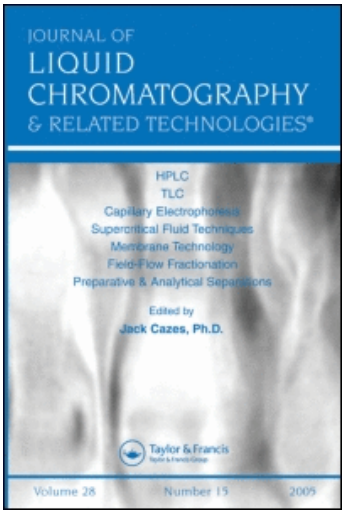

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T. Sheshashena Reddy[a]; P. Sita Devi[a]
[a] Analytical Chemistry Division, Indian Institute of Chemical Technology, Tarnaka Hyderabad-, India

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Simultaneous Determination of Mirtazapine and its Three Main Impurities by a High Performance Thin Layer Chromatography/ Densitometry Method

T. Sheshashena Reddy and P. Sita Devi

Analytical Chemistry Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad, India

Abstract: A quantitative densitometric high performance thin layer chromatography (HPTLC) method was developed for the simultaneous separation and quantification of Mirtazapine and its structurally related impurities. The three different impurities accounting for 3.3% were identified using TLC aluminium plates precoated with silica gel 60 F254 as the stationary phase. The mobile phase consisted of a saturated solution of toluene-acetone-methanol solution (6:2:2 v/v/v) and UV detection by absorbance reflectance at 285 nm facilitated the quantitative identification of the impurities along with the drug molecule. The minimum amount of Mirtazapine that could be authentically detected and quantified was 22.34 ng/spot and 74.47 ng/spot, respectively. The results of the present work showed that scanning densitometric HPTLC with online UV detection was simple, selective, accurate, and proved to be a valuable complementary method for quantitative evaluation of bulk drugs in the presence of their impurities.

Keywords: Mirtazapine, HPTLC, Densitometry, Impurities

INTRODUCTION

Mirtazapine (1,2,3,4,10,14b-hexahydro-2-methyl-pyrazino[2,3-c][2-benzazepine]) (Figure 1) performs effectively in the short term and prolonged treatment of moderately depressed, as well as, severely depressed patients

Correspondence: P. Sita Devi, Analytical Chemistry Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad – 500007, India. E-mail: sitadevi@iictnet.org

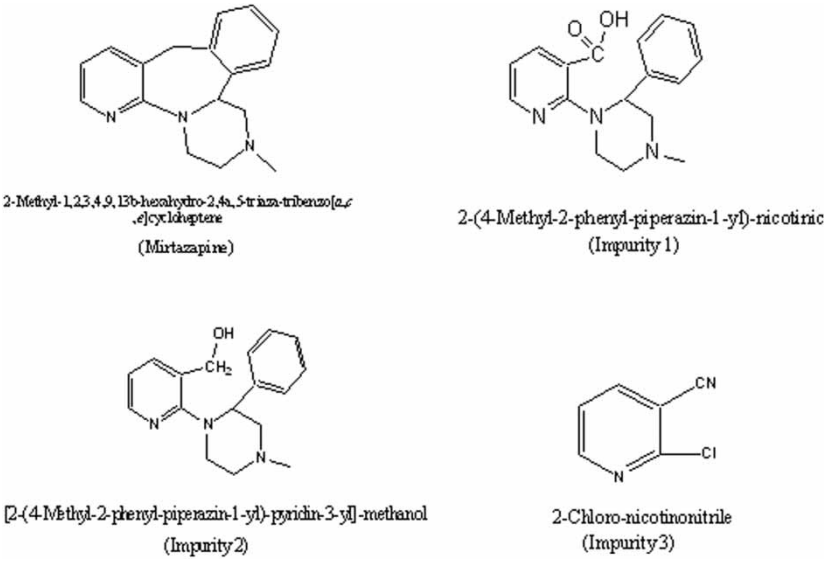

Figure 1. Structures of Mirtazapine and its three impurities.

with hospitalization. Mirtazapine (Mzp) blocks directly presynaptic $\propto_2$-adreno receptors ($\propto_2$ auto receptors) resulting in an increased release of noradrenaline and, subsequently, enhanced noradrenergic neurotransmission.[1–4]

Samples of Mzp may contain some structurally related impurities (Figure 1) derived from the manufacturing process, such as 2-[4-methyl-2-phenyl-piperazin-1-yl]-nicotinic acid (Impurity 1), 2-[4-methyl-2-phenyl-piperazin-1-yl]-pyridin-3-yl]-methanol (Impurity 2), and 2-chloro-nicotinonitrile (Impurity 3). An impurity is a component of a drug product, which is not the active drug substance or an excipient in the drug product, which should not be present beyond certain threshold limits as defined by ICH guidelines.[5–7] Therefore, care is exercised from initial stages of the drug development of a potential bulk drug to the quality control of a marketed pharmaceutical product[8] to fulfill the specified requirements of regulatory agencies with respect to toxicity and safety aspects.

The literature survey reveals that various analytical methods have been reported for the analysis of Mzp in pharmaceutical formulations.[9–11] However, no available chromatographic method for the simultaneous separation of Mzp from its structurally related impurities has been reported. HPTLC has a potential which meets the demands of a routine analytical technique due to its advantages of low operating cost, high sample throughput, and need for minimum sample clean up. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase, unlike HPLC, thus lowering the analytical run times and cost per analysis. In pharmaceutical laboratories, there is always a need for

faster, simpler, cheaper, and better performing analytical methods. Further, TLC and HPTLC in instrumentalized mode using scanning densitometry have been included as general methods in European Pharmacopoeia, permitting the use of planar chromatography for quantification at different stages of pharmaceutical research, development, and production.[12]

Furthermore, one of the main advantages of planar chromatography is its potential ability to facilitate separations, which can be successfully utilized to evaluate very different drug molecules, their impurities, and the metabolites. Generally, the separations are discrete and very often complementary to other classified techniques, such as HPLC. Therefore, HPTLC can be a viable alternative for impurity profiling and characterization of newer drugs and the unknown compounds. Although, many reports are available for the determination of Mzp, the impurities of this anti depressant drug have not been separated and quantified till date. Hence, the objective of the present study is to develop a new HPTLC method for simultaneous separation and identification of Mzp and the impurities in bulk drug. The optimization of the method separation, evaluation, and quantification of the process components of Mzp in bulk drug, and advantages of the HPTLC approach are reported in the following sections.

EXPERIMENTAL

Materials

Mirtazapine (Mzp) and its related impurities were gifted by Neuland Laboratories Ltd, Hyderabad, India. All chemicals & reagents used were of analytical grade & were purchased from Merck.

Standard Solutions

Standard solutions of Mzp and related impurities were prepared by dissolving each of the compounds in methanol to obtain a concentration of 1 mg/mL. Before analysis, the required concentrations of Mzp (4000–9000 ng/spot) and related impurities (100–600 ng/spot) were prepared. Standard stock solutions of all the compounds were stored at 4°C until further analysis.

HPTLC Instrumentation

The samples were spotted in the form of bands of width 4 mm with a Camag microlitre syringe on precoated silica gel aluminium plate 60F-254, (20 × 10 cm with 0.25 mm thickness; Merck, Darmstadt, Germany) using a Linomat IV sample applicator (Camag, Muttenz, Switzerland, supplied by Anchrom technologists, Mumbai). A constant application rate of 6 μL/sec

was employed with 6 mm space between two bands. The slit dimension was kept at 4 × 0.45 mm and 20 mm/sec scanning speed was employed. The mobile phase consisted of toluene-acetone-methanol (6:2:2 v/v/v) and 20 mL of mobile phase were used per chromatography.

Linear ascending development was carried out in a 20 × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 25 min at room temperature. The length of the chromatogram run was 8 cm and subsequent to the development, the TLC plates were dried in a current of air with the help of an air dryer in a wooden chamber with adequate ventilation. The flow of air in the laboratory was maintained unidirectional (laminar flow, towards exhaust).

Densitometric quantification was performed (Camag TLC scanner III) at 285 nm by reflectance scanning and operated by cats 4 software (v 4.05, Camag) resident in the system. The source of radiation utilized was a deuterium lamp emitting a continuous UV spectrum between 190 and 360 nm. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was via peak areas with linear regression.

RESULTS AND DISCUSSION

Mirtazapine is a tetra cyclic piperazinoazepine, which has a different structure from any other currently used antidepressants and the impurities are structurally related. Nevertheless, TLC effectively facilitated well resolved separation of the main compound (Mzp) from its impurities, which is clearly

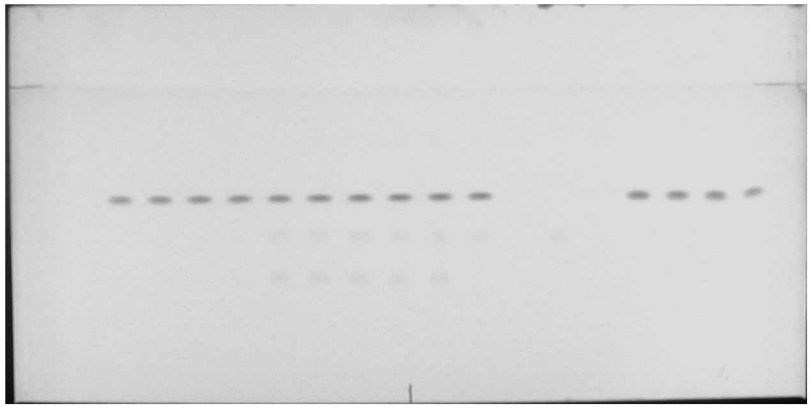

Figure 2. Video image showing separation of Mirtazapine and its impurities at lower concentrations (100 ng/spot of impurity) where the peak at Rf 0.81 is not visible.

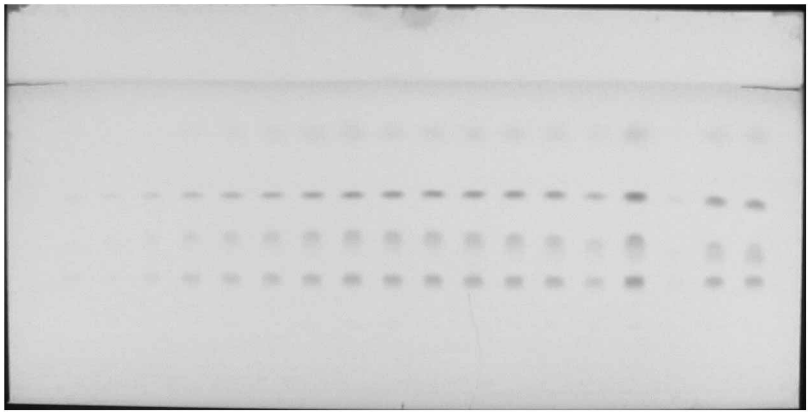

Figure 3. Video image showing separation of Mirtazapine and its impurities at higher concentrations.

visible under UV light, and the same has been reproduced as video images (Figures 2 and 3) of the developed TLC plates.

In order to optimize the conditions for appropriate mobile phase, various ratios of toluene:acetone:methanol were attempted. However, toluene: acetone:methanol (6:2:2 v/v/v) facilitated a sharp and well resolved peaks for Mzp (0.52 $\pm$ 0.02), Impurity 1 (0.21 $\pm$ 0.02), Impurity 2 (0.37 $\pm$ 0.02) and Impurity 3 (0.81 $\pm$ 0.02), respectively. R_f values and wavelength of absorption maxima (λ_{max}) of Mzp and related impurities are given in Table 1. Scanned profiles of chromatographic separation are shown in Figures 4 and 5. In situ spectra taken for Mzp and the impurities were measured from 190 to 360 nm. (Figure 6). The wavelength at 285 nm was selected for detection because it resulted in better detection sensitivity for Mzp and related impurities compared to the well accepted 254 nm.

Linearity

Linear regression data for the calibration curves (n = 3) as depicted in Table 2, showed a good linear relationship over the concentration range of 4000–

Table 1. R_f and λ max value of Mirtazapine and impurities

Compound name	Substance	R_f	λ max
Mirtazapine acid	Impurity 1	0.21 $\pm$ 0.02	277 nm
Mirtazapine alcohol	Impurity 2	0.37 $\pm$ 0.02	278 nm
Mirtazapine	Bulk drug	0.51 $\pm$ 0.02	299 nm
2-Cl 3-Cyanopyridine	Impurity 3	0.81 $\pm$ 0.02	278 nm

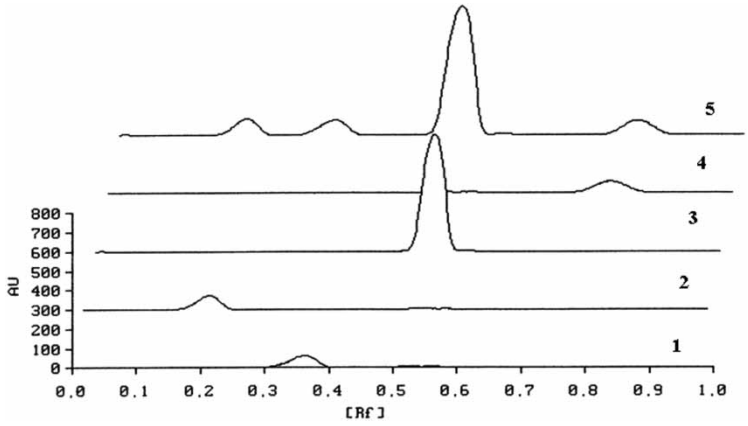

Figure 4. Densitogram showing all tracks of Mirtazapine and its impurities. 1-Impurity 2, 2-Impurity 1, 3-Mirtazapine, 4-Impurity 3, 5-Mirtazapine spiked with all the three impurities.

9000 ng per spot, 100–600 ng per spot, 100–600 ng per spot, and 100–600 ng per spot for Mzp, Impurities 1, 2, and 3, respectively, with respect to peak area.

Limit of Detection and Limit of Quantification

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

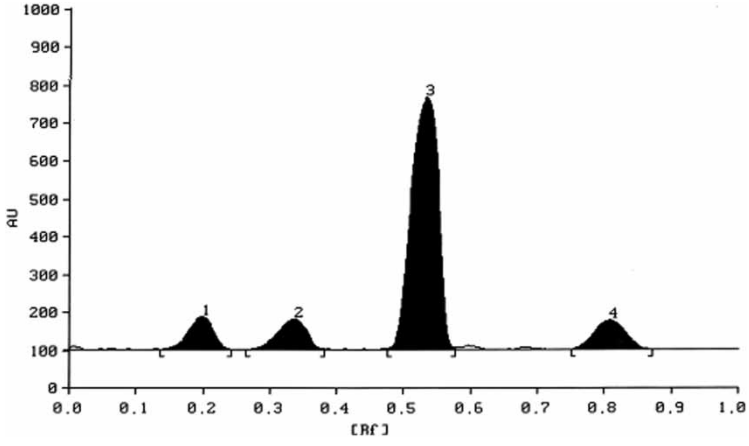

Figure 5. Densitogram showing the separation of Mirtazapine and its impurities using toluene:acetone:methanol (6:2:2 v/v/v) at 285 nm.

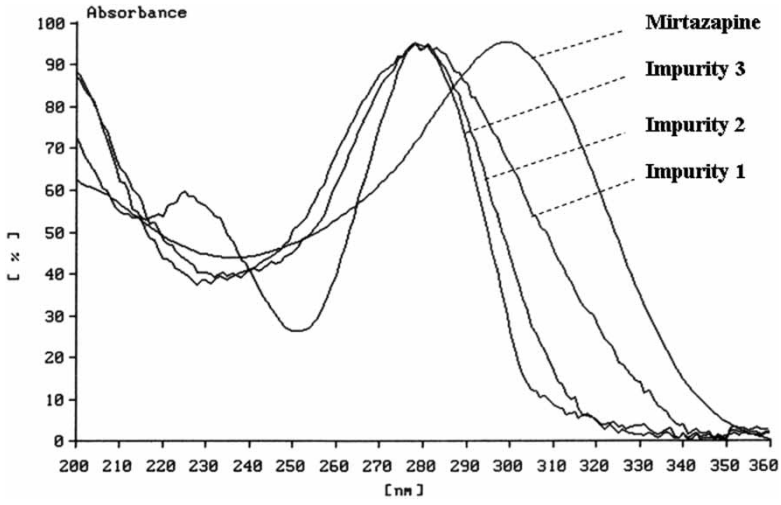

Figure 6. In situ UV spectra procured at 285 nm by HPTLC.

formula LOD = 3.0 S.D/S and for LOQ = 10 S.D/S[13] and LOD, LOQ of Mzp and the impurities are shown in Table 2.

Specificity

The specificity of the method was ascertained by spiking experiments. Thus, Mzp bulk drug was spiked with known quantities of related impurities where it

Table 2. Linear regression data for the calibration curves (n = 3)

Parameter	Mirtazapine	Impurity 1	Impurity 2	Impurity 3
Linear range	4000–9000 ng/spot	100–600 ng/spot	100–600 ng/spot	100–600 ng/spot
r	0.9990	0.9990	0.9995	0.9991
Slope ± S.D	7.540	4.278	4.708	5.433
Confidence limit of slope[a]	7.364–7.717	4.178–4.378	4.630–4.786	5.309–5.557
Intercept[b]	10897	249.97	110.76	153.55
Confidence limit of intercept	10778–11016	211.04–288.91	105.22–201.88	105.22–201.88
LOD (ng/spot)	24.007	23.9607	16.975	23.418
LOQ (ng/spot)	80.028	79.869	56.584	78.060

[a]95% confidence limit.
[b]Percentage of bias of intercept = 95%.

Table 3. Recovery studies (n = 6)

Compound	Amount taken (ng)	Amount found (ng)	RSD (%)
Mirtazapine	9000	8859.4	1.89
Impurity 1	100	97.099	2.450
Impurity 2	100	96.43	1.944
Impurity 3	100	96.06	3.12

was observed that all the impurities and the main compound were well resolved and did not interfere with the retention factor of Mzp.

Accuracy and Precision

A synthetic mixture containing known quantities of all the three impurities (3.3%) and Mzp, was prepared and chromatographed to ensure that these quantities were accurately reflected in their peak areas. All the estimations were carried out six times and the percentage R.S.D. was accordingly calculated (Table 3). The precision of the method was determined on 6 replicate spottings of Mzp, and reported as the relative standard deviations (RSD, 0.63%.)

CONCLUSION

A simple and rapid HPTLC method has been described for the separation of Mzp from its related impurities. The developed HPTLC method is suitable not only for separation and determination of related impurities for monitoring of synthetic reactions, but also for quality assurance of Mzp in the presence of its structurally related impurities.

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