necrosis and other toxic effects.[8] Paracetamol is chemically designated as N-(4-hydroxyphenyl) acetamide. Its molecular formula is $C_8H_9NO_2$ and its molecular weight is 151.16256. Paracetamol is a weak inhibitor of PG synthesis of COX-1 and COX-2 in broken cell systems and The analgesic effect of Paracetamol is central and is due to activation of descending serotonergic pathways, but its primary site of action may be inhibition of PG synthesis.[9] Paracetamol is used to treat many conditions such as headache, muscle aches, arthritis, backache, toothaches, colds, and fevers. It relieves pain in mild arthritis but has no effect on the underlying inflammation and swelling of the joint. [10]

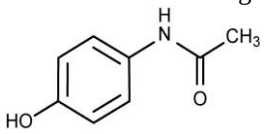

Fig. 3: Chemical Structure of Paracetamol[11]

A literature survey reveals analytical methods like UV spectrophotometer, HPTLC, HPLC, RP-HPLC, LC-MS for simultaneous determination of most of the drugs in multicomponent pharmaceutical dosage forms were reported. However, no reference was reported so far for the simultaneous determination of said drugs by RP-HPLC method. It was concluded to determine the intrinsic stability of drug substances in formulation.

MATERIALS AND METHODS

Pharmaceutical grade Paracetamol and Flupirtine were kindly supplied as a gift sample by Lara Drugs private limited, Hyderabad, Andhra Pradesh, India. Methanol was of HPLC grade and collected from E. Merck, Darmstadt, Germany. Potassium dihydrogen phosphate and Ortho Phosphoric Acid were AR (analytical reagent) grade supplied by Fischer Scientific Chemicals. Water HPLC grade was obtained from a Milli-QRO water purification system. Paracetamol and Flupirtine Tablets are available in the market as Ketoflam-P in composition of Paracetamol (325mg) and Flupirtine Maleate (100mg). The samples were properly checked for their manufacturing license numbers, batch numbers, production, expiry dates and stored properly. All the solutions for analysis were prepared and analyzed freshly.

INSTRUMENTS

Waters e2695Alliance HPLC system (Acquity Waters, USA) connected with PDA Detector 2998 and Empower2 Software. The drug analysis data were acquired and processed using Empower2 software running under Windows XP on a Pentium PC. Electronic balance (Shimadzu AUX220, Shimadzu corporation, Kyoto, Japan), Sonicator(Ultrasonic Cleaner Power sonic 420, Hwashin technology co, Korea), 0.45µ membrane filter from ELICO
(Mmbai. Maharasthra, India) were also used.

RP-HPLC Method development and validation for simultaneous determination of Paracetamol and Flupirtine in the drug products Chromatographic conditions.

PREPARATION AND SELECTION OF MOBILE PHASE

Preparation of Potassium dihydrogen Phosphate (0.1N)

Buffer was prepared by dissolving 13.6g of KH2PO4 in 250ml of water and final volume was made up to 1000ml.

Selection

The preliminary isocratic studies on a reverse phase C18 column with different mobile phase combination of Potassium dihydrogen phosphate buffer and Methanol were studied for simultaneous separation of both the drugs. The optimal composition of mobile phase determined to be Potassium dihydrogen phosphate (Buffer)**:** Methanol (70:30 v/v) and filtered through 0.45µ membrane filter.

PREPARATION OF STANDARD SOLUTION

325mg Paracetamol and 100mg Flupirtine was dissolve in 100 ml of Diluent and was further diluted to get stock solution of Paracetamol(162.5 µg/ml) and Flupirtine(50 µg/ml). This is taken as a 100% concentration and Solutions containing mixture of Paracetamol and Flupirtine of five different concentrations (50%, 75%, 100% 125%, and 150% of target concentration) were prepared in the same way.

PREPARATION OF SAMPLE SOLUTION

Sample solution containing both the drugs was prepared by dissolving tablet powder into diluent. Ten tablets were weighed separately. Their average weights were determined. Powder of tablets equivalent to one tablet weight were weighed and taken in a 100 ml volumetric flask, dissolved in diluent and shaken and sonicated for about 10 minutes then filtered through 0.45µ membrane filter. The filtered solution was further diluted to make the final concentration of working sample equivalent to 100% of target concentration.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

A simple, rapid, specific, accurate and precise reverse phase high performance liquid chromatographic

method was developed for the simultaneous estimation of Paracetamol and Flupirtine in Tablet dosage form. A YMC C18 5mm column having 250 x 4.6mm id in Isocratic mode with mobile phase containing 0.1M Potassium dihydrogen phosphate : methanol (70:30) was used. The flow rate was 0.8ml/min and effluents were monitored at 271nm. The retention times of Paracetamol and Flupirtine was 1.8min and 3.0min respectively.

Optimized Chromatographic Conditions

Column	C_{18} 250X4.6 mm, 5µ,YMC
Flow rate	0.8ml / minute.
Wavelength	271nm
Temperature	30
Injection volume	10µl.
Run time	10min
Mobile phase	Buffer : Methanol(70:30)

METHOD VALIDATION

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of Paracetamol and Flupirtine in tablet dosage form. The experiment was carried out according to the official specifications of USP–30, ICH- 1996 and Global Quality Guidelines-2002.The method was validated for the parameters like system suitability , specificity, linearity, accuracy, precision ,LOD, LOQ, and robustness.

SPECIFICITY

Specificity test determines the effect of excipients on the assay result.

Procedure:

To determine the selectivity of the method, standard sample of Paracetamol and Flupirtine were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another.

Acceptance criteria: The chromatograms of Placebo and Blank should not show any peak at the Retention time of the analyte peak

SYSTEM SUITABILITY

Procedure:

System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of Paracetamol and Flupirtine. Various chromatographic parameters such as retention time, peak area tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

Acceptance Criteria

1. RSD should not be more than 2.0% for five replicate injections of standard
2. USP Tailing of the peak should not more than 2.0
3. The column efficiency as determined for standard, USP Plate Count should not be less than 2000.

PRECISION

Procedure:

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

Acceptance criteria: %RSD should not be more than 2.

Repeatability: Six preparations were prepared individually using single batch of drug as per test method and injected each solutions.

Intermediate precision: Standard solution were prepared as per test method and injected six times.

ACCURACY (RECOVERY STUDIES)

Procedure:

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard Paracetamol and Flupirtine were added to pre-analyzed samples and were subjected to the proposed HPLC method. Prepared solutions in triplicate at levels 50%, 100% and 150% of test concentration using Standard as per the test method and injected each solution in triplicate.

$$\% \text{ Recovery} = \frac{\text{Sample Area}}{\text{Standard Area}} \times \frac{100}{\text{conc. in \%}} \times 100$$

Acceptance criteria: Assay recovery should be between 98%-102%.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ)

LOD: Lowest amount of analyte in a sample that can be detected but not necessarily quantities, under the stated experimental conditions.

Method Procedure:
The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The various concentrations ranging from 0.1 to 1 ppm of drugs were injected and peaks were recorded. 0.1 ppm concentration was detected.
LOQ: Lowest amount of analyte in a sample, which can be quantitatively, determined with suitable precision and accuracy.

Method Procedure:
The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The various concentrations ranging from 0.1 to 1 ppm of drugs were injected and peaks were recorded. 0.5 ppm concentration was detected.

Table 1: Acceptance criteria for LOD and LOQ

RSD Criteria	Concentration at which RSD < 3.0% (LOD)	Concentration at which RSD<10.0 (LOQ)

LINEARITY
The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample.
Procedure: Linearity of the method was determined by constructing calibration curves. Standard solutions of Paracetamol and Flupirtine of different concentrations level (50%, 75%, 100%, 125%, and 150%) were used for this purpose. Each measurement was carried out in six replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients.
Acceptance criteria: The correlation coefficient should be not less than 0.9990.

RANGE
The range of an analytical procedure is the interval between the upper and lower concentrations (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.
Acceptance criteria: Linearity, Precision and Recovery should be shown.
The logic behind this parameter was – typical concentration range was essential between which the actual concentration should fall when performing real sample analysis.

ROBUSTNESS OF METHOD
Measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides indication of its reliability during its normal usage.

Procedure:
To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, temperature, on the retention time and tailing factor were studied. The method was found to be unaffected by small changes ± 0.2 change in flow rate and ± 5ºc change in temperature.
1. Flow: the standard solution was prepared and injected for the two times with (±2) flow rate.
2. Temperature: The standard solution was prepared and injected for two times with (±2º C)
Acceptance Criteria: Overall RSD should not be more than 2.0 %.

RUGGEDNESS
Method/Procedure: samples were prepared as per the test method the test method and injected in duplicate using different system, analyst on different day.
Calculated Mean area, SD, %RSD.

RESULTS AND DISCUSSION:

HPLC METHOD DEVELOPMENT
TRIAL 1:

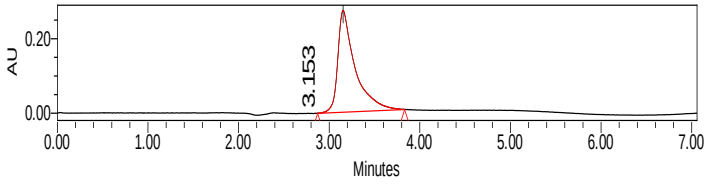

Fig. 4: Chromatogram of Trial 1

Table 2: System Suitability Parameters - Trial 1

Retention Time	USP Tailing	USP Plate Count
3.153	3.5	1000

Discussion: only one peak is eluted, so the column was changed for the next Trial.

TRIAL 2:

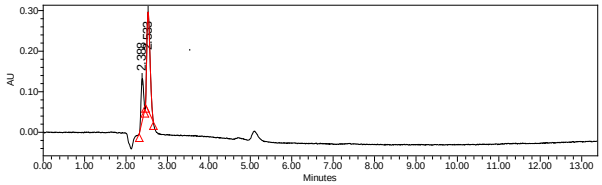

Fig. 5: Chromatogram of Trial 2

Table 3: System Suitability Parameters - Trial 2

Retention Time	Resolution	USP Tailing	USP Plate Count
2.359		1.2	1396
1.890	0.9	1.3	1584

Discussion: There was low retention and resolution between the peaks and the plate count was also less, hence the flow rate was decreased in the next Trial.

TRIAL 3:

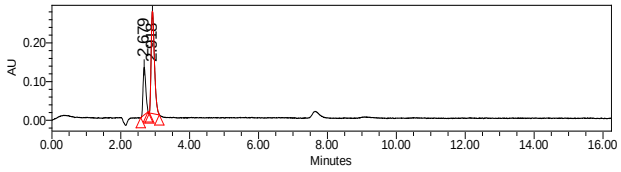

Fig. 6: Chromatogram of Trial 3

Table 4: System Suitability Parameters - Trial 3

Retention Time	Resolution	USP Tailing	USP Plate Count
2.679		1.1	1234
2.910	1.5	1.5	1643

Discussion: resolution between the peaks was poor and also the plate count was also less, so the buffer ratio was increased in the next Trial.

TRIAL 4:

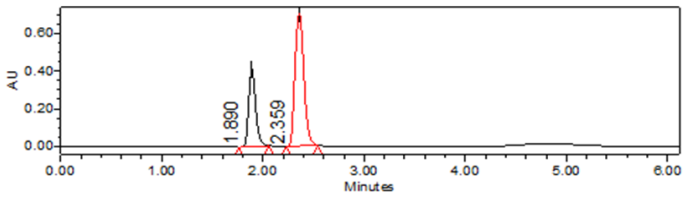

Fig. 7: Chromatogram of Trial 4

Table 5: System Suitability Parameters –Trial 4

Retention time	Resolution	USP Tailing	USP Plate Count
1.890		1.2	3396
2.359	3.2	1.3	3584

Discussion: resolution was still poor, so the buffer ratio was further increased in the next Trial

TRIAL 5:

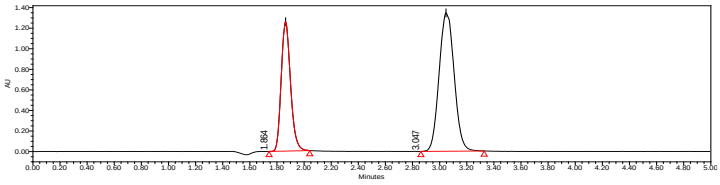

Fig. 8: Chromatogram of Trial 5

Table 6: System Suitability Parameters -Trial 5

Retention time	Resolution	USP Tailing	USP Plate Count
1.864		1.3	3600
3.047	5.3	1.1	3498

Discussion: good resolution between the peaks was achieved, but the peaks were broad, so the sample concentration was decreased for the final Trial.

TRIAL 6: (Dilution changed)

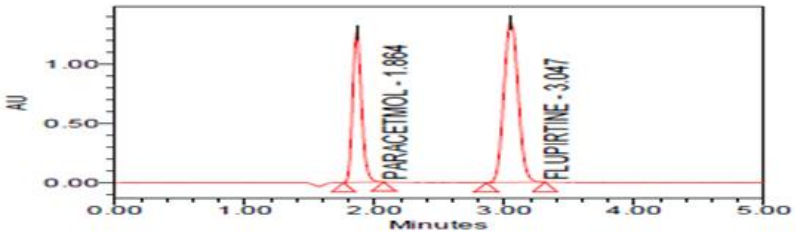

Fig. 9: Chromatogram of Trial 6

Table 7: System Suitability Parameters –Trial 6

Retention time	Resolution	USP Tailing	USP Plate Count
1.864		1.1	3619
3.047	7.1	1.1	3485

Discussion: The peaks were symmetrical with all the System Suitability parameters within the limits and the method was found to be stable

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Optimized Chromatographic Conditions

Parameters	Method
Stationary phase (column)	YMC C_{18} (250 × 4.6 mm, packed with 5 µm)
Mobile Phase	KH2PO4 : Methanol (70:30)
Flow rate (ml/min)	0.8
Run time (minutes)	10
Column temperature (°C)	Ambient
Volume of injection loop (µl)	10
Detection wavelength (nm)	271
Drugs RT (min)	1.0 and 3.4

METHOD VALIDATION
SPECIFICITY

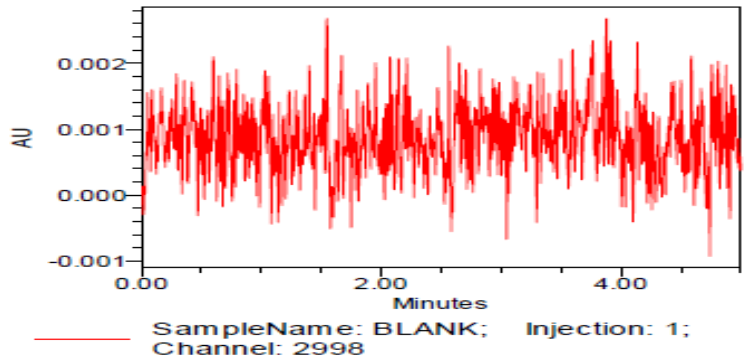

Fig. 10: Chromatogram of Blank

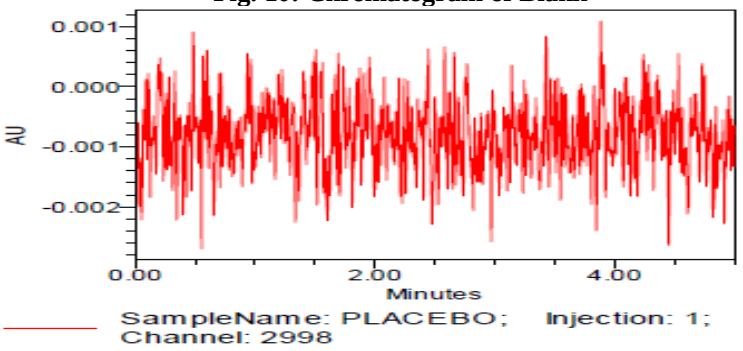

Fig. 11: Chromatogram of Placebo

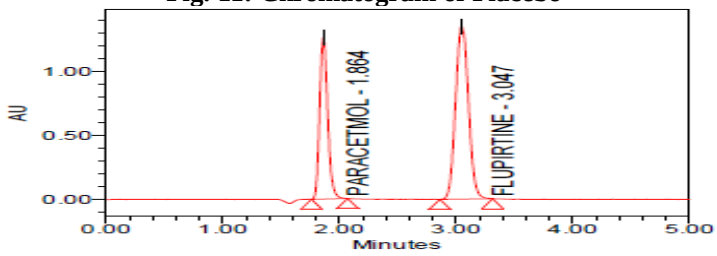

Fig. 12: Chromatogram of Analyte

Discussion: There is no interference at the retention time of the analyte peak

SYSTEM SUITABILITY

Table 8: System Suitability Test Parameters-- Paracetamol

S. No.	Injection	Area	Retention time	USP Tailing	USP Plate Count
1	1	6095935	1.864	1.193	3324
2	2	6034788	1.864	1.195	3271
3	3	6048143	1.861	1.264	3214
4	4	6078651	1.864	1.199	3100
5	5	6057870	1.863	1.213	3997
Mean		6063078			
Std.Dev.		24355			
%RSD		0.4			

Table 9: System Suitability Test Parameters -- Flupirtine

S. No.	Injection	Area	Retention time	USP Resolution	USP Tailing	USP Plate Count
1	1	10376306	3.052	6.952	1.047	3340
2	2	10407752	3.045	6.844	1.111	3379
3	3	10390066	3.050	6.833	1.076	3362
4	4	10408079	3.043	6.671	1.126	3139
5	5	10378709	3.042	6.639	1.152	3049
Mean		10392182				
Std. Dev.		15274				
%RSD		0.1				

Discussion: The %RSD value, plate count, tailing factor and resolution results were found to be satisfactory

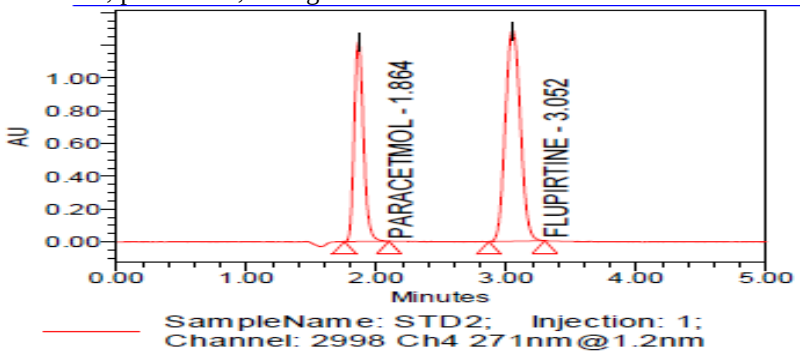

Fig. 13: Chromatogram of System Suitability injection 1

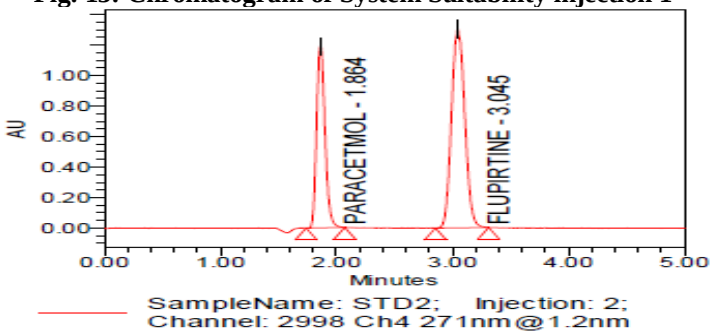

Fig. 14: Chromatogram of System Suitability injection 2

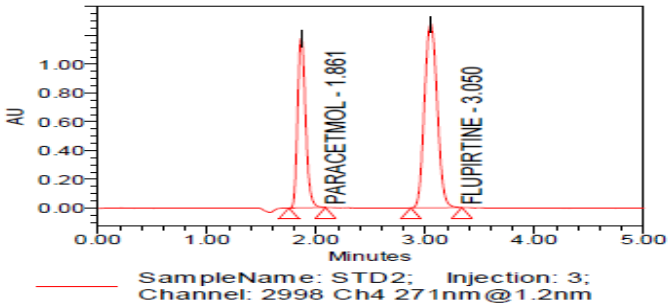

Fig. 15: Chromatogram of System Suitability injection 3

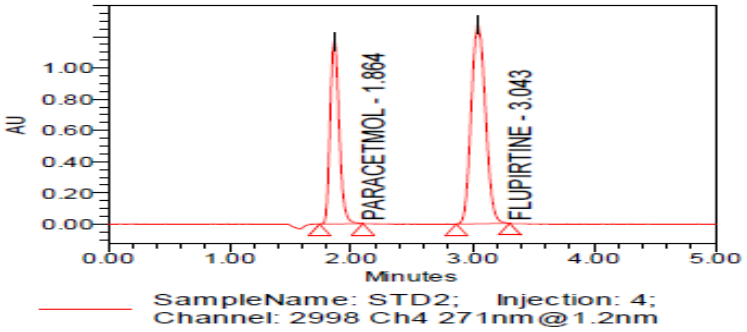

Fig. 16: Chromatogram of System Suitability injection 4

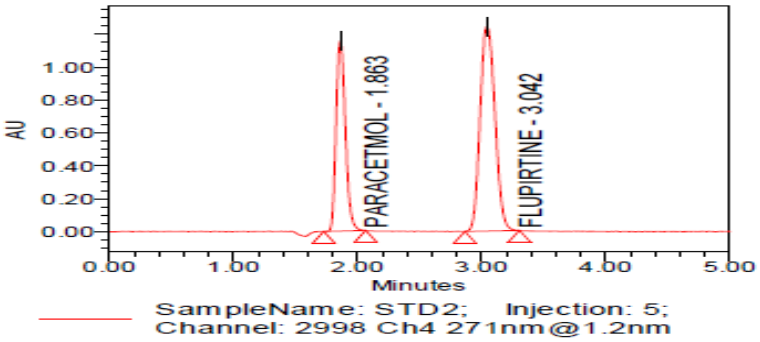

Fig. 17: Chromatogram of System Suitability injection 5

PRECISION

Table 10: Method Precision Values-- Paracetamol

S. No.	Area	%Assay
1	6069103	99
2	6065893	99
3	6069077	99
4	6063043	99
5	6061349	99
6	6067718	99
Avg. assay		99
%RSD		0.05

Table 11: Method Precision Values-- Flupirtine

S. No.	Area	%Assay
1	10312795	99
2	10316990	99
3	10334225	99
4	10347820	99
5	10364416	99
6	10313982	99
Avg assay		99
%RSD		0.20

Discussion: The %RSD value from six preparations is observed to be less than 2 and hence the result is found to be satisfactory.

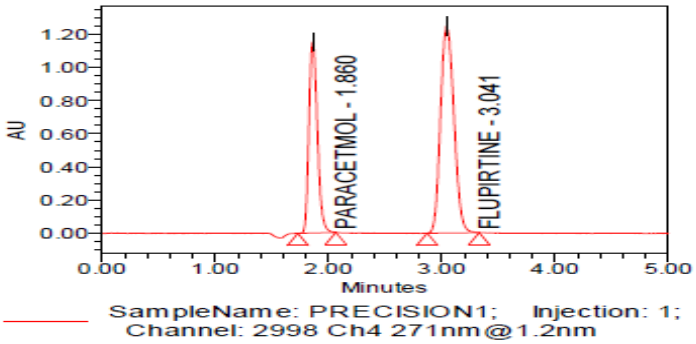

Fig. 18: Chromatogram of Precision 1

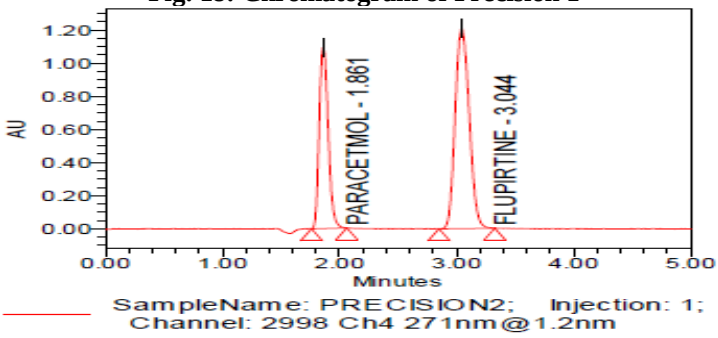

Fig. 19: Chromatogram of precision 2

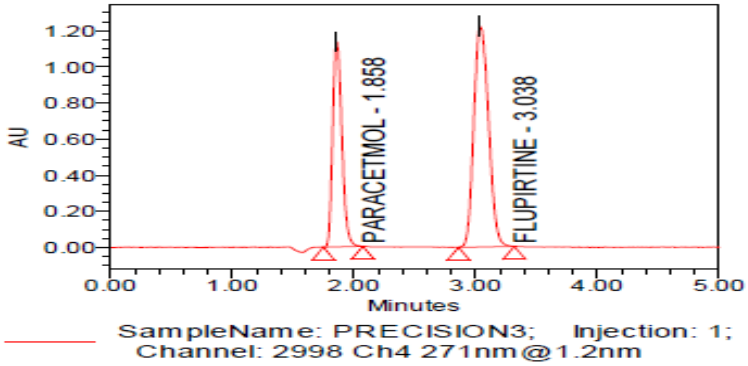

Fig. 20: Chromatogram of precision 3

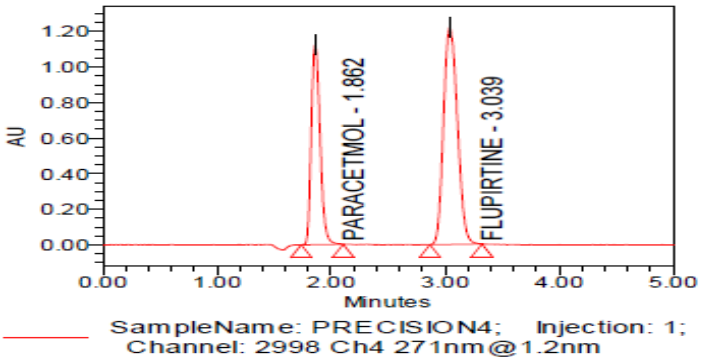

Fig.. 21: Chromatogram of precision 4

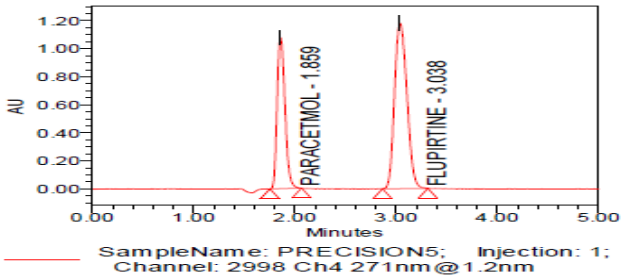

Fig.. 22: Chromatogram of precision 5

Intermediate Precision

Table 12: Intermediate Precision values-- Paracetamol

Injection	Area	%Assay
1	6095935	100
2	6034788	99
3	6048143	99
4	6078651	100
5	6057870	99
Mean	6063078	99
Std. Dev.	24355	
%RSD	0.4	

Table 13: Intermediate Precision values-- Flupirtine

Injection	Area	%Assay
1	10376306	99
2	10407752	100
3	10390066	99
4	10408079	100
5	10378709	99
Mean	10392182	99
Std Dev	15274	
%RSD	0.1	

Discussion: The %RSD value from six injections was observed to be less than 2 and hence the result found to be satisfactory

ACCURACY

Table 14: Summary of results of Accuracy parameter for Paracetamol

Spiked level	Sample wt (mg)	Sample area	Amount added	Amount found	%recovery	mean
50%	404	3034715	80.797	80.60	100	
50%	404	3033256	80.797	80.56	100	
50%	404	3037123	80.797	80.67	100	100
50%	404	3039518	80.797	80.73	100	
50%	404	3032820	80.797	80.55	100	
50%	404	3032527	80.797	80.54	100	
100%	804	6060356	160.795	160.97	100	
100%	804	6064904	160.795	161.09	100	100
100%	804	6062926	160.795	161.03	100	
150%	1206	9098924	241.193	241.62	100	
150%	1206	9099797	241.193	241.67	100	
150%	1206	9099397	241.193	241.69	100	100
150%	1206	9096842	241.193	241.6	100	
150%	1206	9098988	241.193	241.67	100	
150%	1206	9096341	241.193	241.60	100	

Table 15: Summary of results of Accuracy parameter for Flupirtine

Spiked level	Sample wt (mg)	Sample area	Amount added	Amount found	%recovery	mean
50%	404	5198727	24.861	24.94	100	
50%	404	5198914	24.861	24.94	100	100
50%	404	5191906	24.861	24.90	100	
50%	404	5192315	24.861	24.91	100	
50%	404	5192841	24.861	24.94	100	
50%	404	5199509	24.861	24.91	100	
100%	804	10366711	49.475	49.73	101	
100%	804	10362700	49.475	49.71	100	101
100%	804	10383499	49.475	49.81	101	
150%	1206	15589467	74.2	74.78	101	
150%	1206	15590916	74.2	74.76	101	101
150%	1206	15585098	74.2	74.79	101	
150%	1206	15591870	74.2	74.79	101	
150%	1206	15589259	74.2	74.78	101	
150%	1206	15595235	74.2	74.81	101	

Discussion: The recovery results indicating that the test method has an acceptable level of accuracy for the assay of drugs was from 50% to 150% of test concentration.

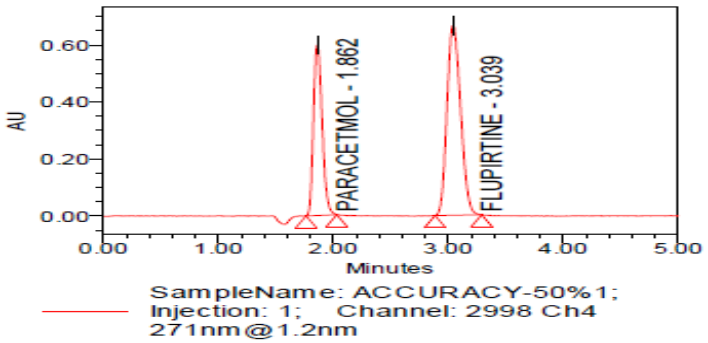

Fig. 23: Chromatogram of Accuracy-50%- 1

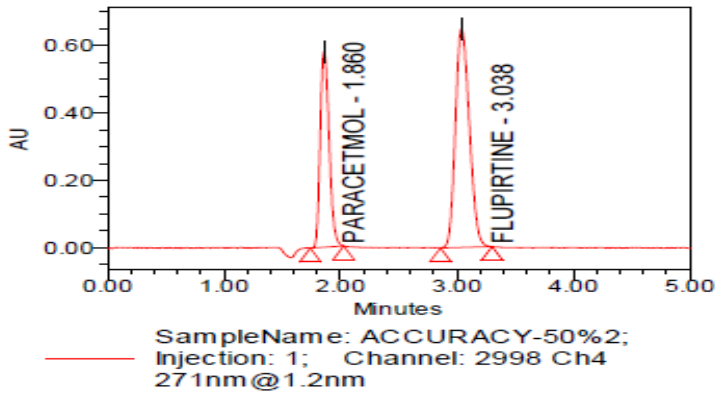

Fig. 24: Chromatogram of Accuracy-50% - 2

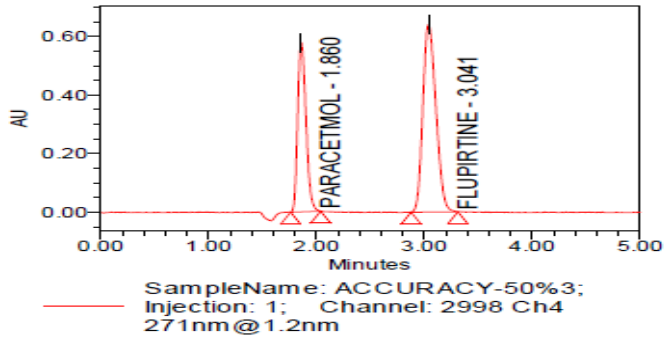

Fig. 25: Chromatogram of Accuracy-50% -3

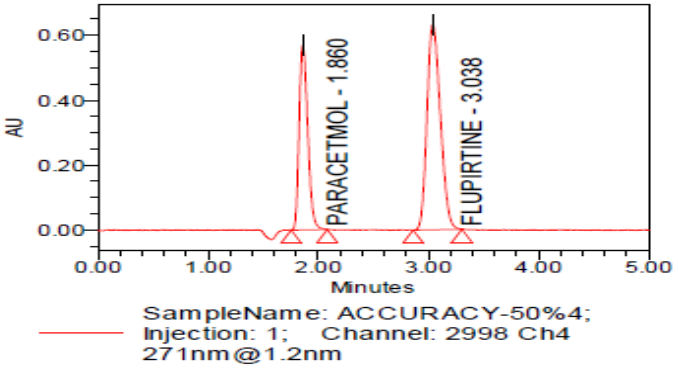

Fig. 26: Chromatogram of Accuracy-50%-4

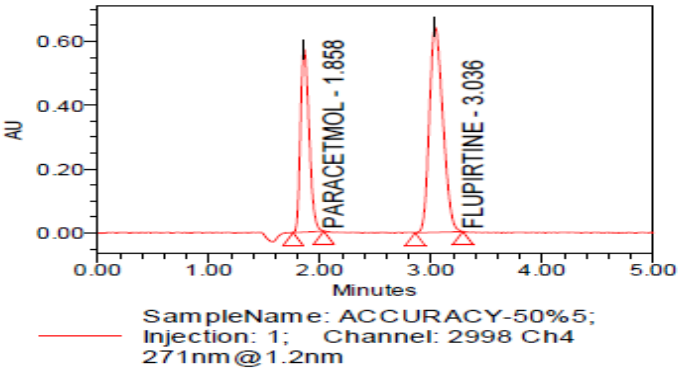

Fig. 27: Chromatogram of Accuracy-50% -5

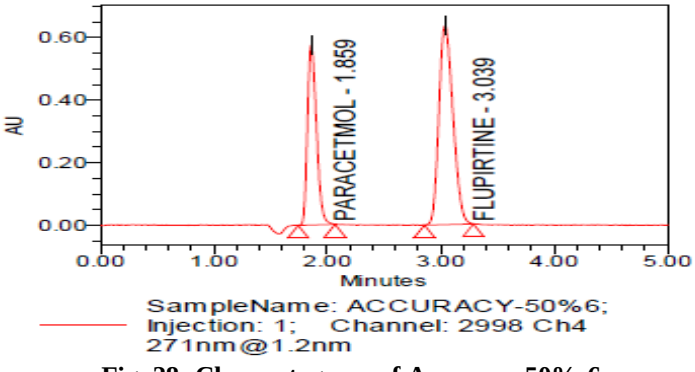

Fig. 28: Chromatogram of Accuracy-50%-6

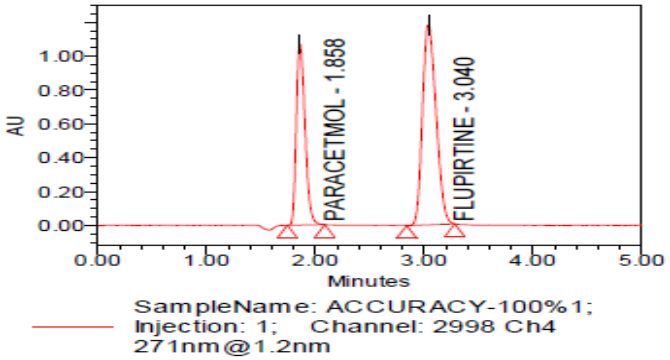

Fig. 29: Chromatogram of Accuracy-100%-1

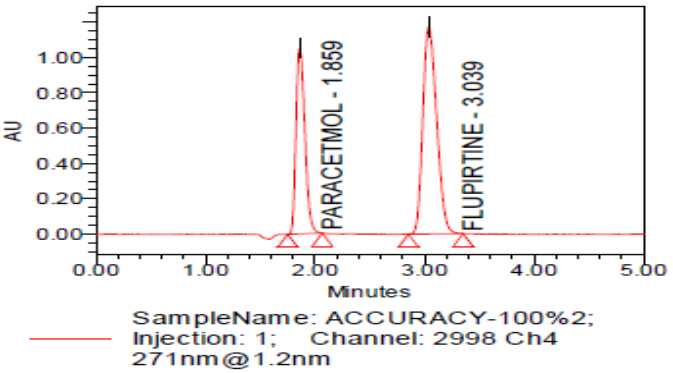

Fig. 30: Chromatogram of Accuracy-100%-2

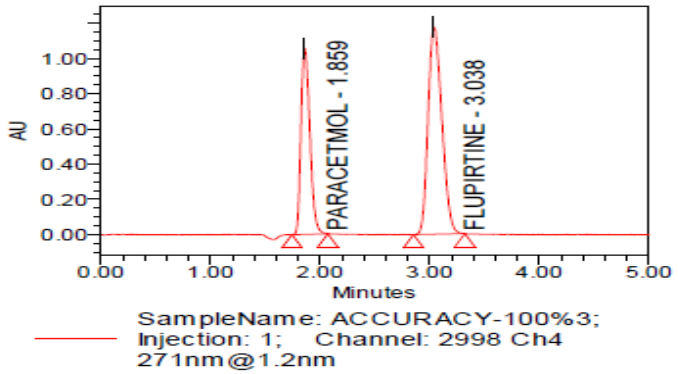

Fig. 31: Chromatogram of Accuracy-100%-3

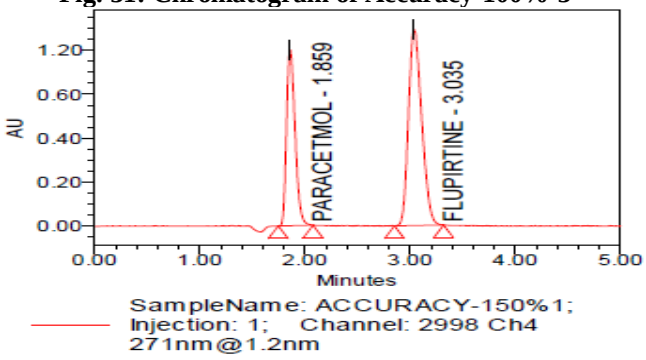

Fig. 32: Chromatogram of Accuracy-150% -1

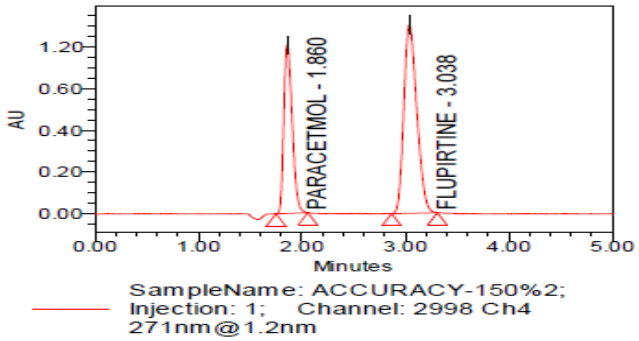

Fig. 33: Chromatogram of Accuracy-150%-2

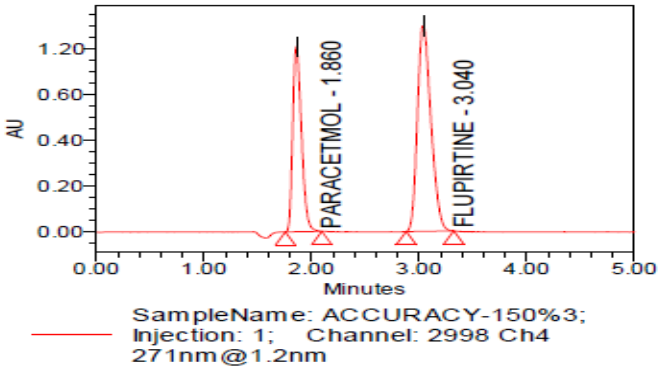

Fig. 34: Chromatogram of Accuracy-150%-3

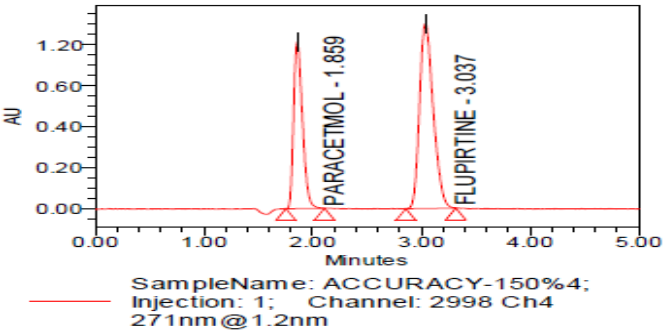

Fig. 35: Chromatogram of Accuracy-150%-4

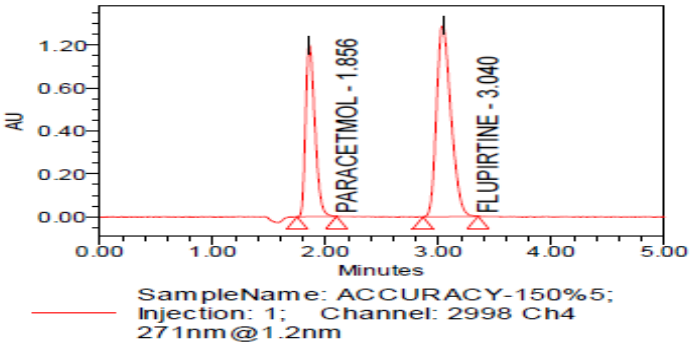

Fig. 36: Chromatogram of Accuracy-150%-5

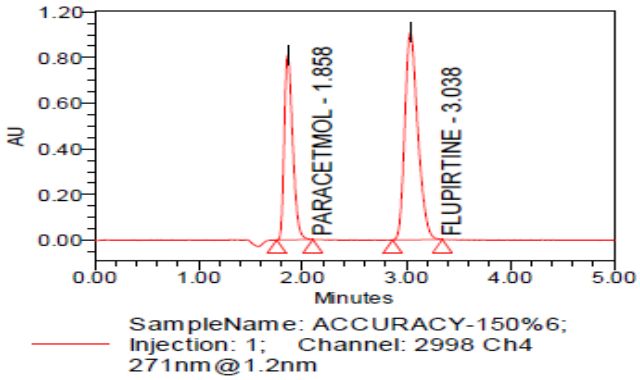

Fig. 37: Chromatogram of Accuracy-150%-6

LIMIT OF DETECTION

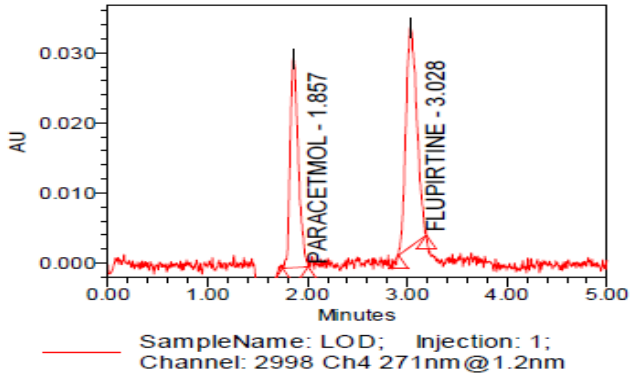

Fig. 38: Chromatogram of LOD

LIMIT OF QUANTIFICATION

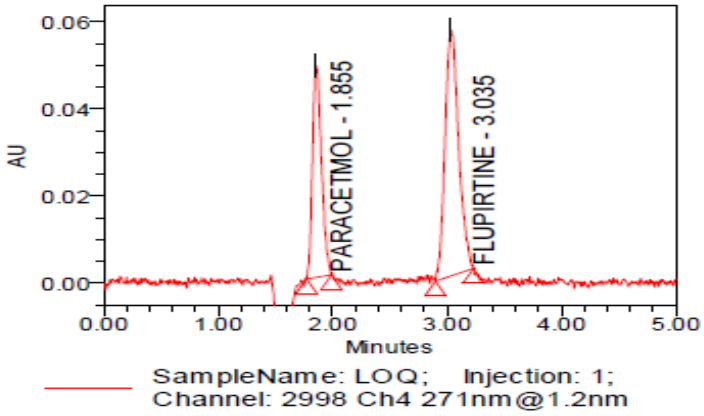

Fig. 39: Chromatogram of LOQ

LINEARITY & RANGE

Acceptance Criteria: R value should be not more than 1

Table 16: Linearity values-- Paracetamol

Conc.%	ppm	Area
50	81.25	3039011
75	121.88	4542901
100	162.50	6064807
125	203.12	7574063
150	243.75	9098205

Table 17: Linearity values-- Flupirtine

Conc.%	ppm	Area
50	25	5195195
75	37.5	7795994
100	50	10395507
125	62.5	12990227
150	75	15588273

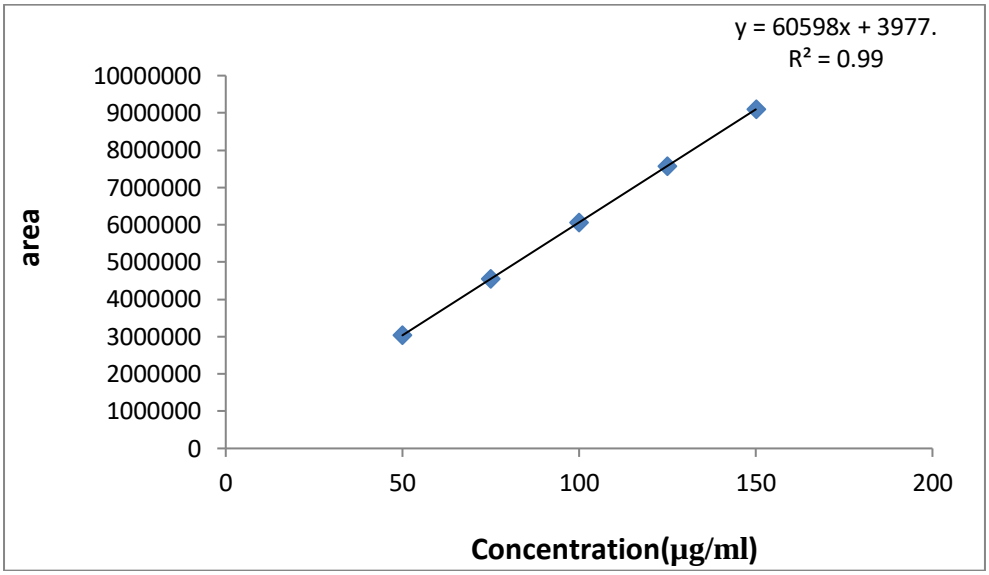

Fig. 40: Linearity Curve for Paracetamol

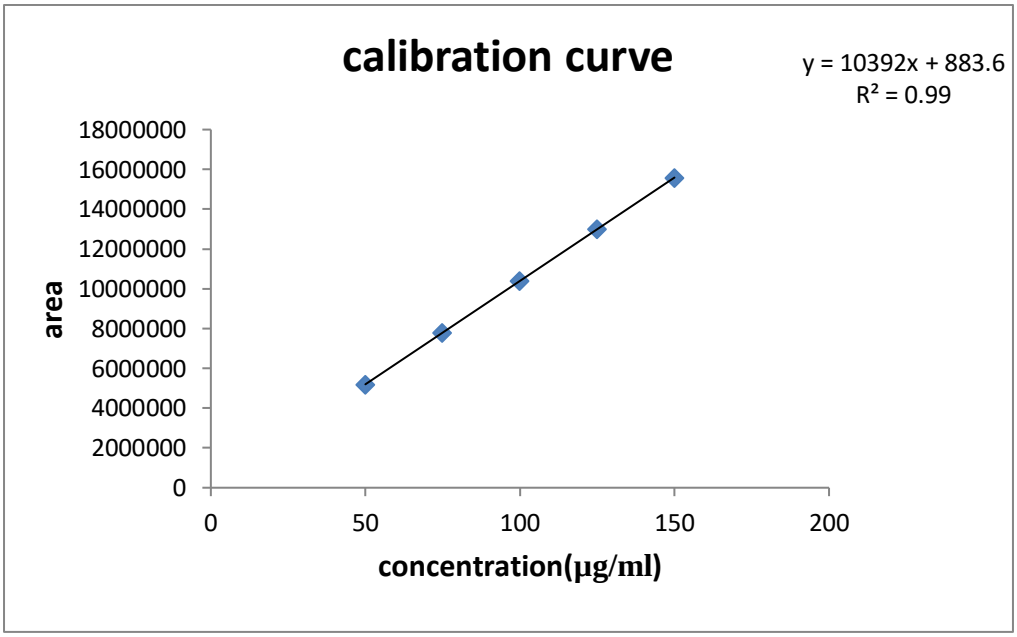

Fig. 41: Linearity Curve for Flupirtine

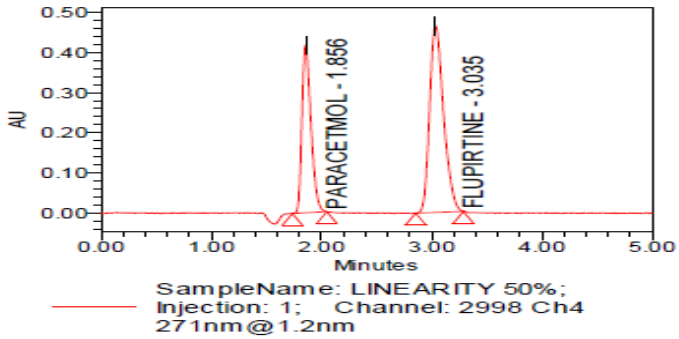

Fig. 42: chromatogram of Linearity-50%

Table18: System Suitability values—Linearity 50%

S. No.	Name	R_t	Area	USP Tailing	Plate count
1	Paracetamol	1.856	3039011	1.21	3997
2	Flupirtine	3.035	5198195	1.15	3041

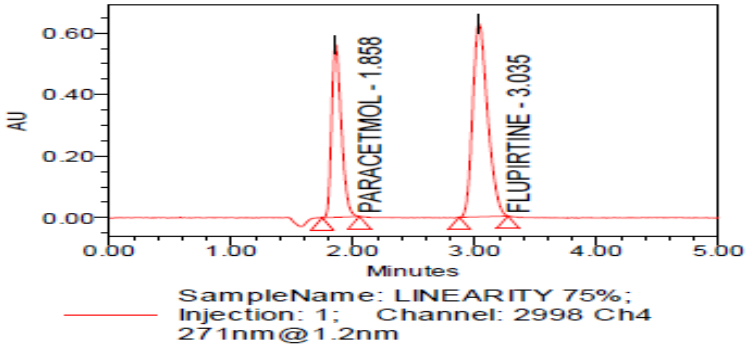

Fig. 43: chromatogram of Linearity-75%

Table 19: System Suitability values—Linearity 75%

S. No.	Name	R_t	Area	USP Tailing	Plate count
1	Paracetamol	1.858	4542901	1.21	3897
2	Flupirtine	3.035	7795994	1.15	3401

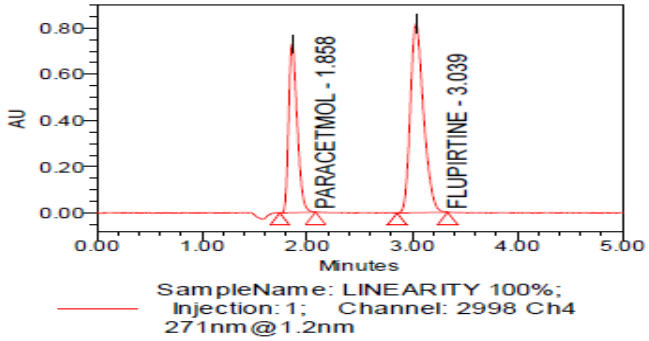

Fig. 44: chromatogram of Linearity-100%

Table 20: System Suitability values—Linearity 100%

S. No.	Name	Rt	Area	USP Tailing	USP Plate count
1	Paracetamol	1.858	6064807	1.21	3369
2	Flupirtine	3.039	10395507	1.15	3584

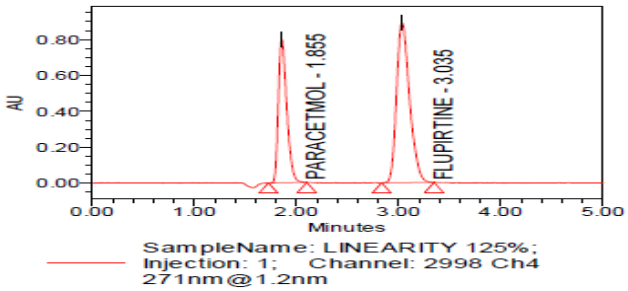

Fig. 45: chromatogram of Linearity-125%

Table21: System Suitability values—Linearity 125%

S. No.	Name	Rt	Area	USP Tailing	Plate count
1	Paracetamol	1.855	7574063	1.21	3997
2	Flupirtine	3.035	12990227	1.15	3041

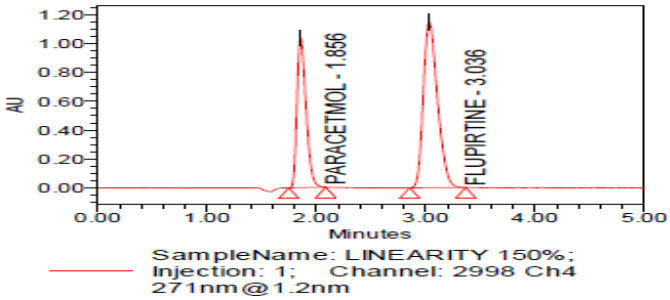

Fig. 46: Chromatogram of Linearity-150%

Table22: System Suitability Values—Linearity 150%

S. No.	Name	R_t	Area	USP Tailing	Plate count
1	Paracetamol	1.856	9098205	1.21	3600
2	Flupirtine	3.036	15588273	1.15	3498

ROBUSTNESS

Table 23: System Suitability value of Robustness--Paracetamol

S. No.	Sample name	Retention time	Area	USP Tailing	USP Plate Count
1	Flow 1	1.912	5999103	1.250	3938
2	Flow 2	1.238	5865893	1.238	3913
3	Temp 1	1.958	6009077	1.289	3830
4	Temp 2	1.662	6053043	1.256	3812
Mean			5891779		
Sts Dev			80733		
%RSD			1.3		

Table 24: System Suitability value of Robustness--Flupirtine

S. No	Sample name	Retention time	Area	USP Tailing	USP Plate Count
1	Flow 1	3.241	10312795	1.173	3123
2	Flow 2	2.994	10116990	1.138	3009
3	Temp 1	3.238	10334225	1.185	3963
4	Temp 2	2.839	10347820	1.186	3975
Mean			10277957		
Sts Dev			108276		
%RSD			1.1		

1. Flow rate

Discussion: Not much variation in the R_t, Tailing, Plate Count was found.% RSD value was less than 2 hence the result was found to be satisfactory.

2. Column temp (+0.2 and -0.2)

Discussion: Not much variation in the R_t, Tailing, Plate Count and % RSD value was less than 2, hence the result found to be satisfactory

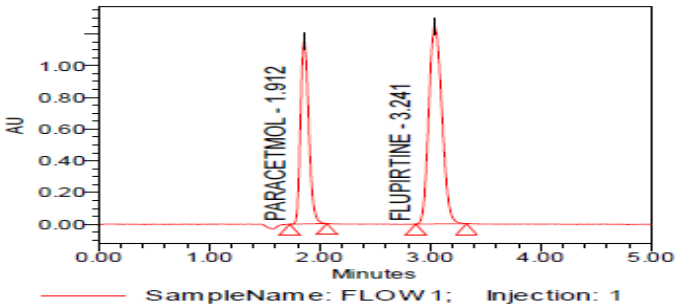

Fig. 47: chromatogram of Robustness (Decreased flow-0.6ml/min)

Table 25: System Suitability value of Robustness—Decreased flow

S. No.	Name	Rt	Area	USP Tailing	Plate count
1	Paracetamol	1.912	5999103	1.25	3938
2	Flupirtine	3.241	10312795	1.17	3123

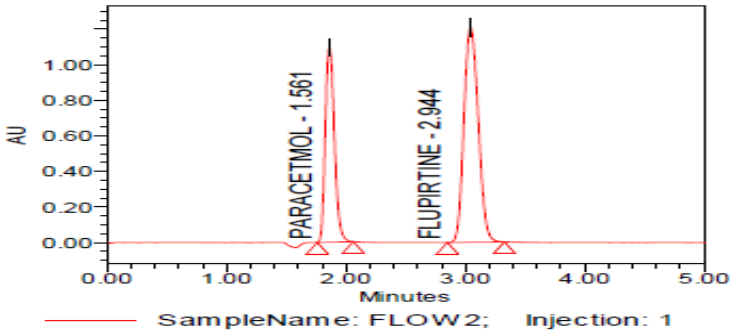

Fig. 48: chromatogram of Robustness (Increased flow1.0ml/min)

Table 26: System Suitability value of Robustness—Increased flow

S. No.	Name	Rt	Area	USP Tailing	Plate count
1	Paracetamol	1.561	5865893	1.23	3913
2	Flupirtine	2.994	10116990	1.13	3009

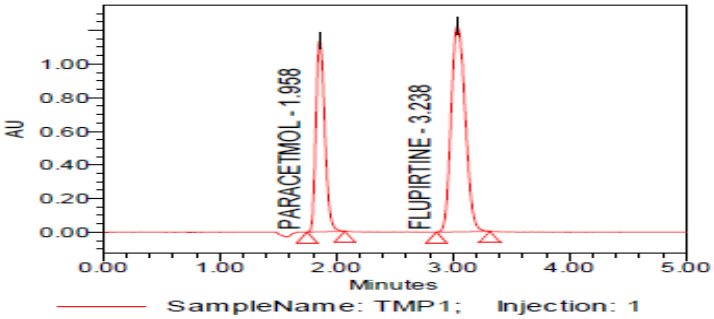

Fig. 49: chromatogram of Robustness (Low Temperature-25°C)

Table 27: System Suitability value of Robustness—low temperature

S. No.	Name	Rt	Area	USP Tailing	Plate count
1	Paracetamol	1.958	6009077	1.28	3830
2	Flupirtine	3.238	10334225	1.18	3963

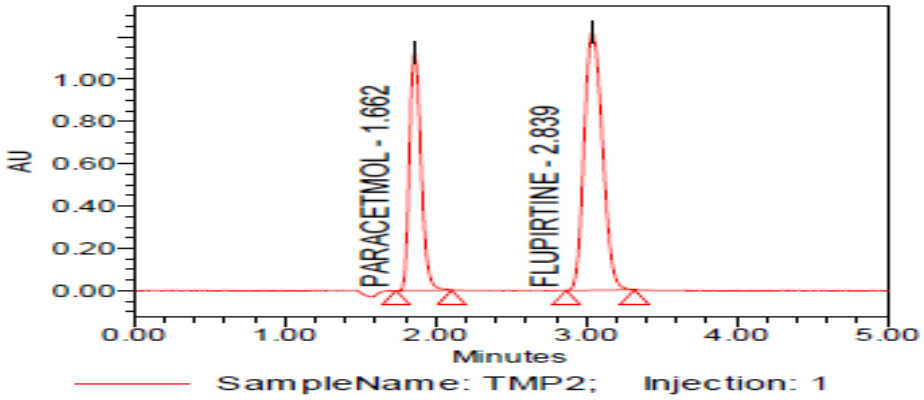

Fig. 50: chromatogram of Robustness (High Temperature-35°C)

Table 28: System Suitability value of Robustness—high temperature

S. No.	Name	Rt	Area	USP Tailing	Plate count
1	Paracetamol	1.662	6053043	1.256	3812
2	Flupirtine	2.839	10347820	1.186	3975

SUMMARY AND CONCLUSION:

SUMMARY

Parameter	Acceptance criteria	Result
Capacity Factor	Capacity Factor should be greater than 2	Capacity Factor was found to be more than 2
Resolution	Resolution between the peaks must be greater than 2	Resolution was found to be greater than 2
Tailing Factor	Tailing of the peak should be less than 2.	Tailing Factor was found to be less than 2
Column Efficiency	The Plate Count should be more than 2500	Column Efficiency was found to be more than 2500

METHOD VALIDATION

Parameter	Acceptance criteria	Result
Specificity	The chromatograms of Placebo and Blank should not show any peak at the Retention time of the analyte peak	No interference from Blank and Placebo was observed.
Linearity	Correlation coefficient value for plot between concentration vs area of peak should not be less than 0.999.	Correlation coefficient value for impurities and related substance was found to be 0.999.
Accuracy	Percentage recovery values should be between 97-103% for 3 replicate injections at 3 concentrations.	Percentage recovery value for accuracy was found to be between 97-103%
Method Precision	The %RSD should not more than 2.0	The %RSD values were found to be less than 2.0.
Limit of detection	The signal to noise ratio should be not more than 3.	Signal to noise ratio was found to be between 2-3.
Limit of quantification	The signal to noise ratio should be not more than 10.	Signal to noise ratio was found to be between 9-10.
System suitability	Resolution between the peaks should not be less than 1.5, tailing factor should not be greater than 2 and theoretical plates should not be less than 2500.	Resolution was greater than 1.5, tailing factor was less than 2 and theoretical plates were greater than 2500.
Robustness	Resolution between the peaks should not be less than 1.5, tailing factor should not be greater than 2 and theoretical plates should not be less than 5000.	Resolution was greater than 1.5; tailing factor was less than 2and theoretical plates were greater than 5000.

CONCLUSION:

The new HPLC method developed and validated for simultaneous determination of Paracetamol and Flupirtine pharmaceutical dosage forms assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form by RP-HPLC method. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

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