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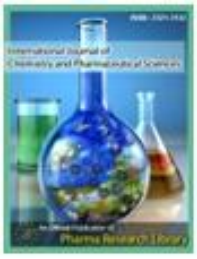

R E S E A E C H A R T I C L E

RP-HPLC Method Development and Validation for Simultaneous Estimation of Paracetmol and Flupirtine Maleate in Tablet Doses Form

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A B S T R A C T

A new method was established for simultaneous estimation of Paracetamol and Flupirtine maleate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Paracetamol and Flupirtine maleate by using Symmetry C18 Agilent (250*4.6mm,5µ), flow rate was 0.8ml/min, mobile phase ratio was OPA buffer pH3.0: methanol (65:35). Detection wave length was 280nm. The instrument used was WATERS HPLC Auto Sampler, Separation module Aliance 2695, PDA Detector, Empower-software version-2.The retention times of Paracetamol and Flupirtine maleate were found to be 4.7 mins and 3.5 mins. The assay of Paracetamol and Flupirtine maleate was performed with tablets and the % assay was found to be 100.18 and 100.00 which shows that the method is useful for routine analysis. The linearity of Paracetamol and Flupirtine maleate was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.1 and 0.1 for Paracetamol and Flupirtine maleate which shows that the method is precise. The acceptance criteria of precision is RSD should be not more than 2.0% and the precision data for Paracetamol and Flupirtine maleate was found to be 0.62 and 0.19 . The accuracy limit is the percentage recovery should be in the range of 97 - 103.0%. The total recovery was found to be 101.00% and 100.00% for Paracetamol and Flupirtine maleate. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQ for Paracetamol was found to be 4.724 and 3.356 and LOD and LOQ for Flupirtine maleate was found to be 4.724 and 3.555 The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

Keywords: Paracetamol, Flupirtine maleate, HPLC

A R T I C L E I N F O

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1. Introduction

Mechanism of action of paracetamol:

Acetaminophen is believed to act primarily in the Central nervous system, increasing the pain threshold by inhibiting both isomers of cyclooxygenase, COX-1, COX-2, and COX-3 enzymes that are involved in prostaglandin (PG) synthesis [1]. Contrary to NSAIDs, acetaminophen doesn't inhibit cyclooxygenase in peripheral tissues and hence has no peripheral anti-inflammatory affects. Meanwhile aspirin acts as an irreversible inhibitor of COX that directly blocks the enzyme's active site, studies have revealed that acetaminophen indirectly blocks Cyclooxygenase, this blockade is ineffective in presence of peroxides [2]. This could brief why acetaminophen is effective in central nervous system and also in endothelial cells but not in platelets and immune cells that have high levels of peroxides. Studies also found 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 not properly understood, but further research may provide scientific insight on how it works[3,4]. The antipyretic properties of acetaminophen are majorly due to direct effect on heat-regulating centres of hypothalamus which results in peripheral vasodilation, sweating and so heat dissipation [5,6].

Metabolism of paracetamol:

Acetaminophen primarily undergoes glucuronidation (45-55% of the dose) in which this process is facilitated by UGT1A1, UGT1A6, UGT1A9, UGT2B15 in the liver or UGT1A10 in the gut. 30-35% of the dose undergoes sulfation [7]. This biotransformation is facilitated by SULT1A1, SULT1A3, SULT1A4, SULT1E1 and SULT2 A1 [8]. A small percentage of acetaminophen is oxidized by CYP2E1 to form N-acetyl-p-benzo-quinone imine (NAPQI), a toxic metabolite which is then conjugated to glutathione and excreted renaly[9]. Studies suggest that CYP3A4 and CYP2E1 are the primary cytochrome P450 isozymes responsible for the generation of toxic metabolites [10,11]. Accumulation of NAPQI may occur if primary metabolic pathways are saturate

Pharmacodynamics of paracetamol:

Acetaminophen (USAN) or Paracetamol (INN) is one of the widely used analgesic and antipyretic drug used in treating fever, headaches and other minor aches and pains[12]. It is also a major ingredient in numerous cold and flu medications and many prescription analgesics [13]. It is extremely safe in standard doses, but because of its wide availability, deliberate or accidental overdoses are not uncommon. Acetaminophen is used singly or in combination with dextromethorphan, pseudoephedrine, diphenhydramine, chlorpheniramine, codeine, doxylamine, hydrocodone, or oxycodone [14].

Mechanism of action of Flupiritine maleate:

Flupirtine upregulates Bcl-2, increases glutathione levels, activates an inwardly rectifying potassium channel, and delays loss of intermitochondrial membrane calcium retention capacity. Flupirtine acts like NMDA receptor antagonists, but does not bind to receptor [15,16]. One study concluded that the discriminative effects of flupirtine are neither of opioid nor of alpha-1 adrenergic type, but are basically mediated through alpha-2 adrenergic mechanisms.

Metabolism of Flupiritine maleate: Hepatic to 2-amino-3-acetylamino-6-(para-fluorobenzylamino) pyridine (which has 20-30% the analgesic potential of its parent compound) and Para-fluorohippuric acid [17].

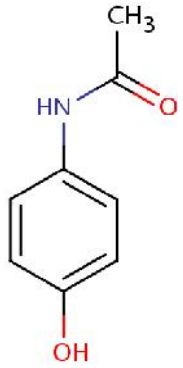

Figure 1: Paracetamol

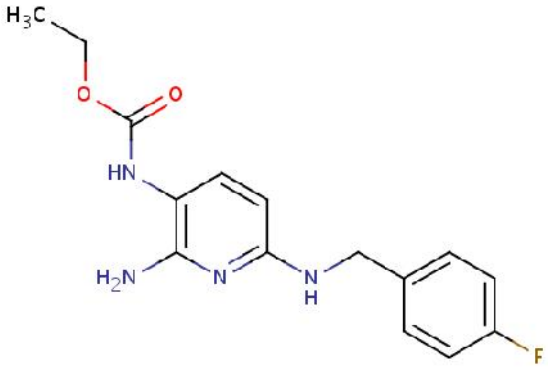

Figure 2: Flupiritine maleate

2. Materials and Methods

Apparatus

The instrument used for the study was WATERS HPLC, Model: Aliance 2695, Photo diode array detector (PDA), with an automated sample injector [19,20]. The output signal was monitored and integrated using Empower 2 software [21].

Preparation of mobile phase:

Transferred 1000ml of HPLC water into 1000ml of beaker and adjust pH 3.0 with ortho phosphoric acid(OPA)[22,23]. Transferred the above solution 650ml and 350ml of buffer and methanol is used as mobile phase. They are mixed and sonicated for 20min [24].

Preparation of Standard Solution:

Accurately weighed quantity of 100mg paracetmol and 100mg flupirtine was transferred into 100 ml of volumetric

flask[25,26]. 10ml of mobile phase was added and sonicated for 10min. shook for 5min and made up the volume with mobile phase [27]. Transferred 5 ml of above solution into 25ml volumetric flask diluted the volume with the mobile phase up to the mark [28].

Preparation of standard stock solution for flupirtine maleate: Accurately weighed quantity of 100mg flupirtine maleate was transferred into 100 ml of volumetric flask [29]. 10ml of mobile phase and sonicated for 10min. shook for 5min and made up the volume with mobile phase [31]. Transferred 5 ml of above solution into 25ml volumetric flask diluted the volume with the mobile phase up to the mark [33,34].

Optimization Chromatographic trials for Simultaneous Estimation of Paracetamol and Flupiritine maleate by RP- HPLC.

Optimization chromatographic conditions

Mobile phase : OPA buffer pH3.0: methanol (65:35)
Auto sample temperature: 25°c
Injection volume : 10µL
Column : C18 AgIlent (250*4.6mm,5µ)
Detector wavelength: 280nm
Flow rate : 0.8ml/min

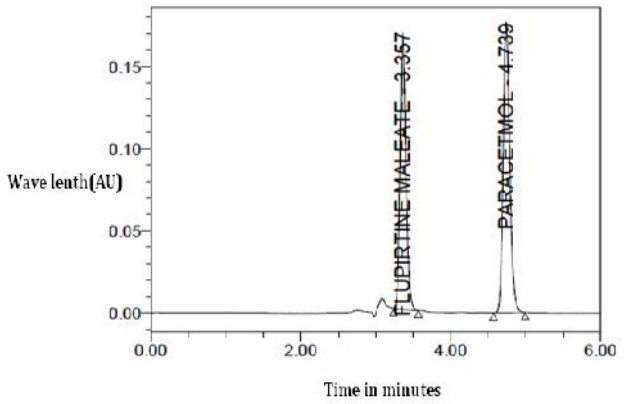

Figure 3: Optimization Chromatogram

Observation: Peeks are well separated all the parameters are within the limits.

Identification of wavelength by UV method:

The wavelength for detection was selected by scanning standard paracetmol and flupirtine maleate over a wide range of wavelength 200nm to 400nm. UV spectra at 280nm was selected as the detection wavelength for liquid different mobile phase were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained on a agilent C18 column with the mobile phase OPA: Methanol (65:35).

HPLC-Method:

Method development process:

In the process of HPLC method development the optimization was done by changing the mobile phase, mobile phase ratio, column and flow rate. Method development focuses on identifying buffer type, strength and PH of organic solvent. Implementing small changes to optimize selectivity enhanced resolution. Different trials were performed and finally the optimized method was found to be suitable. The mobile composition of OPA : Methanol (65:35), agilent column of C18 ($250_X4.6$ ID) $5\mu m$ and flow rate 0.8 ml/min, run time 40 min efficient and reproducible method was developed for determination of paracetmol and flupirtine maleate in tablet doses form and optimized chromatogram is obtained with good resolution.

3. Results and Discussions

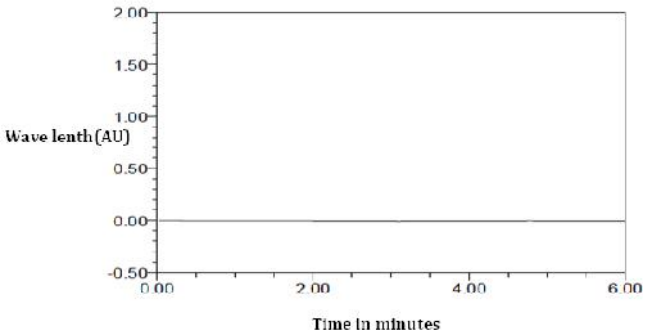

Figure 4: Chromatogram of the blank

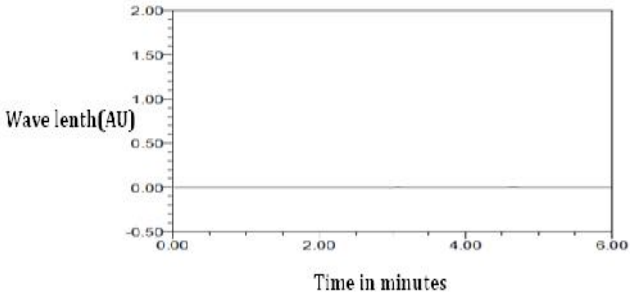

Figure 5: Chromatogram of the Placebo

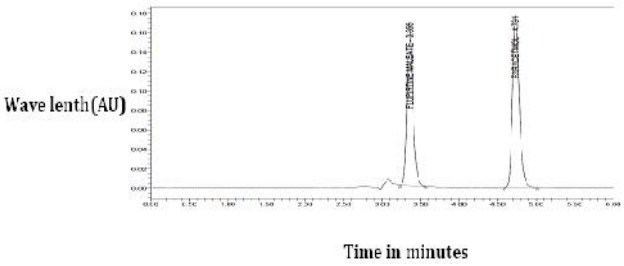

Figure 6: Chromatogram for specificity of standard

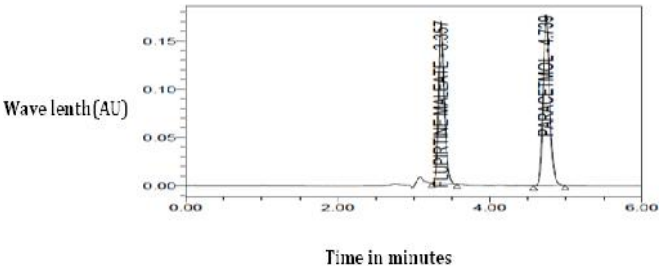

Figure 7: Chromatogram for specificity of sample

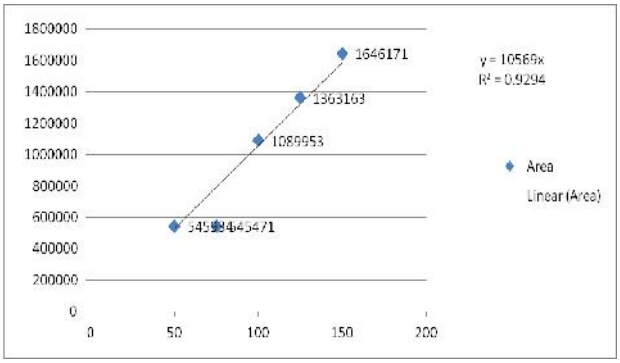

Figure 8: Linearity plot of Paracetmol

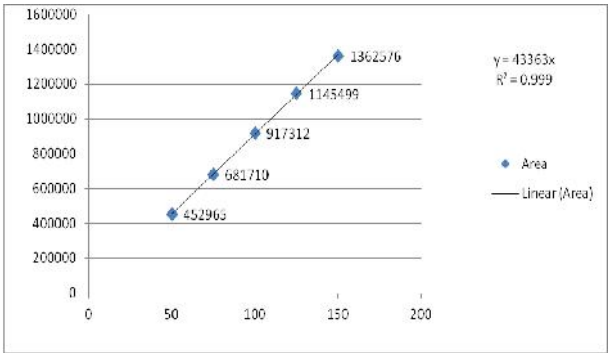

Figure 9: Linearity plot of Flupirtine maleate

Discussion: When a method has been used, it must validation before practical use. By following the ICH guidelines for analytical method validation, the system suitability test was performed and the validation characteristics were addressed. The system suitability test ensures the validity of the analytical procedure as well as confirms the resolution. System suitability parameters like retention time, tailing factor, efficiency, capacity factor and resolution was performed. The results were found to be within the limits. The specificity study was performed and the acid, base, oxidative, photolytic and thermal degradation studies were done to the mother sample. The precision study for system as well as method was conducted for paracetmol and flupirtine maleate and the standard stock solutions. The samples were injected six times in to the HPLC system and the retention time was recorded.

The accuracy was confirmed by recovery studies by adding known amount of pure drug to the previously analyzed formulation and the mixture was analyzed by the proposed method and chromatograms were shown in figures 23 to 25. The percentage recovery of paracetmol and flupirtine maleate was 101.00 % to 100.00% respectively. The data was shown in the table.

The linearity studies were conducted for Paracetomol and Flupirtine standard stock solutions. For the constructions of calibration curves, five different known concentrations of standard solutions of Paracetamol and Flupirtine were selected. The absorbance of those solutions over the concentration range of 50 to 150 μg/ml were observed and recorded. Linearity was observed in the range 50 to 150 μg/ml for Paracetamol (r^2 = 0.999) and 50 to 150 μg/ml Flupirtine (r^2=0.999).

The robustness was performed by changing the flow rate and wavelength. Prepared the sample solution having the concentration of 10μg/ml solution was injected in to the HPLC system by changing the flow rates and observed the retention time. Therefore the developed method was specific, accurate, precise, linear, robust, simple and rapid. Hence, the RP-HPLC method may be applied for paracetmol and flupirtine maleate.

Table 1: Specificity data for Paracetmol and Flupirtine maleate

S.No	Sample name	Paracetmol area	Retention time (min)	Flupirtine maleate Area	Retention time (min)
1	Standard	2251240	1.950	21020626	3.858
2	Sample	2252365	1.944	21107354	3.838
3	Blank	-	-	-	-
4	Placebo	-	-	-	-

Table 2: Linearity data for Paracetmol

S.No	Conc (µg/ml)	Retention time (min)	Area (µV.Sec)
1.	50	4.741	545934
2.	75	4.745	545471
3.	100	4.738	1089953
4.	125	4.733	1363163
5.	150	4.729	1646171

Table 3: Linearity data for Flupirtine maleate

S.No	Conc(µg/ml)	Retention time (min)	Area (µV.Sec)
1.	50	3.362	452965
2.	75	3.359	681710

3.	100	3.359	917312
4.	125	3.357	1145499
5.	150	3.356	1362576

Table 4: Precision data for Paracetmol

S.No	Retention time(min)	Area(µV.Sec)	%Assay
Sample A	4.739	1078871	99
Sample B	4.734	1064983	98
Sample C	4.746	1083323	99
Sample D	4.727	1080612	99
Sample E	4.725	1078487	99
Sample F	4.740	1072778	98
Mean	4.735	1076509	99
Std. Dev.	0.003	950	0.61
% RSD	0.059	0.200	0.62

Table 5: Precision data for Flupirtine maleate

S.No	Retention time(min)	Area(µV.Sec)	%Assay
Sample A	3.357	911468	99
Sample B	3.356	910692	99
Sample C	3.365	913771	99
Sample D	3.354	914557	99
Sample E	3.342	910623	99
Sample F	3.359	910986	99
Mean	3.355	912016	99
Std. Dev.	0.009	0.980	0.19
%RSD	0.041	0.008	0.19

Table 6: Accuracy data for Paracetmol

S.NO	Accuracy level	Injection	Sample area	Retention time
1	50%	1	541598	4.733
		2	546689	4.726
		3	541728	4.709
2	100%	1	1089965	4.728
		2	1080210	4.732
		3	1085854	4.715
3	150%	1	1667553	4.755
		2	1666002	4.743
		3	1666117	4.732

Table 7: Accuracy data for Flupirtine maleate

S.NO	Accuracy level	Sample name	Sample area	Retention time
1	50%	1	459327	3.348
		2	456469	3.358
		3	450190	3.335
2	100%	1	910952	3.345
		2	916393	3.366
		3	916445	3.337
3	150%	1	1367985	3.370
		2	1364944	3.367
		3	459327	3.348

Table 8: LOD data for Paracetamol and flupirtine maleate

S.NO.	Sample name	Retention Time (min)	Area(µV.Sec)
1	paracetamol	4.724	31978
2	Flupirtine maleate	3.356	61493

Table 9: LOQ data for paracetamol and flupirtine maleate

S.NO.	Sample name	Retention Time(min)	Area(µV.Sec)
1	paracetamol	4.724	55203
2	Flupirtine maleate	3.555	106409

Table 10: Robustness data for Paracetmol

parameter	Retention time (min)	Theoretical plates	Asymmetry
Decreased flow rate(0.6ml/min)	5.270	14731	1.27
Increased flow rate(1.0ml/min)	4.321	13704	1.21
Decreased temperature(20^0c)	5.237	13997	1.25
Increased temperature(30^0c)	4.310	13146	1.22

Table 11: Robustness data for Flupirtine maleate

parameter	Retention time (min)	Theoretical plates	Asymmetry
Decreased flow rate(0.6ml/min)	3.726	9384	1.31
Increased flow rate(1.0ml/min)	3.050	8891	1.26
Decreased temperature(20^0c)	3.710	9499	1.31
Increased temperature(30^0c)	3.049	9005	1.26

4. Conclusion

The study is focused to develop and validate HPLC methods for estimation of Paracetmol and Flupirtine maleate in tablet dosage form. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation steps. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. The method shows good reproducibility and good recovery. From the specificity studies, it was found that the developed methods were specific for Paracetmol and Flupirtine maleate.

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