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Spectrophotometric, spectrofluorimetric, HPLC and CZE determination of mirtazapine in pharmaceutical tablets

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Abstract

Four analytical methods have been developed for the quality control of tablets containing mirtazapine: spectrophotometry, spectrofluorimetry, high performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE). All the methods only require a simple extraction procedure of mirtazapine from the tablets before analysis. The concentration of mirtazapine in solutions was determined in the linearity range of 5–25 μg/ml at $\lambda = 315$ nm for spectrophotometry and at $\lambda = 220$ nm for HPLC and CZE. Spectrofluorimetric determinations were achieved at $\lambda_{excitation} = 328$ nm and $\lambda_{emission} = 415$ nm in the linearity range of 2–25 ng/ml. All the methods gave similar results and were validated for selectivity, linearity, precision and sensitivity. Spectrometric methods gave slightly higher RSD values (up to 2.54%). The four methods were directly and easily applied to the pharmaceutical preparation with accuracy, resulting from recovery experiments between 99.72% in HPLC and 101.47% in spectrofluorimetry. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Mirtazapine; Tablets; Analysis; UV spectrophotometry; Spectrofluorimetry; HPLC; CZE

1. Introduction

Mirtazapine (1,2,3,4,10,14b-hexahydro-2-methyl-pyrazino[2,1-a]-pyrido[2,3-c][2-benzazepine]) is the pharmacologically active constituent of Norset®, a recently introduced antidepressant in France. It is available in the form of tablets for oral administration, containing 15 mg of mirtazapine. It is efficacious in the short-term and continuation treatment of moderately and severely depressed hospitalized and out-patients.

Mirtazapine has a unique pharmacological profile, not related to any known class of psychotropic drugs. It combines two synergistic mechanisms of action with enhancement of both noradrenergic and serotonergic neurotransmission [1]. The biotransformation of mirtazapine includes 8-hydroxylation, *N*(2)-demethylation, *N*(2)-oxidation of 8-OH mirtazapine and conjugation with sulphate or glucuronic acid.

Several procedures for the determination of mirtazapine and metabolites in biological fluids are reported in literature; they include gas chro-

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matography with nitrogen sensitive detection [2,3] and high-performance liquid chromatography (HPLC) with UV or fluorescence detection [4,5]. To our knowledge, for the determination of mirtazapine in pharmaceutical forms, only one UV spectrophotometric method [6] and time consuming HPLC and capillary zone electrophoresis (CZE) procedures have been described in the literature [7]. No single extraction procedure has been proposed for the different methods.

In pharmaceutical laboratories, there is always a need for faster, simpler, cheaper and better performance analytical methods. For this reason, we developed four rapid analytical methods for a reliable quality control of Norset® tablets: UV-spectrophotometric, spectrofluorimetric, isocratic HPLC and CZE with UV detection have been investigated. The aim for the analyst was to allow the choice of one of the four methods with a rapid procedure and with the same simple extraction method. The two spectrometric methods without derivation were proposed for their simplicity, and HPLC and CZE methods have been developed for their capability to separate mirtazapine and possible impurities present in the tablets. Because of their differences with respect to selectivity, HPLC and CZE are often complementary [7] and it may be of great interest for the analyst to develop these two techniques simultaneously.

Validation parameters of the four methods have been calculated and compared in terms of specificity, linearity, precision, detection limit and accuracy.

2. Materials and methods

2.1. Reagents

Mirtazapine (laboratory code Org 3770) was kindly supplied by Analytical Control labs. (N.V. Organon, Oss, The Netherlands). The internal standard (I.S.) for the HPLC and the CZE procedures was pirenzepine (Sigma, Saint Quentin Fallavier, France).

Acetonitrile was purchased from Baker (Deventer, The Netherlands), H_3PO_4, KH_2PO_4 and K_2HPO_4 from Merck (Darmstadt, Germany) and TEA (triethylamine) from Lancaster (Morecambe, UK). Water was doubly distilled from all glass apparatus. All chemicals were of analytical grade.

The pharmaceutical formulation analysed was Norset® (Organon, Oss, The Netherlands). A tablet contains 15 mg of mirtazapine and excipient substances (corn starch, hydroxypropyl cellulose, magnesium stearate, anhydrous colloidal silicone, lactose monohydrate, titanium dioxide and yellow iron oxide).

2.2. Apparatus and conditions

A Jasco V 530 UV–vis spectrophotometer (Tokyo, Japan) with a data processing system was used. Spectrophotometric determinations were achieved in 1 cm quartz cells at 315 nm.

Fluorimetric measurements were performed on a SAFAS FL 200 (Monaco). An excitation wavelength of 328 nm and an emission wavelength of 415 nm were used.

HPLC was performed using a Merck liquid chromatograph (Darmstadt, Germany) equipped with a L-6200 solvent delivery pump, a L-4000 ultraviolet detector operating at 220 nm and a D-2000 Chromato-Integrator. Injections were performed manually through a 20 μl loop with a Rheodyne model 7125 injector (Rheodyne, Cotati, USA). A reversed-phase Satisfaction RP18 AB® column (120 Å, 150 mm × 4.6 mm I.D., 5 μm) was used (C.I.L., Sainte Foy La Grande, France). The mobile phase was prepared by mixing acetonitrile and an aqueous solution of 25 mM H_3PO_4–15 mM TEA (18:82, v/v). It was degassed and 0.45 μm filtered prior to use. The pH of the aqueous phase was near 2.5. The flow rate was 1.0 ml/min. The determinations were performed at room temperature.

CZE analysis was carried out on a Spectra Phoresis 1000 (ThermoQuest, Les Ulis, France) capillary electrophoresis system. Fused-silica capillary tube (ThermoQuest) (34 cm × 75 μm I.D) was used with an effective length of 27 cm. Temperature of the capillary tube during electrophoresis was maintained at 25 °C by a thermostating system. Samples were injected using the hydrodynamic mode with an injection time of 15 s. All the analysis were performed at an applied voltage of

15 kV (with a typical current of 50 μA). The electrophoretic zones were detected at 220 nm with an UV detector. The electropherograms were recorded and integrated with a Spectraphoresis software (ThermoQuest). The running buffer was a 25 mM phosphate buffer (pH 7). It was filtered through a 0.45 μm membrane filter. Each day before starting analysis, the capillary column needed a phases conditioning cycle [8,9]. Before each injection, the capillary was washed with 0.1 M NaOH (1 min) and running buffer (1 min).

2.3. Solutions

Stock solutions (1 mg/ml) were prepared by dissolving appropriate amounts of mirtazapine and pirenzepine (I.S.) in methanol. These solutions were stable for at least 2 months at −20 °C. For UV spectrophotometry, working solutions were prepared by diluting the stock solution of mirtazapine with methanol and H_3PO_4 (50 mM) as appropriate. The final content of methanol was 2% in the working solutions. For spectrofluorimetry, the working solutions were dilutions in H_3PO_4 (50 mM) of the stock solution of mirtazapine. For HPLC and CZE studies, the stock solutions of mirtazapine and pirenzepine (I.S.) were diluted as appropriate with mobile phase or with running buffer, respectively.

2.4. Extraction from tablets and quantification:

Ten tablets were accurately weighed and powdered. An amount equivalent to one tablet was weighed and transferred into a 20-ml volumetric flask. Ten millilitres of methanol were added. The flask was sonicated for 10 min and then filled up to the volume with methanol. For spectrophotometry, 1 ml volume of the filtered extract was transferred into a 50 ml measuring flask and completed with H_3PO_4 (50 mM). For spectrofluorimetry, two additional dilutions (final dilution: 1/100) in H_3PO_4 were necessary to obtain the working solution. For HPLC and CZE, filtered 0.2 ml volume of the extract was transferred into a 10 ml measuring flask, 0.5 ml of internal standard solution (100 μg/ml in mobile phase for HPLC and 200 μg/ml in running buffer for CZE) was added and the flask was filled up either with mobile phase for HPLC or running buffer for CZE.

All measurements were repeated six times for each concentration.

2.5. Validation method

2.5.1. Selectivity

The selectivity of the four methods was investigated by observing interferences between mirtazapine and the excipients.

2.5.2. Linearity

For spectrophotometric measurements, the solutions were prepared by dilution of the stock solution of mirtazapine to reach a concentration range of 5–25 μg/ml. For fluorimetric measurements, a 100 ng/ml working solution was used to prepare calibration solutions at concentrations ranging from 5 to 25 ng/ml. For HPLC and CZE measurements, a 100 μg/ml working solution was prepared. Volumes of 0.5–2.5 ml of this solution were introduced into 10 ml measuring flasks and 0.5 ml of I.S. (100 μg/ml in HPLC or 200 μg/ml in CZE) was added to each sample. The flasks were made up to 10 ml with mobile phase or running buffer.

In spectrophotometry and spectrofluorimetry, the calibration curves were set up by plotting absorbance or emission fluorescence versus the respective drug concentrations. In HPLC the mirtazapine/I.S. peak area ratios, in CZE the mirtazapine/I.S. normalized area ratios (i.e. area/migration time) [9] were plotted as a function of mirtazapine concentrations.

2.5.3. Precision

The precision of the four methods was estimated by the relative standard deviation of six determinations of mirtazapine in powdered tablets.

2.5.4. Accuracy

The accuracy of the methods was verified by analysing model mixtures obtained by adding known amounts of mirtazapine standard solution to known amounts of the various excipients (see

Section 2.1). The model mixtures contained 10 (A), 15 (B) and 20 (C) mg of mirtazapine. Six determinations has been made for each model mixture. The recoveries were calculated with respect to the amount of mirtazapine added.

2.5.5. Limit of detection and quantification

The limit of detection (LOD) and the limit of quantification (LOQ) were estimated as 3.3 and 10 times standard deviation intercept/slope ratio, respectively [10].

3. Results and discussion

A spectrophotometric method was proposed for the analysis of mirtazapine in Norset®. It was realised in different conditions from those defined in the literature [4,6]. Karasen et al. [6] described spectrophotometric assays performed at a wavelength of 293.8 nm for mirtazapine dilutions in methanol. Our spectrometric measurements were performed in an acidic medium (H_3PO_4 50 mM) at pH 1.6. In these conditions, the wavelength of maximum absorption for mirtazapine was 315 nm. The amino-2-pyridine and *N*-methyl pyrazine functional groups were actually fully ionised, leading to a bathochromic shift and a hyperchromic change in the spectrum of mirtazapine, and to reproducible absorbance values. When operating at higher pH values, this spectrum was modified due to the effect of the acid–base equilibrium of the amino-2-pyridine group. The corresponding pKa value obtained from both spectrophotometric and potentiometric measurements was 3.6 ± 0.05 (data not shown). The *N*-methyl pyrazine group exhibited a more elevated ionisation constant. A pK_a value of 7.6 ± 0.05 was determined by potentiometric titration (data not shown) (a value of 7.1 was reported by Kelder et al. [11] in a hydro-methanolic medium). In the acidic conditions (pH 1.6), it had no incidence on the spectral features of mirtazapine.

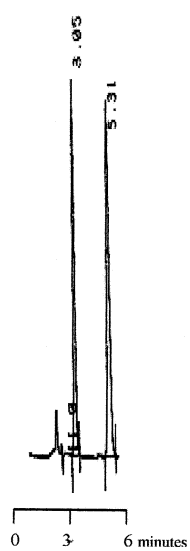

Fig. 1. Typical chromatogram of mirtazapine ($t = 3.05$ min) and internal standard ($t = 5.31$ min) under the described HPLC conditions.

The chromatographic and the electrophoretic conditions were optimised with respect to selectivity and time of analysis. In the development stage, different mobile phases in HPLC and running buffers in CZE were first tested. For the two methods, the most influent parameter was the pH of the aqueous phase. Then, others parameters showed to be very important, e.g. the choice of a short capillary tube or a temperature maintained at 25 °C in CZE shortened short time of analysis.

The proposed RPLC isocratic procedure was found to be suitable for a rapid separation of mirtazapine and the internal standard (retention times 3.05 and 5.31 min, respectively). A typical chromatogram is shown Fig. 1: the substances were eluted forming well shaped, symmetrical single peaks, well separated from the solvent front. Wynia et al. reported a determination of mirtazapine and related substances in tablets by a HPLC–UV procedure showing a retention time of 9.78 min for mirtazapine [7]. The method proposed in this paper is simpler and faster. The run time is less than 6 min and the mobile phase is stable and easier to prepare. An electropherogram obtained from CZE is reported Fig. 2. The migration time of mirtazapine and pirenzepine (I.S.) were $t_M = 3.52$ min and $t_M = 3.65$ min, respectively. The run time for each analysis was 6 min (migration time = 4 min and conditioning time prior to injection = 2 min). The time for the determination of mirtazapine with the HPLC method is similar to the CZE method.

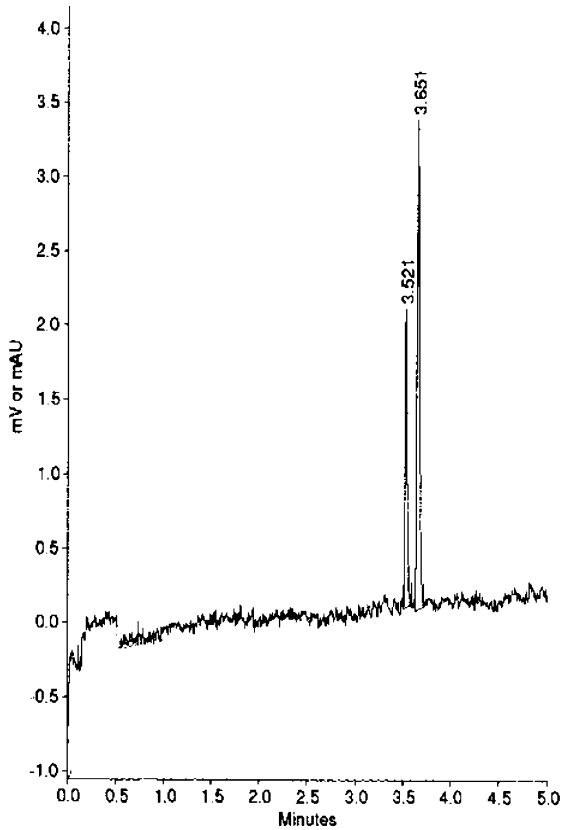

Fig. 2. Typical electropherogram of mirtazapine (migration time = 3.52 min) and internal standard (migration time = 3.65 min) under the described CZE conditions.

Our extraction procedure presents advantages in comparison with those described in the literature [7]. The extraction medium matched simultaneous the running buffer used for the CZE experiments and the mobile phase for the HPLC ones. It results no current fluctuation in CZE and satisfactory validation parameters for the two methods.

The four methods described for the determination of mirtazapine in tablets are specific. No interferences with excipients were encountered.

For quantification, linear calibration curves were obtained over the working concentration range 5–25 μg/ml for spectrophotometric, HPLC and CZE methods and 5–25 ng/ml for the spectrofluorimetric method. The range was different for spectrofluorimetry owing to the better sensitivity of the method. The equations of the calibration curves were obtained using the least square regression method. The slopes and the correlation coefficients (r) are listed in Table 1. Correlation coefficients for the linear fit are higher than 0.993. For the four methods, the results indicate a good linearity between the responses and the concentrations of mirtazapine.

The precision of the four methods is given in Table 2. Mirtazapine was determined six times in tablet forms. The RSDs values show that all the methods exhibit a good repeatability ($< 2.54\%$). No significant differences at 95% confidence level were found for the four methods (F-test).

Recovery values for the study of accuracy were carried out by analysing model mixtures of mirtazapine with the four methods. The recoveries are given in Table 3. The found values of mirtazapine are in good agreement with the theorical values in the three model mixtures. The recovery data ranged from 99.72% in HPLC to 101.47% in spectrofluorimetry.

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

Features	UV Spectrophotometry	Spectrofluorimetry	HPLC–UV	CZE–UV
Regression equation[a]	$y = 0.057x - 0.019$	$y = 3.993x + 1.053$	$y = 0.101x + 0.052$	$y = 0.057x - 0.018$
S.D. of slope	5×10^{-4}	4.1×10^{-2}	1.6×10^{-3}	1.6×10^{-3}
S.D. of intercept	1.9×10^{-2}	6.8×10^{-1}	2.4×10^{-2}	2.4×10^{-2}
Regression coefficient (r^2)	0.995	0.998	0.993	0.998
Linear range (μg/ml)	5–25	5–25	5–25	5–25

S.D. = standard deviation.

[a] $y = ax + b$ where x is the concentration in μg/ml for UV spectrophotometry, HPLC and CZE and the concentration in ng/ml for spectrofluorimetry, and y is the absorbance in UV spectrophotometry, the fluorescence intensity in spectrofluorimetry, mirtazapine/I.S. area ratio in HPLC–UV and normalized mirtazapine/I.S. area ratio in CZE–UV.

Table 2
Precision of the methods

	Amount found (mg in one tablet)			
	UV spectrophotometry	Spectrofluorimetry	HPLC–UV	CZE–UV
n	6	6	6	6
Mean	15.05	14.79	15.15	15.12
S.D.	0.28	0.37	0.09	0.09
RSD(%)	1.89	2.54	0.61	0.59

n = number of experiments, S.D. = standard deviation and RSD = relative standard deviation in percentage.

Table 3
Accuracy of the method

Model mixture (%)	UV spectrophotometry		Spectrofluorimetry		HPLC–UV		CZE–UV	
	Found (%)	RSD (%)	Found (%)	RSD (%)	Found (%)	RSD (%)	Found (%)	RSD (%)
A (10 mg)	100.42	1.42	100.45	0.98	101.02	0.69	99.76	0.86
B (15 mg)	101.46	0.94	101.47	0.93	99.85	0.42	100.86	0.51
C (20 mg)	101.21	1.95	100.31	1.42	99.72	0.67	100.90	0.17

Mean percentage recoveries and RSD of mirtazapine in model mixtures ($n = 6$).

Table 4
Limit of detection and limit of quantification of the four methods

	UV spectrophotometry (μg/ml)	Spectrofluorimetry (ng/ml)	HPLC–UV (μg/ml)	CZE–UV (μg/ml)
LOD	1.10	0.56	0.78	1.38
LOQ	3.30	1.70	2.37	4.21

The LOD and the LOQ of each method are given in Table 4. Spectrofluorimetry is the more sensitive one, with unit values estimated in ng/ml. Among the three other methods, HPLC is more sensitive than spectrophotometry and CZE.

As we observed the ruggedness of the four methods during the early stage of their development; we did not implement afterwards during validation [7]. The four methods have been proved to be rugged, particularly the HPLC and the CZE ones with respect to small changes in mobile phase and running buffer composition during method development.

From these validation results, it can be concluded that the four methods allow a selective quantitative determination of the mirtazapine content in Norset® tablets with the extraction procedure described herein.

4. Conclusion

We have developed four specific, selective, precise, sensitive and accurate analytical procedures, suitable for a reliable determination of mirtazapine in Norset® tablets for routine quality control. The extraction procedure is rapid and easy since a simple dissolution of the drug in methanol is necessary. The two spectrometric procedures are suitable for rapid and not very expensive quality control testing of Norset®, but they cannot be used for the quantitative determination of mir-

tazapine and impurities simultaneously. HPLC and CZE can be used for the quality control of mirtazapine and could also allow the detection and determination of some impurities, often present in pharmaceutical formulations. These methods allow to determine mirtazapine in the very short time of 6 min, compared with the existing methods in the literature [5,6]. Furthermore, as expected, the running costs of CZE caused by low solvent consumption and the use of cheap columns, are lower than for HPLC. However, the reproducibility of CZE is generally not as good as that of HPLC. So, in this study a combination of a constant capillary temperature, an appropriate electrolyte system, a hydrodynamic injection and the use of an internal standard improved the precision and allowed the use of CZE in quality control of pharmaceutical formulations.

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