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Method Development and Validation for the Simultaneous Estimation of Pitofenone Hydrochloride, Diclofenac Potassium and Fenpiverinium Bromide In Pharmaceutical Dosage Forms by UPLC

Pulagurtha Bhaskararao[1]*, Gowri Sankar Dannana[1]
1.Department of Pharmaceutical Analysis, College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India-530 003.

ABSTRACT

The present study is carried out using the UPLC as the analytical technique in developing and validating an accurate, precise, linear and robust analytical method for the simultaneous estimation of Pitofenone hydrochloride, Diclofenac potassium and Fenpiverinium bromide in tablets. The method is optimized with a mixture of 0.01M phosphate buffer (P^H4.8) and Acetonitrile in the ratio of 40:60 (V/V) as mobile phase and Agilent SB C18 (250 X 4.6) mm, 5µm as stationary phase. The Chromatographic peaks were detected and measured at 215nm. The retention times of Pitofenone hydrochloride, Diclofenac potassium and Fenpiverinium bromide were found to be 1.0, 1.66 and 2.0 respectively. The developed method was demonstrated to access its suitability for meeting its intended purpose by the Validation with a set of validation parameters as per ICH and USP guidelines. The method is found to be precise with %RSD - 0.48, 0.69, 0.66 for Pitofenone hydrochloride, Diclofenac potassium and Fenpiverinium bromide respectively: accurate with the recoveries of 99.6 to 101.6, 100.51 to 101 and about 100% for Pitofenone hydrochloride, Diclofenac potassium and Fenpiverinium bromide respectively. The method is proved to be linear from the conc.12.5 to 75ppm for Pitofenone, 250 to750 ppm for Diclofenac and 0.5 to 1.5ppm for Fenpiverinium bromide with the correlation coefficients of 0.999, 0.999 and 0.999 respectively. Hence the developed method could be used for the routine analysis purpose in the evaluation of Pitofenone, Diclofenac potassium and Fenpiverinium bromide Tablets.

Keywords: Pitofenone hydrochloride, Diclofenac potassium, Fenpiverinium bromide, UPLC, Validation

*Corresponding Author Email: bhaskarmph@gmail.com
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INTRODUCTION

Pitofenone is antichollenergic agent which is used as antispsasmodic and The IUPAC name is Methyl 2-[4-(-piperidin-1-ylethoxy) benzoyl] benzoate and molecular formula C22 H25 NO4, Molecular weight -367.45g/mol. Chemical structure is given below Figure 1.

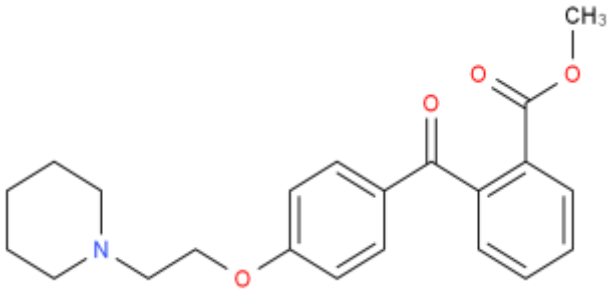

Figure 1: Structure of Pitofenone

Diclofenac potassium[1-3] is (official in I.P, USP) belonging to a nonsteroidal anti-inflammatory agent and class II drug category (high permeability, low solubility). It is benzene acetic acid derivative, showing cyclooxygenase -2 enzyme inhibitions. Usually found in two salts form but individually launched as immediate release tablet in order to give quick pain relief from the site (degenerative joint). Used as an antipyretic and analgesic agent. The chemical name Benzene acetic acid [(2, 6-dichlorophenyl) amino]-monopotassium salt and molecular formula $C_{14}H_{10}CL_2KNO_2$ (Mwt: 334.24) [1-3]. It is sodium salt of an aryl acetic acid derivative. Chemical structure is given below Figure 2.

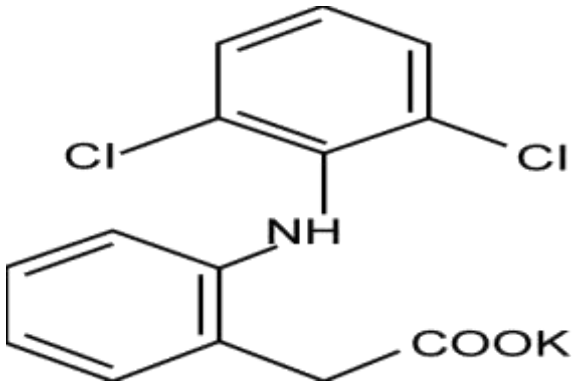

Figure 2: Structure of Diclofenac potassium

Fenpiverinium bromide is synthetic antichollenergic and quaternary ammonium compound antispasmodic agent. The IUPAC name is 1-(4-amino -4-oxo-3,3-diphenylbutyl)-1-methylpiperidinium, molecular formula C_{22} H_{29} N_2O and molecular weight -337.48g/mol. Chemical structure given below Fig no.03.

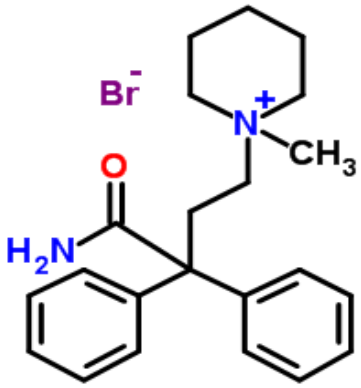

Figure 3: Structure of Fenpiverinium Bromide

The Ultra Performance Liquid Chromatography is the most emerging analytical tool in the field of Pharmaceutical technology in evaluation of the Pharmaceutical Products both for the human and veterinary use.

Literature Survey reveals that there are several analytical methods, existing for individual and combination dosage forms of containing Pitofenone, diclofenac potassium, Fenpiverinum bromide by HPLC[4-10], Ion pair chromatography [11] and Spectroscopy[12-15]. There is no reported method of analysis by Ultra Performance Liquid Chromatography for determination of tablets containing Pitofenone, diclofenac potassium, Fenpiverinum bromide. Hence, UPLC methods in the present work and validated.

MATERIALS AND METHOD

Pitofenone, Diclofenac potassium, Fenpiverinium bromide standards were obtained from SL Drugs and pharmaceuticals, Hyderabad, India. All reagents used were HPLC grade, procured from Merck, Co, Mumbai, India. Nylon membrane filters of 0.45µ pore size were used to filter the mobile phase and its components.

Instrumentation

Analysis was carried out in waters acquity with binary UPLC pump equipped with UV detector and empowers 2 software. Separation has been carried out using Agilent SB C18 (250X4.6) mm, 5µm column.

Method Development

Various analytical development trials has been performed by using different chemicals and reagents, organic solvents at different pH ranges and strengths in different proportions of buffer and Organic solvents to separate the three peaks with acceptable resolution and with good peak shape. Various stationary phases of multiple makes were used to check the chromatography with acceptable peak shape, tailing factor and plate count for reproducibility at 30^{o} C.

Based on the observations and conclusions obtained from the number of chromatographic trials performed on UPLC, a particular set of chromatographic conditions were optimized to be suitable for estimation of the Pitofenone, Diclofenac potassium and Fenpiverinium bromide in the tablets. The optimized chromatographic conditions which are found to be suitable for the estimation of the Pitofenone, Diclofenac potassium, Fenpiverinium bromide are given below. Table 1.

Table 1

Chromatographic Conditions	
Column	Agilent SB C18 250X4.6mm,5µm
Flow rate	0.3ml/mint
Column Temperature	30°C
Injection Volume	0.50µL
Detector wavelength	215 nm
Separation Mode	Isocratic
Run Time	03minutes

Preparation of Diluent

Prepared a mixture of water and Acetonitrile in the ratio of 50:50 (v/v). Mixed well.

Preparation of Phosphate Buffer preparation

Accurately weighed 1.36gm of Potassium dihydrogen ortho phosphate in a 1000ml of volumetric flask, added about 900ml of milli-Q water to it and dissolved the content. pH adjusted to 4.8 with dil. orthophosphoric acid and finally made up to the mark with milli-Q water. Filtered through the 0.45µm pore size nylon filter and sonicated for 10minutes

Preparation of Mobile Phase

Prepared a mixture of Buffer and Acetonitrile in the ratio of 40:60 (v/v). Mixed well. Sonicated for 10minutes.

Preparation of working Solutions

Preparation of Pitofenone Standard stock

Weighed and transferred 50.1 mg of pitofenone into a 20 ml volume metric flask, added 15ml of diluent to it and sonicated for 5 minutes to dissolve the content. Volume made up to the mark with diluent.

Preparation of Diclofenac Standard stock

Weighed and transferred 200.0 mg of Diclofenac into a 20 ml volume metric flask added 15ml of diluent to it and sonicated for 5minutes to dissolve the content. Volume made up to the mark with diluent.

Preparation of Fenpiverinium Standard stock

Weighed and transferred 19.8 mg of pitofenone into a 100 ml volume metric flask added 75ml of diluent to it and sonicated for 7mintes to dissolve the content. Volume made up to the mark with diluent.

Preparation of Standard solution

Transferred 1ml from preparation of pitofenone standard stock and 2.5ml from preparation of diclofenac standard stock and 0.25ml from Preparation of Fenpiverinium Standard stock into a 50ml volumetric flask and mixed well. Volume made up to the mark with diluent.

Calibration curve (25-150%)

Prepared calibration solutions of pitofenone in the following concentrations:

12.5, 25, 37.5, 50.0, 62.5 and 75.0 ppm

Prepared calibration solutions of diclofenac in the following concentrations:

125,250,375,500,625 and 750 ppm

Prepared calibration solutions of fenpiverinium in the following concentrations:

0.25, 0.5, 0.75, 1.00, 1.25 and 1.50 ppm

All the above prepared solutions of respective component were analysed to calculate the correlations coefficient of the individual components i.e. pitofenone, diclofenac and fenpiverinium.

Accuracy

Accuracy was performed with 50 to 150% of the test concentration of the respective individual components. The sample solutions prepared with the concentrations are given below for each component.

Pitofenone: 25, 50 and 75 ppm

Diclofenac: 250, 500 and750 ppm

Fenpiverinium: 0.5, 1, and 1.5 ppm.

Sample Stock Preparation

Twenty tablets were weighed (each contains 5mg of pitofenone HCL, 50mg of diclofenac potassium and 0.1mg of fenpiverinium bromide) and calculated average weight. Tablets were crushed into fine powder by using motor and pestle. Weighed ten tablet equivalent powder and transferred into 200ml volumetric flask and added 15ml of diluent to it. Allowed to sample extraction by 60mints using sonicator and volume made up to mark with diluent. Filtered through 0.45µm nylon filter.

Sample Preparation

Transferred 5ml of Sample Stock Preparation to 25ml volumetric flask and volume made up to the mark with diluents and injected into system.

Method Precision

The Precision of the method was determined (within day variation) by sample preparation. Calculated % of assay using formula given below.

$$\frac{At}{As} \times \frac{Cs}{Ct} \times P$$

At = Area response of testing sample preparation

As =Average area response of standard preparation

Ds = Dilution of standard preparation

Dt = Dilution of assay sample preparation

Ws = Weight of working standard

Wt = Weight of test sample preparation

P = purity of standard

Avg.wt = Average weight of sample preparation

LC = Label claim

Forced Degradation Study

Acid Degradation

Transferred 5 ml of Sample stock preparation and added 5 ml of 2N Hydrochloric acid solution to it and refluxed for 30mins at 60^0c .Neutralized with 2N NaoH solution. The resultant solution was diluted to obtain test concentration and injected into the system and the chromatograms were recorded to evaluate the degradation of sample.

Alkali Degradation

Transferred 5 ml of Sample stock preparation and added 5 ml of 2N sodium hydroxide solution to it and refluxed for 30mins at 60^0c.Neutralized with 2N hydrochloric acid. The resultant solution was diluted to obtain test concentration with diluent and injected into the system and the chromatograms were recorded to evaluate the degradation of sample.

Oxidation Degradation

Transferred 5 ml of Sample stock preparation and added 5 ml of 20% (v/v) hydrogen peroxide (H2O2) to it. Refluxed for 30 min at 60^0c. For UPLC study, the resultant solution was diluted to obtain the test concentration with diluent and injected into the system and the chromatograms were recorded to evaluate the degradation.

Dry Heat Degradation

Transferred 5ml of Sample stock preparation into 25ml volumetric flask separately and placed

in oven at 70°C for 1 hour to study dry heat degradation. For UPLC study, the resultant solution was diluted with diluent up to mark and injected into the system and the chromatograms were recorded to evaluate the degradation of the sample**.**

Photolytic Degradation

The photochemical stability of the drug was also studied by exposing the sample stock preparation to UV Light by keeping the beaker in UV Chamber for 1day or 200 Watt hours/m^2 in photo stability chamber For UPLC study, the resultant solution was diluted to obtain test concentration and injected into the system and the chromatograms were recorded to evaluate the degradation of sample.

RESULTS AND DISCUSSION

Method Optimization

The developed method was optimized after many trials. The optimized method developed on SB C18 (250 x 4.6) mm, 5µm as stationary phase. Using 0.01M phosphate buffer (P^H4.8) and Acetonitrile in the ratio of (40:60v/v) as mobile phase. The column temperature was maintained constantly at 30°C. Mobile phase pumped with a flow rate of 0.3ml/minute and injection volume is 0.5µL. The chromatographic peaks were detected and measured at 215 nm. The retention times of Pitofenone, diclofenac potassium and Fenpiverinium bromide were found to be 1.0, 1.66 and 2.0.

Method Validation

System suitability

All system suitability parameters were passed which include the USP plate count, USP tailing USP resolution for pitofenone. diclofenac and fenpiverinium respectively. Figure 4 and Table 2.

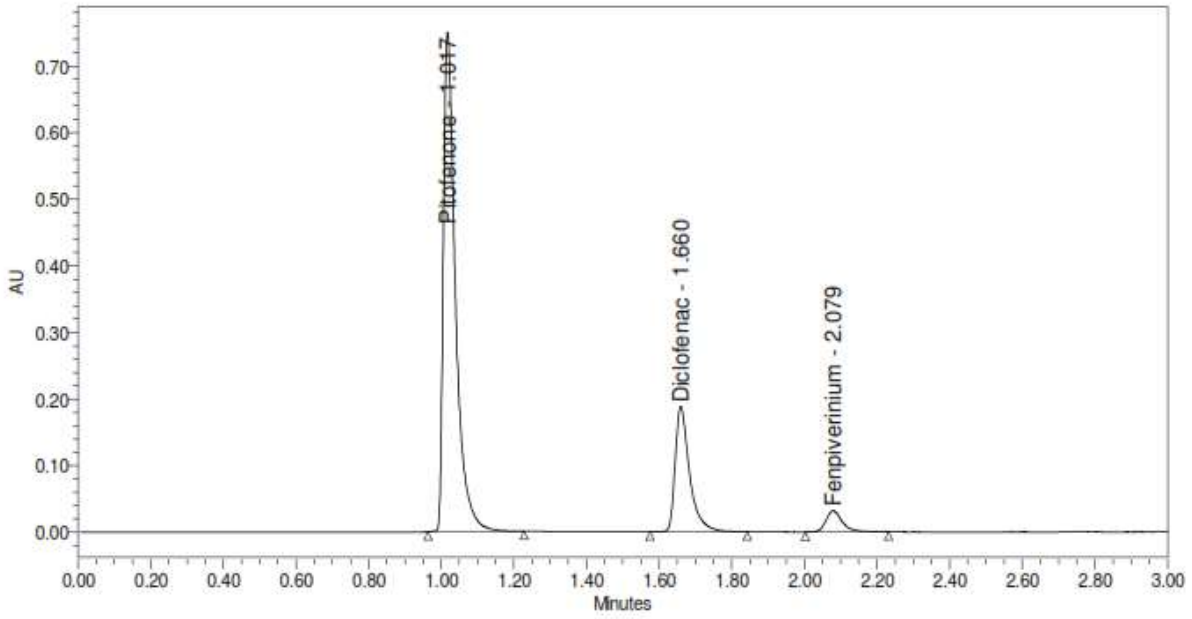

Figure 4: System Suitability Chromatogram

Table 2: System Suitability

Peak Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
Pitofenone	1.0	1760232	4958	0.4	
Diclofenac	1.66	503855	9574	0.3	10
Fenpiverinium	2.0	98583	11364	1	5.6

Linearity

The best fit line was obtained with regression coefficient between the peak response vs concentration. Results are given below .Table 3 and Figure 5a, 5b and 5c.

Result **Table: 3**

Linearity		
Name of the Analyte	**Regression Equation**	**Regression coefficient**
Pitofenone	Y =34646 +50162	0.9994
Diclofenac	Y= 971.45 x +17153	0.9992
Fenpiverinium	Y= 99215 x + 1529.9	0.999

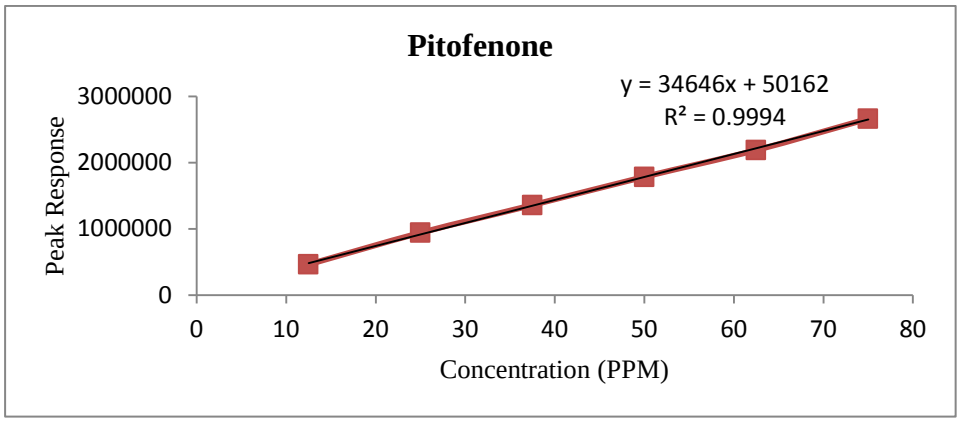

Figure 5a Pitofenone linearity graph

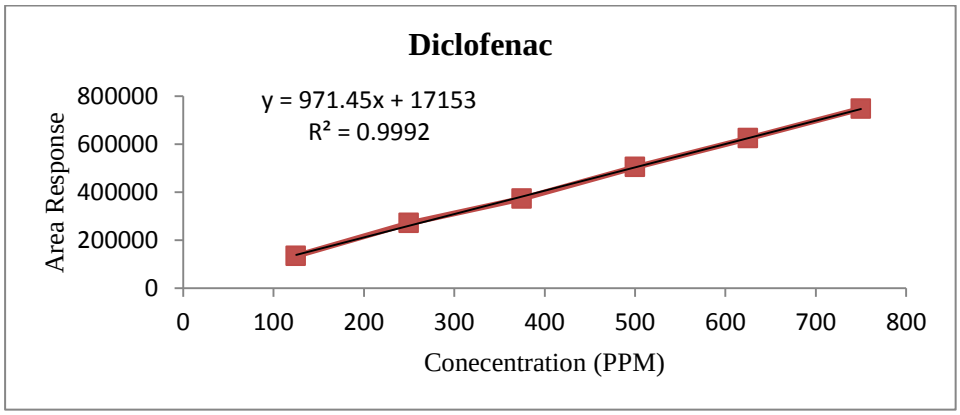

Figure 5b Diclofenac linearity graph

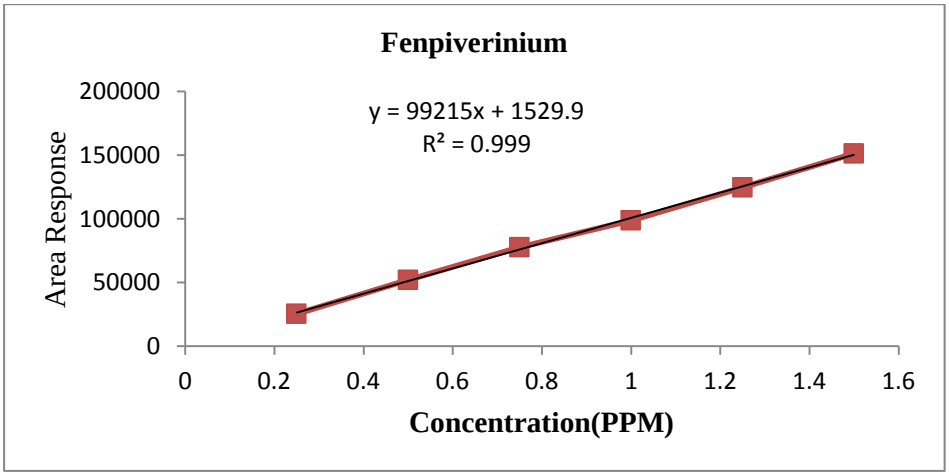

Figure 5c Fenpiverinium bromide linearity graph

Specificity

It was assessed by injecting blank along with drug product, no interference was found at individual components respective retention timings. Chromatogram depicted below Figure 6a and 6b.

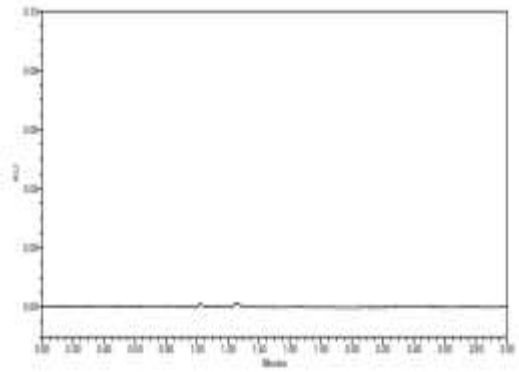

Figure 6: Blank Chromatogram

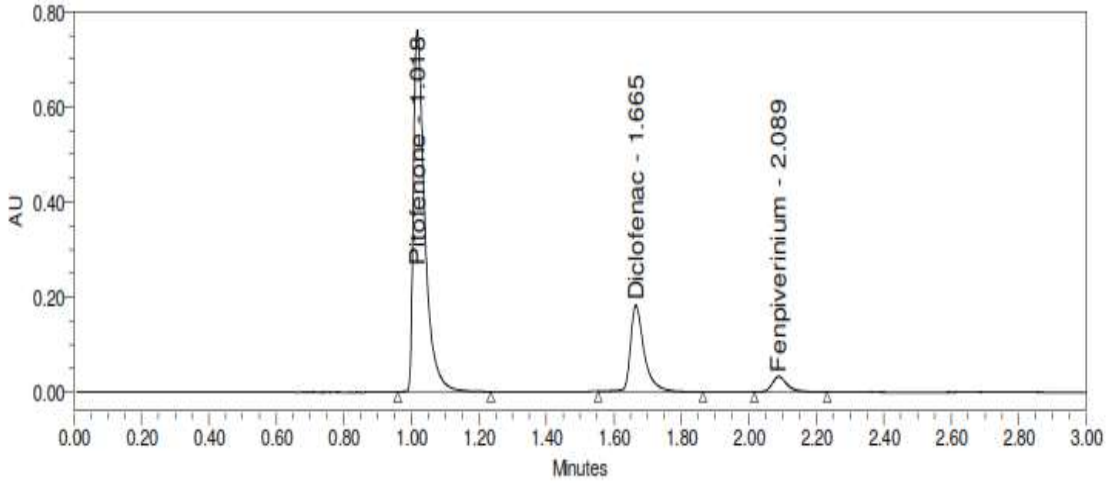

Figure 6b typical chromatogram

Method Precision

The Precision of the method was determined (within day variation) by injecting Pitofenone hydrochloride, Diclofenac potassium, Fenpiverinium bromide with sample solution 6 times respectively. Method precision was expressed in terms of % RSD. Results are given in Table 4.

Table 4

Method Precision	
Pitofenone hydrochloride	0.48% RSD
Diclofenac potassium	0.69%RSD
Fenpiverinium bromide	0.66%RSD

Accuracy

Prepared accuracy at 3 levels in triplicate at 50% 100% and 150% with matrix and achieved satisfactory results and at each level of recovery was calculated. Results are given below table 5.

Table 5

Pitofenone				**Diclofenac**			**Fenpiverinium**		
Level	**Added**	**Found**	**%Mean Recovery**	**Added**	**Found**	**%Mean Recovery**	**Added**	**Found**	**%Mean Recovery**
50	25	25.4	101.6	250	251.6	100.92	0.5	0.5	100
50	25	25.4		250	252.1		0.5	0.5	
50	25	25.4		250	253.2		0.5	0.5	
100	50	50	99.6	500	504.4	100.51	1	1	100
100	50	49.8		500	498.5		1	1	
100	50	49.6		500	504.7		1	1	
150	75	74.7	99.6	750	743.3	101	1.5	1.5	100
150	75	74.3		750	762.7		1.5	1.5	
150	75	75.1		750	766.6		1.5	1.5	

Robustness

Measure of the method capability to remain unaffected by small variations such as flow rate, mobile phase, temperature. Given below Table 6.

Table 6

Robustness								
Pitofenone			**Diclofenac**			**Fenpiverinium**		
Flow Variation-0.9ml								
RT	AREA	USP Plate Count	RT	Area	USP Plate Count	RT	Area	USP Plate Count
1.1	1722561	5116	1.76	504127	9544	2.22	98429	11318
Flow Variation-1.1ml								
1	1616086	4992	2	482827	9311	2.07	91937	11069
Buffer:Acetonitrile(33:67)								
1.03	1737473	4991	1.64	507628	9725	2.08	97901	9750
Buffer:Acetonitrile(37:63)								
1.03	1728137	4987	1.64	508031	9750	2.08	99632	11572
Column Temperature(29°C)								
1.03	1711978	5103	1.64	504729	9598	2.08	98032	11606
Column Temperature(31°C)								
1.02	1730109	5276	1.66	505913	9496	2.09	99027	11194

Forced Degradation

It was studied by using the different stress condition to Evaluate the degradation. Calculated their degradation in terms of %.Results are given below Table 7.

Table 7

Pitofenone HCL	**Diclofenac Potassium**	**Fenpiverinium Bromide**
Acid Degradation		
%Degradation		

4.73	4.96	4.21
Base Degradation %Degradation		
2.83	3.29	2.65
Oxidative Degradation %Degradation		
1.24	1.87	1.16
Photolytic Degradation %Degradation		
0.46	0.15	0
Thermal Degradation %Degradation		
0.49	0.67	0

CONCLUSION

The developed method is new and simple which met the within acceptance criteria as system suitability, tailing factor, resolution among the individual components and reproducibility. Method is specific, précised, robustness and reliability. Drugs are separated within in short span of time. The method and economically also very less over than conventional method .So that based on obtained results from the method and can be use in routine analysis of quality control.

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