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RESEARCH ARTICLE

Ion-association Methods For The Determination of Mirtazapine in Pure and Pharmaceutical Formulations

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ABSTRACT

Two simple and sensitive extractive visible spectrophotometric methods (A and B) for the assay of Mirtazapine in pure and pharmaceutical Formulations based on the formation of colored chloroform soluble ion-association associates under specified experimental conditions are described. Two dyes namely acidic dye Tropaeoline ooo (TPooo, method A), Azocarmine-G (ACG, method B) are utilized. The extracts of the ion-associates exhibit absorption maxima at 480nm and 540nm for methods A and B respectively. Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges (1-3) µg/ml for method A, (2-10) µg/ml for method B respectively. The proposed methods are 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. These methods are applied successfully for the estimation of the Mirtazapine in the presence of other ingredients that are usually present in formulations. These methods offer the advantages of rapidity, simplicity and sensitivity and low cost without the need for expensive instrumentation and reagents.

Key Words: Anti fungal, Azocarmine-G (ACG), Assay, Ion-Association methods, Statistical analysis, Tropaeolineooo(TPOOO)

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INTRODUCTION

Mirtazapine[1-6]is 1,2,3,4,10,14b-hexahydro-2-methylpyrazino [2,1-a] pyrido [2,3-c] benzazepine. Mirtazapine 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. Its molecular weight is 265.36. The structural formula is the following and it is the racemic mixture. Mirtazapine is a white to creamy white crystalline powder which is slightly soluble in water. It disintegrates in the mouth within seconds after placement on the tongue allowing its contents to be subsequently swallowed with or without water. REMERON SoITab also contains the following inactive ingredients: aspartame, citric acid, crosprovidone,hydroxypropyl methylcellulose, magnesium stearate, mannitol, microcrystalline cellulose, natural and artificial orange flavour, poly-methacrylate, povidone, sodium bicarbonate, starch, sucrose. Mirtazapine enhances central noradrenergic and serotonergic activity. These studies have shown that mirtazapine acts as an antagonist at central presynaptic $(alpha)_2$ adrenergic inhibitory autoreceptors and heteroreceptors, an action that is postulated to result an increase in central noradrenergic and serotonergic activity. Mirtazapine is a potent antagonist of 5-HT_2 and 5 –HT_3 receptors. Mirtazapine has no significant affinity for the 5 – HT $_{1A}$ and 5 – HT $_{1B}$ receptors. Mirtazapine is 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.

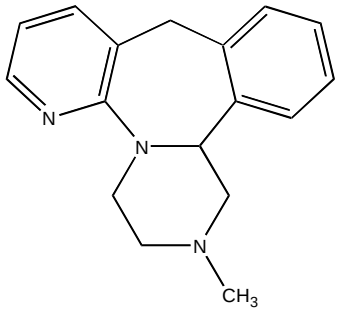

Figure 1: Chemical structure of Mirtazapine

Several analytical techniques like HPLC[7-12], HPLC-TDMS[13-16], LC methods [17-18], RP-HPLC[20], LC-MS[21-23], and UV derivative spectrophotometry [24-27] , have been reported in the literature. Upon thorough survey of literature 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 dyes namely TPooo or ACG 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 two simple and sensitive extraction visible spectrophotometric methods for the determination of MIRT, based on its tendency to form chloroform extractable ion-associates with acidic dyesTPooo[28-33] belonging to azo category dye (method A) or AzocarmineG[34] belonging to Phenazine category dye (method B) under experimental conditions by exploiting the basic nature(nitrogen in triazole linked to 1-butan-2-ol) of the drug molecule. According to the literature, it is the first time for MIRT determination in formulations by visible spectrophotometry. The proposed methods for MIRT determination have 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 in expensive and available in any analytical laboratory. The proposed methods report a new for the determination of MIRT in pharmaceuticals. These methods can be extended for the routine assay of MIRT formulations.

MATERIALS AND METHODS:

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.Tropaeolin000(Fluka,0.2%,5.7x10-3M prepared by dissolving 200mg of Tropaeolin000 in 100ml distilled water and subsequently washed with chloroform to remove chloroform soluble impurities),0.1MHCl (prepared by diluting 8.7ml of Conc. Hydrochloric acid to1000ml with distilled water and standardized) ACG solution(Gurr,0.05%,8.75x10-4M prepared by dissolving 50mg of Azocarmine G in 100ml of distilled water containing traces of sodium hydroxide and subsequently washed with chloroform to remove chloroform soluble impurities), pH 1.5 Buffer solution (prepared by mixing 289ml of 0.1M glycine solution(7.507g of glycine and 5.85g NaCl was dissolved in 100ml of distilled water) with 711ml of 0.1M HCl and the pH of the solution was adjusted to1.5) were prepared.

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.

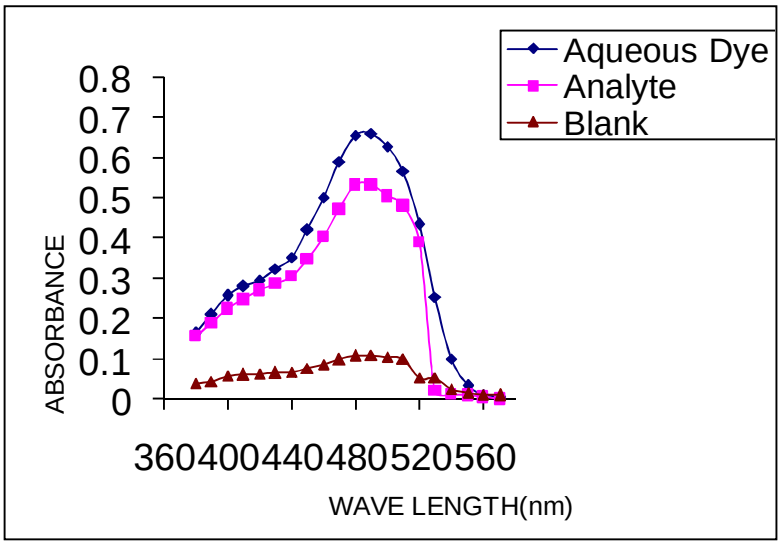

Fig.2: Absorption spectra of MIRT- TPOOO

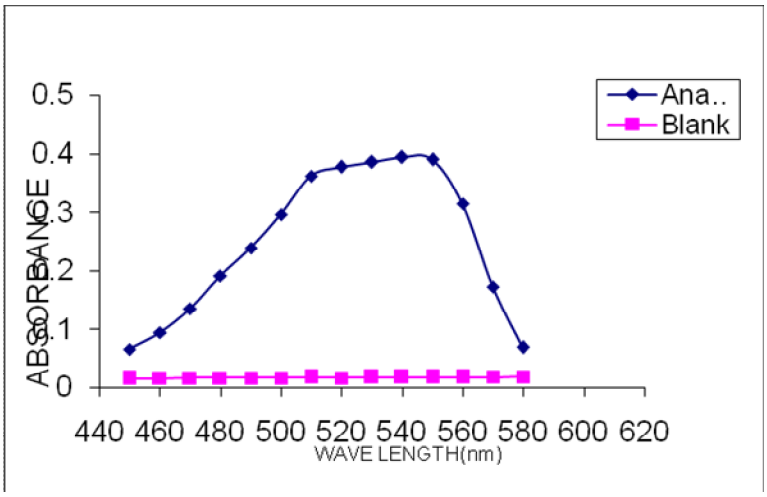

Fig.3: Absorption spectra of MIRT-ACG

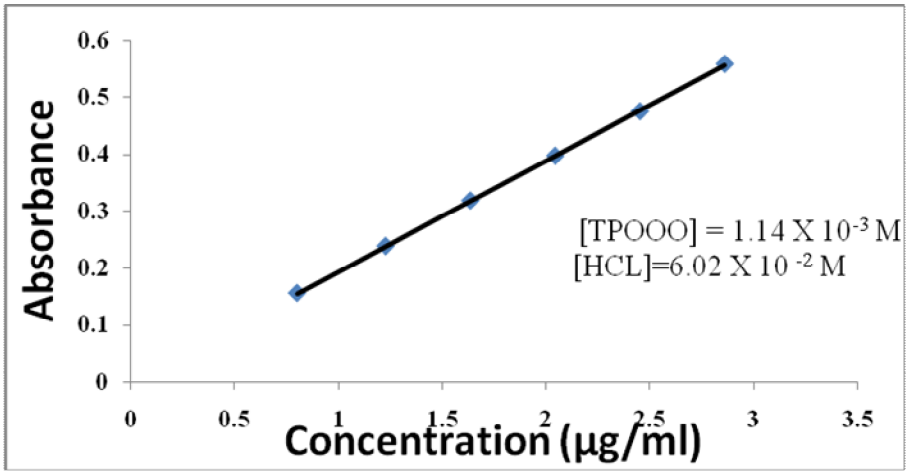

Fig.4: Beer's plot off MIRT- TPOOO

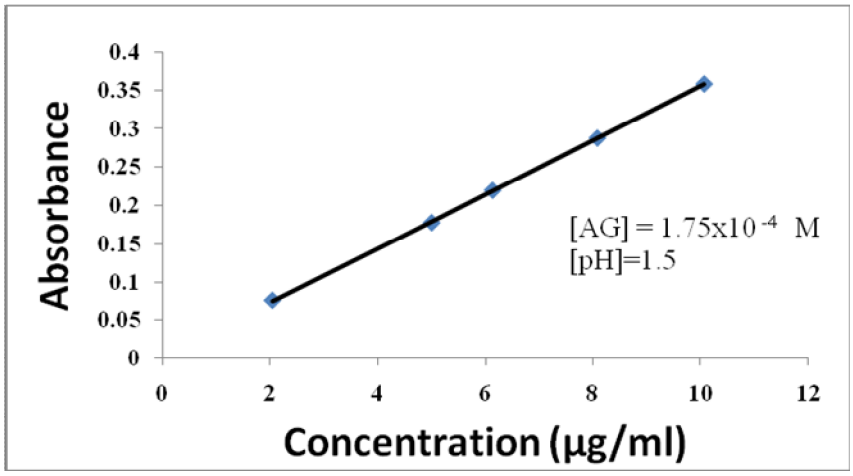

Fig.5: Beer's plot off MIRT-ACG

Assay:

Aliquots of the standard MIRT solution [1.0-5.0 ml,50µg/ml (method A) and 1.0-5.0ml, 100µg/ml (method B)]were placed in a series 125ml separating funnels. Then6.0ml of 0.1M HCl and 2.0ml of TPooo solution $5.70x10^{-3}$M(for method A) or 6.0 ml of pH 1.5 buffer solution and2.0 ml of ACG solution (8.75x10-4M)(for method B) were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0ml with distilled water. Then 10.0ml of chloroform was added to each separating funnel and the contents were shaken for 2minutes. The two phases were allowed to separate. The absorbance of the separated chloroform layer were measured at 500nm (method A) or 550 nm (method B) (**Fig**.**2**and**3** showing

absorption spectra) against a reagent blank within the stability period (5 minutes to 1hour). The amount of drug was computed from its calibration graph.(**Fig.4**,**5**)

Chemistry of colored species:

The protenated 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(**Fig.6,7**).

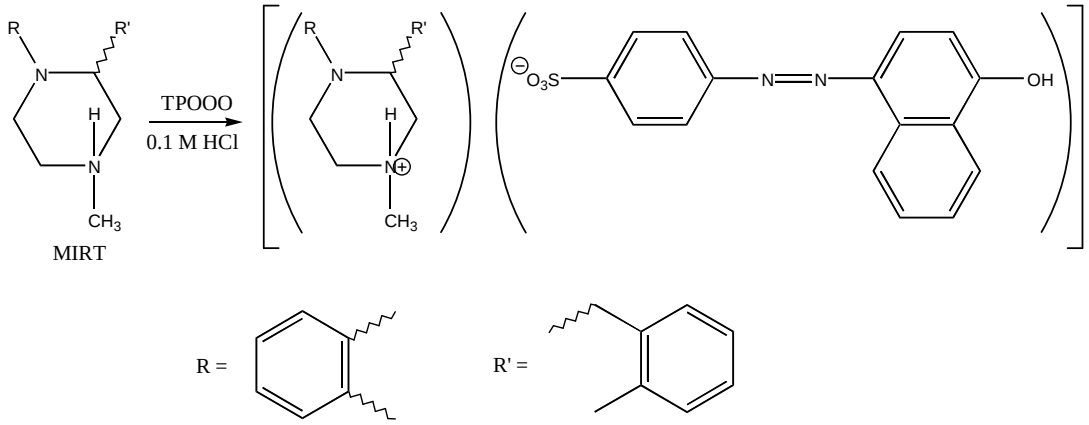

Fig. 6: Probable scheme of reaction for method A

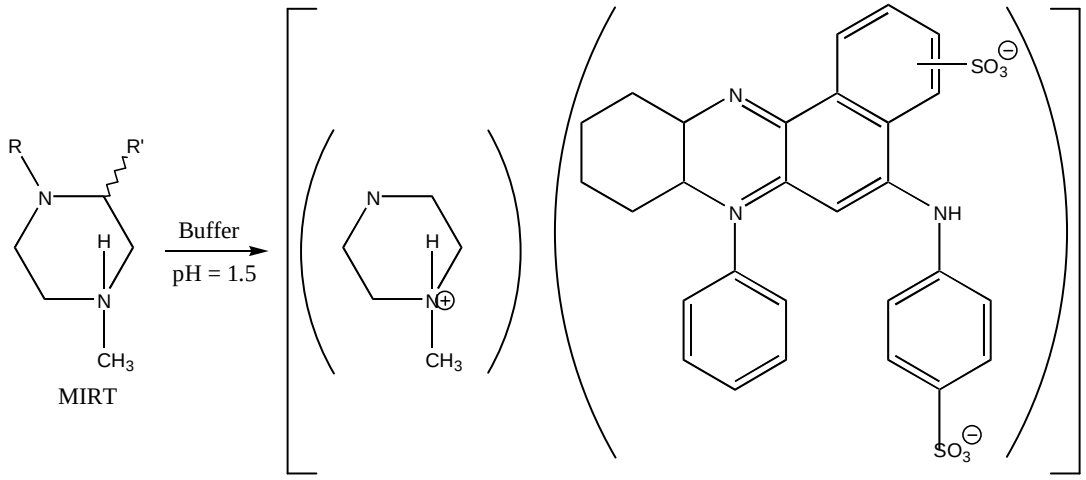

Fig.7: Probable scheme of reaction for method B

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 TPooo, ACG reagents, 0.1MHCl, pH buffer solutions and solvent for final dilution of the colored species were studied. TPooo and ACG were 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 1:1 for methods A and B respectively. 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 0.01confidence limits) were calculated and the results are summarized in Table-1.Commercial formulations containing MIRT were successfully analyzed by the proposed methods. 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. 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. These results are summarized in Table-2.

Table.1: OPTICAL AND REGRESSION CHARECTERISTICS, PRECISION AND ACCURACY OF PROPOSED METHODS

S.No	OPTICAL CHARACTERISTICS	METHOD.A	METHOD.B
1	λ_{max}(nm)	480	540
2	Beer's Law limits(µg/ml)	1-3	2-10
3	Molar absorptivity (l $mol^{-1}cm^{-1}$)	$3.70x10^4$	$2.37x10^4$
4	Correlation coefficient (r)	0.9996	o.9999
5	Sandell's sensitivity (µg/cm^2/0.001absorbance unit)	$2.0x10^{-4}$	$1.2x10^{-3}$
6	Regression equation(y=a+bc) (i)slope (b)	-3.3775	0.035210
	(ii) Standard deviation on intercept(S_b)	0.097	0.097
	(iii)intercept (a)	-3.3775	-3.3775
	(iv) standard deviation (S_a)	0.3760	0.1386
	(v)Standard error of estimation(S_e)	0.1675	
7	Optimum photometric range (µg/ml)	1.2-2.9	4.89-10.4
8	Relative Standard deviation	0.3842	2.098
9	Detection limit	0.0277	0.1828
10	% of range of error(confidence limit) (i)0.05 level	0.4032	2.4125
	(ii)0.01 level	0.6678	4.3502

*Y= a + b c; Where Y= absorbance, c= concentration of MIRT in µg/ml.

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

SAMPLE	LABELLED AMOUNT(mg)	% RECOVERY BY PROPOSED METHODS		% RECOVERY BY REFERENCE METHOD
Tablets-T_1	200mg	99.02 ± .32 t = 0.98 F =1.63	99.98 ± 0.98 t = 0.48 F =1.51	99.41 ± 0.25
Tablets-2	200mg	100.92 ± 0.37 t = 0.32 F = 3042	99.89 ± 0.39 t = 0.98 F = 1.57	99.66 ± 0.26
Tablets – T_3	200mg	99.01 ± 0.47 t = 0.27 F = 1.39	100.37± 0.16 t = 1.95 F = 3.18	99.46 ± 0.49
Tablets – T_4	200mg	99.95 ± 0.34 t = 0.40 F = 2.16	100.30± 0.27 t = 0.26 F = 1.98	99.76 ± 0.38

*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.

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 methods applicable for the assay of drug and the advantage of wider range under Beer's law limits. The proposed extractive visible spectrophotometric methods are validated as per ICH guide lines and possess reasonable precision, accuracy, simple, sensitive and the proposed methods report a new for the determination of MIRT in pharmaceuticals. These methods can be extended for the routine assay of MIRT formulations

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