

Research Article

Studies on Paliperidone in OROS Tablets: Extraction Procedure and Chromatographic Analysis

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A stability-indicating liquid chromatographic (LC) method was studied for the determination of paliperidone in osmotic-controlled release oral delivery system (OROS) tablets. A tablet extraction procedure was developed by testing the efficiency of solvents (water, HCl, NaOH, acetonitrile, methanol) and techniques (ultrasonic bath, magnetic stirrer), and evaluating the release of the drug with respect to time. A forced degradation study was conducted to demonstrate the stability-indicating power of the method. Chromatographic separation was achieved using an isocratic elution in a reversed-phase system with a mobile phase prepared from a mixture of phosphate buffer and acetonitrile. The use of an ultrasonic bath demonstrated paliperidone release from OROS tablets in a total time of 60 min. Verifying the efficiency of the chromatographic procedure, the theoretical plates ($N = 12634.21$) and tailing factor ($tf = 1.31$) were constant during repeated injections. The retention time of paliperidone was 4.8 min, and the method was validated within the concentration range of $10\text{--}50\ \mu\text{g mL}^{-1}$ ($r = 0.9999$). Adequate reproducibility ($\text{RSD}\% = 0.30\text{--}0.59$), interday precision ($\text{RSD}\% = 1.81$), and accuracy were obtained. The proposed method was successfully applied to paliperidone determination in the presence of degradation products, and an efficient extraction procedure from the OROS tablets was developed.

1. Introduction

Paliperidone (Figure 1), (*RS*)-3-[2-[4-(6-fluorobenzo[*d*]isoxazol-3-yl)-1-piperidyl]ethyl]-7-hydroxy-4-methyl-1,5-diazabicyclo[4.4.0]deca-3,5-dien-2-one, is the major active metabolite of risperidone (9-hydroxy-risperidone) and is an atypical antipsychotic that belongs to the chemical class of benzisoxazole derivatives [1–5]. The drug was recently approved by the United States Food and Drug Administration (FDA) for the treatment of schizophrenia [6]. It binds to both D2 and serotonin-2A (5-HT_{2A}) receptors; antagonism at these receptors is thought to account for the therapeutic activity of this drug in schizophrenia, as demonstrated in both *in vitro* and *in vivo* animal and human studies [5, 7–9].

Paliperidone is marketed as an extended-release tablet (paliperidone ER, Invega) that uses a patented osmotic-controlled release oral delivery system (OROS, ALZA Cor-

poration, California, USA; Janssen-Cilag, 2007) in a once-daily dose [7, 10] and exists as a mixture of two equally potent enantiomers that interconvert [11]. The pharmacologic activity of the two enantiomers is qualitatively and quantitatively similar *in vitro* [2]. A series of studies have evaluated the pharmacodynamics, efficacy, and tolerability of paliperidone. The results obtained from randomized, double-blinded, placebo-controlled studies indicate that the drug is effective and safe at all doses, resulting in significant improvements in the symptoms of schizophrenia and related disorders [3, 12–16].

Paliperidone has been recently investigated with regard to its determination as a main metabolite of risperidone. Previous studies have described the analysis of risperidone and 9-hydroxy-risperidone in plasma, urine and serum using LC, LC/MS-MS and MEPS-LC-UV [17–19]. Locatelli et al. [20]

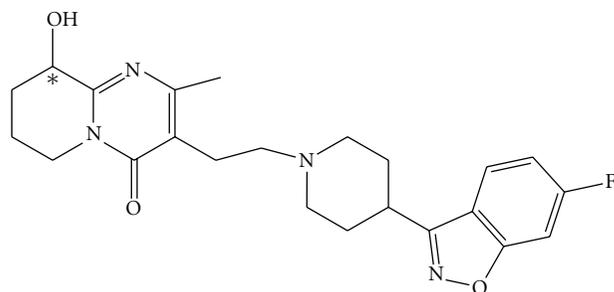


FIGURE 1: Chemical structure of paliperidone. *indicates position of chiral carbon atom.

proposed the determination of these substances in biological fluids using HPLC methods, allowing for the simultaneous analysis of enantiomers. The method combines reverse-phase chiral separation and electrochemical detection, which guarantee sufficient sensitivity to investigate stereoselectivity. Paliperidone has also been determined in bulk using enantioselective analytical methods, liquid chromatography, and capillary electrophoresis [21–23]. Using a dual cyclodextrine mode in the chiral capillary electrophoretic method, the kinetics of racemization under acidic and basic conditions (a process related to the configurational stability of the drug) have been described [23].

Literature relating to the techniques used to determine the drug in routine analysis is scarce, as well as OROS system. The United States Pharmacopoeia has released a monograph on nifedipine extended-release tablets (Adalat OROS, Bayer Schering Pharma) [24] that describes a method to determine the drug involving extraction by stirring in water and an organic solvent in a high-speed blender cup for 30 min, followed by chromatographic separation. In guidelines established by the FDA, paliperidone extended-release tablets are mentioned as approved drug products based on an evaluation of therapeutic equivalence, and a dissolution method for paliperidone testing has also been established [25]. Paliperidone remains absent from the pharmacopoeia to date. In a recent report, various stimuli, such as temperature, magnetic field, and ultrasound, have been described in relation to an action in polymer and interaction with the drug to release the drug [26]. Thus, the complexity involved in these osmotic pump delivery systems requires the establishment of reproducible conditions to guarantee reliable determination during assays.

The aim of the present research was to develop a stability-indicating RP-HPLC method with a DAD detection system to determine paliperidone in OROS system. Extraction of the drug from the osmotic system was investigated, as it is considered an important step in reliable analysis. The purpose was to determine the amount of paliperidone (both enantiomers) present in the tablet rapidly and reproducibly.

2. Material and Methods

2.1. Chemicals. Paliperidone reference standard (98.9%) was purchased from AK Scientific, Inc. (Mountain View, CA, USA). Invega (Janssen-Cilag Pharmaceutica) tablets

containing 3 mg of paliperidone were purchased over the counter at a retail store. The excipients listed on the dosage form (cellulose acetate, stearic acid, butylhydroxytoluene, carnauba wax, sodium chloride, hyetelose, macrogol, opadry II, iron oxide, polioxiethylene, and povidone) were acquired from various suppliers and were of pharmaceutical grade. All other chemicals used were of analytical grade, and all solvents used were of HPLC grade. Methanol and acetonitrile were purchased from Tedia (Fairfield, OH, USA). Hydrochloric acid (35%) was purchased from Merck (Darmstadt, Germany). Sodium hydroxide, monobasic sodium phosphate and hydrogen peroxide (30%) were purchased from Synth (São Paulo, Brazil). Purified water was prepared using a Milli-Q Plus system (Millipore, Bedford, USA).

2.2. Apparatus. HPLC analysis was performed on a Prominence Liquid Chromatograph Shimadzu instrument equipped with an LC-20AT pump, SIL-20A autosampler, SPD-20AT PDA detector, and CTO-20A column oven (Shimadzu Corporation, Kyoto, Japan). LC Solution V. 1.24 SP1 system software was used to control the equipment and to evaluate the data and system response from the LC system. A Unique Ultrasonic bath (Indaiatuba, SP, Brazil) was used to extract the drug. Photodegradation stability studies were performed in a photostability UV chamber Farma 424CF equipped with internal mirrors and a UV fluorescent lamp emitting radiation with a spectral distribution from 320 nm to 400 nm (Nova Ética, São Paulo, Brazil). A dry-air oven (Nova Ética, São Paulo, Brazil) was used for thermal degradation. A Gehaka PG1800 pH meter (São Paulo, Brazil) was used to measure pH values.

2.3. Chromatographic Conditions. HPLC analysis was conducted using a reverse-phase technique. Paliperidone was eluted isocratically at a flow rate of 1.0 mL min^{-1} using a mobile phase consisting of 50 mM monobasic phosphate buffer containing 0.3% triethylamine (pH 3.8): acetonitrile in the proportions 70:30 (v/v). The PDA detector was set to 280 nm. The mobile phase was prepared daily, filtered through a $0.45 \mu\text{m}$ membrane filter (Millipore), and sonicated before use. A Shim-pack CLC (M) ODS ($250 \times 4.0 \text{ mm}$ i.d., $5 \mu\text{m}$ particle size, Shimadzu Corporation, Kyoto, Japan) was used as the analytical column, and the HPLC system was operated at $25 \pm 1^\circ\text{C}$.

2.4. Extraction Procedure of Paliperidone from the OROS System. An extraction test was performed to determine the adequate conditions for accelerating drug release from the tablets during sample preparation. Two techniques were tested: magnetic stirring and the use of an ultrasonic bath. Several solvents (aqueous and organic) were tested: water, 0.1 N HCl, 0.01 N HCl, 0.1 N NaOH, acetonitrile, and methanol. Extraction time was considered an important characteristic for quantitative determination, and this factor was also considered during the development phase to avoid long analysis times. At predetermined times (30, 60, 90, and 120 min), aliquots were collected and analyzed.

For the assay, five tablets were transferred to 250 mL volumetric flasks containing each solvent and subjected to the extraction procedure. At each time point, aliquots were collected, filtered through quantitative paper, and diluted in the mobile phase to yield a final concentration of $30.0 \mu\text{g mL}^{-1}$. Each sample was analyzed in triplicate using HPLC, and the results were compared to the reference standard solution at the same concentration. The results were expressed as % drug release.

To evaluate the efficiency of extraction as a function of the number of tablets inserted into the extractive solution, the extraction procedure was also conducted using one tablet in a 50 mL volumetric flask, maintaining the same proportions of drug and solvent and the other conditions described above.

The % of drug released was graphed as a function of time for both conditions tested.

2.5. Sample Preparation for HPLC Analysis. The paliperidone reference standard was accurately weighed and dissolved in a 200 mL volumetric flask containing 0.01 N HCl to obtain a concentration of $60.0 \mu\text{g mL}^{-1}$. This solution was appropriately diluted 1 : 2 in the mobile phase to yield a final concentration of $30.0 \mu\text{g mL}^{-1}$ (pH 3.00 ± 0.02).

Twenty Invega paliperidone extended-release OROS tablets were weighed, and the mean weight was obtained. Five tablets (previously weighed) were transferred into a 250 mL volumetric flask containing 0.01 N HCl and placed in an ultrasonic bath for 60 min. An aliquot of this solution was diluted 1 : 2 in a 10 mL volumetric flask in the mobile phase to yield a final concentration of $30.0 \mu\text{g mL}^{-1}$ (pH 3.00 ± 0.02).

Sample and standard solutions were filtered through $0.45 \mu\text{m}$ Millex HV syringe filters (Millipore, Bradford, USA) before injection.

2.6. System Suitability. A system suitability test of the chromatographic assay was performed. A reference standard and sample solutions containing $30 \mu\text{g mL}^{-1}$ of paliperidone were injected in triplicate. Chromatographic parameters including peak area, retention time, theoretical plates, and tailing factor were measured, and the relative standard deviation (RSD) for each parameter was determined.

2.7. Validation Procedure. The chromatographic method was validated based on specificity, linearity, precision, accuracy, and robustness [24, 27]. The stability-indicating capability was determined under forced degradation conditions, including light, heat, oxidation, and acidic and basic degradation [28].

2.8. Specificity. The specificity experiments were assessed by testing analytical interference from excipients and degradation products. The influence of tablet composition was determined by analyzing a placebo solution and comparing the chromatograms to the reference standard solution. The concentration of the excipients was established from the literature [29]. Suitable analytical conditions for the stability

studies were determined by observing the chromatographic profile resulting from a forced degradation assay. Specific details of the experimental conditions are described below.

- (a) *UV Light.* 1 mL of a standard solution containing $60.0 \mu\text{g mL}^{-1}$ of paliperidone was placed in a closed 1 cm quartz cell, which was exposed to a UV chamber for 60 min. The same procedure was performed for the sample solution extracted from commercial tablets as described above. Samples that were protected from light under identical conditions and at the same time were used as controls. After the degradation period, the samples were diluted in the mobile phase to $30.0 \mu\text{g mL}^{-1}$ and immediately analyzed.
- (b) *Heat.* A standard solution of $60.0 \mu\text{g mL}^{-1}$ of paliperidone was prepared in 0.01 N HCl and stored in transparent glass in a dry-air oven at 60°C for 96 hours. After this period, an aliquot of this solution was diluted with the mobile phase to $30.0 \mu\text{g mL}^{-1}$. The same procedure was performed for sample solutions prepared from commercial tablets, as cited above.
- (c) *Oxidation.* The paliperidone standard solution was diluted in 30% hydrogen peroxide in a volumetric flask to a concentration of $60.0 \mu\text{g mL}^{-1}$ and left at room temperature for 6 hours. For commercial tablets, the paliperidone was extracted with 0.01 N HCl to a concentration of $100.0 \mu\text{g mL}^{-1}$ and then diluted with hydrogen peroxide to $60.0 \mu\text{g mL}^{-1}$ at room temperature. After 6 hours, an aliquot of each solution was diluted with the mobile phase to $30.0 \mu\text{g mL}^{-1}$ and immediately analyzed. A control solution containing hydrogen peroxide was also prepared and analyzed.
- (d) *Acid and Alkaline Hydrolysis.* The paliperidone reference standard was diluted to $100.0 \mu\text{g mL}^{-1}$ in volumetric flasks containing either a 0.1 N HCl solution for acidic degradation or a 0.1 N NaOH solution for alkaline degradation. After 1 hour, one aliquot of the solution was neutralized and diluted with the mobile phase to $30.0 \mu\text{g mL}^{-1}$.

2.9. Linearity. Paliperidone reference solutions were prepared in triplicate at concentrations of 10.0, 20.0, 30.0, 40.0, and $50.0 \mu\text{g mL}^{-1}$. Standard plots were constructed, and linearity was evaluated statistically by linear regression analysis using least-square regression and analysis of variance (ANOVA).

2.10. Precision. The repeatability (intraday) and intermediate precision (interday) of the method were determined as follows. Paliperidone sample solutions were prepared (see Section 2.5 above). From the extractive solution ($60.0 \mu\text{g mL}^{-1}$), six $30 \mu\text{g mL}^{-1}$ solutions were prepared on three different days. The analyses were performed in triplicate, and the results were expressed as the RSD of the analytical measurements.

2.11. Accuracy. The accuracy was determined based on the recovery of known amounts of the paliperidone reference standard added to samples at levels of 10, 20, and 30% of the sample concentration ($30.0 \mu\text{g mL}^{-1}$). The results were expressed as the percentage of the paliperidone reference standard recovered from the sample. All solutions were prepared in triplicate.

2.12. Robustness. Robustness was tested to evaluate the susceptibility of the measurements to deliberate variations in selected analytical conditions, including aqueous phase pH, mobile phase proportions, flow rate, and column supplier. The variations are listed in Table 1. Each condition was assayed separately, while all other conditions were held constant at the selected values.

2.13. Stability of Analytical Solutions. The analytical solutions were evaluated to verify their behavior under standard analysis conditions. The paliperidone reference standard and the extended-release tablets were prepared according to the methods described (see Section 2.5 above). Samples of each solution were stored at 4°C , room temperature (25°C) and at 40°C in triplicate. After 24, 48, and 72 h, the paliperidone reference standard and the extended-release tablet solutions were analyzed, and the chromatographic patterns were compared to freshly prepared samples and standard solutions. RSDs (%) were calculated for these stability studies.

3. Results and Discussion

Analytical assays were developed by comparing a series of procedures for sample preparation and their resulting performance during analysis. The formulation, composition, and processing of the drug must be considered. According to Chandran and Singh [30], analytical methods are used to aid in drug synthesis, screening potential drug candidates, supporting formulation studies, monitoring the stability of bulk pharmaceuticals and formulated products, and the testing of final products for release. Validated analytical methods for the qualitative or quantitative testing of drug molecules assume greater importance when they are employed to generate quality and safety compliance data during the development and postapproval of drug products [30].

Currently, several technologies are available to improve the efficacy of drugs. Novel oral therapeutic systems have been invented, and drug delivery technologies, including controlled-release preparations with an emphasis on osmotic systems (such as the elementary osmotic pump, OROS push-pull system and OROS delayed push-pull system from ALZA Corporation), have developed appreciably [31]. Osmotic pump tablets generally consist of a drug-containing core, an osmotic agent, other excipients, and a semipermeable membrane coating [32]. OROS tablets are nondisintegrating, osmotically driven tablets that release drugs over time using a delivery technology that usually uses polymers such as polyethylene oxide or poloxamer as critical excipients to achieve the desired drug release profile and functionality

TABLE 1: Factors used during robustness testing applied to LC analysis of paliperidone in extended-release OROS tablets.

Condition	Reference	Variation
pH of aqueous solvent	3.8	3.6; 4.0
Concentration of organic solvent (%)	30.0	28.0; 32.0
Flow rate (mL min^{-1})	1.0	0.8; 1.2
Column supplier	Shim-pack C18, 25 cm	ODS-Hypersil, 25 cm

[33–35]. The active pharmaceutical is contained in a laser-drilled reservoir surrounded by a semipermeable membrane harboring a delivery orifice, the whole being formulated into a tablet [26, 36].

Currently, paliperidone and other CNS drugs are administered by an osmotic-controlled release oral delivery system; these drugs include methylphenidate (methylphenidate ER, Concerta, Janssen-Ortho Inc.) and hydromorphone (OROS hydromorphone ER, Exalgo, ALZA Corporation) [37, 38]. Paliperidone-ER (Invega) is embedded in a polymeric compartment containing the drug and excipients as well as hydrophilic polymers [8]. For accurate analysis, qualitative and quantitative methods must be developed that consider the complexity of the tablet compositions, including the use of various excipients such as viscous polymers. Depending on the polymer composition, drug release samples can become very viscous, and this may impact injection accuracy and reproducibility during analysis [35]. Therefore, sample preparation is important and requires particular attention during analysis. In this case, the difficulty in accessing the drug due to its location in a polymeric compartment led us to consider an extractive technique that allows drug release.

In the present work, a recently released osmotic system was studied with the aim of developing an analytical technique to determine the drug content using a reverse phase system and an ODS column. We began with the osmotic pump, developed a suitable extraction procedure, and then developed a dilution protocol for preparing solutions for LC analysis. Due to their hardness, it was first necessary to break the tablets to determine how to access the drug. The tablet contained a moist, hard material that was difficult to work with using a traditional analysis routine. Thus, samples were prepared using an extraction procedure to accelerate drug release. Previous experiments with aqueous and organic solvents indicated that there might be difficulty in obtaining a well-defined chromatographic peak and that this would depend on the solvent used to dissolve the drug and extract it from the tablets. In general, the chromatograms exhibited a peak with a shoulder and a tail. The same peak asymmetry was observed when using an unbuffered mobile phase, which was immediately corrected in tests by using phosphate buffer. When water : methanol or water : acetonitrile mixtures were tested in different proportions, a large tailing peak for paliperidone was observed. Using phosphate buffer, the peak had improved in symmetry; by adjusting the proportion of the organic solvent, rapid analysis was achieved. In any case, the pH of the final solution had to be controlled because

TABLE 2: Results from system suitability determination applied to LC analysis of paliperidone in extended-release OROS tablets.

Parameter	Mean ^a	RSD (%)
Peak area	768367	1.81
Retention time	4.88	0.25
Theoretical plates	12643.21	1.59
Tailing factor	1.31	1.22

^a Mean of nine replicates, three a day.

our results indicated the need to extract the drug using acidic solutions. Because the paliperidone reference solutions were prepared using organic solvents such as acetonitrile and methanol, reproducible analysis was not achieved. Basic and acidic media were tested, and high pH values did not guarantee good analytical performance. Using 0.1 N HCl, the performance was improved, although the low pH (1.8) of the extractive solution still required adjustment. To achieve this correction, the extractive solution was prepared using 0.01 N HCl diluted 1:2 with the mobile phase, thereby obtaining an analytical solution of pH 3.0. Figure 2 illustrates a chromatogram for the paliperidone reference standard with a retention time of 4.8 min at 280 nm. UV spectra were observed from the chromatographic peak, which contained two absorption maxima (236 and 276 nm). The system suitability was analyzed, and the data are presented in Table 2. Analytical results are only valid if the defined system suitability criteria are fulfilled [39]. The results indicate that the method performed well, and the average number of theoretical plates ($N = 12643.21$) indicated that the separation was efficient, considering that both enantiomers of paliperidone were present and that the pH of the samples probably interfered in the analysis. Based on previous research, pH (under strongly acidic or basic conditions) can affect the configurational stability of paliperidone, causing racemization and thereby affecting the analysis [23]. In this work, the experimental results indicated that the chromatographic system was suitable for the intended analysis. The chromatographic profile obtained is typical of that obtained in drug analysis, with a clear chromatogram and a rapid run. The RSD values calculated for the peak area and the retention time were 1.81 and 0.25%, respectively, indicating good reproducibility for these parameters. The tailing factor observed was 1.31 (RSD 1.22%), indicative of peak symmetry.

The investigation performed here was intended to study the conditions that can be used to determine the concentration of paliperidone in osmotic tablets, considering the formulation as the limiting factor of the assay. The extraction procedure was tested using two conditions: magnetic stirring and the use of an ultrasonic bath. In both cases, the osmotic system is forced to accelerate the release the drug, modifying the forces involved in the interaction between drug and polymer [26]. Various solvents were evaluated for their effect on the release of the drug. Figure 3 shows the drug release profile after 30, 60, 90 and 120 min under both extraction conditions. Using the ultrasonic bath, extraction with water or 0.1 N HCl allows for release of all of the

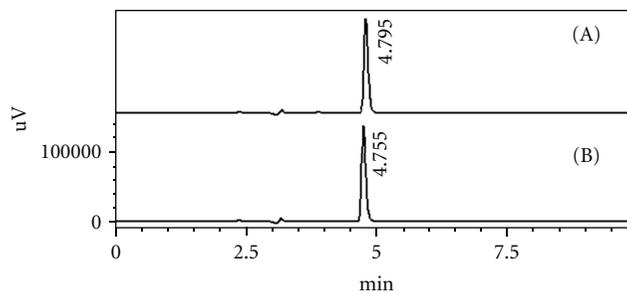


FIGURE 2: LC chromatograms of paliperidone standard (A) and drug product (B). Concentration of analysis: $30.0 \mu\text{g mL}^{-1}$.

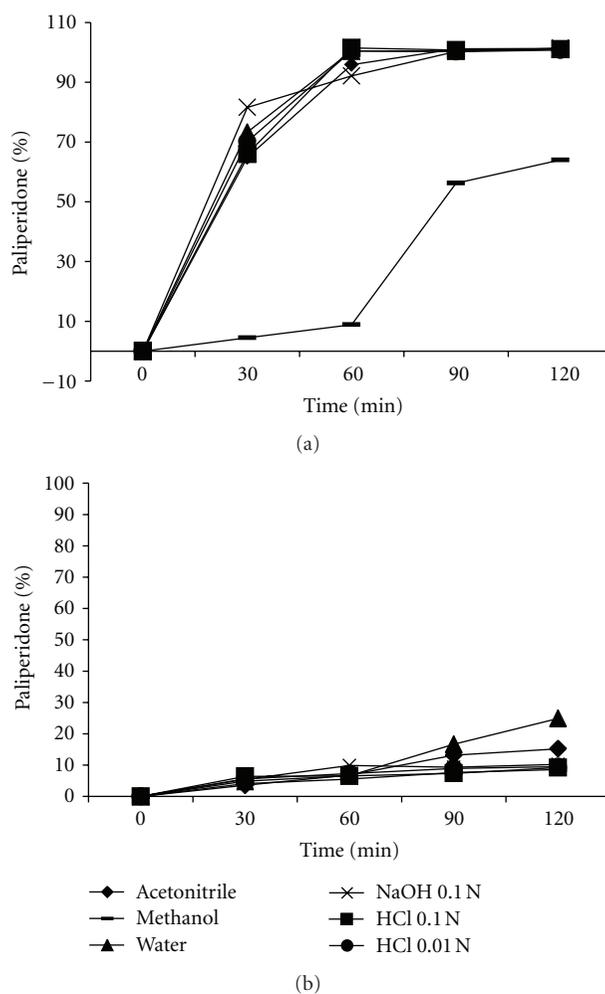


FIGURE 3: Drug release profiles of paliperidone OROS tablets obtained from extraction procedure using ultrasonic bath (a) and magnetic stirrer (b).

paliperidone from the oral system after 60 min, the time chosen to perform the analysis. For methanol, only 8.83% of the drug was released after 60 min. In 0.1 N NaOH and acetonitrile, all of the paliperidone was released after 90 min. The results indicated that magnetic stirring was inadequate because the magnetic force was not sufficient to promote efficient release under any of the conditions tested.

In water, only 24.90% of paliperidone was released after 120 min. This observation led us to consider the importance of using an efficient extraction procedure to access the drug. Observing the procedure described for nifedipine extended-release tablets, the technique officially recommended for extraction suggests the use of a stirrer at high blending speeds for 30 minutes and details an exhaustive procedure for preparation of the analytical solution [24]. Acetonitrile, water, NaOH, and HCl allowed for the complete extraction of paliperidone, indicating the efficiency of these solvents in disrupting the interaction between the drug and the polymers to allow for drug release from the osmotic system. When the internal structure of the osmotic pump was examined, it was clear that the polymer had expanded into the pump. According to the manufacturer, the polymers in the nucleus of paliperidone OROS form hydrates in water, resulting in the formation of a drug-containing gel, which is subsequently released from the porous tablet [8].

The development of methods for the measurement of paliperidone in osmotic tablets depends on the development of improved extraction conditions while aiming for the most reliable results. According to ICH [27], the analytical procedure refers to the method of performing the analysis, describing in detail the steps necessary to perform each analytical test. The goal of any analytical method is to produce analytical results that reflect the content of the samples with an acceptable standard of accuracy [40]. The absence of interferences in the analysis is an important start to the development of methods. In this work, the parameters were specified by determining possible interference from excipients and degradation products in the chromatographic run. Because of the complex qualitative and quantitative formulation of the drug system, many excipients are present and need to be controlled. A placebo was prepared using data from the literature [29]. Previous experiments were performed using UV spectrophotometry, and the results demonstrate that the matrix excipients interfere with the measurement of absorption, impeding this measurement in the assay. In addition to evaluating a placebo solution, we also performed stress testing by exploring various extrinsic conditions. The method must have the ability to unambiguously assess the analyte of interest in the presence of all expected components, which may consist of degradants, excipients, sample matrix, and sample blank peaks [41]. The demonstration of the specificity and the ability of the method to monitor changes in the chemical properties of the drug over time invariably calls for a forced degradation (stress testing) study to be performed on the drug substance and drug product [42]. According to FDA guidelines [43], stress testing of a drug substance can aid in the identification of likely degradation products, which can help to establish degradation pathways and reveal the intrinsic stability of the molecule. Stability indication refers to a process whereby it is demonstrated that, after degradation of the test article, the analytical assay method is capable of producing reliable results for its active substances and preservatives, if applicable, without interference from degradation products [41]. Figure 4 illustrates a chromatographic analysis for the placebo solution and degraded samples. For the placebo

TABLE 3: Results of drug analysis obtained from precision determination in LC assay for paliperidone in extended-release OROS tablets.

	Mean area	Intra-day precision	
		Label claim ^a (%)	RSD (%)
Day 1	758709	101.25	0.30
Day 2	785177	104.21	0.45
Day 3	758951	100.79	0.59
Inter-day precision	767612.3 ^b	102.08 ^b	1.81

^aMean of six amounts, assayed in triplicate.

^bMean obtained from precision in different days.

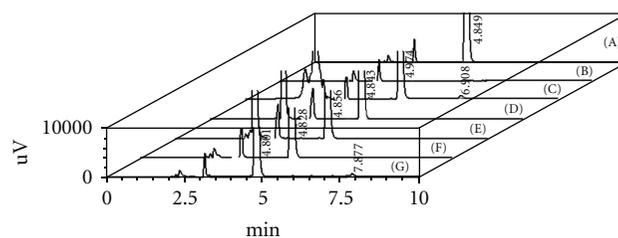


FIGURE 4: LC chromatograms of paliperidone sample solution, placebo and degraded samples. (A) paliperidone sample solution ($30 \mu\text{g mL}^{-1}$); (B) placebo solution; (C) paliperidone sample solution after basic degradation (NaOH 0,1N, 1 h); (D) acid degradation (HCl 0,1N, 1 h); (E) oxidative degradation (H_2O_2 30%, 6 h); (F) photodegradation (UV chamber, 60 min); (G) thermal degradation (60°C , 96 h).

(Figure 4(B)), it is possible to observe peaks at 2.5 and 2.6 min, retention times that are different than those for paliperidone and are probably attributable to HCl. The same profile is observed in degraded samples, demonstrating low levels of decomposition under all conditions tested. When submitted to acid and photodegradation (Figures 4(D) and 4(F)), no decomposition was observed under the established conditions. In the basic and heat decomposition tests (Figures 4(C) and 4(G)), minor products were detected at 6.9 and 7.8 min, respectively, times that do not interfere in the analysis of paliperidone. This result was confirmed by peak purity determination, which demonstrated an absence of interference in the drug peak at 4.8 min. For an identification test based on high-performance liquid chromatography, peak purity evaluation should be used to assess the homogeneity of the peak corresponding to the analyte of interest [41]. For future studies, the presented data could be useful for understanding the stability of this drug, the main degradation products, and the decomposition pathway involved. Thus, chromatograms of the placebo solution and degraded samples allowed us to conclude that excipients and degradation products do not interfere with the analysis of paliperidone, indicating that the developed LC method was selective for the determination of the drug in the pharmaceutical formulation.

To assess linearity, three standard curves for paliperidone were constructed by graphing the concentration of drug (x) versus peak area (y), data not shown. Over the concentration

TABLE 4: Results from accuracy evaluation of the LC method for determination of paliperidone in extended-release OROS tablets.

Level (%)	Concentration of sample ($\mu\text{g mL}^{-1}$)	Amount of drug added ($\mu\text{g mL}^{-1}$)	Amount of drug found ($\mu\text{g mL}^{-1}$)	Mean of recovery ^a (%)	RSD (%)
10	30	3.0	2.98	99.36	1.37
20	30	6.0	3.00	100.07	1.52
30	30	9.0	3.00	100.11	0.13

^a Mean of three amounts, assayed in triplicate.

TABLE 5: Results from robustness testing of the LC method for paliperidone in extended-release OROS tablets.

	Nominal condition ^a	Robustness condition							
		pH of aqueous solvent		Concentration of organic solvent (%)		Column supplier		flow rate (mL min^{-1})	
		3.6	4.0	28	32	Shim-pack C18	ODS-Hypersil	0.8	1.2
Amount (%)	99.67	100.24	100.18	99.93	99.51	99.67	100.38	99.99	98.67
RSD (%)	1.23	0.33	0.53	1.31	0.73	1.23	1.21	1.34	1.40
Retention time	4.83	4.67	4.79	5.63	4.18	4.83	5.89	8.96	4.03
Theoretical plates	12767.9	12355.3	13047.4	13366.2	11544.6	12767.9	6316	14810.2	10581.1

^a nominal condition: pH 3.8; flow rate 1.0 mL min^{-1} ; concentration of organic solvent 30.0%; column Shim-pack C18.

TABLE 6: Results from stability of analytical solutions applied to LC analysis of paliperidone in extended-release OROS tablets.

Time (h)	Amount of paliperidone ^a (%)					
	4°C		25°C		40°C	
	Standard solution	Sample solution	Standard solution	Sample solution	Standard solution	Sample solution
0	100.11	99.97	100.45	100.07	101.12	100.34
24	100.05	99.88	99.50	99.44	99.94	99.74
48	101.09	99.80	100.78	98.94	100.87	99.03
72	99.15	99.71	98.92	98.51	100.95	99.11

^a Mean of three amounts, assayed in triplicate. Solutions stored at $30.0 \mu\text{g mL}^{-1}$.

range of $10.0\text{--}50.0 \mu\text{g mL}^{-1}$, the correlation coefficient was 0.9999, indicating an excellent correlation between the parameters cited above. The statistical results obtained using ANOVA demonstrated that the regression equation was linear ($F_{\text{calculated}} = 1901.14 > F_{\text{critical}} = 4.96$; $P = 0.05$) with no deviation from linearity ($F_{\text{calculated}} = 0.753 < F_{\text{critical}} = 3.71$; $P = 0.05$). The reproducibility of the method was determined by performing six replicate analyses in various solutions prepared from a matrix of OROS tablets. The results are shown in Table 3. For intra-assay precision, the RSD value for the peak area of paliperidone was between 0.30–0.59%. The intermediate precision was determined by analyses performed on three different days, and the results indicated an amount of paliperidone of 102.08%. The low values of RSD for both the repeatability and the intermediate precision tests demonstrate the high precision of the method proposed.

The accuracy of the method was studied by applying the recovery test, which was performed by adding the reference standard to the sample solution and analyzing the resulting mixture. Accuracy was calculated as the percentage of drug recovered from the test samples, and the results are summarized in Table 4. The mean recovery of the paliperidone reference from the sample was calculated

as between 99.36 and 100.11 ($n = 3$), indicating that the developed method was able to determine the drug amount accurately. To guarantee the reliability of the drug determination when small variations occur, the robustness of the method was tested. The LC method developed indicated good performance, demonstrating robust determination of the drug. The chromatographic pattern was maintained under conditions that were variable with respect to pH, column used, proportions of the mobile phase, and flow rate; small changes in the retention time were observed. A major variation in the retention time occurred when the proportions of the mobile phase and flow rate were modified. Despite this disparity in retention time, paliperidone was quantified accurately under all conditions, as demonstrated by the low RSD values. When the effects of using different columns were evaluated, the theoretical plates and retention times were modified, although the drug amount was found to be adequate. The results are shown in Table 5.

Evaluation of the stability of the analytical solutions indicated that the reference standard and samples presented good stability at 4°C, 25°C and 45°C after sample preparation. Table 6 illustrates the values obtained for drug quantitation. Both retention time and peak area were not modified. The RSD obtained was less than 1.0%, and no

significant degradation was observed during storage (72 h). This result is important in improving reliability during the chromatographic run, as it allows the samples to be stored during the evaluation time.

4. Conclusions

A simple, fast, and reliable stability-indicating RP-HPLC method for the quantitative analysis of paliperidone was developed and validated in osmotic pump tablets. An extraction procedure using an ultrasonic bath was established and allowed for drug release during sample preparation. Tests of system suitability illustrate good performance and reproducibility. The degradation products formed during the stress testing were well separated from the drug in the chromatograms, indicating that the method is capable of indicating stability. In addition, the sample preparation procedure is adequate for routine quality control. The proposed method for determining paliperidone in OROS tablets represents an important reference study of this modern pharmaceutical delivery system.

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