

A simple and rapid spectrophotometric method for the determination of *trans*-chalcone in raw-material and topical formulation

Método espectrofotométrico simples e rápido para determinação de *trans*-chalcona em matéria-prima e em formulação tópica

Renata Martinez¹, Nathalia Yurika Fukuda², Jenifer Freitas da Silva², Clara Ruiz de Souza³, Sandra Regina Georgetti⁴, Marcela Maria Baracat⁴, Rúbia Casagrande⁴

Abstract

Trans-chalcone (TC) is a flavonoid precursor characterized by a wide spectrum of action, with anti-inflammatory and antioxidant effects. However, no validated methods are available in official compendia for the analysis of this substance. Thus, the aim of this work was to develop and validate a simple, fast, and reproducible spectrophotometric method for the analysis of TC in raw material, and in topical pharmaceutical formulation containing TC. The established conditions were: methanol as extracting solvent, and detection wavelength of 309 nm by UV spectrophotometer. All tests followed the rules of Resolution RDC 166, 2017. The proposed method was selective. Linearity was demonstrated in the concentration range of 1 to 8 µg/mL ($r = 0.999$). Repeatability and intermediate precision were confirmed by low relative standard deviation values of 1.53% and 2.70% for TC, and of 1.73% and 2.91% for formulation containing TC. Accuracy, evaluated through recovery test, was adequate, with minimum of 98.24% and maximum of 100.23% of recovery. It was observed that the small deliberate modifications done did not interfere with the results, demonstrating the method is robust. The results showed that the method was considered suitable for the intended purpose, inexpensive, easy to apply, selective, linear, precise, accurate, and robust for the determination TC, and pharmaceutical formulation containing TC. Thus, the method developed satisfies the need for an analytical method for the determination of TC, and topical formulation containing TC, being effective, innovative and able to aid in the development of the pharmaceutical field.

Keywords: Chalcone; UV spectrophotometry; Quality control; Topical formulation; Validation.

¹ Doutorado em Ciências da Saúde pela Universidade Estadual de Londrina (UEL), Londrina, Paraná, Brasil. Professora do Departamento de Ciências Farmacêuticas da Universidade Estadual de Londrina (UEL), Londrina, Paraná, Brasil. *E-mail:* renatamartinez@gmail.com

² Graduação em Farmácia pela Universidade Estadual de Londrina (UEL), Londrina, Paraná, Brasil.

³ Mestrado em Ciências Farmacêuticas pela Universidade Estadual de Londrina (UEL), Londrina, Paraná, Brasil.

⁴ Doutorado em Ciências Farmacêuticas pela Universidade de São Paulo (USP), Ribeirão Preto, São Paulo, Brasil. Professora do Departamento de Ciências Farmacêuticas da Universidade Estadual de Londrina (UEL), Londrina, Paraná, Brasil.

Resumo

Trans-chalcona (TC) é um precursor de flavonoides caracterizado por um amplo espectro de ação, como efeitos anti-inflamatórios e antioxidantes. No entanto, não há método validado disponível em compêndio oficial para análise deste composto. Então, o objetivo deste trabalho foi desenvolver e validar um método espectrofotométrico, simples, rápido e reprodutível para análise de TC em matéria-prima, e em formulação farmacêutica tópica contendo TC. As condições estabelecidas foram: metanol como o solvente de extração, e detecção no comprimento de onda de 309 nm por espectrofotometria no UV. Todos os testes seguiram as normas da RDC 166, 2017. O método proposto foi seletivo. A linearidade foi demonstrada na faixa de concentração de 1 a 8 µg/mL ($r = 0.999$). A repetibilidade e a precisão intermediária foram confirmadas pelos valores baixos de desvio padrão relativo de 1,53% e 2,70% para a TC, e de 1,73% e 2,91% para a formulação contendo TC. A exatidão, avaliada por meio de testes de recuperação, foi adequada, com mínimo de 98,24% e máximo de 100,04% de recuperação. Observou-se que pequenas modificações no método não interferiram nos resultados, demonstrando que o método é robusto. Os resultados demonstraram que o método foi adequado para a finalidade pretendida, barato, de fácil aplicação, seletivo, linear, preciso, exato e robusto para determinação de TC, e de formulação contendo TC. Então o método desenvolvido satisfaz as necessidades de um método analítico para determinação de TC, e de formulação tópica contendo TC, e é eficaz, inovador e pode contribuir para o desenvolvimento da área farmacêutica.

Palavras-chave: Chalcona; Espectrofotometria no UV; Controle de qualidade; Formulação tópica; Validação.

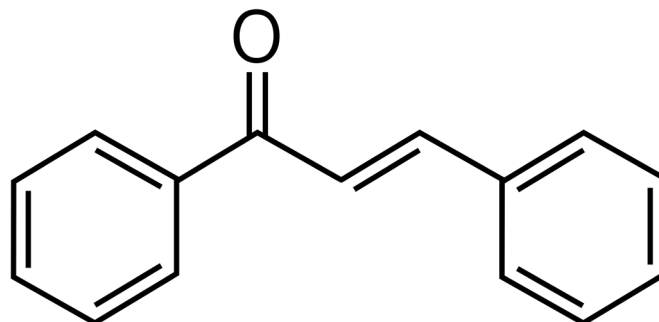
Introduction

Topical administration of anti-inflammatory and antioxidants compounds is an efficient way to enrich the endogenous cutaneous system of protection, and thus, a successful strategy to decrease damage of the skin induced by ultraviolet irradiation.⁽¹⁻⁵⁾ Lately, there has been increasing attention dedicated to chalcones as they have shown diverse biological activities,⁽⁶⁾ including anti-inflammatory and antioxidants.⁽⁷⁾ Chalcones consist of two aromatic rings joined by a three-carbon enone moiety and may exist in two isomeric forms, *cis*

and *trans*, being the latter thermodynamically favorable (Figure 1).

Trans-chalcone (TC), or 1,3-diphenyl-2-propen-1-one, is a plant flavonoid precursor, that presents anti-inflammatory and antioxidant effects.^(5,6,8-11) TC is a promising compound to combat inflammatory conditions, by mechanisms that include inhibition of pro-inflammatory and activation of antioxidant pathways.^(5,6,8,11) Furthermore, it was demonstrated that topical formulation containing TC inhibited inflammation, improved antioxidant and detoxification systems in the skin, therefore, protecting mice skin from UVB radiation.⁽⁵⁾

Figure 1 - Chemical structure of *trans*-chalcone.



Source: Martinez et al.⁽⁸⁾

To this date, no validated methods are available in official compendia for the analysis of *trans*-chalcone, a flavonoid precursor, despite of available monograph for flavonoids as quercetin, rutin, and hesperidin at The United States Pharmacopoeia.⁽¹²⁾ This fact, aligned with the importance of TC to combat inflammatory conditions,^(5,6,8-11) and of topical formulation containing TC to treat and/or prevent UV irradiation-induced skin damage,⁽⁵⁾ which affects millions of people around the world, justify the conducting of a validation test for TC in raw-material and topical formulation.

There is a great interest in developing rapid and efficient analytical methods that provide precise and accurate parameters for the quantitative analysis of drugs, important for routine analysis during quality control and development of new drug forms,^(13,14) with spectrophotometric analysis being the most likely candidate, since offers low cost and easy implementation.^(15,16)

Once TC is still not cited in any pharmacopoeia and there is the necessity of alternative methods which can be easily adopted and applied in quality control laboratories and research centers, the aim of this manuscript was to develop and validate a simple and fast spectrophotometric method to determine TC in raw material, and in topical formulation.

Material and Methods

Chemicals

Trans-chalcone (TC) was obtained from Santa Cruz Biotechnology (Dallas, Texas, United States), with an assigned purity of 99.6%, and used as standard. Materials for formulations were obtained from Galena (Campinas, São Paulo, Brazil). Methanol was provided by JT Baker (Xalostoc, Edo. de Mexico, Mexico) and Nuclear (Diadema, São Paulo, Brazil). All other reagents were of analytical grade.

Formulation

Formulation was prepared using the self-emulsifying wax Polawax[®] (cetostearyl alcohol and polyoxyethylene derived of a fatty acid ester of sorbitan 20 0E) (2%), and anionic hydrophilic colloid (carboxypolymethylene, Carbopol[®] 940) (0.18%) as stabilizing agent. Caprylic/capric triglyceride (5%) was used as emollient, and propylene glycol (5%) as solubilizing agent and moisturizer. Phenonip (0.4%) was used as preservative and deionized water was used for the preparation of formulation to complete 100%. TC (1%) was solubilized in propylene glycol and then added to the formulation at room temperature. Formulation base (placebo) did not contain TC. We used 1% of TC in formulation considering previous evidence using the same concentration of this compound in topical formulation.^(1,3-5) Formulations were allowed to equilibrate for 24 hours prior to use.

Equipment and conditions

A Thermo Scientific Evolution 60 UV-VIS spectrophotometer with 1 cm quartz cells was used to perform the absorbance measurements. Prior to use, background correction was carried out using the matrix solvent (methanol). For the analysis, the wavelength was set at 309 nm.

Standard and sample preparation

Formulation containing 1% of TC (5), and TC (99.6%), were used. 10 mg of TC, and 1 g of formulation containing 1% of TC (equivalent of 10 mg of TC) were weighed and transferred to a volumetric flask of 50 mL, dissolved with 40 mL of methanol, and the volume completed with the same solvent. Magnetic stirring (10 min) was used for the formulation. The solution was filtered through filter paper for the retention of insoluble particles, and 250 μ L was transferred to a volumetric flask of

10 mL, completed to volume with methanol, and then homogenized, to obtain the theoretical working concentration of 5 µg/mL.

Method validation

The validation process was performed according to the guidelines contained in resolution RDC 166 (ANVISA, 2017).⁽¹⁷⁾

Selectivity

Selectivity was demonstrated through the analysis of the spectra obtained between 220 nm-400 nm. Topical formulation base (placebo), TC, and topical pharmaceutical formulation containing TC, at the working concentration (5 µg/mL) were determined through analysis, using UV grade methanol as the diluent, since TC is insoluble in water.

Linearity and Matrix Effect

A stock solution was prepared containing 200 µg/mL of TC, in that 10 mg of TC, and 1 g of formulation containing 1% of TC (equivalent of 10 mg of TC) were weighed and dissolved in 50 mL of methanol. Aliquots of these solution were transferred to volumetric flasks to obtain final concentrations of 1, 2, 3, 4, 5, 6 and 8 µg/mL (range of 20 – 160%). Each solution was prepared in triplicate. Statistical analysis such as equation of least square, Analysis of Variance (ANOVA) and residue distribution were performed to confirm the linearity of both curves considering a significance of 5%.

The same process to prepare TC and formulation containing TC samples as well as their respective curves were used to determine if a Matrix Effect was present. Thus, the angular coefficients were used to perform an unpaired T-student test to determine if there was equivalency between the curves.

The Minitab® Statistical Software was used to perform the Analysis of Variance, residue distribution plots and normality test for linearity evaluation, in addition to the T-student test to detect if a matrix effect was occurring.

LOD and LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated directly using the slope and the standard deviation of the intercept of the mean of the calibration graphs (n =3) determined by a linear regression model the calibration graph.⁽¹⁷⁾ For LOD and LOQ were applied the factors of 3.3 and 10.0, respectively, which were multiplied by the ratio of the residual standard deviation and the slope.

Precision

The repeatability was evaluated on the same day for intra-day precision in six vessels independently. The evaluation of inter-day precision (intermediate precision) was performed on two different days by two analysts in six vessels independently. The precision was determined by preparation, under the same conditions, of six analytical solutions of the TC, and of the formulation containing TC at working concentration (5 µg/mL). Relative standard deviation (RSD) of up to 5% was considered as acceptable, according to RDC 166 (ANVISA, 2017).⁽¹⁷⁾

Accuracy

Accuracy was assessed based on the degree of recovery, as percentage of formulation base (placebo) solutions fortified with known amounts of TC, and of known amounts of TC dissolved in methanol. Solutions were prepared in triplicate, at concentrations of 80%, 100% and 120% of the working concentration, reaching the final concentrations of 4, 5, and 6 µg/mL.

Robustness

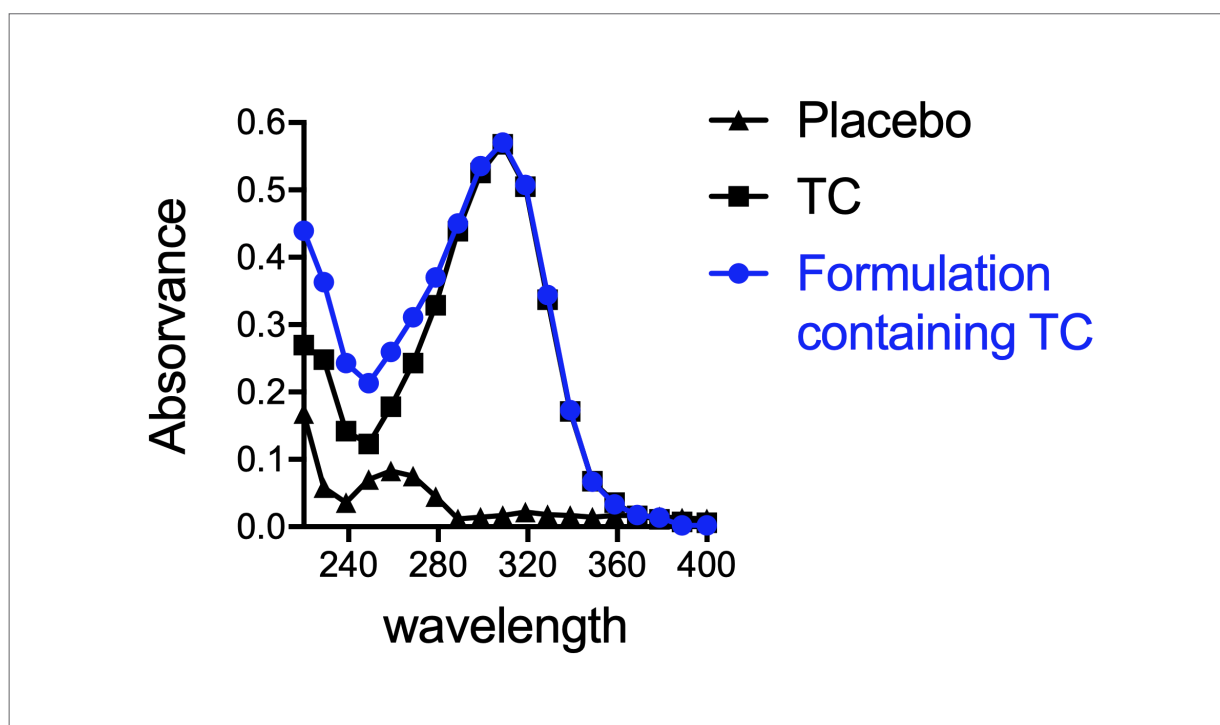
Robustness of the method was determined with TC and formulation containing TC at working concentration of 5 µg/mL, in triplicate. The variation among solvent manufacturers was evaluated with two different solvent brands (usually JT Baker and now Nuclear). Robustness was also determined by the change of the wavelength (308, 309, and 310 nm). Furthermore, the stability of the solutions was evaluated at 0 and 24 hours.

Results

Selectivity

The method used was selective for TC through comparative analysis of formulation base (placebo), TC and formulation containing TC, prepared under the same conditions. The shapes of formulation containing TC and TC methanol solution were similar, and the absorbances at 309 nm were close, indicating that TC was extracted from the formulation successfully. There was no significant interference (< 3%) from other components (placebo) at a wavelength of 309 nm (Figure 2).

Figure 2 - UV spectra of formulation base (placebo), *trans*-chalcone (TC) and formulation containing TC (5 µg/mL), in absolute methanol.



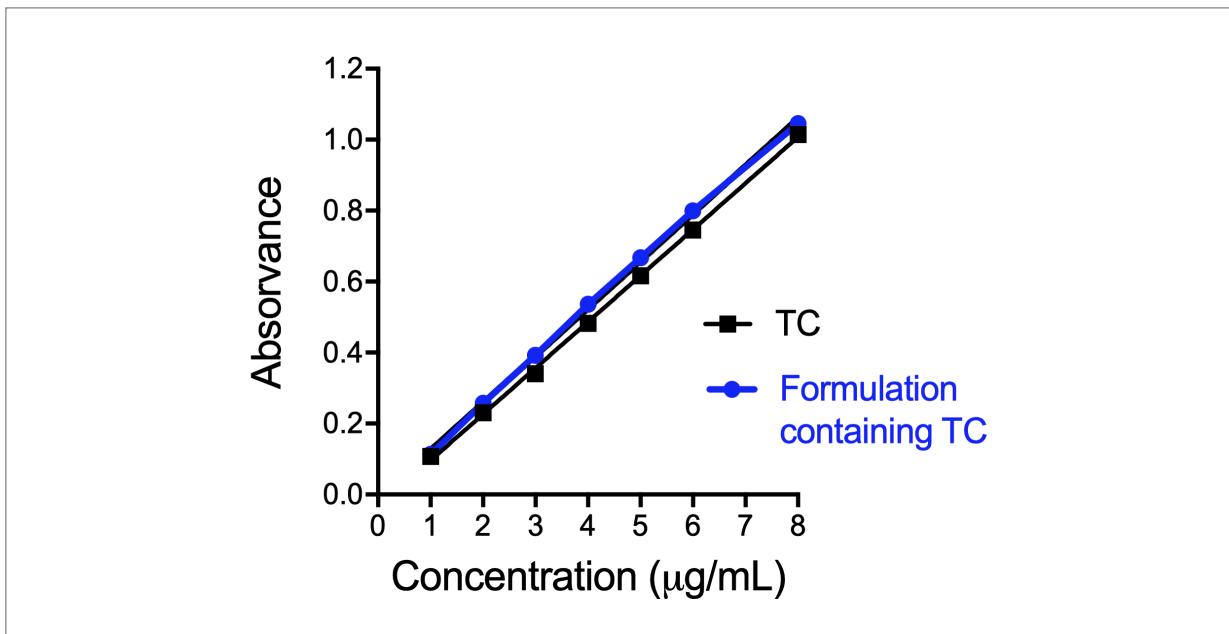
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Linearity

Linearity was confirmed in concentration range of 1 to 8 µg/mL. The regression equations obtained were $y = 0.1302x - 0.03378$ and $y = 0.1336x - 0.0088$ for TC, and formulation contain-

ing TC, respectively. The correlation coefficients (r) of the calibration curves were 0.999 both for TC, and for formulation containing TC, according to RDC 166 (ANVISA, 2017)^(17,18,19) minimum acceptable criterion (Figure 3).

Figure 3 - Calibration curves of *trans*-chalcone (TC) and for formulation containing TC through absorption spectrophotometry in the UV region at 309 nm.



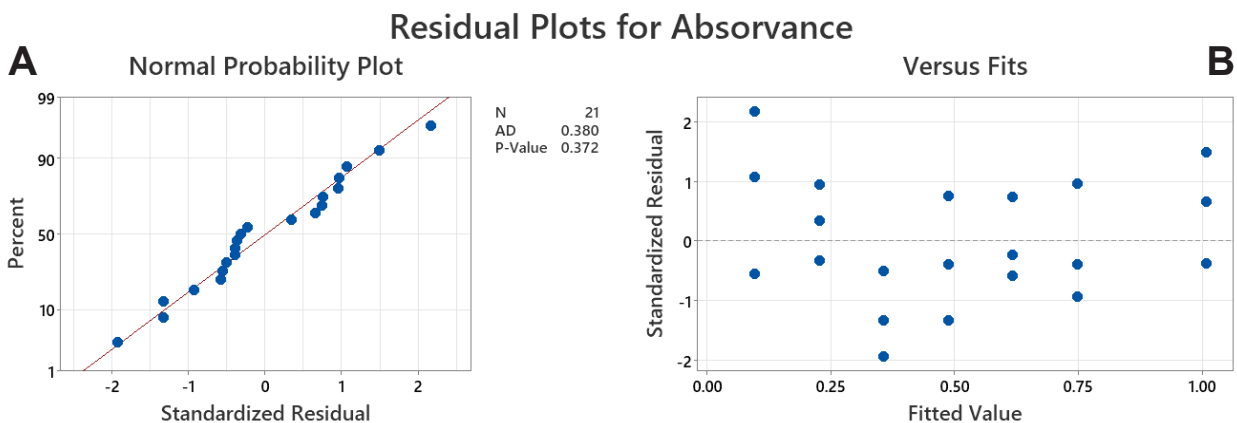
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In addition, through an Analysis of Variance the linear regression of the absorbances obtained and their respective concentrations was confirmed since the F calculated for TC (9364.83) and Formulation containing TC (11920.95) were greater than the F tabulated (4.381).

The residual dispersion graphs of the curves (Figures 4 and 5 A-D) prepared with TC and for-

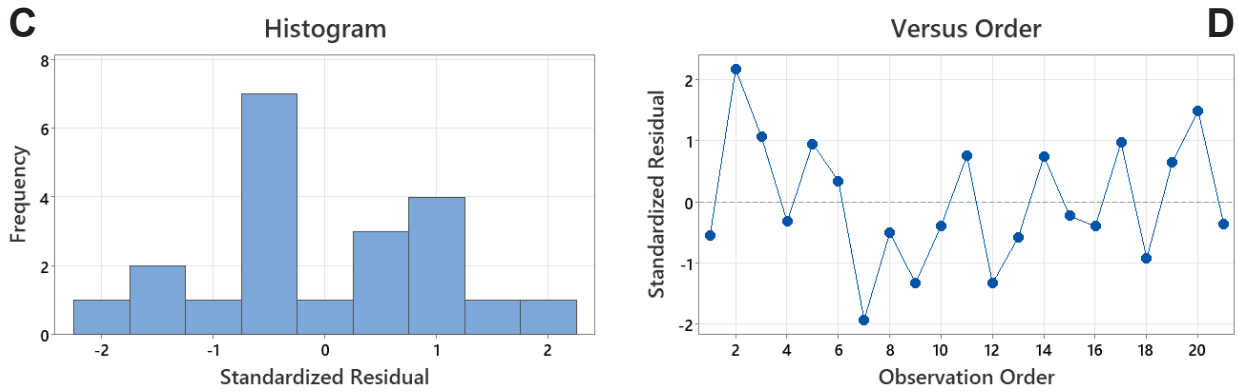
mulation containing TC demonstrated a normal (Figures 4 and 5 A) random, unbiased and without points in extreme areas (Figures 4 and 5 B-D) distribution, indicating a homoscedastic model. Moreover, the Anderson Darling analysis was used to confirm that the residues found in both curves follow a normal distribution (p-value >0.05, Figure 4 and 5 A).

Figure 4 - Residual Plots obtained for TC curve, (A) Normal Distribution Probability; (B) Residuals Distribution versus Fit; (C) Residuals Distribution Histogram; (D) Residuals Distribution versus Order.



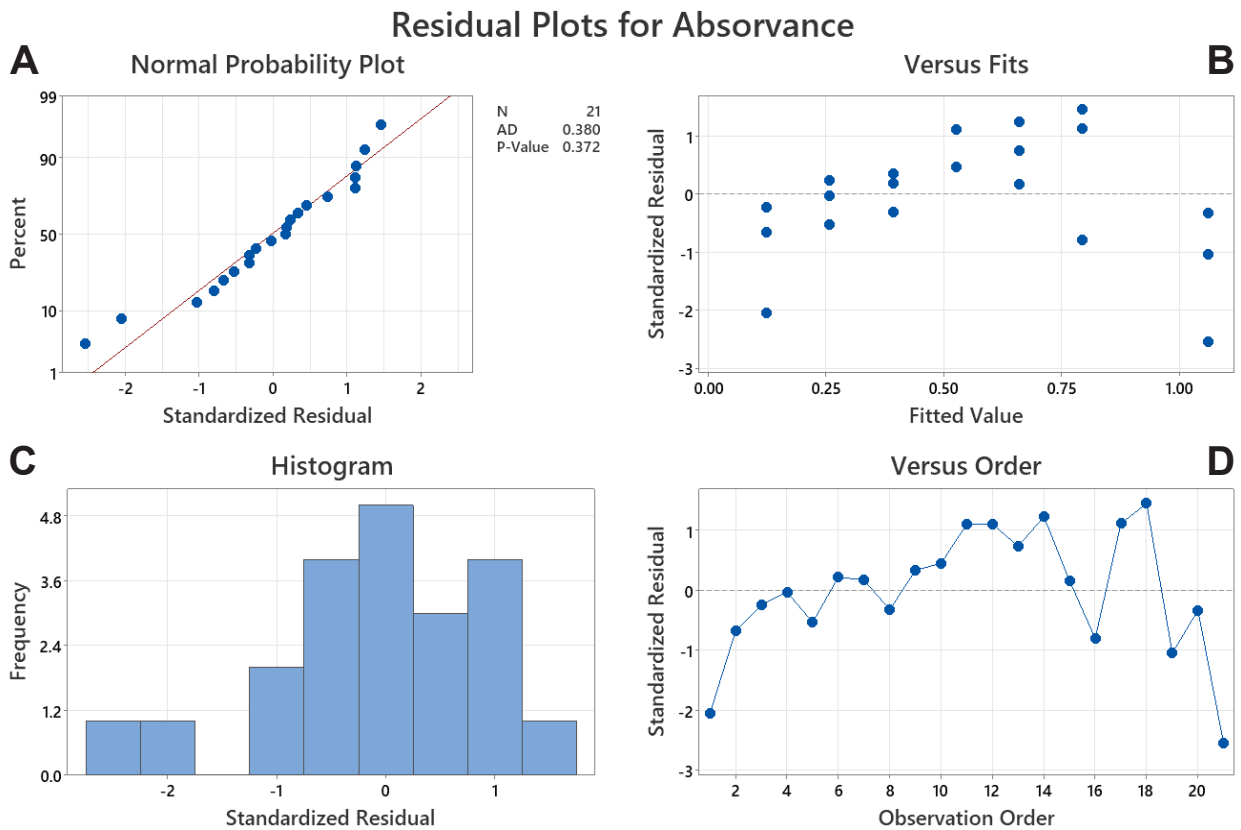
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Source: the authors.

Figure 5 - Residual Plots obtained for Formulation containing TC curve, (A) Normal Distribution Probability; (B) Residuals Distribution versus Fit; (C) Residuals Distribution Histogram; (D) Residuals Distribution versus Order.



Source: the authors.

Finally, an ANOVA for equal variances analysis was made to demonstrate that the response data (absorbances) has equal variance. For both curves the p-value obtained was higher than

0.05 (TC p-value= 0.938 and formulation p-value =0.621), demonstrating that the null hypothesis shall be accepted and there is no statistical difference between the variances.

Matrix Effect

To evaluate if matrix effects might be interfering the absorbance results, an unpaired T-student test with 95% of confidence level was performed to compare the slopes of both TC and formulation containing TC curves. The angular coefficients of the TC curves were 0.1324, 0.1301 and 0.1281; and the angular coefficients of the formulation containing TC curves were 0.1340, 0.1354 and 0.1313. Therefore, the p-value obtained for this analysis was 0.1234, being greater than 0.05 and thus demonstrating that there is no statistical difference between the curves and indicating that the matrix effect is not relevant in this case.⁽¹⁷⁾

LOD and LOQ

The estimated limit of detection and limit of quantitation⁽¹⁷⁾ of the method were 0.13 and 0.39 µg/mL for TC, and 0.20 and 0.69 µg/mL for formulation containing TC, respectively.

Precision

Considering data obtained from the six analytical solutions prepared, the method demonstrated repeatability with an RSD of up to 1.53% and 1.73% for TC, and formulation containing TC, respectively, among samples prepared on the same day under the same conditions. The method also showed intermediate precision with RSD% of

2.70% and 2.91 for TC and formulation containing TC, respectively, among results of six analytical solutions prepared by two different analysts on different days. These results indicate that the proposed method presents good precision.⁽²⁰⁾

Accuracy

The results for accuracy, at concentrations of 80%, 100% and 120%, showed a mean recovery percentage of 98.43; 99.98 and 100.23 for TC, and of 98.24; 100.04 and 99.71 for formulation containing TC, respectively. Mean recoveries are within the 98% to 102% range, which is in agreement with the literature.⁽¹³⁾

Robustness

It was observed that the small deliberate modifications done did not interfere with the results, demonstrating the method is robust (Table 1). For the same sample, both TC solutions, and formulation containing TC solutions analyzed using different solvent brands (methanol), JT Baker (nominal) and Nuclear, revealed no significant variations (Table 1). The method also was shown to be robust by the change of the wavelength 308, 309 (nominal) and 310 nm since the results of TC content were not significantly different (Table 1). Moreover, the data obtained in the experiments proved that the sample solutions used during assays were stable up to 24 hours.

Table 1 - Robustness evaluation of UV spectrophotometric method applied to *trans*-chalcone (TC), and formulation containing TC.

	Amount** (%)	
	TC	Formulation containing TC
Normal condition	100.36	99.37
Methanol Nuclear	99.57 ($p = 0.88^*$)	100.04 ($p = 0.82^*$)
λ 308 nm	99.77 ($p = 0.94^*$)	99.02 ($p = 0.90^*$)
λ 310 nm	99.77 ($p = 0.94^*$)	100.70 ($p = 0.64^*$)
24 hours after preparation	101.05 ($p = 0.93^*$)	101.11 ($p = 0.48^*$)

Caption: * $p > 0.05$, no statistically significant difference as compared to the normal condition (methanol JT Baker, 309 nm, 0 hours after preparation). **Mean of 3 replicate analyses.

Source: the authors.

Discussion

UV spectrophotometry is a very useful technique, already employed for identification of flavonoids as quercetin and rutin,⁽¹²⁾ but there is no description for *trans*-chalcone (TC). TC, a flavonoid precursor, is characterized by a wide spectrum of action, as anti-inflammatory and antioxidant effects, which reinforces the importance of quality control of TC. However, there is still a lack of methods for the analysis of TC in raw material, and in pharmaceutical formulation containing TC, aiming quality control applications. The data obtained in the present study showed that the developed and validated method was considered suitable for the intended purpose, offering a low cost, easy implementation, being consistent and within the limits required by the official guideline.

The selective features of the proposed method is the primary principle of a high-accuracy procedure,⁽²¹⁾ as demonstrated for the method in this study, since no significant interference was observed from other components of formulation containing TC at wavelength of 309 nm, because the excipients present in the base formulation (placebo) did not exhibit relevant absorption at a wavelength of 309 nm.

The linearity of an analytical method is defined as its ability (in a certain range) to get analyte responses which are proportional to the content of the sample solutions. The linearity must be obtained by the correlation coefficient (r) of the calibration curve. According to RDC 166 (ANVISA, 2017),⁽¹⁷⁾ the minimum acceptable criterion is 0.99. Herein, the concentration range tested for linearity is in accordance, with value of $r = 0.999$ in the calibration curves of the TC and formulation containing TC through absorption spectrophotometry in the UV region at 309 nm. In addition, the residues originated from the linear regression analysis should follow the normality distribution, be independent, with no bias or present significant variance. Therefore, the residues study was performed for both TC and formulation containing TC curves

and all criteria were met to respect the RDC 166 (ANVISA, 2017). The Matrix Effect evaluation was made to determine if any formulation components might interfere with the active molecule quantification, and thus the curves for TC and formulation containing TC should be equivalents. The statistical analysis performed demonstrated that this requirement was met, and the method developed can quantify TC without interference of the formulation. The detection and quantitation limits were calculated based on the standard deviation and the slopes of the calibration curves, and the values found, indicate the high sensitivity of the method.⁽¹⁷⁾

Intraday and interdays precisions could be demonstrated by low RSD values obtained in the performed determinations, that this value should not exceed 5%. Therefore, the method to determine TC, and pharmaceutical formulation containing TC content is precise under the conditions of this experiment and could be easily performed by trained professionals.

Moreover, a method must be exact, that is the proximity of measurement results to the true value. The data of this work show a recovery degree of between a minimum of 98.24% and maximum of 100.23%, indicating the suitability of the developed method in quantifying the amount of TC in raw material, and in topical pharmaceutical formulation.

The results obtained by quantification of the analytical solution of the same sample, analyzed with different solvent brands (methanol), and with different wavelength (308, 309, and 310 nm) revealed insignificant variations in the results. These results indicate high and satisfactory robustness of the method for small variations. Regarding the stability of TC, and formulation containing TC analytical solutions, a maximum variation of 1.74% was detected in 24 hours, thus not exceeding the maximum recommended limit of 2%.^(13,17)

The method to determine TC, and formulation containing TC content through spectrophotometry in the ultraviolet region was validated

according to RDC 166 (ANVISA, 2017).⁽¹⁷⁾ The results showed that the method developed to quantification of TC in methanol and incorporated to a topic formulation is simple, inexpensive, easy to apply, selective, linear, precise, accurate, and robust. Moreover, the method provides the reliability required by an analytical method, as well as the practicality required by a quality control laboratory routine.

In conclusion, the spectrophotometric method developed met the needs for an effective and rapid quantification method for the antioxidant TC in solvent and also when incorporated to a topic formulation, representing a relevant aid to the pharmaceutical field.

Acknowledgments

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References

- 1 Casagrande R, Georgetti SR, Verri WA Jr, Dorta DJ, Santos AC, Fonseca MJ. Protective effect of topical formulations containing quercetin against UVB-induced oxidative stress in hairless mice. *J Photochem Photobiol B*. 2006;84(1):21-7. doi: 10.1016/j.jphotobiol.2006.01.006.
- 2 Campanini MZ, Custódio DL, Ivan AL, Martins SM, Paranzini MJ, Martinez RM, et al. Topical formulations containing *Pimenta pseudocaryophyllus* extract: *In vitro* antioxidant activity and *in vivo* efficacy against UV-B-induced oxidative stress. *AAPS PharmSciTech*. 2014;15(1):86-95. doi: 10.1208/s12249-013-0049-8.
- 3 Martinez RM, Pinho-Ribeiro FA, Steffen VS, Silva TCC, Caviglione CV, Bottura C, et al. Topical formulation containing naringenin: efficacy against ultraviolet b irradiation-induced skin inflammation and oxidative stress in mice. *PloS one*. 2016;11(1):e0146296. doi: 10.1371/journal.pone.0146296.
- 4 Martinez RM, Pinho-Ribeiro FA, Steffen VS, Caviglione CV, Pala D, Baracat MM, et al. Topical formulation containing hesperidin methyl chalcone inhibits skin oxidative stress and inflammation induced by ultraviolet B irradiation. *Photochem Photobiol Sci*. 2016;15(4):554-63. doi: 10.1039/c5pp00467e.
- 5 Martinez RM, Pinho-Ribeiro FA, Vale DL, Steffen VS, Vicentini FTMC, Vignoli JA, et al. *Trans*-chalcone added in topical formulation inhibits skin inflammation and oxidative stress in a model of ultraviolet B radiation skin damage in hairless mice. *J Photochem Photobiol B*. 2017;171:139-46. doi: 10.1016/j.jphotobiol.2017.05.002.
- 6 Sikander M, Malik S, Yadav D, Biswas S, Katare DP, Jain SK. Cytoprotective activity of a *trans*-chalcone against hydrogen peroxide induced toxicity in hepatocellular carcinoma (HepG2) cells. *Asian Pac J Cancer Prev*. 2011; 12(10):2513-6.
- 7 Komoto TT, Lee J, Lertpatipanpong P, Ryu J, Marins M, Fachin AL, Baek SJ. *Trans*-chalcone suppresses tumor growth mediated at least in part by the induction of heme oxygenase-1 in breast cancer. *Toxicological research*. 2021;37(4):485-93. doi: 10.1007/s43188-021-00089-y.
- 8 Martinez RM, Pinho-Ribeiro FA, Steffen VS, Caviglione CV, Fattori V, Bussmann AJC, et al. *trans*-Chalcone, a flavonoid precursor, inhibits UV-induced skin inflammation and oxidative stress in mice by targeting NADPH oxidase and cytokine production. *Photochem Photobiol Sci*. 2017;16(7):1162-73. doi: 10.1039/c6pp00442c.
- 9 Sahu NK, Balbhadra SS, Choudhary J, Kohli DV. Exploring pharmacological significance of chalcone scaffold: a review. *Curr Med Chem*. 2012;19(2):209-25. doi: 10.2174/092986712803414132.
- 10 Silva G, Marins M, Fachin AL, Lee SH, Baek SJ. Anti-cancer activity of *trans*-chalcone in osteosarcoma: Involvement of Sp1 and p53. *Mol Carcinog*. 2016t;55(10):1438-48. doi: 10.1002/mc.22386.
- 11 Lamoke F, Labazi M, Montemari A, Parisi G, Varano M, Bartoli M. *Trans*-Chalcone prevents VEGF expression and retinal neovascularization in the ischemic retina. *Exp Eye Res*.

- 2011;93(4):350-4. doi: 10.1016/j.exer.2011.02.007.
- 12 The United States Pharmacopeial Convention. Pharmacists' Pharmacopeia: USP 49. Rockville: United States Pharmacopeial; 2023.
- 13 Nascimento GNL, Rosa DL, Nishijo H, Aversif-Ferreira TA. Validation of a spectrophotometry method to determine ciprofibrate content in tablets. BJPS. 2011;47(1):23-9.
- 14 Casagrande R, Baracat MM, Georgetti SR, Verri WA, Vicentini FTMC, Rafael JA, *et al.* Method validation and stability study of quercetin in topical emulsions. Quím Nova [Internet]. 2009 [cited 2023 Oct 20];32(7):1939-42. Available from: <https://www.scielo.br/j/qn/a/F9gdX75WPfF7dCnKTzLpVCN/?format=pdf&lang=en>
- 15 Rapalli VK, Kaul V, Gorantla S, Waghule T, Dubey SK, Pandey MM, Singhvi G. UV Spectrophotometric method for characterization of curcumin loaded nanostructured lipid nanocarriers in simulated conditions: Method development, *in-vitro* and *ex-vivo* applications in topical delivery. Spectrochim Acta A Mol Biomol Spectrosc. 2020;224:117392. doi: 10.1016/j.saa.2019.117392.
- 16 Hong L, Han D, Li MX, Zhang P, Liu CG. Development and validation of an ultraviolet-visible spectrophotometric method for determination of phenylethyl resorcinol in new topical nanoemulsions. Int J Cosmet Sci. 2017;39(3):337-43. doi: 10.1111/ics.12381.
- 17 Agência Nacional de Vigilância Sanitária (BR). Resolução da Diretoria Colegiada - RDC N° 166, de 24 de Julho de 2017. Dispõe sobre a validação de métodos analíticos e dá outras providências. Diário Oficial da República Federativa do Brasil. 2017; Seção 1.
- 18 Lima IA, Reis RR, Melo EB. Desenvolvimento e validação de método seguro e ambientalmente adequado para quantificação de atenolol em matéria-prima e comprimidos. Biosci J [Internet]. 2012 jun 29 [citado 2023 out 20]; 28(3):484-493. Disponível em: <https://seer.ufu.br/index.php/biosciencejournal/article/view/13653>
- 19 Anacleto SS, Teixeira LS, Borges MMC, Moraes CT, Borges WS, Pereira AC, *et al.* A simple and rapid HPLC method for the multidetermination of enrofloxacin, ciprofloxacin, and oxytetracyclin in raw materials and veterinary pharmaceutical formulations. Curr Pharm Anal [Internet]. 2018 [cited 2023 Oct 20];14(4):397-404. Available from: https://www.researchgate.net/publication/324928516_A_Simple_and_Rapid_HPLC_Method_for_the_Multidetermination_of_Enrofloxacin_Ciprofloxacin_and_Oxytetracyclin_in_Raw_Materials_and_Veterinary_Pharmaceutical_Formulations
- 20 Silva-Buzanello RA, Ferro AC, Cardozo-Filho L, Bona E, Araújo PHH, Leimann FV, *et al.* Validation of an Ultraviolet-visible (UV-Vis) technique for the quantitative determination of curcumin in poly(L-lactic acid) nanoparticles. Food Chem. 2015;172:99-104. doi: 10.1016/j.foodchem.2014.09.016.
- 21 Bitencourt AS, Oliveira SS, Mendez ASL, Garcia CV. UV Spectrophotometric method for determination of posaconazole: Comparison to HPLC. Rev Ciênc Farm Básica Apl [Internet]. 2015 [cited 2023 Oct 20];36(4):491-5. Available from: <https://rcfba.fcfar.unesp.br/index.php/ojs/article/view/14/13>

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