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Research Paper

Light effect on the stability of β-lapachone in solution: pathways and kinetics of degradation

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Abstract

Objectives The purpose of this work was to study the chemical stability of the new antitumoral β-lapachone (βLAP) to determine the degradation pathway/s of the molecule and the degradation kinetics in addition to identifying several degradation products.

Method Samples of βLAP in solution were stored under conditions of darkness and illumination at 40°C at which the pseudo-first order rate constants for the βLAP degradation were determined. Furthermore, drug degraded solutions were concentrated and purified using Sephadex LH-20 and preparative thin-layer chromatography and degradation products were identified by nuclear magnetic resonance spectroscopy.

Key findings The results revealed that βLAP shows two different degradation routes: hydrolysis in the dark and photolysis under the light. The βLAP exposure to light accelerated the drug degradation about 140 fold, compared with the samples stored in the absence of light. The hydrolysis produced hydroxylapachol as the main degradation product. The photolysis yielded phthalic acid, 6-hydroxy-3methylene-3*H*-isobenzofuran-1-one and a benzomacrolactone together with a complex mixture of other phthalate-derivatives such as 2-(2-carboxy-acetyl)-benzoic acid.

Conclusions This study provides useful information for the development of βLAP dosage forms, their storage, manipulation and quality control.

Keywords beta-lapachone; cancer chemotherapy; chemical photostability; degradation products; preformulation

Introduction

β-Lapachone (βLAP) is a naphthoquinone obtained on a small scale from South American trees of the Bigoniaceae and Verbenaceae families.[1] On a larger scale it can be produced following the method developed by Hooker and co-workers[2] through cyclization of lapachol in sulfuric acid by an intramolecular nucleophilic attack of the oxygen at C-4 on the carbocation of the isoprenyl side chain formed, and purified by further recrystallization.

Over the last few years βLAP has been reported to possess a wide range of pharmacological properties, including antineoplastic activity, which has certainly generated greater expectations from the molecule. In-vitro and in-vivo studies have shown that βLAP inhibits conventional therapy-resistant tumours, particularly the proliferation of neoplasms of slow cell cycles, like prostate, pancreatic, colon and lung cancer and some ovarian and breast cancers.[3–9] Clinical trials on this molecule have recently been launched.[10–12]

It has been reported that βLAP is unstable under light irradiation;[13] however, as far as we know, the degradation mechanism of βLAP is not yet fully understood and the kinetics of the process have neither been established nor have the degradation products been identified, which is important for manipulating formulations and for predicting possible side effects and toxicity.

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On this basis, the objective of this work is to study the stability properties of βLAP in solution both in the dark and light under accelerated conditions, to determine the pathway/s of degradation of the molecule, identify some degradation products and establish the kinetics of the processes.

Materials and Methods

Materials

βLAP (3,4-dihydro-2,2-dimethyl-2*H*-naphthol[1,2-b]pyran-5,6-dione; $C_{15}H_{14}O_3$; MW 242.3) was supplied by the Laboratorio Farmacêutico do Estado de Pernambuco/LAFEPE (Recife, Brazil) with purity estimated by differential scanning calorimetry (DSC) and high-performance liquid chromatography (HPLC) to be 99.9%. Reference standard of β-lapachone (083K1337) and lapachol (00509KN) were purchased from Sigma-Aldrich (Steinhin, Germany). Methanol for HPLC was obtained from Merck (Darmstadt, Germany). The solutions were prepared using pure water (Millipore Milli Q Plus quantification system; Massachusetts, USA). The solvents and reagents used were of high-purity grade.

Stability of β-lapachone in solution

βLAP aqueous solutions (30 μg/ml) prepared from a stock ethanolic solution (20 mg/ml) were enclosed in glass ampoules and stored at 40 ± 2°C under the two conditions of illumination (darkness and light). Light conditions were established in accordance with option 2 of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH),[14] and were applied using a modified oven equipped with two white fluorescent lamps (Hitachi F8T5; Hitachi, Tokyo, Japan) and two emission mercury UV lamps (370 nm) (Philips 08F8T5/BLB; Philips, Eindhoven, Holland). Total brightness (1800 lx) and radiation UV (0.0078 W/m^2) were measured by a luximeter (HD9221; Delta Corps, Padua, Italy) equipped with a probe (P912151). Both lamps were switched on simultaneously. The dark conditions were achieved by wrapping the ampoules in foil and were included in the same environment.

Throughout one year, the percentage of the remaining βLAP was determined in triplicate by HPLC at different preset times. The logarithm of the percentage of the remaining βLAP against time followed a linear trend making it possible to calculate pseudo-first-order rate constants for the βLAP degradation. Prediction of the drug shelf life was calculated from the kinetics studied as the time that the drug retained 90% potency.

Purification and identification of the degradation products of βLAP

The solutions of βLAP under degradation in the presence or absence of light showed the formation of several compounds upon TLC analysis. In the case of degradation under dark conditions, the solvent was removed under reduced pressure and the corresponding residue was purified by preparative TLC using dichloromethane–methanol (9 : 1) as the eluent. In the case of degradation under light conditions, the solvent was also removed under reduced pressure and the corresponding residue was purified by Sephadex LH-20 column with hexane–dichloromethane–methanol (2 : 2 : 1) followed by preparative TLC using dichloromethane–methanol (9 : 1) as eluent.

The identification of the isolated products was carried out by proton nuclear magnetic resonance spectroscopy (^{1}H NMR), carbon-13 nuclear magnetic resonance spectroscopy (^{13}C NMR), electron ionization mass spectrometry (EIMS) and high-resolution electron ionization mass spectrometry (HREIMS) analysis and by comparison with the data published in chemical literature. ^{1}H and ^{13}C NMR spectra were recorded in deuterated chloroform ($CDCl_3$) on a Bruker instrument at 300 and 75 MHz, respectively, with tetramethylsilane (TMS) as the internal reference. High and low-resolution mass spectra were obtained on a VG Autospec spectrometer. Macherey-Nagel polygram Sil G/UV_{254} foils were used for TLC and SIL G-100UV254 foils for preparative TLC. Sephadex LH-20 was used for column chromatography.

High-performance liquid chromatography (HPLC) assay

βLAP assays were performed on a Waters M600 apparatus, equipped with a C18 cartridge column (125 mm × 45 mm × 5 μm) (Waters, Massachusetts, USA) based on a previously reported method.[15] The mobile phase consisted of methanol–water 65% (v/v). The isocratic flow rate was 1 ml/min at room temperature. The injection volume was 20 μl. Chromatographic detection was set at 253 nm with a photodiode array detector. The βLAP test concentration was 40 μg/ml with a retention time around 5 min. The mobile phase and samples were filtered using a 0.45 μm nylon membrane (Waters, USA). A system suitability test was evaluated by obtaining the chromatographic parameters; capacity factor, number of the theoretical plates and tailing factor.[16]

The validation of the analytical method was carried out according to the ICH Q2.[17] Linearity in the range of 20–60 μm/ml was established (A = 112 967.C – 13 944; correlation coefficient r = 0.9991 with degrees of freedom = 14, F = 7078.5, $\alpha > 0.01$). Precision and accuracy were studied, the values being within the acceptable USP limits. The detection limit and quantification limit for the βLAP assay were 0.04 and 0.07 μg/ml respectively

To obtain complete drug decomposition for validation purposes and for purification of the βLAP degradation products, samples of βLAP solution (30 μg/ml) were placed in an oven at 50°C in light and darkness conditions until complete decomposition. The selectivity of the analytical method for βLAP against its photo and thermal degradation products was tested.

Results and Discussion

βLAP stability in solution

Saturated aqueous solutions of βLAP present an intense orange colour related to the naphthoquinone structure, which progressively became colourless as a consequence of exposure to the light. However, the samples stored in darkness became red.

The chromatograms of βLAP solutions before storage and after storage in light and darkness are presented in Figure 1. βLAP eluted at around 5 min under the HPLC conditions selected in this study (Figure 1b). Irradiated βLAP solution

chromatograph (Figure 1a) showed the appearance of multiple peaks in the range of 0.9–3.8 min and the disappearance of the βLAP peak. The chromatograph did not maintain the βLAP peak in the sample stored in the dark (Figure 2c) and presented two new earlier peaks at 0.9 and 1.6 min.

Table 1 summarizes data of the remaining drug during the experiment under light and dark conditions as well as the degradation kinetic results. Drug shelf-life in the dark was 50 days but only 0.4 days if the drug is stored under light conditions. Light accelerated βLAP degradation about 140 fold, confirming the photo instability of this drug, as described in the literature.[13]

These facts, together with the βLAP naphthoquinone structure, suggest the existence of two different degradation routes: hydrolysis in the dark, and photolysis in the light.

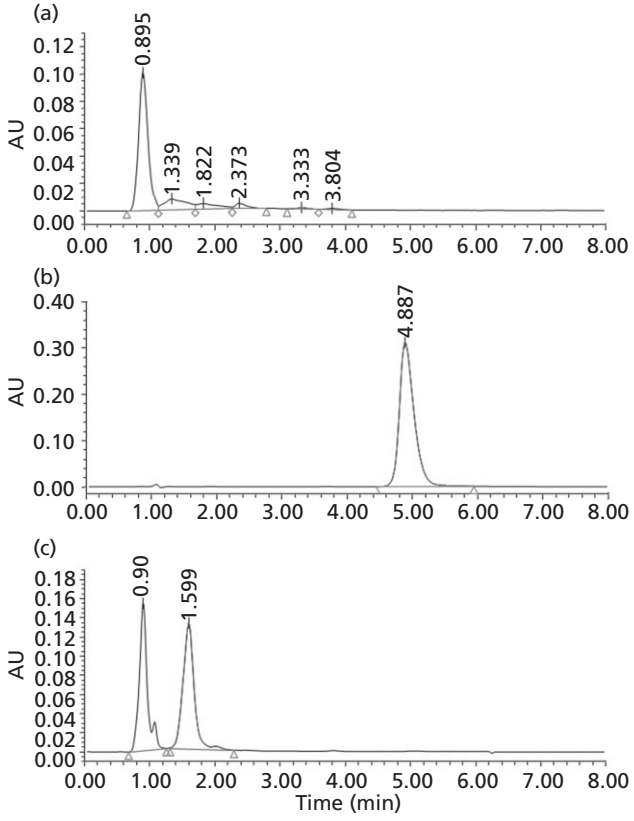

Figure 1 HPLC chromatograms of β-lapachone solutions before storage (b) and in completely degraded solutions after storage in light (a) and darkness (c).

Purification and identification of the degradation products of βLAP

Aiming to investigate the degradation processes and identify the main degradation products, we studied the crude materials obtained after elimination of the solvent of the corresponding solutions under light and dark conditions (Figure 2).

A dry sample of 71 mg stored in the dark was chromatographed on two preparative-TLC systems with dichloromethane–methanol (9 : 1). Two products were detected but just the major one could be isolated as an amorphous red wine solid (31 mg) and its molecular formula was identified by high-resolution mass spectrometry (HRMS) as $C_{15}H_{16}O_4$. The main differences in the ^{1}H NMR spectrum, with respect to βLAP, were the chemical shifts of the aromatic and the aliphatic hydrogens. The ^{13}C NMR spectrum revealed the presence of a 1,4-naphthoquinone system instead of 1,2-naphthoquinone moiety, as appears in βLAP. All these data suggested that the product isolated was hydroxylapachol (Figure 2, compound **2**) which has been previously obtained by synthesis from 2-hydroxy-1,4-naphthoquinone[18] or from 2-hydroxy-3-(3-oxobutyl)-1,4-naphthoquinone by reductive acetylation and treatment with methyl magnesium iodide.[19]

Compound **2** has been identified as the only unconjugated metabolite of βLAP in hepatocytes, a major metabolite in dogs and a minor one in rats and humans.[20] This compound showed low activity against carcinoma walker 256.[21]

13-Hydroxy-lapachol (2-hydroxy-3-(3-hydroxy-3-methylbutyl)-naphthalene-1,4dione): ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.05 (bs, 2H, H-5 + H-8), 7.69 (bs, 1H, H-6), 7.63 (bs, 1H, H-7), 2.67 (bs, 2H, CH_2-11), 1.68 (bs, 2H, CH_2-12), 1.29

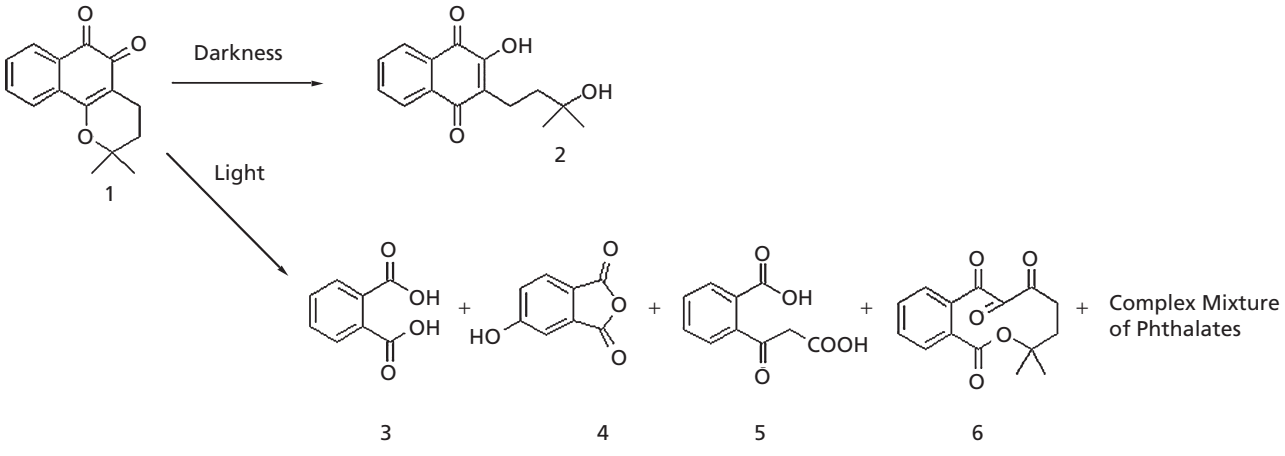

Figure 2 Degradation routes of β-lapachone (**1**) under light and dark conditions. **2**, Hydroxylapachol; **3**, phthalic acid; **4**, 6-hydroxy-3methylene-3*H*-isobenzofuran-1-one; **5**, (2-carboxy-acetyl)-benzoic acid; **6**, benzomacrolactone.

Table 1 Kinetics of β-lapachone in aqueous solution degradation at 40°C in light and dark conditions

Light		Darkness	
Time (days)	Remaining drug % (standard deviation)	Time (days)	Remaining drug % (standard deviation)
0	100.0 (0.0)	0	100.0 (0.0)
3	56.7 (3.5)	14	99.5 (0.1)
4	43.2 (0.2)	32	95,6 (0.3)
5	27.7 (2.1)	80	87.0 (0.2)
6	23.7 (2.0)	105	84.2 (1.2)
7	18.9 (1.3)	166	73.8 (3.5)
8	12.1 (1.2)	250	60.4 (0.1)
10	6.2 (1.1)	320	51.8 (0.1)
K^a (10^{-3}/day)	280.0	K^a (10^{-3}/day)	2.1
r^b	0.9908	r^b	0.9979
Shelf-life (days)	0.4	Shelf-life (days)	50.0

[a]Pseudo-first-order degradation rate constant. [b]Correlation coefficient

(s, 6H, CH_3-14 and 15). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 184.7 (C, C-4), 181.4 (C, C-1), 153.1 (C, C-2), 134.8 (CH, C-6), 132.8 (C, C-10), 132.8 (CH, C-7), 129.5 (C, C-9), 126.6 (CH, C-5), 126.1 (CH, C-8), 124.7 (C, C-3), 71.0 (C, C-13), 41.5 (CH_2, C-12), 29.1x2 (CH_3, C-14 and 15), 18.3 (CH_2, C-11). EIMS: 260 (1, M^+), 245 (11, M^+-Me), 242 (24, M^+-H_2O), 227 (100, M^+-Me-H_2O). HREIMS: 260.1055 ($C_{15}H_{16}O_4$ calcd. 260.1049).

The solution of βLAP under light degradation conditions was concentrated by the elimination of the solvent. The ^1H NMR spectrum of the resulting crude material showed the existence of a complex mixture of phthalates. A dry sample of 48 mg was first purified by a Sephadex LH-20 column. The different fractions obtained were chromatographed on preparative TLC. Four compounds were identified together with several inseparable mixtures of products (Figure 2, compounds **3**, **4**, **5** and **6**). Three showed a retention factor (Rf) in TLC lower than 0.1 using dichloromethane–methanol (9 : 1). This indicated the presence of polar functional groups in the corresponding structures. The first one showed a molecular formula of $C_8H_5O_4$ in HRMS and two aromatic signals from the ^1H NMR and ^{13}C NMR spectra. The analysis of these data revealed that the isolated product was phthalic acid (Figure 2, compound **3**). The other compound (Figure 2, compound **4**) showed a similar EIMS spectrum to phthalic acid but it presented significant differences in the ^1H NMR and the ^{13}C NMR spectra. The most significant difference was the presence of a three-substituted aromatic ring (δ 7.97 (d, 1H, 8.6 Hz), 7.38 (d, 1H, 2.6 Hz), 6.85 (dd, 1H, 8.6 and 2.6 Hz)). From these NMR data and the existence of a molecular ion peak at *m/z* 164 in the EIMS spectra with the molecular formula $C_8H_4O_4$ established by HREIMS, this product was identified as 6-hydroxy-3methylene-3*H*-isobenzofuran-1-one.[22] Another compound isolated at a lower amount was (2-carboxy-acetyl)-benzoic acid[23] (compound **5**), which was obtained as a mixture together with compound **4**. The formation of these phthalic derivatives can be explained on the basis of an oxidative photolysis of βLAP. This process starts with the oxidative cleavage of the C2-C3 bond to obtain a phthalic-type intermediate, which suffers different radical decarboxylations to yield the other products. Similar compounds have been proposed to explain the Hooker's oxidation mechanism for 1,4-naphthoquinones.[24–26]

6-Hydroxy-3methylene-3*H*-isobenzofuran-1-one: ^1H NMR (MeOD, 300 MHz) δ: 7.97 (d, 1H, 8.6 Hz), 7.38 (d, 1H, 2.6 Hz), 6.85 (dd, 1H, 8.6 and 2.6 Hz). ^{13}C NMR (MeOD, 75 MHz) δ: 160.1 (C), 134.2 (CH), 131.4 (C), 130.4 (C), 119.16 (CH), 117.4 (CH). EIMS: 164 (65, M^+), 119 (100) 103 (4) 91.(83) HREIMS: 164.0111 ($C_8H_4O_4$ calcd. 164.0110).

The ^1H NMR spectrum of the fourth product (Figure 2 compound **6**) showed four signals corresponding to four aromatic hydrogens of a 1,2-disubstituted aromatic system, several complex signals for benzyl CH_2 and aliphatic CH_2, and two methyl groups. The ^{13}C NMR analysis confirmed the presence of the 1,2-disubstituted aromatic ring and revealed the existence of various carbonyl groups. It showed a molecular formula of $C_{15}H_{14}O_5$, which seems to correspond with a benzomacrolactone as one of the less polar intermediates in the degradation of βLAP under light conditions.

Benzomacrolactone: ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.22 (m, 1H), 8.05 (m, 1H), 7.81 (m, 2H), 3.03 (m, 1H), 2.29 (m, 2H), 2.01 (m, 1H), 1.46 (s, CH_3) 1.23 (s,CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 197.1 (C), 192.6 (C), 187.3 (C), 182.3 (C), 134.9 (CH), 134.2 (CH), 131.4 (C), 130.1 (CH), 128.7 (C), 126.4 (CH), 88.3 (C), 36.4 (CH_2), 32.6 (CH_2), 29.0 (CH_3), 27.4 (CH_3). EIMS: 260 (1, M^+-CH_2), 230 (9, M^+-CO), 189 (10) 104 149 (20) 132 (63) 104 (100) 76 (25) HREIMS: 260.1065 ($C_{15}H_{16}O_4$, M^+-CH_2, calcd 260.1049), 230.0944 ($C_{14}H_{14}O_3$, M^+-CO, calcd. 230.0943).

Isolated degradation products were dissolved in distilled water and qualitatively analysed by HPLC using the same conditions as previously described. Chromatograms of compounds **2**, **3**, **4**, **5** and **6** presented main peaks at shorter retention times (0.9–3.8 min) than βLAP (4.9 min) suggesting that the degradation products did not interfere on βLAP resolution using the selected HPLC method. Moreover, UV spectra profiles of main peak of the compounds **2**, **4** and **6** matched their corresponding peaks of the chromatograms of completely degraded solutions after storage in light (Figure 1a) and darkness (Figure 1c). Compound **3** is probably overlapped and cannot be distinguished under the conditions of the study.

Conclusions

βLAP in solution showed two different degradation routes: hydrolysis in the dark and photolysis in light. Hydrolysis produced, as the main degradation product, hydroxylapachol (2-hydroxy-3-(3-hydroxy-3-methyl-butyl)-[1,4] naphthoquinone). Photolysis yielded phthalic acid, 6-hydroxy-3methylene-3*H*-isobenzofuran-1-one, 2-(2-carboxy-acetyl)-benzoic acid and a benzomacrolactone, together with a complex mixture of other phthalate derivatives.

Light highly accelerates βLAP degradation in comparison with dark conditions. These results provide useful information for establishing the storage, manipulation and quality control conditions for this drug.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Burnett AR, Thomson RH. Naturally occurring quinones. XII. Extractives from *Tabebuia chrysantha* and other Bignoniaceae. *J Chem Soc C* 1968; 850–853.
2. Hooker SC *et al*. Constitution of lapachol and its derivatives. *J Am Chem Soc* 1936; 58: 1190–1197.
3. Li CJ *et al*. β-Lapachone, a novel DNA topoisomerase I inhibitor with a mode of action different from camptothecin. *J Biol Chem* 1993; 268: 22463–22468.
4. Li CJ *et al*. Potent inhibition of tumor survival in vivo by β-lapachone plus taxol: combining drugs imposes different artificial checkpoints. *Proc Natl Acad Sci USA* 1999; 96: 13369–13374.
5. Li Y *et al*. Selective killing of cancer cell by β-lapachone: direct checkpoint activation as a strategy against cancer. *Proc Nat Acad Sci USA* 2003; 100: 2674–2678.
6. Pink JJ *et al*. NAD(P)H: quinone oxidoreductase activity is the principal determinant of β-lapachone cytotoxicity. *J Biol Chem* 2000; 275: 5416–5424.
7. Boorstein RJ, Pardee AB. Coordinate inhibition of DNA synthesis and thymidylate synthase activity following DNA damage and repair. *Biochem Giophys Res Commun* 1983; 117: 30–36.
8. Dong Y *et al*. Intratumoral delivery of β-lapachone via polymer implants for prostate cancer therapy. *Clin Cancer Res* 2009; 15: 131–139.
9. Blanco E *et al*. β-lapachone micellar nanotherapeutics for non–small cell lung cancer therapy. *Cancer Res* 2010; 70: 3896–3904.
10. Pardee AB *et al*. Cancer therapy with β-lapachone. *Curr Cancer Drug Targets* 2002; 2: 227–242.
11. Khong HT *et al*. A phase 2 study of ARQ 501 in combination with gemcitabine in adult patients with treatment naïve unresectable pancreatic adenocarcinoma. *J Clin Oncol* 2007; 25: 15017.
12. Hartner LP *et al*. Phase 2 dose multi-center, open-label study of ARQ 501, a checkpoint activator, in adult patients with persistent, recurrent or metastatic leiomyosarcoma (LMS). *J Clin Oncol* 2007; 25: 20521.
13. Glen VL *et al*. Quantitation of β-lapachone and 3-hydroxy-β-lapachone in human plasma samples by reversed-phase high performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 1997; 692: 181–186.
14. ICH Q1B. Photostability testing of new drug substances and products. International Conference on Harmonization, 1996.
15. Soares da Cunha Filho MS *et al*. Beta-lapachone: development and validation of analytical method for the new therapeutic antineoplastic alternative. *Rev Bras Farm* 2005; 86: 39–43.
16. USP 29. *The United States Pharmacopoeia*, 29th edn. Rockville, MD: United States Pharmacopoeial Convention, 2006.
17. ICH Q2 (R1). Validation of analytical procedures: text and methodology. International Conference on Harmonization, 2005.
18. Pettit GR, Houghton LE. Synthesis of hydroxyhydrolapachol and lapachol. *J Chem Soc* 1971; 3: 509–511.
19. Cassis R *et al*. Studies on quinones. IV Synthesis of hydroxylapachol and related products. *Anales Química* 1977; 73: 1512–1513.
20. Miao XS *et al*. *In vitro* metabolism of β-lapachone (ARQ 501) in mammalian hepatocytes and cultured human cells. *Rapid Commn Mass Spectrom* 2009; 23: 12–22.
21. Subramanian S, Ferreira MMC. A Structure-activity relationship study of lapachol and some derivatives of 1,4-naphthoquinones against carcinosarcoma Walker 256. *Struct Chem* 1998; 9: 47–57.
22. Leblois D *et al*. Pyrophthalones. VII. Synthesis and anti-inflammatory activity of 2-(4-dihydropyridinylidene)indan-1,3-diones diversely substituted on the benzene and/or heterocyclic ring. *Eur J Med Chem* 1987; 22: 229–238.
23. Grotzinger E, Campbell IM. 4-(2′-Carboxyphenyl)-4-oxobutyrate. Obligatory intermediate in lawsone biosynthesis. *Phytochemistry* 1974; 13: 923–926.
24. Hooker SC. On the oxidation of 2-hydroxy-1,4-naphthoquinone derivatives alkaline potassium permanganate. *J Am Chem Soc* 1936; 58: 1174–1179.
25. Hooker SC, Steyermark A. On the oxidation of 2-hydroxy-1,4-naphthoquinone derivatives with alkaline potassium permanganate. Part II. Compounds with unsaturated side chains. *J Am Chem Soc* 1936; 58: 1179–1181.
26. Fieser IF, Fieser M. Naphthoquinones antimalarials XII. The Hooker oxidation reaction. *J Am Chem Soc* 1948; 70: 3214–3222.