Validation of analytical method by HPLC for determination of dapsone in polymeric nanocapsules based on crude rice brain oil

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ABSTRACT

Dapsone is a sulfone used for the treatment of skin diseases such as psoriasis. Its clinical use by the oral route is limited. For counteract this hindrance, innovative systems based on polymeric nanocapsules containing rice bran oil and dapsone have been developed. Thus, an analytical method using HPLC for quantification of dapsone in polymeric nanocapsules based on rice brain oil was developed and validated. The separation was achieved on a C18 reverse phase column (Phenomenex, 4.6×150 mm, 5 μm) using a mobile phase composed of acetonitrile–1.5 % acetic acid (25:75 v/v) at a flow rate of 0.7 mL.min^{-1}. The injection volume was 20 μL and the wavelength of detection was 230 nm. The method was linear in the range of 5-25 μg.mL^{-1}, with a correlation coefficient of 0.9999. Precision and accuracy analysis showed low relative standard deviation (lower than 2) and recovery percentage in the range of 99.72 − 106.25%. The limits of detection and quantification were 0.41 and 1.24 μg.mL^{-1}, respectively. The analytical method was specific, linear, precise and exact, showing that this procedure can be applied in quantification of dapsone-loaded polymeric nanocapsules based on rice brain oil.

INTRODUCTION

Dapsone (4,4'diaminodiphenylsulfone, $C_{12}H_{12}N_2O_2S$) (Figure 1), molecular weight of 248.31 g.mol-1 , is a white, crystalline and odorless powder. It is sparingly soluble in water, easily soluble in acetone and lightly soluble in ethanol and methanol (Wozel *et al*., 2010).
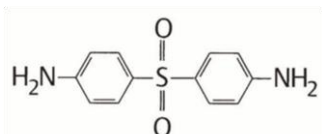

Fig. 1: Chemical structure of dapsone

This drug belongs to the class of sulfones, being the drug of choice in the treatment of leprosy due to its antibiotic activity. Dapsone is also used for other chronic skin diseases such as psoriasis that is a chronic inflammatory disease that has a predilection for skin and joints (Zhu and Stiller, 2001). When used orally, dapsone presents some undesirable effects such as hemolytic anemia and methemoglobinemia (Wozel *et al*., 2010). Due to the high incidence of side effects, the development of a topical formulation of the drug becomes interesting, however, the clinical efficacy of a drug, including dapsone, administered topically is limited since the most of drugs have unsuitable physico-chemical characteristics for overcoming the skin barriers, in particular the stratum corneum (Schafer-Korting *et al*., 2007).

Thus, the development of carrier systems in order to improving the therapeutic effect of drugs administered by the topical route is an interesting alternative. Among these systems, the polymeric nanoparticles are noteworthy. Polymeric nanoparticles are vesicular or matricial colloids based on polymers. These vesicular carriers constituted of an oil core surrounded by a polymeric wall are named nanocapsules (Bernardi *et al*., 2011).

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In this work, dapsone loaded-nanocapsules were developed using crude rice bran oil as the oily core and poly (ε-caprolactone) as the polymer. Unsaponifiable matter of crude rice bran oil contains high levels of components with antioxidant properties, such as tocopherols, tocotrienols and gamma-oryzanol. Besides, the crude rice bran oil presents moisturizing, humectants and UV protection properties. Thus, it is a multifunctional component for the formulation of cosmetic and dermatological products (Juliano *et al.*, 2005), including the innovative dapsone-loaded polymeric nanocapsules intended for the treatment of psoriasis. Several methods for the quantification of dapsone have been related in the literature. These studies were focused on identification of degradation products (Bardsley *et al.*, 2011), evaluation of stability (Nahata *et al.*, 2000), quantification of dapsone and its metabolites in muscle, plasma (Kwadijk and Torano, 2002) and urine (Abuirjeie *et al.*, 1991) in biological evaluation of the drug in humans or animals (Hela *et al.*, 2003). Apart of these, a method for quantification of dapsone in theospheres, a special lipid nanoparticulated system, has been developed (Santos *et al.*, 2012). The drug extraction from nano-particles for quantification purposes is extremely difficult, and it requires the use of different solvents that allow the disruption of the carrier and the extraction of the nanoencapsulated substance, take into account the specific characteristics of the carrier system. Therefore, the development of a simple, fast and accurate method for the extraction and quantification of dapsone from this particular kind of nanocapsules, based on rice bran oil is highly desirable due to complexity of this system that include polymer, surfactants and a vegetal component. Thus, this study aimed to develop and validate an analytical method for quantification of dapsone in polymeric nanocapsules based on crude rice brain oil by high performance liquid chromatography (HPLC).

MATERIAL AND METHODS

Dapsone (99.7%, w/w) was obtained from Fragon (Brazil). Poly-(ε-caprolactone) (PCL) (MW=10.000 g.mol-1) and sorbitan monooleate (Span® 60) was purchased from Sigma Aldrich (Brazil). Polysorbate 80 (Tween 80®) was supplied by Delaware (Brazil). Crude rice bran oil was kindly donated by Camil foods (Brazil). HPLC-grade acetonitrile was acquired from Tedia (Brazil), acetic acid was adquired from Vetec (Brazil). All other solvents and reagents were analytical grade and used as received.

Chromatographic equipment and conditions

The analyses were carried out on a Proeminence® (Shimadzu) high performance liquid chromatograph equipped with binary pump and diode-array detector.

The separation was achieved on a C18 reverse phase column (Phenomenex, 4.6×150 mm, 5 μm) using a mobile phase composed of acetonitrile–1.5 % acetic acid (25:75 v/v) at a flow rate of 0.7 mL.min^{-1}. The injection volume was 20 μL and the wavelength of detection was 230 nm.

Preparation and characterization of dapsone-loaded nanocapsules

The nanocapsules were prepared by interfacial deposition method of the preformed polymer (Jager *et al.*, 2009). The organic phase, consisting of acetone (30 mL), dapsone (0.01 g), PCL (0.1 g), sorbitan monostearate (0.077 g) and crude rice bran oil (0.15 g), was poured into the aqueous phase (50 mL) containing polysorbate 80 (0.077 g), under moderate magnetic stirring. After 10 min acetone was eliminated and the suspension was concentrated under reduced pressure. Empty nanocapsules (without dapsone) were prepared and used as a control.

The nanoparticles were characterized in terms of mean diameter and size distribution by laser diffractometry (Mastersizer® 2000, Malvern Instruments). In the drug content evaluation, stock solution of dapsone (1000 μg.mL^{-1}) was prepared by dissolving accurately weighed 10 mg of the drug in acetonitrile using 10 mL volumetric flask. Standard solution was prepared by dilution of the diluted stock solution with mobile phase to obtain solutions in a final concentration of 15 μg.mL^{-1}. To prepare the sample solutions, 0.15 mL of nanocapsule suspension (1 mg.mL^{-1} of dapsone) was transferred into a 10 mL volumetric flask, with 10 mL of acetonitrile, obtaining the final concentration of 15 μg.mL^{-1}. This flask was kept in an ultrasonic bath for 30 min, causing partial dissolution of the polymer and release of the active contained into the nanostructure. The solutions were filtered through a 0.45 μm membrane filter and analyzed by HPLC, determining the total concentration of dapsone. Free drug was also determined by HPLC in the ultrafiltrate after the separation of the nanoparticles by an ultrafiltration-centrifugation technique (Ultrafree-MC® 10,000MW, Millipore), at 12000 rpm for 5 min. In this case, the ultrafiltrate was directly injected in HPLC. Encapsulation efficiency (%) was calculated by the difference between the total and free drug concentrations. In addition, the pH value of the nanocapsule suspension was determined.

Validation of analytical method

The validation of the analytical method was performed according to the International Conference on Harmonization Guidelines (ICH) (ICH, 2005) by validation of analytical procedures. The parameters evaluated in the development of this work were: specificity, linearity, intermediate precision, repeatability, accuracy, detection and quantification limits. The specificity of the method was evaluated through comparative analyzes of the suspensions of empty nanocapsules (without dapsone) and the suspensions containing the drug.

To study the linearity,calibration curves (n=3) (area as a function of dapsone concentration) were obtained. Stock solution of dapsone (1000 μg.mL^{-1}) was prepared by dissolving accurately weighed 10 mg of the drug in acetonitrile using 10 mL volumetric flask. Samples solution was prepared by dilution of the diluted stock solution with mobile phase to obtain solutions in a final concentration 5, 10, 15, 20 and 25 μg.mL^{-1}. Precision was assessed by the analysis of dapsone-loaded nanocapsule suspension (n=6) in one single day (repeatability) and through analysis of samples in

three different days (intermediate precision). For this, 0.15 mL of nanocapsule suspension (1 mg.mL^{-1} of dapsone) was transferred into a 10 mL volumetric flask, with 10 mL of acetonitrile, obtaining the final concentration of 15 μg.mL^{-1}. This flask was kept in an ultrasonic bath for 30 min. The samples were filtered through a 0.45 μm membrane filter and analyzed by HPLC. The accuracy was determined in three different concentrations (low, intermediate and high). A standard solution of dapsone was prepared (1000 mg.mL^{-1}) and aliquots of 50 μL, 150 μL, and 250 μL of this solution were transferred to a 10 mL volumetric flask containing 100 μL of empty nanocapsule suspension (without drug) and acetonitrile was used to reach the final volume. Thus, the evaluated concentrations were 5 μg.mL^{-1}, 15 μg.ml^{-1} and 25 μgml^{-1} performed in triplicate. The limits of detection (LOD) and quantitation (LOQ) were calculated using the calibration line directly. The factors (3.3 and 10, respectively) were multiplied by the ratio from the residual standard deviation and the slope of the standard curve, according to the guidelines (ICH, 2005).

RESULTS AND DISCUSSION

The dapsone-loaded nanocapsules prepared using crude rice bran oil presented aspect similar to a milky bluish opalescent fluid. The empty nanocapsules presented mean diameter of 167±0.1 nm and the presence of dapsone did not change the particle size which was 168 ± 0.1 nm. Size distributions showed monomodal profiles with Span values of 1.66 and 1.69 for empty and dapsone-loaded nanocapsules, indicating narrow size distributions for both suspensions. The pH values of the suspensions were close to 6.0. The dapsone-loaded nanocapsule presented drug content close to their theoretical value (1 mg.mL^{-1}) and the drug encapsulation efficiency was 83.2%.

Regarding the validation of analytical method, Figure 2 shows the chromatogram of the nanocapsules suspensions of dapsone and the chromatogram of the suspensions without the drug. Prior the analysis, the sample of empty nano capsule was processed in the same manner as nanocapsules containing dapsone, using the same solvent (acetonitrile). As shown in the Figure 2, there was no interference in the chromatogram for the evaluation of the drug in the nanocapsule formulations. Thus, the analytical method presented is specific to quantify dapsone in nanocapsule based on rice brain oil, which is consistent with the official specifications. In linearity assay, the straight line equation (y = 106073.99x + 40990.53) was obtained by linear regression studies between the concentration of dapsone and their respective responses, yielding a coefficient of determination of 0.9999, which is characterized linear and suitable for such use over the 5-25 μg.mL^{-1} range. The slope and intercept (± SD, n = 3) were 40990.53 ± 312.04 and 106073.99 ± 6007.53, respectively. The ANOVA results showed that the regression equation was linear ($F_{calculated}$ = 1.4.10^4 > $F_{critical}$ = 4.96; P = 0.05) with no deviation from linearity ($F_{calculated}$ = 2.05 < $F_{critical}$ = 3.71; P = 0.05). The values obtained for the LOD and LOQ were 0.41 and 1.24 μg.mL^{-1}, respectively, indicating a good sensitivity of the method for determining of dapsone. From the results obtained in the development of the method, it is concluded that the calibration curve can be used for quantification of experimental values of dapsone, whereas the determination coefficient was higher than 0.99, confirming the quality of the obtained calibration curve (ICH, 2005). In the performed intra-day tests the values were between 75.02 and 78.18% with RSD of 1.68 on the first day of the analysis. Already on the second day of the test, recovery values were between 77.25 and 78.74% with RSD of 1.00. Finally, on the third day of the test, the values were between 77.73 and 79.95% with RSD of 1.54. Thus, the parameter has been confirmed by accurate alignment of the different results obtained for the same sample. Regarding the accuracy test, the results obtained are shown in Table 1. Values of recovery percentages were satisfactory, since it remained between 99.72 and 106.25%. Thus, the method can be considered accurate for quantification of dapsone in polymeric nanocapsules based on crude rice brain oil.
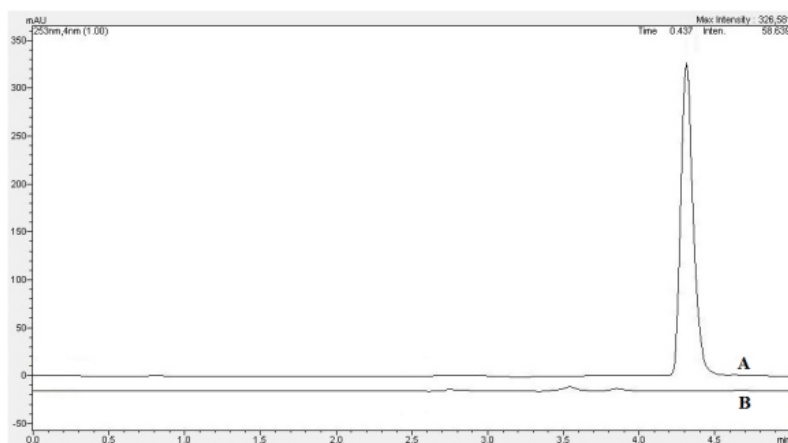

Fig. 2: Comparison of chromatograms obtained from the analysis of the dapsone-loaded nanocapsules (A) and empty nanocapules (B).

Table 1: Experimental values of accuracy of the method for dapsone quantification in polymeric nanocapsules.

Theoretical Concentration (μg mL^{-1})	Recovered Concentration (μg mL^{-1})	Recovery (%)
5	5.12	99.72
15	15.75	105.6
25	26.25	106.25

CONCLUSION

The analytical method for the detection and quantification of the dapsone was linear, accurate, precise and specific in the concentration range from 5 to 25 $\mu g.mL^{-1}$. The obtained limits of quantification and detection indicated that the method is effective to quantify the dapsone for a minimum concentration of 1.24 $\mu g.mL^{-1}$. Thus, the validated method was suitable for quantification of dapsone in polymeric nanocapsules based on crude rice brain oil.

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Conflict of Interests: There are no conflicts of interest.

REFERENCES

Abuirjeie MA, Irshaid YM, al-Hadidi HF, Rawashdeh NM. Simultaneous high performance liquid chromatographic determination of dapsone and monoacetyldapsone in human plasma and urine. J Clin Pharm Ther, 1991; 16:247-255.

Bardsley B, Barry SJ, Drozdz MA, Hancock SA, Okafo GN, Szafran, MM. Formation and identification of a degradant in chlorproguanil-dapsone-artesunate (Dacart) tablets. J Pharmaceut Biomed, 2011; 54:610-613.

Bernardi DS, Pereira TA, Maciel NR, Bortoloto J, Viera GS, Oliveira GC, Rocha-FilhoPA. Formation and stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments. J Nanobiotechnolo*gy*, 2011;9, 44.

Hela W, Brandtner M, Widek R, Schuh R. Determination of sulfonamides in animal tissues using cation exchange reversed phase sorbent for sample cleanup and HPLC DAD for detection. Food Chem, 2003; 83: 601-608.

ICH 2005. Harmonised Tripartite Guideline; Validation of Analytical Procedures: Text and Methodology. Geneva: IFPMA.

Jager E, Venturini CG, Poletto FS, Colome LM, Pohlmann JP, Bernardi A, Battastini AM, Guterres SS, Pohlmann AR. Sustained release from lipid-core nanocapsules by varying the core viscosity and the particle surface area. J Biomed Nanotechnol, 2009; 5:130-140.

Juliano C, Cossu M, Alamanni MC, Piu L. Antioxidant activity of gamma-oryzanol: mechanism of action and its effect on oxidative stability of pharmaceutical oils. Int J Pharmaceut, 2005; 299: 146-154.

Kwadijk S, Torano JS. High-performance liquid chromatographic method with ultraviolet detection for the determination of dapsone and its hydroxylated metabolite in human plasma.Biomed Chromatogr : BMC,2002; 16: 203-208.

Nahata MC, Morosco RS, Trowbridge JM. Stability of dapsone in two oral liquid dosage forms. ***Pharmacother Ann***, 2000; 34: 848-850.

Santos GS, Pereira GG, Bender EA, Colomé LM, Guterres SS. Desenvolvimento e caracterização de nanopartículas lipídicas destinadas à aplicação tópica da dapsona. Quim Nova, 2012; 35(7): 1388-1394, 2012

Schafer-Korting M, Mehnert W, Korting HC.Lipid nanoparticles for improved topical application of drugs for skin diseases. Adv Drug Deliver Rev, 2007; 59: 427-443.

Siqueira NM, Contri RV, Paese K, Beck RC, Pohlmann AR, Guterres SS. Innovative sunscreen formulation based on benzophenone-3-loaded chitosan-coated polymeric nanocapsules. Skin Pharmacol Phys, 2011; 24: 166-174.

Wozel G, Sticherling M, Schon MP. Cutaneous side effects of inhibition of VEGF signal transduction. J Dtsch Dermatol Ges : JDDG, 2010; 8: 243-249.

Zhu YI, Stiller MJ. Dapsone and sulfones in dermatology: overview and update. J Am Acad Dermatol, 2001; 45: 420-434.

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