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Journal of Pharmaceutical and Biomedical Analysis
24 (2000) 11–17

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Determination of mirtazapine in tablets by UV spectrophotometric and derivative spectrophotometric methods

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Received 15 November 1999; received in revised form 22 May 217; accepted 27 May 2000

Abstract

Mirtazapine, 1, 2, 3, 4, 10, 14b-hexahydro-2-methyl-pyrazino [2, 1-a] pyrido [2, 3-c] [G.L. Stimmel, J.A. Dopheide and S.M. Stahl, Pharmacotheraphy 17(1) (1997) 10] benzazepine, is a new and well tolerated antidepressant. It blocks pre-synaptic α_2-adrenergic receptors and postsynaptic serotonin type 2 and type 3 receptors. The drug is rapid and completely absorbed after oral administration. Mirtazapine was analyzed by HPLC and gas chromatography with nitrogen-sensitive detection. In this study, mirtazapine was analyzed by using UV spectrophotometry, first and second order derivative spectrophotometry. The type of solvent, the degree of derivation, range of wavelength and n value were chosen in order to optimize the conditions. The concentration of mirtazapine in its methanolic solutions were determined between the wavelength range of 225–360 nm in the linearity range of 1–100, 2–100 and 1–120 μg ml^{-1} by using the values obtained from UV, first-order derivative ($n = 5$, $\Delta\lambda = 17.5$ nm) and second-order derivative ($n = 9$, $\Delta\lambda = 31.5$ nm) spectrum of the substance, respectively. The developed UV Spectrophotometric, first-order and second-order derivative spectrophotometric methods were applied to a pharmaceutical preparation as tablet form. Developed UV and derivative UV spectrophotometric method in this study are accurate, sensitive, precise, reproducible and can be directly and easily applied to the pharmaceutical preparations.

Keywords: Mirtazapine; Analysis; Tablets; UV spectrophotometry; Derivative spectrophotometry

1. Introduction

Mirtazapine is a new antidepressant agent its' chemical formula is 1, 2, 3, 4, 10, 14b-hexahydro-2-methyl-pyrazino [2, 1-a] pyrido [2, 3-c] [2] benzazepine (Fig. 1). It is a member of a chemical series of compounds known as piperazinoazepines that are not related to any known class of psychotropic drugs. Mirtazapine is available in the form of tablets (Remeron®) for oral administration, containing 15 or 30 mg of mirtazapine, red and yellow FeO, TiO_2 and other excipients.

Mirtazapine is efficacious in the short-term and continuation treatment of moderately and severely depressed hospitalized and out-patients.

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0731-7085/00/$ - see front matter
PII: S0731-7085(00)00394-0

It has a unique pharmacological profile, different from all other currently used antidepressants. Mirtazapine blocks directly presynaptic α_2-adrenoreceptors (α_2-autoreceptors) resulting in an increased release of noradrenaline and subsequently enhanced noradrenergic neuro-transmission [1–3]. Adverse effects are somnolence, weight gain, dizziness, increased appetite and edema [4]. The pharmacologic profile of mirtazapine [5,6] and a review about its usage in major depression were given [7]. pK_a value of mirtazapine was found 7.1 by using potentiometric titration, oxidation potential was given as +0.83 V and lots of physicochemical and biological properties of mirtazapine were given [8].

The biotransformation of mirtazapine includes 8-hydroxylation, *N*(2)-demethylation, *N*(2)-oxidation of 8-OH mirtazapine with sulphate or glucoronic acid. The main metabolites found in human experiments were *N*-desmethyl-mirtazapine and 8-hydroxy-mirtazapine-glucuronide [9–11]. A number of pharmacokinetic studies on mirtazapine have been published elsewhere [12–15]. Mirtazapine was analyzed from biological materials by using high performance liquid chromatography [9–11,16], gas chromatography with nitrogen-sensitive detection [12–15,17] and gas chromatography-mass spectrometry [18].

To our knowledge, there is no spectrophotometric method for the determination of mirtazapine in pharmaceutical forms in literature. We wanted to develop a new spectrophotmetric method for the determination of mirtazapine in pharmaceutical preparation. After developing UV spectrophotometric method, derivative UV spectrophotometric methods were also carried out and all optimization parameters were also considered.

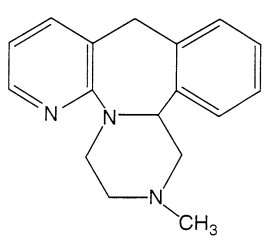

Fig. 1. Chemical structure of mirtazapine.

This study was conducted in order to develop two alternative derivative spectrophotometric methods to UV spectrophotometry not only for analysis mirtazapine in tablets, but also for comparison.

2. Experimental

2.1. Apparatus

A Shimadzu UV-160 recording double-beam UV-Visible Spectrophotometer with a data processing system was used. UV spectra of reference and sample solutions were recorded in 1 cm quartz cells at a scan speed of 60 nm min^{-1}. UV, first-order derivative ($n=5$, $\Delta\lambda=17.5$ nm) and second-order derivative ($n=9$, $\Delta\lambda=31.5$ nm) curves were recorded over the range of 225–360 nm and with a fixed slit width of 3 nm.

2.2. Reagents

Mirtazapine standard was obtained from the Central Institute of Hygiene of Turkey. It was tested for purity by controlling its melting point, UV and IR spectrum. No impurities were found. Analytical grade methanol was purchased from Carlo Erba.

2.3. Standard solutions of mirtazapine

Stock solutions of mirtazapine were prepared at a concentration of 1000 μg ml^{-1} in methanol and kept at +4°C. Stability of mirtazapine stock solutions were tested during more than 8 months and the results showed that methanolic mirtazapine solutions were stable. Working standard solutions were daily prepared by diluting stock solutions at the concentrations of 5, 10, 20, 40 μg ml^{-1} in methanol and methanol was used as a reference. Pharmaceutical tablets contain 15 or 30 mg of mirtazapine and excipients (corn starch, magnesium stearate, lactose, hydroxypropyl cellulose, colloidal silicone, polyethylene glycol 8000, titanium dioxide (E171), yellow iron oxide (E172) and red iron oxide (E172)).

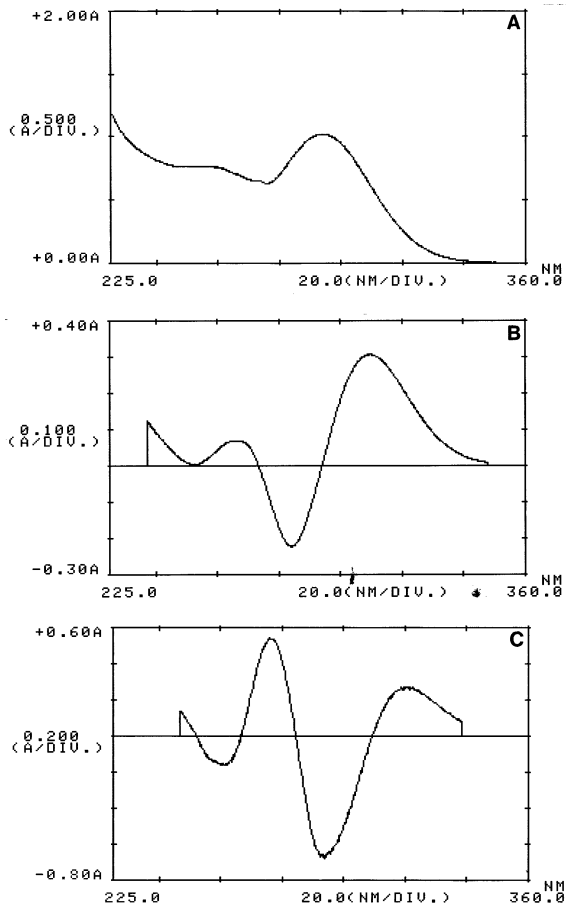

Fig. 2. (A) Zero-order derivative, (B) First-order derivative and (C) Second-order derivative spectra of 50 μg ml^{-1} standard mirtazapine solution in methanol.

2.4. Procedure

Ten tablets of mirtazapine were accurately weighed and powdered. A simple equivalent to one tablet was weighed and transferred to a 50 ml volumetric flask. Thirty mililitres of methanol was added and the flask sonicated for 30 min, then filled up to the volume with methanol. After filtration with Advantec 5A (110 mm) filter paper appropriate dilutions were made in the range of 5–40 μg ml^{-1} with methanol. UV spectra were recorded against methanol as a reference substance. From calibration curves the average content of one tablet was calculated.

3. Results and discussion

The original UV (zero-order), first-order and second-order derivative spectrum of standard mirtazapine solution of 50 μg ml^{-1} are shown in Fig. 2A, B and C, respectively. Each spectra can be used for the determination of this drug. In the original spectrum, mirtazapine shows a single well-defined peak at 293.8 nm. First-order and second-order derivative spectrum have three peaks and the opposite peak at 293.8 nm that can be useful for determination of mirtazapine. Zero-order, first-order and second-order derivative UV spectrum of Remeron® tablet solution in methanol are shown in Fig. 3A, B and C, respec-

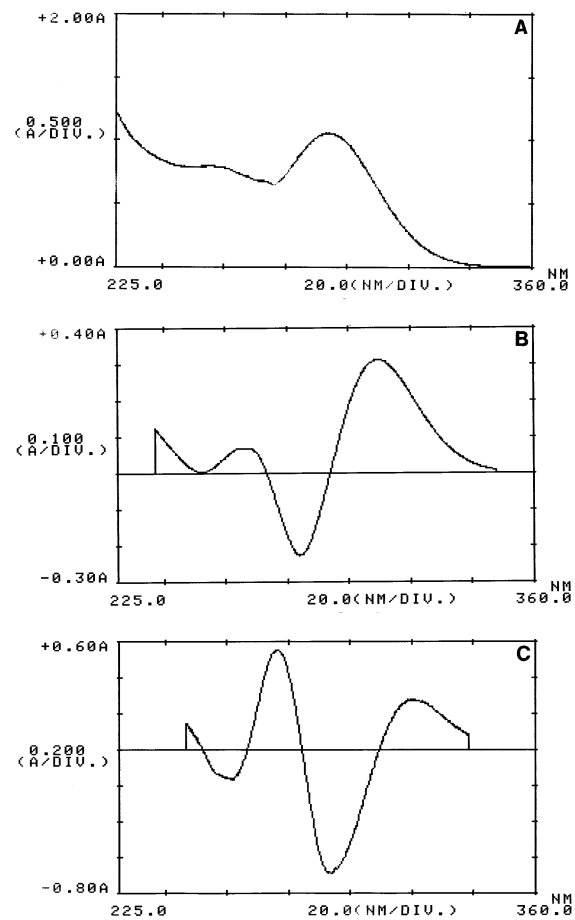

Fig. 3. (A) Zero-order derivative, (B) First-order derivative and (C) Second-order derivative spectra of Remeron® tablet solution in methanol.

Table 1
Features of the calibration curves of three spectrophotometric methods ($n = 6$)

Features	Zero-order	First-order	Second-order
Regression equation[a]	$y = -0.005 + 0.022x$	$y = -0.003 + 0.008x$	$y = -0.009 + 0.024x$
Standard error of slope	3.42×10^{-4}	1.40×10^{-4}	4.22×10^{-4}
Standard error of intercept	1.26×10^{-3}	7.35×10^{-4}	1.76×10^{-4}
Correlation coefficient	0.99991	0.99998	0.99997
Determination coefficient	0.99982	0.99996	0.99994
Linear range (μg ml^{-1})	1–100	2–100	1–120

[a] $y = a + bx$ where x is the concentration in μg ml^{-1}, y is the absorbance for zero-order and amplitude for first- and second-order derivative, a is the intercept and b is the slope.

tively. No difference was observed in the maximum wavelength of all spectra.

3.1. Optimization of conditions

The derivative wavelength difference ($\Delta\lambda$) depends on the measuring wavelength range and N values (smoothing factor). Generally, the noise decreases by increasing $\Delta\lambda$. Optimal wavelength range should be chosen since the broad peaks become sharper, the ratio of signal/noise elevates and the sensitivity of the method increases by controlling the degree of low pass filtering or smoothing. Therefore a series of n values ($n =$ 1–9) were tested in the first and second-order UV spectrum of mirtazapine in methanol. Optimum results were obtained in the measuring wavelength range 225–360 nm, $n = 5$ ($\Delta\lambda =$ 17.5 nm) for first-order derivative and $n = 9$ ($\Delta\lambda = 31.5$ nm) for second-order derivative spectrophotometry.

3.2. Linearity of calibration curves

In quantitative analysis, the calibration curves for zero-, first- and second-order derivative spectrophotometry in methanol were plotted and evaluated by using peak to peak, peak to zero and tangent methods for derivative spectrophotometric methods. The slopes of tangent calibration curves were higher than that of calculated for the others. Thus it has been found that the tangent method is more preferable. The regression equations, standard errors of slopes, intercepts, correlation coefficients and linear ranges were given in Table 1.

3.3. Sensitivity

The limit of quantitation (LOQ) for mirtazapine was found as 1 μg ml^{-1} both in zero and second-order derivative spectrophotometry and 2 μg ml^{-1} first-order derivative spectrophotometry. The limit of detection (LOD) was 0.2 μg ml^{-1} ($S/n = 2.6$) for methods.

3.4. Quantitation, accuracy and precision

The developed UV and derivative spectrophotometric methods were applied to three serials of film tablets and results were given in Table 2.

Recovery studies were performed by using 25 μg ml^{-1} standard mirtazapine solutions in methanol by zero-order, first-order and second-order derivative UV spectrophotometric methods ($n = 10$). The mean mirtazapine values and the standard deviations were 24.78 ± 0.60 (1.30%), 24.94 ± 0.19 (2.34%), 25.07 ± 0.17 (2.02%), respectively. Mean recovery and relative standard deviations were found to be 99.12 ± 0.43 (1.30%), 99.78 ± 0.80 (2.41%) and 100.23 ± 0.67 (2.02%), respectively. Another recovery study was performed on the synthetic mixtures (it is included not only mirtazapine but also all excipient substances). The percentage recovery of mirtazapine was calculated by comparing the found and added concentrations ($C_{found}/C_{added} \times 100$) in each case (Table 3). These data suggested that the developed methods have a good precision.

3.5. Selectivity

Commonly drugs were tested for possible interference in the standard addition method. In order to detect interactions of excipients in this method (Potential interfering compounds were titanium dioxide, yellow and red iron oxide, lactose, magnesium stearate), the standard addition technique was applied to the same preparations which were analyzed by the calibration curve without standard addition. In the standard addition method increasing amounts of mirtazapine were added to six different tubes, containing the same amount of the sample. Zero, first and second-order derivative spectrum of each tube were recorded. Absorbance values of tangent measurements were plotted against the added mirtazapine concentrations to the samples. The amount of mirtazapine in the sample was calculated from the intercept. The regression equation of standard addition curves were found zero-order, first-order and second-order as $y=0.317+0.022x$ ($r=0.99981$), $y=0.118+0.008x$ ($r=0.99978$) and $y=0.324+0.024x$ ($r=0.99800$), respectively. y is absorbance for zero-order, and amplitude for first- and second-order derivatives. x is the concentration of mirtazapine in μg ml^{-1} and r is the coefficient of correlation. There was no difference between the relative standard deviations and slopes of three methods with and without standard addition. The

Table 2
The results of pharmaceutical preparations containing mirtazapine (30 mg) in combination ($n=6$)[a]

S.No.	Zero-order found (mg)	First-order found (mg)	Second-order found (mg)
A	*X*: 29.82 SD: 0.34 CV: 1.15%	*X*: 29.46 SD: 0.68 CV: 2.31%	*X*: 29.73 SD: 1.32 CV: 4.44%
B	*X*: 29.95 SD: 0.83 CV: 2.78%	*X*: 29.87 SD: 0.41 CV: 1.37%	*X*: 29.91 SD: 0.49 CV: 1.65%
C	*X*: 30.22 SD: 0.43 CV: 1.42%	*X*: 29.82 SD: 1.19 CV: 3.98%	*X*: 30.18 SD: 0.65 CV: 2.16%

[a] *X*, mean; SD, standard deviation; CV, coefficient of variation.

Table 3
Recovery data of mirtazapine which were obtained by three spectroscopic methods in added 30 mg synthetic mixtures ($n=6$)[a]

Zero-order		First-order		Second-order	
Found (mg)	Recovery (%)	Found (mg)	Recovery (%)	Found (mg)	Recovery (%)
29.63	98.73	28.75	95.83	29.91	99.69
30.19	100.63	29.50	98.33	29.91	99.69
31.44	104.76	30.25	100.83	31.55	105.15
31.52	105.08	30.25	100.83	31.27	104.24
30.86	102.86	30.25	100.83	31.91	106.36
27.43	91.43	30.75	102.50	27.45	91.51
	X: 100.58		*X*: 99.86		*X*: 101.11
	SD: 5.10		SD: 2.38		SD: 5.47
	CV: 5.07%		CV: 2.38%		CV: 5.41%

[a] X, mean; SD, standard deviation; CV, coefficient of variation.

Table 4
Statistical evaluation of obtained data from three spectrophotometric methods (30 mg mirtazapine in one tablet of Remeron®)[a]

Statistical values	Zero-order derivative	First-order derivative	Second-order derivative	F values
n	18	18	18	
X	30.01 ± 0.14	29.72 ± 0.19	29.94 ± 0.20	F_C: 0.75
SD	0.58	0.80	0.86	F_T: 19.48
CV	1.92%	2.67%	2.88%	
CI	29.71–30.30	29.32–30.12	23.86–36.02	

[a] n, Number of determination; X, mean; SD, standard deviation; CV, coefficient of variation; CI, confidence intervals ($\alpha = 0.05$); F_C, calculated F value; F_T, tabulated F value.

present data shows that there was no interaction of excipients in the analysis of mirtazapine.

In order to investigate, whether the developed methods were used as stability-indicating method or not, drug solutions were exposed to light, different pH and temperatures. It cannot be said anything about the methods to use as stability-indicating procedures, because of not decomposing the drug solutions.

3.6. Statistical analysis of the results which were obtained from three spectrophotometric methods

The performance of zero-, first- and second-order derivative spectrophotometric methods was judged by F-values. At 95% confidence level, the calculated F-values do not exceed the theoretical values (Table 4). Therefore, there is no significant difference between zero-, first- and second-order derivative spectrophometric methods. This is suggested that the three methods are equally applicable.

4. Conclusion

The liquid chromatographic and gas chromatographic determination of mirtazapine in plasma has been reported in literature [8–14]. Chromatographic methods need expensive equipment and time-consuming sample preparation steps for determination. There is no spectrophotometric method for the analysis of mirtazapine in pharmaceutical preparations has been reported in literature.

The methods described here are direct methods for the analysis of mirtazapine without any extraction process to eliminate the excipients, do not use time consuming procedure such as standard addition method and also there is no need any expensive equipment. Due to the results of the present study the developed spectrophotometric methods are concluded as accurate, sensitive, precise, reproducible and can be easily and directly applied to the pharmaceutical preparations. Additionally, the short analysis time and low costs are the other advantages of these methods for routine analysis.

It should be noted that all methods gave similar and favourable results with respect to precision and accuracy. The all RSD values are lower than 10% and the recovery as a measure of the accuracy is close to 100% in all cases. These criteria were given in literature for validation of methods [19]

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