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Accelerated chemical stability data of O/W fluid emulsions containing the extract of *Trichilia catigua* Adr. Juss (and) *Ptychopetalum olacoides* Bentham

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Development of topical dosage forms requires physical, physicochemical and chemical assays that provide, as soon as possible, the formulation with the best stability profiles. This study evaluated the stability of O/W fluid emulsions, by total flavonoids determination, expressed in rutin, containing the standardized extract of Trichilia catigua *Adr. Juss (and)* Ptychopetalum olacoides *Bentham. Samples were evaluated for 90 days stored at 24.0 ± 2.0 °C, 5.0 ± 0.5 °C and 40.0 ± 0.5 °C, following a protocol for the assessment of accelerated chemical stability assay, also known as Normal Stability Test. A sensitive UV-spectrophotometric method at 361.0 nm was previously validated for the determination of the active substance. By Normal Stability Test, the O/W fluid emulsions presented acceptable chemical stability, for at least 90 days, when the samples were stored at 24.0 ± 2.0 °C and 5.0 ± 0.5 °C. The storage condition at 40.0 ± 0.5 °C has accelerated the degradation process of the total flavonoids, consequently, those O/W emulsions containing this kind of natural active substance or a similar preparation must not be stored at elevated temperatures.*

Uniterms
- Stability test
- Accelerated stability test
- O/W emulsion
- *Trichilia catigua*
- *Ptychopetalum olacoides*
- Rutin

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INTRODUCTION

Recently, attention has been drawn to the utilization of pharmaceutical products based on natural active substances due to the presumable safe utilization, ecological orientation and preservation, and reduced ambient impact (Rolim *et al.*, 2006).

Flavonoids exert a wide range of biochemical, physiological and pharmacological activities, including antioxidant, vasodilatory, antiinflammatory, antibacterial, immune-stimulating, antialergic and antiviral effects. Their capability to inhibit a broad spectrum of enzymes, such as tyrosine protein kinase and topoisomerase II, encouraged the researchers to evaluate these natural compounds as potential anticarcinogens and cardioprotective agents (Gao *et al.*, 2003; Harborne, Williams, 2000; Walle, 2004).

Rutin (quercetin-3-rutinoside) is one of the major flavonoids found in a variety of plants. The attention regarding on the application of this flavonol-type is due to its well-recognized health benefits. This natural active substance application includes its utilization as natural pigment, antioxidant, stabilizer, food preservative, UV absorver in food, animal feed, pharmaceutical, cosmetic and chemical industries (Kim *et al.*, 2005; Calabrò *et al.*, 2005).

Stability tests represent an indispensable part of the testing program for pharmaceutical or cosmetic products since the instability of the preparation modifies requisites, like: quality, efficacy and safety (Bilia *et al.*, 2001). Stability can be affected by environmental factors such as temperature, pH, light and air, which can provoke severe damages on the constituents of the product.

Trichilia catigua Adr. Juss (Meliaceae) – Catuaba – (and) *Ptychopetalum olacoides* Bentham (Olacaceae) – Muirapuama – standardized extract in total flavonoids content, expressed in rutin, also contains alkaloids, tannins, aromatic oils, saponins, terpenes, steroids, fatty resins, behenic acid and lupeol (Drewes, George, Khan, 2003; Rolim *et al.*, 2005).

The purpose of this study was to evaluate the stability of topical pharmaceutical dosage forms, as O/W fluid emulsions, containing the extract of *Trichilia catigua* Adr. Juss (Meliaceae) (and) *Ptychopetalum olacoides* Bentham (Olacaceae), standardized in total flavonoids. The research employed a validated UV-spectrophotometric method at 361.0 nm; a low-cost, precise, accurate and sensitive analytical method for total flavonoids determination, expressed in rutin. The accelerated chemical stability was assessed with the Normal Stability Test, being carried out in 90 days.

MATERIAL AND METHODS

Material

Ethanol 95.0% and acetic acid 0.02 M were purchased both from LabSynth (Brazil). Standard rutin (96.1%, NF XI standard) was purchased from PVP (Brazil). All chemicals and reagents were of analytical or pharmaceutical grade obtained from commercial sources and used without any further purification.

The commercial extract of *Trichilia catigua* Adr. Juss (Meliaceae) (and) *Ptychopetalum olacoides* Bentham (Olacaceae), standardized in total flavonoids content 1.02% w/w (expressed in rutin), was a gift from Chemyunion (Brazil). The preparations were developed as O/W fluid emulsions presenting the following components: glycerin and butylated hydroxitoluene (BHT) purchased from LabSynth (Brazil); oleyl alcohol, ceteth-10 phosphate, pentaerythrityl tetraisostearate, 2-bromo-2-nitropropane-1,3-diol and sodium gluceptate were gifts from Croda (Brazil); glyceryl stearate, behenyl alcohol, palmitic acid, stearic acid, lecithin, lauryl alcohol, myristyl alcohol, cetyl alcohol, diisopropyl adipate and octyldodecyl stearoyl stearate were donated by ISP (Brazil); xanthan gum was a gift from Rhodia (Brazil); dimethicone/vinyl dimethicone crosspolymer (and) C12-14 pareth-12 was obtained from Dow Corning (Brazil); and lecithin was obtained from Polytechno (Brazil). Quantitative composition of O/W fluid emulsions is presented in Table I.

Equipments

A Spectrophotometer Beckman DU 640 UV-Visible with a 1 cm quartz cell was performed to record absorbance measurements at 361.0 nm. Adams SafetyHead centrifuge, Sartorius BL 2106 analytical balance, Mettler P-120 and Scientech SA210 semi-analytical balances, Fanem and Fabbe chambers, Ecoplus 370 refrigerator and Labsystem 4500 pipette (100 – 1000 μL) were employed.

Quantification assay for total flavonoids, expressed in rutin

The UV-spectrophotometric method at 361.0 nm was previously validated (Rolim *et al.*, 2005; Baby *et al.*, 2006) for linearity, specificity, precision, accuracy, recovery, limit of detection (LOD) and limit of quantification (LOQ) (ICH Q2B, 1996; Feinberg, Raguènès, 1999; Analytical Procedures and Methods Validation, 2000; United States Pharmacopeia, 2004; Ramos *et al.*, 2005; Kedor-Hackmann *et al.*, 2006). As solvent and blank, the mixture of ethanol 95.0% and acetic acid 0.02 M (99:1) was used.

Stability assay

The O/W fluid emulsions had their chemical stability evaluated by the Normal Stability Test. Samples were weighted (30 g) and packaged in opaque white polyethylene flasks with 50 g of content capacity, respecting the head-space (Brasil, 2004). Replicates of two were employed.

Samples were analyzed after the resting period of 24 h of preparation (t_0), thus, permitting the finalization of emulsification process (De Navarre, 1993; Lachman, Lieberman, Kanig, 2001). All assays were conducted at room temperature (24.0 ± 2.0 °C).

TABLE I - Composition (% w/w) of the O/W fluid emulsions, in grams (Baby *et al.*, 2006).

Components	Function	FE [a]	FEL [b]
Ceteth-10 phosphate	Emulsifying agent	2.5	2.5
Diisopropyl adipate	Emollient	1.0	1.0
Octyldodecyl stearoyl stearate	Skin-conditioning agent	2.0	2.0
Pentaerythrityl tetraisostearate	Skin-conditioning agent	2.0	2.0
Glyceryl stearate (and) behenyl alcohol (and) palmitic acid (and) stearic acid (and) Lecithin (and) lauryl alcohol (and) myristyl alcohol (and) cetyl alcohol	Emulsifying agent	1.0	1.0
Oleyl alcohol	Emollient	3.0	3.0
Butylated hydroxitoluene	Antioxidant	0.1	0.1
Soy lecithin	Surfactant	NA	2.0
Dimethicone/vinyl dimethicone crosspolymer (and) C12-14 pareth-12	Emollient	1.0	1.0
Glycerin	Humectant	6.0	6.0
Xanthan gum	Emulsion stabilizer	0.3	0.3
Sodium gluceptate	Chelating agent	0.1	0.1
2-Bromo-2-nitropropane-1,3-diol	Preservative	0.005	0.005
Trichilia catigua Adr. Juss (Meliaceae) (and) *Ptychopetalum olacoides* Bentham (Olacaceae) commercial extract	Biological product/natural active substance	5.0	5.0
Distilled water	Vehicle	75.95	73.95

[a] O/W fluid emulsion lecithin-free; [b] O/W fluid emulsion containing lecithin; *NA:* Not added

Storage conditions were: room temperature with exposition or not to sunlight (24.0 ± 2.0 °C), low temperature (5.0 ± 0.5 °C – standard or reference sample/storage condition of reference) and high temperature (40.0 ± 0.5 °C). Samples had their chemical stability obtained by total flavonoids determination achieved with the previously validated UV-spectrophotometric method.

The interval of analysis was the 3rd, 7th, 15th, 30th, 60th and 90th day after the resting period. For each day of study there were replicates of two of the samples for all the storage conditions.

At the pre-determined times, samples were removed from the storage conditions and allowed to achieve room temperature (24.0 ± 2.0 °C) prior to the evaluation of the chemical stability. Samples were accurately weighed (500.0 mg) and transferred to 25 ml volumetric flasks. Volumes were completed with ethanol 95.0% and acetic acid 0.02 M (99:1). Solutions were centrifuged at 863 G (3500 rev/min) for five minutes at room temperature. Supernatants were discarded. Analytical determinations were realized in replicates of three.

RESULTS AND DISCUSSION

The UV-spectrophotometric method for the determination of the chemical stability of total flavonoids has provided a linear relationship of absorbances measured at 361.0 nm *versus* concentrations of standard rutin ranging from 5.0 to 15.0 µg/mL. Analytical method validation data are summarized in Table II.

Interval was chosen as the corresponding to the usual content that is expected of total flavonoids at the O/W fluid emulsions employed to quantitative assay of the active substance and stability assay.

O/W fluid emulsions (FE and FEL) had their physical, physicochemical (data summarized in Table III) and chemical characteristics evaluated during the accelerated stability assay, also known as Normal Stability Test.

TABLE II - UV-spectrophotometric analytical validation data (Rolim *et al.*, 2005; Baby *et al.*, 2006)

Analytical parameters						
Regression line	Slope	0.0293				
	Y-intercept	0.0129				
	r^2 [a]	0.9995				
Interval	TC [b] (µg/mL)	5.0	8.0	10.0	12.0	15.0
	CR [c] (µg/mL)	5.06	7.92	10.07	11.90	15.07
	RSD [d] (%)	0.41	0.47	0.20	0.27	0.15
	E [e] (%)	101.24	98.98	100.66	99.18	100.50
Precision (%)	TC µg/mL)	8.0	10.0	12.0		
	Intra-day	0.51*/1.79**	0.50*/1.42**	0.75*/0.34**		
	Inter-days	0.38*/1.14**	0.79*/1.29**	1.25*/1.24**		
Accuracy (%)	TC (µg/mL)	8.0	10.0	12.0		
	Intra-day	99.00*/97.88**	100.20*/98.90**	100.67*/99.00**		
	Inter-days	98.79*/98.38**	100.85*/100.79**	99.65*/100.61**		
Recovery (%)	TC (µg/mL)	8.0	10.0	12.0		
	R [f] (%)	97.87*/100.74**	100.14*/99.95**	100.13*/98.64**		
LOD [g] (µg/mL)	0.20					
LOQ [h] (µg/mL)	0.30					

$y = 0.0293x + 0.0129$, where y is absorbance and x is concentration of rutin (µg/mL)

[a] Coefficient of linear correlation; [b] theoretical concentration of rutin; [c] Concentration of rutin calculated; [d] Precision (relative standard deviation); [e] Accuracy; [f] Recovery; [g] Limit of detection; [h] Limit of quantification; * O/W fluid emulsion lecithin-free (FE); ** O/W fluid emulsion containing lecithin (FEL)

TABLE III - Physical and physicochemical properties of the O/W emulsions evaluated after the resting period of 24 h (t_0) of preparation and at the finalization of the Normal Stability Test (90th day of analyses).

Parameters	FE				FEL			
	t_0	RT	LT	HT	t_0	RT	LT	HT
pH	5.8 ± 0.05	5.5 ± 0.00	5.5 ± 0.10	5.0 ± 0.12	6.1 ± 0.01	5.7 ± 0.06	6.0 ± 0.00	4.9 ± 0.06
Viscosity (cP)	353 ± 11	490 ± 0	365 ± 7	270 ± 0	255 ± 7	220 ± 14	225 ± 7	150 ± 0
Aspect	Homogeneous	M	S	S	Homogeneous	S	S	M
Color	Brown-red	S	S	S	Brown-red	S	S	M
Odor	Characteristic	S	S	S	Characteristic	S	S	S

FE: O/W fluid emulsion lecithin-free; *FEL:* O/W fluid emulsion containing lecithin; t_0*:* resting period of 24 h after preparation; *RT:* room temperature (24.0 ± 2.0 °C); *LT:* low temperature (5.0 ± 0.5 °C); *HT:* high temperature (40.0 ± 0.5 °C); *M:* modified parameter (slightly modification); *S:* unaffected parameter (stable). Values were presented as mean ± standard deviation (n = 3)

Usually, the Normal Stability Test is conducted in an interval of 90 to 120 days, maintaining the test samples stored, at minimum, in three distinct storage conditions involving temperature, humidity and luminosity. A standard or reference sample should be stored at a condition which conserves its physical, physicochemical and chemical characteristics or a condition that worthless modifications are known and expected. The packaging material must be the final one for the future market accessibility or a neutral glass and all the stability assays must be realized, at least, in replicates of two. The results obtained from the Normal Stability Test, after interpretation and statistical treatment, may be extrapolated, suggesting valuable theoretical data about the shelf life of the product (Brasil, 2004).

Aspect, color and odor for both formulations stored at low temperature (5.0 ± 0.5 °C) did not suffer perceptible alterations during the study period of 90 days and, at room temperature (24.0 ± 2.0 °C), FE presented itself with a vaguely alteration of the aspect at the end of the experiment, being classified as slightly modified, but still considered stable; color and odor remained unaffected. FEL preparation appeared to be more susceptible to the high temperature (40.0 ± 0.5 °C) possessing modifications of the aspect and color. According to the results, the presence of soy lecithin at the formulation containing the extract of *Trichilia catigua* Adr. Juss (Meliaceae) (and) *Ptychopetalum olacoides* Bentham (Olacaceae) reduced its stability, concerning the aspect and color parameters.

The pH values of FE and FEL resulted in apparent variations of higher magnitude at 40.0 ± 0.5 °C with reduction of 13.8 and 19.7%, respectively. At high temperatures, alterations were expected for this physicochemical parameter since the temperature elevation possess direct influence on the stability of cosmetic and pharmaceutical dosage forms, as well, the active substances (Maia *et al.*, 2006). Both formulations presented tendency to reduce the pH value at high temperature storage condition, probably, due to the deterioration of the components of the fluid emulsions and the active substances from the extract of *Trichilia catigua* Adr. Juss (Meliaceae) (and) *Ptychopetalum olacoides* Bentham (Olacaceae).

Apparent viscosity for FE resulted in increased values of 37.1 and 5.7% when stored at 24.0 ± 2.0 °C and 5.0 ± 0.5 °C, respectively, and at 40.0 ± 0.5 °C there was a reduction of 22.9%. At all conditions of storage, FEL apparent viscosity suffered decrease of 13.7 (24.0 ± 2.0 °C), 11.8 (5.0 ± 0.5 °C) and 40.0% (40.0 ± 0.5 °C). Due to the soy lecithin presence at FEL, it seemed that the rheology profile was possibly modified in comparison with the FE (lecithin-free), in addition, viscosity for FEL generated values apparently inferior from FE.

The chemical stability of the O/W fluid emulsions was obtained in function of the total flavonoids determination, expressed in rutin, employing the UV-spectrophotometric method at 361.0 mm, previously validated.

Samples stored at room temperature (24.0 ± 2.0 °C) and at low temperature (5.0 ± 0.5 °C) suffered a reduced degradation of the total flavonoids content, data showed in Tables IV and V, compared with the concentrations evaluated after the resting period of 24 h after preparation (t_0).

FE samples presented reduction of the active substance of 3.36% for the room temperature condition and 0.68%, for the low temperature, after the 90th day of the stability assay. Although, there was a relevant decreasing pH variation at 5.0 ± 0.5 °C storage condition, the concentration of total flavonoids has maintained its stability, indicating that the pH alterations (reduction of -5.2%) did not provoke an increase of the degradation of the active. For the FEL samples stored at 24.0 ± 2.0 °C and 5.0 ± 0.5 °C, the decrease of total flavonoids was 6.38% and 1.31%, respectively, after the finalization of the assay. According to those data, all samples in analysis showed satisfactory chemical stability evaluated by the Normal Stability Test.

At the high temperature condition (40.0 ± 0.5 °C), the concentration of total flavonoids presented on the O/W fluid emulsions had greatest decrease attributed to the effect of the elevated temperature of storage which caused an acceleration of the chemical degradation rate of the active substance for the lotions (Lachman, Lieberman, Kanig, 2001). The reduction of the content of total flavonoids was 34.16% for the FE samples and 35.12%, for the FEL.

TABLE IV - Chemical stability data of the O/W fluid emulsion lecithin-free (FE) by total flavonoids determination, expressed in rutin

Storage conditions	Time (days)						
	t_0	3	7	15	30	60	90
	Total flavonoids (%)						
Room temperature (24.0 ± 2.0 °C)		97.76 ± 2.24	99.17 ± 5.22	98.91 ± 0.80	97.33 ± 3.65	96.54 ± 3.62	96.02 ± 1.78
Low temperature (5.0 ± 0.5 °C)	99.38 ± 1.46	98.63 ± 1.34	99.89 ± 5.29	98.95 ± 1.27	99.40 ± 3.54	98.99 ± 4.06	98.70 ± 3.34
High temperature (40.0 ± 0.5 °C)		95.33 ± 2.06	92.92 ± 3.56	87.46 ± 2.58	81.81 ± 2.93	75.51 ± 3.28	65.22 ± 2.97

t_0: resting period of 24 h after preparation; values were presented as mean ± standard deviation (n = 3)

TABLE V - Chemical stability data of the O/W fluid emulsion with addition of lecithin (FEL) by total flavonoids determination, expressed in rutin

Storage conditions	Time (days)						
	t_0	3	7	15	30	60	90
	Total flavonoids (%)						
Room temperature (24.0 ± 2.0 °C)		96.65 ± 2.21	96.64 ± 3.83	95.95 ± 2.09	95.29 ± 4.06	92.99 ± 4.55	90.27 ± 4.11
Low temperature (5.0 ± 0.5 °C)	96.65 ± 4.02	97.25 ± 3.62	97.50 ± 3.96	98.01 ± 1.97	95.97 ± 3.03	96.64 ± 4.09	95.34 ± 3.28
High temperature (40.0 ± 0.5 °C)		94.16 ± 8.05	91.20 ± 2.14	84.54 ± 3.19	78.44 ± 2.54	67.38 ± 2.47	61.53 ± 2.66

t_0: resting period of 24 h after preparation; values were presented as mean ± standard deviation (n = 3)

Flavonoids are phenolic compounds with moderate solubility in water and sensitive to the presence of metal ions, ultraviolet radiation, elevated temperatures and to the hydrolysis, which is accelerated direct and proportionally to an increase of the temperature (Bruneton, 1999; Friedman, Jürgens, 2000).

Bilia *et al.* (2001) evaluated the chemical stability of flavonols, a class of flavonoids that rutin is part of, as dry extract added or not with the mixture of ascorbic and citric acid (200:1), as antioxidants, at 2.85%. Samples were stored at room temperature (25 °C) for 90 days and at high temperature (40 °C) for 45 days. The authors observed a satisfactory chemical stability of the flavonols and the presence of the antioxidants did not affect the stability of the extract, compared with the samples free of the mixture of ascorbic and citric acid. This research suggested that the absence of water in a pharmaceutical dosage form maintained the stability of the active substance and the concentration of the natural material, avoiding the degradation reaction by hydrolysis (Bilia *et al.*, 2001; Bruneton, 1999).

Another possible interpretation of the elevated kinetic of deterioration of the total flavonoids at 40.0 ± 0.5 °C would be the presence the chelating agent sodium gluceptate in inadequate concentration (0.1% w/w). As an alternative to prevent the total flavonoids degradation, formulations containing water, as emulsions and gels, could be added of chelating agent associated to an antioxidant agent, like disodium ethylenediaminitetraacetate (disodium EDTA) and butylated hydroxitoluene (BHT), since these excipients act synergistically at pH values usually used in cosmetic forms, slightly acid. Both agents could be employed in association at concentrations ranging from 0.1 to 0.4% w/w (Banov, 2002).

Banov (2002) verified the stability of total flavonoids from *Ginkgo biloba* L. extract added to aqueous gels and O/W emulsions containing disodium EDTA, as chelating agent. Total flavonoids from aqueous gel suffered a content decrease of 5.0% after 90 days of analyses with the samples stored at 40.0 °C and, those presented at the O/W emulsion, decreased 4.0%. The author's results indicated an acceptable chemical stability of the natural active in the presence of the chelating-antioxidant system (disodium EDTA and BHT) evaluated by the Normal Stability Test at 40.0 ± 0.5 °C (Banov, 2002).

CONCLUSIONS

A validated, rapid and sensitive UV-spectrophoometric method was used to obtain information about the chemical stability of O/W fluid emulsions containing the extract of *Trichilia catigua* Adr. Juss (Meliaceae) (and) *Ptychopetalum olacoides* Bentham (Olacaceae), by total flavonoids determination, expressed in rutin, employing the accelerated aging assay, known as Normal Stability Test.

Since emulsion stability is, usually, evaluated by experimental means on a case-by-case basis (Civan, Alarcon, Campbell, 2004), the protocol of Normal Stability Test presented in this research work possesses advantage applicability by establishing systematic assays and acceptance/rejection criteria of interpretation of the results reached in a short period of time.

The O/W fluid emulsions presented acceptable chemical stability, for at least 90 days, when the samples were stored at room temperature with exposition or not to sunlight (24.0 ± 2.0 °C) and at low temperature (5.0 ± 0.5 °C). The storage condition at high temperature (40.0 ± 0.5 °C)

has greatly accelerated the degradation rate of the total flavonoids, thus, those O/W emulsions containing this kind of natural active substance or a similar preparation must not be stored at elevated temperatures.

RESUMO

Estabilidade química de emulsões O/A fluidas contendo o extrato de *Trichilia catigua* Adr. Juss (e) *Ptychopetalum olacoides* Bentham

O desenvolvimento de formas farmacêuticas tópicas necessita ensaios físicos, físico-químicos e químicos que selecionem rapidamente a formulação de melhor desempenho de estabilidade. Este estudo avaliou a estabilidade de emulsões O/A fluidas, por meio da determinação de flavonóides totais, expressos em rutina, contendo o extrato padronizado de Trichilia catigua *Adr. Juss (e)* Ptychopetalum olacoides *Bentham. As amostras foram armazenadas a 24,0 ± 2,0 °C; 5,0 ± 0,5 °C e 40,0 ± 0,5 °C durante 90 dias e foram avaliadas segundo o protocolo para a determinação da estabilidade acelerada, conhecida como Teste de Estabilidade Normal. A quantificação da substância ativa foi determinada por espectrofotometria na região do ultravioleta a 361,0 nm, previamente validado. Após os ensaios de estabilidade, as emulsões O/A fluidas apresentaram estabilidade adequada, pelo menos, no período de 90 dias, quando armazenadas a 24,0 ± 2,0 °C e 5,0 ± 0,5 °C. A condição de armazenamento a 40,0 ± 0,5 °C acelerou a cinética de degradação dos flavonóides totais, expressos em rutina, portanto, preparações possuindo esta categoria de substância ativa natural ou formulações similares não devem ser armazenadas em temperaturas elevadas.*

UNITERMOS: Teste de estabilidade acelerada. Emulsão O/A. Trichilia catigua. Ptychopetalum olacoides. *Rutina*

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