

DEVELOPMENT OF NEW ANALYTICAL METHODS FOR THE ESTIMATION OF FLUPIRTINE MALEATE IN BULK AND IN FORMULATIONS

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ABSTRACT

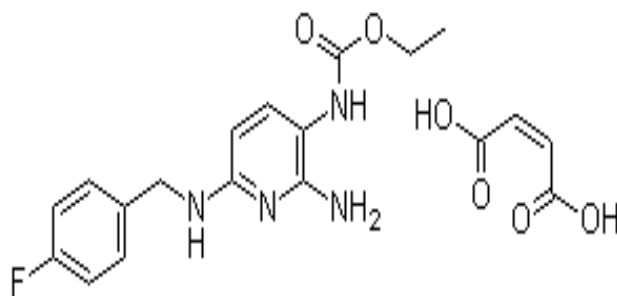
Two simple, sensitive, specific UV - spectroscopic and RP-HPLC methods are developed for the estimation of Flupirtine Maleate in bulk and pharmaceutical formulation. The first method was based on UV – spectroscopic determination of the drug. It involves absorbance measurements at 319nm (λ max of Flupirtine Maleate) in acetonitrile:water. Calibration curve was linear with the correlation coefficient was 0.9999 over a concentration range of 2 to 35 μ g/ml for the drug. The second method was based on HPLC separation of the drug in reverse phase mode using C₁₈ column (150 mm \times 4.6 mm i.d. 5 μ). The mobile phase constituted of Acetonitrile: water: Methanol

(40:40:20 v/v) and flow rate 1.0ml/min. Detection was performed at 249nm. Separation completed with in 5minutes. Calibration curve was linear with the correlation coefficient was 0.9998 over a concentration range of 5 to 30 μ g/ml for the drug. The relative standard deviation (R.S.D) was found <2.0% for UV – spectroscopic and RP-HPLC methods. Both these methods have been successively applied to bulk and pharmaceutical formulation. The present methods were validated according to ICH guidelines.

Key Words: Flupirtine Maleate, UV-spectroscopy, High performance liquid chromatography.

INTRODUCTION

Flupirtine Maleate belongs to the class Antidote. Chemical name is Ethyl 2-amino-6-[(4-fluorobenzyl) amino] pyridine-3-carbamate compound with maleic acid (1:1). Flupirtine Maleate is an amino pyridine derivative. Flupirtine maleate is a centrally acting analgesic mainly used to treat pain. It appears to work by suppressing pain perception transmission to the brain and spinal cord through stimulation of descending monoaminergic pathways. The drug appears to work by affecting noradrenaline. The drug works best for pain associated with noninflammatory conditions. In addition to alleviating pain, Flupirtine relaxes muscles through GABA-ergic mechanisms (it inhibits reflexes in the spinal cord) and works against seizures. It is not official in any of the pharmacopoeia. It is listed in the Merckindex 14th edition [2] and Martindale the complete drug reference 35th edition [3].



From the survey of the literature available for Flupirtine maleate their physical and chemical properties pharmacological pharmacokinetic studies knowledge were gained for the development of several analytical methods were reported. In this present work study an attempt was made to developed rapid and economical spectroscopy and RP-HPLC method for estimation of Flupirtine Maleate in bulk and pharmaceutical formulation with better sensitivity, precision and accuracy using C₁₈ column and UV detector.

MATERIALS AND METHODS

Flupirtine Maleate was procured from Lupin Laboratories Ltd., Chennai, India. All chemicals and reagents used were of HPLC grade and AR grade. Tablets were purchased from Indian market, containing Flupirtine Maleate 100mg/per tablet (Katadol 100mg, Lupin Laboratories Ltd., Chennai). Shimadzu - 1700 Double Beam UV - Visible spectrophotometer with pair of 10mm matched quartz cells, Shimadzu HPLC system(LC – 10 ATvp solvent deliver module, SPD – 10 Avp UV - Visible detector,) using Phenomenax Luna C₁₈ column (150 mm × 4.6 mm i.d. 5μ), apparatus was used for the analysis. The mobile phase constituted of Acetonitrile: water: Methanol (40:40:20 v/v) and flow rate was 1.0ml/min. Detection was performed at 249 nm.

UV – spectroscopic method

Standard solution

Standard solution of Flupirtine Maleate was prepared by dissolving 10 mg of the drug in 100 ml of Acetonitrile:water (1:4 ratio). This solution contains 100 mg/ ml concentration.

Selection of wavelength

The standard stock solution was further diluted with Acetonitrile:water to get the concentration of 10 µg/ ml and the solution was scanned between 200 and 400 nm using Acetonitrile:water as blank. From the spectra, λ_{max} was found to be 319 nm and was selected as an analytical wavelength. The spectrum are shown in fig 1

Preparation of calibration graph

Aliquots of standard stock solution (0.2ml, 0.5ml, 1.0ml, 1.5ml, 2.0ml, 2.5ml, 3.0ml, and 3.5ml) were taken in to a 10ml volumetric flask and Make up to mark with Acetonitrile:water to obtain series in the concentration range of 2-35µg/ml. The absorbances were measured at 319nm and calibration curve was plotted using absorbance Vs concentration. The value of slope and correlation coefficient were found to be 0.026857 and 0.9999 respectively. The optical characteristics of the method is listed in table 1.

Assay of tablet formulation

Six tablets of Flupirtine Maleate were accurately weighed and average weight of tablet formulation was determined. The tablets were crushed, the tablet powder equivalent to 25 mg of Flupirtine Maleate was weighed and dissolved in to 25ml of Acetonitrile:water. The content of flask was sonicated for 15 mintues centrifuged for another 15 minutes. The supernatant liquid was filtered through Whatmann filter paper No. 41. Further dilutions were made by diluting 5 ml into 50 ml to produce 200µg/ml. From that 1 ml was taken in 10ml standard flask and made up to mark with acetonitrile: water (1:4 ratio) to produce 10µg/ml solution theoretically. Absorbance was measured at 319nm using Acetonitrile:water as a blank. The amount of Flupirtine Maleate per tablet was calculated using the calibration curve. The results are shown in table 2.

RP-HPLC method

Optimized Chromatographic Conditions

| | | |
|-------------------|---|--|
| Mode of operation | - | Isocratic |
| Stationary phase | - | C ₁₈ column (150 mm □ 4.6 mm i.d. 5□) |

| | | |
|----------------------------|---|--------------------------------------|
| Mobile phase | - | Acetonitrile: water: Methanol |
| Proportion of mobile phase | - | 40:40:20% v/v |
| Detection wavelength | - | 249 nm |
| Flow rate | - | 1 ml/ min |
| Temperature | - | Ambient |
| Sample load | - | 20 μ l |
| Operating pressure | - | 80 kgf |
| Method | - | External Standard Calibration method |

The solution of Flupirtine Maleate was injected and the respective chromatogram was recorded. The chromatogram are shown in fig 2.

Standard solution

Standard stock solution of Flupirtine Maleate was prepared by dissolving 25mg of the drug in 25 ml of mobile phase. Further dilution was made by pipetting 2.5 ml of stock solution into 25 ml to get the concentration of 100 μ g/ml with mobile phase from this pipetting 1 ml to 10 ml of standard flask make up to mark with mobile phase to obtain 10 μ g/ml solution (working standard solution). Aliquots of working standard solution (0.5 – 3 ml) were taken and diluted with mobile phase to obtain series of solution in the concentration range of 5 - 30 μ g/ml. All the solutions were injected and the chromatograms were recorded at 249 nm and calibration curve was plotted using peak area Vs concentration. The values of slope and correlation coefficient were found to be 359803.6571 and 0.9998 respectively.

Assay of tablet formulation

Six tablets of Flupirtine Maleate were accurately weighed and average weight of tablet formulation was determined. The tablets were crushed, the tablet powder equivalent to 25 mg of Flupirtine Maleate was transferred to a 25 ml volumetric flask. Dissolve the active ingredients and volume was made up to 50ml with methanol, the contents were sonicated for 15 minutes, centrifuged at 2000 rpm for 15 minutes and filtered through a 0.2 μ membrane filter. From the clear solution, further dilutions were made by diluting 5 ml into 50 ml with mobile phase to obtain 100 μ g/ml concentration. This solution was used for further analysis. 2 ml of test solution was transferred into six 10 ml volumetric flasks and made up to the mark with mobile phase the concentration of the resulting solution is 20 μ g/ml. A 20 μ l volume of each sample solution was injected into the sample injector of HPLC. The peak area was measured at 249nm. The amount of drug present in the sample solutions were determined

using calibration curve of standard Flupirtine Maleate. The results are shown in table 5.

METHOD VALIDATION

Linearity

The plot of absorbance against concentration is shown in fig 3 and 4 for uv and HPLC methods, respectively. It can be seen that plot is linear over the concentration range of 2 to 35 µg/ml for UV – Spectroscopy and for HPLC 5 – 30 µg/ml Flupirtine Maleate with a correlation coefficient (r^2) 0.9999 and 0.9998, respectively.

Precision

Intra day and inter day precision was determined by repeating assay three times on the same day for intra day and on different days for inter day precision. The relative standard deviation for six replicates of sample solution was less than 2.0%, which met the acceptance criteria established for spectroscopic method. The obtained results were presented in table 3.

Accuracy

To check the accuracy of the proposed method, recovery studies were carried out at 80,100,120% of the test concentration as per ICH guidelines and low relative standard deviation value show the accuracy of the Spectroscopy and HPLC methods. The data were presented in table 4 and table 6 for uv and HPLC methods respectively..

LOD and LOQ (sensitivity)

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The relative standard deviation of the regression lines and slope of the calibration curve were used to calculate LOD and LOQ.

Standard and sample solution stability

Standard and sample solution stability was evaluated at room temperature for 48 hours. The relative standard deviation was found below 2.0%. It shows that standard and sample solution were stable up to 48 hours at room temperature.

RESULTS AND DISCUSSION

In this study a simple, fast and reliable UV spectroscopy and HPLC methods were developed and validated for the determination of Flupirtine Maleate in bulk and pharmaceutical formulation. As these proposed methods have the lowest LOD values and wider linearity range is more sensitive method. From the results obtained, we conclude that the suggested

methods showed high sensitivity, accuracy, reproducibility and specificity. Moreover, these methods were simple and in expensive and this can be employed for the routine quality control of Flupirtine Maleate in bulk and pharmaceutical formulation.

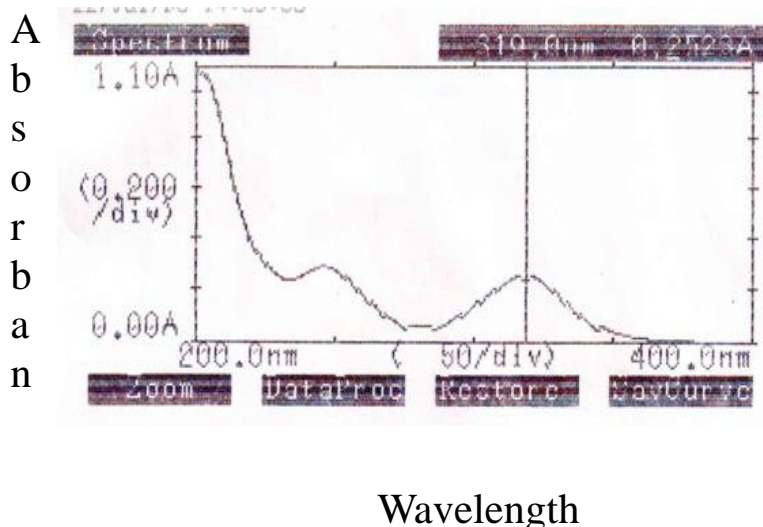


Fig.1. UV spectrum for Flupirtine Maleate in Acetonitrile: Water (1:4 Ratio)

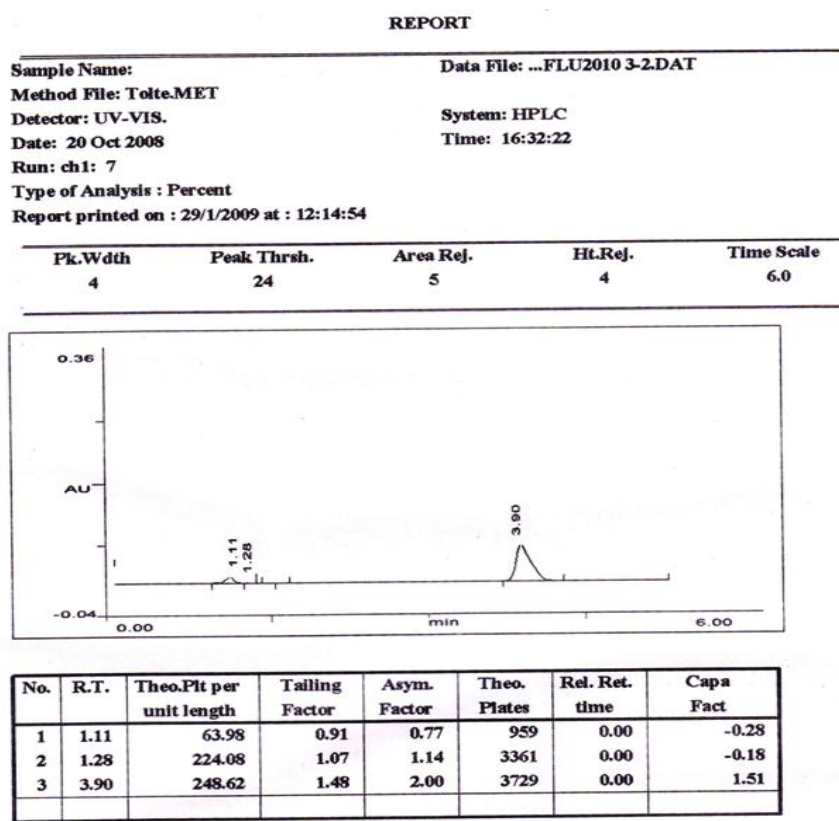


Fig. 2. Chromatogram of Flupirtine Maleate

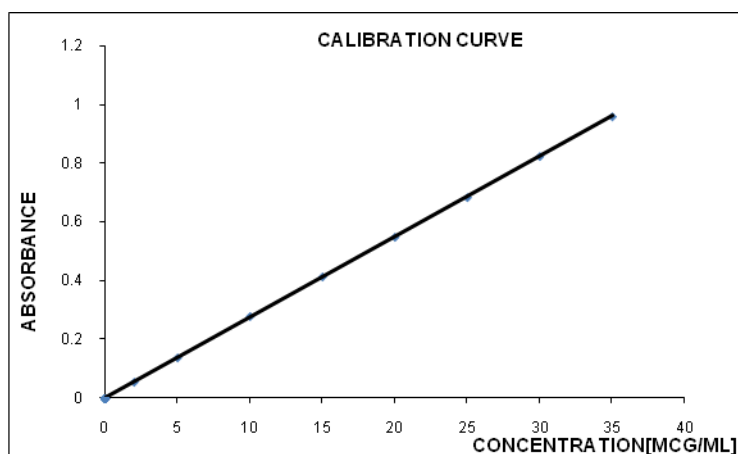


Fig. 3. Calibration curve of Flupirtine Maleate in Acetonitrile:water at 319 nm By uv method

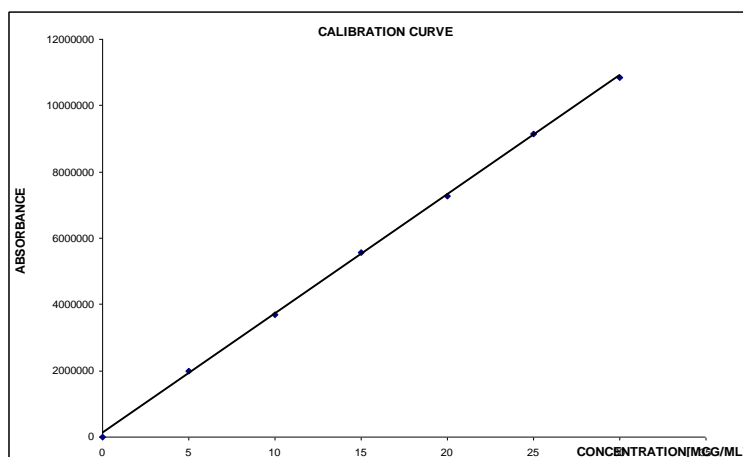


Fig. 4. Calibration curve of Flupirtine Maleate by RP – HPLC method

Table 1. Optical characteristics of Flupirtine Maleate by UV – spectroscopy and RP-HPLC method

| METHOD | PARAMETERS | VALUES* |
|-----------------|--|--------------------------|
| UV-spectroscopy | □ max (nm) | 319 |
| | Beer's law limit (□ g/ ml) | 2 – 35 |
| | Sandell's sensitivity (□ g/cm ² /0.001 A.U) | 0.03725 |
| | Molar absorptivity (L mol ⁻¹ cm ⁻¹) | 1.1410 × 10 ⁴ |
| | Correlation coefficient (r) | 0.9999 |
| | Regression equation (y=mx+c) | Y=0.026857x+0.0036031 |
| | Slope(m) | 0.026857 |
| | Intercept(c) | 0.0036031 |
| | Precision | <2 |

| | | |
|---------|---|------------------------------|
| | Inter day, intraday (RSD) | |
| | LOD ($\mu\text{g/ml}$) | 0.464015 |
| | LOQ ($\mu\text{g/ml}$) | 1.406106 |
| | Standard error | 0.000853 |
| | λ_{max} (nm) | 249 |
| | Beer's law limit ($\mu\text{g/ml}$) | 5-30 |
| | Correlation coefficient (r) | 0.9998 |
| | Regression equation ($y = mx + c$) | $Y=359803.6571x+101462.1429$ |
| RP-HPLC | Slope (m) | 359803.6571 |
| | Intercept (c) | 101462.1429 |
| | LOD ($\mu\text{g/ml}$) | 0.5690674 |
| | LOQ ($\mu\text{g/ml}$) | 1.724469 |
| | Standard error | 27342.78672 |

Table 2. Quantification of formulation - katadolon by UV methods

| S.No | Labelled Amount (mg/tab) | Amount found (mg/tab) | Percentage obtained | Average % | S.D | %RSD | S.E |
|------|--------------------------|-----------------------|---------------------|-----------|--------|----------|---------|
| 1 | 100 | 97.77 | 97.77 | 99.1271 | 0.8288 | 0.836198 | 0.33839 |
| 2 | 100 | 99.57 | 99.57 | | | | |
| 3 | 100 | 99.03 | 99.03 | | | | |
| 4 | 100 | 99.03 | 99.03 | | | | |
| 5 | 100 | 100.3 | 100.3 | | | | |
| 6 | 100 | 99.03 | 99.03 | | | | |

*Mean of six observations

Table 3. Inter day and Intra day analysis of formulation katadolon by UV method

| Drug | Sample No. | Labelled amount (mg/tab) | Percentage obtained* | | \pm S.D | | % R.S.D. | | S.E | |
|------|------------|--------------------------|----------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| | | | Intra day | Inter day | Intra day | Inter day | Intra day | Inter day | Intra day | Inter day |
| FLP | 1 | 100 | 100.48 | 97.77 | 0.779 | 0.794 | 0.7817 | 0.7960 | 0.317 | 0.3230 |
| | 2 | 100 | 100.11 | 99.58 | | | | | | |
| | 3 | 100 | 100.3 | 99.04 | | | | | | |
| | 4 | 100 | 99.39 | 99.04 | | | | | | |
| | 5 | 100 | 99.04 | 100.30 | | | | | | |
| | 6 | 100 | 98.31 | 99.03 | | | | | | |

* Mean of six observations

Table 4. Recovery Studies For Formulation- Katadolon By Uv Method

| Drug | Sample No. | Amount present (µg/ml) | Amount added (µg/ml) | Amount estimated* (µg/ml) | Amount recovered (µg/ml) | % Recovery* | ±S.D | % R.S.D | S.E. |
|------|------------|------------------------|----------------------|---------------------------|--------------------------|-------------|---------|---------|-------|
| FLP | 1 | 10.02 | 5 | 15.13 | 5.11 | 102.75 | ±1.4464 | 1.4292 | 0.837 |
| | 2 | 10.02 | 10 | 20.01 | 9.99 | 99.88 | | | |
| | 3 | 10.02 | 15 | 26.12 | 16.12 | 101.00 | | | |
| | Mean | | | | | 101.21 | | | |

* Mean of six observations

Table 5. Quantification of formulation - Katadolon by RP – HPLC method

| Drug | Sample No | Labeled amount (mg/tab) | Amount found (mg/tab)* | Percentage Obtained* | Average (%) | S.D | % R.S.D | S.E |
|------|-----------|-------------------------|------------------------|----------------------|-------------|-------|---------|--------|
| FLP | 1 | 100 | 104.017 | 104.01 | 103.469 | 1.154 | 1.1153 | 0.4729 |
| | 2 | 100 | 105.29 | 105.29 | | | | |
| | 3 | 100 | 103.116 | 103.112 | | | | |
| | 4 | 100 | 102.516 | 102.51 | | | | |
| | 5 | 100 | 103.786 | 103.78 | | | | |
| | 6 | 100 | 102.088 | 102.08 | | | | |

* Mean of six observations

Table 6. Recovery analysis of formulation –katadolon RP-HPLC method

| Drug | Sample No. | Amount present (µg/ml) | Amount added (µg/ml) | Amount estimated* (µg/ml) | Amount recovered (µg/ml) | % Recovery* | ±S.D | % R.S.D | S.E. |
|------|------------|------------------------|----------------------|---------------------------|--------------------------|-------------|---------|---------|--------|
| FLP | 1 | 16.56 | 5 | 21.61 | 5.048 | 100.97 | ±0.36.4 | 0.3583 | 0.2080 |
| | 2 | 16.56 | 10 | 26.60 | 10.099 | 100.39 | | | |
| | 3 | 16.56 | 15 | 31.60 | 15.046 | 100.31 | | | |
| | Mean | | | | | 100.75 | | | |

*Mean of three observations

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