ANALYTICAL VALIDATION AND THE INFLUENCE OF NANOENCAPSULATION IN A FORCED DEGRADATION STUDY OF DIHYDROMYRICETIN[1]

VALIDAÇÃO ANALITICA E A INFLUÊNCIA DA NANOENCAPSULAÇÃO EM ESTUDO DE DEGRADAÇÃO FORÇADA DE DIHIDROMIRICETINA

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ABSTRACT

A simple and rapid high-performance liquid chromatographic method was developed and validated for the determination of dihydromyricetin (DMY) in nanocapsule suspensions. The suspensions containing DMY showed mean particle diameter of 161 nm, polydispersity index less than 0.100, positive zeta potential between 11 and 14 mV, pH about 5.0, drug content of 100% and encapsulation efficiency of 80%. The method is carried out an RP-18 column with a mobile phase composed of acetonitrile-water (80:20 v/v) and a photodiode array detector at 290 nm. The method validation yields adequate results on linearity, specificity, precision, accuracy, and robustness. The samples (free and nanoencapsulated DMY) were subjected to following degradative conditions: acidic, alkaline, oxidative, thermal, and photolytic. Free DMY was most sensitive to alkaline degradation followed by oxidation, photolysis, thermal degradation and acidic hydrolysis. DMY nanocapsules suspensions were able to resist the thermal and photolytic degradation and reduced the extent of oxidative, alkaline and acidic hydrolysis. In conclusion, this method is suitable for measuring the DMY content of polymeric nanocapsules, it avoids the use of a buffer solution in the mobile phase, and it has a short retention time. Furthermore, this study showed for the first time that DMY can be protected from degradation by nanoencapsulation in polymeric nanocapsules. These results suggest that the use of DMY nanocapsule formulations have a great potential, and its use appears to be a promising strategy.

Keywords: liquid chromatography, nanocapsule, polymeric nanoparticles, stability-indicating.

RESUMO

Um método simples e rápido de cromatografia líquida de alta eficiência foi desenvolvido e validado para a determinação de dihidromiricetina (DMY) em suspensões de nanocápsulas. As suspensões contendo DMY apresentaram diâmetro médio de partícula de 161 nm, índice de polidispersão inferior a 0,100, potencial zeta positivo entre 11 e 14 mV, pH próximo de 5,0, teor de fámaco de 100% e eficiência de encapsulação de 80%. O método é realizado em uma coluna RP-18 com fase móvel composta por acetonitrila-água (80:20 v/v) e detector de arranjo fotodiodo a 290 nm. A validação do método produz resultados adequados em linearidade, especificidade, precisão, exatidão e robustez. As amostras (DMY livre e nanoencapsulada) foram submetidas às seguintes condições de degradação: ácida, alcalina, oxidativa, térmica e fotolítica. A DMY livre foi mais sensível à degradação alcalina seguida de oxidação, fotólise, degradação térmica e hidrólise ácida. As suspensões de nanocápsulas


DMY foram capazes de resistir à degradação térmica e fotolítica e reduziram a extensão da hidrólise oxidativa, alcalina e ácida. Em conclusão, este método é adequado para medir o teor de DMY em nanocápsulas poliméricas, evitando o uso de uma solução tampão na fase móvel e com um tempo de retenção curto. Além disso, este estudo mostrou pela primeira vez que a DMY pode ser protegida da degradação por nanoencapsulação em nanocápsulas poliméricas. Estes resultados sugerem que o uso de formulações de nanocápsulas DMY tem um grande potencial, e seu uso parece ser uma estratégia promissora.

Palavras-chave: *cromatografia líquida, nanocápsulas, nanopartículas poliméricas, indicativo de estabilidade.*

INTRODUCTION

Dihydromyricetin (DMY, Figure 1), is a flavonoid and is the main bioactive compound extracted from Ampelopsis grossedentata, a vine from the south of China (ZHANG et al., 2007; XIONG; ZHU; LIU et al., 2009; KOU; CHEN, 2012). The DMY content of the plant's leaves is about 20-30%, which has led to their widespread use as a health tea in China (LIU et al., 2009).

Figure 1 - Chemical structure of Dihydromyricetin (3,5,7,3`,4`,5`-6 hydroxy-2,3-dihydro).
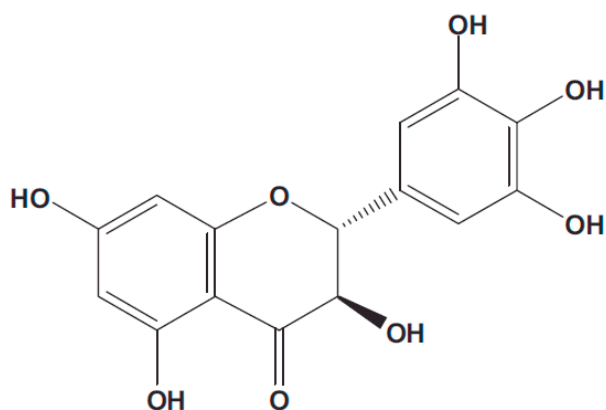
Source: Image drawn in the ChemGraph software.

DMY has many pharmacological functions, such as antibacterial (DALCIN et al., 2017) antioxidant (MA et al., 2004; SONG et al., 2017), antitumor (ZHENG; LIU, 2003; KOU; CHEN, 2012), antihypertensive (KOU; CHEN, 2012), hypoglycemic (ZHONG et al., 2002; KOU; CHEN, 2012), anti-inflammatory (KOU; CHEN, 2012; QI et al., 2012), hepatoprotective (MURAKAMI et al., 2004; KOU; CHEN, 2012), and neuroprotective effects (YE et al., 2017). However, DMY has high lipid solubility, which contributes to their poor tissue penetration leading to low bioavailability (YOO; MUN; KIM, 2006; LIU et al., 2009).

The encapsulation of drugs is a possibility that has been widely studied by different areas of science such as neurological, oncological, cardiovascular, dermatological, among others. (BOISSEAU; LOUBATON, 2011; NEWMAN; CRAGG, 2012; BREGOLI et al., 2016). The use of nanotechnology brings a new approach to solving problems primarily associated with the pharmaceutical field (SARAF, 2010). With a decreased particle size there is a larger surface so that the chemical interactions occur. Thus, the use of this new technology is aimed at reducing pharmacological doses can decrease the incidence of adverse effects, promote prolonged release of the drug at its site of action, increase the solubility and the interval between the doses and getting the best efficiency (ASLAM, 2008; SARAF, 2010; BOISSEAU; LOUBATON, 2011; FRANK et al., 2015).

Nanoencapsulation is one of the approaches that have been studied in an attempt to overcome microbial resistance to DMY, yet there are no reports in the literature that discuss the nanoencapsulation of DMY. Therefore, we developed polymeric nanoparticles loaded with DMY to enhance its antimicrobial effects. Nanocapsules are vesicular structures consisting of a thin polymeric shell and an oil core cavity; the drug can be dissolved in the oily core or can be adsorbed onto the polymer wall (SOPPIMATH et al., 2001; SCHAFFAZICK et al., 2003; BULCÃO et al., 2014; FRANK et al., 2015).

The high-performance of liquid chromatography (HPLC) is a useful and rapid technique and it has been widely used in validating analytical methods for the quantitation of drugs (LANÇAS, 2009). Several techniques have been used to determine DMY content. Studies have reported the quantification of DMY in both the plasma of rats and the extracts of plants using methods based on HPLC (Table 1) (YOO; MUN; KIM, 2006; ZHANG et al., 2007; ZHONG et al., 2014; YE et al., 2017; JIN et al., 2014; PARK et al., 2016).

We developed DMY-loaded nanocapsules for antimicrobial studies but were unable to find a literature method describing the use of HPLC to measure the DMY content of nanocapsule suspensions. Therefore, the aim of this study was to develop and validate a simple and reliable stability-indicating HPLC method to assay DMY in nanocapsule suspensions. The method was to be isocratic and avoid the use of a buffer solution in the mobile phase. This study also evaluates the stability of DMY in different degradative conditions to verify the influence of nanoencapsulation.

Table 1 - Literature methods used to assay DMY content using HPLC. Abbreviation: DMY, dihydromyricetin.

Analyte	Column	Mobile Phase	Flow	Wavelength	Ref
Rat plasma	YMC C18 column (250 mm × 4.6 mm, 5 μm)	Gradient elution using acetonitrile and water containing 0.04% (v/v) phosphoric acid starting at 12:88, (v/v), increasing linearly to 80:20, (v/v) at 60 min	1.0 mL/min	290 nm	Zhang et al. (2007)
Ampelopsis grossedentata extracts	C18 column (250 mm × 4.6 mm)	Methanol:water:acetic acid (50:50:1 v/v/v)	1.0 mL/min	292 nm	Zhong et al. (2014)
Hovenia dulcis extracts	C18 column (250 mm × 4.6 mm, 5 μm)	Gradient elution using water and acetonitrile from 90:10 (v/v) to 30:70 (v/v)	1.0 mL/min	254 nm	Yoo, Mun e Kim (2006)
Ampelopsis grossedentata extracts	Agilent Porshell 120 EC C18 column (5 cm × 0.46 cm, 2.7 μm)	Water 0.1% (v/v) acetic acid and acetonitrile 0.1% (v/v) acetic acid consisting of 90:10 (v/v)	0.5 mL/min	290 nm	Ye et al. (2017)
Dried ground part of *Ampelopsis sinica*	Dikma C18 column (250 × 4.6 mm, 5 μm)	Gradient elution of acetonitrile and 0.1% phosphoric acid	1 mL/min	292 nm	Jin et al. (2014)
Ampelopsin (purity >98%)	Xbridge™ Shield RP18 column (4.6 mm I.D. × 150 mm, 3.5 μm)	The mobile phase consisted of 0.1% acetic acid (Solvent A) and 100% acetonitrile (Solvent B). The percentage composition of Solvent B was maintained at 20% for 3 min, gradually increased to 38% for 24 min, further increased to 90% for 1 min and maintained at 90% for 5 min, which was followed by equilibration to the initial composition for 6 min.	1 mL/min	365 nm	Park et al. (2016)

Source: author.

MATERIAL AND METHODS

DMY reference substance (98% purity) was obtained from Sigma-Aldrich (St. Louis, MI, USA). HPLC-grade ethanol was acquired from Panreac (Barcelona, Spain) and acetonitrile was acquired from Sigma-Aldrich (St. Louis, MI, USA). Eudragit® RS 100 was kindly provided by Evonik (Germany), caprylic/capric triglyceride mixture was obtained from Alpha Química (São Paulo, Brazil), Polysorbate 80 (Tween 80) and acetone were supplied by Synth (Diadema, Brazil). All chemicals and solvents were of pharmaceutical grade and were used as received.

APPARATUS AND CHROMATOGRAPHIC CONDITIONS

A Shimadzu HPLC system (Kyoto, Japan) was used and was equipped with LC-20AT pump, an SPD-M20A photodiode array (PDA) detector, a CBM-20A system controller, an RP-18 Phenomenex column (150 mm × 4.0 mm, 5 μm particle size, 100 Å pore diameter), and a C18

Phenomenex (4 × 3.0 mm) precolumn. The mobile phase was acetonitrile-water (20:80 v/v) at pH 4.0 (adjusted with acetic acid) at an isocratic flow rate (0.6 mL/min). Each run was 10.0 min at room temperature. The injection volume was 20 μL. Detection was performed at 290 nm.

PRODUCTION OF NANOCAPSULE SUSPENSION

An aqueous suspension of nanocapsules was prepared by interfacial deposition of pre-formed polymer as described by our research group (DALCIN et al., 2017), through the preparation of two separate phases. The organic phase was prepared by dissolving the polymer Eudragit® RS 100 (250 mg) and capric/caprylic triglyceride mixture (825 μL) in acetone (68 mL) at 40°C, with magnetic stirring. An aqueous phase was prepared by dissolving polysorbate 80 (190 mg) in ultrapure water (132 mL). The organic phase was then injected into the aqueous phase, at a controlled rate, and nanocapsules were formed and maintained with stirring for 10 min. The organic solvent and some of the water were evaporated under reduced pressure. The final volume was 25 mL, and the suspension was stored at room temperature and protected from light. The nanocapsule suspensions that were used for the evaluation of all parameters were prepared at a concentration of 1000 μg•mL^{-1}.

Nanocapsule suspensions (1.0 mL) were diluted with acetonitrile to a concentration of 100.0 μg•mL^{-1} and subjected to ultrasonication (Unique®, Brazil) for 30 min. Subsequently, a portion of the solution (2.0 mL) was diluted with the mobile-phase and again ultrasonicated for 30 min to yield a final concentration of 20.0 μg•mL^{-1}. The resulting solution was filtered through a 0.45-micron membrane (Millipore®, Brazil) and injected into the HPLC system (n = 3).

PHYSICOCHEMICAL CHARACTERIZATION OF NANOCAPSULE SUSPENSIONS

The suspensions containing nanocapsules were characterized in mean particle diameter, polydispersity index, zeta potential, pH, encapsulation content and encapsulation efficiency. The mean particle diameter and polydispersity index were determined by dynamic light scattering (Zetasizer® Nano-ZS model ZEN 3600, Malvern Instruments, UK), and the zeta potential by electrophoretic mobility (Zetasizer® Nano-ZS model ZEN 3600, Malvern Instruments, UK), the pH was measured directly from the formulations using a previously calibrated potentiometer (Digimed® DM - 20, Brazil). In addition, the encapsulation efficiency was determined by methodology provided by Santos *et al.*, 2014, using ultrafiltration/centrifugation devices (Amicon®10 kDa, Millipore) at 7000 × g for 10 min, followed by determination by HPLC according to the method described below.

METHOD VALIDATION

Validation was performed according to International Conference on Harmonization (ICH) guidelines to assess the following parameters: specificity, linearity, precision, accuracy, robustness, and detection and quantification limits (ICH, 2005; ANVISA, 2017). Theoretical plates, peak symmetry, tailing factor, retention factor, and resolution in relation to the excipient peak were calculated according to official guidelines (LANÇAS, 2009; ICH, 2005; ANVISA, 2017; USP, 2008).

STANDARD SOLUTION

A standard solution of DMY (400.0 µg•mL^{-1}) was prepared by dissolving DMY standard (4.0 mg) in ethanol (10.0 mL) with 15 min ultrasonic agitation. In addition, the stock standard solution was diluted, with mobile phase to give five standard solutions containing different concentrations of DMY (10.0, 15.0, 20.0, 25.0, and 30.0 µg•mL^{-1}), which were used for the linearity study (3 curves in 3 consecutive days). All solutions were filtered (0.45-µm) before being injected into the HPLC system (n = 3).

Specificity

In order to evaluate possible interference from the nanocapsule constituents, specificity for DMY was assessed by preparing and analyzing blank nanocapsule (B-NC) - suspensions containing all of the nanocapsule components except the drug. The system response was examined for the presence of interference or overlap with the DMY responses. In addition, the specificity of the control injection was also evaluated by examining the purity of peaks.

Linearity, limits of detection, and quantification

Analytical curves (n = 3) were obtained for five concentrations of the reference solution in the range of 10.0, 15.0, 20.0, 25.0, and 30.0 µg•mL^{-1}. Three independent curves were plotted and linearity was evaluated by linear regression analysis using the least-square regression method, which was used to calculate the correlation coefficient, y-intercept, and slope of the regression line. The limit of detection (LOD) and the limit of quantification (LOQ) were determined directly from the calibration plot, according to the equations in the ICH guidelines (ICH, 2005; ANVISA, 2017).

Precision

The precision of the method was determined by repeatability (intra-day precision) and inter-mediate precision (inter-day precision). Repeatability was evaluated by measuring, in triplicate, six

different samples of the same concentration (20.0 μg•mL-1) under the same experimental conditions and on the same day. Intermediate precision was calculated from the results obtained by the analysis of samples with the same concentration (20.0 μg•mL-1) on three different days. On one of the days, the experiments were conducted by a different analyst. Precision (repeatability and intermediate precision) was expressed as relative standard deviation (RSD).

Accuracy

Accuracy was evaluated by assaying, in triplicate, nanocapsules suspensions of known concentrations (15 μg•mL^{-1}) spiked with three different concentrations of standard solution (3.75, 7.5, and 11.25 μg•mL^{-1}) at three different levels (lower, medium, and upper concentration), giving sample solutions with final concentrations of 18.75, 22.5, and 26.25 μg•mL^{-1}, respectively. The accuracy was calculated as the percentage of drug recovered from the formulations and expressed as RSD between the replicates (n = 3).

Robustness

Robustness was studied by evaluating the effect of small and deliberate variations in the chromatographic conditions. The conditions that changed included the flow rate (±0.2 mL•min^{-1}), pH, composition of mobile phase (acetonitrile ± 5.0%), and wavelength (±5.0 nm). Sample solutions (n = 3) were evaluated with each variation of the conditions. The results were compared to the recommended conditions.

DEGRADATION STUDIES

Samples of DMY were exposed to degradative conditions in order to evaluate the stability indicating properties and specificity of the method (ICH, 2003; ANSEL et al., 2007; BLESSY et al., 2014; FONTANA et al., 2010). This study was conducted with both free DMY and DMY-loaded nanocapsules. The acidic and alkaline degradation of DYM were studied in HCl (0.1 M) and NaOH (0.01 M) solutions, for 5 minutes at room temperature. Oxidation used 3% H_2O_2 solution, for 5 minutes at room temperature. Photolysis was initiated by exposing the drug to UV light (365 nm) for 24 hours in a UV light chamber and a thermal stability study was performed by heating the samples in an oven at 60°C for 24 hours. All DMY solutions that were used in degradation studies were prepared at an initial concentration of 400 μg•mL^{-1}. All samples were diluted with mobile phase to give a final concentration of 20.0 μg•mL^{-1} and filtered before injection.

RESULTS AND DISCUSSION

HPLC-based pharmaceutical analysis methods have been studied in the context of nanotechnological drug vectors (FONTANA et al., 2010; OLIVEIRA et al., 2011; SARAJI; GHANI, 2014; GITE; PATRAVALE, 2015) Nanocapsule suspensions are complex mixtures of surfactant, polymer, and oil; thus, the methods used to analyze the drug content of such systems must take into account their chemical complexity. But even with a complex structure, DMY was possible to extract only with the use of ultrasound. Thus, the analysis and the drug testing method for such systems should be developed and validated with many criteria to demonstrate their suitability.

The results of the physicochemical characterization showed a mean particle diameter of 161 ± 2.50 nm, polydispersity index of 0.079 ± 0.01, zeta potential of $+11.4 \pm 0.60$, pH of 5.63 ± 0, 04, drug content of 100.5 ± 0.2 and encapsulation efficiency of 80.8%. These results are in agreement with the results already published by our research group (DALCIN et al., 2017).

In this work, we assessed the feasibility of developing a simple stability-indicating method using HPLC using UV/Vis PDA detection to assay the DMY content of nanocapsules. Firstly, the maximum absorption wavelength of the standard reference solution was evaluated. The absorbance at 290 nm was chosen for our method since it had the highest absorption intensity; this result is in agreement with the literature (ZHANG et al., 2007; YE et al., 2017).

Different conditions were tested during the development of the method, including the use of methanol in the mobile phase; however, this did not result in a symmetrical peak. The pH of the mobile phase was also varied (2.5, 4.0, and 7.0) using different solutions (phosphoric acid, acetic acid, trifluoroacetic acid, and triethylamine); a pH of 4.0 obtained using acetic acid was chosen owing to its chromatographic profile. Acetonitrile and water at different ratios were tested, and ultimately an isocratic method using 20% acetonitrile and 80% water was chosen owing to it having the best chromatographic profile and involving a low concentration of organic solvent.

The method provided an acceptable number of theoretical plates (N = 4,633), a peak symmetry of 1.58, an acceptable peak tailing factor of 1.43, and an adequate retention factor (K´ = 0.554) over a short run (5.6 min). All of the chromatographic parameters calculated were in accordance with reference values indicating the suitability of the proposed method (LINDSAY, 1992; FONTANA et al., 2010; OLIVEIRA et al., 2011; GITE; PATRAVALE, 2015; PARK et al., 2016).

The retention time of DMY was around 5.6 min. This allowed a run time of 10 min to be used, which represents a dramatic improvement in comparison with other methods described (retention time around 17.65 min and run time of 60 min (ZHANG et al., 2007)) and retention time after 12 min and run time of 35 min for simultaneous quantification of DMY and Resveratrol (JIN et al., 2014). The new method also avoids the use of a buffer solution in the mobile phase, which decreases wearing the columns and components of the apparatus. The new method is also isocratic which is

advantageous compared to methods using gradient elution (YOO; MUN; KIM, 2006; ZHANG et al., 2007; JIN et al., 2014; PARK et al., 2016).

The specificity of the test method was evaluated by comparative analysis of the DYM in free form, DYM-loaded nanocapsules suspensions, and blank nanocapsules suspensions (placebo formulation) at a concentration of 20 µg.mL^{-1} (Figure 2).

Specificity was assessed by visualization of chromatograms shown (Figure 2), which confirmed the specificity of the validated method. PDA analysis showed a peak purity of 100%, indicating that the other components in the formulations did not interfere with the DMY assay, and demonstrating the specificity of the proposed method. Furthermore, the chromatograms obtained by injecting a sample containing only the components of the nanocapsule did not influence the DMY assay.

Figure 2 - Chromatograms obtained from solutions (20 µg.mL^{-1}) (A) DYM reference substance, (B) DYM-loaded nanocapsule suspensions, and (C) blank-nanocapsule (placebo formulation).
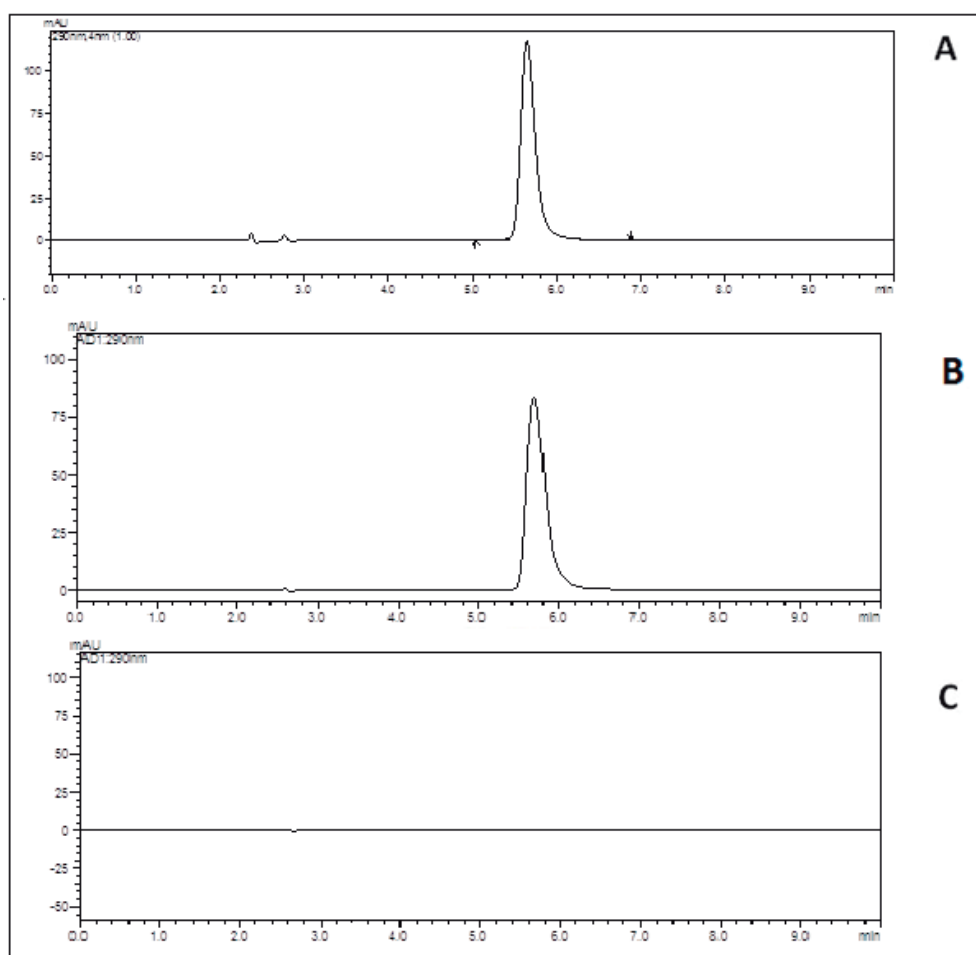
Source: author.

The analytical curves for DMY were constructed by plotting concentration against peak area. A good linearity was observed in the range 10-30 µg•mL^{-1}. A linear equation (y = 69204x - 54772) was obtained using the least-square method (n = 3) and showed an acceptable correlation coefficient

(r = 0.999). LOD and LOQ were 1.23 and 3.69 µg•mL^{-1}, respectively indicating that the limit for detection of DMY by this method is 1.23 µg•mL^{-1} and the limit of quantification of DMY is 3.69 µg•mL^{-1}.

The repeatability (intra-day precision) and intermediate precision (inter-day precision) are shown in table 2. All data are lower than the acceptance criterion of 1,2%, indicating the accuracy of the proposed method. Regarding the accuracy evaluation, good recoveries (101-113%) were obtained (Table 3). The percentage of recovery indicated moderate accuracy and, consequently, an agreement between the theoretical and real concentration values. The values found are in accordance with the recommended by ANVISA (2017), considering that the suspensions of nanocapsules constitute a complex matrix.

Table 2 - Repeatability (intra-day) and intermediate precision (inter-day) of the method used to assay DMY content of nanocapsule suspensions (sample solutions at a theoretical concentration of 20.0 µg•mL^{-1}). Abbreviations: DMY, dihydromyricetin; RSD, relative standard deviation.

Parameter	*n*	RSD (%)
Intra-day	6	0.69
Inter-day		
Day 1	3	0.67
Day 2	3	1.86
Day 3	3	1.06
Day 1 + 2 + 3	9	1.2

Source: author.

Table 3 - Determination of the accuracy of the method (sample solutions at a theoretical concentration of 15·0 µg.mL-1). Abbreviation: RSD, relative standard deviation.

Reference sample (µg·mL^{-1})	Added (µg·mL^{-1})	Found (µg·mL^{-1})	Recovery (%)	RSD (%)
16.2 ± 0.40	3.75	20.24 ± 1.12	101.67 ± 1.05	1.04
	7.50	25.31 ± 1.51	109.79 ± 2.34	2.13
	11.25	28.67 ± 1.18	113.53 ± 3.44	3.03
		Mean	108.33	

Source: author.

The method was shown to have high robustness because a deliberate variation of the method conditions had no significant effect on assay data or chromatographic performance (LANÇAS, 2009). The results from the robustness testing are shown in table 4. Regarding the composition of the mobile phase, no significant influence in the percentage content of DMY was found when changing the composition of the mobile phase to 15:85 (acetonitrile: water) or 25:85, changing the detection wavelength to 285 nm or 295 nm, or changing the pH of the mobile phase to 3.85 and 4.25 (Table 4).

Stability studies were conducted to assess conditions that may influence the quality, safety, and efficacy of the pharmaceutical product. In this context, exposing the drug to degradative conditions can help to visualize the degradation products and ensure the integrity of the drug (ICH, 2005; ANSEL et al., 2007; BLESSY et al., 2014). Several studies have demonstrated that DMY has diverse

pharmacological activity, but, at the moment, it is unknown how it behaves under degradative conditions. For this reason the drug - both free and nanoencapsulated - was subjected to different stress conditions. The results can be seen in figure 3.

Table 4 - Determination of the robustness of the method. The recommended chromatographic conditions were: RP-18 Phenomenex column (150 mm × 4.0 mm, 5 μm particle size, 100 Å pore diameter) and pre column C18 Phenomenex (4 × 3.0 mm) with acetonitrile-water pH 4.0 80:20 (v/v) as mobile phase at a flow rate of 0.6 mL/min and UV detection at 290 nm. Abbreviation: RSD, relative standard deviation.

Conditions	%	RSD (%)
Recommended conditions	101.17	0.34
Mobile phase (acetonitrile-water)		
15-75	103.17	3.17
25-75	107.88	0.59
Wavelength (nm)		
285	105.27	0.18
295	105.27	0.18
pH water		
3.85	103.88	4.39
4.25	107.06	5.77

Source: author.

Figure 3 - Degradation of drug under different stress conditions. (N = 3) **$P < 0.01$, ***$P < 0.001$.
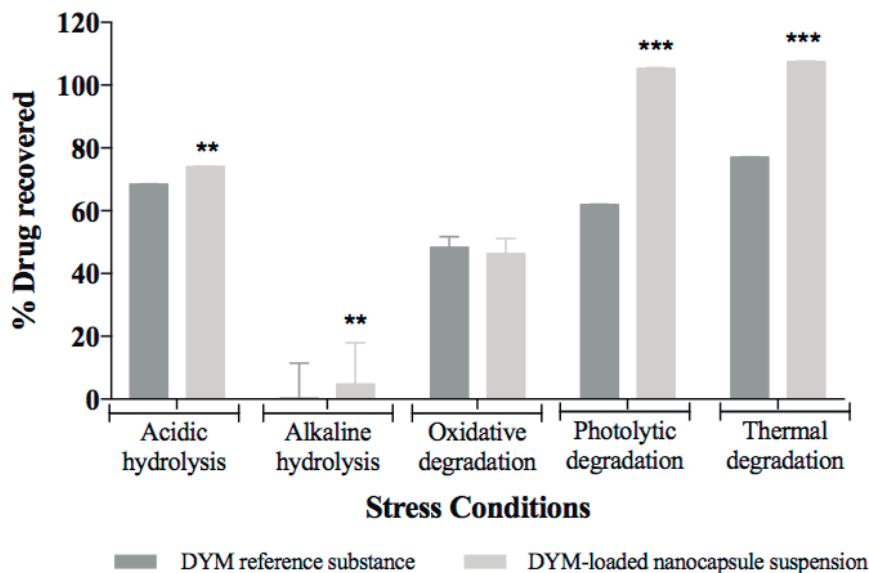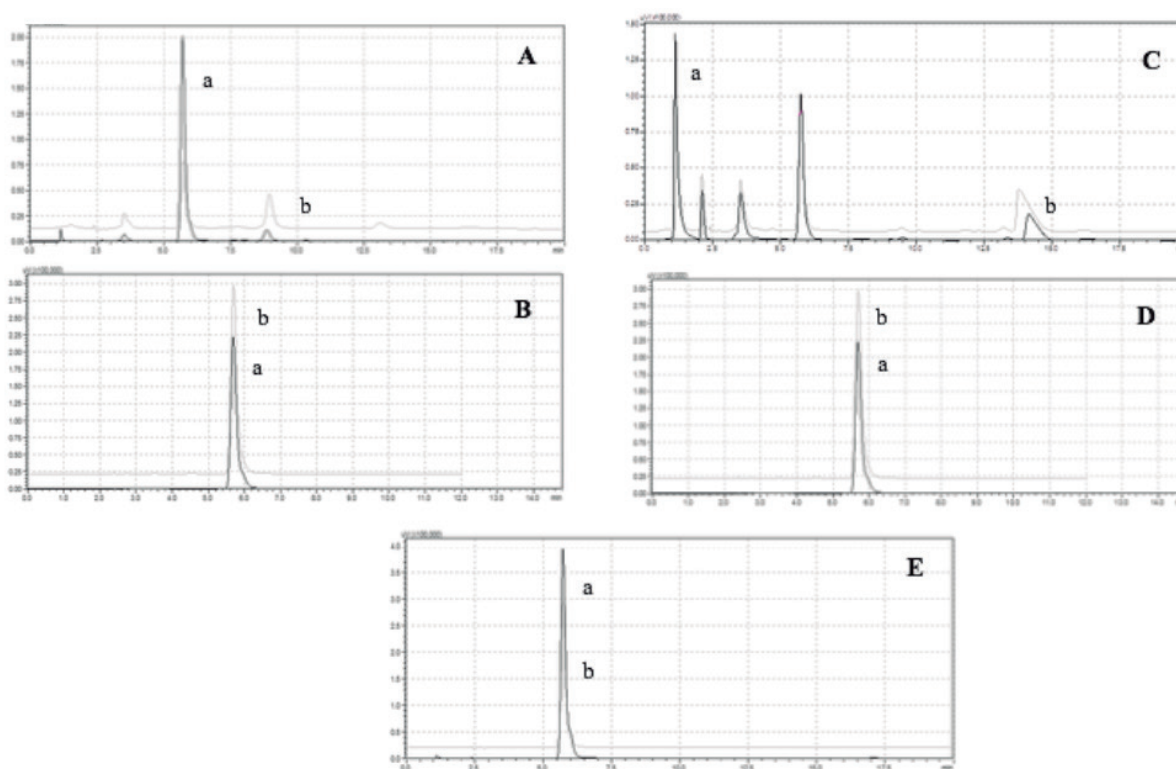

Source: author.

Under alkaline conditions, the free drug was degraded around 85%; however, nanoencapsulation protected the drug to degradation extreme (Figure 4A). In the acid hydrolysis degradation occurs both free form DMy, and in nanoencapsuled form (Figure 4B), however, the nanoencapsulation again showed a lower degradation compared to the reference substance in free form (Figure 3). A similar result was found by Mazzarino et al. (2010), where the nanocapsules reduced hydrolysis of curcumin at pH 5.0.

Oxidative degradation was apparent in both forms (Figure 4C); however the drug in free form showed significantly higher degradation compared to the nanoestructured drug. This type of degradation can be prevented by pharmaceutical industry by removing air from the vessel in which drugs are stored (SILVA et al., 2009).

Results from the photolytic and thermal degradation experiments demonstrated significant degradation of the free substance and no degradation in the case of DMY-loaded nanocapsules (Figure 3 and Figure 4D and E); this indicates that the nanocapsules protect against photolytic and thermal degradation. The results corroborate with other studies (OURIQUE et al., 2008; FRANK et al., 2015; SANTOS et al., 2013; SANTOS et al., 2014), in which the nanocapsules were able to protect or better the photostability of the UV degradation of the drug. Frank et al. (2015) conclude that the photoprotection observed is due to the nanocapsule polymer used in the interface which protects the drug from photolytic degradation.

Figure 4 - Chromatogram obtained after study of forced degradation. A) alkaline degradation;
B) acidic hydrolysis; C) oxidative degradation; D) photolytic degradation; E) Thermal degradation.
Where a= Free DMY and b= DMY-loaded nanocapsules suspension.

Source: author.

Importantly, temperature and lighting conditions are very relevant conditions to indicate the stability of drugs and dosage forms in the pharmaceutical industry (SILVA et al., 2009). Photolytic and thermal degradation may be affected during transport, storage and handling, pharmaceutical promoting instabilities.

CONCLUSIONS

A rapid, specific, and reliable stability-indicating HPLC method for the determination of the DMY content of nanocapsule suspensions has been developed and validated. The analytical methodology proposed is simple, precise, accurate, and linear in the concentration range of 10.0-30.0 µg•mL-1. The method developed is innovative and has advantages over methods previously described in the literature such as using a smaller proportion of organic solvent, a shorter running time, isocratic elution. It is also the first method that indicates stability.

Degradation was induced in free DMY, and DMY loaded nanocapsule suspensions, and the resulting materials were assayed. Encapsulated DMY was observed to be less susceptible than free DMY to acid hydrolysis, alkaline hydrolysis, and oxidative degradation. Encapsulated DMY was found to be able to resist completely thermal and photolytic degradation. Under the conditions tested greater stability was evidenced the DMY nanocapsules formulation as compared to free DMY, suggesting a protective nanostructure of the degradations. Therefore, nanoencapsulation is a promising strategy for the development of new formulations containing DMY.

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REFERENCES

ANSEL, H. C.; POPOVICH, N. G.; ALLEN-JR, L. V. **Farmacotécnica:** formas farmacêuticas & sistemas de liberação de fármacos. 8. ed. Porto Alegre: Artmed, 2007.

ANVISA. Resolução RDC nº 166, de 24 de julho de 2017. Dispõe sobre a validação de métodos ana-líticos e dá outras providências. **Diário Oficial [da] República Federativa do Brasil**, 25 de julho de 2017, ed. 141, seção 1, p. 87.

ASLAM, S. Effect of antibacterials on biofilms. **American Journal of Infection Control**, v. 36, n. 10, p. 9-11, 2008.

BLESSY, M. et al. Development of forced degradation and stability indicating studiesofdrugs - a review. **Journal of Pharmaceutical Analysis**, v. 4, n. 3, p. 159-165, 2014.

BOISSEAU, P.; LOUBATON, B. Nanomedicine: nanotechnology in medicine. **Comptes Rendus Physique**, n. 12, p. 620-636, 2011.

BREGOLI, L. et al. Nanomedicine applied to translational oncology: a future perspective on cancer treatment. **Nanomedicine: Nanotechnology, Biology, and Medicine**, v. 12, p. 81-103, 2016.

BULCÃO, R. et al. In vivo toxicological evaluation of polymeric nanocapsules after intradermal administration. **European Journal of Pharmaceutics and Biopharmaceutics**, v. 86, n. 2, p. 67-77, 2014.

DALCIN, A. J. F. et al. Anti biofilm effect of dihydromyricetin-loaded nanocapsules onurinary catheter infected by *Pseudomonas aeruginosa*. **Colloids and Surfaces B: Biointerfaces**, v. 156, p. 282-291, 2017.

FONTANA, M. C.; BASTOS, M. O.; BECK, R. C. R. Development and Validation of a Fast RP-HPLC Method for the Determination of Clobetasol Propionate in Topical Nanocapsule Suspensions. **Journal of Chromatographic Science**, v. 48, p. 637-640, 2010.

FRANK, L. A. et al. Improving drug biological effects by encapsulation into polymeric nanocapsules. **Nanomedicine and Nanobiotechnology**, v. 7, p. 623-639, 2015.

GITE, S.; PATRAVALE, V. Validation of RP-HPLC method and stress degradation for combination of Metformin HCl, Atorvastatin Calcium and Glimepirine: Application to nanoparticles. **Journal of Chromatographic Science**, v. 53, n. 10, p. 1654-1662, 2015.

ICH - International Conference on Harmonization. **Validation of Analytical Procedures:** Text and Methodology. Q2 (R1), Current Step 4 version Parent Guideline dated 27 October 1994 (Complementary Guideline on Methodology dated 6 November 1996 incorporated in November 2005).

JIN, M. et al. Simulaneous determination of Dihydromyricetin and resveratrol in Ampelopsis sinica (Miq) W. T. Wang by High-Perfomance Liquid Chromatografy Coupled with a diode array detection method. **Journal of Chromatographic Sciences**, v. 52, p. 339-343, 2014.

KOU, X.; CHEN, N. Pharmacological potential of ampelopsin in Rattan tea, **Food Science and Human Wellness**, n. 1, p. 14-18, 2012.

LANÇAS, F. M. **Cromatografia líquida moderna:** HPLC/CLAE. Campinas, SP: Átomo, 2009.

LIU, B. et al. Characterization and antioxidant activity of dihydromyricetin-lecithin complex. **European Food Research and Technology**, v. 230, n. 2, p. 325-331, 2009.

LINDSAY, S. **High performance liquid chromatography.** 2nd. Wiley: New York, 1992.

MA, J. et al. Analysis of polyphenolic antioxidants from the fruits of three pouteria species by selected ion monitoring liquid chromatography-mass spectrometry. **Journal of Agricultural and Food Chemistry**, v. 52, p. 5873-5878, 2004.

MAZZARINO, L. et al. Curcumin-Loaded Polymeric and Lipid Nanocapsules: Preparation, Characterization and Chemical Stability Evaluation. **Latin American Journal of Pharmacy**, v. 29, n. 6, p. 933-40, 2010.

MURAKAMI, T. et al. Hepatoprotective activity of tocha, the stems and leaves of *Ampelopsis grossedentata*, and ampelopsin. **Biofactors**, v. 21, p. 175-178, 2004.

NEWMAN, D. J.; CRAGG, G. M. Natural Products as Sources of New Drugs over the 30 Years from 1981 to 2010. **Journal of Natural Products**, v. 23, n. 75, p. 311-335, 2012.

OLIVEIRA, L. T. et al. HPLC-FLD methods to quantify chloroaluminum phthalocyanine in nanoparticles, plasma and tissue: application in pharmacokinetic and biodistribution studies**. Journal of Pharmaceutical and Biomedical Analysis**, v. 56, n. 1, p. 70-77, 2011.

OURIQUE, A. F. et al. Tretinoin-loaded nanocapsules: Preparation, physicochemical characterization, and photostability study. **International Journal of Pharmaceutics**, v. 352, p. 1-4, 2008.

PARK, J. S. et al. HPLC determination of bioactive flavonoids in *Hovenia Dulcis* Fruit Extracts. **Journal of Chromatographic Science**, v. 54, n. 2, p. 130-5, 2016.

QI, S. et al. Ampelopsin reduces endotoxic inflammation via repressing ROS-mediated activation of PI3K/Akt/NFkappaB signaling pathways. **International immunopharmacology**, v. 12, p. 278-287, 2012.

SANTOS, S. S. et al. Clotrimazole-loaded Eudragit® RS100 nanocapsules: Preparation, characterization and in vitro evaluation of antifungal activity against Candida species. **Materials Science and Engineering C**, v. 33, p. 1389-1394, 2013.

SANTOS, S. S. et al. Formulation and in vitro evaluation ofcoconutoil-corecationic nanocapsules Intended for vaginal delivery of clotrimazole. **Colloids and Surfaces B: Biointerfaces**, v. 116, p. 270-276, 2014.

SARAF, A. S. Applications of novel drug delivery system for herbal formulations. **Fitoterapia**, v. 81, p. 680-689, 2010.

SARAJI, M.; GHANI, M. Dissolvable layered double hydroxide coated magnetic nanoparticles for extraction followed by high performance liquid chromatography for the determination of phenolic acids in fruit juices. **Journal Chromatography A**, v. 1366, p. 24-30, 2014.

SCHAFFAZICK, S. R. et al. Caracterização e estabilidade físico-química de sistemas poliméricos nanoparticulados para administração de fármacos. **Química Nova**, v. 26, n. 5, p. 726-737, 2003.

SILVA, K. E. R. et al. Modelos de Avaliação da Estabilidade de Fármacos e medicamentos para a Indústria Farmacêutica. **Revista de Ciências Farmacêuticas Básica e Aplicada**, v. 30, n. 2, p. 129-135, 2009.

SONG, Q. et al. Dihydromyricetin attenuated Ang II induced cardiac fibroblasts proliferation related to inhibitory of oxidative stress. **European Journal of Pharmacology**, v. 87, p. 159-167, 2017.

SOPPIMATH, K. S. et al. Biodegradable polymeric nanoparticles as drug delivery devices. **Journal of Controlled Release,** v. 70, p. 1-20, 2001.

USP 33. **Pharmacopoeia The United States**. 30. ed. Rockville: United States Pharmacopoeial Convention, 2008.

XIONG, D. et al. Effect of ampelopsis extract from young stems and leaves on antimicrobial activities. **Food Science**, v. 21, p. 48-50, 2009.

YE, X. et al. Oral administration of ampelopsin protects against acute brain injury in rats following focal cerebral ischemia. **Experimental And Therapeutic Medicine**, v. 13, n. 5, p. 1725-1734, 2017.

YOO, S. M.; MUN, S.; KIM, J. Recovery and pre-purification of (+)-dihydromyricetin from Hovenia dulcis. **Process Biochemistry**, v. 41, p. 567-570, 2006.

ZHANG, Y. et al. Simultaneous determination and pharmacokinetic studies of dihydromyricetin and myricetin in rat plasma by HPLC-DAD after oral administration of *Ampelopsis grossedentata* decoction. **Journal of Chromatography B**, v. 860, p. 4-9, 2007.

ZHENG; H.; LIU, D. Anti-invasive and anti-metastatic effect of ampelopsin on melanoma. **Ai Zheng**, v. 22, p. 363-367, 2003.

ZHONG, Z. et al. Experimental studies of hypoglycemic action on total flavone in *Ampelopsis grossedentata* from Guangxi. **Zhongguo Zhong Yao Za Zhi**, v. 27, p. 687-689, 2002.

ZHONG, S. et al. Efficient conversion of myricetin from Ampelopsis grossedentataextracts and its purification by MIP-SPE. **Journal of Chromatography B**, v. 39, p. 945-946, 2014.