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Analysis of reboxetine, a novel antidepressant drug, in pharmaceutical tablets by capillary electrophoresis and derivative spectrophotometry

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Abstract

The recent antidepressant drug reboxetine was quantified in pharmaceutical tablets by derivative spectrophotometry and capillary zone electrophoresis. The feasible sample pretreatment consists of a single extraction with a pH 2.5 phosphate buffer, centrifugation and dilution. For the spectrophotometric assay, the fourth derivative of the absorbance was used which gave satisfactory results in terms of accuracy (mean recovery 99.7%) and precision (mean RSD 3.4%). The electrophoretic experiments were carried out using the shortest effective length of the capillary (8.5 cm) in order to obtain a very rapid separation of reboxetine and dibenzepine used as the internal standard. Using a pH 2.5, 50 mM phosphate buffer as the background electrolyte, each analysis lasted less than 2.5 min. Accuracy (101.3%) and precision (1.5%) were very good.

Keywords: Reboxetine; Derivative spectrophotometry; Capillary electrophoresis; Pharmaceutical formulations

1. Introduction

Reboxetine, (RS)-2-[(RS)-α-(2-ethoxyphenoxy)-benzyl]morpholine (Fig. 1), is a novel antidepressant drug which is highly efficient in the treatment of major depression and has a low incidence of adverse effects.

The antidepressant activity of reboxetine is due to its selective inhibition of norepinephrine reuptake at the presynaptic level [1].

With respect to other older drugs such as tricyclic antidepressants (TCAs), reboxetine is reported to have several advantages in terms of safety [2]. In fact, reboxetine causes a low incidence of anticholinergic, cardiovascular and proconvulsant effects [3,4] since it has a lower affinity for the different receptors responsible for those side effects [5]. This is not to say that reboxetine

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does not cause side effects; the most recurrent side effects of reboxetine treatment are insomnia, diaphoresis, stypsis, tachycardia and urinary retention [6].

Reboxetine, however, has a short latency time for the onset of the therapeutic effect [7] and has minimal interaction with the cytochrome P450 system [8,9], which leads to little or no interference with the metabolism of other drugs, and is thus suitable for long-term treatment [10].

The antidepressant effect is obtained with daily dosages as low as 8–10 mg, which is about 15 times lower than that required by treatment with TCAs (150–200 mg/day) [11] and comparable to those required by treatment with SSRI (selective serotonin reuptake inhibitor) antidepressants drugs such as fluoxetine [12].

Reboxetine has been recently introduced on the market; it is present in Italy as Edronax® (Pharmacia & Upjohn) and Davedax® (Bracco) tablets. Each tablet contains 2 or 4 mg of reboxetine methansulfonate.

The aim of this study is the development of new analytical methods for the quality control of pharmaceutical formulations containing reboxetine. To our knowledge, no paper is present in the literature that deals with the determination of reboxetine in pharmaceutical preparations. Furthermore, none of the main Pharmacopoeias (United States Pharmacopeia XXIV, 2000, and Supplements; British Pharmacopoeia 2000; European Pharmacopoeia Supplement 2001) report any monograph on the subject.

On the contrary, some papers on the analysis of the drug in biological fluids are available. These papers propose analytical methods based on the use of HPLC with fluorimetric detection after derivatisation of reboxetine with suitable fluorogenic reagents such as 1-(9-fluorenyl)ethyl chloroformate [13–15]. The use of high-sensitivity methods is necessary for the determination of the very low reboxetine levels (a few nanograms per millilitre) in the plasma of patients under therapy. For the quality control of tablets, however, this high sensitivity is not required. For this reason we studied and implemented two feasible methods based on derivative spectrophotometry (DS) and capillary zone electrophoresis (HPCE).

2. Experimental

2.1. Chemicals

Reboxetine methansulfonate was kindly provided by Pharmacia & Upjohn S.p.A. (Milan, Italy). Dibenzepine used as the internal standard (I.S., Fig. 1) for the HPCE method was kindly provided by Novartis AG (Basel, Switzerland). Methanol, phosphoric acid (85%, v/v) and potassium hydroxide pellets were pure for analysis from Carlo Erba (Milan, Italy). Ultrapure water

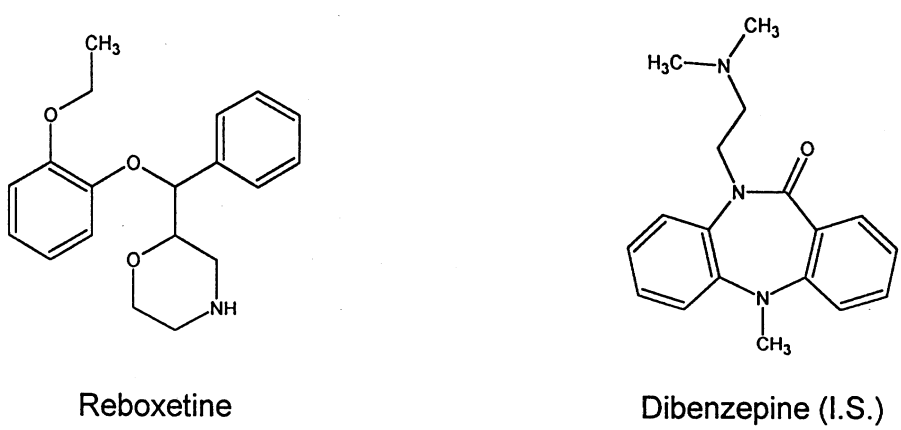

Fig. 1. Chemical structures of reboxetine and dibenzepine (I.S.).

(18.2 MΩ cm) was obtained from a Millipore (Milford, Mass.) MilliQ apparatus.

2.2. Extraction procedure of reboxetine from pharmaceutical formulations

The commercial pharmaceutical formulations analysed were tablets of Davedax® (Pharmacia & Upjohn S.p.A.) and Edronax® (Bracco S.p.A., Milan, Italy). Each tablet had a reboxetine declared content (calculated as free base) of 4 mg.

Both kinds of tablets contained microcrystalline cellulose, calcium hydrogen phosphate dihydrate, crospovidone, silicon dioxide and magnesium stearate as excipients.

Reboxetine was extracted from the tablets using the following procedure. At first, ten tablets were accurately weighed, finely ground to a powder and thoroughly mixed. Then, an aliquot of this powder corresponding to 4 mg of reboxetine (calculated as free base) was weighed and then transferred to a centrifuge vial. A 10-ml volume of pH 2.5, 50 mM phosphate buffer was added, the mixture was agitated for 5 min and then centrifuged for 15 min at 3000 rpm. The supernatant was filtered through a paper filter (55 mm, Whatman, Maidstone, UK). The solution thus obtained (stock sample solution) had a nominal concentration of 400 μg/ml of reboxetine free base. The working solutions (sample solutions) were obtained by diluting this stock solution with the pH 2.5, 50 mM buffer (spectrophotometric method) or with water (HPCE method).

2.3. DS method – apparatus and leading conditions

A Jasco (Tokyo, Japan) UVIDEC-610 double-beam spectrophotometer was used. Stock solutions of reboxetine were 1 mg/ml in methanol. Standard solutions were prepared daily by diluting stock solutions with a pH 2.5, 50 mM phosphate buffer. The stock solutions in methanol were stored at −20 °C and were stable for at least 3 months.

Measurements were carried out using the difference between the fourth derivative of the absorbance at 282 and 287 nm (with a 5× magnification), against a blank of pH 2.5, 50 mM phosphate buffer.

2.4. HPCE method – apparatus and electrophoretic conditions

An Agilent Technologies (Palo Alto, CA) ^{3D}CE electrophoretic system with diode array detection was used. The electrophoretic runs were monitored at 206 nm. The analyses were carried out on a Supelco (Bellefonte, PA) CElect-F50 uncoated fused silica capillary with a total length of 33.0 cm (effective length: 8.5 cm) and an internal diameter of 50 μm (outer diameter: 363 μm). The background electrolyte (BGE) was a pH 2.5, 50 mM phosphate buffer. Samples were injected by pressure (−50 mbar for 10 s) at the cathodic end of the capillary, followed by water (−50 bar for 5 s). Then a potential of 20 kV was applied. The capillary was thermostatted at 25 °C.

The capillary was rinsed (pressure: 90 kPa) with water (10 min) and BGE (20 min) at the beginning of every working day. After each electrophoretic run the capillary was washed with BGE (5 min), water (5 min) and BGE (5 min). At the end of the day the capillary was washed with water (15 min) and air-dried for 5 min.

Stock solutions of reboxetine and dibenzepine (I.S.) were 1 mg/ml in BGE. These stock solutions were stored at −20 °C and were stable for at least 1 month. Standard solutions were prepared daily by diluting stock solutions with water.

2.5. Method validation

A ten-point calibration curve on standard solutions was set up for DS in the 20–100 μg/ml concentration range plotting the difference of the fourth derivative of the absorbance at 287 and 282 nm against the corresponding reboxetine concentration (expressed as μg/ml).

A ten-point calibration curve on standard solutions was set up for HPCE in the 1–50 μg/ml concentration range analysing sample solutions and plotting the reboxetine/I.S. peak height ratio (a dimensionless number) against the corresponding reboxetine concentration (expressed as μg/ml).

The amount found of declared was calculated analysing sample solutions from tablets having a nominal reboxetine concentration of 20, 40 and 80 μg/ml (DS) or or 5, 10 and 30 μg/ml (HPCE) and interpolating the measurements on the respective calibration curve. The reboxetine concentration thus found was then compared with the nominal concentration.

Accuracy was evaluated by means of recovery studies. Known amounts of reboxetine pure compound powder were added to known amounts of formulation powder (whose reboxetine content had already been determined), to obtain final reboxetine additions of 20, 40 and 80 μg/ml (DS) or 5, 10 and 30 μg/ml (HPCE). The mixture was then analysed and the percentage recovery of added reboxetine was calculated.

Precision was evaluated by repeating the same assay six times in the same day (to obtain repeatability) and six times over six different days (to obtain intermediate precision).

The limit of quantitation (LOQ) and limit of detection (LOD) were calculated according to USP XXIV Edition guidelines [16].

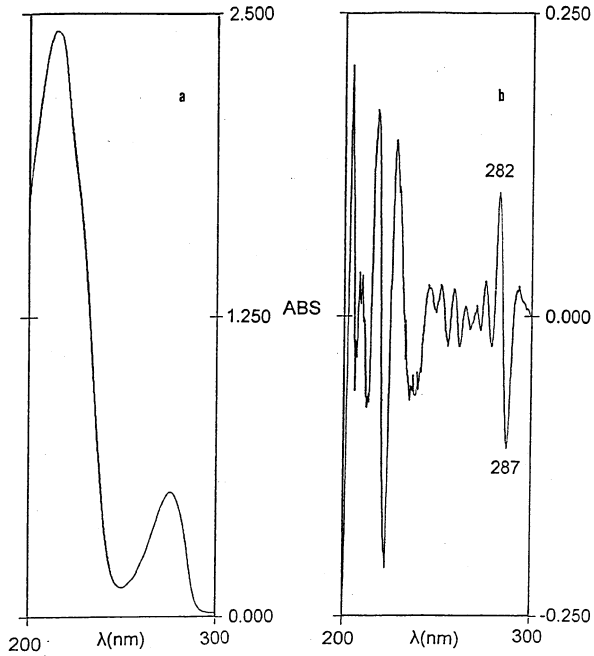

Fig. 2. (a) Ultraviolet spectrum of a reboxetine standard solution (80 μg/ml) and (b) fourth derivative spectrum of an Edronax® sample solution (nominal concentration: 80 μg/ml).

3. Results and discussion

One simple and fast extraction procedure of reboxetine from commercial tablets was implemented for both analytical methods. In fact, reboxetine was completely extracted from the powdered formulations with a one-step extraction using a pH 2.5 phosphate buffer, following the same procedure already reported in our previous paper [17] on the analysis of neuroleptic drugs in pharmaceutical formulations. This feasible extraction procedure of reboxetine from tablets did not lead to any interference for the HPCE method, and to only a small interference for the spectrophotometric method; the interference was eliminated using the fourth derivative spectra.

3.1. DS method

The spectrum of a 40 μg/ml reboxetine standard solution in a pH 2.5 phosphate buffer (against a blank of the same buffer) is shown in Fig. 2a. Two intense absorbance bands in the UV region, with maxima at 206 and 274 nm, are apparent. Preliminary assays however demonstrated that neither maximum allowed for a reliable determination of reboxetine in pharmaceutical formulations (amounts found of declared were higher than 120%, probably because of interference from excipients). For this reason, several assays were carried out using the first, second, third and fourth derivative spectra; best results were obtained when using the fourth derivative of the absorbance, namely the difference between the fourth derivative values at 287 and 282 nm, so these were used for all subsequent assays.

Good linearity was obtained on standard solutions over the 20–100 μg/ml concentration range. The linearity equation was $y = -0.0007 + 0.00174x$ ($r_c = 0.9998$), where x is the reboxetine concentration (expressed as μg/ml) and y is the difference between the values of fourth derivative at 287 and 282 nm. Standard errors for intercept

and slope were 1.3×10^{-3} and 2.7×10^{-5}, respectively. Precision assessed on standard solutions was satisfactory: RSD% values of 2.3% (repeatability) and 2.6% (intermediate precision) were found for six replicates at a concentration of 40 μg/ml.

The fourth derivative spectra of formulation sample solutions (Fig. 2b) are morphologically identical to those of standard solutions. The amount of reboxetine found of declared and the precision of the method were calculated on sample solutions at nominal concentrations of 20, 40 and 80 ng/ml. Accuracy was calculated adding known amounts of reboxetine pure substance to powdered formulations, obtaining additions of 20, 40 and 80 μg/ml (total concentrations: 40, 60, 100 μg/ml). As can be seen from Tables 1 and 2, all assays gave satisfactory results: the mean amount found of declared was always between 97.9 and 103.4% for both formulations, while precision RSD% values were always under 4.4% and accuracy above 98.2%. The LOQ was 20 μg/ml and the LOD 7 μg/ml, according to USP XXIV guidelines [16].

3.2. HPCE method

A paper on the determination of 11 CNS drugs by means of capillary electrophoresis has been recently published by us [18]. This paper represented the starting point for the electrophoretic analysis of reboxetine in commercial tablets. The same BGE was used at first, and namely a pH 2.5, 35 mM phosphate buffer; however, the addition of polyvinylpyrrolidone was not deemed necessary because only two compounds (reboxetine and the I.S. dibenzepine) had to be separated. Broad and tailing peaks were obtained under these conditions, thus higher BGE concentrations were tried; at 100 mM the current intensity value was too high (> 80 μA); a BGE concentration of 50 mM was found optimal. Furthermore, different dilution solvents (BGE, diluted BGE, water) were tried. Best results in terms of peak shape and reproducibility were obtained diluting stock solutions with water. Three different wavelenghts were monitored: those corresponding to the absorbance maxima of reboxetine (206 and 274 nm) and 200 nm; then 206 was selected for all measurements, because it gave the highest sensitivity and reproducibility. The effective length of the capillary was 8.5 cm (total length 33.0 cm) and its internal diameter 50 μm; the voltage applied was 20 kV and the capillary temperature was 25 °C. Under these leading conditions, reboxetine and the I.S. (dibenzepine) are positively charged, thus they run towards the cathode. Reboxetine is detected as a neat electrophoretic peak at migration time (t_m) = 1.74 min, while the I.S. is detected at 1.56 min. Good linearity was obtained on standard solutions in the 1–50 μg/ml concentration range (r_c = 0.9995) plotting the reboxetine/I.S. peak height ratio (a dimensionless number) against the correspondent reboxetine concentration (expressed as

Table 1
Assays on commercial tablets

Method	Reboxetine nominal concentration (μg/ml)	Edronax® found/declared% (RSD%)		Davedax® found/declared% (RSD%)	
		Repeatability[a]	Intermediate precision[a]	Repeatability[a]	Intermediate precision[a]
Fourth DS	20	97.9 (4.1)	101.9 (4.3)	101.9 (4.3)	102.4 (4.4)
	40	99.6 (3.7)	102.1 (3.9)	102.2 (4.0)	101.0 (4.1)
	80	98.4 (2.0)	102.3 (2.1)	101.4 (2.3)	103.4 (2.4)
HPCE–UV	5	98.9 (1.6)	98.8 (1.8)	99.3 (1.7)	98.5 (1.8)
	10	99.5 (1.5)	99.5 (1.9)	98.5 (1.6)	99.8 (1.7)
	30	99.4 (1.5)	99.7 (1.8)	100.5 (1.6)	100.9 (1.7)

[a] $n = 6$.

Table 2
Accuracy of the method

Method	Reboxetine concentration added (μg/ml)	Edronax®		Davedax®	
		Recovery (%)[a]	RSD%[a]	Recovery (%)[a]	RSD%[a]
Fourth DS	20	98.7	3.8	101.8	4.2
	40	98.2	3.6	100.7	3.5
	80	99.6	2.9	99.0	2.5
HPCE–UV	5	101.2	1.9	101.3	1.3
	10	99.4	1.7	102.3	1.4
	30	100.7	1.5	102.9	1.3

[a] $n = 6$; RSD% corresponds to intermediate precision.

μg/ml). The regression equation was $y = 0.01495 + 0.20688x$. Standard errors for intercept and slope were 9.8×10^{-3} and 2.3×10^{-5}, respectively. Precision was evaluated on 5 μg/ml standard solutions: RSD% values of 1.1% for repeatability and 1.5% for intermediate precision were found. Application of the method to pharmaceutical formulations gave good results. The electropherogram of a 20 μg/ml sample solution (nominal concentration) obtained from Davedax® tablets is reported in Fig. 3. The analyte and I.S. peaks are neat and apparent at $t_m = 1.74$ and 1.56 min, respectively; no interference from the formulation matrix is present.

The amount found of declared was always very near to 100% (98.8–100.9%), with good precision values ($1.5 \leqslant \text{RSD}\% \leqslant 1.9$), as can be seen from Table 1.

Accuracy was evaluated by means of recovery studies at three concentration levels (5, 10 and 30 μg/ml). Results were good: recovery values were between 99.4 and 102.9% (Table 2).

The LOQ was 1.0 μg/ml and the LOD 0.3 μg/ml, according to USP XXIV guidelines [16].

4. Conclusions

The two proposed methods, one based on DS and one based on HPCE, are suitable for the determination of reboxetine in commercial tablets which are the only kind of formulation currently available on the Italian drug market.

The methods are simple, reliable and fast: in fact, the spectrophotometric method requires only a wavelength scan and the automatic calculation of the fourth derivative, while the electrophoretic run lasts less than 2.5 min.

Of the two methods, the electrophoretic one appears to be the most precise: RSD% values between 1.5 and 1.9% were obtained, while the spectrophotometric method gave RSD% values between 2.0 and 4.4%.

Furthermore, the proposed methods are inexpensive and non-polluting, because small volumes of buffer are needed for the preparation and analysis of samples and because organic solvent are not used at all.

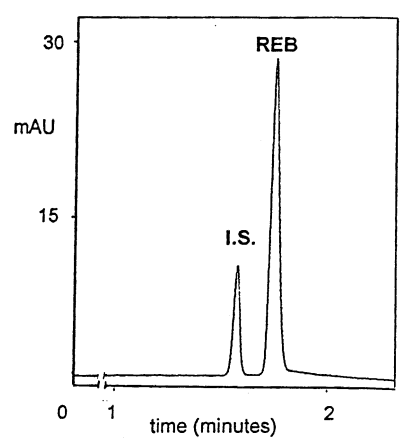

Fig. 3. Electropherogram a Davedax® sample solution (nominal concentration: 20 μg/ml) containing 5 μg/ml I.S. Legend: REB = Reboxetine.

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