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Bioequivalence between two extended Release Tablets of Oxycodone Hydrochloride in Healthy Subjects under Fasting and Fed Conditions

Vespasiano CFP[*1], Accennato VAC[1], Costa F[1], Riccio MF[2], Bernasconi G[2] and Noboli AC[2]

[1]Zodiac Produtos Farmacêuticos S/A, São Paulo, Brazil
[2]CAEP - Centro Avançado de Estudos e Pesquisas, Campinas, Brazil

[*]**Corresponding author:** Vespasiano CFP, Zodiac Produtos Farmacêuticos S/A, São Paulo, Brazil, CEP 04794-000, Tel: 011 5501-5584, E-mail: celso.vespasiano@zodiac.com.br

Abstract

Bioavailability of different formulations of oxycodone 20 mg extended release tablets was compared in two bioequivalence studies, one under fasting conditions and the other one after a high-fat breakfast. Both studies were single dose, randomized, open label, and two-period crossover, with Brazilian male and female healthy subjects. Blood samples were taken during 48 h and plasmatic concentrations were determined using a validated UPLC-MS/MS method. Confidence intervals (CI90%) for the peak plasma concentration (Cmax) and area under the concentration-time curve (AUC0-t) were determined by calculating LN-transformed data. In the fasting study, the ratios and 90% CI for the geometric mean test/referencewere93.36% (87.62-99.48%) for Cmax and 102.10% (96.71-107.79%) for AUC0-t. In the fed study, the ratios and 90% CI for the geometric mean was 89.26% (85.05-93.69%) for Cmax and 100.84% (95.95-105.98%) for AUC0-t. Under fasting and fed conditions, the test (oxycodone hydrochloride20 mg extended release tablets, Zodiac Produtos Farmacêuticos S.A.) and reference (Oxycontin®20 mg extended release tablets, Purdue Pharmaceuticals L.P.) formulations were considered bioequivalent since the 90% CIs for the geometric mean test/reference ratios were within the ANVISA and FDA predetermined range of 80% to 125%.

Keywords: Oxycodone Hydrochloride; Extended Release Tablets; Fasting and Fed Conditions; Bioequivalence; Chromatography

Introduction

Oxycodone hydrochloride (oxycodone; 14-hydroxy-7,8-dihydrocodeinone) is a semisynthetic strong opioid agonist, that provides effective relief for moderate to severe pain in cancer and post-operative patients (1, 2, 3).Studies have shown oxycodone to be effective in alleviating malignant pain, postoperative pain, osteoarthritis and neuropathic non-malignant pain [1].

Dose proportionality has been established for the 10 mg, 20 mg, 40 mg, and 80 mg tablet strengths for both Cmax and AUC. Food has no significant effect on rate and extent of exposure of oxycodone [1].

Oral bioavailability of oxycodone in humans is 60% (range, 50%-87%) and the terminal elimination half-life is not affected by dose, with modest inter-individual variations [2].The time to peak plasma concentration (tmax) of oxycodone after single oral administration of controlled release tablets is approximately between 2.1 and 3.2 hours [3,4].

Oxycodone is extensively metabolized by liver CYP3A enzymes, to a primary inactive metabolite, noroxycodone, and to a much lesser extent to the active metabolite oxymorphone, via CYP2D6 [1,3]. Both oxymorphone and noroxycodone are transformed to another possibly active metabolite, noroxymorphone, by CYP3A4 and CYP2D6, respectively. CYP2D6 genotypes caused expected differences in pharmacokinetics, but they did not influence pain control in patients treated with oxycodone for cancer pain [3].

Oxycodone and its metabolites are excreted primarily via the kidney, but less than 10% of the dose is excreted unchanged [1,3]. The elimination half-life ($t_{1/2}$) of oxycodone is between 4.5 and 8 hours after controlled release formulation [1,4].

Opioid-naïve females subjects demonstrate up to 25% higher mean plasma concentrations and greater frequency of typical opioid adverse events than males, even after adjustments of body weight. The clinical relevance of this finding is low for a drug intended for chronic usage at individualized dosages, also there was no male/female difference detected for efficacy or adverse events in clinical trials [5].

The objective of the studies in this paper was to compare, in healthy volunteers of both genders, the pharmacokinetic profiles of oxycodone, aiming at assessing the bioequivalence between two formulations: oxycodone hydrochloride 20 mg extended release film-coated tablet, registered by Zodiac Produtos Farmacêuticos S.A. (test drug) and Oxycontin® 20 mg extended release film-coated tablet, imported by Purdue Pharmaceutical LP (reference drug) under fasting and fed conditions.

Although food does not influence the bioavailability of oxycodone, this paper is presenting studies under both fasting and fed conditions because it is an extended release formulation product. This is in accordance with sanitary regulations (including ANVISA) that require both studies for modified/extended release formulations.

Material and Methods

Population

Thirty-two (32) volunteers of both genders (16 female and 16 male subjects) aged 18 to 50 years were screened for each study (fasting and fed conditions). All volunteers were considered as being eligible to participate in the studies based on the inclusion and exclusion criteria defined in the protocols.

All volunteers showed good health conditions or the absence of significant diseases after assessment of medical history, verification of vital signs, physical examination, electrocardiogram, and routine laboratory tests. All subjects enrolled in the studies showed negative tests for hepatitis B (HBsAg and Anti-HBc IgM), hepatitis C and HIV and urine HCG (pregnancy test only for female subjects).

Both studies were conducted in compliance with guidelines and standards for researches involving human beings from Resolutions no. 466/12 and 251/97 by the National Health Council - Ministry of Health, Good Clinical Practices according to ICH, and the Document of the Americas and in compliance with the Declaration of Helsinki (adopted by the 18th WMA General Assembly in Helsinki/ Finland, 1964, and with the last amendment by the 64th WMA General Assembly in Fortaleza/ Brazil, 2013). The protocols (fasting and fed conditions) were submitted and approved before study initiation by the Ethics Committee of Faculdade de Jaguariúnain Jaguariúna, São Paulo, Brazil. After explaining the nature and purpose of the studies, all volunteers provided their written informed consent for participation.

Study Treatments

The test formulation was oxycodone hydrochloride 20 mg extended release film-coated tablets (batch number L001/16), manufactured by Zodiac Produtos Farmacêuticos S.A. Brazil, and the reference formulation was Oxycontin®, oxycodone hydrochloride 20 mg extended release film-coated tablets (batch number WSS52-1), manufactured by Purdue Pharmaceuticals L.P, North Caroline, USA. Before starting the clinical study, test and reference formulations were evaluated in vitro to check if they could be considered pharmaceutical equivalents. Besides the tests described in the pharmacopeia, comparative dissolution profile was also performed. The similarity factor found in the comparative dissolution profile was 56, 99. Test and reference drugs presented similar performance *in vitro* and were considered pharmaceutical equivalents.

Study Design

The purpose of the studies was to compare the bioavailability of two formulations of oxycodone hydrochloride 20 mg extended release film-coated tablets, one study under fasting and the other one under fed conditions.

The studies were conducted using an open-label, randomized, two-period, crossover, and balanced design, with a washout period of 7days between administrations. In each of the study periods, the volunteers received annex tended release film-coated tablet containing 20 mg of oxycodone hydrochloride from one of the two formulations mentioned above orally, as a single dose with a 200-mL glass of water at room temperature. Also, 8h before and 12h after study drug intake, naltrexone chloride 50 mg film-coated tablet was orally administered, to avoid adverse events caused by oxycodone administration such as effects on central nervous system, respiratory depression and constipation. In the fasting study, the drugs were administered after a minimum fasting of 8 hours. In the fed study, volunteers fasted for at least 8 hours and received the study drug 30 minutes after starting a high-fat breakfast (bread, butter, mozzarella cheese, ham, vanilla strawberry cake, strawberry yogurt and juice). In both studies, volunteers fasted for 4 hours after drug administration. To maintain the standardization of treatment groups, the diet (food and drink) followed the same standard for all volunteers and in both periods.

The intake of alcoholic beverages, food or beverages containing caffeine or xanthine (such as coffee, tea, chocolate and cola- or guarana-based soft drinks) was not permitted. In addition, the use of nicotine was prohibited from 48 hours before hospitalizations until the last blood draw, as well as any regular drugs (for at least 14 days) or occasional drugs (up to 7 days) before study initiation.

Blood samples (7.5 mL) were collected in coated tubes, containing EDTA as anticoagulant. The schedule in both studies included collections before (pre-dose), at 00:15, 00:30 and 00:45 minutes and at 1:00; 1:20; 1:40; 2:00; 2:20; 2:40; 3:00;3:20; 3:40; 4:00; 4:20; 4:40; 5:00; 5:20; 5:40; 6:00; 7:00; 8:00; 12:00; 24:00 and 48:00 hours after the administration of each drug. A total of 25 blood samples were collected from each volunteer in each period.

Immediately after collection, blood samples were centrifuged at 1,646g (3,500 rpm) for 10 minutes at approximately 4°C. After centrifugation, the plasma was separated and transferred to two previously labeled cryotubes. The tubes were stored in freezer at -20 °C and were maintained at this temperature until the analysis.

Clinical, analytical, and statistical stages of the study were conducted by Centro Avançado de Estudos e Pesquisas Ltda. (CAEP), located in the city of Campinas, São Paulo, Brazil.

Quantification of Oxycodone in Human Plasma

Plasma concentrations of oxycodone were determined using reversed-phase ultra-performance liquid chromatography with tandem mass spectrometry (RP-UPLC-MS/MS). The analytes were extracted from plasma using liquid-liquid extraction with ether/hexane (60:40) solvent. Deuterated oxycodone was used as the internal standard. To avoid inter-assay variations, all the samples from the same volunteer were assessed in the same analytical run.

The detection parameter used was the mass-to-charge ratio (m/z) between precursor ions and product, and the quantification parameter was the ratio of areas under chromatogram peak identified in the retention time between analyte and internal standard. Oxycodone concentrations in volunteer samples were calculated using interpolation in the calibration curve.

The chromatographic analysis was conducted in an UPLC Acquity (Waters) with Waters column Acquity UPLC BEH Phenyl 2.1 x 50 mm, with a flow rate of 0.15 mL/min. The column was maintained at a temperature of 50°C, while the autoinjector was maintained at 10 °C. The mobile phase used was water plus 0.1% formic acid and100% methanol at a 50:50 ratio (v/v). The injection volume was 5 μL and the total run time set as 2 minutes. The mass spectrometry detection was conducted using electrospray ionization source in positive mode. The multiple reaction monitoring (MRM) method was used, and the transitions monitored were m/z 316.2>298.3and m/z 322.2>304.4 for oxycodone and oxycodone-d6, respectively.

The method was validated in compliance with ANVISA guidance for bioanalytical method validation, RDC Resolution no. 27, dated May 17, 2012 [6]. The validation parameters assessed were selectivity, linearity, intra- and inter-run precision, intra- and inter-run accuracy, matrix effect, residual effect, and stability of oxycodone under different conditions.

Pharmacokinetic and Statistical Analysis

The pharmacokinetic parameters were obtained from the oxycodone plasma concentration-time curves. These parameters were statistically assessed for bioequivalence determination using Phoenix WinNonLin version 6.4 software and Microsoft Excel version 2007. The area under the plasma concentration-time curve was calculated using the linear trapezoidal method, from time zero to the last measurable concentration (AUC_{0-t}). The area under the plasma concentration-time curve was also calculated from time zero to infinity ($AUC_{0-\infty}$), where $AUC_{0-\infty} = AUC_{0-t} + Ct/z$, with Ct being the last drug concentration experimentally defined and z being the terminal phase elimination constant rate. The peak of maximum plasma concentration (Cmax) of oxycodone and the time to reach this peak (tmax) were obtained directly with no data interpolation. The elimination half-life ($t_{1/2}$) was defined using the equation $t_{1/2} = \ln(2)/z$.

For the bioequivalence assessment between the formulations, AUC and Cmax were used. The model included a fixed effects term for sequence, period and treatment (ANOVA). Subjects (nested in sequence) were treated as a random effect. A 90% Confidence Interval (CI) was generated for the difference in averages of LN-transformed data from test and reference drugs. The antilog of obtained CI comprised the 90% CI for geometric mean ratio of primary parameters. The drug products are considered as bioequivalent if the bounds of the 90% CI generated for the geometric mean ratio for both primary parameters are equal or higher than 80% and equal or lower than 125%, as established by ANVISA and FDA [7,8].

Results

The validation method used allowed for the selective determination of oxycodone in a linear range within 0.5 ng/ml to 40 ng/ml. UPLC method developed was robust, selectivity, the accuracy and precision (%CV) observed for the calibration curve standards ranged from 94.7 to 109.7% and 1.8 to 7.8%, respectively. There was no carryover effect observed during autosampler carryover experiment. Further, the extent of matrix effect in different lots of plasma was within the acceptable limit. The stability of the oxycodone and its internal standard in human plasma and stock solutions were evaluated at different storage conditions. Oxycodone and oxycodone-d6 were found to be stable in all tested condition, including in plasma samples stored below -20 °C for 100 days.

In the fasting study, out of 32 participant volunteers, 25 completed the two study periods. Subjects 05, 06, 07, 16, 17 and 18 were prematurely dropout during Period 1 (personal reasons, vomiting after study drug administration and positive answer to an exclusion criteria). Subject 12 was prematurely dropout at Period 1, before drug dose administration, due to adverse event (vomiting). In the fed condition study, out of 32 enrolled volunteers, 27 completed the study. Subjects 04, 18, 21 and 30 were prematurely dropout during Period 1 (positive answer to an exclusion criterion, vomiting after study drug administration and use of prohibited concomitant medication). Subject 08 was prematurely dropout at Period 1, before drug dose administration, due to adverse event (vomiting and diarrhea).The volunteers participating in the bioequivalence study under fasting conditions had mean

age of 31.87 years, ranging from 19 to 48 years; mean weight of 68.9 kg (51.1 to 100 kg); mean height of 1.66 m (1.46 to 1.84 m) and mean BMI of 24.85 kg/m² (19.52 to 29.54 kg/m²). The volunteers participating in the bioequivalence study after feeding had mean age of 29.65 years, ranging from 18 to 49 years; mean weight of 73.6 kg (50 to 98 kg); mean height of 1.68 m (1.53-1.85 m) and mean BMI of 25.99 kg/m² (19.05 to 29.91 kg/m²).

Oxycodone was well tolerated at the administered dose in both studies. No serious adverse events were seen or reported, and no pregnancies were detected during the studies. The most common adverse event was headache, reported by 28.1% of the volunteers in the fasting study and by 31.25% of the volunteers in the fed study. Also, 21.8% subjects in the fasting condition reported nausea as most common adverse event [9].

The high incidence of dropout on both studies was related to oxycodone and naltrexone side effects on the gastrointestinal system.

The mean plasma concentration-time curves for test and reference drugs are shown in Figure 1 (fasting) and 2 (fed). The curves were shown to be overlapped, showing a similar pharmacokinetic profile between the drugs in both conditions. None subject had concentration on the pre-dose collection time in the second period of study, showing adequate washout period.
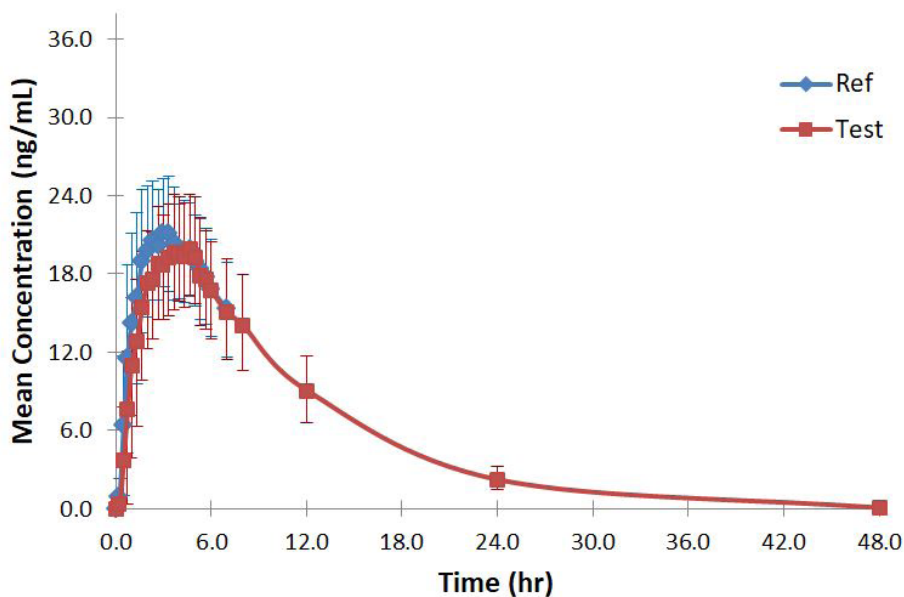

Figure 1: Mean curves of oxycodone plasma concentration vs. time obtained for test and reference drugs under fasting conditions
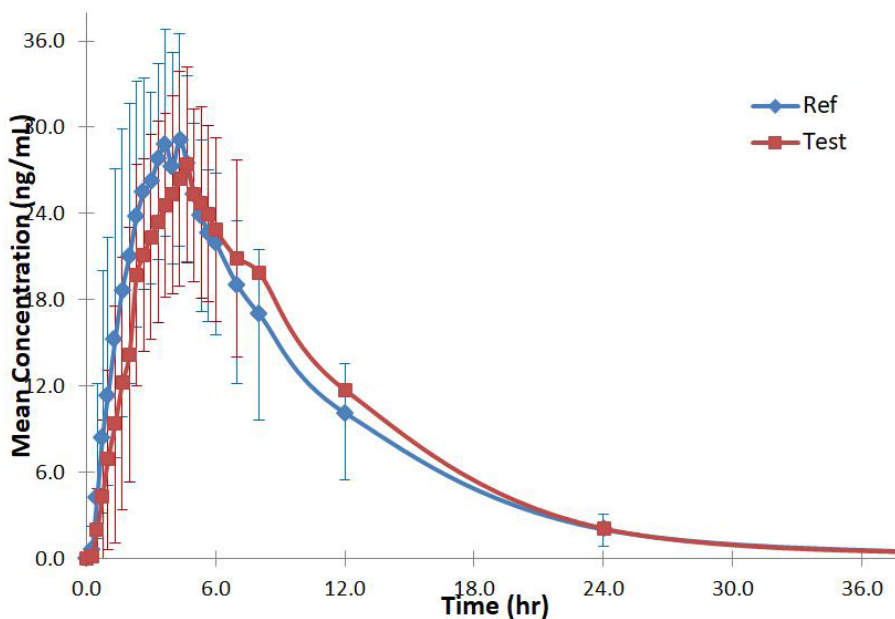

Figure 2: Mean curves of oxycodone plasma concentration vs. time obtained for test and reference drugs under fed conditions

The central location and dispersion measures for all pharmacokinetic parameters from both formulations are shown in Table 1 (fasting) and Table 2 (fed).

Parameter	Test Drug	Reference Drug
Cmax (ng/mL)	22.32 ± 20.61	23.92 ±17.62
tmax (h)*	4 (1.33-5.667)	2.667 (0.75-6)
AUC_{0-t} (ng*h/mL)	236.68 ± 20.23	231.04 ± 19.22
$AUC_{0-\infty}$ (ng*h/mL)	256.34 ±20.77	245.55 ±20.24
$t_{1/2}$ (h)	6.10 ± 29.62	5.48± 19.28

Cmax: Maximum Plasma Concentration; ***tmax:** Time To Reach The Maximum Plasma Concentration (Median And Range); **AUC_{0-t}:** area under the plasma concentration vs. Time curve from time 0 to t; **$AUC_{0-\infty}$:** Area Under the Curve of Plasma Concentration vs. Time from Time 0 to Infinity; **$t_{1/2}$:** Elimination Half-Life

Table 1: Pharmacokinetic parameters (geometric mean ± **CV**%) of oxycodone obtained after oral administration under fasting conditions

Parameter	Test Drug	Reference Drug
Cmax (ng/mL)	29.71 ± 20.33	33.12 ± 22.93
tmax (h)*	4.33 (2-6)	3 (0.75-4.667)
AUC_{0-t} (ng*h/mL)	283.07 ±29.18	280.28 ± 25.44
$AUC_{0-\infty}$ (ng*h/mL)	297.36± 30.70	296.44 ± 26.57
$t_{1/2}$ (h)	4.84 ± 20.59	5.05± 15.29

Cmax: Maximum Plasma Concentration; ***tmax:** Time To Reach The Maximum Plasma Concentration (Median And Range); **AUC_{0-t}:** area under the plasma concentration vs. Time curve from time 0 to t; **$AUC_{0-\infty}$:** Area Under the Curve of Plasma Concentration vs. Time from Time 0 to Infinity; **$t_{1/2}$:** Elimination Half-Life

Table 2: Pharmacokinetic parameters (geometric mean ± **CV**%) of oxycodone obtained after oral administration under fed conditions

Results of parameter tmax after test drug treatment under fed conditions are higher than the results obtained after administration of reference drug. This could be related to the fact that the uptake of active substance from test drug is slower than from reference drug. However; this had no influence on the AUC results.

After test treatment, Cmax was significantly below when compared with the results after reference treatment. However; this fact did not affect similarity between treatments at fast and fed conditions, as treatments were considered bioequivalent under both conditions.

Table 3 and Table 4 show the test/reference geometric mean ratios for pharmacokinetic parameters Cmax, AUC_{0-t}, and $AUC_{0-\infty}$ and the respective 90% CIs for the bioequivalence analysis of the studies. All 90% CIs were within the range of 80% to 125%.

Parameters	Geometric mean ratio (%)	Confidence interval (90%)	Intra-subject coefficient of variation (%)
Ln(Cmax)	93.36	87.62-99.48	13.13
Ln(AUC_{0-t})	102.10	96.71-107.79	11.21
Ln($AUC_{0-\infty}$)	104.01	97.95-110.45	12.43

Table 3: Geometric mean ratio and confidence intervals (90%) of oxycodone test and reference drugs for assessment of bioequivalence under fasting conditions

Parameters	Geometric mean ratio (%)	Confidence interval (90%)	Intra-subject coefficient of variation (%)
Ln(Cmax)	89.26	85.05-93.69	10.42
Ln(AUC_{0-t})	100.84	95.95-105.98	10.72
Ln($AUC_{0-\infty}$)	100.13	95.62-104.86	9.95

Table 4: Geometric mean ratio and confidence intervals (90%) of oxycodone test and reference drugs for assessment of bioequivalence under fed conditions

Discussion

Two drugs are considered bioequivalent if their rate and extent of absorption do not show statistically significant differences when administered at the same molar dose of the active ingredient, under the same experimental conditions (-9). In this paper, the relative bioavailability of two formulations of oxycodone was assessed after single-dose administration under fasting and fed conditions. Single dose studies are considered more sensitive to assess bioequivalence when compared to multiple-dose study, even for extended release formulations. The use of multiple-dose study for bioequivalence is recommended in Brazil only when the study is conducted in patients and/or the quantification of the drug in plasma is not possible after single dose.

With the purpose of obtaining a highly sensitive and rapid method for quantification of oxycodone in plasma, a method by UPLC-MS/MS was developed and validated in this project. In the presented method the lower limit of quantification was 0.5ng/mL, which allowed for a sensitive and efficient analysis of oxycodone plasma concentrations.

The pharmacokinetic results (C_{max}, AUC, t_{max} and $t_{1/2}$) found in the studies for oxycodone (Tables 1 and 2) were very similar to those reported on the literature under fasting [1,3,4] and fed conditions [1,3]. Test drug had a delay in tmax, both under fasting and fed condition (Table 1 and 2), showing a slightly slower absorption when compared to reference. As extended release oxycodone is indicated for treatment of chronic pain, the difference in tmax between the two formulations would not provide a clinically meaningful difference in the treatment of pain. As shown in Tables 3 and 4, 90% CIs obtained for pharmacokinetic parameters defining bioequivalence (Cmax, AUC_{0-t}, and $AUC_{0-\infty}$) of formulations of oxycodone 20 mg were shown to be within the bioequivalence limits defined by ANVISA (80%-125%) in RE Resolution no. 1170, dated April 19, 2006 [7].

Since the adverse events and laboratory test results were similar for both drugs in the two conditions evaluated (fast and fed), it was also possible to verify that test and reference drugs have similar safety profile and are well tolerated by patients.

Conclusion

Based on the pharmacokinetic and statistical results obtained in both bioequivalence studies, one conducted with administration of oxycodone under fasting conditions and the other one with administration of oxycodone under fed conditions (high-fat breakfast), we conclude that the test drug product (oxycodone hydrochloride 20 mg - Zodiac Produtos Farmacêuticos S.A.) and the reference drug product (Oxycontin® 20 mg - Purdue Pharmaceuticals L.P.) are bioequivalent. Thus, oxycodone 20 mg extended release film-coated tablets may be considered as being interchangeable in medical practice, since they have the same efficacy and safety profile for the patients.

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