Pharmacogn. Mag.
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ORIGINAL ARTICLE

Innovative Phytoformulation Containing Capsaicinoids: Microparticles Development, Analytical Method Validation, and Anti-ulcer Effect

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Submitted: 03-05-2017 Revised: 13-04-2018 Published: 14-08-2018

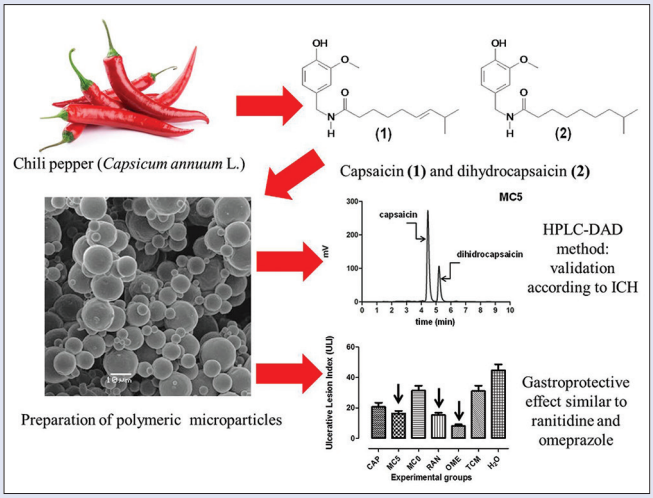
ABSTRACT

Background: *Capsicum annuum* L. and *Capsicum frutescens* L., of family *Solanaceae*, contain capsaicinoids with several pharmacological effects. However, their pungency limits the long-term use through the gastrointestinal tract. **Objective:** To obtain phytoformulations of microparticles containing capsaicinoids for reducing their pungency by oral route to achieve an anti-ulcer effect. **Materials and Methods:** Microparticles of poly(ε-caprolactone) containing capsaicinoids were prepared by simple emulsion/solvent evaporation. An optimized reverse-phase high-performance liquid chromatography method with ultraviolet (UV) detection for quantifying capsaicinoids into microparticle phytoformulations was then developed and validated. Chromatographic conditions consisted of a C18 reverse-phase analytical column (250 mm × 4.60 mm, 5 μm) using a mixture of acetonitrile and water (70:30 v/v) adjusted to pH 4.5 as mobile phase at a flow rate of 0.75 mL/min with UV detection at 280 nm. The developed method was validated as per the ICH guidelines with respect to specificity, linearity, limit of quantification, limit of detection, accuracy, precision, and robustness. The gastroprotective activity of pure capsaicinoids and loaded microparticles against ethanol-induced gastric ulcer in rats was performed. **Results:** Microparticles were successfully obtained. Analytical validation provided suitable results regarding all parameters investigated. These phytoformulations presented suitable encapsulation efficiency higher than 90%. Regarding the ulcerative lesion index (ULI) scores, poly(ε-caprolactone) (PCL) microparticles containing 5% of capsaicinoids (ULI = 16.3 ± 1.8 points) was statistically similar ($P > 0.05$) to ranitidine (ULI = 15.3 ± 1.4 points) and omeprazole (ULI = 8.0 ± 1.2 points). **Conclusion:** Capsaicinoids-loaded PCL microparticles reveal the potential to be suitable candidates as controlled drug delivery system for the therapeutic management of ulcer.

Key words: Anti-ulcer activity, capsaicin, dihydrocapsaicin, gastric protection, low-irritative oral preparation, poly(ε-caprolactone)

SUMMARY

- Capsaicinoids-loaded poly(ε-caprolactone) microparticles were successfully prepared by simple emulsion/solvent evaporation
- A reverse-phase high-performance liquid chromatography method using acetonitrile and water (70:30 v/v) adjusted to pH 4.5 at a flow rate of 0.75 mL/min with ultraviolet detection at 280 nm was used for quantifying capsaicinoids into phytoformulations
- Analytical validation provided a linear calibration curve in the range of 10.0–50.0 μg/mL with a correlation coefficient higher than 0.999
- High encapsulation efficiency (>90%) was obtained for microparticles containing capsaicinoids
- Capsaicinoids-loaded poly(ε-caprolactone) microparticles showed gastroprotective activity statistically similar to ranitidine and omeprazole ($P > 0.05$) by the ethanol-induced gastric ulcer method.



Abbreviations used: CAP: Pure capsaicin; EE: Encapsulation efficiency; HPLC: High-performance liquid chromatography; LOD: Limit of detection; LOQ: Limit of quantitation; MC0: Unloaded microparticles; MC3: Capsaicinoids-loaded poly(ε-caprolactone) microparticles (3%); MC5: Capsaicinoids-loaded poly(ε-caprolactone) microparticles (5%); MC10: Capsaicinoids-loaded poly(ε-caprolactone) microparticles (10%); OME: Omeprazole; PCL: Poly(ε-caprolactone); PVA: Poly (vinyl alcohol); PTFE: Polytetrafluoroethylene; RAN: Ranitidine; RSD: Relative standard deviation; SD: Standard deviation; SEM: Standard error of mean; TCM: Caprylic/capric triglycerides; TRPV: Transient receptor potential vanilloid; TRPV1: Transient receptor potential vanilloid 1; ULI: Ulcerative lesion index

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DOI: 10.4103/pm.pm_160_17

Access this article online
Website: www.phcog.com
Quick Response Code:

Cite this article as: Almeida MA, Nadal JM, Klein T, de Paula JP, Budel JM, Novatski A, *et al*. Innovative phytoformulation containing capsaicinoids: Microparticles development, analytical method validation, and anti-ulcer effect. Phcog Mag 2018;14:290-6.

INTRODUCTION

Chili peppers or red peppers (*Capsicum annuum* L. and *Capsicum frutescens* L., *Solanaceae*) are widely used all over the world because of their spicy taste. These species contain capsaicinoids which are naturally occurring alkaloids that are responsible for spicy and pungent taste. Capsaicinoids are primarily comprised of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homohydrocapsaicin, homodihydrocapsaicin, and nonivamide. In general, capsaicin and dihydrocapsaicin [Figure 1] account for almost 90% of capsaicinoids and they are the main compounds of commercial *C. annuum* extracts.[1,2] Their pungency is related to the binding of capsaicinoids to transient receptor potential vanilloid (TRPV) ion-channel receptors.[3] Capsaicin shows a high affinity to TRPV1 receptor[4] however other capsaicinoids also have heat sensation through TRPV1 receptor. Moreover, some of other biological activities of capsaicin such as its antineoplastic and cardioprotective effects have been reported to be independent of TRPV1 receptor. Capsaicinoids can also be topically used for relief of chronic pain and some works describe their use for weight loss.[5-7]

In spite of these pharmacological properties and their biomolecular effects, capsaicinoids are very irritating substances causing pain and burning on skin and mucosae. In particular, capsaicinoids may produce increased salivation, gastric secretion, and gastrointestinal disorders when administered orally.[8] In that sense, a long-term use on skin or through the gastrointestinal tract is restricted due to pungency of capsaicinoids.[9]

To improve the use of capsaicinoids, this paper designed the formulation of innovative microparticles containing capsaicinoids aiming at reducing pungency by oral route due to a controlled release pattern. In recent years, polymeric microparticles have aroused increasing concern as drug delivery systems. Many authors reported that microparticles can be used to deliver active compounds and natural products by oral route and these formulations are particularly interesting for the development of controlled release dosage forms. They also play an important role as drug carriers aiming at improved drug stability and minimizing side effects as pungency. Various methods are readily available for microencapsulation of active compounds and one of the most commonly used is the simple emulsion/solvent evaporation approach.[10]

In our laboratories, experiments are being carried out to formulate capsaicinoids-loaded polymeric microparticles using the simple emulsion/solvent evaporation technique and poly(ε-caprolactone) (PCL), a biocompatible and biodegradable polyester. However, an analytical validation procedure is required for assaying capsaicinoids from these formulations at the same time.

The literature reports few high-performance liquid chromatography (HPLC) methods for determining capsaicinoids from different sample types. These papers are mainly devoted to describing HPLC methods for isolation and analytical and bioanalytical quantification of capsaicinoids from foods, herbal extracts, pharmaceutical formulations, and plasma.[11-17] In that sense, no work was found on validation of analytical methods for determining capsaicinoids from microparticles formulations as proposed in this paper.

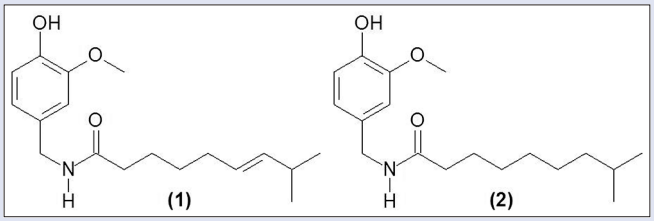

Figure 1: Chemical structures of capsaicin (1) and dihydrocapsaicin (2)

In addition, a classical paper demonstrated that capsaicin given into pylorus-ligated stomachs at small doses and low concentrations remarkably reduced ulcer formation in rats.[1] Ward *et al.*[18] established that capsaicin demonstrated TRPV1 activation on nerves of gastrointestinal (GI) tract and acted in nonnervous tissues like gastric epithelial cells as well and stimulate the secretion of gastrin.[19] Activated TRPV1 releases calcitonin gene-related peptide and activates cyclooxygenase-1 enzymes, which have gastroprotective effects.[20,21] However, gastrointestinal effects of capsaicin are dose-dependent and there is evidence of negative effects of high doses of capsaicin.[2] In our hypothesis, microencapsulation of capsaicinoids using PCL can provide a prolonged release in the upper region of GI tract providing a gastroprotective effect against gastric mucosal injuries.

Taking all the above into account, the aim of this paper was to obtain capsaicinoids-loaded PCL microparticles by simple emulsion/solvent evaporation method and to develop and validate a fast, simple, and optimized reverse-phase HPLC method with ultraviolet (UV) detection for quantifying capsaicinoids in microparticle formulations. Other experiments were also performed to explore the potential gastroprotective activity of free and microencapsulated capsaicinoids against ethanol-induced gastric ulcer model in rats.

MATERIALS AND METHODS

Reagents and chemicals

Capsaicinoids were purchased from Valdequímica Produtos Químicos (São Paulo, Brazil) as a mixture of 73.1% capsaicin and 26.9% dihydrocapsaicin. PCL (Mw 70,000–90,000 g/mol, Sigma Aldrich, St. Louis, MO, USA) and poly (vinyl alcohol) (Mw 72,000 g/mol, 88.5 mol% of hydrolysis, Vetec, Rio de Janeiro, Brazil) were used as received. As an internal standard, *E*-capsaicin, matrix substance ≥99.0% (HPLC), was purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile was also provided by Sigma-Aldrich (St. Louis, MO, USA). Water was purified in a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA). All others reagents and solvents were of analytical grade.

Equipment

HPLC analysis was performed using a Merck-Hitachi Lachrom (Tokyo, Japan) system equipped with an Interface D-7000, a UV detector module L-74,000, a quaternary pump L-7100, and an integral degasser. Chromatography software platform was ChromQuest 5.0 (Thermo Fisher Scientific, San Jose, CA, USA). A manual injector (Rheodyne) system equipped with a 20 µL injector loop and a 100 µL syringe (Hamilton, Microliter 710) was used.

Preparation of capsaicinoids-loaded poly(ε-caprolactone) microparticles

Microparticles containing capsaicinoids were obtained by simple emulsion/solvent evaporation method.[22] Three formulations [Table 1] were prepared depending on the amount of capsaicinoids in their compositions (3, 5 and 10%). Briefly, the organic phase was added into the aqueous phase under mechanical stirring (3500 rev/min) for 5 min. Emulsion was kept under mechanical stirring (1000 rev/min) at room temperature for 6 h. After evaporating organic solvent, microparticles were separated by centrifugation (2500 rev/min, 10 min), washed twice with purified water and dried under vacuum at 40°C ± 1°C for 6 h. The samples were stored into a desiccator under vacuum at room temperature. All formulations were obtained in triplicate from independent batches. Unloaded-microparticles (MC0) were also prepared as negative control.

Table 1: Composition of poly(ε-caprolactone) microparticles

Composition	Formulation			
	MC0	MC3	MC5	MC10
Aqueous phase				
Polysorbate 80 (g)	0.25	0.25	0.25	0.25
PVA (g)	4.00	4.00	4.00	4.00
Purified water (mL)	200.0	200.0	200.0	200.0
Organic phase				
Capsaicinoids (g)	–	0.06	0.10	0.20
PCL (g)	2.00	1.94	1.90	1.80
Methylene chloride (mL)	40.0	40.0	40.0	40.0

PCL: Poly(ε-caprolactone); PVA: Poly (vinyl alcohol); MC0: Unloaded microparticles; MC3: Capsaicinoids-loaded PCL microparticles (3%); MC5: Capsaicinoids-loaded PCL microparticles (5%); MC10: Capsaicinoids-loaded PCL microparticles (10%)

Chromatographic conditions

Validation and drug-loading experiments were performed in the previously described HPLC system using a LiChrospher® RP-18 analytical column (Thermo Fisher Scientific, Waltham, MA, USA) with 5-μm particle size, 4.6-mm internal diameter, and 250 mm length at 30°C ± 2°C. Mobile phase consisted of acetonitrile: water (70:30 v/v), pH 4.5, adjusted with acetic acid at an isocratic flow rate of 0.75 mL/min. The sample injection volume was 20 μL. Capsaicinoids were monitored at 280 nm. The method run time was 10 min and all experiments were carried out in triplicate.

Preparation of standard solutions

A stock standard solution (1 mg/mL) was daily prepared by dissolving 50 mg of capsaicinoids into a 50-mL volumetric flask using ethanol. This solution was further diluted in ethanol to prepare seven different working standard solutions ranging from 10.0 to 50.0 μg/mL. These solutions were filtered through a polytetrafluoroethylene membrane filter (Cromafil® Xtra, 0.45 μm × 25 mm, Macherey-Nagel, Düren, Germany) before injection into the HPLC system.

Method development

Detection wavelength for HPLC study was selected as 280 nm. Chromatographic conditions were optimized for resolution of the peaks of capsaicin and dihydrocapsaicin by varying the composition and proportion of the mobile phase. Samples of different formulations were used to optimize the chromatographic conditions for resolving capsaicinoids. An appropriate negative control was injected before the analysis of all samples. Method was then validated and used for determination of capsaicinoids into PCL microparticles.

Method validation

Validation studies were performed using the optimized chromatographic conditions based on the principles of validation described in the International Conference Harmonization guidelines.[23] The method was validated for specificity, linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision, and robustness.

The specificity was determined by analyzing the chromatograms of unloaded microparticles (MC0) in comparison with those obtained for capsaicinoids-loaded PCL microparticles (MC3, MC5, and MC10) aiming at confirming that none of the excipients interfere with the quantitation of capsaicinoids.

The linearity was determined by calculating a regression line from plotting peak area versus concentration of the working standard solutions prepared at seven concentration levels (10.0, 20.0, 25.0, 30.0, 35.0, 40.0, and 50.0 μg/mL) using least-squares linear regression analysis. The linearity test was performed for 3 consecutive days in the same concentration range. The solutions were injected in triplicate into the HPLC column keeping the injection volume constant (20 μL) and chromatograms were recorded. The standard deviation (SD) value for the slope and Y-intercept of the calibration curve were calculated.

LOD and LOQ were calculated based on the SD of the response (δ) and the slope (S) of the calibration curve and were expressed as 3.3 δ/S and 10 δ/S for LOD and LOQ, respectively.

The accuracy of the analytical method was investigated by spiking unloaded microparticles (MC0) with known concentrations of the stock solution to achieve final theoretical drug concentrations of 22, 32, and 42 μg/mL. The accuracy value was determined by calculating the percent recovery of capsaicinoids for these three concentration levels and then determining the relative SD (RSD).

The precision was assessed at two levels: Repeatability (intraday precision) and intermediate precision (inter-day precision) using MC5. The repeatability was investigated by testing three different sample solutions at 10, 30, and 50 μg/mL on the same day. Three samples solutions at 30 μg/mL were also evaluated in other two different days to determine intermediate precision. Results were reported in terms of RSD.

To determine robustness, experimental conditions were purposely changed to check the reproducibility of the method. Robustness was evaluated by analyzing drug content of the microparticles (MC5) with variations in the temperature of analytical column (35°C and 45°C), flow rate (0.65 and 0.85 mL/min), and chromatographic column was replaced by a similar Waters XTerra C_{18} column (250 mm × 4.6 mm, 5 μm). Samples were evaluated in triplicate for each variation of the method conditions. Chromatograms were recorded and compared with the previously reported chromatographic conditions.

Evaluation of drug-loading and encapsulation efficiency

To demonstrate the applicability of the validated method, drug content and encapsulation efficiency (EE) of capsaicinoids into PCL microparticles was performed.

For capsaicinoids quantification, the amount of capsaicin and dihydrocapsaicin into capsaicinoids-loaded PCL microparticles was directly determined. In brief, an amount of microparticles, equivalent to 3 mg of capsaicinoids, was weighted and magnetic stirred (1000 rev/min) with 7 mL ethanol for 24 h in order to completely extract the drug. The volume was made up to 10 mL and filtered through a poly (vinylidene fluoride) membrane filter (Durapore membrane, 0.45 μm pore size, Millipore, Bedford, MA, USA). After suitable dilution in ethanol, the concentration of capsaicinoids was determined by HPLC system in triplicate as previously described. EE was then calculated using Equation 1.

$$EE = \left(\frac{\text{mass of capsaicinoids in microparticles}}{\text{theoretical mass of capsaicinoids}} \right) \times 100 \quad (1)$$

In vivo gastroprotective assay

All *in vivo* experimental protocol was carried out in accordance with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health.[24] This protocol was previously approved by the Ethics Committee on Animal Use of the State University of Ponta Grossa (CEUA/UEPG) under number 14/2012.

Animals

Antiulcer activity experiments were performed using male Wistar rats, approximately 90 days, weighing between 250 and 300 g, obtained from the Central Animal Facility of State University of Ponta Grossa. Animals

were kept in air-controlled environment (22°C ± 2°C) with *ad libitum* access to food and water in light-dark cycle of 12/12 h.

Experimental protocol

Ethanol-induced gastric lesions

Forty-two rats were randomly divided into seven groups of six animals each. Pure capsaicin (CAP) group received pure capsaicinoids (15 mg/kg, orally); capsaicinoids-loaded PCL microparticles (MC5) was administered to MC5 group in an amount equivalent to 15 mg/kg of capsaicinoids (orally); MC0 group received unloaded-microparticles MCO in mass equivalent to MC5 group (orally); ranitidine (RAN) group received RAN (100 mg/kg, orally); omeprazole (OME) group received OME (20 mg/kg); 0.5 mL of medium chain triglycerides was administered to caprylic/capric triglycerides (TCM) group since TCM were used as vehicle for dispersing all formulations; and H_2O group received 0.5 mL of water as control. After an hour, 0.5 mL of ethanol P. A. was administered orally to rats by gastric gavage to induce gastric ulcers.[25] One hour after this treatment, the animals were slaughtered by anesthetic overdose (55 mg/kg ketamine and 8 mg/kg xylazine). The stomachs were then removed, opened along the greater curvature, and gently rinsed with 0.9% saline. The lesions were identified, qualified, and quantified by ulcerative lesion index (ULI).

Ulcerative lesion index

Ulcerative lesions were qualitative and quantitatively evaluated by receiving a score according to their extensive area (large >1 mm and small <1 mm), number of petechial lesions (up to 10 petechiae, 1 point; up to 20, 2 points; and up to 30, 3 points), presence of hemorrhagic lesion, loss of folds, and loss of color (equal to 1 point). The results were expressed as an ULI using Equation 2.[26]

$$ULI=(3xA)+(2xB)+C+D+E+F \quad (2)$$

Where, A: ulcerative lesion >1 mm; B: Ulcerative lesion <1 mm; C: Hemorrhagic lesion; D: Loss of folds; E: Loss of color; and F: Number of petechial lesions.

Considering ULI obtained for all experimental groups, it was possible to calculate the percentage of ulceration inhibition in relation to the negative control (H_2O group) according to Equation 3:[27]

$$\%\,\text{Ulceration inhibition} = \left(\frac{ULI_{\text{negatitve control}} - ULI_{\text{test or positive control}}}{ULI_{\text{negative control}}}\right)\times 100 \quad (3)$$

Statistical analysis

The anti-ulcer activity was expressed as mean ± standard error of mean. Comparisons among groups were tested by one-way ANOVA with Bonferroni's *post hoc* test. The significance level was set at $\alpha = 5\%$ ($P < 0.05$). The analyses were performed using GraphPad Prism software (version 5.00, San Diego, CA, USA).

RESULTS AND DISCUSSION

Preparation of capsaicinoids-loaded poly(ε-caprolactone) microparticles

The capsaicinoids-loaded PCL microparticles were successfully obtained by the simple emulsion/solvent evaporation procedure. After drying, all the formulations showed powder aspect and white color similar to the polyester PCL.

Method development

During the development of HPLC method for quantifying the capsaicinoids present in microparticles, different mobile phases using methanol:water and acetonitrile:water were tested.[12,13,15] Various gradient systems and analysis times were investigated, as well as several proportions in isocratic mode.

The best result was achieved with a mobile phase of acetonitrile:water (70:30 v/v), pH 4.5, adjusted with acetic acid at an isocratic flow rate of 0.75 mL/min. Considering these chromatographic parameters previously set for the quantification of capsaicinoids by HPLC-diode-array detector (DAD), retention times of 4.43 and 5.18 min were, respectively, obtained for capsaicin and dihydrocapsaicin in the chromatograms of samples from loaded PCL microparticles. These reduced retention times provide a rapid determination of capsaicinoids, which is important in the routine analysis of R and D and quality control laboratories of phytoformulations.[28]

Method validation

The analytical method was validated by determining its specificity, linearity, LOD, LOQ, precision, accuracy, and robustness.[23]

Specificity

Specificity was investigated by comparing the chromatograms of unloaded (MC0) and capsaicinoids-loaded microparticles (MC5) prepared as per test method. Results demonstrated that there was no interference at the retention time of capsaicinoids from the other formulation components. In that sense, the specificity of the proposed method was confirmed [Figure 2].

Linearity

A linear relationship between peak area and concentration of capsaicinoids at the concentration range of 10.0–50.0 µg/mL was verified [Figure 3]. The linear equation obtained by the least-square method was y = 12650*x*–35050, where *y* is the peak area and *x* is the standard solution concentration in µg/mL. A suitable correlation coefficient ($r = 0.9996$) was recorded which demonstrates that the method was linear with an *r* value of nearly 1[23] at the purposed range.

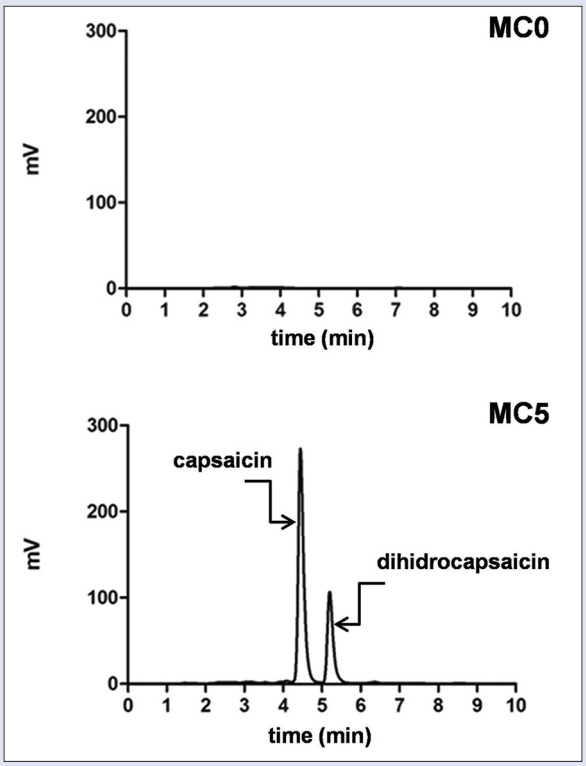

Figure 2: High-performance liquid chromatography chromatograms (λ = 280 nm) obtained from unloaded and capsaicinoids-loaded microparticles: MC0 and MC5

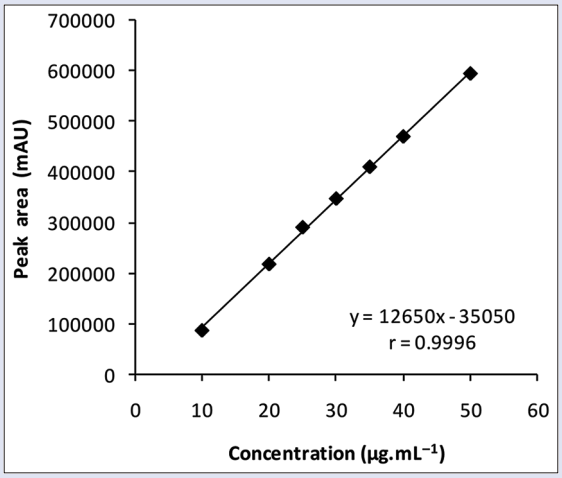

Figure 3: Mean calibration curve obtained for capsaicinoids using working standard solutions at the concentration range of 10.0–50.0 µg/mL (n = 3)

Limit of detection and limit of quantitation

The lowest concentration where capsaicinoids can be detected (LOD) and quantified (LOQ) with adequate precision and accuracy was 18.49 and 56.04 ng/mL, respectively. These results denote that the chromatographic method was suitable enough to detect and to quantify capsaicinoids at the range of 10.0–50.0 µg/mL.

Accuracy

Accuracy was analyzed using nine determinations at three concentration levels (22.0, 32.0, and 42.0 µg/mL). The percentage obtained was in the range of 98.92 ± 0.11 and 99.93% ±0.29%. These data show that the HPLC method was accurate since the results are consistent with the recommended range (98%–102%).[29]

Precision

Precision was confirmed by repeatability and intermediate precision as depicted in Table 2. The RSD value was < 0.88% and 2.03% for intra- and inter-day precision, respectively. These experimental data were lower than 5%[23] and indicate a good precision of the analytical method.

Robustness

Robustness demonstrates the reliability of analysis to deliberate variations in the parameters of the methods.[23] There were no significant differences in the peak area and the retention time of capsaicinoids when the flow rate, temperature, and analytical column were changed at a 5% level. Therefore, the method proved to be robust for the determination of the phytocompounds[28] under the conditions studied.

Evaluation of encapsulation efficiency

Table 3 summarizes the results for drug content and EE. All formulations showed suitable EE values higher than 90%. These data can be related to the low aqueous solubility of capsaicinoids (capsaicin has solubility of 10.3 mg/L in water at 25°C)[30] which provides enhanced drug entrapment into PCL microparticles. In addition, these data are similar to those previously reported, in which Eudragit RS100 nanocapsules containing capsaicinoids were obtained by the interfacial deposition procedure and presented EE close to 100%.[31]

Therefore, the validated method was successfully used to the determination of capsaicinoids into PCL microparticles and can be considered an important tool for the quality control of these novel phytoformulations.

Table 2: Repeatability and intermediate precision data for capsaicinoids analysis

Concentration (µg/mL)	RSD			Mean (%)
	Day 1 (%)	Day 2 (%)	Day 3 (%)	
10	2.03			
30	0.88	1.05	0.92	0.95
50	1.75			

RSD: Relative standard deviation

Table 3: Capsaicinoids-loaded* and encapsulation efficiency for poly(ε-caprolactone) microparticles

Microparticles	Capsaicinoids (mg/g)	EE (%)
MC0	-	-
MC3	27.77±1.08	92.6
MC5	47.5±2.11	95.3
MC10	95.23±1.52	95.2

*Mean (n=3)±SD. EE: Encapsulation efficiency; PCL: Poly(ε-caprolactone); MC0: Unloaded microparticles; MC3: Capsaicinoids-loaded PCL microparticles (3%); MC5: Capsaicinoids-loaded PCL microparticles (5%); MC10: Capsaicinoids-loaded PCL microparticles (10%); SD: Standard deviation

Table 4: Ulcerative lesion index values and percentage of ulceration inhibition observed for the experimental groups in ethanol-induced model of gastric ulcers

Experimental groups	ULI (mean±SEM)	Ulceration inhibition (%)
CAP	20.7±2.7[e]	53.73
MC5	16.3±1.8[a,d,e]	63.43
MC0	31.3±3.2[b,c]	29.85
RAN	15.3±1.4[d,e]	65.67
OME	8.0±1.2[d,e]	81.34
TCM	31.0±3.6	30.60
H_2O	44.7±3.9	-

[a]P<0.05 versus MC0, [b]P<0.05 versus RAN, [c]P<0.05 versus OME, [d]P<0.05 versus TCM, [e]P<0.05 versus H2O. ULI: Ulcerative lesion index; CAP: Pure capsaicin; MC0: Unloaded microparticles; MC5: Capsaicinoids-loaded PCL microparticles (5%); RAN: Ranitidine; OME: Omeprazole; TCM: Caprylic/capric triglycerides; SEM: Standard error of the mean

In vivo anti-ulcer effect

In contrast to the irritation that capsaicinoids provide when in contact with mammalian tissues, these vanillylamides have a clinical use as anti-ulcerogenic drugs. This activity can be explained by the fact that capsaicinoids perform vasodilation of gastric mucosae, promote the release of nitric oxide and activate TRPV1 receptors on sensory nerve fibers, abundant in stomach, which lead to desensitization, and loss of ability of sensory neurons to release mediators involved in the inflammatory process.[9] In that sense, they can act as phytocompounds against indomethacin- and ethanol-induced gastric ulcer as previously described.[32,33]

Thus, the *in vivo* anti-ulcer study was carried out using the ethanol-induced model of gastric ulcers to investigate the anti-ulcerogenic effect of capsaicinoids-loaded PCL microparticles comparing to pure drug and controls.

Figure 4 shows the morphological aspect of rat stomachs after treatment with all experimental groups. It was observed that PCL microparticles containing 5% of capsaicinoids (MC5 group) reduced the number of ulcerative lesions, hemorrhagic and petechial lesions caused by ethanol compared to CAP group (pure drug), MC0 (unloaded PCL microparticles), TCM (vehicle), and H_2O (negative control). However, this gastroprotective effect was lower than that verified for RAN and OME that were used as positive controls.

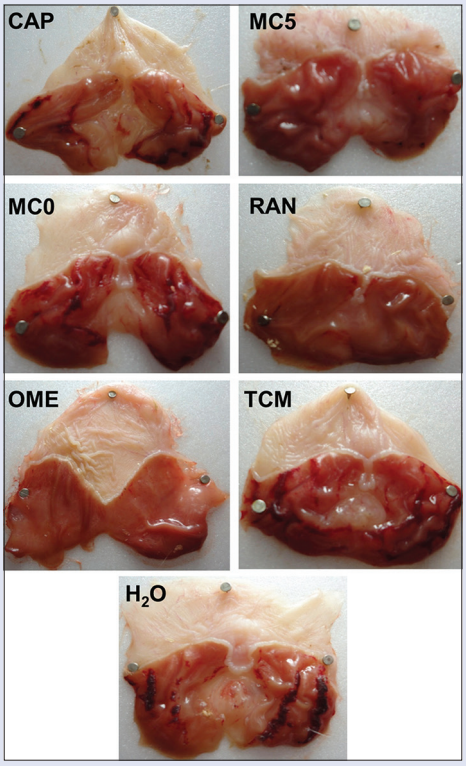

Figure 4: Morphology of rat stomachs after treatment with the experimental groups using the ethanol-induced model of gastric ulcers. Legend: CAP: Pure capsaicinoids (15 mg/kg); MC5: Capsaicinoids-loaded poly(ε-caprolactone) microparticles (amount equivalent to 15 mg/kg of capsaicinoids); MC0: Unloaded microparticles; RAN: Ranitidine (100 mg/kg); OME: Omeprazole (20 mg/kg); TCM: vehicle (0.5 mL of medium chain triglycerides); and H_2O: Water (0.5 mL)

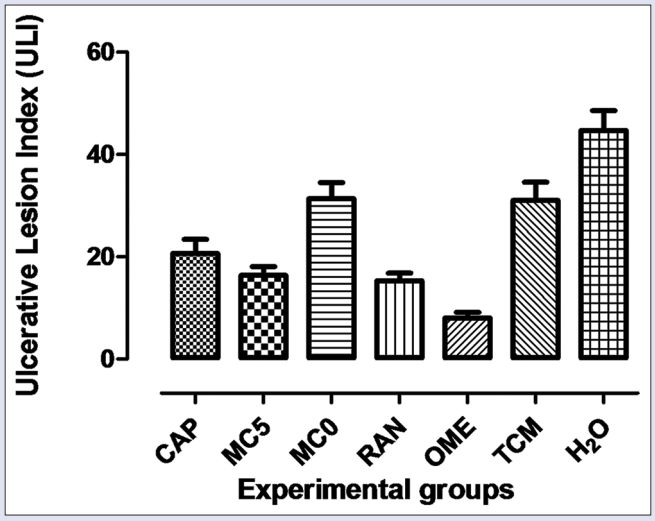

Figure 5: Ulcerative lesion index scores obtained for the experimental groups in the ethanol-induced model of gastric ulcers. Legend: CAP: Pure capsaicinoids (15 mg/kg); MC5: Capsaicinoids-loaded poly(ε-caprolactone) microparticles (amount equivalent to 15 mg/kg of capsaicinoids); MC0: unloaded microparticles; RAN: Ranitidine (100 mg/kg); OME: Omeprazole (20 mg/kg); TCM: Vehicle (0.5 mL of medium chain triglycerides); and H_2O: Water (0.5 mL)

To provide a quantitative analysis, the ULI score and the percentage of ulceration inhibition were calculated as shown in Figure 5 and Table 4. The MC5 group had an ULI score of 16.3 points and a percentage inhibition of ulcers of 63.43%, while pure capsaicinoids exhibited values of 20.7 points and 53.73%, respectively.

MC5 formulation had a ULI score statistically lower ($P < 0.05$) than MC0 groups (ULI = 31.3 points), TCM (vehicle, ULI = 31.0 points), and H_2O (negative control, ULI = 44.7 points) as expected. Furthermore, the result obtained for PCL microparticles containing 5% of capsaicinoids was statistically similar ($P > 0.05$) to those observed for RAN (ULI = 15.3 points) and OME (ULI = 8.0 points) groups, which demonstrates the higher potential of this phytoformulation as a therapeutic strategy to obtain a gastroprotective effect. However, the results of MC5 group were not statistically different to that seen to pure drug (CAP group, ULI = 20.7 points). Thus, the advantage of using PCL microparticles is in the fact that this formulation provides a prolonged release of capsaicinoids[6] which possibly could result in a lower pungency on the gastric mucosae and better acceptance by the patients.

CONCLUSION

Innovative phytoformulations containing capsaicinoids as PCL microparticles were successfully obtained by simple emulsion/solvent evaporation method. A simple and efficient reversed-phase HPLC-DAD method was developed and validated according to the ICH guidelines. In this study, the method proved to be simple, sensitive, accurate, linear, precise, specific, and robust. These results confirm that the method can be a suitable technique to quantify capsaicinoids in polymeric microparticles encouraging its application for quality control. In addition, PCL microparticles containing 5% of capsaicinoids showed a gastroprotective activity similar to RAN and OME against ethanol-induced gastric ulcers in rats. Thus, capsaicinoids-loaded PCL microparticles may be further used as controlled drug delivery system for the therapeutic management of ulcer.

Acknowledgements

The authors are thankful to CAPES/Brazil for the financial support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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