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ANALYTICAL METHOD DEVELOPMENT AND SIMULTANEOUS ESTIMATION OF FLUPIRTINE MALEATE AND PARACETAMOL BY USING RP-HPLC

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ABSTRACT

A simple, selective, rapid, precise and economical reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the simultaneous estimation of Paracetamol (PARA) and Flupiritine Maleate (FLU) from pharmaceutical formulation. The method is carried out on Agilent C18 (25 cm x 4.6 mm i.d., 5 µ) column with a mobile phase consisting of Methanol Water (0.2% TEA, adjusted to pH 3 using orthophosphoric acid) in the ratio of 50:50 v/v. The retention time of Paracetamol and Flupiritine Maleate is 6.3 min and 4.2 min respectively with the flow rate of 1mL/ min with PDA detection at 240 nm. The linear regression analysis data for the linearity plot showed good linear relationship with correlation coefficient value for Paracetamol and Flupiritine Maleate were R2=0.9998 and R2=1.0000 in the concentration range of 1.25-60.08 µg. mL-1 , 6.25-300.03 µg. mL-1 respectively. The relative standard deviation for intra-day precision has been found to be lower than 2.0 %. The method is validated according to the ICH guidelines. The developed method is validated in terms of specificity, selectivity, accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method can be used for simultaneous estimation of these drugs in marketed dosage forms.

KEY WORDS: RP-HPLC, Paracetamol and Flupiritine Maleate

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INTRODUCTION

A drug is a substance, which has a physiological effect when ingested or otherwise introduced into the body. It is a natural or synthetic substance, which when taken into a living body, affects its functioning Or structure and is used in the diagnosis, mitigation, treatment or prevention of a disease or relief of discomfort. Pharmaceutical analysis is a branch of chemistry, which involves the series of processes for the identification, determination, quantitation, and purification of compounds. This is mainly used for the separation of the components from their mixtures and for the determination of the structure of the compounds. Chromatographyis the collective term for the set of laboratory techniques used for the separation of mixtures. It is a physical method of separation that distributes components to separate between two

phases. The mixture of components is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. Components of the mixture are separated by the relative attraction of each component to stationary phase while mobile phase passes through the stationary phase. A species, which is more strongly attracted to the mobile phase than to the stationary phase, is swept along with the mobile phase more rapidly than a species, which is more strongly attracted to the stationary phase.At the end of the process, separated components emerge in order of increasing interaction with the stationary phase. The least retarded component emerges first; the most strongly retained component elutes last. Chromatographic separation in HPLC is the result of specific interactions between sample molecules with both the stationary and mobile phases. When a mixture of components is introduced into the HPLC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent, travels slower and the component with less affinity to stationary phase travels faster. Since no two components have same affinity towards the stationary phase, the components are separated from a mixture. HPLC is able to separate macromolecules and ionic species, labile natural products, polymeric materials, and a wide variety of other high-molecular-weight polyfunctional groups. HPLC offers a greater variety of stationary phases, which allow a greater variety of these selective interactions and more possibilities for separation. Sample recovery is easy in HPLC, as the separated fractions are easily collected by placing an open vessel at the end of the column (1-2).

Flupirtine (fig-1) is an aminopyridine that functions as a centrally acting non-opioid analgesic. It first became available in Europe in 1984, and is sold mainly under the names Katadolon, Trancolong, Awegal, Efiret, Trancopal Dolo, and Metanor. Like nefopam, it is unique among analgesics in that it is a non-opioid, non-NSAID, non-steroidal centrally acting analgesic. In 2010 the chemically related drug (the difference being that the pyridine group in flupirtine is replaced with a phenyl group) retigabine (INN; ezogabine [USAN]) was approved by the FDA as an anticonvulsant for the treatment of refractory partial-onset seizures in treatment-experienced patients. Retigabine also works by opening the neuronal KCNQ/Kv7 potassium channel, just like flupirtine. Flupirtine 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 orthopedic uses, but it is also used for migraines, in oncology, postoperative care, and gynecology. Flupirtine has been noted for its neuroprotective 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. Flupirtine underwent a clinical trial as a treatment for multiple sclerosis and fibromyalgia. Flupirtine showed promise for fibromyalgia due to its different action than the three approved by U.S. FDA drugs: Lyrica (pregabalin), Savella (milnacipran), and Cymbalta (duloxetine).

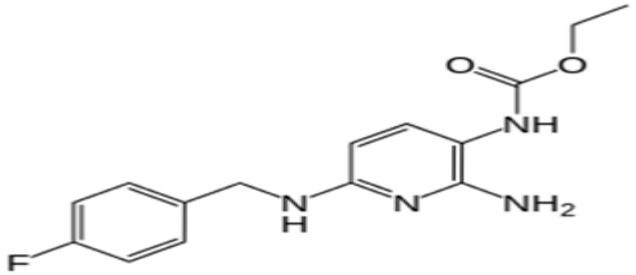

Fig-1 Structure of Flupirtine Maleate

Paracetamol (also called acetaminophen) is a widely used analgesic and antipyretic agent. Paracetamol is well absorbed in the gastrointestinal tract. Oral bioavailability is dose dependant: with larger doses, the hepatic first pass effect is reduced due to overwhelming of the liver enzymatic capacity; and therefore, bioavailability is increased. Rectal administration of paracetamol is also feasible. In this case, bioavailability is inconsistent and in overall reduced, due to incomplete dissolution of the suppository in the rectum. The absorption rate through this route of administration is elongated. Paracetamol is distributed throughout the body fluids in a homogeneous way. The analgesic activity is attributable to the small fraction that penetrates into the brain. Paracetamol given at therapeutic doses

binds to plasma proteins at less than 20%. In case of intoxication, this proportion may increase to up to 50%. Paracetamol is essentially metabolized in the liver by conjugation with glucuronic acid (55%) and sulfuric acid (35%). Hepatotoxic metabolites are produced in small amounts by the cytochrome P450 (isoenzyme CYP2E1). In the therapeutic plasma concentration range, this metabolite is detoxified by conjugation with glutathione. In case of intoxication the amount of this toxic metabolite increases and outweighs the amount of available glutathion, which can lead to hepatic failure and renal tubular necrosis. Metabolites are excreted through the kidneys in the urine. Only 2-5% of the dose is excreted in an unchanged form in the urine. As a consequence of its short elimination half-life (1-3h), 24 hours after the ingestion of a single dose of paracetamol, 98% of the dose is eliminated. aracetamol belongs to a group of medicines known as analgesics, or painkillers. It is used to relieve mild-to-moderate pain. It is also useful for lowering a raised temperature (fever), such as after childhood immunisation. Paracetamol is a common painkiller and is available to buy from many retail outlets as tablets/capsules and as liquid medicine. Many brands of 'over-the-counter' combination painkillers contain paracetamol, as do many cold and flu remedies. It is important that you check the label on any preparation that you buy to make sure that you are not taking more than one preparation containing paracetamol (3-4).

This analytical method validation of Simultaneous Estimation of Paracetamol and Flupirtine maleate assay in its dosage forms has been carried out at GLORESINE LIFE SCIENCES, Pune. The same method of analysis has been adopted or the finished product method of analysis. Few chromatographic methods for the determination of Paracetamol and Flupirtine maleate have been described. The following parameters have been considered for the validation of proposed assay method for Paracetamol and Flupirtine maleate.

MATERIALS AND METHODS (5-8)

Preparation of Buffer pH 5.0-Dissolve 0.5ml of orthophosphoric acid in 500ml of water. Filter through 0.45 µ or finer porosity membrane filter.

Preparation of mobile phase- Prepare a degassed mixture of buffer pH 5.0 and Methanol in the ratio of 50:50 v/v

Preparation of Diluent:- 100% Methanol is used as diluent.

Reference solution- Weigh accurately 100mg of Flupirtine maleate and 10 mg of Paracetamol and dissolve in 10 ml of diluents into 10 ml volumetric flask and make up to the volume with diluent making 9980µg/ml of Flupirtine maleate and 997µg/mlof Paracetamol solution.

Sample preparation-Weigh accurately 100mg of Flupirtine maleate and 10mg of Paracetamol and dissolve in 100 ml of diluents into 100 ml volumetric flask and make up to the volume with diluent making of 9980µg/ml and 997µg/ml of Paracetamol solution.

RESULTS AND DISCUSSION

Optimized Chromatographic Conditions

Optimized Chromatographic Conditions is shown in table-1 and fig-2.

Table-1 Optimized Chromatographic Conditions

Parameters	Description
Stationary phase(Column)	AZILENT, C18, 250×4.6 mm, 5µ.
Mobile phase A	Orthophosphoric acid
Mobile phase B	Methanol
Mobile phase A:B	50:50
Elution mode	Isocratic

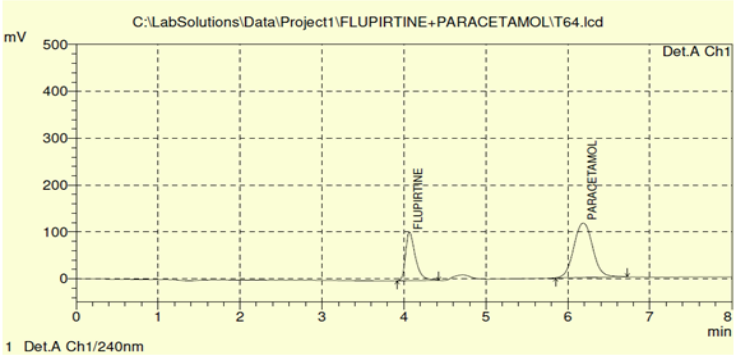

Fig-2 optimized Chromatogram

The % Assay for Flupirtine maleate and Paracetamol was found to be 101.385% &100.59 %. The acceptance limits should be between 98 -102% (Table-2 and 3).

Table-2 Data of standard

Drug name	Flupirtine maleate	Paracetamol
Average RT	4.18	6.35
AveragePeak area	603249	2956910

Table- 3 Data of sample

Drug name	Flupirtine maleate	Paracetamol
Average RT	4.30	6.47
AveragePeak area	606502	297340

After satisfactory development of method, it was subjected to method validation as per ICH guideline. The method was validated to demonstrate that, it is suitable for its intended purpose by the standard procedure to evaluate adequate validation characteristics in terms of parameters like linearity, precision, accuracy, robustness, ruggedness, specificity, LOQ and LOD.

For accuracy determinations prepare separately 50%, 100%, 150% (i.e. 3 different concentrations) of the Analyte and inject separately 3 replicate volumes of 10 µl for each solution and calculate the % recovery by comparison with standard preparation (Table-4 and 5).

Table-4 Accuracy for Flupirtine maleate

S.NO	Spike level	amount added 1(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 for 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	

The % RSD values of peak area for five replicate injections of Flupirtine maleate and Paracetamol were found to be 0.47 and 0.67 respectively which are well within the acceptance criteria limit of NMT 2%. The % RSD values of peak area for five replicate injections of Flupirtine maleate and Paracetamol were found to be 0.39 and 1.77 respectively which are well within the acceptance criteria limit of NMT 2%.

For Linearity calculations, prepare a series of solutions of different concentrations in a given range using a stock solution. Linearity results for Flupirtine maleate and Paracetamol is given in table-6

Table–6 Linearity results for Flupirtine maleate and Paracetamol

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

LOD values of Flupirtine maleate and Paracetamol are 0.02390 and 0.01520 respectively. LOQ values of Flupirtine maleate and Paracetamol are 0.072 and 0.046 respectively. From the intermediate precision data, the % RSD for Flupirtine maleate andParacetamol were found to be 0.38 and 0.07 respectively which is well within theacceptance criteria indicating that the method is rugged. As both sample and standard got peaks at nearly same Retention times and no other peaks are eluted and interrupted.

CONCLUSION

A simple and reproducible HPLC procedure was developed and validated as per ICH guidelines for the estimation of Paracetamol and Flupirtine maleate. Quantitative estimation of Paracetamol and Flupirtine maleate.was estimated by RP- HPLC using MeOH: 0.1% Ortho phosphoric acid (50:50 %v/v) as a mobile phase and Azilent column (150mm×4.6mm, 5µ) as a stationary phase and the peaks were observed at 240nm which was selected as a wavelength for quantitative estimation. After development of the method it was validated for specificity, system suitability, accuracy, linearity, precision, ruggedness and robustness. The value of theoretical plates, tailing factor, retention time and peak area was found to be within limits, hence it is concluded that the system is suitable to perform assay. The method was found to be specific because it did not show any interference with placebo and blank. The linearity studies were performed for the standard and found to be linear. From the linearity studies, the specified range was found to be 1.25µg/mL to 60µg/mL of the target concentration of Paracetamoland 6.5µg/mL to 300µg/m of Flupirtine maleate. The precision was checked and found to be within limits, hence the method is precise. The accuracy has been determined from LQC to HQC and the prescribed limits for recovery are 90%-102%. From accuracy studies, % recovery was calculated and found to be within limits. The ruggedness of the method was checked on different analysts and by different columns and standard was able to give same results which indicate that the method is rugged. The robustness of the method was checked by changing flow rate and mobile phase compositions, and standard was able to give system suitability parameters within limit, which indicates that the method is robust. Therefore it was concluded that the proposed method can be used for routine analysis of Paracetamol and Flupirtine maleate tablet dosage forms.

REFERENCES

1. *Practical HPLC Method Development* by Lloyd R. Snyder second edition
2. *Indian Pharmacopoeia*, Indian Pharmacopoeia commission, Ghaizabad, 2007; page no.718-19, 722- 23, 725-26.
3. *British pharmacopoeia*, British Pharmacopoeia commission, London SW8 5NQ,2008; Pg no. 688-89
4. Shabir GA. Validation of High-Performance Liquid Chromatographyethod spharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the USPharmacopeia and the International

conference on Harmonization. *J Chromatograph. A*. 2003; 987 (1-2) : 57-66.

5. ICH, "*Validation of Analytical Procedures*: Text and Methodology," InternationalConference on Harmonization Guidance Documents, Q2 (R1), 2005.
6. Singh S, Bakshi M. Development of validated stability-indicating assay methodscritical review. *J. Pharm. Biomed. Anal*. 2002; 28: 1011-40.
7. Acharya MM. *Stability Testing and Studies,* Eastern Pharmmacist 1999; 5: 31-35.
8. Dhoka M, Gawande V, Joshi P. High-performance liquid chromatographic method for determination of Flupirtine maleate and paracetamolin oral dosage forms. *Inter J of Pharm Tech Res*. 2010; 2:129-133.