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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF FLUPIRITINE MALEATE AND PARACETAMOL BY USING RP-HPLC

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Abstract:
A new RP-HPLC method for the quantitative determination of Flupiritine Maleate and Paracetamol was developed and validated as per ICH guidelines. The drugs were injected into Azilent HPLC system with SPD-20A Detector and C_{18} column (250×4.6, 5 µm) maintained at ambient temperature and effluent monitored at 240 nm. The mobile phase consisted of 0.1% Orthophosphoric acid: Methanol (50:50 V/V). The flow rate was maintained at 1.0 ml/min. The calibration curve for flupiritine maleate and paracetamol was linear from 6-300.03µg/ml and 1.25 - 60.08µg/ml (r^2 for Flupiritine Maleate =1.0 and r^2 for Paracetamol = 0.99). The proposed method was adequate, sensitive, reproducible, accurate and precise for the determination of Flupiritine Maleate and Paracetamol in bulk and pharmaceutical dosage form.

Keywords: *Flupiritine Maleate and Paracetamol, Linearity, Validation.*

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INTRODUCTION:

Flupiritine maleate is Ethyl 2-amino-6-[(4-fluorobenzyl)amino]pyridine-3yl- carbamate maleate with molecular formula of C15H17FN4O2.C4H4O4 and molecular weight of 304.32g/mol . It belongs to the class 'selective neuronal potassium channel openers. It displays indirect N-methyl-D-aspartate(NDMA) receptor antagonism via activation of potassium channels .The generation of the M-current is facilitated by flupirtine via the opening of neuronal Kv7 potassium channels. The opening of these channels inhibits exaggerated neuronal action potential generation and controls neuronal excitability.It is used as an analgesic for acute and chronic pain, in moderate to severe cases. Its muscle relaxant properties make it popular for back pain and other orthopaedic uses, but it is also used for migraines, in oncology, postoperative care, and gynecology. It has been noted for its neuro-protective properties, and it is being investigated for possible use in Creutzfeldt-Jakob disease, Alzheimer's disease, and multiple sclerosis. It has also been proposed as a possible treatment for Batten disease. Paracetamol is *N*-(4-hydroxyphenyl)acetamide , with a molecular formula of C8H9NO2 and molecular weight: 151.17 g/mol. It acts primarily in the CNS, increasing the pain threshold by inhibiting both isoforms of cyclooxygenase, COX-1, COX-2, and COX-3 enzymes involved in prostaglandin (PG) synthesis. Unlike NSAIDs, it does not inhibit cyclooxygenase in peripheral tissues and, thus, has no peripheral anti-inflammatory affects. Studies also report data suggesting that acetaminophen selectively blocks a variant of the COX enzyme that is different from the known variants COX-1 and COX-2. This enzyme is now referred to as COX-3. Its exact mechanism of action is still poorly understood, but future research may provide further insight into how it works. The antipyretic properties of acetaminophen are likely due to direct effects on the heat-regulating centres of the hypothalamus resulting in peripheral vasodilation, sweating and hence heat dissipation.It is used for pain and fever.[1-5]. Various analytical methods have been reported for the estimation of Flupiritine Maleate and Paracetamol, including spectrophotometric methods and HPLC. HPLC is the most widely used technique for the estimation of Flupiritine Maleate and Paracetamol in human plasma, saliva, cerebrospinal fluid, and human blood cells, as well as for studying the drug metabolites in the urine. Different reagents are used for assay of Flupiritine Maleate and Paracetamol which are quite expensive and need complex and sophisticated instrumentation. The present research work describes a HPLC and UV spectrophotometric method for estimation of Flupiritine Maleate and Paracetamol in API [6-10]. The present method aims at developing a simple, accurate and precise RP-HPLC method for the estimation of Flupiritine Maleate and Paracetamol in bulk and Pharmaceutical dosage forms.

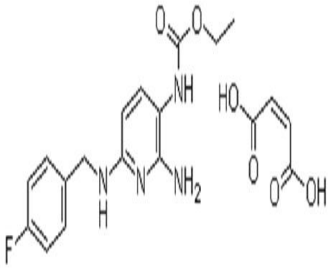

Fig 1: Chemical structure of Flupiritine Maleate

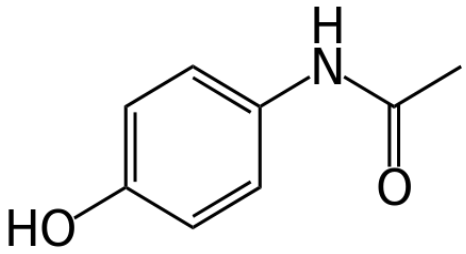

Fig 2: Chemical structure of Paracetamol

MATERIALS AND METHODS:

Chemicals and solvents:

The API of Flupirtine maleate and Paracetamol was obtained from A R&D, Gloresine Life Sciences, Unit II, Pune, India. HPLC grade water (prepared by using 0.45 Millipore Milli –Q) was procured from Standard Reagents, Hyderabad. HPLC grade Methanol, Potassium dihydrogen orthophosphate, 0.1% orthophosphoric acid was bought from Merck, Mumbai.

Instrumentation:

An Azilent HPLC system with SPD-20A Detector and C_{18} column (250×4.6, 5 µm) maintained at ambient temperature and effluent monitored at 240 nm. for finding out the λmax values of the drugs was used throughout this study. The mobile phase consisted of 0.1% Orthophosphoric acid: Methanol (50:50 V/V). The flow rate was maintained at 1.0 ml/min. The analytes were monitored by UV detection at 240 nm using an isocratic mode with Orthophosphoric acid: Methanol in the ratio 50:50 as mobile phase. The flow rate was set at 1.0 ml/min and effluent was monitored at 240 nm. The temperature and run time were maintained at 25°C and 8 min. respectively. Solubility of the compounds was enhanced by sonication on an ultrasonicator .

Selection of mobile phase:

The objective of this experiment was to optimize the method for estimation of Flupirtine maleate and Paracetamol based on the literature survey. Various mobile phases were tested to select the best possible system. The various mobile phases used included water : Acetonitrile (50:50), water: methanol (70:30) , Acetonitrile : water (40:60). Better peak resolution and adequate retention time were obtained with the ratio of 0.1% Orthophosphoric acid: Methanol (50:50).100% methanol was used as diluent.

Preparation of Mobile Phase

The mobile phase was prepared by mixing 500 ml of Methanol and 500 ml of Orthophosphoric acid in a 1000 ml clean and dry flask. The mobile phase was then degassed using Ultra-Sonicator to remove dissolved gases and the resultant mobile phase was filtered through a 0.45 µm membrane filter under vacuum.

Preparation of Standard solution:

100mg of Flupirtine maleate and 10 mg of Paracetamol were weighed and dissolved in 10 ml of diluents into 10 ml volumetric flask and made up to the volume with diluent making 9980µg/ml of Flupirtine maleate and 997µg/ml of Paracetamol solution.

Preparation of Sample solution:

Sample solution was prepared by taking 20 tablets and weighing a powder equivalent 100mg of Flupirtine maleate and 10mg of Paracetamol and dissolve in 100 ml of diluents into 100 ml volumetric flask and made up to the volume with diluent making of 9980µg/ml and 997µg/ml of Paracetamol solution.

Prior to validation studies blank solution was injected and chromatogram was noted. System suitability studies were performed using the standard solution of Flupirtine maleate and Paracetamol .Optimized conditions maintained where the drugs were eluted with good retention time and peak area which was shown in the fig 4.

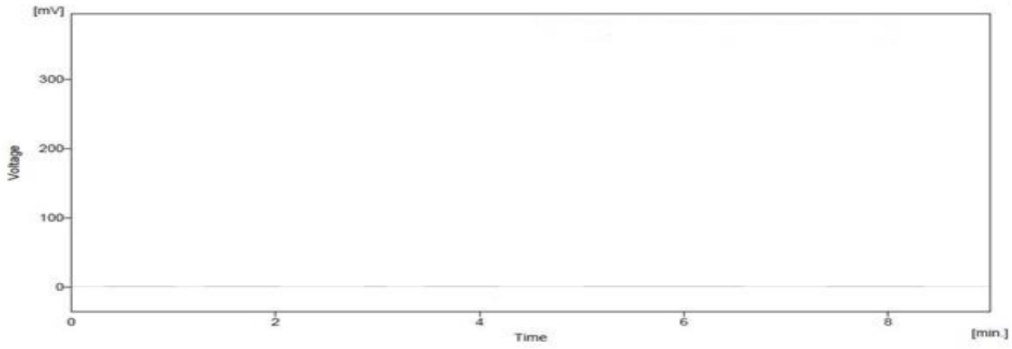

Fig 3: Blank chromatogram

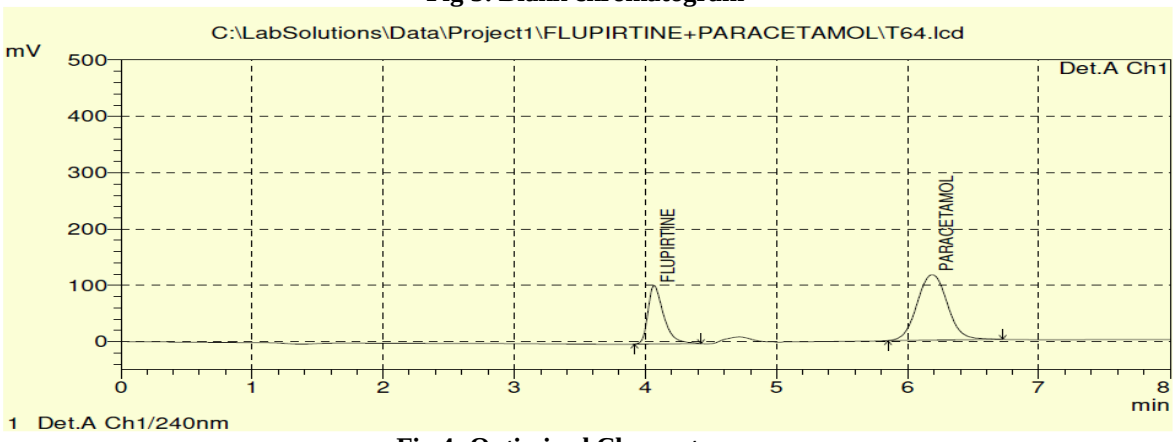

Fig 4: Optimized Chromatogram

System suitability:

The standard solution of 20mcg/ml was used as system suitability solution which was injected for about 5 times .The peak area ,tailing factor and number of theoretical plates were noted and results are tabulated below .

Table 1: System suitability data of Flupirtine maleate

Injection	RT	Peak area	Theortical plates	Tailing factor
1	4.04	784259	14713	1.45
2	4.03	785550	14800	1.45
3	4.11	782478	15406	1.42
4	4.11	782437	15564	1.42
5	4.08	780777	15596	1.43
Mean	4.074	785700.2	15215.8	1.43
SD	0.0378	7288.06		
%RSD	0.93	0.93		

Table 2: System suitability data of Paracetamol

Injection	RT	Peak area	Theortical plates	Tailing factor
1	6.1	3579431	9223	1.04
2	6.08	3589474	9314	1.04
3	6.22	3496823	9825	1.05
4	6.24	3565602	9626	1.06
5	6.19	3658820	9094`	1.04
Mean	6.16	3578130	9416.4	1.046
SD	0.0720	57902.04		
%RSD	1.17	1.62		

Linearity:

The linearity of the method was established by determining the absorbance of different concentrations of Flupirtine maleate and Paracetamol over a range of 6.25-300.03µg/ml and 1.25-60.08 µg/ml respectively.

Table 3: Linearity data of Flupirtine maleate and Paracetamol

Linearity	Conc. of Flupirtine maleate (µg/ml)	Conc. of Paracetamol (µg/ml)	Peak area of Flupirtine maleate	Peak Area of Paracetamol
CC-01	6.25	1.25	41808	334639
CC-02	25.0	5.01	143331	953346
CC-03	50.01	10.01	285605	1725245
CC-04	100.01	20.03	568558	3424782
CC-05	200.02	40.05	1126815	6632197
CC-06	300.03	60.08	1676917	9710250

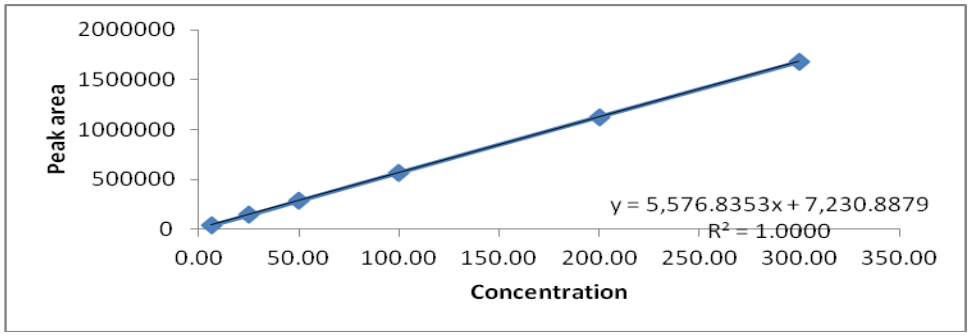

Fig 5: Calibration curve of Flupirtine maleate

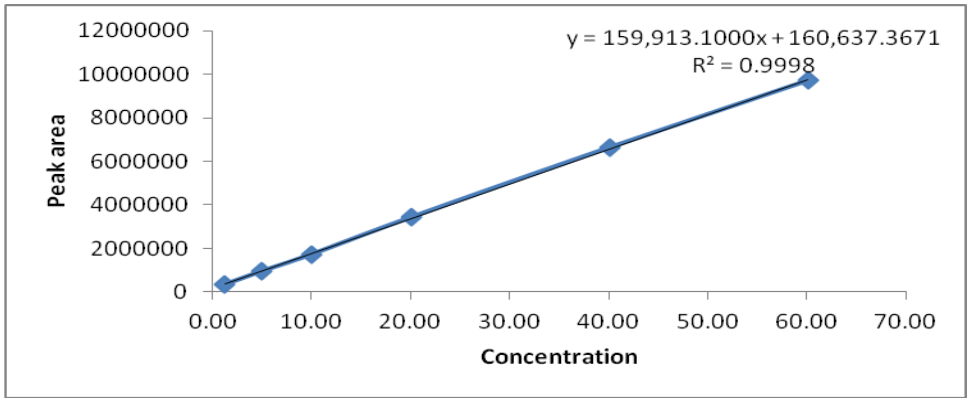

Fig 6: Calibration curve of Paracetamol

Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by analyzing the samples were carried out by analyzing the measured concentration and the added concentration of the drug. Each sample was injected thrice .The percent recoveries of the drugs were estimated.

Table 4: Accuracy data of Flupirtine maleate

S.NO	Spike level	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Mean %recovery
1	50%	124.75	126.45	101.36	100.97
2	50%	124.75	127.14	101.92	
3	50%	124.75	124.30	99.64	
1	100%	249.50	249.74	100.10	100.57
2	100%	249.50	252.11	101.05	
3	100%	249.50	250.90	100.56	
1	150%	374.25	376.90	100.71	100.93
2	150%	374.25	379.95	101.52	
3	150%	374.25	376.38	100.57	

Table 5: Accuracy data of Paracetamol

S.NO	Spike level	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Mean %recovery
1	50%	24.925	24.94	100.07	100.23
2	50%	24.925	24.92	99.98	
3	50%	24.925	25.08	100.64	
1	100%	49.85	49.63	99.55	100.32
2	100%	49.85	50.23	100.75	
3	100%	49.85	50.18	100.67	
1	150%	74.77	73.44	98.22	98.80
2	150%	74.77	74.51	99.64	
3	150%	74.77	73.67	98.52	

Precision:

It is one of the important factors which determine the reliability of an analytical method. The precision of the developed method was tested and was found to be suitable. Both system and method precision were performed and are given in table 6,7.

Table 6: Data for method precision

Parameter	Flupirtine maleate	Paracetamol
Average area	1406599	8228888.33
SD	1.18	0.34
%RSD	0.47	0.67
Retention time	4.17	6.3

Table 7: Data for system precision

Parameter	Flupiritine maleate	Paracetamol
Average area	782061.8	3563419.7
SD	3030.34	63091.05
%RSD	0.39	1.77
Retention time	3.87	5.75

Robustness: The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like volume of injection, wavelength which may differ but the responses were still within the limits of the assay.

Table 8: Data for Robustness

Name of the drug	Flow variation		pH variation	
	0.9ml	1.1ml	Acid	Alkaline
Flupirtine maleate	0.64	1.71	1.17	1.31
Paracetamol	0.56	0.94	1.46	1.60

Assay:
Assay of different formulations available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system and recovery studies were carried out. The average % Assay for Flupirtine maleate and Paracetamol in the different formulations was found to be 100.47% & 98.78 %.

DISCUSSION:

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and economical RP-HPLC method. It was successfully applied for the determination of Flupirtine maleate and Paracetamol in pharmaceutical dosage forms without the interferences of other constituents in the formulations. Different mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was done based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The drugs were injected into Azilent HPLC system with SPD-20A Detector with C_{18} column (250×4.6, 5 µm) maintained at ambient temperature and effluent monitored at 240 nm. The mobile phase consisted of 0.1% Orthophosphoric acid: Methanol (50:50 V/V). The flow rate with 1.0 ml/min flow rate was quite robust.

The optimum wavelength for detection was 240 nm at which better detector response for drug was obtained. The average retention time for Flupiritine maleate and Paracetamol was found to be 4.06 mins and 6.23 mins . The % RSD values in the system suitability were found to be less than 2%. The low values of % RSD indicate the method is precise and accurate.

Sample to sample precision and accuracy were evaluated using, three samples of five and three different concentrations respectively, which were prepared and analyzed on same day. These results show the accuracy and reproducibility of the assay. The proposed method was validated in accordance with ICH parameters and the results of all methods were very close to each other as well as to the label value of commercial pharmaceutical formulation. There was no significant difference in the results achieved by the proposed method.

CONCLUSION:

The proposed method for the assay of the Flupiritine maleate and Paracetamol in the commercially available tablet formulation was found to be precise, simple, accurate, economical, and rapid. It can be easily adopted for routine quality control for monitoring the assay in the bulk drug samples, in-process samples, and for the finished tablet formulation.

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