Validation of high performance liquid chromatography method for determination of meloxicam loaded PEGylated nanocapsules

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A method to ensure that an analytical method will produce reliable and interpretable information about the sample must first be validated, making sure that the results can be trusted and traced. In this study, we propose to validate an analytical high performance liquid chromatography (HPLC) method for the quantitation of meloxicam loaded PEGylated nanocapsules(M-PEGNC). We performed a validation study, evaluated parameters including specificity, linearity, quantification limit, detection limit, accuracy, precision and robustness. PEGylated nanocapsules were prepared by interfacial deposition of preformed polymer, and the particle size, polydispersity index, zeta potential, pH value and encapsulation efficiency were characterized. The proposed HPLC method provides selective, linear results in the range of 1.0-40.0 µg/mL; quantification and detection limits were 1.78 µg/mL and 0.59 µg/mL, respectively; relative standard deviation for repeatability was 1.35% and intermediate precision was 0.41% and 0.61% for analyst 1 and analyst 2, respectively; accuracy between 99.23 and 101.79%; robustness between 97.13 and 98.45% for the quantification of M-PEGNC. Mean particle diameters were 261 ± 13 nm and 249 ± 20 nm, polydispersity index was 0.15 ± 0.07 and 0.17 ± 0.06, pH values were 5.0 ± 0.2 and 5.2 ± 0.1, and zeta-potential values were -37.9 ± 3.2 mV e -31.8 ± 2.8 mV for M-PEGNC and placebo(B-PEGNC), respectively. In conclusion, the proposed analytical method is suitable for the quality control of M-PEGNC. Moreover, suspensions showed monomodal size distributions and low polydispersity index indicating high homogeneity of formulations with narrow size distributions, and appropriate pH and zeta potential. The extraction process was efficient for release of meloxicam from nanostructured systems.

Uniterms: High performance liquid chromatography/quantitative analysis. Meloxicam/determination. PEGylated nanocapsules/quality control. Nanoparticles. Poly(ethylene glycol).

Para se assegurar que um método analítico produzirá informação confiável e interpretável sobre a amostra este deve ser inicialmente validado, tornando claro que os resultados podem ser confiados e rastreados. Neste estudo, propomos validar um método de cromatografia líquida de alta eficiência (CLAE) para a quantificação do meloxicam encapsulado em nanocápsulas PEGuiladas (M-PEGNC). Efetuamos a validação, avaliando parâmetros de especificidade, linearidade, limite de quantificação, limite de detecção, exatidão, precisão e robustez. As nanocápsulas PEGuiladas foram preparadas por deposição interfacial do polímero pré-formado e caracterizaram-se o tamanho da partícula, índice de polidispersão, potencial zeta, pH e eficiência de encapsulação. O método de CLAE proposto fornece resultados seletivos e lineares na faixa de 1,0-40,0 mg/mL; limites de quantificação e detecção de 1,78 mg/mL e 0,59 mg/mL, respectivamente; desvio padrão relativo para a repetibilidade de 1,35% e precisão intermediária de 0,41% e 0,61% para o analista 1 e analista 2, respectivamente; exatidão entre 99,23 e 101,79%; robustez entre 97,13 e 98,45% para a quantificação de M-PEGNC. Os diâmetros médios das partículas foram 261 ± 13 nm e 249 ± 20 nm; índice de polidispersão de 0,15 ± 0,07 e 0,17 ± 0,06, valores de pH de 5,0 ± 0,2 e 5,2 ± 0,1 e valores do potencial zeta de -37,9 ± 3,2 mV e -31,8 ± 2,8 mV para o M-PEGNC e o placebo(B-PEGNC), respectivamente. Concluindo, o método analítico proposto é adequado para o controle de qualidade do M-PEGNC. Além disso, suspensões mostraram distribuição

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de tamanho monomodal e baixo índice de polidispersão, indicando alta homogeneidade das formulações com distribuição estreita de tamanho, pH e potencial zeta apropriados. O processo de extração foi eficiente para a liberação do meloxicam dos sistemas nanoestruturados.

Unitermos: Cromatografia líquida de alta eficiência/análise quantitativa. Meloxicam/determinação. Nanocápsulas PEGuiladas/controle de qualidade. Nanopartículas. Poli(etileno glicol).

INTRODUCTION

Biodegradable polymers, such as poly (ε-caprolactone) (PCL) and their copolymers diblocked or multiblocked with poly(ethylene glycol) (PEG), have been commonly used to form polymeric nanoparticles and to encapsulate a variety of therapeutic compounds (Chan *et al.*, 2010; Plapied *et al.*, 2011). Nanoparticles feature dimensions from one to hundreds of nanometers, and remarkable applications in biomedicine due to the unique way in which they interact with matter (Wagner *et al.*, 2006; Kim, Rutka, Chan, 2010). However, several challenges hamper the use of nanoparticles in clinical practice, such as their uptake by the reticuloendothelial system (Knop *et al.*, 2006). The addition of PEG to the surface of nanoparticles (PEGylation) can reduce many of these challenges. Furthermore, PEG is one of few polymers approved for internal use in humans by the FDA, which is an important consideration in developing new pharmaceutical formulations (USP-NF, 2013).

PEGylated nanoparticles increase the potential for drug delivery and targeting system, besides crossing the blood-brain barrier interacting with microvascular transport proteins. Also, PEG chains can create a barrier layer with the finality of camouflage the phagocytic cells, thus prolonging the circulation period in the blood (Owens III, Peppas, 2006; Grover, Hirani, Sutariya, 2013; Rabanel, Hildgen, Banquy, 2014). Moreover, PEG provides a system with adequate stability in physiological media, which is useful to target tumoral and inflamed regions (Beduneau *et al.*, 2007; Diaz-Lopez *et al.*, 2010; Hervella *et al.*, 2014). Accordingly, the nanoencapsulation of anti-inflammatory drugs is very promising for use as adjuvant treatment of various diseases. In this context, meloxicam [4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide] is a nonsteroidal anti-inflammatory drug with analgesic and antipyretic effects that preferentially inhibits cyclooxygenase-2 (Megan, 2006).

However, nanoencapsulation requires considerable quality control. The choice of an appropriate analytical methodology is crucial for efficient quality control of an active substance, as such, or in a certain pharmaceutical form (Ermer, 2001). In order to ensure that an analytical method will generate reliable and interpretable information about a sample, this method must first be validated, ensuring the reliability and traceability of results (Ribani *et al.*, 2004). In fact, according to Brazilian legislation, an analytical methodology for analysis of a drug or pharmaceutical form should be validated when it is not described in pharmacopeias or official forms recognized by the National Health Surveillance Agency (ANVISA, 2003). No previous data in the literature demonstrated the development and validation of a quantitative bioanalytical high-performance liquid chromatography (HPLC) method for the quantification of meloxicam-loaded PEGylated nanocapsules(M-PEGNC).

Moreover, experimental studies should ensure that the method meets the requirements of analytical applications, demonstrating the reliability of the results (ANVISA, 2003). The characterization of nanostructured materials is carried out by different methods. These methods are essential to determine the drug content incorporated into them. Some methods for the quantification of meloxicam in pharmaceutical forms by HPLC method are described in the literature (Zhang, Choi, 2008; Bandarkar, Vavia, 2009; Sahoo *et al.*, 2014). However, there is no specific methodology for nanostructured systems containing meloxicam. Therefore, in this study, we revalidate the analytical HPLC method for quantitation of M-PEGNC prepared according to the interfacial deposition of preformed polymer. This method was validated according to the official guidelines (ANVISA, 2003; ICH, 2005).

MATERIAL AND METHODS

Reagents and chemicals

Meloxicam (Henrifarma® - Brazil). Caprylic/capric triglyceride and polysorbate 80 (Delaware® - Brazil). Span 60® (sorbitan monostearate) (Sigma Aldrich® - USA). Polyethylene glycol 4000 (PEG) (Galena® – Brazil). Poly(ε-caprolactone) (PCL) (70000) (Sigma-Aldrich® - USA). HPLC-grade methanol (Sigma-Aldrich® - Venezuela). Milli-Q Water (Millipore

Corporation®). All other chemicals and solvents used were of analytical or pharmaceutical grade. All reagents were used as received.

Preparation of loaded PEGylated nanocapsules

M-PEGNC were prepared by interfacial deposition of preformed polymer according to Fessi *et al.* (1989). Meloxicam was dissolved in acetone with the polymer (PCL), the oil (caprylic/capric triglyceride) and sorbitan monostearate at 40 ºC. This organic phase was poured into an aqueous phase composed of polysorbate 80 and PEG under stirring, leading to the immediate formation of a milky dispersion. Lastly, the organic solvents were removed under vacuum, and the M-PEGNC was concentrated to 0.3 mg/mL (w/v) and a fixed volume of 25 mL. The placebo suspension (drug-unloaded B-PEGNC) was prepared as described above without adding meloxicam.

Physico-chemical characterization of loaded PEGNC

The particle size, polydispersity index and zeta potential of M-PEGNC and B-PEGNC were measured by photon correlation spectroscopy. Samples were diluted in Milli-Q water and the analysis was performed at 25 ºC, using a Zetasizer® (Nanoseries, Malvern, UK). The pH values of the suspensions were determined using a Denver® (Ultrabasic) potentiometer. Each sample was analyzed in triplicate.

Extraction of meloxicam from the nanocapsules

After preparation, several tests were performed for drug extraction from nanocapsules with change of solvents, ultrasound, agitation, heating and centrifugation time (Table I). The amount of meloxicam was determined by the straight-line equation of the standard curve obtained.

Encapsulation efficiency of M-PEGNC

The amount of the encapsulated drug was determined in the clear supernatant following separation of the M-PEGNC from the aqueous medium by a combined filtration and centrifugation technique. The meloxicam content in the M-PEGNC was calculated by the difference between the total and free estimated drug concentrations. Measurements were performed in triplicate for all prepared batches.

TABLE I - Altered parameters for drug extraction from the nanocapsules

Parameters	Variations
Solvents (used alone or mixed in various proportions)	methanol acetonitrile ethanol tetrahydrofuran NaOH
Initial ultrasound time and after shaking	5 minutes 10 minutes 15 minutes 20 minutes
Shaking time	30 minutes 60 minutes
Centrifugation time	15 minutes 20 minutes
Heating time	37 ºC e 40 ºC - 10 minutes 37 ºC e 40 ºC - 15 minutes 37 ºC e 40 ºC - 30 minutes

Chromatographic conditions and instruments

The assay was developed and validated on a Shimadzu LC system (Kyoto Japan), with an LC-20AT pump, SIL-20A ht automatic injector, CTO-20AC column oven, SPD-M20A photodiode array detector (PDA) and CBM-20A controller with LC solution software. Chromatographic separations were achieved using a Phenomenex® Luna C18 (250 x 4.6 mm, 5 µm) column. The mobile phase and diluent contained a mixture of methanol:aqueous phosphoric acid (1%, v/v) pH 2.5 (70:30 v/v), 1.0 mL/min flow, PDA detection at 360 nm (Zhang, Choi, 2008; Patel *et al.,* 2011). The injection volume was 20 µL and the column temperature was set at 25 ºC.

Validation study

Parameters evaluated included specificity, linearity, quantification limit, detection limit, accuracy, precision and robustness.

System suitability

System suitability was evaluated by five replicate analyses of a meloxicam reference substance and sample at a concentration of 10.0 µg/mL. The parameters calculated were: number of theoretical plates, tailing factor and asymmetry.

Specificity

To assess the method specificity, a solution of

meloxicam reference chemical substance (RCS) and placebo (B-PEGNC) was prepared. All analyses were performed in triplicate. The purity of the chromatographic peak with respect to meloxicam was determined with the aid of PDA.

Linearity and range

Method linearity was evaluated through a calibration curve in the 1.0-40.0 µg/mL concentration range. An initial solution of meloxicam (1000 µg/mL) was prepared in a volumetric flask with methanol and 1 mL NaOH. This solution was diluted appropriately in the range from 1.0 to 40.0 µg/mL, with an average concentration of 10.0 µg/mL. The linear working range was obtained by plotting the area ratio of meloxicam as a function of the meloxicam concentration, and a least square linear regression analysis was conducted.

Quantification limit and detection limit

The quantification and detection limits were based on standard deviation of response and mean slope of three calibration curves.

Precision and accuracy

The precision assay was investigated with respect to repeatability (intra-day) and intermediate precision (inter-day). The repeatability was evaluated by assaying six determinations at a concentration of 10.0 µg/mL, during same day and under the same experimental conditions. The same analysis was performed on 3 different days to assess intermediate precision. Precision was expressed as % of relative standard deviation (RSD).

Accuracy was evaluated by the amount of meloxicam RCS. The study was performed by adding a known amount of meloxicam standard solution to sample solution, to obtain the final concentrations of 5.0, 7.0, 10.0, 12.0 and 15.0 µg/mL. The experiments were repeated three times.

Robustness

Four analytical parameters were selected and small variations were induced in nominal values of the method. The parameters employed, as well as the variations introduced are shown in Table II.

An appropriate amount was transferred into an individual 10 mL volumetric flask, diluted to volume with diluents, and filtered through a 0.45 µm membrane filter (Millipore, Bedford, USA), obtaining the final concentration of 10.0 µg/mL of the active pharmaceutical ingredient. The concentrations of meloxicam presented in samples were determined from the standard curve.

TABLE II - Analytical parameters and variations to evaluate the robustness of the chromatographic method for meloxicam quantification

Parameter	Variation	
Mobile phase flow rate(mL min^{-1})	1.2	0.8
Column supplier	Phenomenex	Gemini
Column temperature (ºC)	25	30
Mobile phase pH	2.3	2.8

RESULTS AND DISCUSSION

In this study, we developed a suspension that enables controlled release through nanocarriers. M-PEGNC consisting of an oily core stabilized with a PEG, M-PEGNC was characterized with respect to their mean particle diameter, polydispersity index, pH, zeta potential and encapsulation efficiency. Furthermore, the analytical HPLC method was validated for quantitation of M-PEGNC. In fact, nanostructured systems coated with polymers, such as PEG, can increase drug half-life in the blood circulation, prevent opsonizing proteins from adhering to the nanomaterial surface, and reduce rapid metabolism and clearance (Kim, Rutka, Chan, 2010; Bender *et al.*, 2012).

Physico-chemical characterization of loaded PEG-NC

We designed M-PEGNC based on a PCL and capric/caprylic triglyceride materials known to be useful for the encapsulation of drugs (Jornada *et al.*, 2012). In fact, PCL and PEG meet drug delivery device requirements of biodegradability, biocompatibility and absence of immunogenicity (Chan *et al.*, 2010; Lepeltier, Bourgaux, Couvreur, 2014). M-PEGNC and B-PEGNC suspensions were prepared by interfacial deposition of preformed polymer and did not require subsequent purification. Both suspensions (M-PEGNC and B-PEGNC) were macroscopically homogeneous bluish-white opalescent liquids.

After preparation, the mean particle diameters (Z-average) were 261 ± 13 nm for M-PEGNC and 249 ± 20 nm for B-PEGNC (Figure 1). In fact, the mean particle sizes of nanocapsules prepared from pre-formed polymers are generally between 250 and 500 nm (Mora-Huertas, Fessi, Elaissari, 2010). Furthermore, particle sizes smaller than 500 nm and composed of biocompatible materials are very important characteristics for therapeutic applications (Bender *et al.*, 2012). However, the size of

individual particles tested for drug delivery of therapeutic and imaging agents may range from 2 to 1000 nm (Plapied *et al.*, 2011). According to Kreuter (2014), for pharmaceutical purposes nanoparticles ranging in size from 1 to 1000 nm consist of macromolecular materials in which the active principle is dissolved, entrapped, or encapsulated, or to which the active principle is adsorbed or attached.

The suspensions showed monomodal size distributions and a polydispersity index of 0.15 ± 0.07 for M-PEGNC and 0.17 ± 0.06 for B-PEGNC, indicating that the formulations were highly homogeneous with narrow size distributions. Accordingly, nanoparticles produced by the nanoprecipitation method feature a well-defined size and are characterized by an instantaneously formed narrow distribution (Rao, Geckeler, 2011). The pH values were 5.0 ± 0.2 for M-PEGNC and 5.2 ± 0.1 for B-PEGNC.

In general, pH values of nanocapsule dispersion are within a range of 3.0–7.5 when nanoprecipitation occurs. Furthermore, the medium pH may play an important role in relation to the size of the nanoparticles as well as their biodistribution (Mora-Huertas, Fessi, Elaissari, 2010).

Zeta potential is an indicator of surface charge, which determines particle stability in dispersion (Win, Feng, 2005). The zeta-potential values were -37.9 ± 3.2 mV e -31.8 ± 2.8 mV, for M-PEGNC and B-PEGNC respectively (Figure 2). The zeta-potential of nanocapsules depends mainly on the chemical nature of polymer and stabilizing agent, and medium pH (Mora-Huertas, Fessi, Elaissari, 2010). Physicochemical analyses demonstrated that suspensions showed homogeneity with a narrow distribution of mean diameter and low polydispersity. Additionally, suspensions demonstrated appropriate zeta potential and pH. According to a study by Win and Feng
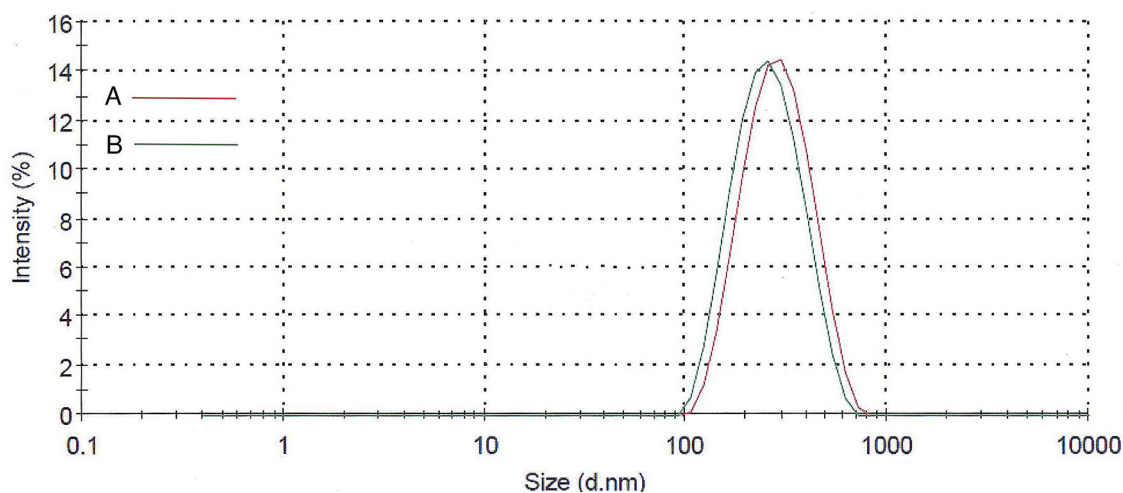

FIGURE 1 - Particle diameters of placebo and M-PEGNC.(A) M-PEGNC; (B) Placebo(B-PEGNC).
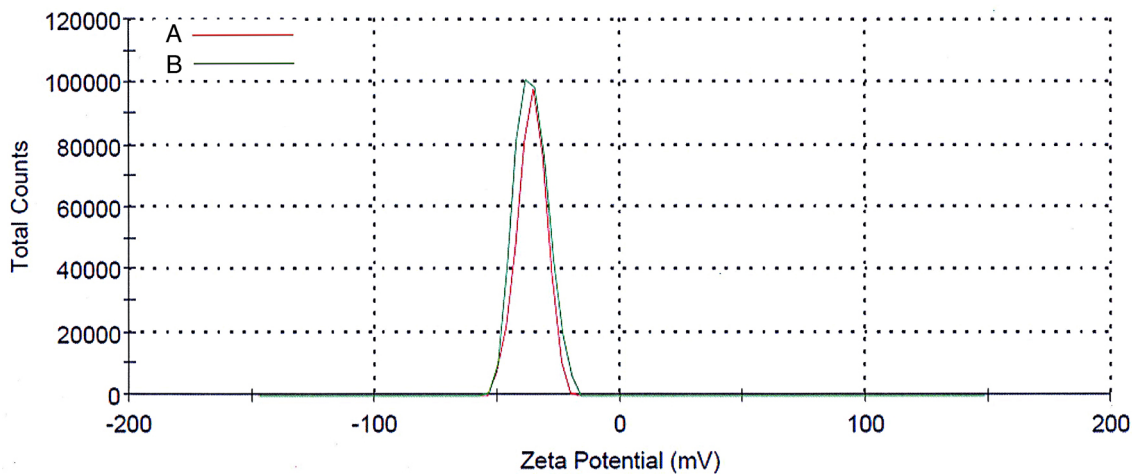

FIGURE 2 - Zeta-potential placebo and M-PEGNC. (A) Placebo B-PEGNC; (B) M-PEGNC.

(2005), stable nanoparticules were found in a state of dispersion, with high absolute values of zeta potential and negative surface charges.

Extraction of M-PEGNC

Several tests were performed for the extraction of meloxicam from the nanocapsules. The best way to mine meloxicam in several tests was by adding nanocapsules in suspension in a volumetric flask with 3 mL methanol and subjecting it to ultrasound for 15 minutes, followed by heating at 40 °C for another 15 minutes. Next this solution was subjected to shaking for 60 minutes. To this solution were added 1 mL NaOH 0.5 M methanolic and 6 mL tetrahydrofuran and it was subjected to ultrasound for another 15 minutes. Then, this solution was added in another volumetric flask and supplemented with methanol. This solution was centrifuged at 4000 rpm for 10 minutes in order to extract the drug present in the nanostructures. The changes in some method parameters, such as solvents, ultrasound, agitation, heating and centrifugation during the optimization of drug extraction process from nanocapsules were rejected for different reasons, e.g. precipitation of the polymer or drug degradation. The extraction process proved to be efficient and the proposed method was simple and efficient for quantification and quality control of M-PEGNC.

Quantification of M-PEGNC

The extraction method described above was used to determine meloxicam in nanocapsules. After extraction,

the samples were filtered and then analyzed by HPLC, using the previously validated method. The theoretical concentration obtained was 10.0 mg/mL.

Encapsulation efficiency of M-PEGNC

The calculation of the difference between total amount of drug present in the suspension and free drug found in the supernatant after centrifugation was used to check the encapsulation efficiency of M-PEGNC. The encapsulation efficiency was close to 99% for all batches. Agreement about nanoprecipitaton methods generated good results for nanocapsule encapsulation (80% or more). In addition, drug encapsulation efficiency is determined by different factors, such as chemical nature and polarity of the drug (Mora-Huertas, Fessi, Elaissari, 2010).

Validation method

System suitability

To obtain the best chromatographic method, the mobile phase containing methanol and aqueous phosphoric acid (70:30 v/v, pH 2.5) was utilized to provide adequate peak and satisfactory results. A 250 mm length octylsilane column was able to obtain a resolution and peak asymmetry and short analysis time.

Specificity

The chromatograms obtained with the specificity test (Figure 3), showed that none of the formulation excipients eluted in the same retention time as the
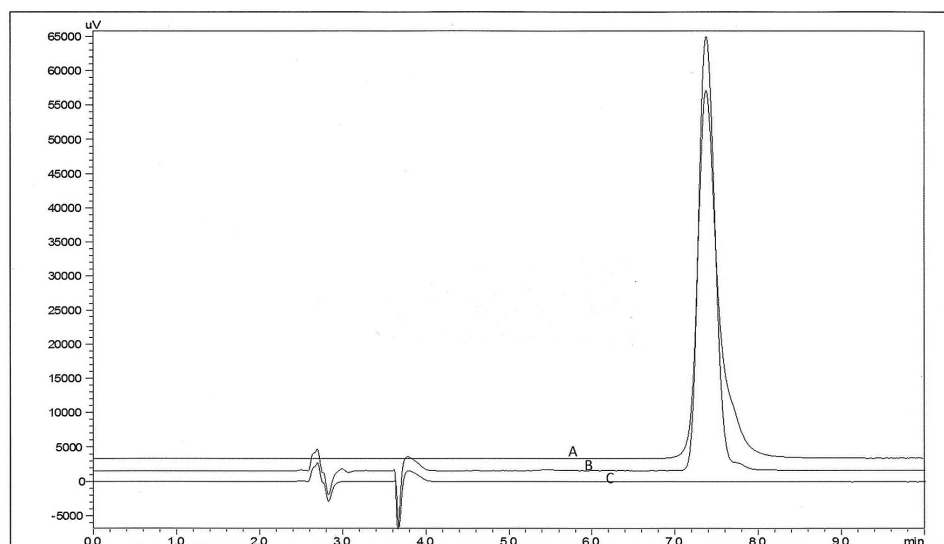

FIGURE 3 - Chromatograms corresponding to: (A) solution of meloxicam reference substance; (B) M-PEGNC; (C) Placebo (B-PEGNC).

meloxicam. Therefore none of the excipients interfered in the quantitative analysis of the meloxicam.

Linearity and range

Linearity corresponds to the ability of the methodology to demonstrate that values obtained are directly proportional to the drug concentration in the sample within a specified range. In fact, it is recommended that it be composed of at least five concentration levels within a range corresponding to at least 80 to 120 % of the working concentration (ANVISA, 2003; ICH, 2005).

Linearity was observed over the concentration range of 1.0 - 40.0 µg/Ml (Figure 4), with a correlation coefficient of r = 1.0000 according to the criteria established for r = 0.99 and the linear regression equation y = 72888x –2717 (where, x is concentration and y is the peak absolute area). Assay validity was verified by means of the ANOVA. According to the statistical data, there is a linear relationship between the variables and there is no deviation from linearity (p<0.05). It was shown that it could be used for interpolation of the experimental values, aiming at quantitative determination of this substance. Quantification and detection limits were 1.78 µg/mL and 0.59 µg/mL respectively, showing method sensibility.
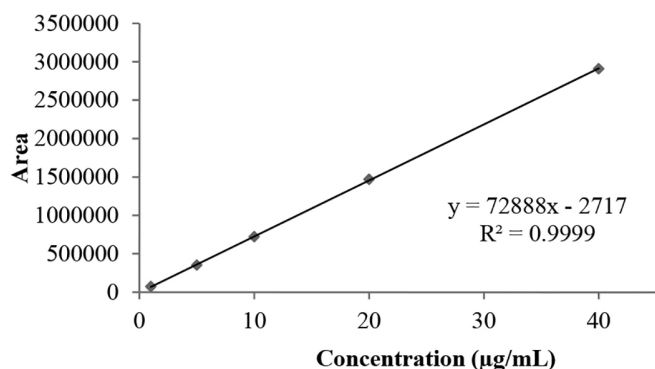

FIGURE 4 - Graphic representation of the average standard curve reference chemical substance(RCS) meloxicam.

Precision and accuracy

Precision is the scattering of results among different studies, repeated from the same sample, or samples of similar patterns under defined conditions (ANVISA, 2003).

Method precision was evaluated as repeatability and intermediate precision and was expressed as RSD %. Mean results were 1.35% and 0.51% of RSD respectively. The limit is RSD maximum of 2.0% (USP, 2012).

Experimental results for intermediate precision were evaluated statistically by analysis of variance (ANOVA) in order to determine whether there is a significant difference

in the values obtained on different days. As shown in Tables III and IV, the value obtained for calculated F is lower than the tabulated value F, indicating no significant difference between the results for p<0.05.

TABLE III - Analytical parameters and variations to evaluate the repeatability of the chromatographic method for meloxicam quantification

Day	n	Repeatability Content of the sample (%)
		Analyst 1
	1	98.90
	2	96.41
	3	95.51
1	4	96.43
	5	95.90
	6	95.33
Mean (%) ± SD		96.41 ± 1.30
RSD (%)		1.35

**p<0,05; S*D – standard deviation; RSD – relative standard deviation.

From RSD values obtained, it can be seen that the developed method provides an accurate determination to show when it was performed on different days and by different analysts, and adequate repeatability for analysis on the same day.

The test was carried out accurately by retrieving the amounts of RCS added to the sample, obtaining a mean value of 100.25% (Table V). The recommended value for different levels of concentration must be in the range of 98.0% to 102.0% (ANVISA, 2003; ICH, 2005). The method showed adequate strength when subjected to small variations in the parameters established.

Robustness

After variation of some parameters of the method proposed, these variations were not able to significantly change the meloxicam content in the sample, average levels of meloxicam ranging from 97.13 to 98.45%.

The validation methods ensure credibility during routine use and, sometimes, they are referred to as the process that provides documented evidence of method (USP, 1999). In addition, ICH, ANVISA and other agencies require the validation of analytical methods as a key requirement in the accreditation for quality assurance and demonstration of technical competence (ANVISA, 2003; ICH, 2005). The proposed liquid chromatography method provides selective, linear, precise, accurate and

TABLE IV - Analytical parameters and variations to evaluate the intermediate precision of the chromatographic method for meloxicam quantification

Day	n	Intermediate precision Content of the sample (%)		Mean ± SD	RSD (%)
		Analyst 1	Analyst 2		
1	1	97.11	96.78		
	2	97.12	97.69	97.15 ± 0.45	0.47
	3	97.65	96.56		
2	1	97.18	97.72		
	2	96.94	96.70	97.00 ± 0.44	0.45
	3	96.44	97.03		
3	1	97.14	98.32		
	2	97.27	97.77	97.42 ± 0.44	0.45
	3	97.84	97.36		
Mean Overall (%) ± SD			97.26 ± 0.49		
RSD Overall (%)			0.51		
ANOVA		F *calculated*	F *tabulated**		
Between days		3.13	3.68		

*$p<0,05$; SD – standard deviation; RSD – relative standard deviation.

TABLE V - Experimental values obtained for the accuracy test

Samples	Final concentration (µg/mL)	Average number of RCS recovered*	
		(µg/mL)	(% ±SD)
R1	7	6.95	99.23 ± 0.12
R2	10	10.07	100.70 ± 0.29
R3	12	12.41	99.30 ± 0.12
R4	15	15.27	101.79 ± 0.47

*average of 3 determinations; SD – standard deviation.

robust results. Furthermore, the method is linear over a wide range, inexpensive and utilizes a mobile phase that can be easily prepared. Moreover, some studies indicated that use of methanol as an organic solvent is less harmful for the environment and it is even considered favorable when a methanol-water mixture is used (Capello, Fischer, Hungerbuhler, 2007). A 250 mm length octylsilane column was able to obtain a resolution, peak asymmetry and short analysis time. All these factors make this method suitable for quantification of M-PEGNC.

CONCLUSION

In conclusion, the suspensions showed mean particle sizes according to the literature, monomodal size distributions and low polydispersity index indicating highly homogeneous formulations with narrow size distributions and appropriate pH and zeta potential. The extraction process proved to be efficient for the release of meloxicam nanostructured systems. The proposed liquid chromatography method provides selective, linear, precise, accurate and robust results for the quantification of M-PEGNC. The proposed method is suitable to determine meloxicam in the quality control for M-PEGNC.

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