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DERIVATIVE ULTRAVIOLET SPECTROPHOTOMETRIC METHOD TO DETERMINATE SYNEPHRINE IN LIPID NANOCARRIER[1]

ESPECTROFOTOMETRIA ULTRAVIOLETA DERIVADA PARA DETERMINAR SINEFRINA EM NANOCARREADOR LIPÍDICO

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ABSTRACT

Gynoid lipodystrophy (GLD) is a common dermatological condition that affects especially women after the puberty. GLD is concentrated mainly in the pelvic region, lower limbs, and abdomen. Several therapies for physical and mechanical methods are used for the treatment of GLD. However, cosmetics products are distinguished by ease of access and practicality of application. The nanotechnology has contributed to improving the performance of cosmetics and consumer acceptance, providing an alternative for increasing the stability, controlled liberation and, mainly, obtaining of products more aesthetically pleasing. The objective this work was the development and validation of a method by the spectrophotometry ultraviolet derived for determination of synephrine (SFN) specifically in a lipid carrier containing dihydromyricetin, diosgenin, and synephrine. The method showed selectivity for SFN at 239 nm in the presence of other active compounds using second order derivative, $\Delta\lambda$ 10.000 and scale factor 50. The parameters, such as linearity, precision, accuracy and robustness were analyzed during its development. The results were analyzed statistically using the GraphPad Prism software version 5.0. The standard curve showed a correlation coefficient of 0.9983. The precision showed good repeatability (RSD = 2.13) and intermediate precision (RSD = 1.93). The accuracy of the method was confirmed by the mean recoveries of 100.79%. The robustness was evaluated by small changes in the conditions of sample analysis, and it was observed that using of ethanol grade per analysis was essential for this analysis. Thus, the developed and validated methodology demonstrated to be specific, linear, accurate, precise and robust. Furthermore, its execution is quick and straightforward, offering an alternative less expensive for determination of SFN in lipid nanocarrier.

Keywords: analytical determination, gynoid lipodystrophy, nanoparticles, nanostructured lipid carriers.

RESUMO

A lipodistrofia ginoide (LDG) é uma afecção dermatológica comum entre mulheres após a puberdade. LDG está concentranda preferencialmente na região pélvica, membros inferiores e abdomen. Várias terapias por métodos físicos e mecânicos são empregadas para o tratamento da LDG. Entretanto, os cosméticos se destacam pela facilidade de acesso e praticidade de aplicação. A nanotecnologia tem contribuído para melhorar o desempenho dos produtos cosméticos e sua aceitação pelo consumidor, proporcionando uma alternativa para aumento da estabilidade, liberação controlada e, principalmente, obtenção de produtos esteticamente

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mais agradáveis. O objetivo do presente trabalho foi o desenvolvimento e validação de um método por espectrofotometria ultravioleta derivada para determinação da sinefrina (SFN) de forma específica em um carreador lipídico contendo dihidromiricetina, diosgenina e sinefrina. O método apresentou seletividade para SFN em 239 nm, na presença dos outros compostos ativos, utilizando a segunda ordem de derivada, Δλ 10.000 e fator escala 50. Os parâmetros de linearidade, precisão, exatidão e robustez foram analisados durante seu desenvolvimento. Os resultados foram analisados estatisticamente através do software GraphPad Prisma versão 5.0. A curva padrão do método apresentou um coeficiente de correlação de 0.9983. A precisão mostrou boa repetibilidade (DPR=2.13) e precisão intermediária (DPR=1.93). A exatidão do método foi confirmada pelo valor médio de recuperação de 100.79%. A robustez foi avaliada por pequenas mudanças nas condições de análise da amostra e observou-se que o uso do etanol grau PA foi essencial para esta análise. Assim, a metodologia desenvolvida e validada demostrou ser específica, linear, precisa exata e robusta. Além disso, sua execução é rápida e simples, oferecendo uma alternativa menos dispendiosa para determinação de SFN em nanocarredor lipídico.

Palavras-chave: *carreadores lipídicos nanoestruturados, determinação analítica, lipodistrofia ginóide, nanopartículas.*

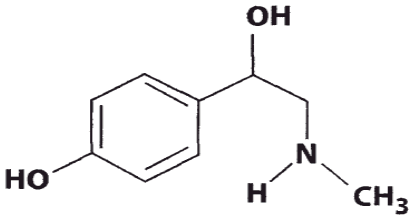
INTRODUCTION

Gynoid lipodystrophy (GLD), also known as cellulite, involves changes in the skin aspect and occurs in approximately 85% of women after puberty, appearing in regions of the pelvis, lower limbs, and abdomen. Some authors consider it attribute proper of sex and not a pathological condition because there is no mortality or morbidity. However, it can cause aesthetic disturbances, of order psychosocial, originated by charging the aesthetic standards of society. The skin becomes shaped appearance of "orange peel" with irregular depressions (KHAN et al., 2010; RAWLINGS, 2006; SANTOS et al., 2011).

There are several methodologies for the treatment of GLD. The physical and mechanical methods may be lymphatic drainage, vacuum therapy, mesotherapy, liposuction, subcision, ultrasound, whirlpool, infrared radiation, laser lipoplasty, among others (AVRAM, 2004; KHAN et al., 2010; SANTOS et al., 2011). The cosmetics are distinguished by ease of access, convey actives with vasopressor action, anti-inflammatory, stimulating peripheral circulation and lipolytic agents (SANTOS et al., 2011). Assets can be methylxanthines, retinoic acid, catecholamines, and botanical extracts, as chestnut India, Ginkgo biloba, Centella asiatica, red grapes, green tea, wild yam, bitter orange, bayberry and ivy (DAVID et al., 2011; HEXSEL et al., 2005; RAWLINGS, 2006; SANTOS et al., 2011).

The SFN (Figure 1) is a substance of polar character and water soluble (ANDRADE, 2008). It is extracted from the fruits of Citrus aurantium L.var. Amara, a small fruit tree, a native of the Southeast Asia, being grown in orchards throughout Brazil, known as bitter orange. The SFN is used in the treatment of GLD based at a presupposed stimulation specific of receptors β-3 adrenergic, found mainly in the liver and adipocytes, which when stimulated, cause an increase in metabolic rate, leading to the stimulation of lipolysis and burning of calories (LINK et al., 2006).

Figure 1 - Chemical structure of synephrine.



The quantification of a drug in a formulation and in its isolated form is a key aspect of quality assurance of medicinal products (BORBA et al., 2013). The development and validation of analytical methods are intended check whether the drug is present properly for its intended purpose and if it is able to provide reliable results that can be satisfactorily interpreted (BRASIL, 2003).

Among the different techniques, the high performance liquid chromatography (HPLC) is a technique widely explored, especially for quality control of pharmaceutical laboratories (PASCHOAL et al., 2003; TAVARES et al., 2011). The HPLC has some limitations, such as the high cost of instrumentation and operation time relatively long for analysis and the need of experience in handling equipment and samples. Furthermore, the disadvantages associated with the use of solvents, such as discarding, operator health and environmental damage, encourage the development of simpler and faster methods, with small amounts of extraction solvents and without the need of prior extraction from the sample (TAVARES et al., 2011).

The spectrophotometry in the ultraviolet region stands out for the speed, robustness, low operating cost and high reliability of results (BORBA et al., 2013; TREVISAN; POPPI, 2006). However, the spectra overlay reduces significantly the use of this method in the analysis of drug combination without prior separation of the constituents. An alternative for the analysis of multicomponents systems is the use of ultraviolet derivated spectrophotometry (UDS) (DONATO et al., 2010).

Several methodologies have been developed and validated for quantification of SFN in products of plant origin. Arbo et al. (2009) developed and validated methodology by HPLC for quantification of SFN in extracts of *C. aurantium.* The method showed a correlation coefficient of 0.9999, precision of 0.62% (expressed as RSD) and accuracy ranging 98-107%. Andrade et al. (2009) quantified the same active employing gas chromatography. The correlation coefficient was 0.9997, repeatability of 3.60%, intermediate precision of 3.59% and average recovery of 78.1%.

Nanotechnology has contributed to Cosmetology in developing innovative delivery systems actives, as the NLC. Despite being used by different routes of administration, the NLC are widely employed in cosmetics and medicines for dermatological use because of its advantages which include excellent biocompatibility, efficient transdermal delivery and high skin hydration (DIMER et al., 2013).

Taking into consideration that there are no official methods for the determination of drugs carried by lipid nanostructures and the limited availability of scientific articles aiming at the

determination the synephrine by spectrophotometric technique in the UV region, This study aimed to the development and validation of an analytical method for the quantification of active synephrine conveyed in NLC by UDS.

EXPERIMENTAL

The experimental part of this work was developed in the Nanotechnology Laboratory Centro Universitário Franciscano. The Lipid nanoparticles used in the experiments were developed, characterized and its stability demonstrated by Inventive Company, which kindly provided samples and raw materials for this study.

Monographs not found described in pharmacopeia for analysis of these assets in pharmaceutical formulations. In the UV-VIS spectrophotometric method diosgenin and dihydromyricetin were quantified at 434 nm and 561 nm, respectively, using reagents such as sulfuric acid and ρ-anisaldehyde (results not shown here). For quantification of synephrine, it was necessary to use the derivative ultraviolet spectrophotometry as described in this work.

REAGENTS AND EQUIPMENT

NLC containing the active dihydromyricetin 0.1% (DHM), diosgenin 0.1% (DGN) and synephrine 0.1% (SFN) (Inventiva, Brazil), NLC without the actives (Inventiva, Brazil), DHM reference standard (96%), DGN reference standard (93%) and SFN reference standard (98%) (Sigma-Aldrich, Germany), acetonitrile analytical grade (Synth, Brazil), ethanol (Synth, Brazil), ethanol HPLC (JT. Baker, Brazil), methanol analytical grade (Synth, Brazil), ultrapure water (Millipore, France) and membrane filters with a 0.45 μm pore size (Macherey-Nagel GmbH & Co. KG, Germany).

UV-VIS double-beam spectrophotometer 1650 PC (Shimadzu®, Japan) using 1.0 cm quartz cell, ultrasound Ultra Cleaner USC5000 of 270 watts RMS power and frequency of 40 KHz (Unique®, Brazil), analytical balance AUW220D (Shimadzu Corporation, Japan), Zetasizer Nano ZS® (Malvern Instruments, England), potentiometer Digimed® model DM-22 (Digimed, Brazil).

SOLUTIONS PREPARATION

Standard solutions preparation

To prepare for standard solution containing 10 μg/mL of SFN, weighed 10 mg of the active and transferred to a 25 mL volumetric flask, using a small amount of ethanol (10 mL) as the solvent.

Then was placed in an ultrasound bath for 15 minutes. The volume was completed with the same solvent, obtaining a solution of concentration 400 µg/mL. A 250 µL aliquot of this solution was transferred to a 10 mL volumetric flask to obtain a final solution containing 10 µg/mL of SFN. The same procedure was performed for the standard solutions of others actives, DHM and DGN.

Sample preparation

To prepare of samples of NLC with the actives (DHM, DGN, SFN), a 1 mL aliquot of the same to a 10 mL volumetric flask, using ethanol as solvent and was placed in an ultrasound bath for 15 minutes. Then the volume was completed with the same solvent, obtaining a suspension of concentration 100 µg/mL of each active. A 1 mL aliquot of this suspension was transferred to 10 mL volumetric flasks to get a final suspension containing 10 µg/mL. To obtain the blank suspension (NANO BL), the same procedure was performed with the NLC without the actives.

CHARACTERIZATION OF SAMPLES

Particle size, polydispersity index, zeta potential and pH determination

For characterization of NLC, particle size and polydispersity index (PDI) analyzes were performed by dynamic light scattering and the zeta potential by electrophoretic mobility, which were determined using Zetasizer Nano ZS equipment. To determine the particle size and PDI, an aliquot of the sample was diluted ultra purified water (1:500,v/v). To determine the zeta potential, the sample was diluted in the solution of sodium chloride at a concentration of 10 mM (1:500, v/v). The pH was determined using potentiometer previously calibrated (using standard solutions of pH 4.0 and 7.0) directly in samples. Results were expressed from the reading of three different samples.

DEVELOPMENT OF THE ANALYTICAL METHOD BY DERIVATED ULTRAVIOLET SPECTROPHOTOMETRY

Firstly a test of solubility was performed with differents solvents (acetonitrile, water, methanol, and ethanol). In this study, we observed the behavior of actives in differents solvents, with the objective of define the best diluent for the analytical method developed. For such, took into consideration the power of solubilization of actives, cost, and toxicity of the solvent and the absorbance of the actives front of the wavelength used. Facing this, we carried out a scan in the range of 200 to 400 nm in different solvents proposed, to identify the wavelength that presents the absorbance value most suitable for the method.

The absorption spectra of standard solutions were determined in UV-VIS double-beam spectrophotometer, using quartz cells of 1 cm optical path. For the derivation of the zero-order spectra was used 2.21 UVProbe program the same spectrophotometer, seeking to identify the wavelength, scanning speed, Δλ, derivative order and the scale factor appropriates for the development of the methodology.

Validation of the analytical method by derivate ultraviolet spectrophotometry

The method was validated as recommended in Resolution RE n° 899, of 29/05/2003, and ICH (The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) (BRASIL, 2003; ICH, 2005a; ICH, 2005b). The evaluated parameters were specificity, linearity, precision, accuracy and robustness.

Specificity

The specificity of the method was evaluated by the analysis of NLC suspension as well as standard solutions of actives (DHM, DGN, SFN), all in the concentration of 10 μg/mL. Zero-order spectra were obtained over the 200 - 400 nm range. From this, first, second and third-order derivate spectra were obtained.

Linearity

The linearity was established by the average of three standard curves, which were obtained in seven different concentration levels of actives DGN, DHM, and SFN. To get the stock solution, were weighed 10 mg of each active and transferred to 25 mL volumetric flask, using ethanol as solvent, and was placed in an ultrasound bath for 15 minutes. The volume was completed with the same solvent, obtaining the actives solution concentration of 400 μg/mL. The above solution was systematically diluted in 10 mL volumetric flasks (in triplicate) to obtain final solutions containing 7, 8, 9, 10, 11, 12 and 13 μg/mL of actives. The diluted solutions were analyzed at a wavelength of 239 nm, in the second-order derivate, medium scanning speed, Δλ 10.000 and scale factor 50. The spectrophotometer was zeroed with NANO BL.

Precision

The intra-day precision (repeatability) was assessed by measuring (in triplicate) of solutions of three concentration levels (7, 10 and 13 μg/mL) on the same experimental conditions. The inter-day precision (intermediate precision) was assessed by the results obtained by the analysis of samples

in the same concentrations on different days and by different analysts. The analysis evaluated by relative standard deviation (RSD). Values will be considered up to 5%, as recommended by the RE 899 (BRAZIL, 2003).

Accuracy

To determination of the accuracy, were added aliquots of known concentrations of the standard solutions of the actives in the suspension of NLC (5 µg/mL), to obtain suspensions the final concentrations of 7.5, 8.75 and 10 µg/mL. The spectrophotometer was zeroed with NANO BL, and the procedure was performed in triplicate.

Robustness

The robustness of the method was determined in triplicate, from the working solution at a concentration of 10 µg/mL, varying the use of ethanol PA to ethanol HPLC, use of different wavelengths (237, 239 and 241 nm) and the employment or not of membrane filter 0.45 µm during the extraction process (Table 1).

Table 1 - Changes assigned to the samples to determine the robustness.

Filter*	Grade of solvent (ethanol)	Wavelength (nm)
P	PA	239
P	HPLC	239
P	PA	237
P	HPLC	237
P	PA	241
P	HPLC	241
A	PA	239
A	HPLC	239
A	PA	237
A	HPLC	237
A	PA	241
A	HPLC	241

*P=present, A=absent

STATISTICAL ANALYSIS

The results were expressed as the mean and standard deviation. Statistical analyses were performed by analysis of variance (ANOVA) followed by Tukey test complement, while $p < 0.05$

considered statistically significant addition to linear regression testing. The data were generated using GraphPad Prism® software.

RESULTS AND DISCUSSIONS

CHARACTERIZATION OF SAMPLES

The results of the characterization of samples of nanostructured lipid carriers with actives (NLCA) and nanostructured lipid carriers without actives (NANO BL) (Table 2) enable them for use in the development and validation of the method. Showed pH compatible for local use (pH 3 to 10) (LEONARDI et al., 2002), average diameter below 130 nm, appropriate for the purpose of nanoparticles, can career the actives to the deeper layers of the skin (VERMA et al., 2003). The values obtained for dispersion index of less than 0.3 demonstrate sufficient homogeneity in the distribution of particle size (PAESE, 2008). The negative value found in this potential enables the particles to keep yourself apart, avoiding aggregate formation and precipitation of the nanostructure (DINIZ, 2008).

Table 2 - Physicochemical characteristics of NLCA and NANO BL.

Parameters	NANO BL*	NLCA*
Particle diameter (nm)	129.8	120.6
Polydispersity index	0.211	0.159
Zeta Potential (mV)	-12.6	- 12.9
pH	5.30	6.23

***** Each value represents the mean of three determinations.

DEVELOPMENT OF THE ANALYTICAL METHOD BY DERIVATE ULTRAVIOLET SPECTROPHOTOMETRY

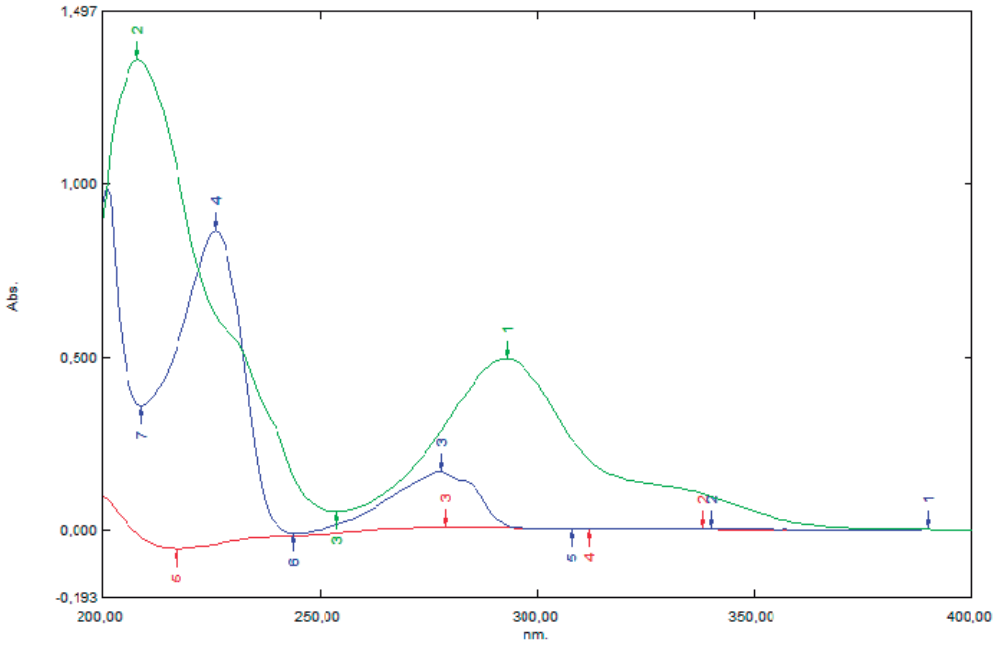
For method development, initially was employed water, acetonitrile, ethanol and methanol as initial solubilization solvent. Spectrophotometric scans were performed in the range 200-400 nm of actives in each solvent and were observed that they showed higher absorptivity when tested with the solvent ethanol.

Adding to this fact, to the ultimate choice of the solvent to be used, took into account the toxicity and the cost of it. The ethanol has a lower toxicity and is more affordable when compared to methanol and acetonitrile (CASSINI et al., 2013). Despite the low cost and free toxicity of water, its use has not been possible due to the low solubility of the active in the solvent. Moreover,

among the alcohols, the actives have a higher solubility when used ethanol (CHEN et al., 2014; CONTRERAS-PACHECO et al., 2013; RUAN et al., 2005). Taking into consideration such criteria, we opted for the ethanol solvent for the development of the methodology.

In the analysis of chemical reference substances (CRS) in the concentration of 10 µg/mL of the three actives separately prepared in solvent ethanol in the ultraviolet region showed absorption peaks in the range of 200 - 400 nm (Figure 2), occurring overlap of electron transition bands, precluding a quantitative analysis of zero order spectrophotometry. As an alternative becomes necessary to use the method derivated ultraviolet spectrophotometry to better individualize the constituents and until eliminate the interference of one component over the other, making possible the quantification of SFN.

Figure 2 - Spectrum scan using standard solutions of concentration of 10 µg/mL of actives dihydromyricetin (green), diosgenin (red) and synephrine (blue) using ethanol as solvent.

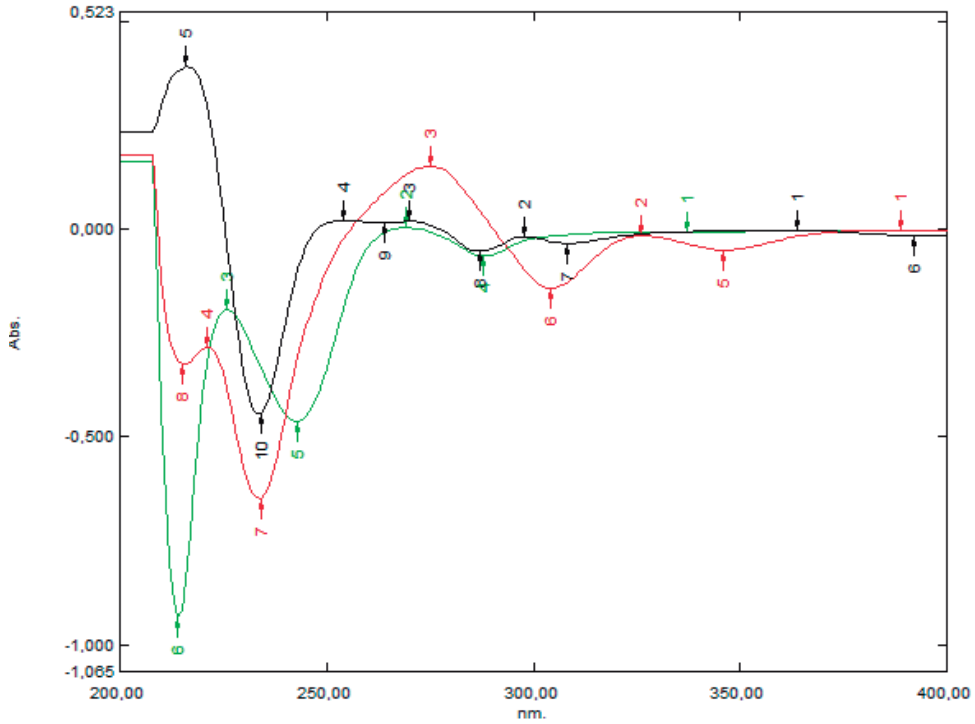


VALIDATION OF ANALYTICAL METHOD

Specificity

Several changes were performed to determine a wavelength where synephrine could be quantitated in a specific way without interference from nanostructure components. In this sense, we tested different orders of derivatization. With the use of the first order, and Δλ 10,000 scaling factor 10, as shown in figure 3, there was no point of cancellation.

Figure 3 - Spectra of solutions SQR (red), lipid nanocarrier (green) and lipid containing the active nanocarrier (black) in ethanol solvent, first order derivative, Δλ 10,000 and scaling



In the evaluation of specificity, worked up with a solution containing the three CRS (DHM, DGN, SFN), a suspension of NLCA, all at a concentration of 10 µg/mL, and a suspension de NANO BL, performing a scanning between 200 and 400 nm, zeroing the spectrophotometer with the solvent ethanol. When plotted and overlaid the spectra of the solution de CRS, of the suspension de NLCA and NANO BL, it was observed an overlap of the spectra, precluding detachment and quantification of SFN. In the derivation of the zero-order spectrum in the second order derivative, Δλ of 10.000 and scale factor 10, was observed a point of annulment at a wavelength of 239 nm, however, there was no overlap of peaks of the suspension synephrine of NLCA with the peak of synephrine of solution CRS (Figure 4), probably due to interference exerted by the NLC. This interference was eliminated with the use of the suspension of NANO BL to zero the spectrophotometer, maintaining the same order of derivative, Δλ and scale factor, revealing a selectivity to the active synephrine at a wavelength of 239 nm (Figure 5).

Figure 4 - Scanning spectra of the solutions of CRSs in the concentration of 10 µg/mL (DGN (red), SFN (black), DHM (Green)) and a mixture of CRSs (blue), in solvent ethanol, second-order, Δλ of 10.000 and scale factor 10. The spectrophotometer was zeroed with ethanol.

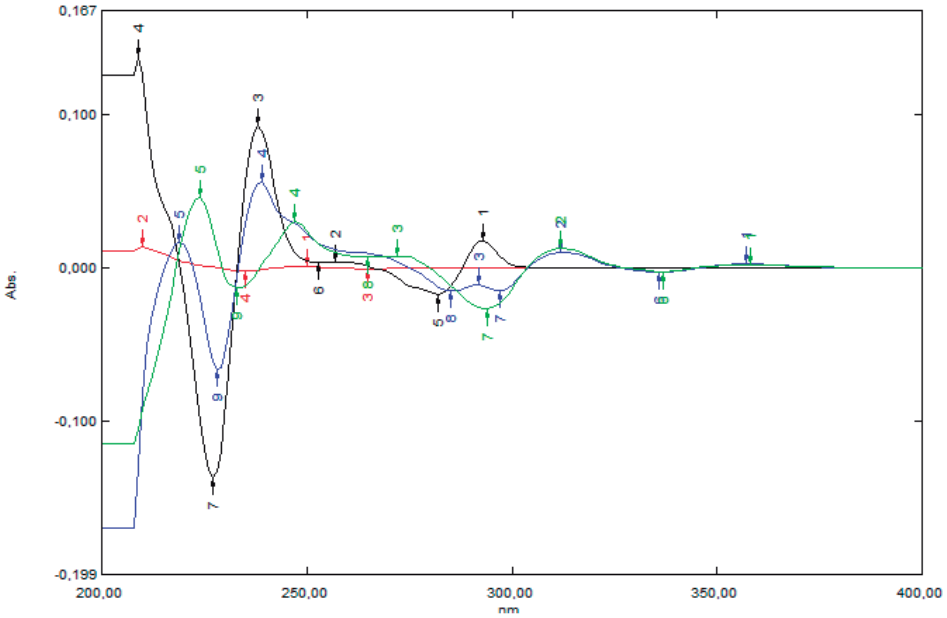
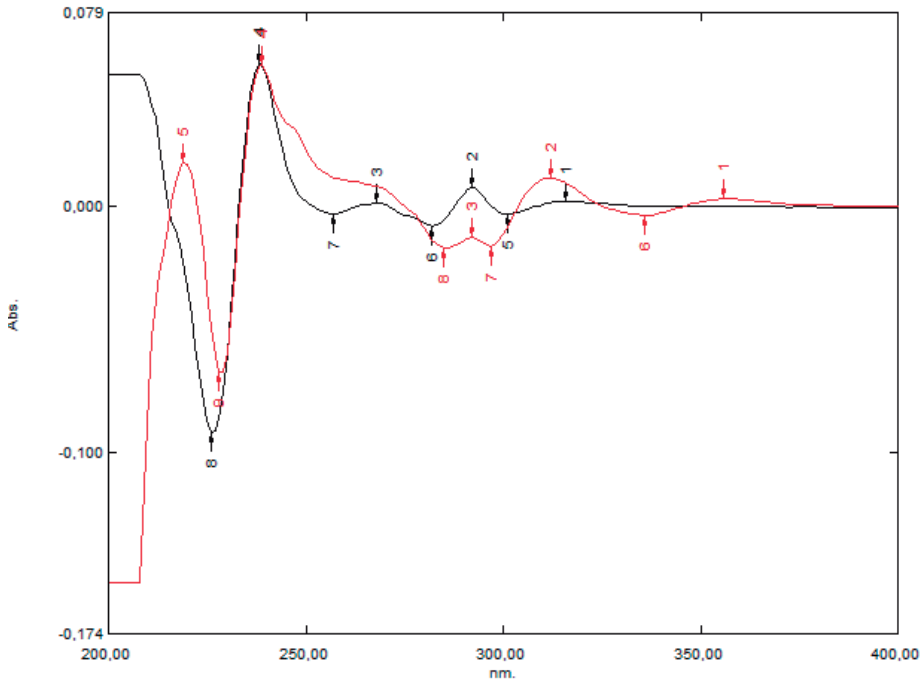


Figure 5 - Scanning spectra of the solutions CRSs (mixture of the three actives) (red) and NLCA (black), in solvent ethanol, second-order derivative, Δλ of 10.000 and scale factor 10. The spectrophotometer was zeroed with suspension de NANO BL.



The value of the scale factor of the method was evaluated by subjecting the variations of 10, 50, 100 and 150, being the scale factor of 50 proved to be the most suitable for a good resolution of the spectrum. Therefore, taking into account all the analyzes, it is concluded that the optimum conditions for quantification of synephrine are a wavelength of 239 nm, average speed scanning, second-order derivative, $\Delta\lambda$ of 10.000 and scale factor 50.

Linearity

The analysis of results of the linearity for ANOVA demonstrated $P< 0,0001$ for synephrine be a significant difference in readings between the concentrations analyzed. From the standard curve found of the active ($y= 0.02814x + 0.0005$), calculated the linear correlation coefficient of Pearson, thereby obtaining the value of 0.9983, being by the recommendations by ANVISA, establishing a correlation coefficient equal or greater than 0.99 (BRASIL, 2003). Linear results for simultaneous and specific quantification of different markers were also observed by Roggia et al. (2016), where a linear correlation coefficient of Pearson was observed from 0.9993 for catechin and value of 1 for caffeine. Likewise, Tavares et al. (2011) obtained linear correlation values of 0.9999 for the quantification of aciclovir in polymeric nanoparticles. Stanisz et al. (2012) showed linearity in the method for the quantification of moexipril hydrochloride in the pure form, pharmaceutical formulations, showing a correction coefficient of 0.999.

Precision

The precision of the method assesses the closeness of the results obtained in a multiple sampling references to the same sample. The results allusive repeatability (intra-day precision) and intermediate precision (inter-day precision) showed of 2.13 and 1.93%, respectively. The values contemplate the maximum limit accepted 5.0%, as recommended by RE 899, of 29/05/2013 (BRASIL, 2003).

The precision of the method for the quantification of different drugs was also observed in the works found in the literature and previously described. Roggia et al. (2016) showed values with the relative standard deviation (RSD) lower than 2.0% for all analyzed conditions, thus indicating a good Intra-day and inter-day precision of the method for the quantification of catechin and caffeine in guarana. RSD values below 2.0% were also observed by Stanisz et al. (2012) in the quantification of moexipril hydrochloride in the pure form and pharmaceutical formulations. Likewise, Tavares et al. (2011) showed intra-day and inter-day precision in the quantification of acyclovir in polymeric nanoparticles, with RSD values below 5%. In all these studies, the ultraviolet derivative showed

to be an accurate method for the quantification of different active compounds that normally it is present in the samples and it interferers in their quantification when the conventional (zero-order) spectrophotometric form is used.

Accuracy

With the purpose to ascertain the accuracy of the proposed analytical method, was used the method of standard addition. The resulting accuracy calculated as an average percentage was of 100.79% (Table 2), being in agreement with ICH, 2005, which orients that the content in recovered assets must be between 98 and 102% (ICH, 2005a; ICH, 2005b)

Table 2 - Results concerning the recovery test using the DUS.

	Concentration		Recuperation (%)	Average (%)
	Added (µg/mL)	Recovered (µg/mL)*		
R_1	2.50	2.60	103.97	
R_2	3.75	3.68	98.20	100.79
R_3	5.00	5.01	100.20	

* Each value represents the mean of three determinations.

The accuracy of the method using the ultraviolet derivative spectrophotometric method, with accuracy values ranging from 98 to 102%, were also observed by Roggia et al. (2016), Stanisz et al. (2012) and Tavares et al. (2012). Indicating that all methods show to be accurate for the quantification of different compounds using the derivative ultraviolet spectrophotometric method.

Robustness

The analysis of the robustness (Table 3) of the method by ANOVA and Tukey posttest demonstrated that the change in wavelength and the whether or not the membrane filter does not interfere in the analysis of the same. However, the variation of the degree PA to HPLC of solvent ethanol showed significant difference, therefore, the use of ethanol PA in the solubilization of actives was considered necessary condition for the analysis.

Table 3 - Robustness Evaluation of ultraviolet method derived by synephrine termination on nanocarriers lipid.

Filter*	Grade of solvent (ethanol)	Wavelength (nm)	Concentration (%)
A	PA	239	98.94
P	PA	239	94.87
P	HPLC	239	61.75
P	PA	237	104.66
P	HPLC	237	73.64
P	PA	241	86.36
P	HPLC	241	54.55
A	HPLC	239	59.64
A	PA	237	105.93
A	HPLC	237	71.02
A	PA	241	92.21
A	HPLC	241	52.74

*P=present, A=absent

The results show that the degree of purity of the solvent used in the solubilization of the active influenced the recovery percentage of the same. In contrast to the expected higher purity solvent, that is, the HPLC grade, showed a lower rate of recovery, when using ethanol grade PA, the recovery percentage was around 100%. No scientific explanation was found for this result.

In the other articles cited above, the robustness parameter was not evaluated, except for Roggia et al. (2016), but no significant difference was observed in the altered parameters in relation to the recommended condition, which were sample filtration, water purity and different ultrasonic times for the solubilization of the sample.

In our study, in general, all validation parameters were satisfactory for the quantification of a specific form of synephrine in lipid nanocarrier. Karpinska (2004) reports that the main disadvantage of the derivative method is the low reproducibility, a problem not reported in our research.

CONCLUSIONS

The presented method was developed and validated in accordance with the ICH (ICH, 2005a; ICH, 2005b) and the ANVISA (RE n° 899, 2003), presented the reliability required for an analytical method, besides showing as a quick alternative, safe and inexpensive when compared with other techniques. The method derivative ultraviolet spectrophotometry showed to be specific linear, precise and accurate for the quantitative analysis of synephrine in lipid nanocarrier (contend dihydromyricetin and diosgenin). As to the degree of ethanol solvent, the use of PA degree was considered condition necessary for the analysis. The method presented results of accuracy and precision similar when

compared with some techniques described in the literature to quantify SFN in extracts of *Citrus aurantium*, such as HPLC and gas chromatography. It is a simple tool than can be used for routine quality control in laboratories.

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