Development and Validation of a Reversed-Phase HPLC Method for Separation and Simultaneous Determination of Process-Related Substances of Mirtazapine in Bulk Drugs and Formulations

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Abstract

A simple and rapid reversed-phase high-performance liquid chromatographic method has been developed for the separation and simultaneous determination of related substances of mirtazapine in bulk drugs and pharmaceutical formulations. Six impurities, including one degradation product of mirtazapine, have been separated on a BDS Hypersil (4.6 × 250 mm; particle size 5 µm) column with a mobile phase consisting of 0.3% triethylamine (pH 3.0)–acetonitrile (78:22 v/v) eluted in an isocratic mode and monitored with a photo diode array detector at 215 nm. The chromatographic behavior of all the analytes was studied under variable compositions of different solvent systems, temperatures, buffer concentrations, and pH values. The method was validated in terms of accuracy, precision, and linearity. The inter- and intra-day assay precision was found to be < 0.98% [relative standard deviation; (RSD)] and the recoveries were in the range 95.54–102.22% with RSD < 2.21%. The correlation coefficients for calibration curves for mirtazapine as well as impurities were in the range of 0.9941–0.9999. The method was successfully applied to the analysis of commercial formulations and the recoveries of mirtazapine were in the range of 99.38–100.73% with < 0.52% RSD. The method is useful not only for rapid evaluation of the purity of mirtazapine, but also for the simultaneous determination of related substances in bulk drugs and pharmaceutical formulations.

Introduction

Mirtazapine, 1,2,3,4,10,14b-hexahydro-2-methylpyrazino [2,1-a]-pyrido [2,3-c][2] benzazepine, is a tetracyclic antidepressant used in the treatment of patients with severe depression (1). It is a member of the piperazinoazepines, structurally not related to any known class of psychotropic drugs. It has a unique pharmacological profile combining dual action on both the noradrenergic and serotonergic neurotransmitter systems by blocking a_2 receptors and selectively antagonizing 5-HT_2 and 5-HT_3 receptors (2). Mirtazapine is extensively metabolized by the liver, and the biotransformations include hydroxylation, demethylation, oxidation, and conjugation with sulphate or glucuronic acid (3). Des-methylmirtazapine, is a pharmacologically active metabolite which contributes 3–6% of the total pharmacodynamic profile of mirtazapine (4). While studying the synthetic process in our laboratory, we observed I, II, III, IV, and VI (Figure 1) as process-related impurities likely to be present in the finished products. Thus, there is a great need for development of analytical methods for separation and determination of related substances not only for evaluation of quality of mirtazapine but also pharmacological profiles for clinical studies.

Several methods have been described in the literature for the determination of mirtazapine and demethylmirtazapine in biological fluids. The techniques used include capillary electrophoresis (5), gas chromatography (6,7), and high-performance liquid chromatography (HPLC) with UV (8,9), diode-

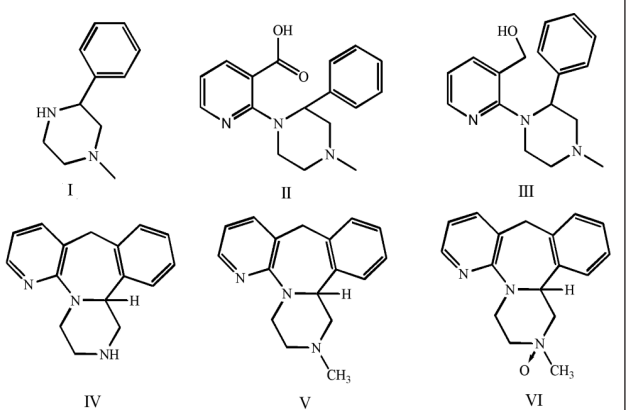

Figure 1. Process-related impurities (I, II, III), side products (IV and VI), and degradation product (VI) of mirtazapine (V).

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array (10,11), tandem mass spectrometry (12), and flourimetry (13–15). Recently, HPLC with UV detection has been used for the separation of enantiomers of mirtazapine in human plasma (16–18). Spectrometric and spectrofluorimetric methods have been used for the determination of mirtazapine in pharmaceutical formulations (19,20). Wynia et al. have developed a capillary zone electrophoresis method for the determination of mirtazapine and five of its structurally related substances in pharmaceutical dosage forms, and compared the results with those obtained by HPLC using an ion pair reagent as a mobile phase additive (21). The use of ion pair reagents in the mobile phase decreases the column life and needs a long time for equilibration. Furthermore, the method was not validated thoroughly.

This paper describes a reversed-phase (RP) isocratic HPLC method for the simultaneous separation and determination of mirtazapine, degradation products, and process related impurities on a C_{18} column using triethylamine (TEA) (pH adjusted to 3 with H_3PO_4) and acetonitrile as a mobile phase. The method was validated and found to be suitable for the quality assessment of mirtazapine in pharmaceutical formulations in the presence of process-related impurities and degradation products. Forced degradation of mirtazapine was carried out under thermal, photo, and acidic, basic, and peroxide conditions.

Experimental

Materials and reagents

All reagents were of analytical-reagent grade unless stated otherwise. Glass-distilled and de-ionized water (Barnstead Nanopure, Thermo Fisher; Waltham, MA), HPLC-grade acetonitrile, methanol, triethylamine, and orthophosphoric acid (S.D. Fine Chem; Mumbai, India) were used. The investigated samples of mirtazapine bulk drug materials and impurities were obtained from the process development department of IICT (Hyderabad, India).

Apparatus

The HPLC system was composed of two LC-10AT VP pumps, an SPD-M10AVP photo diode array detector, an SIL-10AD VP auto injector, a DGU-12A degasser, an SCL-10A VP system controller, and CTD-6AS column oven (all from Shimadzu, Kyoto, Japan). An RP BDS Hypersil C_{18} (250 × 4.6 mm i.d; particle size 5 µm) column (Thermo Electron Corporation, Reading, U.K.) was used for separation. The chromatographic and the integrated data were recorded using an HP-Vectra (Hewlett Packard, Waldbronn, Germany) computer system.

Chromatographic conditions

The analysis was carried out on a BDS Hypersil C_{18} column using a mobile phase consisting of 0.3% TEA (pH adjusted to 3.0 with ortho-phosphoric acid) and acetonitrile in the ratio (78:22 v/v). Before delivering the mobile phase into the system, the solvent was degassed and filtered through 0.45 µm PTFE filter using vacuum. The flow rate was kept constant at 1.0 mL/min and the column maintained at 40ºC. The injection volume was 20 µL. The detection was performed at 215 nm using a photodiode array detector (PDA).

Analytical procedures

Stock solutions (1000 µg/mL) of mirtazapine and its process impurities were prepared by dissolving known amounts of the components in the mobile phase. The solutions were adequately diluted to determine the accuracy, precision, linearity, and limits of detection and quantitation (LOD and LOQ). The specific concentration of mirtazapine was taken as 100 µg/mL.

Preparation of drug matrix and formulation solutions

An excipient matrix containing starch, methylcellulose, calcium phosphate, and calcium carbonate in the ratio 1:1:1:1 (w/w/w/w) was prepared. It (100 mg) was transferred to a 100-mL volumetric flask and dissolved in methanol. It was fortified with mirtazapine (1000 µg/mL), ultra-sonicated for approximately 10 min, and made up to the mark with the mobile phase. The supernant liquid was collected and filtered through a 0.45-micron filter for use. The solution was adequately diluted to determine the accuracy, precision, and LOD and LOQ of mirtazapine. The solutions of mirtazapine formulation were prepared in a similar way. Ten weighed tablets of mirtazapine were finely powdered and homogenized in a mortar. An amount equivalent to 200 mg of mirtazapine was taken into a 100-mL volumetric flask, dissolved in methanol, ultra-sonicated for approximately 10 min, and made up to the mark with the mobile phase. The supernant liquid was collected and filtered through a 0.45-micron filter for use.

Method validation

The developed method was validated with respect to specificity, accuracy, precision, and linearity.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of all potential impurities. For specificity determination, all known impurities were added to mirtazapine and its concentration was determined using the developed method. The specificity was also checked by stressing mirtazapine under (*i*) UV light at 254 nm and 60ºC temperature for 15 days, and (*ii*) extreme conditions such as 1N HCl, 1N NaOH, and 3% H_2O_2. Interference of excipients was studied by spiking a known amount of mirtazapine into matrix solution, and extracted in methanol, and the recovery was measured by the developed method.

Accuracy

Accuracy of the method was performed by recovery experiments using standard addition technique. The recoveries of I, II, III, IV, and VI were determined by spiking each impurity at five different levels ranging from 0.5–2.0 µg/mL into mirtazapine formulation solution, extracting into methanol, and using them for recovery studies. Similarly, recovery studies were carried out for mirtazapine by fortifying mirtazapine bulk drug solution with matrix solution at five concentration levels (i.e., 50, 75, 100, 120, and 150 µg/mL) in triplicate for 3 days. The extraction of fortified drug matrix was carried out as described under the "Preparation of drug matrix and formulation solutions" section.

Precision

The fortified drug matrix solution was prepared at different concentrations of mirtazapine, and precision of the method was studied using repeatability, intermediate precision, and reproducibility (ruggedness). Repeatability was the intra-day variation in assay obtained at different concentration levels of mirtazapine and expressed in terms of relative standard deviation (RSD) calculated for each day. The intermediate precision was the inter-day variation at the same concentration level determined on successive days. The ruggedness of the method was defined as the degree of reproducibility obtained by the analysis of the same sample under a variety of conditions at different labs, with different analysts using different instruments and different lots of reagents.

Linearity

The linearity of detector response to different concentrations of impurities was studied in the range from 0.5–5.0 µg/mL. Similarly, the linearity of mirtazapine was also studied by preparing standard solutions at ten different levels ranging from 25 to 200 µg/mL. The data were subjected to statistical analysis using a linear-regression model.

LOD and LOQ

LOD and LOQ represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. LOD and LOQ were determined by measuring the magnitude of analytical background, by injecting matrix solution and calculating the signal-to-noise ratio for each compound by injecting a series of solutions until the S/N ratio reached 3 for LOD and 10 for LOQ.

Method development

All impurities and mirtazapine were subjected to separation by RP-HPLC on different commercial C_{18} columns. Initially, buffers such as acetate and phosphate were tried using methanol and acetonitrile as organic modifiers. However, the compounds exhibited a characteristic tailing on all columns. This could be due to their ability to form strong hydrogen bonding with the residual silanols of the C_{18} materials. To minimize the tailing and to improve peak shapes, TEA in the range 0.1%–0.3% (concentration) was used as a buffering system. The effect of pH (6.0–3.0) of the buffer on separation was studied. To minimize the peak tailing, the column temperature was increased (25ºC–40ºC). Among the studied columns, BDS Hypersil C_{18} (250 mm × 4.6 mm) had shown promising separation of the test compounds with minimum tailing, so it was chosen for method development.

Results and Discussion

The chemical structures of all compounds are shown in Figure 1. The present study was aimed at developing a chromatographic system capable of eluting and resolving mirtazapine and its impurities.

Optimization of the chromatographic conditions

The HPLC conditions were optimized by studying the effects of concentration of organic modifier, concentration of TEA, buffer pH, and column temperature.

Effect of organic modifier

Methanol and acetonitrile were tried as organic modifiers with TEA buffer (pH = 3.0) on a BDS Hypersil C_{18} (250 mm × 4.6 mm) column maintained at 40ºC. With methanol, compounds were eluted at higher retention times with broad peak shapes, while with acetonitrile, sharp peaks were found. So acetonitrile was selected as organic modifier. Further studies were carried out to

Table I. The Effect of Organic Modifier on Resolution and Retention Factors in Optimized Conditions

	Retention factor (*k*)						Resolution (R_s)					
Organic modifier (%)	I	II	III	IV	V	VI	I	II	III	IV	V	VI
25	0.11	0.38	0.47	1.32	1.53	1.89	3.50	1.61	10.76	1.67	2.12	–
22	0.13	0.46	0.61	1.84	2.13	2.59	5.42	2.43	12.52	1.92	2.47	–
20	0.16	0.76	1.00	2.99	3.37	4.01	7.82	3.12	16.72	2.13	2.94	–

Table II. The Effect of TEA and Temperature on Resolution and Tailing in Optimized Conditions

		Resolution (R_s)						Tailing factor (*As*)					
TEA (%)	Temperature (°C)	I	II	III	IV	V	VI	I	II	III	IV	V	VI
0.1	40	3.91	5.15	15.67	1.14	1.57	–		1.21	1.17	1.25	1.31	2.64
2.55													
0.2	40	5.92	2.89	14.49	1.47	2.46	–		1.20	1.17	1.23	1.21	2.07
1.69													
0.3	40	5.42	2.43	12.52	1.92	2.47	–	1.13	1.17	1.15	1.27	1.55	1.35
0.3	25	5.91	2.22	12.76	2.13	2.59	–	1.08	1.21	1.32	1.71	2.21	1.71
0.3	30	5.79	2.27	12.66	2.08	2.55	–		1.09	1.21	1.26	1.59	1.87

determine the effect of acetonitrile concentration on separation of the test compounds. The effect of acetonitrile on retention and resolution is given in Table I. From Table I, it can be clearly seen that as the concentration of acetonitrile decreased, the resolutions and retentions increased, but peaks became broad. At 22% of acetonitrile, optimum resolutions were found, with sharp and symmetrical peaks.

Concentration of TEA

The effect of TEA buffer concentration on the system was studied by varying its concentration from 0.1% to 0.3%. The pH of the buffer was adjusted to 3.0 with diluted H_3PO_4, and the BDS Hypersil C_{18} column was maintained at 40ºC. As the concentration of TEA increased, retentions decreased (Figure 2A). But resolution was increased for (I, II), (IV, V), and (V, VI) and decreased for (II, III) and (III, IV) (Table II). Tailing was reduced (Table II) with improved peak shapes by increasing the concentration of TEA. At 0.3% of TEA, sharp symmetrical peaks with good resolution were obtained. So 0.3% of TEA was used for further optimization of other variables. The tailing of the mirtazapine peak compared to other peaks was higher, probably due to its higher concentration.

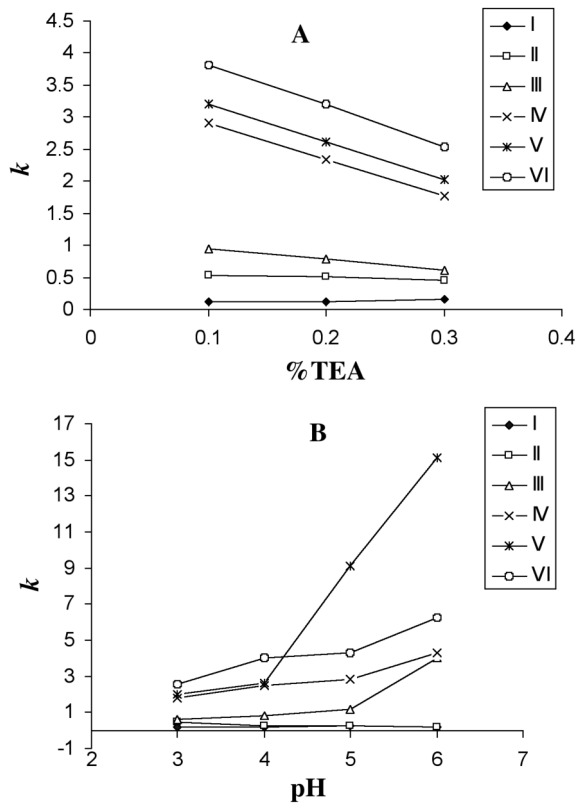

Figure 2. The effect of TEA (A) and pH (B) on retention factors (*k*).

Effect of pH

Further studies were carried out to determine the effect of buffer pH on resolution and retention. On increasing the pH, retention of test compounds was increased (Figure 2B) and it was more for compounds III, IV, V, and VI. At pH 5.0 to 6.0, compounds I and II were co-eluted and V was eluted after VI. On decreasing the pH, resolution increased for I and II and decreased for other compounds (Table III). But with a decrease in pH, peaks became sharp and tailing was reduced drastically for all compounds (Table III), allowing accurate quantitation. At pH 3.0, symmetrical peaks with optimum resolutions were obtained.

Effect of temperature

The column was maintained at different temperatures ranging from 25ºC–40ºC in a thermostated oven. Retention times

Table IV. Specificity Data

S. No.	Assay of mirtazapine (%) Without spiking the impurities	Assay of mirtazapine (%) Spiked with impurities
1	99.97	99.96
2	99.92	99.83
3	99.86	99.85
Mean	99.92	99.86
SD	0.055	0.068
RSD	0.055	0.068

Table V. System Suitability Data*

Sample	t_R (± RSD)† (min)	RRT	R_s	*As*	RRF
I	3.49 ± 0.08	0.371	5.42	1.08	0.29
II	4.38 ± 0.18	0.466	2.43	1.17	0.65
III	4.83 ± 0.14	0.514	12.52	1.15	0.39
IV	8.53 ± 0.21	0.907	1.92	1.22	0.85
V	9.40 ± 0.25	1.00	2.47	1.55	1.00
VI	10.79 ± 0.28	1.148	–	1.35	0.47

* Abbreviations: t_R, retention time; RRT, relative retention time; R_s, resolution; As, tailing factor; RRF, relative response factor; RSD, relative standard deviation.
† Average of five determinations.

Table III. The Effect of pH on Resolution and Tailing in Optimized Conditions

pH	Resolution (R_s) I	II	III	IV	V	VI	Tailing factor (*As*) I	II	III	IV	V	VI
3	5.42	2.43	12.52	1.92	2.47	–	1.13	1.17	1.15	1.27	1.55	1.35
4	0.51	6.92	13.39	6.40	6.84	–	1.07	1.19	1.25	1.59	1.88	1.72
5	0.00	10.45	11.40	3.34	9.95	–	1.09	1.21	1.39	2.10	2.36	2.17
6	–		–	–	–	–	–	1.12	1.29	1.45	2.55	3.08

decreased slightly with increasing temperature, but peaks became very sharp, and resolutions increased for compounds II and III and slightly decreased for the remaining compounds (Table II). But tailing was reduced with increasing temperature for IV, V, and VI; at 40ºC, minimum tailing was found. Finally, separation was carried out on a BDS Hypersil C_{18} (250 mm × 4.6 mm) column maintained at 40ºC using a mobile phase consisting of 0.3% TEA (pH = 3.0) and acetonitrile (78:22 v/v) with PDA detector set at 215 nm. These were the final optimized conditions. A typical chromatogram of mirtazapine (V) spiked with 5% (w/w) of each of the related substances is shown in Figure 3. It is clear from Figure 3 that all compounds were eluted and separated with good peak shapes and resolution. The method was applied to pharmaceutical formulations. The HPLC chromatogram of a typical formulation is shown in Figure 4. The peaks were identified by injecting and comparing with the retention times of the individual compounds and also by the absorption spectra recorded by the PDA detector. Unknown impurity UK1 and excipients (EX1) in the formulations were well-separated under the optimized conditions (Figure 4). A monograph of mirtazapine was recently approved by the European Pharmacopeia (E.P.) of 2007. It describes a method that utilizes a mobile phase comprising of a very high concentration of tetramethyl ammonium hydroxide as a buffer and tetrahydrofuran, methanol, and acetonitrile as organic modifiers for separation of related substances. The use of high concentrations of such ion pair reagents decreases the column lifetime and needs a long time for equilibration. Furthermore, the present work describes a method for the separation of six impurities, including two new substances which were not reported by the E.P. Moreover, the run time of the present method was half of that of the E.P., and the detection limits of mirtazapine and impurities were quite low compared to E.P.

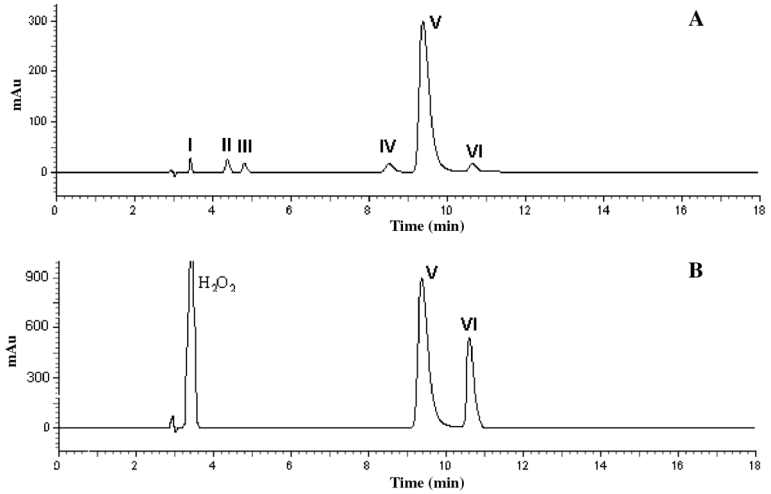

Figure 3. Typical chromatograms of mirtazapine (V) spiked with 5% (w/w) each of the impurities (I, II, III, IV, and VI); degradation of mirtazapine (V) with H_2O_2 (B).

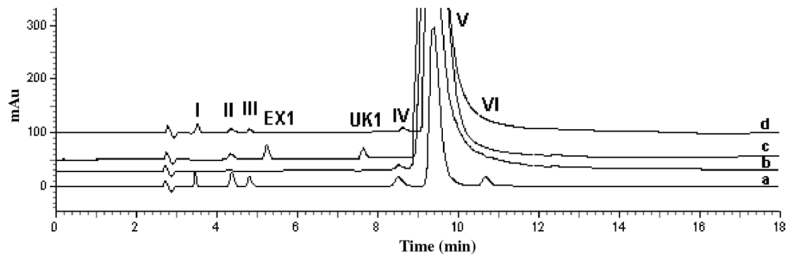

Figure 4. Typical chromatograms of mirtazapine: spiked with 5% (w/w) each of impurities (A); pharmaceutical formulations of two different manufacturers (B and C); bulk drug (D).

Validation

Specificity

Specificity was performed by spiking impurities into mirtazapine, and the assay results for mirtazapine were not changed in the presence of impurities (Table IV). Stressed degradation studies were also carried out to check the specificity of the method with respect to degradation products. Under UV light, thermal, acidic, and

Table VI. Recovery Data

Sample	Recovery* (%)	RSD†	Recovery* (%)	RSD	Recovery* (%)	RSD	Recovery* (%)	RSD	Recovery* (%)	RSD
Amount added (µg/mL)	0.5		0.7		1.0		1.5		2.0	
I	97.19	0.91	98.32	1.02	96.83	1.05	98.55	0.66	98.91	0.79
II	96.44	1.79	96.81	1.68	97.69	0.92	95.76	2.15	98.51	0.83
III	95.54	1.61	95.97	1.37	97.95	0.72	98.39	0.63	99.09	0.48
IV	97.05	1.06	96.48	0.95	99.55	0.58	100.61	0.72	99.59	0.33
VI	101.51	2.21	96.77	1.61	102.22	1.27	98.15	0.93	100.25	0.67
Amount added (µg/mL)	50		75		100		125		150	
V	99.55	0.33	99.42	0.55	100.32	0.28	100.17	0.36	99.79	0.61

* Average of three determinations.
† RSD: Relative standard deviation.

alkaline conditions, no change in the sample purity was observed; but in the presence of peroxide, one degraded product was formed, was well-separated from mirtazapine, and matched with impurity VI in the present conditions (Figure 3). The recoveries for the mirtazapine fortified drug matrix solutions were in the range 99.31%–100.75%, indicating specificity of the method with respect to matrix solutions. In pharmaceutical formulations, it was observed that the excipient peaks did not interfere with the peaks of interest (Figure 4), and excipients (EX1) and unknown impurity (UK1) were well-separated from mirtazapine and other impurities.

System suitability

The system suitability was conducted using 1.0% (w/w) of all the impurities spiked into mirtazapine (100 µg/mL) and evaluated by making five replicate injections. The system was suitable for use if the tailing factors for mirtazapine and its impurities were not more than 1.55 and the resolution was not less than 1.92. Synthetic mixtures and process samples were analyzed under identical conditions. The quantities of impurities and assay of V were calculated from their respective peak areas. System suitability data is given in Table V.

Accuracy

The recoveries of I, II, III, IV, and VI were determined by spiking each impurity at five different levels ranging from 0.5–2.0 µg/mL into mirtazapine formulation solution at the specified level (100 µg/mL). The recovery range and RSD for all impurities were found to be 95.54–102.22% and 0.33–2.21%, respectively (Table VI). Similarly, the recovery studies for mirtazapine were carried out by fortifying drug solutions at five concentration levels each (i.e., 50, 75, 100, 120, and 150 µg/mL) in triplicate for 3 days and recording the percentage recoveries in Table VI. The RSD values were found to be below 0.61%.

Precision

The precision of the method was tested using six (n = 6) injections of mirtazapine spiked with 0.5% (w/w) of each impurity, and the RSD of retention time (t_R) and peak area were determined. The RSD ranged from 0.22 to 0.89%. The precision in determination of the assay was studied by repeatability, intermediate precision, and reproducibility (ruggedness). The RSD values were found to be below 1.0%, indicating a good repeatability (Table VII). The intermediate precision was calculated for three concentration levels using the data of 3 days and are expressed in terms of RSD values (Table VII). At each concentration level, the RSD values were below 1.0%, indicating a good intermediate precision. The ruggedness of the method was studied by the analyzing the same samples under a variety of conditions at different labs, with different analysts using different instruments and different lots of reagents. The same samples at three concentrations were analyzed in triplicate on 2 days using another instrument (LC-10A Module HPLC system containing pump and UV-visible detector) by a different analyst with different lots of reagents and columns. The data obtained were within 2% RSD.

Linearity, LOD, and LOQ

The linearity of detector response to different concentrations of impurities was studied in the range from 0.5–5.0 µg/mL. Similarly, the linearity of mirtazapine was also studied by

Table VII. Inter- and Intra-Day Assay of Mirtazapine

Intra-day			
Day 0			
Mean of concentration (mg/mL), n = 3	0.051	0.102	0.205
SD*	0.0004	0.0010	0.0015
RSD†	0.78	0.98	0.73
Day 1			
Mean of concentration (mg/mL), n = 3	0.055	0.104	0.202
SD	0.0005	0.0009	0.0019
RSD	0.91	0.87	0.94
Day 2			
Mean of concentration (mg/mL), n = 3	0.053	0.105	0.206
SD	0.0003	0.0006	0.0013
RSD	0.57	0.57	0.63
Inter-day			
Mean of concentrations of three days	0.053	0.104	0.204
SD	0.0004	0.0007	0.0016
RSD	0.76	0.67	0.78

* SD: standard deviation.
† RSD: relative standard deviation.

Table VIII. Calibration Data

Sample	Range (µg/mL)	Regression equation	r^2	LOD (µg/mL)	LOQ (µg/mL)
I	0.5–5.0	$y = 16406x - 1075$	0.9979	0.087	0.279
II	0.5–5.0	$y = 36993x - 2486$	0.9962	0.083	0.252
III	0.5–5.0	$y = 30893x - 5287$	0.9941	0.090	0.265
IV	0.5–5.0	$y = 47795x + 1226$	0.9974	0.099	0.291
V	25–200	$y = 54441x + 141167$	0.9999	0.110	0.315
VI	0.5–5.0	$y = 37309x - 13918$	0.9960	0.131	0.352

Table IX. Results of Analysis of Bulk Drugs and Formulations*

	(%) Impurities (w/w)				
Sample	I	II	III	IV	VI
Bulk-1	0.05	0.04	0.03	0.03	–
Bulk-2	0.03	0.05	–	–	0.03
Bulk-3	0.04	0.05	0.05	–	–
Form-1	–	0.06	0.03	0.05	–
Form-2	–	–	–	0.04	–
Form-3	–	0.05	–	–	–

* Form: formulation; bulk: bulk drug.

preparing standard solutions at ten different levels ranging from 25 to 200 µg/mL. The data were subjected to statistical analysis using a linear-regression model; the regression equations and coefficients (r^2) are given in Table VIII. The results have indicated good linearity. LOD and LOQ were calculated as given in the "Experimental" section and the results are given in Table VIII. The LODs and LOQs were in the range 0.087%–0.131% and 0.252%–0.352% for mirtazapine.

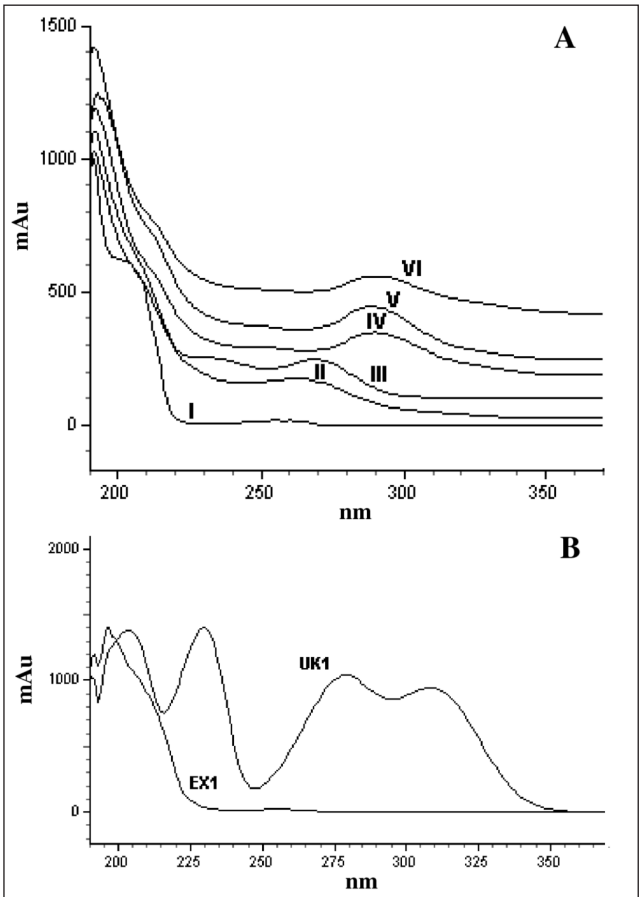

Figure 5. Overlay UV spectra of known impurities (I, II, III, IV and VI) and mirtazapine (V) (A), and unknown impurity (UK1) and excipients (EX1) (B).

Table X. Results of Analysis of Mirtazapine in Bulk Drugs and Formulations

Sample	Concentration of mirtazapine* Theoretical value (mg)	Found† value (mg)	(%) Recovery	RSD
Bulk-1	15.23	15.28	100.33	0.35
Bulk-2	29.65	29.68	100.10	0.43
Bulk-3	30.51	30.32	99.38	0.27
Form-1	45.0	44.80	99.56	0.41
Form-2	30.0	30.22	100.73	0.52
Form-3	15.0	14.95	99.67	0.25

* Form: formulation; bulk: bulk drug.
† Average of three determinations.

The results of analysis of bulk drugs and formulations

High-low chromatographic technique was employed for detecting trace level impurities present in bulk drugs and formulations of mirtazapine. Accordingly, a very high concentration (1000 µg/mL) of formulation and bulk drug sample solutions were prepared as described in the "Experimental" section to increase the concentration of the impurities above their detection limits. The previously mentioned solutions were analyzed by the developed method and the results are recorded in Table IX. Almost all of the impurities were found in different amounts in all the formulations and bulk drugs studied. Unknown impurity UK1 and excipients (EX1) in the formulations were well-separated under the optimized conditions (Figure 4). The peaks were identified by injection and comparison with the retention times of the individual compounds, and also by the absorption spectra recorded by PDA detector (Figure 5). The assay for determining mirtazapine was carried out by diluting the previously mentioned solutions to 100 µg/mL with the mobile phase. The results of analysis of bulk drugs and formulations are given in Table X.

Conclusions

An isocratic RP-HPLC method has been developed and validated for determining the process-related substances and degradation products of mirtazapine in bulk drugs and pharmaceuticals. The separation of mirtazapine and its process-related impurities on different commercial C_{18} columns was studied. The chromatographic conditions were optimized by studying the effects of temperature of the column, concentration of organic modifier, and concentration and pH of TEA buffer. The developed method was found to be selective, sensitive, precise, linear, accurate, and reproducible in determining mirtazapine and its potential impurities which may be present at trace levels in finished products. Thus, the method can be used for process development as well as quality assurance of mirtazapine in bulk drugs as well as pharmaceutical formulations.

Acknowledgments

The authors thank Dr. J.S. Yadav, Director, Indian Institute of Chemical Technology for encouragement and permission to communicate the results for publication. (The IICT communication number is 061025).

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Manuscript received May 2, 2007;
Revision received July 18, 2007.