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Validation of a HPTLC densitometry method for the quantitative determination of mirtazapine in tablet formulations

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ABSTRACT

A sensitive, selective and precise HPTLC method of analysis of mirtazapine in formulations was developed and validated. The method employed TLC aluminium plates precoated with silica gel 60 F254 as the stationary phase. The solvent system consisted of toluene-acetone-methanol solution (6:2:2v/v/v). This system was found to give compact spots for mirtazapine (Rf value of 0.50±0.02). Densitometric scanning of mirtazapine was carried out in the absorbance/reflection mode at 298 nm. The linear regression analysis data for the calibration plots showed good linear relationship with r=0.999, and respectively. The method was validated for precision, robustness, ruggedness and recovery. The limits of detection and quantification were 24.37ng per spot and 81.25ng per spot, respectively.

KEYWORDS

Mirtazapine;
HPTLC;
Method validation;
2-[4-methyl-2-phenyl-piperazin-1-yl]-nicotinic acid.

INTRODUCTION

Mirtazapine(1,2,3,4,,10,14b-hexahydro-2-methyl-pyrazino[2,3-c][2-benzazepine]) (Figure 1).It is efficacious in the short-term and continuation of moderately and severely depressed hospitalized and out-patients. Mirtazapine blocks directly presynaptic $\propto_2$-dreno receptors($\propto_2$ auto receptors)resulting in an increased release of nonadrenaline and subsequently enhanced noradrenergic neuro-transmission[1-3].

Literature survey reveals individual determination of mirtazapine in pharmaceutical formulations using HPLC, CZE, spectrophtometric, and spectrofluorimetric[4], UV spectrophotometric method[5], HPLC and CE[6]. However, no HPTLC method has yet been reported for the determination and validation of mirtazapine in pharmaceutical formulations. Therefore, we have chosen quantitative densitometric HPTLC for the determination and validation of mirtazapine in pharmaceutical formulations and here in we describe the details of our investigative study.

EXPERIMENTAL

Materials

Mirtazapine(99.53 %) was gifted from Neuland Laboratories ltd, Hyderabad, India. All chemicals and reagents used were of analytical grade & were purchased from Merck. Mirtaz-15(label claimed mirtazapine 15mg) tablets were procured commercially.

Standard solutions and calibration curves

A stock solution of mirtazapine was prepared by dissolving the authentic sample in methanol to obtain a

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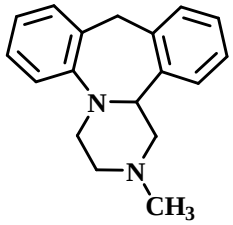

Figure 1 : Chemical structure of mirtazapine

concentration of 1mg/ml. This was used to construct a calibration plot by applying 3,4,5,6,7 and 8µl in the concentration range of 300-800ng per spot. Linear regression data for the calibration curves(n=3) as shown in TABLE 1 showed a good linear relationship over the concentration range 300-800ng per spot with respect to peak area. Further, significant difference was observed in the slope of standard curves($p<0.01$).

Sample preparation

To determine the concentration of mirtazapine in tablets(labeled claim 5.45mg of NB per tablet), the content of 20 tablets was weighed, powdered, the powder equivalent to10 mg of mirtazapine was weighed in 10ml volumetric flask, and the drug from powder was extracted with methanol. To ensure the completion of extraction of the drug it was further sonicated for 30minutes at room temperature ($25^0C\pm2$). (Fast Clean Ultrasonic Cleanar, Enertech Electronics pvt,ltd, Mumbai). The volume was made up to 10ml. The supernatant layer(1ml) was (filtered through a whatman no.42 filter paper) collected and further dilutions were made by adding methanol at the time of analysis. A sample solution (1µl, containing of 600ng) was applied to the plates for assay of Mirtazapine.

HPTLC instrumentation

TLC was performed on 20×10cm HPTLC plates pre coated with 60F-254 (With 0.25mm thickness; Merck, Darmstadt, Germany) and the plates were washed with methanol before use and activated at 110^0C for 5minutes. The samples were spotted in the form of bands of 4mm width using Linomat IV applicator (Muttenz, Switzerland, supplied by Anchrom technologists, Mumbai) equipped with 100µl syringe. A constant application rate of 6µl/sec was employed and the space between two bands was 6mm.The slit dimension was kept at 4×0.45mm and a scanning speed of 20 mm/sec was employed. The mobile phase consisted of toluene: acetone: methanol in a ratio of (6:2:2 v/v/v) and 10 ml of mobile phase was used for chromatography. Linear ascending development was carried out in 20×10cm twin trough glass chamber (Camag, Muttenz, Switzerland). The chamber was saturated, prior to placing the TLC plate, with mobile phase at room temperature for 20minutes. The optimized chamber saturation time for mobile phase was 20min. at room temperature($25^0C\pm2$) at relative humidity of 60%±5. The length of chromatogram run was 8cm and subsequent to the development, the TLC plates were dried in a current of air with the help of a dryer in wooden chamber with adequate ventilation. Densitometric scanning was performed on Camag TLC scanner III in the reflectance-absorbance mode at 298nm and operated by CATS 4 software(v 4.05, Camag) resident in the system. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 190 and 360nm and concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light.

TABLE 1: Linear regression data for the calibration curves(n=3)

Linear range	300-800ng/spot
Correlation coefficient (r)	0.9990
Slope±S.D	4.516
Confidence limit of slope[a]	4.408-4.623
Intercept[b]	3107.9
Confidence limit of intercept	3046.1-3169.8

[a]95% confidence limit, [b]percentage of bias of intercept=95%

RESULTS AND DISCUSSION

Development of the optimum mobile phase

In an attempt to optimize the mobile phase, varying concentrations of toluene: acetone: methanols were tried. However, toluene: acetone: methanol in a ratio of (6:2:2 v/v/v) offered a sharp and well-defined peak of mirtazapine at R_f value of 0.50±0.02. It has also been observed that well defined spots were obtained only when the chamber was saturated with the mobile phase for 20min at room temperature prior to plate development.

Validation of the method

Precision

The intra day and inter day precision of the method was estimated by means of six determinations of mirtazapine in low(300ng/spot), middle(500ng/spot) and high (800ng/spot) concentrations of sample solution. The results of precision study are depicted in TABLE 2[7].

Robustness of the method

By introducing small changes in the mobile phase composition and plate run length, the effects on the results were examined in sample solution. Mobile phase having different compositions of toluene-acetone-methanol(6:2:2 and 5.5:2.5:2.0 v/v/v) were tried along with two different run lengths(8cm and 9cm) at two different concentration levels of 400 and 600 ng per spot[7]. The low values of %RSD obtained after introducing small changes in mobile phase composition and plate run length indicated robustness of the method as indicated in TABLE 3.

TABLE 2 : Intra- and inter-day precision by HPTLC method[a]

Amount	Intra-day precision	Inter-day precision		
(ng/spot)	Area	%R.S.D	Area	%R.S.D.
300	4471.15	0.31	4468.58	0.51
500	5420.36	0.36	5418.26	0.55
800	6654.55	0.42	6654.73	0.50

[a]n=6.

TABLE 3: Robustness of the method[a]

Parameter	% Recovery[b]	%R.S.D.[b]
Mobile phase composition		
I. T-A-M(6:2:2)	100.30	0.55
II.T-A-M(5.5:2.5:2)	99.25	1.13
Plate run length		
I. 8cm	100.12	0.50
II. 9cm	99.89	0.69

[a]n=6, [b]Average of two concentrations 400 and 600ng/spot

TABLE 4 : Recovery studies(n=3)

Excess of drug added to the analyte (%)	theoretical content(ng)	Recovery(%)	%R.S.D.
0	300	100.46	1.07
50	450	98.77	1.34
100	600	99.15	1.28
150	750	99.64	1.52

TABLE 5 : Ruggedness of the method[a]

	% Recovery	% R.S.D
Analyst I	99.86	0.55
Analyst II	99.76	0.53

[a]n=6, [b]Average of two concentrations 400 and 600ng/spot

LOD and LOQ

The limit of detection and quantification were calculated based on the slope(s) of the calibration curve and the standard deviation of response(S.D) using the formula LOD=3.0 S.D/S and for LOQ=10 S.D/S[8]. Detection limit and quantification limit were calculated by the method as described above and found to be 26.02ng and 86.74ng, respectively, which establishes the adequate sensitivity of the method.

Specificity

The specificity of the method was ascertained by analyzing reference standard and samples. The spots for mirtazapine in pharmaceutical formulations were confirmed by comparing the R_f and spectra of the respective separated spots with that of standard(Figure 3). The peak purity of mirtazapine was assessed by comparing the spectra at three different levels, i.e. peak start(S), peak apex(M) and peak end(E) positions of the spot i.e. r(S,M)=0.9992 and r(M,E)=0.9990.Good correlation(r=0.9994) was also obtained between standard and sample spectra of mirtazapine as depicted in figure 4[7].

Recovery studies

The analyzed samples were spiked with an additional 50,100 and 150ng of the standard mirtazapine and the mixtures were reanalyzed by the proposed method. The experiment was carried out in triplicate. This had to be done to check the recovery of the drug at different levels in the formulations and drug afforded recovery of 98% -101% as listed in TABLE 4.

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating conditions from instrument to instrument and from analyst to analyst. The ruggedness was tested by applying 400 and 600 ng/spot and the results are presented in TABLE 5 [7].

Assay of mirtazapine in tablets

The applicability of the proposed method was examined by assay determination of miratazapine in tablets by spotting 600ng/spot. The spots at R_f 0.50±0.02

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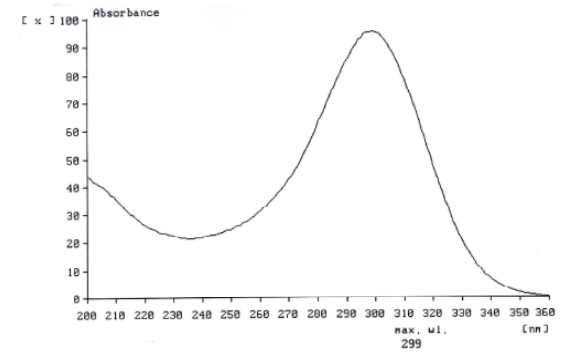

Figure 2 : In-situ UV spectrum of standard mirtazapine

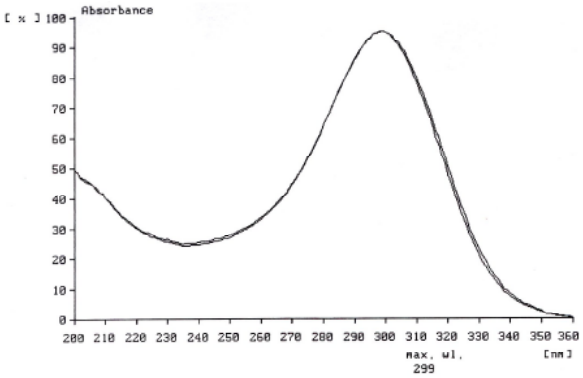

Figure 3 : In-situ UV spectrum of standard mirtazapine and sample mirtazapine

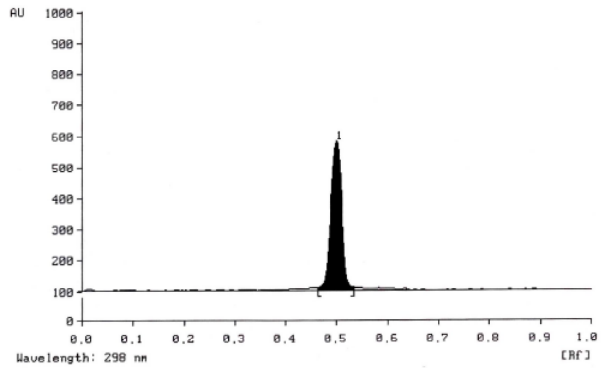

Figure 4 : HPTLC Densitogram of sample mirtazapine 600 ng(Mobile phase:Toluene: Acetone:Methanol 6:2:2v/v/v)

for mirtazapine were observed in the densitogram of the drug samples extracted from tablets(Figure 4) and it is evident that there was no interference from the excipients commonly present in the tablets. The drug content was found to be 98.58%(%R.S.D. of 1.15)(n=6) for mirtazapine. The low % R.S.D. value indicated the suitability of this method for routine analysis of mirtazapine in pharmaceutical dosage form as it could be validated in accordance with the specifications stipulated by regulatory standards for pharmaceuticals.

CONCLUSION

The developed HPTLC technique is precise, specific, robust and accurate and finds a potent application in routine quality control analysis of pharmaceutical formulations.

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