Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 871–876
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Stability-indicating methods for the determination of piretanide in presence of the alkaline induced degradates

Nadia F. Youssef *

National Organization for Drug Control and Research (NODCAR), 6 Abou Hazem St., Pyramids Ave., P.O. Box 29, Cairo, Egypt

Accepted 23 February 2005
Available online 22 April 2005

Abstract

Stability-indicating high performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and first-derivative of ratio spectra (1DD) methods are developed for the determination of piretanide in presence of its alkaline induced degradates. HPLC method depends on separation of piretanide from its degradates on μ-Bondapak C_{18} column using methanol:water:acetic acid (70:30:1, v/v/v) as a mobile phase at flow rate 1.0 ml/min and UV detector at 275 nm. TLC densitometic method is based on the difference in Rf-values between the intact drug and its degradates on thin-layer silica gel. *Iso*-propanol:ammonia 33% (8:2, v/v) was used as a developing mobile phase and the chromatogram was scanned at 275 nm. The derivative of ratio spectra method (1DD) depends on the measurement of the absorbance at 288 nm in the first-derivative of ratio spectra for the determination of the cited drug in the presence of its degradates. Calibration graphs of the three suggested methods are linear in the concentration ranges 0.02–0.3 μg/20 μl, 0.5–10 μg/spot and 5–50 μg/ml, with mean percentage recovery 99.27 ± 0.52, $99,17 \pm 1.01$ and $99.65 \pm 1.01\%$, respectively. The three proposed methods were successfully applied for the determination of piretanide in bulk powder, laboratory-prepared mixtures and pharmaceutical dosage form with good accuracy and precision. The results were statistically analyzed and compared with those obtained by the official method. Validation of the method was determined with favourable specificity, linearity, precision, and accuracy was assessed by applying the standard addition technique.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Stability-indicating; HPLC; TLC; Ratio spectra; Piretanide

1. Introduction

Piretanide, 4-phenoxy-3-(pyrrolidin-1-yl)-5-sulphamoyl benzoic acid, affects renal and cardiovascular function. It is used as a diuretic and antihypertensive drug [1,2]. Piretanide exhibits a native fluorescence so spectrofluorimetric methods were published [3,4]. A few techniques have been applied for its determination in the biological fluids and pharmaceutical preparation including HPLC [5–7], electrochemical [8], and radio-immunoassay [9].

However, TLC and spectrophotometric methods have not been reported previously, although they are simple, direct and economical in cost, time and chemicals. Hence the proposed methods are suitable for routine analysis in quality control laboratories.

The aim of this work was to introduce stability-indicating methods for the determination of piretanide in presence of its degradates without any interference.

2. Experimental

2.1. Apparatus

- HPLC system: waters LC equipped with 600 controller; 486 Tunable absorbance detector, 600 pump, injector valve with constant 20 μl loop; integrator 746 Data Module; column μ-Bondapak C_{18}, 10 μm, 250 × 4.6 mm.
- Densitometer dual wavelength SHIMADZU flying spot CS-9301PC.

* Tel.: +20 2 5851278; fax: +20 2 585582.
E-mail address: gomagdy@yahoo.com.

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.02.035

- UV lamp short wavelength 254 nm.
- Thin-layer chromatographic plates precoated with silica gel GF, 10 cm × 10 cm, 0.25 mm thickness, fluorescent at 254 nm (E-Merck, Germany).
- UNICAM UV 300, Thermo spectronic, with vision 32 software, with 1 cm quarts cuvettes, connected to IBM PC computer used for all the absorbance measurements and treatment of data and hp laser Jet 1000 series printer.

2.2. Materials

Piretanide, working standard, was kindly supplied by Hoechst Orient S.A.E. Co., Cairo, Egypt, its purity was found to be $99.29 \pm 0.55\%$ according to the European Pharmacopoeia [10].

Arelix tablets (Japan Hexal, 3-1-10 Mita, Minatoku, Tokyo, Japan), each tablet was labeled to contain 6 mg piretanide.

2.3. Reagents

Chromatographic grade, methanol:water:glacial acetic acid (70:30:1, v/v/v) as a mobile phase for HPLC.

Spectroscopic grade, *iso*-propanol:ammonia 33% (8:2, v/v) as a developing mobile phase and methanol as a solvent for TLC and ratio spectra.

2.4. Preparation of the degradates

Fifty milligrams of piretanide was dissolved in 50 ml aqueous sodium hydroxide (pH 9) and transferred into a screw-capped tube. The solution became dark brown with pH 7 after exposure to sunlight for 10 days. Decomposition was assessed by applying TLC using *iso*-propanol:ammonia 33% (8:2, v/v) and by disappearance of the native fluorescence of the intact drug at 335 nm excitation and 415 nm emission in methanol. The solution of the degradates (1 mg/ml) was kept in a refrigerator and used for the preparation of laboratory-prepared mixtures.

2.5. Standard stock solutions

- Piretanide (0.1 mg/ml) in the mobile phase for HPLC or in methanol for ratio spectra.
- Piretanide (1 mg/ml) in methanol for TLC.
- Degradates (0.1 mg/ml) in the mobile phase for HPLC or in methanol for ratio spectra.
- Degradates, (1 mg/ml) in methanol for TLC.

All solutions were stable for at least 1 month if stored in a refrigerator at 4 °C.

2.6. Procedure

2.6.1. Calibration for HPLC method

Aliquots of standard stock solution (0.1 mg/ml) equivalent to 1–15 μg piretanide were transferred into a series of 10 ml volumetric flasks and diluted up to the mark with the mobile phase methanol:water:acetic acid (70:30:1, v/v/v). Twenty microlitres of each solution was injected at flow rate 1.0 ml/min. The effluent was monitored at 275 nm, AUFs equal 0.05 and the chromatograms were recorded. The calibration curve representing the relationship between the recorded area under the peak and the corresponding concentration was plotted and the regression equation was computed.

2.6.2. Calibration for TLC method

Aliquots of standard stock solution (1 mg/ml) equivalent to 0.25–4.5 mg piretanide were transferred into a series of 5 ml volumetric flasks and diluted up to the mark with methanol. Ten microlitres of each solution was applied to TLC plate. The chromatographic chamber was equilibrated with the developing mobile phase *iso*-propanol:ammonia 33% (8:2, v/v) for 10 min prior to use. The plates were developed over a distance of 8 cm and air dried at room temperature. The spots were visualized under UV lamp at 254 nm and the chromatogram was scanned with spectrodensitometer at 275 nm using photo mode: reflection and scan mode: zigzag. The calibration curve representing the relationship between the recorded area under the peak and the corresponding concentration was plotted and the regression equation was computed.

2.6.3. Calibration for 1DD method

Aliquots of standard stock solution (0.1 mg/ml) equivalent to 0.05–0.5 mg piretanide were transferred into a series of 10 ml volumetric flasks and diluted up to the mark with methanol. The zero-order spectrum of each solution was divided by the spectrum of the degradates (45 μg/ml). All spectra were stored in the IBM PC. The first-derivative of each ratio spectra was recorded at range 200–400 nm; band width 1.5 nm; scan speed: intelliscan; data interval: normal; smoothing: high and 1DD values were measured at 288 nm. The calibration curve was plotted and the regression equation was computed.

2.7. Laboratory-prepared mixtures

2.7.1. HPLC method

Aliquots equivalent to 1–15 μg of piretanide solution (0. 1 mg/ml) were transferred into a series of 10 ml volumetric flasks. Ten to ninety percentage of the degradates (0.1 mg/ml) were added to the same flasks and diluted up to the mark with the mobile phase.

2.7.2. TLC method

Aliquots equivalent to 0.25–4.5 mg of piretanide solution (1 mg/ml) were transferred into a series of 5 ml volumetric

flasks. Ten to ninety percentage of the degradates (1 mg/ml) were added to the same flasks and diluted up to the mark with methanol.

2.7.3. 1DD method

Aliquots equivalent to 0.05–0.5 mg of piretanide solution (0.1 mg/ml) were transferred into a series of 10 ml volumetric flasks. Ten to ninety percentage of the degradates (0.1 mg/ml) were added to the same flasks and diluted up to the mark with methanol.

2.8. Assay of pharmaceutical formulation

Ten tablets were accurately weighed and finely powdered. An amount of the powder equivalent to 50 mg piretanide was weighed, dissolved in 40 ml methanol and shaked for 1 h in a mechanical shaker [13]. The solution was filtered and transferred quantitatively into 50 ml volumetric flask and made up to the mark with methanol. The assay was completed as described above. The concentration was calculated using the corresponding regression equation.

3. Results and discussion

3.1. HPLC method

Various mobile phase systems were prepared and used for chromatographic separation but the proposed mobile phase comprising of methanol:water:acetic acid (70:30:1, v/v/v) gave a better resolution of piretanide. The analyte peak was well defined, resolved and free from tailing. The elution order was the degradates at (RT = 2.61–4.07 min) and piretanide (RT = 5.03 min) at 275 nm with flow rate 1.0 ml/min (Fig. 1). Linearity was obtained for the concentration range 0.02–0.3 μg/20 μl with mean percentage recovery

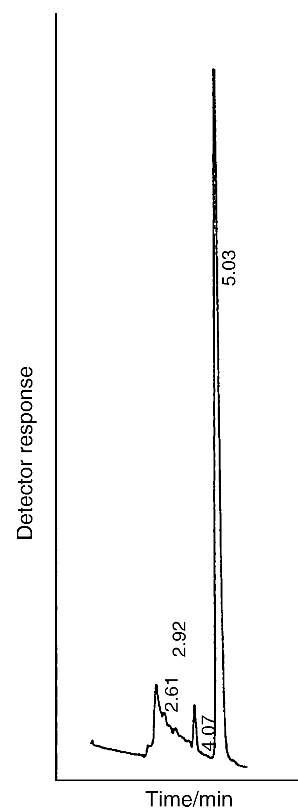

Fig. 1. HPLC chromatogram of piretanide (0.2 μg/20 μl) (RT = 5.03 min) and its degradates (0.2 μg/20 μl) (RT = 2.61–4.07 min).

Table 1
Validation parameters for the determination of piretanide by HPLC, TLC and 1DD methods

Paramters	HPLC method	TLC method	1DD method
Wavelength (nm)	275	275	288
Specificity (S.D.)			
RT or Rf intra-day[a]	0.05	0.005	
RT or Rf inter-day[a]	0.06	0.01	
PA or ABS intra-day[a]	0.71	0.59	0.006
PA or ABS inter-day[a]	1.08	1.25	0.01
Linearity range	0.02–0.3 μg/20 μl	0.5–10 μg/spot	5–50 μg/ml
Regression equation[b]			
Slope (b)	267.52	9.0517	0.1311
Intercept (a)	0.8367	3.057	0.0414
Correlation coefficient (r^2)	0.9992	0.9974	0.9999
S.D. of slope[c]	1.4800	0.0370	0.0013
S.D. of intercept[c]	0.2537	0.6768	0.0429
S.D. of (r^2)[c]	0.0000	0.0004	0.0001
Precision (mean ± R.S.D.%)			
Intra-day[d]	99.01 ± 0.82	99.58 ± 0.91	99.41 ± 0.88
Inter-day[d]	99.39 ± 1.63	99.67 ± 1.33	99.53 ± 0.91

[a] Average of $n = 5$, retention time (RT), Rf-values, peak area (PA) for HPLC and TLC or absorbance (ABS) for 1DD methods.
[b] $y = a \pm bc$ where c is the concentration of drug μg/20 μl for HPLC, μg/spot for TLC and μg/ml for 1DD methods, y the peak area for HPLC and TLC or absorbance for 1DD at the specified wavelength.
[c] Average of $n = 3$.
[d] Average of $n = 9$.

99.27 ± 0.52%. The parameters of regression equation are shown in Table 1.

3.2. TLC method

TLC densitometric method was used for the determination of piretanide in presence of its degradates using the difference in Rf-values. Complete separation was obtained using *iso*-propanol:ammonia 33% (8:2, v/v) as a developing mobile phase. The Rf-value of the drug was 0.54 and those of the degradates were 0 and 0.38. The spots of piretanide were scanned at 275 nm (Fig. 2). A linear calibration curve was obtained in the concentration range 0.5–10 μg/spot with mean recovery 99.17 ± 1.01%. The parameters of regression equation are shown in Table 1.

3.3. 1DD method

The zero-order spectra (D0) of piretanide and its degradates showed a complete overlapping (Fig. 3a). The application of first-derivative of ratio spectra technique resolves the problem of interference in D0 and also in the first-derivative spectra (D1) where no zero-crossing point was observed. Piretanide spectra (D0) were divided by those of its degradates (45 μg/ml) and the obtained ratio spectra (Fig. 3b) were converted to first-derivative (1DD) (Fig. 3c). The measurements at 288 nm showed no interference. Careful choice of the divisor and the working wavelength were of great importance, so different concentration of the degradates (15, 25, 45 μg/ml) were tested as a divisor. The best one was (45 μg/ml) as it produces minimum noise and gives better results. Linearity of the calibration curve was found in the concentration range 5–50 μg/ml with mean percentage recovery 99.65 ± 1.01%. The characteristic parameters of regression equation are given in Table 1.

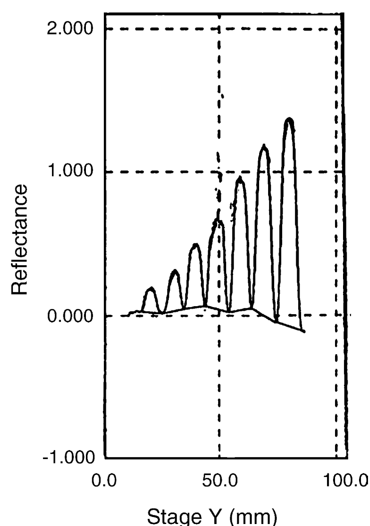

Fig. 2. TLC chromatogram of piretanide at 275 nm.

Physicochemical properties and stability of piretanide were studied by Matsubara et al. and some of the decomposition products were elucidated and identified [11,12].

The accuracy of the proposed methods were checked by analyzing nine laboratory-prepared mixtures of piretanide in presence of its degradates in different ratios, Table 2. Satisfactory recoveries with small relative standard deviations (R.S.D.) were obtained which indicates the high repeatability and accuracy of the proposed methods.

The proposed methods were also successfully applied for the analysis of piretanide in the pharmaceutical dosage form and the results are shown in Table 3. The validity of the methods was assessed by applying the standard addition technique and the results obtained were reproducible with low R.S.D. as shown in Table 3. The mean recovery of tablets was compared with that obtained by the compendial method [13] and there

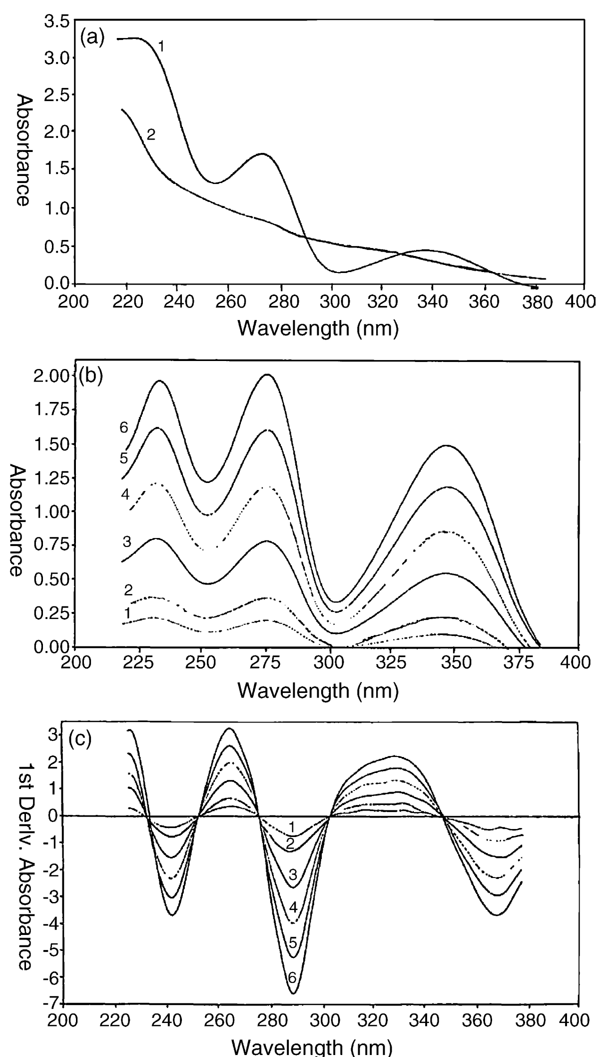

Fig. 3. (a) Zero-order spectra of (1) piretanide (50 μg/ml) and (2) its degradates (45 μg/ml). (b) Ratio spectra. (c) First-derivative of the ratio spectra (1) 5.0 μg/ml; (2) 10.0 μg/ml; (3) 20.0 μg/ml; (4) 30.0 μg/ml; (5) 40.0 μg/ml; (6) 50.0 μg/ml of piretanide using (45 μg/ml) of its degradates as a divisor in methanol.

Table 2
Determination of piretanide by the proposed methods in presence of its degradates

Sample number	Percentage of the degradates[a]	HPLC method found (%)[b]	TLC method found (%)[b]	1DD method found (%)[b]
1	10	99.85	97.73	100.47
2	20	98.16	98.93	99.71
3	30	100.89	101.59	99.38
4	40	101.27	98.93	98.24
5	50	97.55	99.12	100.29
6	60	99.52	100.40	99.38
7	70	98.75	99.37	100.21
8	80	101.49	99.93	99.34
9	90	99.52	101.58	98.54
Mean ±		99.67	99.73	99.51
R.S.D.%		1.37	1.28	0.76

[a] Calculated with respect to total weight (drug–degradates mixture).
[b] Found (%) of pure sample.

Table 3
Determination of piretanide in pharmaceutical dosage form by the proposed methods and by the compendial method [13]

Pharmaceutical preparation	HPLC method		TLC method		1DD method		Compendial method[a]
	Found[b] ± R.S.D. (%)	Recovery[b] ± R.S.D. (%)	Found[b] ± R.S.D. (%)	Recovery[b] ± R.S.D. (%)	Found[b] ± R.S.D. (%)	Recovery[b] ± R.S.D. (%)	Found[b] ± R.S.D. (%)
Arelix 6 mg per tablet	101.80 ± 0.45	99.68 ± 0.77	101.79 ± 0.41	99.35 ± 0.60	101.59 ± 0.41	99.73 ± 0.91	101.35 ± 0.89
S.D.	0.46		0.60		0.42		0.9
S.E.	0.21		0.27		0.19		0.40
Varience	0.21		0.36		0.18		0.81
t (2.306)[c]	1.00		0.91		0.54		
F (5.19)[c]	3.86		2.25		4.5		

[a] Measuring the extinction of tablets and reference standard solutions in 0.1 M methanolic sodium hydroxide at 334 nm using methanol as a blank.
[b] Average of $n = 5$.
[c] The values between parenthesis are corresponding to the theoretical values of t and F at the 95% confidence level.

was no evidence of interference from excipients. However this compendial method for tablets is not a suitable stability-indicating method since the measurements at 334 nm in the zero-order spectra showed a marked interference from the degradates.

Table 4 shows the statistical comparison of results for determination of piretamide by the proposed methods and the official method [10]. Results of the t-test for the accuracy and the F-test for the precision assessment [14] did not exceed the corresponding theoretical values, indicating insignificant differences between the results and supporting the robustness of the proposed methods.

4. Method validation

4.1. Specificity

HPLC, TLC and 1DD methods showed suitable specificity for drug identification. Chromatographic specificity was investigated by RT, Rf and PA of drug using standard solutions.

The ratio spectra method is specific for the determination of piretanide in presence of its degradates, at the selective wavelength 288 nm. The intra- and inter-day variation (S.D.) of RT were between 0.05 and 0.06 and PA, 0.71–1.08 for HPLC method; Rf-values (S.D.) were between 0.005 and 0.01

Table 4
Statistical comparison of results for the determination of piretanide in bulk powder by the proposed methods and the official method

Values	HPLC method	TLC method	1DD method	Official method [10]
Mean[a] ± R.S.D.	99.27 ± 0.52	99.17 ± .01	99.65 ± 1.01	99.29 ± 0.55
S.D.	0.52	1.00	1.01	0.55
S.E.	0.23	0.45	0.45	0.25
Varience	0.27	1.00	1.02	0.30
t (2.306)[b]	0.06	0.23	1.36	
F (5.19)[b]	1.11	3.33	3.40	

[a] Average of $n = 5$.
[b] The values between parenthesis are corresponding to the theoretical values of t and F at the95% confidence level.

and PA, 0.59–1.25 for TLC method; Absorbances (S.D.) were between 0.006 and 0.01 for 1DD method Table 1.

The assay results are unaffected by the presence of extraneous materials (degradates, impurities, excipients). The good results of laboratory-prepared mixtures prove the specificity of the proposed methods.

4.2. Linearity

Three independent calibration equations were obtained. Calibration curves were prepared and analyzed daily. Linear regression analysis was used to calculate the slope, intercept and correlation coefficient (r^2) of each calibration. The standard deviation was calculated for each parameter.

4.3. Precision

Methods precision was assessed by repeatability and reproducibility of HPLC, TLC and 1DD methods. The intra- and inter-day variations expressed by mean $\pm$ R.S.D.%, Table 1 were determined by assaying three samples in triplicate over a period of 3 days. The concentrations (0.02, 0.12, 0.3 μg/20 μl for HPLC); (0.5, 6, 10 μg/spot for TLC); (5, 30, 50 μg/ml for 1DD) represented the entire range of the calibration curves.

4.4. Accuracy

The recovery method was studied where a known amount of standard drug was added to the pharmaceutical product components and the recovered standard was calculated.

4.5. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small variations in method conditions. With respect to the composition of HPLC mobile phase, no significant influence in RT and peak area was found when acetic acid was kept in a 0.5–1% (v/v) range and flow rates at 1 ± 0.2 ml/min. Also no significant changes in Rf and peak area were found when the composition of the TLC mobile phase *iso*-propanol:ammonia 33% was tested in the ratios (7:3, v/v), (7.5:2.5, v/v) and (8:2, v/v), while significant influence was observed upon using (9:1, v/v). Therefore the proposed analytical methods are robust and remain unaffected by small variations in the mobile phase composition and over time of the methods performance with R.S.D. less than 2% Table 1.

5. Conclusion

This paper described HPLC, TLC and first-derivative of ratio spectra methods, which can be used as stability-indicating assay for the determination of piretanide in presence of its degradates and in commercial formulations without interference from tablets excipients. The advantages of the suggested methods were the ease of performance, the reproducibility and the lack of complicated pretreatments before analysis. In addition these methods have a potential for application in quality control laboratories.

References

[1] S.C. Sweetman, Martindale the Complete Drug Reference, 33th ed., Pharmaceutical Press, London, 2002, pp. 501–502.
[2] Goodman and Gilman's, The Pharmacological Basis of Therapeutics, 10th ed., 2001, p. 769 (Chapter 29).
[3] F.G. Sanchez, A.F. Gutierrez, C.C. Blanco, Anal. Chim. Acta 306 (1995) 313–321.
[4] F.G. Sanchez, A.N. Diaz, M.C. Torijas, Quim. Anal. (Barcelona) 20 (2001) 113–116.
[5] H. Spahn-Langguth, G. Hahn, E Mutschler, Arch. Pharm. 324 (1991) 445–447.
[6] S.P.D. Lalljie, M.B. Barroso, D. Steenackers, R.M. Alonso, R.M. Jimenez, P. Sandra, J. Chromatogr., B: Biomed. Appl. 688 (1997) 71–78.
[7] M. Manderscheid, T. Eichinger, J. Chromatogr. Sci. 41 (2003) 323–326.
[8] M.B. Barroso, R.M. Alonso, R.M. Jimenez, Anal. Chim. Acta 305 (1995) 332–339.
[9] W. Heptner, S. Baudner, E.E. Dagrosa, C. Hellstern, R. Irmisch, H. Strecker, H. Wissmann, J. Immunoassay 5 (1984) 13–27.
[10] European Pharmacopoeia, Council of Europe Strasbourg (EDQM), 4th ed., 2002, pp. 1770–1771.
[11] K. Matsubara, Y. Hamachi, T. Asano, T. Kuriki, N. Suzuki, Iyakuhin Kenkyu (Japan) 17 (1986) 595–603.
[12] T. Ohsaki, K. Matsubara, T. Kuriki, T. Asano, N. Suzuki, Iyakuhin Kenkyu (Japan) 17 (1986) 604–608.
[13] Hoechst Orient S.A.E., Quality Control, Egypt, 1996.
[14] A. Osol, Remington's Pharmaceutical Science, 16th ed., 1980, pp. 108–119.