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***Research Paper***

# Determination of Mirtazapine using fast Sulphone BlackF as Chromogenic Reagent

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***Abstract:*** *A simple and sensitive extractive visible spectrophotometric method for the assay of Mirtazapine in pure and pharmaceutical formulations based on the formation of colored chloroform soluble ion-association associate under specified experimental conditions was described. The dye namely acidic dye Fast Sulphone Black F was utilized. The extract of the ion-associates exhibit absorption maxima at 520nm. Regression analysis of Beer-Lambert plots showed good correlation in the concentration range of 5-20µg/ml. The proposed method can be applied to commercial available formulations and the results are statistically compared with those obtained by the UV reference method and validated by recovery studies. The results are found satisfactory and reproducible. The methods can be applied successfully for the estimation of the Mirtazapine in the presence of other ingredients that are usually present in formulations. The method offer the advantages of rapidity, simplicity and sensitivity and low cost without the need for expensive instrumentation and reagents. It is for the first time in the literature that an attempt is been made by the author to use FSBF is used as Chromogenic reagent.*

**Keywords:** Chromogenic reagent, Fast sulphone Black-F (FSBF), Ion-Association methods, Statistical analysis.

## Introduction

Mirtazapine is 1,2,3,4,10,14b-hexahydro-2-methylpyrazino [2,1-a] pyrido [2,3-c] benzazepine.Mirtazapine (Figure 1) has a tetracyclic chemical structure and belongs to the piperazing – azepine group of compounds. It is designated and has the empirical formula of $C_{17}H_{19}N_3$. Mirtazapine is official in (B.P, U.S.P, E.P, M.I, P.D.R, and CIMS), a potent antagonist of histamine ($H_1$) receptors, a property that may explain its prominent sedative effects. Mirtazapine is a moderate peripheral $(alpha)_1$ adrenergic antagonist, a property that may explain the occasional orthostatic hypotension reported in association with its use. Mirtazapine is a moderate antagonist at muscarinic receptors, a property that may explain the relatively low incidence of anti-cholinergic side effects associated with its use. Several analytical techniques like HPLC[1-4], HPLC-TDMS[5,6], LC[7,8], GC-MS[9], GC-TDMS[10], RP-HPLC[11], LC-MS[12,13,14] and UV derivative spectrophotometry[15-16] and GC-MS[13-15] have been reported in the literature. But to the best of our knowledge there is no single method available for the estimation by visible spectrophotometry which is far simpler and economical and less time consuming as compared to above mentioned methods.

So the authors have made some attempts in developing visible spectrophotometric methods and succeeded in developing two methods based on the reaction between the drug and acidic dye namely FSBF under specified experimental conditions. As the extraction spectrophotometric procedures are popular for their sensitivity and selectivity in the assay of drugs, the extractive spectrophotometric acid- dye technique was therefore, utilized in the present work for the estimation of MIRT. The present paper

describes one simple and sensitive extraction visible spectrophotometric method for the determination of MIRT, based on its tendency to form chloroform extractable ion-associate with acidic dye FSBF belonging to azo category dye under experimental conditions by exploiting the basic nature(nitrogen in triazole linked to 1-butan-2-ol) of the drug molecule. As per the literature, it is the first time the author has tried to utilize the reagent for MIRT determination in formulations by visible spectrophotometry. No methods were reported so far in the literature using FSBF as dye for forming ion-Association complex. The proposed method for MIRT determination has many advantages over other analytical methods due to its rapidity, lower cost and environmental safety. Unlike HPLC, LC procedures, the instrument is simple and is not costly. Economically, all the analytical reagents are inexpensive and available in any analytical laboratory. The proposed method report a new for the determination of MIRT in pure and pharmaceuticals.

## Material and Methods

**FSBF**: The Chemical name is Di sodium8-(phenyl amino)-5-[4-(5-sulfonato naphthalene-1-yl) diazenyl napthalenn-1-yl] di azenyl naphthalene-1-sulfonate. Its CAS no is 3682-47-1(Figure 2).

**Apparatus and chemicals:** A Shimadzu UV-Visible spectrophotometer 1601 with1cmmatched quartz cells was used for all spectral measurements. A Systronics digital pH meter mode-361was used for pH measurements. All the chemicals used were of analytical grade. MIRT Pure drug was obtained as a gift sample from Panacea laboratories. Zipdep -30mg tablets and Nassa 30mg tablets were purchased from local market. FSBF solution(0.02%),(sd.Fine) Prepared by dissolving 200mg of FSBF in 100ml of distilled water and subsequently washed with chloroform (A.R grade ) to remove chloroform soluble impurities),0.1MHCl (prepared by diluting 8.7ml of Conc. Hydrochloric acid to1000ml with distilled water and standardized).

**Preparation of Standard stock solution:** The standard stock solution (1mg/ml) of MIRT was prepared by dissolving100mg of MIRT in 100 ml distilled water. The working standard solutions of MIRT were obtained by appropriately diluting the standard stock solution with the same solvent.

**Preparation of Sample solution:** About 10 tablets were pulverized and the powder equivalent to 100mg of MIRT was weighed, dispersed in 25ml of alcohol, shaken well and filtered. The filtrate was evaporated to dryness and the residue was dissolved as under standard solution preparation.

**Recommended procedure:** Aliquots of standard solution (0.5-2.5ml, 200 µg/ml) were placed into a series of 125ml separating funnels, 5.0ml of 0.1M HCl and 2.0ml of FSBF solution were added.

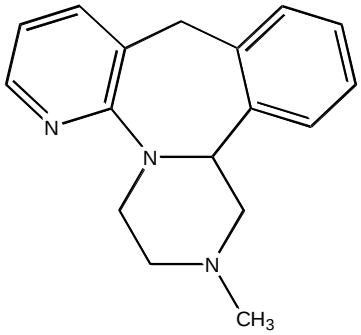


**Figure 1: Chemical structure of Mirtazapine**

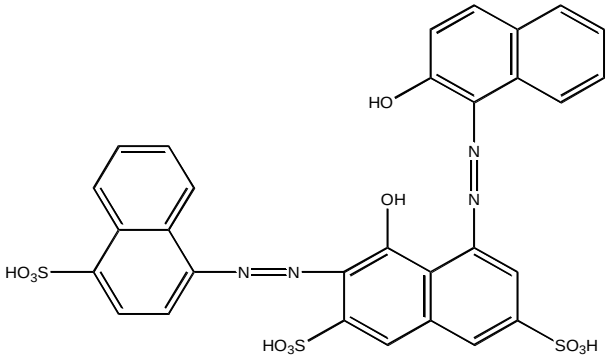


**Figure 2: Chemical structure of FSBF**

## Material and Methods

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The filtrate was evaporated to dryness and the residue was dissolved as under standard solution preparation.

**Recommended procedure:** Aliquots of standard solution (0.5-2.5ml, 200 µg/ml) were placed into a series of 125ml separating funnels, 5.0ml of 0.1M HCl and 2.0ml of FSBF solution were added.

The total volume of aqueous phase in each separating funnel was adjusted to 10ml with distilled water and organic layer to 10ml with $CHCl_3$. The contents were shaken for 5 min. The two phases were allowed to separate and the absorbances of the separated organic layer was measured at 520nm (Figure 3) against a similar reagent blank after 10 min. The amount of MIRT in the sample solution was obtained from Beer-Lambert plot (Figure 4)

**For pharmaceutical formulations:** The tablet powder equivalent to 100mg of MIRT was extracted with 3x25 ml of chloroform and filtered.

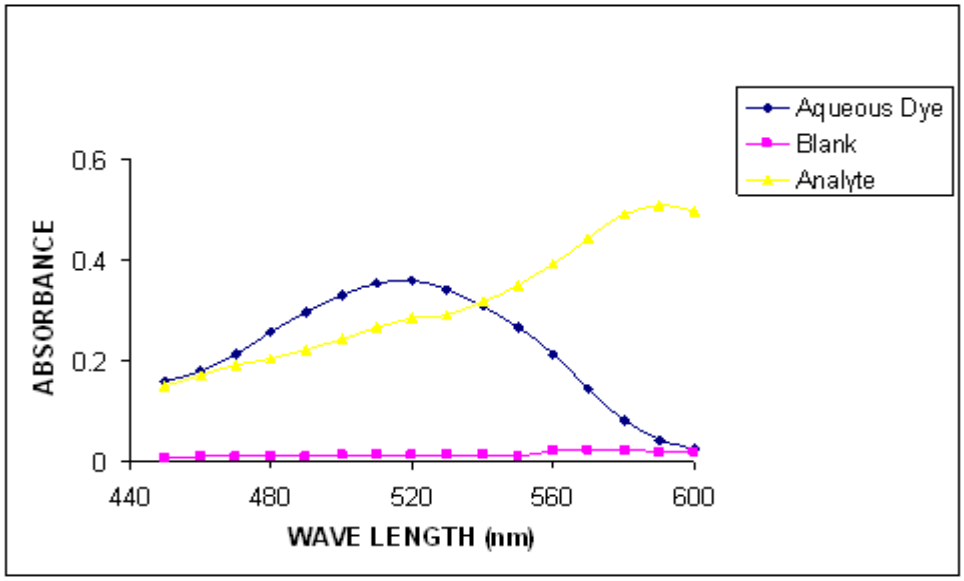


**Figure 3: Absorption spectra of MIRT-FSBF**

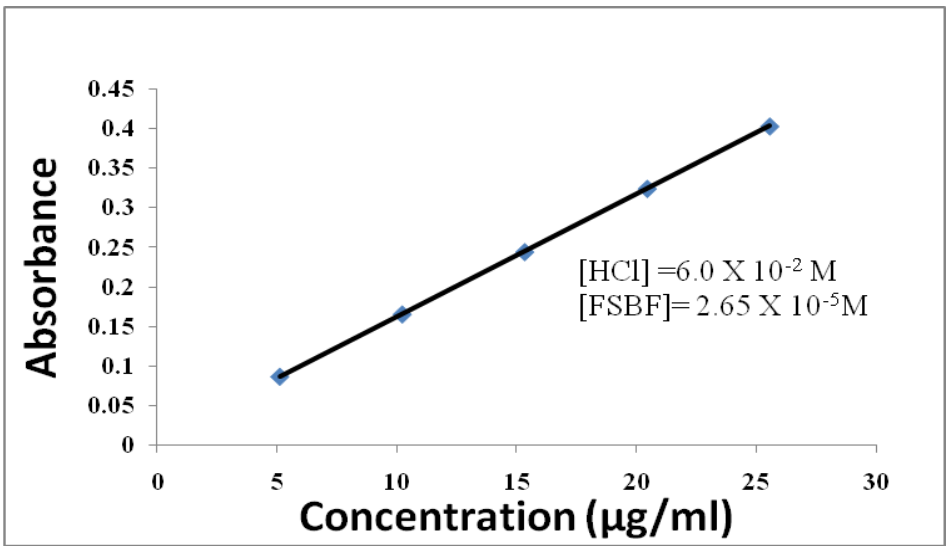


**Figure 4: Beer's law plot of MIRT-FSBF**

The combined filtrate was evaporated to dryness and the residue was dissolved in100ml of distilled water to achieve a concentration of 1mg/ml stock solution. The solution was further diluted step wise with distilled water to get working standard solutions and analysed under procedures described for bulk samples.

## Results and Discussion

Optimum operating conditions used in the procedure were established adopting variation of one variable at a time (OVAT) method. The effect of various parameters such as time, volume and strength of reagents, 0.1MHCl, pH buffer solutions and solvent for final dilution of the colored species were studied. FSBF was preferred for this investigation as they yield high molar absorptivity values among six dyes belonging to different chemical classes.

The water immiscible solvents tested for the extraction of colored complex into organic phase include Chloro Benzene, dichloromethane, carbon tetrachloride, benzene, nitro benzene, n-butanol or chloroform. Chloroform was preferred for its selective extraction of colored drug -dye complex into organic layer from the aqueous phase. The stoichiometric ratio of the dye-drug was determined by the slope ratio method and was found to be 3:1 for method.The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements, Regression characteristics like standard deviation of slope(Sb), standard deviation of intercept (Sa), standard error of estimation (Se) and % range of error (0.05 and 0.01 confidence limits) were calculated and the results are summarized in Table 1.Commercial formulations containing MIRT were successfully analyzed by the proposed methods. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre analyzed formulations at three different concentration levels. The values obtained by the proposed and reference methods for formulations were compared statistically by the t-and F-test and found not to differ significantly. These results are summarized in Table 2.

## Conclusion

A significant advantage of an extraction spectrophotometric determination is that it can be applied to the determination of individual compounds in a multi component mixture. This aspect of spectrophotometric analysis is of major interest in analytical chemistry, since, it offers distinct possibilities in assay of a particular component in a complex dosage formulation. In the present study, MIRT was determined successfully as pure compound as well as a single component in representative dosage formulations. The proposed method applicable for the assay of drug and the advantage of wider range under Beer's law limits. The proposed visible spectrophotometric method is validated as per ICH guide lines and possess reasonable precision, accuracy, simple, sensitive and the proposed method report a new for the determination of MIRT in pharmaceuticals. The method can be extended for the routine assay of MIRT in formulations.

**Table 1**
**Optical and regression characteristics, precision and accuracy of proposed method**

| S. No. | Optical Characteristics | FSBF |
|---|---|---|
| 1 | $\lambda_{max}$(nm) | 520 |
| 2 | Beer's Law limits(µg/ml) | 5-20 |
| 3 | Molar absorptivity(l $mol^{-1}cm^{-1}$) | $2.67x10^{4}$ |
| 4 | Correlation coefficient (r) | o.9999 |
| 5 | Sandell'ssensitivity (µg/$cm^2$/0.001absorbance unit) | $2.52x10^{-3}$ |
| 6 | Regression equation(y=a+bc) (i)slope (b) | 0.0155 |
| | (ii) Standard deviation on intercept($S_b$) | 0.0089 |
| | (iii)intercept (a) | 0.00710 |
| | (iv) standard deviation ($S_a$) | 0.1519 |
| | (v)Standard error of estimation($S_e$) | 0.1449 |
| 7 | Optimum photometric range (µg/ml) | 10.2-25.1 |
| 8 | Relative Standard deviation | 0.5624 |
| 9 | Detection limit | 0.0658 |
| 10 | % of range of error(confidence limit) | 0.5903 |
| | (i)0.05 level | 0.9716 |
| | (ii)0.01 level | |

**Table 2**
**Analysis of Mirtazapine in pharmaceutical formulations by proposed and reference methods**

| Sample | Labeled Amount(mg) | %Recovery | %Recovery |
|---|---|---|---|
| | | **Proposed method** | **Reference Method** |
| Capsule1 | 200mg | 99.76 ± 0.43<br>t = 1.08<br>F = 2.65 | 99.51± 0.70 |
| Capsule2 | 200mg | 99.17 ± 0.67<br>t = 1.21<br>F = 1.32 | 99.93 ± 0.77 |
| Capsule3 | 200mg | 99.58 ± 0.71<br>t = 1.21<br>F = 1.53 | 99.48 ± 0.88 |
| Capsule4 | 200mg | 99.91± 0.22<br>t = 1.23<br>F = 2.53 | 99.19 ± 0.35 |

Two different batches of capsules from two different Pharmaceutical companies
+Average ±Standard deviation of six determinations, the t-and f-tests values
refer to the comparison of the proposed method with the reference method

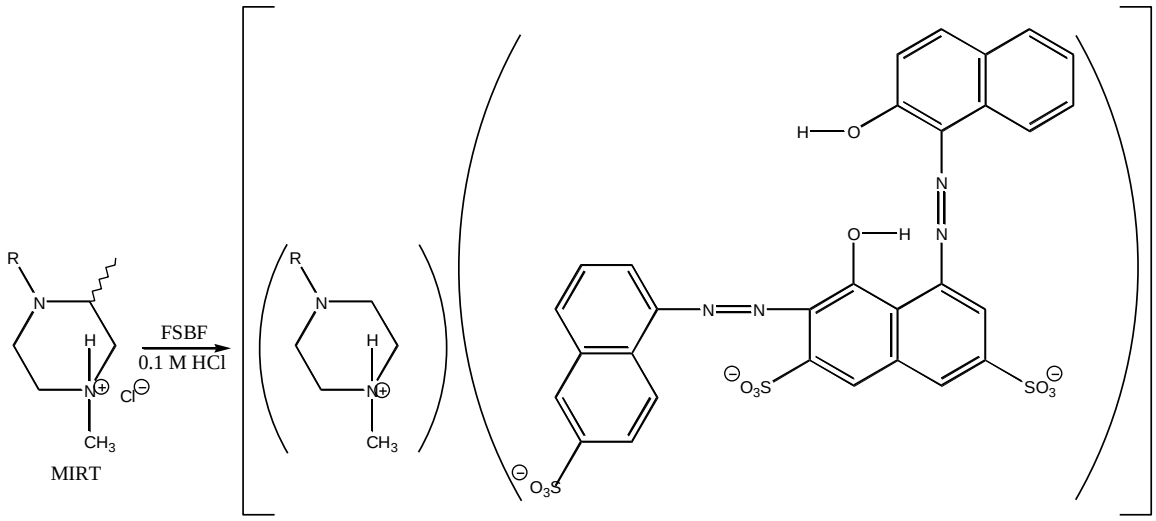


**Figure 5: Ion-Association Complex of MIRT-FSBF**

**Chemistry of colored species:** The protonated Nitrogen (positive charge) of the drug molecule in acid medium is expected to attract the oppositely charged part (negative charge) of the dye and behave as a single unit being held together by electrostatic attraction as given in scheme (Figure 5).

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