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Quantitation of olanzapine in tablets by HPLC, CZE, derivative spectrometry and linear voltammetry

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Abstract

Four analytical methods have been developed for the quality control of pharmaceutical formulations containing the novel antipsychotic drug, olanzapine: high performance liquid chromatography (HPLC), capillary zone electrophoresis (CZE), derivative spectrometry and linear voltammetry. All methods require only a simple extraction procedure of olanzapine from the tablets before analysis. HPLC with ultraviolet detection at 260 nm is carried out with a C8 column and a mobile phase constituted of acetonitrile and aqueous tetramethylammonium perchlorate. CZE is performed in an uncoated capillary with phosphate buffer, pH 3.0, as the background electrolyte, with UV detection at 214 nm. Spectrophotometry uses the derivative of the spectrum at 298 nm. In linear voltammetric method (LSV) the current intensity of the oxidation wave at +495 mV is measured. All methods gave similar results in terms of precision and accuracy. For HPLC and CZE, repeatability and intermediate precision, expressed by the RSD was better than 1.8%. The accuracy, resulting from recovery experiments, was between 99.9 and 101.1%. Spectrometry and voltammetry gave slightly higher RSD values (up to 2.9%) and a larger variation of the accuracy (the recovery was between 97.8 and 102.6%). However, the requirements for quantitative analysis are fulfilled for all methods.

Keywords: Olanzapine; Liquid chromatography; Capillary electrophoresis; Spectrophotometry; Voltammetry; Pharmaceutical formulations

1. Introduction

The last years have seen several improvements in the treatment of psychiatric disorders. In particular, many new drugs other than classical neuroleptics (phenothiazines, butyrophenones) are now available for the treatment of schizophrenia. These new drugs, called 'atypical antipsychotics',

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such as risperidone, clozapine and olanzapine, seem to be more effective than the previously used ones, since they can suppress both, positive and negative symptoms of schizophrenia [1,2], whereas classical neuroleptics are only active against the positive symptoms of the illness. Furthermore, the recent drugs cause less extrapyramidal side effects than the older ones [3,4].

The most recent atypical antipsychotic drug to become commercially available is olanzapine (2-methyl-4-(4-methyl-1piperazinyl)-10H-thieno [2,3b][1, 5]benzodiazepine, Fig. 1).

Olanzapine is a very active drug, and as such is administered at very low dosages [5]. In fact, it is usually administered as one or two daily oral doses, for an overall dosage of 5–20 mg per day [6]. Several techniques are reported in the literature for the determination of olanzapine levels in biological fluids; among these are gas chromatography [7,8], HPLC with electrochemical [9–12], or UV detection [13] and liquid chromatography – tandem mass spectrometry (LC-APCI-MS-MS) [14].

The drug has been introduced in the last year in the Italian market as film-coated, gastro-resistant Zyprexa® tablets containing either 5 or 10 mg of olanzapine each. The need for a reliable quality control of pharmaceutical formulations containing olanzapine is obvious. However, to our best knowledge any method has been described so far in the literature for the quality control of pharmaceutical formulations containing olanzapine, except our paper on the determination of several antipsychotic in pharmaceutical formulations [15]. It is therefore the aim of this paper to develop and compare analytical methods for the determination of olanzapine in tablets commercially available. This was done based on HPLC, CZE, a spectrophotometric method and a voltammetric method, respectively.

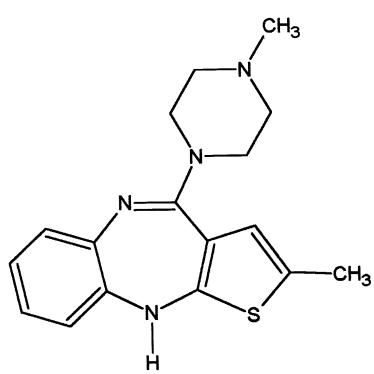

Fig. 1. Chemical structure of olanzapine.

2. Experimental

2.1. Chemicals

Olanzapine reference standard, 2-methylolanzapine (LY170222) and Ziprexa® tablets (10 mg) were kindly donated from Eli Lilly and Co. (Indianapolis, IN). Carbamazepine and tetramethylammonium perchlorate were purchased from Sigma Chemicals (St Louis, MO). Acetonitrile and methanol (HPLC grade), 60% (w/w) perchloric acid, 25% (w/w) ammonia, ethyl acetate (analytical grade) were from Carlo Erba (Milan, Italy). 35% (w/w) hydrochloric acid was from Loba Feinchemie AG (Fischamend, Austria) Sodium hydroxide (analytical grade) and 85% (w/w) ortho-phosphoric acid were from E. Merck (Darmstadt, Germany). Ultrapure water (18.2 MΩ cm) was obtained by means of a Millipore (Milford, MA) MilliQ apparatus.

2.2. Apparatus and experimental conditions

2.2.1. Method A: high-performance liquid chromatography (HPLC)

The chromatographic apparatus consisted of a Varian (Harbor City, CA) Model 9001 chromatographic pump and a Jasco Model 975 spectrophotometric detector set at 260 nm. The separation was achieved on a Varian ResElut reversed phase column (C8, 150 × 4.6 mm i.d., 5 μm), kept at room temperature. The mobile phase was an acetonitrile-tetramethylammonium perchlorate (17 mM; pH 2.6) (45:55, v/v) (pH* 3.1) mixture and was filtered (Millipore membrane filters: nylon, 47 mm diameter, 0.2 μm pore size) and degassed by ultrasonication (Transsonic T-310 apparatus from Elma GmbH, Singen, Germany) before use. Injection was carried out with a loop of 20 μl volume.

The flow rate was 0.7 ml min^{-1}. Data were handled by means of a Waters (Milford) Model 745 integrator.

2.2.2. Method B: capillary zone electrophoresis (CZE)

CZE was carried out with an instrument (Applied Biosystem 270A-HT, Perkin–Elmer Corporation, Foster City, CA) using an uncoated fused-silica capillary (Supelco, PA) with a total length of 43.0 cm, effective length of 23.0 cm and 50 μm i.d. The background electrolyte was phosphate buffer (50 mM; pH 3.0). The sample was injected into the capillary by pressure (165 mbar) for 1 s. The instrument was operated at +15 kV with currents typically less than 35 μA. Detection was carried out at 214 nm (detector placed at cathodic side of capillary). The data were recorded and processed with a dual-channel interface (970 A, PE Nelson, Cupertino, CA).

Before filling with CZE buffer, the capillary was rinsed for 10 min with deionized water, 10 min with 0.1 M sodium hydroxide, 5 min with 1 M sodium hydroxide, 10 min with 0.1 M sodium hydroxide and 30 min with water. This procedure was repeated after every 30 runs. For storage overnight, the capillary was additionally washed with water for 10 min.

2.2.3. Method C: derivative spectrophotometry

For the spectrophotometric assays a Jasco (Tokyo, Japan) Uvidec-610 double-beam spectrophotometer and quartz cuvettes (1 cm optical path) were used. Measurements were carried out using the first derivative of the absorbance spectra, measuring the height above zero of the peak at 298 nm.

2.2.4. Method D: linear scan voltammetry (LSV)

The voltammetric assays were carried out using an AMEL (Milan, Italy) Model 433 voltammeter (working electrode: glassy carbon, reference electrode: Ag–AgCl; auxiliary electrode: stainless steel). Voltammograms were obtained by means of linear scan voltammetry, with the voltage increasing from 0 to 1000 mV at the constant rate of 50 mV s^{-1}, using a phosphate buffer (250 mM; pH 2.5) as the supporting electrolyte The assays were carried out while thermostatting the solutions at 25°C, measuring the current intensity at the potential value of +495 mV.

2.3. Extraction procedures

2.3.1. Method A

An amount of the powder, nominally corresponding to 5 mg of olanzapine, was accurately weighed, added to 10 mg of carbamazepine (the internal standard) and 10 ml of 2% (w/w) ammonia and sonicated for 10 min, to facilitate dissolution. The mixture was then transferred to a separating funnel and extracted with 5 ml of ethyl acetate four times. The collected organic layers were cooled at 4°C for 20 min, then filtered through Whatman (Maidstone, England) 1PS phase separator filters. This solution has an olanzapine nominal concentration of 250 μg ml^{-1}.

2.3.2. Method B

Solutions of olanzapine were prepared by transferring the weighed tablet powder into an appropriate volume of acidic solution (pH ~ 2) obtained with 20 μl of hydrochloric acid 35% (w/w), treating the mixture in an ultrasonic bath for 15 min followed by centrifugation (at 15 000 rpm) for 5 min. The supernatant solutions consisted of olanzapine at a declared concentration of 200 μg ml^{-1}. All solutions were filtered (0.45 μm, Minisart RC25, Sartorious, Göttingen, Germany) prior to use.

2.3.3. Methods C and D

The solution was prepared as for method A, except the internal standard was not added.

2.4. Quantitation

2.4.1. Method A

Stock solutions of olanzapine and carbamazepine were 1 mg ml^{-1} in methanol. Dilutions were made using the mobile phase. Standard solutions of olanzapine at different concentrations and containing 100 ng ml^{-1} of carbamazepine (IS) were injected into the HPLC. The ten-point calibration curves were set up by means of the least square method, plotting the value of the

olanzapine/IS area ratio against the olanzapine concentration. The Zyprexa® solution was diluted with methanol (first dilution) and then the mobile phase (subsequent dilutions).

2.4.2. Method B

Stock solutions of olanzapine were pH 2, 50 mM phosphate buffer. Ultrapure water was used for all dilutions. Standard solutions of olanzapine at different concentrations and containing 25 μg ml^{-1} of 2-methylolanzapine (IS) were injected into the HPCE. The calibration curves were set up plotting the value of the olanzapine/IS area ratio against the olanzapine concentration. The Zyprexa® solution was diluted with water.

2.4.3. Method C

Stock solutions of olanzapine were 1 mg ml^{-1} in methanol. Dilutions were made using a pH 2.5, 250 mM phosphate buffer. The calibration curves were set up analysing standard solutions at different concentrations and plotting the value of the first derivative of absorbance at 298 nm against the olanzapine concentration. The Zyprexa® solution was diluted with methanol (first dilution), then with a pH 2.5, 250 mM phosphate buffer.

2.4.4. Method D

Stock solutions of olanzapine were 1 mg ml^{-1} in methanol. Dilutions were made using the mobile phase of the HPLC method. The calibration curves were set up analysing standard solutions at different concentrations and plotting the value of the current intensity at $+495$ mV against the olanzapine concentration. The Zyprexa® solution was diluted with methanol (first dilution), then with the mobile phase.

For all methods, the values resulting from the analyses of diluted Zyprexa® solutions were interpolated on the respective calibration curves and the percentage of olanzapine found related to the declared amount was expressed as follows: (concentration found/concentration declared) $\times$ 100.

2.4.5. Precision assays

Olanzapine standard and Zyprexa® solutions were prepared and analysed six times within the same day to obtain the repeatability and six times over different days to obtain the intermediate precision, according to USP requirements [16]. Each assay was carried out on a different extraction of olanzapine from the tablets. The percentage relative standard deviations (RSD%) of the data obtained were calculated.

2.4.6. Accuracy

Known amounts of olanzapine powder were added to known amounts of the powdered Ziprexa® tablets, in order to obtain three different levels of addition. The samples were analysed and the mean recovery, as well as the repeatability was calculated on six assays for each concentration added.

3. Results and discussion

Preliminary spectrophotometric assays on Ziprexa® tablets were made by dissolving olanzapine from the powdered formulation either in methanol, methanol-water (50:50, v/v), or isopropanol, filtering and subjecting the resulting solution to spectrophotometric measurements. However, none of these assays gave satisfactory results, due to heavy interference from the excipients. An extraction with water followed by another extraction on the remaining powder with isopropanol gave unsatisfactory results as well. For this reason a modification of the extraction procedure for olanzapine as the free base with ethyl acetate (as reported in the Experimental section) was worked out for methods A, C and D. As CZE uses aqueous background electrolytes, an even simpler extraction with acidified aqueous solvent was found appropriate for method B.

4. HPLC method

4.1. Separation

Our previous work on the determination of several CNS (central nervous system) drugs in plasma [17] prompted us to develop this simple HPLC method with UV detection for the quantitation of olanzapine with carbamazepine chosen

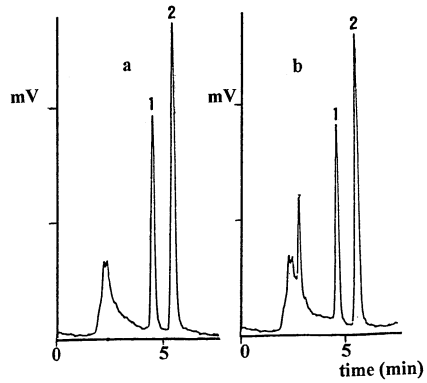

Fig. 2. Chromatograms of a 50 ng ml^{-1} olanzapine standard solution (a) and a 50 ng ml^{-1} (olanzapine nominal concentration) Ziprexa® extract (b), containing 200 ng ml^{-1} of IS (carbamazepine) each. Peak identification: 1, IS; 2, Olanzapine. Conditions: RP C8 column, 150 length, 4.6 mm i.d., 5 μm particle size. Mobile phase: acetonitrile-tetramethylammonium perchlorate (17 mM; pH 2.6) (45:55, v/v, pH* 3.1). Flow rate 0.7 ml min^{-1}. Detection at 260 nm.

as internal standard. A modification of the previous method was necessary for separation, changing the mobile phase composition and the flow rate as well in order to adjust baseline separation of olanzapine from carbamazepine. Increase of sensitivity resulted from the change of the detection wavelength from 270 to 260 nm, which is an absorbance maximum of the UV spectrum when solutions are prepared in the mobile phase instead of methanol. As a result, olanzapine and the I.S. are eluted within 6.5 min on a C8 column with a mobile phase composed of acetonitrile-tetramethylammonium perchlorate (17 mM; pH 2.6) (45:55, v/v) flowing at 0.7 ml min^{-1}. The chromatogram of a 50 ng ml^{-1} olanzapine standard solution containing 200 ng ml^{-1} of carbamazepine obtained is shown in Fig. 2.

4.2. Quantitative analysis

The equation for the calibration line between 10 and 150 ng ml^{-1} olanzapine standard solutions, obtained by the least-square regression was: $y = 0.0330\ x - 0.0241$, where x is the olanzapine concentration, expressed as ng ml^{-1}, and y is the ratio between the peak area value of olanzapine and the peak area value of carbamazepine. The linearity, expressed by the linear correlation coefficient, r, was 0.9997. The LOQ (quantitation limit) value was 10 ng ml^{-1} and the LOD (detection limit) value was 6 ng ml^{-1}. The precision assays gave RSD% values of 1.2 for the repeatability and 1.3 for the intermediate precision on 50 ng ml^{-1} olanzapine standard solutions ($n = 6$). The method was applied to the analysis of olanzapine solutions obtained from Ziprexa® tablets. The chromatogram of an extract having a nominal olanzapine concentration of 50 ng ml^{-1} is shown in Fig. 2b. It can be seen that no interference is present. The percentage of label claim found, reported in Table 1, is near to 100%. Here the precision expressed by the RSD% (percentage relative standard deviation) values was below 2% for both repeatability and intermediate precision. The accuracy of the method was evaluated by means of recovery studies. All data are detailed in Table 2. The results show that the mean recovery value was 100.5%, with a mean repeatability (RSD%) of 1.4%.

4.3. CZE.

4.3.1. Separation

CZE of positively charged analytes in uncoated fused silica capillaries might be problematic due

Table 1
Olanzapine content of Zyprexa® tablets quantified by different analytical methods[a]

Parameter	HPLC	CZE	UV	LSV
% Drug found of declared	99.4	99.3	99.3	98.1
Repeatability (RSD%)	1.4	0.7	1.6	2.3
Intermediate precision (RSD%)	1.8	1.3	2.3	2.9
Concentration (μg ml^{-1})	0.050	25.0	2.5	10.0

[a] Each value is the result of six independent assays ($n = 6$).

Table 2
Results of the recovery experiments for the characterisation of the accuracy of the different analytical methods[a]

Parameter	HPLC	CZE	UV	LSV
Low concentration				
Recovery%	101.1	101.1	102.6	97.8
Repeatability (RSD%)	1.8	0.4	1.6	3.5
Concentration (μg ml^{-1})	0.010	10.0	2.5	6.2
Middle concentration				
Recovery%	100.3	99.9	101.8	98.2
Repeatability (RSD%)	1.3	1.5	1.0	3.2
Concentration (μg ml^{-1})	0.025	20.0	10.0	20.0
High concentration				
Recovery%	100.0	99.9	101.3	97.9
Repeatability (RSD%)	1.0	0.9	0.6	3.0
Concentration (μg ml^{-1})	0.100	40.0	50.0	40.0

[a] Each value is the result of six independent assays ($n = 6$).

to the electrostatic interaction of the analytes with the negatively charged wall. Therefore a too high pH of the background electrolyte must be avoided in many cases for such analytes, otherwise peak distortion or even loss of analytes due to irreversible adsorption can take place. This restriction is obviously counter-productive for the rapid analysis of cations, because at low pH also the electroosmotic flow (migrating to the cathodic side of the separation capillary) is reduced as well. However, under the acidic condition finally adjusted (the pH range between 2 and 4.5 was investigated), olanzapine is baseline separated from the I.S. (2-methylolanzapine), in less than 3 min (see Fig. 3). Very sharp peaks are obtained, reflecting the high separation efficiency of the method.

4.4. Quantitative analysis

In the 10–50 μg ml^{-1} range of olanzapine concentration, the equation of linear regression, $y = ax + b$ (y is the area ratio, and x is the concentration of olanzapine in μg ml^{-1}) was calculated using the least square method. The following equation was obtained: $y = 0.03622x + 0.01643$, and used for further quantitation. The resulting linear correlation coefficient was 0.9994. The LOQ value was 0.9 μg ml^{-1} and the LOD value was 0.3 μg ml^{-1}.

The repeatability of the CE measurement, determined from six consecutive injections of 30 μg ml^{-1} solutions, as expressed by the relative standard deviation, was 1.2%.

An appropriate aliquot of solutions obtained by treatment of the pharmaceutical formulations was diluted with water to a declared concentration of 25 μg ml^{-1} olanzapine. A typical electro-

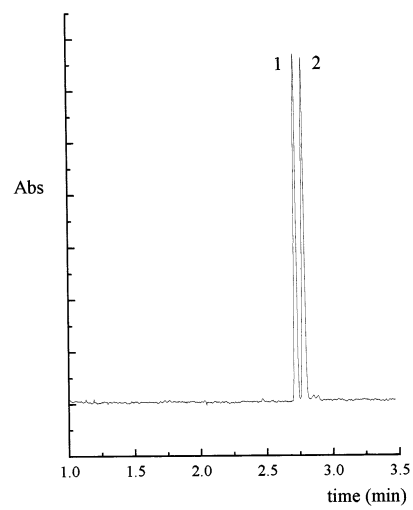

Fig. 3. Capillary zone electropherogram of olanzapine (nominal concentration 25.0 μg ml^{-1}) obtained from Zyprexa® tablets. Peak identification: 1, Olanzapine; 2, IS. Conditions: uncoated fused silica capillary, 43 cm, 23 cm total and effective length, 50 μm i.d. BGE: phosphate buffer (50 mM, pH 3.0). Voltage +15 kV. Detection at 214 nm.

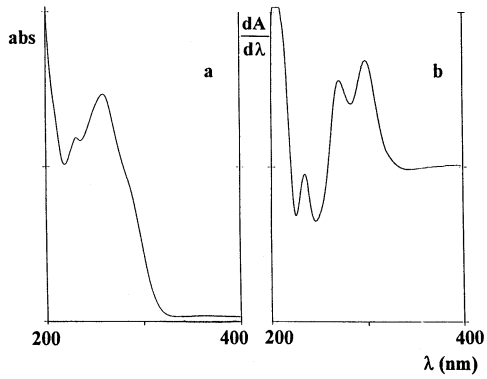

Fig. 4. Ultraviolet spectrum of a 10 μg ml^{-1} olanzapine standard solution in the mobile phase (a); first derivative spectrum of the same solution (b).

pherogram is shown in Fig. 3, obtained from Zyprexa® tablets. It can be seen that no interference from the sample matrix are observed.

The results of quantitation obtained by the ratio of area of olanzapine to IS are depicted in Table 1. It can be seen that for both formulations the content found is about 99% of the declared content. The precision of the method (RSD%) is always better than 1.4 ($n = 6$). The recovery as a measure for the accuracy is about 100% (see Table 2).

4.5. Spectrophotometric method

The spectrum of a standard solution with 10 μg ml^{-1} olanzapine concentration in the mobile phase of the HPLC method as solvent is shown in Fig. 4a; the two main absorbance bands are at 260 and 232 nm. For simplicity we chose the solvent of the standard solutions was identical with the mobile phase for the HPLC assays as well as for the spectrophotometric assays, because this allowed for the preparation of only one series of solutions for each assay.

The spectrum of a solution obtained from the extraction of Ziprexa® tablets is morphologically identical to the spectrum of the standard solution. The percentage of label claim (olanzapine found of declared) is, however, too high (about 130%), probably due to interfering substances not completely eliminated by the extraction step. For this reason the derivative spectra (1st, 2nd, 3rd and 4th derivative) were measured. It was found that the first derivative spectra, namely the derivative value at 298 nm, was best suited for quantitative determinations. In fact, this band shows no interference due to the excipients and is higher than those of the other derivative spectra, thus allowing for accurate quantitation even at relatively low concentrations. The first derivative spectrum of a standard olanzapine solution is shown in Fig. 4b, where the maximum at 298 nm is apparent.

The calibration curve, set up in the 1.0–100.0 μg ml^{-1} concentration range is expressed by the linear regression equation $y = 0.0229x + 0.0007$, where x is the olanzapine concentration, expressed as μg ml^{-1}, and y is five times the first derivative value, expressed as AU nm^{-1}. The linear correlation coefficient, r, is 0.9998. The LOQ value was 1 μg ml^{-1} and the LOD value was 0.5 μg ml^{-1}.

The precision data (expressed as RSD% values) were determined on 2.5 μg ml^{-1} olanzapine standard solutions, and were 1.6 for both repeatability and intermediate precision.

The quality control assays gave the results as shown in Table 1: the percentage of label claim is higher than 99%, and the RSD is better than 2.5%.

Addition of pure olanzapine to the sample at three different concentration for the determination of the recovery studies leads to the following results: mean recovery is 101.9%, with a mean RSD% of 1.1% (Table 2).

4.6. Voltammetric method

Preliminary assays were carried out in order to find most favourable experimental conditions by varying the buffer concentration, its pH and the temperature. The concentration range of the supporting electrolyte (phosphate buffer) was 50 and 250 mM. Variation of the pH of the buffer solution was between 1.5–6.5 units (Fig. 5a). Best results were obtained at pH 2.5. At lower pH values the oxidation wave is less evident, while at higher pH values the oxidation wave rises but shifts to lower values of potential. This indicates an easier oxidation of the molecule, which is thus

less stable and has a negative impact on reproducibility.

From the variation of the temperature between 15–35°C (Fig. 5b) it was found that lower temperatures lead to broader, smaller oxidation waves. Higher temperatures increase the height of the oxidation waves, and shift them toward lower values of potential. Therefore a temperature of 25°C was selected.

As a result the leading conditions used throughout the assays were: phosphate buffer (250 mM; pH 2.5) as the supporting electrolyte; temperature 25°C, and linear scan voltammetry at a constant rate of 5 mV s^{-1}.

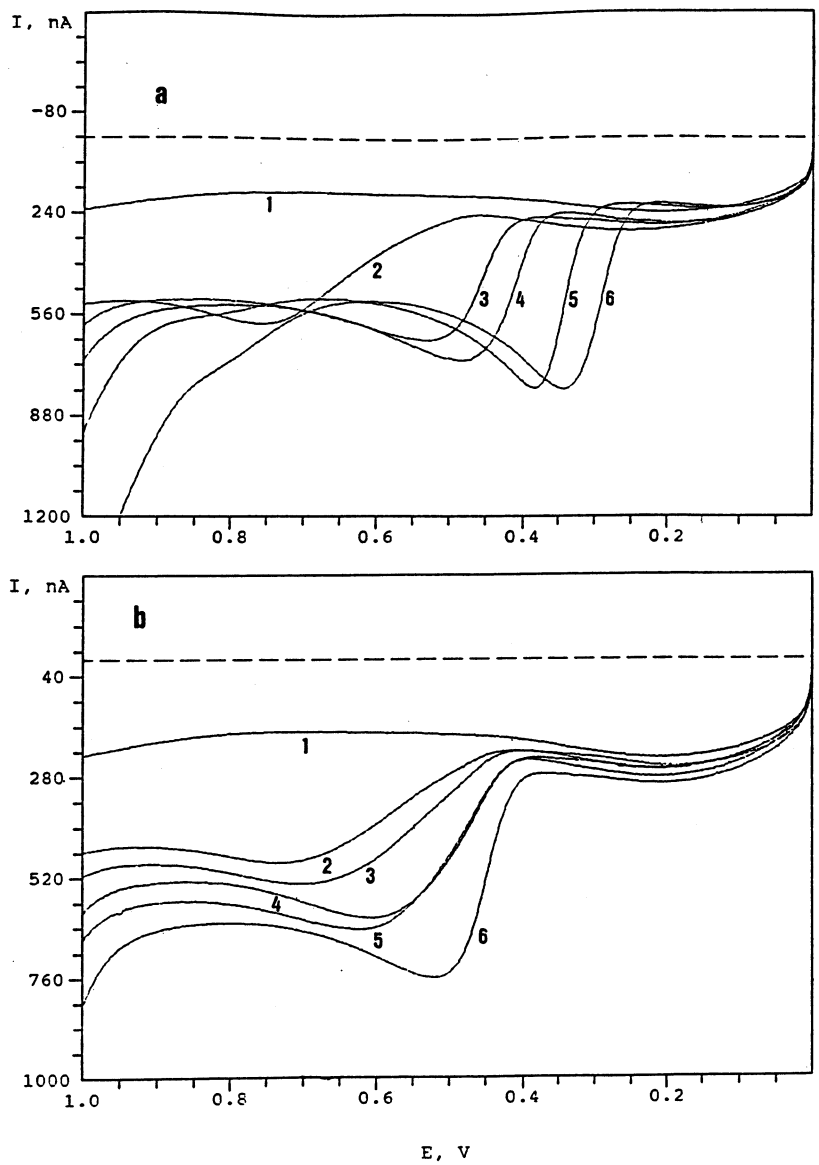

Fig. 5. Voltammograms of a 10 μg ml^{-1} olanzapine standard solution, recorded at several pH values (1 = supporting electrolyte; 2 = pH 1.5; 3 = pH 2.5; 4 = pH 3.5; 5 = pH 4.5; 6 = pH 6.5) (a) and several temperature values (1 = supporting electrolyte; 2 = 15°C; 3 = 20°C; 4 = 25°C; 5 = 30°C; 6 = 35°C) (b).

The calibration curve, set up in the 6.2–50.0 μg ml^{-1} range, is described by the following regression equation (obtained by means of the least square method) $y = 0.040x + 0.039$. y is the height of the oxidation wave, expressed as nA, and x is the concentration of olanzapine, expressed as μg ml^{-1}. The linear correlation coefficient was $r = 0.9990$. The LOQ value was 5 μg ml^{-1} and the LOD value was 3 μg ml^{-1}.

The precision was assessed for 10 μg ml^{-1} standard solutions, and the RSD% values resulted to be 1.5% (repeatability) and 2.8% (intermediate precision), with $n = 6$.

The quality control assays of olanzapine in Zyprexa® tablets, expressed as percentage of the label claim, gave results which were near to 100%, with RSD% values under 3% for both repeatability and intermediate precision (Table 1).

The recovery studies give the following results: mean accuracy 98.0%, RSD 3.2%; the results are detailed in Table 2.

5. Conclusion

HPLC, CZE, derivative spectrometry and voltammetry are suitable methods for a reliable quantitation of olanzapine in the analysed commercial tablets in terms of accuracy and precision. The selectivity of all methods was sufficient, as no apparent interference are present in any assay. The proposed methods have the advantage of using feasible analytical procedures and needing only a very simple pre-treatment of the samples.

It should be noted that all methods gave similar and favourable results with respect to precision and accuracy. The RSD values are all lower than 3%, and the recovery as a measure of the accuracy is close to 100% in all cases.

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