Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 5 pp. 809–819, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

# DEVELOPMENT OF RAPID AND ROBUST STABILITY-INDICATING METHOD FOR ANALYSIS OF ZIPRASIDONE (HYDROCHLORIDE AND FREEBASE) AS DRUG SUBSTANCE AND IN MEDICINES BY UPLC

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**Abstract**: Ultra Performance Liquid Chromatography (UPLC) was employed to develop a rapid and robust method for the analysis of ziprasidone, both as a drug substance and in the final dosage forms. The application of this method in stability analyses was verified. Tests were carried out according to ICH/FDA guidelines, European Pharmacopeia, and United States Pharmacopeia rules, which take into account factors such as specificity, linearity, accuracy, and precision. Separation was performed on an Acquity UPLC BEH phenyl 1.7-µm column with a simple mobile phase, consisting of acetonitrile and water adjusted to pH 2.0 with ortho-phosphoric acid. Using this mobile phase and gradient elution, the separation was completed within 5 min. This method is very sensitive, and allows performing simultaneous identification, assay, and determination of impurities and related substances in one injection.

**Keywords:** UPLC, ziprasidone, ziprasidone hydrochloride, ziprasidone freebase, stability

Ziprasidone is an antipsychotic substance and belongs to a group known as indole derivatives (ATC code: N05AE04). It is used to treat mental disorders such as schizophrenia and manic symptoms of bipolar disorder (manic depression) (1, 2). Ziprasidone is chemically described as 5-{2-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]ethyl}-6-chloro-1,3-dihydro-2*H*-indol-2-one. Its empirical formula is $C_{21}H_{21}ClN_4OS$, and its structural formula is given in Figure 1. Its molecular weight is 412.9 g/mol. It is an insoluble substance, and thus is administered as salt, for example, with hydrochloric acid, in drug products. Ziprasidone hydrochloride is available on the market as Zeldox (Geoden) capsules of different strength, manufactured by Pfizer (3, 4).

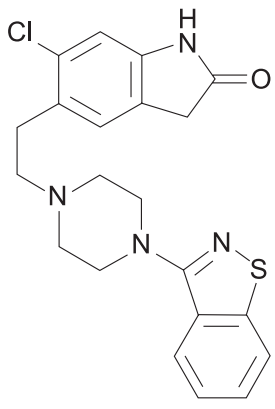


Figure 1. Chemical structure of ziprasidone

A few different analytical methods that describe determination of ziprasidone using high-performance liquid chromatography (RP-HPLC) can be found in the literature (5–8). There are also published applications of ultra performance liquid chromatography combined with tandem mass spectrometry (UPLC-MS-MS) in determination of ziprasidone hydrochloride in plasma (9). These techniques have achieved both desired sensitivity and run time, which is important in drug analysis.

In the present study, a sensitive and inexpensive stability-indicating UPLC-UV method for the related substances determination and quantitative evaluation of ziprasidone is described. The intended uses of the developed method was broad and fulfill main purposes of pharmaceutical analysis, that is

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analysis of drug substance identity, assay, determination, testing of related substances and dissolution rate. In order to verify the reliability of this method in broad concentration range, validation tests were performed according to ICH and FDA guidelines,

UPLC is relatively new chromatographic technique that allows to reduce separation time and requires low flow rates of mobile phase. Unlike traditional HPLC, UPLC technique allows to achieve maximum separation power and sensitivity at a much lower mobile phase flow rate. This is attained using analytical columns packed with smaller particles of the stationary phase. Application of particles with a smaller diameter results in higher efficiency but also increases the pressure of the system significantly. Both UPLC equipment and columns have to be able to withstand such high system backpressure. According to manufacturer's specification, the Acquity UPLC system is able to work under pressures up to 15 000 psi (1000 bar). Analytical columns designed to work with UPLC systems are based on second-generation hybrid material that utilizes a bridged ethylsiloxane/silica hybrid (BEH) structure with particle size of 1.7 µm. BEH technology ensures column stability under high backpressure, over a broad pH range (pH 1–12) (10, 11). Thorough theoretical backgrounds of UPLC and experimental approaches have been reported elsewhere (12–14).

For many years, HPLC has played an important role in the analysis of both active pharmaceutical ingredients (APIs) and final dosage forms (10, 11). Due to the numerous advantages of UPLC, its popularity in pharmaceutical analysis is increasing, and will gradually supplant traditional HPLC.

## EXPERIMENTAL

### Chemicals, reagents and standards

Samples of APIs, ziprasidone hydrochloride and ziprasidone freebase were obtained from Pharmaceutical Works "Polpharma" (Starogard Gdański, Poland). The following impurities were purchased from Teva Pharmaceutical Industries Ltd. (Beer Sheva, Israel):

- A (2,2'-(1,4-piperazinyl)-5-ethyl-6-chloro-1,3-dihydro-2H-indol-2-one);
- C (3-{5'-[2-[4'-(1,2-benisithiasole-3'yl)-1'piperazinyl]ethyl]-6'-chloro-1-H-indol-2,3-dione);
- D (6-chloro-5- (2-chloroethyl)-1,3-dihydro-2H-indole-2-one);
- E (3-{5'-[2-[4'-(1,2-benisithiasole-3'yl)-1'piperazinyl]ethyl]-6'-chloro-3'-hydroxy-1H-indol-2-one-3'-yl}-5-[2-[4-(1,2-benzisothiazloe-3-yl)-1-piperazinyl]ethyl-6-chloro-1-H-indol-2-one).

Impurity B (3-(1-piperazinyl)-1,2-benzisothiazole hydrochloride) was obtained from Cadila Healthcare Ltd. (Gujarat, India). Acetonitrile and methanol of HPLC grade were supplied by Merck (Darmstadt, Germany). High purity water used in analytical procedures was obtained from a Millipore Milli-Q plus ultra-pure water system (Bedford, MA, USA). Ortho-phosphoric acid 85%, used for pH adjustment, was purchased from Merck (Darmstadt, Germany). Spray-dried α-lactose monohydrate (FlowLac100) was supplied by Meggle Pharma (Wasserburg, Germany). Partially pregelatinized maize starch (Starch 1500) was purchased from Colorcon (Idstein, Germany). Magnesium stearate was purchased from Brenntag (Gdynia, Poland). Capsules shells were obtained from the original drug product Zeldox (20 mg) manufactured by Pfizer.

### Apparatus and operating conditions

Separations were carried out using the ACQUITY UPLC system from Waters (Millford, MA, USA) and 50-mm columns with an internal diameter of 2.1 mm, packed with 1.7-µm ACQUITY UPLC BEH phenyl particles from Waters (Wexford, Ireland). The column was thermostated at 25°C. The ACQUITY UPLC system consisted of a Binary Solvent Manager, Sample Manager, Column Manager, and Photodiode

Table 1. UPLC gradient profile.

| Time (min) | Flow rate (mL/min) | Mobile phase A (%, v/v) | Mobile phase B (%, v/v) | Gradient curve |
|---|---|---|---|---|
| initial | | 15 | 85 | - |
| 3.00 | | 35 | 65 | 6 |
| 3.50 | | 35 | 65 | 6 |
| 4.00 | 0.5 | 80 | 20 | 6 |
| 4.10 | | 15 | 85 | 6 |
| 5.00 | | 15 | 85 | 6 |

Array eλ Detector (PDA). UV absorbance data were collected at 209 nm with a data collection rate of 40 points/s. The mobile phase consisted of acetonitrile (mobile phase A) and purified water adjusted to pH 2.0 with ortho-phosphoric acid (mobile phase B). The gradient program ran from 15 to 80% of mobile phase A in 5 min at a flow rate of 0.5 mL/min. The gradient program is presented in Table 1.

In the UV spectral range ziprasidone exhibits strong light absorption at 209 nm, and weaker absorption at 314 nm. Because most of analyzed impurities show maximum of absorption exactly at or close to 209 nm this wavelength was chosen in routine analysis.

The partial loop with needle overfill option was used and the injection volume was 0.5 µL. Additionally, a syringe draw rate of 100 µL/min, needle overfill flush of 15 µL and both air gaps (pre-aspirate and post-aspirate) of 0.3 µL were used. The needle was washed with 400 µL of methanol (as a strong wash) and 600 µL of a mixture of water and acetonitrile (as a weak wash; water and acetonitrile were mixed in a volume ratio of 85:15). The data were acquired and calculated using the Empower Pro 2 software from Waters (Millford, MA, USA).

**Verification of analytical procedures**

The verification of the analytical procedures (analytical method validation) was performed, as a part of the Good Laboratory Practice (GLP). The aim was to demonstrate that the performance of the method was typical of other analytical methods and would be suitable for its intended purpose. Typical validation characteristics include method specificity or selectivity, linearity, estimation of the detection limit (LOD) and quantification limit (LOQ), accuracy, precision, and robustness (15, 16). Specific guidelines regarding analytical procedures validation are published by FDA and ICH. A detailed description of the validation process, requirements, and acceptance criteria can be found elsewhere (17–19).

**Specificity**

Specificity or selectivity is the ability of a method to measure the desired analyte in the presence of other components, without any interference from them. API and drug products can contain impurities from the synthesis route, excipients or undergo substance degradation (15, 16). To demonstrate the selectivity of the method, a set of solutions containing impurities, excipients, and API was prepared and injected into the chromatographic system. The retention time of every component in the mixture was determined and compared. Based on the chromatograms obtained, identification of each component in the solution containing all analytes was performed, and separation data such as the resolution ($R_s$), selectivity factor ($\alpha$) and capacity factor (k') were calculated. Calculations were performed automatically, using software according to the European Pharmacopoeia requirements.

The selectivity of the method was investigated using all five ziprasidone impurities described above, and excipients found in Pfizer's drug product, Zeldox. According to the information published in the Zeldox package insert, the drug product comprised of lactose monohydrate, pregelatinized maize starch, and magnesium stearate (3, 4). The possibility of employing this method in the analysis of the ziprasidone freebase was also investigated.

**Forced degradation studies**

A thorough verification of method selectivity was carried out by forced degradation studies, also known as stress testing. They are performed to determine possible degradation products, and confirm the ability of the developed method to detect and separate impurities, which can possibly arise during the lifetime of an API or drug product. Stress tests are conducted in conditions exceeding those used in accelerated stability testing (20, 21). In the studies, ziprasidone, both in the form of hydrochloride salt and freebase, was subjected to acid, base, and neutral hydrolysis. In addition, it was also exposed to oxidizing agents, and subjected to thermal- and photodegradation. Acid hydrolysis was conducted using 0.5 mol/L of hydrochloric acid for 8 h at 40°C, and base hydrolysis was performed using 0.5 mol/L of sodium hydroxide solution for 8 h at ambient temperature. Oxidation was performed with a 3% hydrogen peroxide solution for 1 h at room temperature. For thermal degradation, the tested substance was heated at about 100°C for 8 h, and for the photostability test, ziprasidone was exposed to UV/Vis light. The illumination intensity at a distance of 10 cm was 1.0–1.2 mW $cm^{-2}$. The illumination was carried out for 8 h.

The solutions obtained were tested by UPLC, and their chromatograms were analyzed. The peaks of impurities arising from the degradation studies were identified and compared with peaks of known impurities. In addition, the occurrence of interference was checked. To detect co-elution with unknown substances, a peak purity test was performed (22). In this test, an advanced detector, such as the photodiode array detector was employed. To automatically evaluate peak spectral purity, the

Table 2. Separation data calculated for components presented in Figure 2.

| Peak identification | Solution containing ziprasidone, impurities A–E and excipients | | | | | |
|---|---|---|---|---|---|---|
| | Retention time [min] | Relative retention time | $R_s$ | $A_s$ | k' | α |
| Impurity A | 0.753 | 0.27 | – | 1.2 | 1.6 | – |
| Impurity B | 1.275 | 0.46 | 16.5 | 1.2 | 3.4 | 2.1 |
| Impurity C | 2.305 | 0.82 | 26.8 | 1.1 | 6.9 | 2.0 |
| Ziprasidone | 2.795 | 1.00 | 8.8 | 2.5 | 8.6 | 1.2 |
| Impurity D | 3.487 | 1.25 | 11.8 | 1.0 | 10.9 | 1.3 |
| Solvent | 3.745 | 1.34 | 3.8 | 1.7 | 11.8 | 1.1 |
| Impurity E | 3.955 | 1.42 | 3.2 | 1.6 | 12.6 | 1.1 |

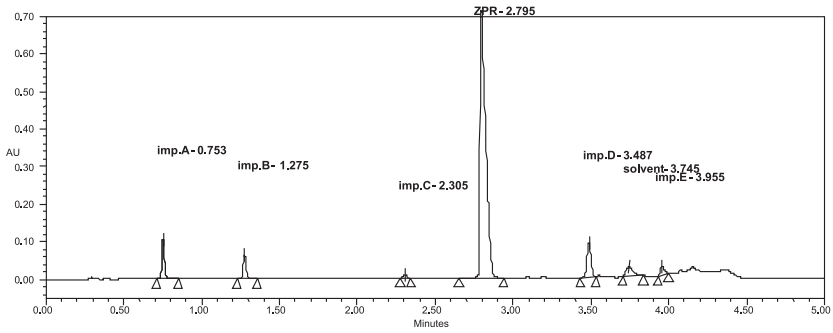


Figure 2. Chromatogram of the solution containing ziprasidone hydrochloride, impurity A, impurity B, impurity C, impurity D, impurity E and excipients (lactose monohydrate, pregelatinized maize starch, magnesium stearate)

Empower Pro 2 software, which has built-in advanced mathematical algorithms, was used. This was to ensure that the observed signal was homogenous and not due to more than one component.

**LOD and LOQ**

LOD of an analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value. LOQ of an analytical procedure is the lowest amount of analyte in a sample, which can be determined with precision and accuracy. The estimation of LOD and LOQ was based on the signal-to-noise ratio. A set of solutions containing ziprasidone at various low concentrations was prepared, and the height of the ziprasidone peak was measured. Baseline noise was estimated from a blank solution, which was the sample solvent without analyte. The concentration, at which the signal-to-noise ratio was about 3:1, was considered as an acceptable LOD, and analyte can be reliably detected. The concentration, at which the signal-to-noise ratio was about 10:1, was considered to be LOQ, and analyte can be reliably quantified (15, 16).

**Linearity**

The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of analyte in the sample within a given range (15, 16). A set of solutions of ziprasidone in the concentration range between 0.125 µg/mL (LOQ) and 300 µg/mL was prepared. The area under each ziprasidone peak in the chromatograms obtained for each solution was measured. The relationship between the concentration of the solution and the area under the ziprasidone peak was subjected to the least-squares linear-regression analysis. The calibration curve was estimated and the calibration equation and correlation coefficients were calculated.

Table 3. Comparison of impurities profile rising in stress tests of ziprasidone hydrochloride and freebase.

| Analyte identification | Relative retention time | Ziprasidone hydrochloride | | | Ziprasidone freebase | | |
|---|---|---|---|---|---|---|---|
| | | Acidic degradation | Base degradation | Oxidative degradation | Acidic degradation | Base degradation | Oxidative degradation |
| | | Amount of analyte [%] | | | | | |
| UI 1 | 0.13 | – | – | 0.06 | – | – | 0.08 |
| UI 2 | 0.15 | – | – | 0.08 | – | – | 0.33 |
| UI 3 | 0.16 | – | – | 0.40 | – | – | 0.66 |
| UI 4 | 0.21 | – | – | 0.18 | – | – | 0.30 |
| UI 5 | 0.26 | – | – | 0.30 | – | – | 0.55 |
| UI 6 | 0.31 | 0.05 | 0.05 | 0.05 | – | – | – |
| UI 7 | 0.36 | – | – | 2.30 | – | – | 4.75 |
| UI 8 | 0.45 | 0.06 | 0.07 | – | 0.12 | 0.11 | 0.15 |
| UI 9 | 0.99 | 0.12 | – | – | 0.24 | – | – |
| UI 10 | 1.04 | 10.47 | 2.37 | – | 15.08 | – | – |
| UI 11 | 1.10 | – | – | 1.45 | – | – | 9.02 |
| UI 12 | 1.13 | – | – | – | 0.22 | – | – |
| UI 13 | 1.19 | 3.48 | 1.69 | – | 4.46 | 1.75 | 0.15 |
| UI 14 | 1.33 | 0.31 | 0.14 | – | 0.39 | 0.24 | – |
| UI 15 | 1.35 | 0.05 | 0.05 | – | 0.06 | 0.30 | – |
| UI 16 | 1.36 | 0.66 | 0.26 | – | 0.27 | 0.19 | – |
| UI 17 | 1.37 | – | – | 0.05 | – | – | – |
| UI 18 | 1.38 | – | 0.09 | – | 0.10 | 0.06 | – |
| UI 19 | 1.39 | 0.09 | 0.20 | – | 0.05 | 0.21 | 0.10 |
| **ziprasidone** | 1.00 | **84.71** | **95.08** | **95.13** | **79.01** | **97.14** | **83.83** |

UI – unknown impurity

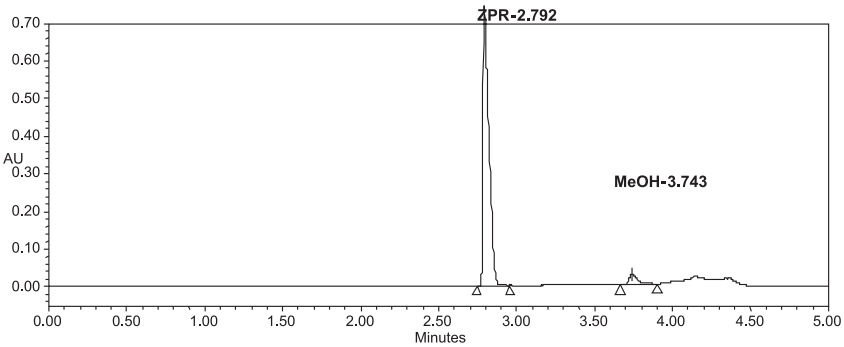


Figure 3. Chromatogram of the solution of ziprasidone freebase

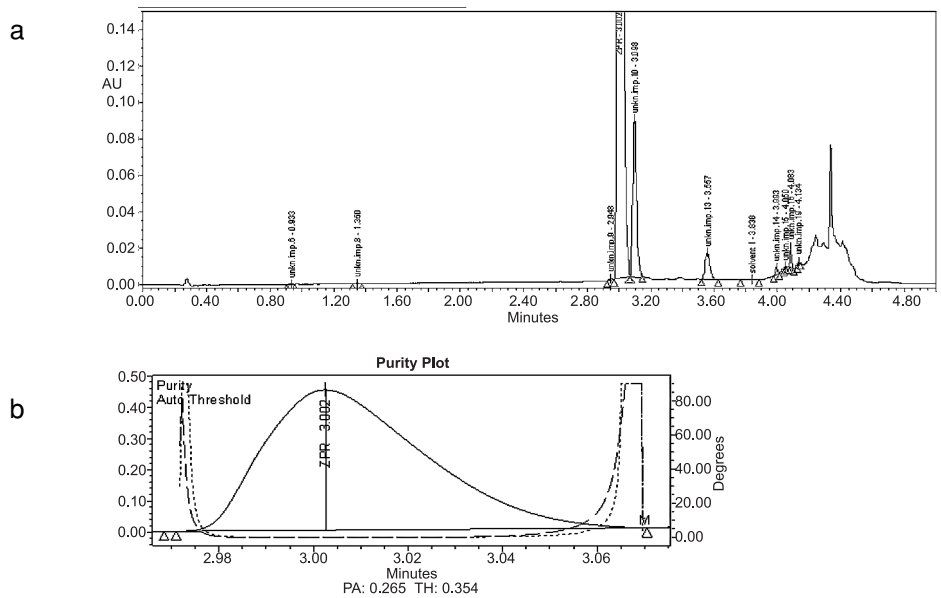


Figure 4. Example chromatogram of the stressed solution of ziprasidone hydrochloride subjected to acidic degradation (a) and peak purity plot for the ziprasidone peak (b)

**Accuracy**

The accuracy of an analytical procedure describes the closeness of the test results, obtained using the method, to their true values (15, 16). Performing accuracy tests allows one to draw conclusions about the influence of formulation components, such as excipients, on measurement reliability. The method was applied to drug samples with known amounts of ziprasidone added. Samples containing amounts of ziprasidone corresponding to 0.05, 0.1, 0.5, 1.0, 20, 50, 100, and 120% of the label claim were prepared and the ziprasidone content was determined. Recovery was determined by comparing the obtained results to the expected values. The recovery results were expressed as percentages. All results were averaged and the relative standard deviation (RSD, %) was calculated.

**Precision**

The precision of an analytical procedure describes the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision, and reproducibility. Repeatability describes the precision under the same operating conditions over a short interval of time. Repeatability is also called intra-assay precision. Intermediate precision describes within-laboratories variations such as different days, different analysts, and different equipment. In addition to these preci-

Table 4. Comparison of the linear regression data for the regression curve in the concentration range 0.05–0.30 mg/mL and 0.125–2.50 $mg^{-3}$/mL of ziprasidone.

| Concentration range | Regression data values | | | |
|---|---|---|---|---|
| | slope | intercept | Syx | r |
| 0.05–0.30 mg/mL | 7024934 | 19979 | 17400 | 0.9998 |
| 0.125–2.50 $mg^{-3}$/mL | 7087961 | 405 | 214 | 0.9996 |

Table 5. Results of the method precision measurement at a concentration of $1.25\times10^{-4}$ mg/mL.

| Repeatability | | Intermediate precision | |
|---|---|---|---|
| SD | RSD | SD | RSD |
| $8.6 \times 10^{-6}$ | 6.9 % | $9.2 \times 10^{-6}$ | 7.2 % |
| Comparison of repeatability and intermediate precision results | | | |
| SD | RSD | The difference of the average values of concentration determination | |
| $8.6 \times 10^{-6}$ | 6.8 % | 2.2 % | |

Table 6. Results of the method precision measurement at a concentration of 0.25 mg/mL.

| Repeatability | | Intermediate precision | |
|---|---|---|---|
| SD | RSD | SD | RSD |
| 0.004 | 1.5% | 0.005 | 1.8% |
| Comparison of repeatability and intermediate precision results | | | |
| SD | RSD | The difference of the average values of concentration determination | |
| 0.004 | 1.6% | 0.6% | |

sion tests, the repeatability of injection of different concentrations of samples was determined. This method determines whether applying even small volumes of injection gives reliable and repeatable results (15, 16).

System repeatability was determined by performing series of injections of different analyte concentrations: LOQ and the concentration equal to 100% of the nominal concentration of ziprasidone in the method (0.25 mg/mL). The active compound peak area and its retention time were measured, and RSD was calculated.

Method repeatability was determined from the RSD obtained from six independent (prepared from different weights) samples. Intermediate precision was assessed by assaying ziprasidone in six independent samples prepared by different analysts on different days.

Analytical procedure precision was considered at two levels: repeatability and intermediate precision. Results obtained from repeatability and intermediate precision were compared. The method precision was performed at two different concentration levels i.e., LOQ and equal to 100% of the nominal concentration of ziprasidone in the method (0.25 mg/ mL).

**Robustness**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, deliberate variations in method parameters. It provides an indication of its reliability during normal usage (15, 16). To evaluate robustness, experimental conditions such as mobile phase flow rate, column temperature, and mobile phase pH were altered. With these varying conditions, a solution containing ziprasidone and its five impurities was injected and the influence of these changes on retention data such as $R_s$ between each component was checked. As a requirement, the resolution between each pair of peaks should not be less than 1.5. This value, according to the European Pharmacopoeia, corresponds to baseline separation. The flow rate of the mobile phase was changed by

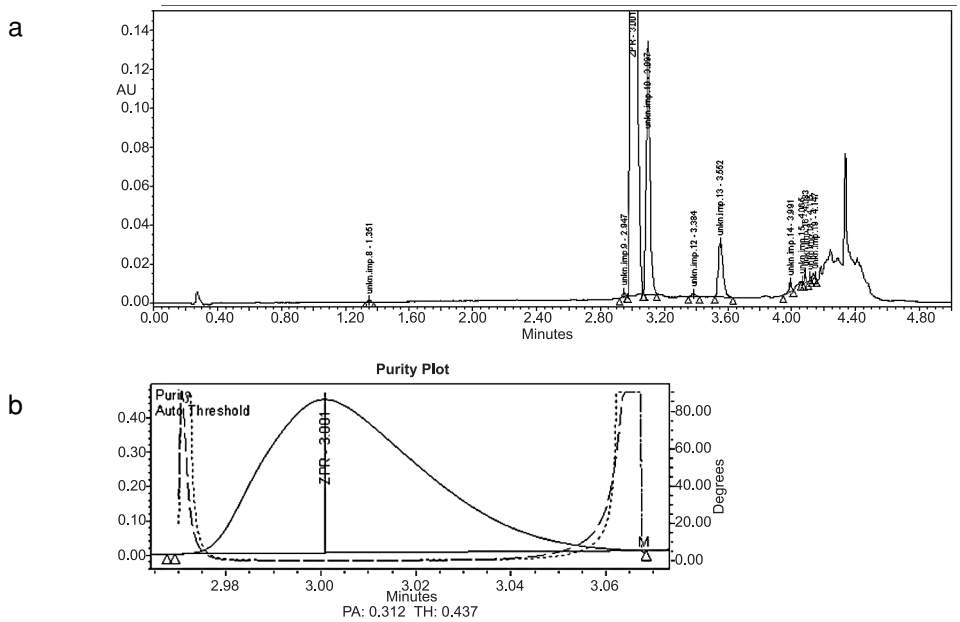


Figure 5. Example chromatogram of the stressed solution of ziprasidone freebase subjected to acidic degradation (a) and peak purity plot for the ziprasidone peak (b)

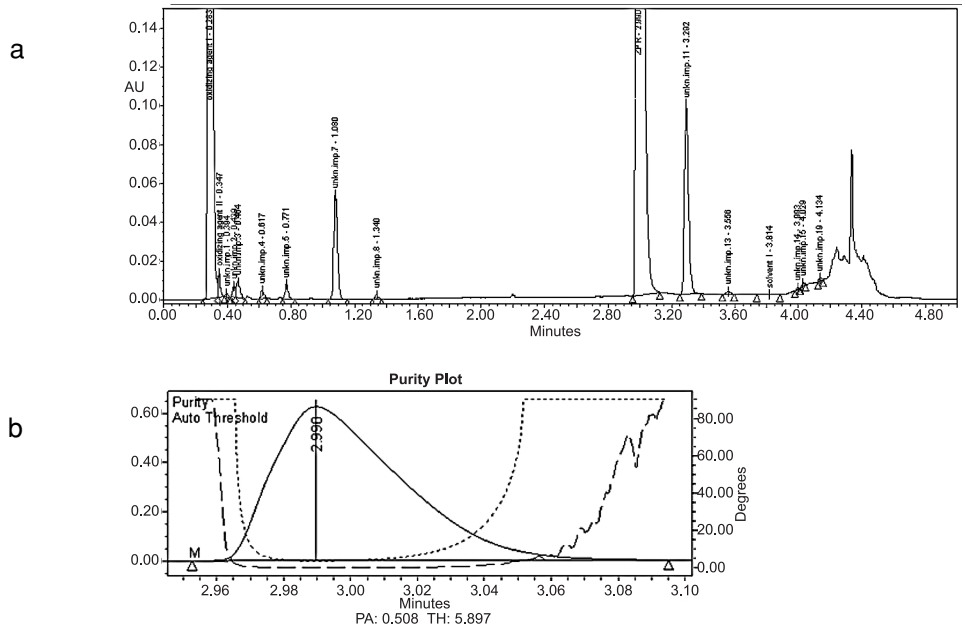


Figure 6. Example chromatogram of the stressed solution of ziprasidone freebase subjected to oxidative degradation (a) and peak purity plot for the ziprasidone peak (b)

± 10% of the nominal value, i.e., from 0.45 to 0.55 mL/min. Column temperature was varied from 20 to 30°C. pH was altered by ± 25%, i.e., from pH 1.5 to pH 2.5.

## RESULTS AND DISCUSSION

### Specificity

The results obtained from specificity test, with calculated separation data such as resolution $R_s$,

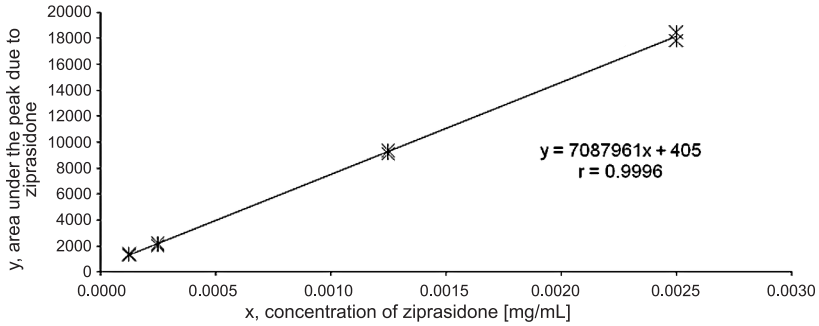


Figure 7. Regression curve for ziprasidone hydrochloride in the concentration range 0.125–2.50 µg/mL

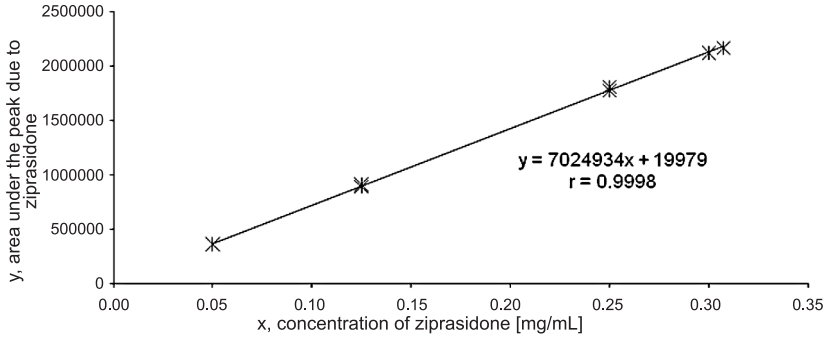


Figure 8. Regression curve for ziprasidone hydrochloride in the concentration range 0.05–0.30 mg $mL^{-1}$

selectivity factor (α) and capacity factor (k') are collected in Table 2. The method was found to be specific. No interference was observed between the peaks due to the sample solvent (methanol), drug substance and its impurities (Fig. 2). No signal from excipients used in Pfizer's drug product, Zeldox capsules, was found. No additional peaks due to capsules shells were detected. The retention time of the principal peak in the chromatogram obtained with the solution of ziprasidone hydrochloride was the same as the retention time of the principal peak in the chromatogram obtained with the ziprasidone freebase solution (Fig. 3). No shift in the retention time was observed.

## Forced degradation studies

### *Stress tests of ziprasidone hydrochloride*

In the presence of water (neutral hydrolysis), insignificant degradation can be observed, while at a high temperature (thermal degradation) or when the sample was exposed to electromagnetic radiation (photodegradation) no degradation occurred. In a basic and acidic environment or in the presence of hydrogen peroxide, considerable degradation was observed and the amounts of a few impurities increased. A significant increase in the amounts of four impurities, which could be notated as main impurities: with relative retention time 0.36 (unknown impurity (UI 7), 1.04 (UI 10), 1.10 (UI 11) and 1.19 (UI 13), can be observed.

Results of stress test of ziprasidone hydrochloride are comparable with these obtained by Singh et al. (5) using RP-HPLC method, which confirms reliability of the presented UPLC method. Other force degradation study results, with identification of some degradation product using ESI-MS (electrospray-mass spectrometry) analysis, were presented by El-Sherif et al. (6).

### *Stress tests of ziprasidone freebase*

In the presence of water (neutral hydrolysis), at a high temperature (thermal degradation), or when the sample was exposed to electromagnetic radiation (photodegradation), mild degradation can be observed. Analogically to ziprasidone hydrochloride, ziprasidone freebase is sensitive to acidic and base conditions, and easily undergoes oxidative degradation. Nevertheless, it can be noticed that in

the same conditions, freebase of ziprasidone is less stable than the form of hydrochloric salt. Similar to ziprasidone hydrochloride, four impurities can be recognized as main impurities: with relative retention time 0.36 (UI 7), 1.04 (UI 10), 1.10 (UI 11) and 1.19 (UI 13).

Comparison of impurity profiles rising during ziprasidone hydrochloride and ziprasidone freebase degradation in stress conditions for the most degraded samples is presented in Table 3. In all tested conditions, degradation pathways of both substances were very similar. Example chromatograms of the most degraded samples are presented in Figures 4–6. Peak purity was checked for the peak of ziprasidone and was based on an estimation method by comparing spectra measured at different points on the peak. When the calculated purity angle (PA) is lower than the purity threshold (TH), the investigated peak was considered homogenous and no coelution with other analytes occurred.

**LOD and LOQ**

The recommended maximum amount of drug substance, which can be safely administrated per day (maximum daily dose) for ziprasidone, is equal to 160 mg/day (23). The reporting threshold of organic impurities in active substance with maximum daily dose = 2.00 g per day is 0.05%. For drug products with maximum daily dose = 1.00 g per day the reporting threshold is 0.1%. The quantification limit for the analytical procedure should not be higher than the reporting threshold (24). The limit of detection found was 0.038 µg/mL of ziprasidone. The limit of quantification was 0.125 µg/mL of ziprasidone, which was 0.05% of the nominal concentration of drug substance used in the test solution (0.25 mg/mL).

**Linearity**

Linearity data were investigated by taking into consideration two ziprasidone concentration ranges, from 0.125 µg/mL (LOQ) to 2.50 µg/mL, and from 50 µg/mL to 300 µg/mL. The correlation coefficients obtained were 0.9996 and 0.9998, respectively. The regression data showed good linearity in each concentration range investigated (Figs. 7 and 8) (Table 4).

**Accuracy**

Accuracy expressed as a percentage of recovered drug substance was determined by comparing calculated values of concentrations of ziprasidone in samples with expected values. The average drug recovery obtained in the concentration range from 0.125 µg/mL (LOQ) to 300 µg/mL (120% of labeled claim) was 100.3%, with RSD of 3.5%. In the lower concentration range from 0.05% to 1%, recovery results fall into the range of 93–109%, while in the higher concentration range, from 20% to 120%, recovery results fall into the range 98–102%. These results demonstrate good accuracy of the method.

**Precision**

Repeatability of injection was checked as a function of RSD calculated for peak area and retention time. Injection precision was checked at two analyte concentrations, 250 µg/ mL (100% of label claim) and 0.125 µg/mL (LOQ). RSD calculated from the areas of the peaks was 1% and 2%, respectively. RSD calculated from the retention times of the peaks was 0.07% and 0.03%, respectively.

The method precision was determined using an assay of ziprasidone in drug samples spiked with known amounts of analyte. From the obtained results, SD and RSD were calculated. Precision was determined at two concentration levels, i.e., 0.125 µg/mL (LOQ) and 250 µg/mL (100% of label claim). The results obtained from repeatability and intermediate precision were compared, and differences were estimated (Tables 5 and 6).

**Robustness**

Under all the altered chromatographic conditions, i.e., mobile phase flow rate, column temperature, pH of the mobile phase, the method was found to be robust. Resolution between each pair of peaks in all cases was greater than the required value of 1.5. The biggest changes in chromatograms were observed when pH of the mobile phase was changed. It was observed that when pH was more acidic $R_s$ value was better. Conversely, when pH of the mobile phase was increased the resolution between all components decreased.

## CONCLUSIONS

A rapid and robust method for the analysis of ziprasidone was developed and its applicability as a method for analyzing stability was checked. The method was found to be specific, accurate, precise, and reproducible. Force degradation studies confirmed its ability to determine stability because no interference from degradation products was observed. Moreover, no influence from excipients was found, allowing it to be used in final drug product analysis. The method was also validated in accordance with ICH and FDA requirements.

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*Received: 14. 07. 2011*