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Total flavonoids quantification from O/W emulsion with extract of Brazilian plants

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Abstract

A new derivative spectrophotometric (DS) method was proposed and validated for quantification of total flavonoids from in O/W emulsion with polyacrylamide (and) C13-14 isoparaffin (and) laureth-7 containing Catuaba (*Trichilia catigua* Adr. Juss) (and) Marapuama (*Ptychopetalum olacoides* Bentham) extract. DS method was optimized to perform the assay in most favorable conditions. Linearity, specificity and selectivity, recovery (Rc, %), precision (R.S.D., %), accuracy (*E*, %), detection (LOD, $\mu g\,ml^{-1}$) and quantification limits (LOQ, $\mu g\,ml^{-1}$) were established for method validation. First-derivative at 388.0 nm (zero-to-peak; amplitude = ± 0.12; wavelength range = 300.0–450.0 nm and $\Delta\lambda = 4$ nm) offered linearity for rutin concentrations ranging from 10.0 to 60.0 $\mu g\,ml^{-1}$ in ethanol 99.5%. Second-derivative provided to be unsuitable for interval evaluation obtaining unacceptable accuracy. Analytical method was validated for first-derivative, according to the experimental results: correlation coefficient ($r = 0.9999$); specificity to total flavonoids quantification, expressed in rutin, at wavelength 388.0 nm and selectivity with elimination of interference from matrix; Rc = 108.78%; intra- and inter-run precision (1.30–3.65% and 3.48–4.68%), and intra- and inter-run accuracy (100.00–112.19% and 101.25–118.44%); LOD = 0.62 $\mu g\,ml^{-1}$ and LOQ = 1.86 $\mu g\,ml^{-1}$.

Keywords: *Trichilia catigua*; *Ptychopetalum olacoides*; Derivative spectrophotometry; Flavonoids; Analytical method validation; Rutin

1. Introduction

Recent interest in botanical extracts has led to an increased require for efficient methodologies for the assurance of standardization, reproducibility, efficacy, safety and quality of those varieties of raw materials. The renewed consideration on the use of substances derived from natural sources occurs due to the presumable safe utilization, ecological orientation and preservation, and reduced ambient impact (Paniwnyk et al., 2001).

There is a great demand on the addition of natural antioxidants in pharmaceutical forms for topical application and flavonoids are known to exert this activity. Flavonoids are a group of polyphenolic compounds broadly distributed as secondary metabolism in plants and pharmaceutical, cosmetic and food industries present interest on their utilization, specially, for their reported biological activity (Bonina et al., 1996; Kontogianni et al., 2003; Rhodes and Price, 1996).

The botanical extract of *Trichilia catigua* Adr. Juss (and) *Ptychopetalum olacoides* Bentham, Catuaba (Meliaceae) and Marapuama (Olacaceae), respectively, was standardized in total flavonoids content, expressed in rutin. Phytochemical trials of those Brazilian exotic plants also indicated the presence of alkaloids, tannins, aromatic oils, saponins, terpenes, steroids, fatty resins, behenic acid, lupeol, flavonoids and flavalignans (Drewes et al., 2003; Siqueira et al., 2003; Souza et al., 2001).

An assortment of methods have been described by scientific literature to quantify total flavonoids presented in pharmaceutical vehicles, as spectrophotometry, liquid chromatography, capillary electrophoresis, fluorimetry, chemiluminescence and mass spectrometry (Baranowska and Raróg, 2001; Kreft et al., 1999; Leite et al., 2001; Pejic et al., 2004; Queija et al., 2001; Rhodes and Price, 1996; Song and Hou, 2002; Stobiecki, 2000; Valenta et al., 1999). However, up to now, no derivative spectrophotometric method has been validated and employed to analytical

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determination of total flavonoids, expressed in rutin, in topical formulations, besides, this analytical procedure presents application advantages comparing to some routine methods, like: simplicity, rapidity, adequate sensitivity, operational convenience, relative low cost of equipment and reagents.

Derivative spectrophotometry is an analytical technique which consists in the differentiating of normal spectrum by mathematical transformation of spectral curve into a derivative (first- or higher derivatives). This technique usually improves resolution bands, eliminates the influence of background or matrix and provides more defined fingerprints than traditional ordinary or direct absorbance spectra, since it enhances the detectability of minor spectral features (Kazemipour et al., 2002; Ojeda and Rojas, 2004). Derivative transformation permits discrimination against broad band interferents, arising from turbidity or non-specific matrix absorption and it tends to emphasize subtle spectra features, allowing the enhancement of the sensitivity and specificity in mixtures analysis (Karpinska, 2004).

The aim of this research work was to propose, develop and validate a UV-derivative spectrophotometric method for total flavonoids (expressed in rutin) quantification from O/W emulsion obtained by cold process with polyacrylamide (and) C13-14 isoparaffin (and) laureth-7 containing the commercial extract of Catuaba (*Trichilia catigua* Adr. Juss) (and) Marapuama (*P. olacoides* Bentham). Analytical method validation was carried out by establishing parameters, like: linearity, interval evaluation, specificity, precision, accuracy, detection and quantification limits (Analytical Procedures and Methods Validation, 2000; Causon, 1997; Jenke, 1996; Karnes and March, 1993; Polesello, 1997; Shabir, 2003; US Pharmacopeia XXVII, 2004; Ye et al., 2000).

2. Experimental

2.1. Apparatus and reagents

A Beckman DU–640 UV–vis spectrophotometer capable of taking first- to fourth-derivative spectra by an internal data processing system with a 1 cm quartz cuvette was performed to record ordinary and derivative spectra of reference standard and sample solutions. Adams SafetyHead centrifuge, Sartorius BL 2106 analytical balance and Labsystem 4500 pipette (100–1000 μl) were employed. Rutin (Merck, Germany), NF XI secondary standard (96.1%), and ethanol 99.5% (LabSynth, Brazil) analytical reagent grade were used without any further purification. Ethanol 99.5% was the blank solution. O/W emulsion was developed with a self-emulsifying agent that permits emulsification procedure at room temperature, technique known as cold process.

Components were of pharmaceutical grade: polyacrylamide (and) C13-14 isoparaffin (and) laureth-7 (Seppic, France); octyldodecanol (Cognis, Germany); isopropyl palmitate (Croda, United Kingdom); phenoxyethanol (and) methylparaben (and) ethylparaben (and) propylparaben (and) butylparaben (Croda, United Kingdom); cyclomethicone (Dow Corning, United Sates); glycerin (LabSynth, Brazil); disodium edetate (LabSynth, Brazil); and distilled water. *Trichilia catigua* Adr. Juss (Meliaceae) (and) *P. olacoides* Bentham (Olacaceae) extract, standardized in 1.02% (w/w) total flavonoids, expressed in rutin, was purchased from Chemyunion (Brazil). O/W emulsion qualitative and quantitative composition (% w/w) is presented in Table 1.

Table 1
Qualitative and quantitative (% w/w) composition of O/W emulsion obtained by cold process containing the commercial extract of *Trichilia catigua* Adr. Juss (Meliaceae) (and) *Ptychopetalum olacoides* Bentham (Olacaceae) based on self-emulsifying agent polyacrylamide (and) C13-14 isoparaffin (and) laureth-7

Chemical name	Composition (% w/w)
Isopropyl palmitate	1
Octyldodecanol	3
Polyacrylamide (and) C13-14 isoparaffin (and) laureth-7	3
Cyclomethicone	1
Phenoxyethanol (and) methylparaben (and) ethylparaben (and) propylparaben (and) butylparaben	0.5
Glycerin	3
Disodium edetate	0.1
Trichilia catigua Adr. Juss (Meliaceae) (and) *Ptychopetalum olacoides* Bentham (Olacaceae) extract, standardized in 1.02% (w/w) total flavonoids	5
Distilled water	83.4

2.2. Reference standard stock solution and serial dilutions

Standard rutin was accurately weighed (25.0 mg) and transferred to a 50 ml volumetric flask followed by addition of ethanol 99.5% to promote total dissolution. Concentration obtained was 500.0 μg ml^{-1}. Serial dilutions were prepared to concentrations 10.0, 20.0, 30.0, 40.0, 50.0 and 60.0 μg ml^{-1} transferring appropriate amounts of the stock solution to 25 ml volumetric flasks.

2.3. Sample solutions

O/W emulsion was accurately weighed (1.0, 1.5 and 2.0 g) and transferred to 25 ml volumetric flasks separately. Volumes were completed with ethanol 99.5%. Solutions were centrifuged at 863 × *g* (3500 rpm) for 5 min at room temperature. Supernatants were discarded. Analytical concentrations of total flavonoids, expressed in rutin, from O/W emulsion containing 5.0% (w/w) *T. catigua* Adr. Juss (and) *P. olacoides* Bentham commercial extract in ethanol 99.5% solutions were 20.0, 30.0 and 40.0 μg ml^{-1}.

2.4. First- and second-derivative analytical parameters

Analytical parameters for first- and second-derivative were set to perform the assay in optimal conditions. Degree of derivation, amplitude (^1D and ^2D), wavelength range (nm), smoothing factor, scan speed (nm min^{-1}) and delta lambda ($\Delta\lambda$) were established.

2.5. Calibration curve and linearity

Linearity was evaluated by visual and statistical analysis (Guide for Validation of Analytical and Bioanalytical Methods,

2003; Jenke, 1996; Rolim et al., 2005). Calibration curves for ordinary, first- and second-derivative spectrophotometric methods were achieved with six standard rutin concentrations ranging from 10.0 to 60.0 μg ml^{-1}. Six replicates were used (Causon, 1997; Fabre et al., 1993). Calibration curve for ordinary spectrophotometric method was constructed with absorbance measurements at 362.0 nm (Rolim et al., 2005); calibration curves for first- and second-derivative spectrophotometric methods were obtained at 388.0 and 402.0 nm, respectively.

Optimal conditions for calibration curves for first- and second-derivative were determined by coefficient correlation (r), precision (R.S.D., %) and accuracy (E, %) of the results (Guide for Validation of Analytical and Bioanalytical Methods, 2003; Shabir, 2003).

2.6. Specificity to total flavonoids expressed in rutin

Standard rutin and *T. catigua* (and) *P. olacoides* commercial extract spectra were recorded individually and overlapped at wavelength range 300.0–450.0 nm. Analytical concentrations of reference standard and sample (total flavonoids from commercial extract, expressed in rutin) were 30.0 μg ml^{-1}.

T. catigua (and) *P. olacoides* commercial extract (1.02% total flavonoids, expressed in rutin) was accurately weighed (2.5 g) and transferred to a 50 ml volumetric flask. Ethanol 99.5% was added to complete the volume. Solution was centrifuged at 863 × g for 5 min at room temperature. Appropriate amount of supernatant was employed to obtain total flavonoids concentration 30.0 μg ml^{-1}, expressed in rutin (Guide for Validation of Analytical and Bioanalytical Methods, 2003; Rolim et al., 2005).

2.7. Derivative spectrophotometric (DS) method justification and interferent assay

Solutions of O/W emulsion analyte-free (60.0 mg ml^{-1}), *T. catigua* (and) *P. olacoides* commercial extract (30.0 μg ml^{-1} total flavonoids, expressed in rutin) and O/W emulsion added with 5.0% (w/w) *T. catigua* (and) *P. olacoides* commercial extract (30.0 μg ml^{-1} total flavonoids, expressed in rutin) spectra was recorded separately and overlapped at wavelength range 300.0–450.0 nm by ordinary spectrophotometric method.

2.8. Recovery

O/W emulsion analyte-free sample was spiked with a known appropriate amount of standard rutin (0.05% w/w). Spiked sample was diluted to analytical concentrations 20.0, 30.0 and 40.0 μg ml^{-1} of standard rutin in ethanol 99.5%. Spectra were recorded at wavelength range 300.0–450.0 nm. Recovery was performed in nine replicates (Q2B Validation of Analytical Procedures: Methodology, 1996).

2.9. Precision and accuracy

Precision and accuracy were evaluated by nine replicates of analytical concentrations (20.0, 30.0 and 40.0 μg ml^{-1} total flavonoids, expressed in rutin) of the O/W emulsion containing 5.0% (w/w) *T. catigua* (and) *P. olacoides* commercial extract solutions in ethanol 99.5% (sample dilution procedure was described at Section 2.3) (Yilmaz et al., 2004). Precision and accuracy were calculated intra- and inter-run assays as R.S.D. (%) and E (%), respectively, according to Eqs. (1) and (2) (Guide for Validation of Analytical and Bioanalytical Methods, 2003; US Pharmacopeia XXVII, 2004).

$$\text{precision} = \text{R.S.D.}(\%) = \frac{\text{S.D.} \times 100}{C} \quad (1)$$

where R.S.D. (%) is the precision; S.D., the standard deviation; C is the mean of calculated concentrations.

$$\text{accuracy} = E(\%) = \frac{C \times 100}{\text{TC}} \quad (2)$$

where E (%) is the accuracy; C, the mean of calculated concentrations; TC is the theoretical concentration.

2.10. Detection (LOD) and Quantification (LOQ) limits

Detection and quantification limits were estimated by the slope and mean standard deviation of standard rutin concentrations employed to construct the calibration curve, according to Eqs. (3) and (4) (Guide for Validation of Analytical and Bioanalytical Methods, 2003; Q2B Validation of Analytical Procedures: Methodology, 1996):

$$\text{LOD} = \frac{3.3\sigma}{S} \quad (3)$$

where LOD is the estimated detection limit (μg ml^{-1}); σ, the standard deviation of y-intercepts of regression lines ($n = 6$); S is the slope of the calibration curve.

$$\text{LOQ} = \frac{10\sigma}{S} \quad (4)$$

where LOQ is the estimated quantification limit (μg ml^{-1}); σ, the standard deviation of y-intercepts of regression lines ($n = 6$); S is the slope of the calibration curve.

3. Results and discussion

Optimal conditions for DS assays were defined by the analytical parameters for first- (^1D) and second-derivative (^2D). Experimentally, amplitudes of ^1D and ^2D were set at values ranging from ±0.12 and ±0.012, respectively. Wavelength range, smoothing factor, scan speed and delta lambda are reported in Table 2. Amplitude ranges for both derivations enclosed the spectra for minimum to maximum concentrations of standard rutin.

Linearity was evaluated by individual calibration curves, drawn with zero-to-peak values for DS method, in addition to visual and statistical analysis (Sözgen and Tüttem, 2004). Ordinary, first- and second-derivative have generated straight lines indicating proportional and linear relationship of absorbances (ordinary ultraviolet spectrophotometric method) or amplitudes (DS method) values versus concentrations of standard rutin ranging from 10.0 to 60.0 μg ml^{-1} in ethanol 99.5%. Regression

Table 2
Optimal conditions (degree of derivation, wavelength, wavelength range, smoothing factor, scan speed and amplitudes) for first- and second-derivative set experimentally

Degree of derivation	Wavelength (nm)	Wavelength range (nm)	Smoothing factor	Scan speed (nm min^{-1})	Delta lambda ($\Delta\lambda$, nm)
^1D[a]	388.0	300.0–450.0	13	2400	4
^2D[b]	402.0				

[a] First-derivative.
[b] Second-derivative.

lines with corresponding coefficients of correlation (r), slopes and y-intercepts, calculated using the least-square fit method (Shabir, 2003; Süslü and Altinöz, 2002), were

$$A = 0.0307C - 0.0323, \quad r = 0.9996 \tag{5}$$

$$^1A_p = -0.0016C + 0.0003, \quad r = 0.9999 \tag{6}$$

$$^2A_p = 0.0001C - 8.0 \times 10^{-5}, \quad r = 0.9999 \tag{7}$$

where regression lines, slopes, y-intercepts and coefficients of correlation obtained by least-square fit method for ordinary (5), first- (6) and second-derivative (7). A is the absorbance; ^1A_p, the amplitude for first-derivative; ^2A_p, the amplitude for second-derivative; C, the standard rutin concentration ($\mu g\,ml^{-1}$); r is the coefficient of correlation.

Fig. 1 demonstrated the proportional and delineated overlapped spectra of standard rutin concentrations ranging from 10.0 to 60.0 $\mu g\,ml^{-1}$ for ordinary, first- and second-derivative at wavelength range 300.0–450.0 nm.

Intervals of reference substance concentration values used to construct calibration curves were evaluated by correlation coefficients (r) and confirmed by precision (R.S.D., %) and accuracy (E, %), Eqs. (1) and (2) (Trabelsi et al., 2002). According to data obtained, DS method for first- and second-derivative resulted in adequate r, near to 1 (acceptable minimal criteria of correlation coefficient must be greater than $r = 0.99$) (Guide for Validation

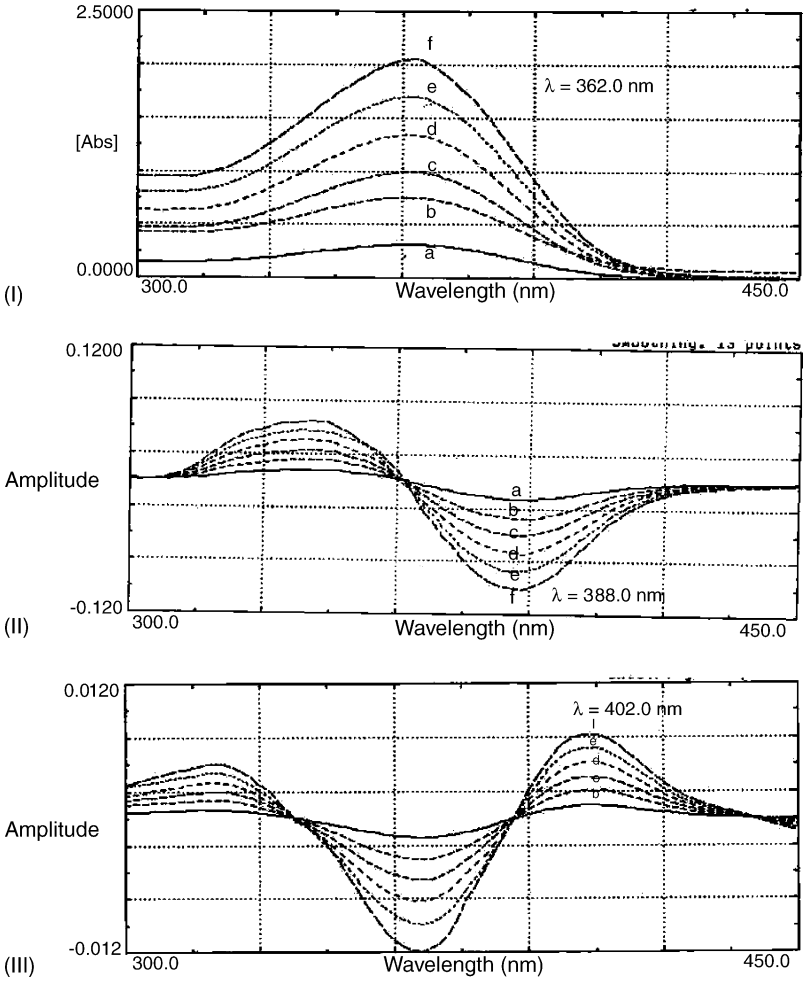

Fig. 1. Ordinary (I), first- (II) and second-derivative (III) overlapped absorbance spectra of standard rutin (96.1% purity) in ethanol 99.5%. Absorbance peaks (λ, nm) was 362.0, 388.0 and 402.0 nm. Concentrations employed were (a) 10.0, (b) 20.0, (c) 30.0, (d) 40.0, (e) 50.0 and (f) 60.0 $\mu g\,ml^{-1}$; Abs: absorbance.

Table 3
Evaluation of interval by precision and accuracy data of standard rutin concentrations used to construct the calibration curves for first- and second-derivative

Standard rutin theoretical concentrations ($\mu g\,ml^{-1}$)	First-derivative ($\lambda = 388.0$ nm)[a]			Second-derivative ($\lambda = 402.0$ nm)[a]		
	Calculated concentrations ($\mu g\,ml^{-1}$)	R.S.D.[b] (%)	E[c] (%)	Calculated concentrations ($\mu g\,ml^{-1}$)	R.S.D.[b] (%)	E[c] (%)
10.0	9.96	1.04	99.58	11.93	0.48	119.33
20.0	20.84	0.78	104.22	25.77	0.22	128.83
30.0	30.27	0.25	100.90	38.17	0.30	127.22
40.0	40.45	0.40	101.12	51.40	0.00	128.50
50.0	50.80	0.67	101.60	64.93	0.62	129.87
60.0	61.13	1.02	101.88	77.30	0.22	128.83

[a] Wavelength (nm).
[b] Precision: relative standard deviation (%).
[c] Accuracy (%).

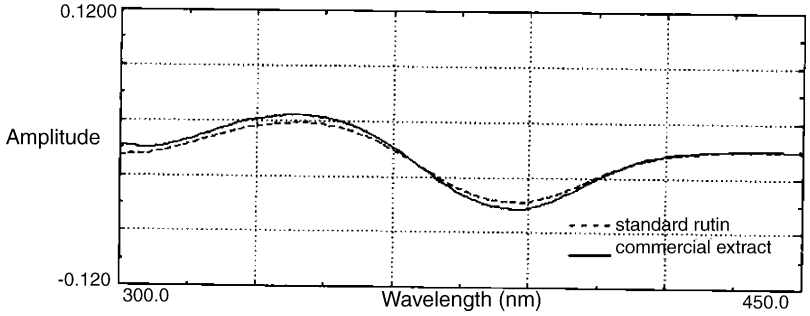

Fig. 2. Specificity of DS method for first-derivative. Overlapped spectra of standard rutin (30.0 $\mu g\,ml^{-1}$) and commercial extract of *Trichilia catigua* Adr. Juss (Meliaceae) (and) *Ptychopetalum olacoides* Bentham (Olacaceae) (30.0 $\mu g\,ml^{-1}$ total flavonoids, expressed in rutin). Ethanol 99.5% was the blank solution. First-derivative provided identical minimum absorbance peaks at 388.0 nm.

of Analytical and Bioanalytical Methods, 2003; Jenke, 1996; Mulholland and Hibbert, 1997), and R.S.D., inferior than 5.0%, although, second-derivative has generated unacceptable E (%) values, ranging from 119.33 to 129.87%, demonstrating that accuracy for total flavonoids (expressed in rutin), at concentration range used, has not represented adequate degree of agreement of results (Bioanalytical Method Validation, 2001; Jenke, 1996). Hence, first-derivative presented to be a functional analytical method for precise and accurate determination of total flavonoids, expressed in rutin (Sözgen and Tüttem, 2004). Table 3 summarizes the results for interval evaluation for first- and second-derivative.

Specificity to total flavonoids, expressed in rutin, was confirmed with the overlapped spectra of standard rutin and *T. catigua* (and) *P. olacoides* commercial extract at identical concentration values, as shown in Fig. 2.

According to the overlapped absorbance spectra of O/W emulsion analyte-free (60.0 $mg\,ml^{-1}$), *T. catigua* (and) *P. olacoides* commercial extract (30.0 $\mu g\,ml^{-1}$ total flavonoids, expressed in rutin) and O/W emulsion containing 5.0% (w/w) *T. catigua* (and) *P. olacoides* commercial extract (30.0 $\mu g\,ml^{-1}$ total flavonoids, expressed in rutin) (Fig. 3), ordinary ultraviolet spectrophotometric method at 362.0 nm has revealed to offer valuable response of interferent from polyacrylamide (and) C13-14 isoparaffin (and) laureth-7, the self-emulsifying concentrate used to obtain emulsions by cold process. Based upon the ordinary spectrophotometric data, there is no wavelength where total flavonoids can be accurately quantified without substantial

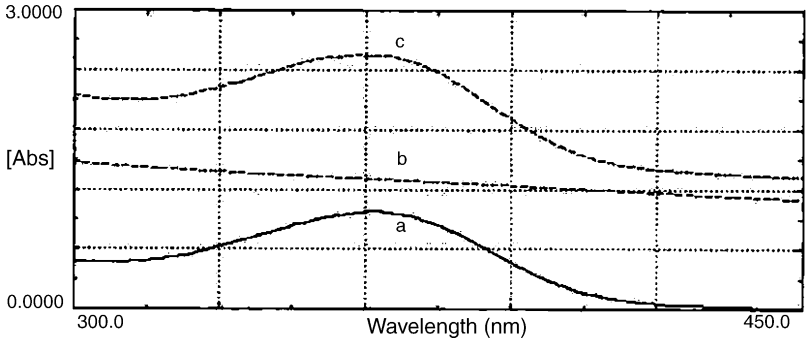

Fig. 3. Ordinary absorbance spectra of (a) standard rutin (30.0 $\mu g\,ml^{-1}$), (b) O/W emulsion analyte-free obtained by cold process with polyacrylamide (and) C13-14 isoparaffin (and) laureth-7 (60.0 $mg\,ml^{-1}$) and (c) spiked O/W emulsion (60 $mg\,ml^{-1}$; 30.0 $\mu g\,ml^{-1}$ equivalent in total flavonoids). Abs: absorbance.

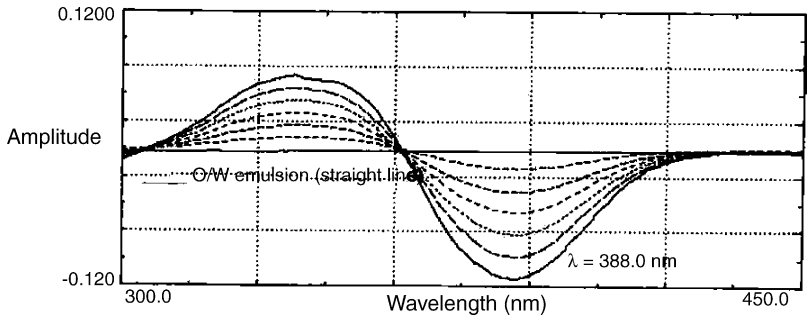

Fig. 4. Selectivity for first-derivative overlapped spectra of standard rutin solutions (concentrations ranging from 10.0 to 60.0 μg ml^{-1}) and O/W emulsion analyte-free (straight line passing through amplitude zero) obtained by cold process with polyacrylamide (and) C13-14 isoparaffin (and) laureth-7 in ethanol 99.5%.

background interference from the matrix (Kazemipour et al., 2002).

Such observation provided to be a concern to pharmaceutic and cosmetic chemists considering the fact that this self-emulsifying agent presents attractiveness to personal and health care industries since production will be reduced in cost by preventing energy spent avoiding emulsification heat process and its compatibility with several raw materials and active substances. Additionally, its profile exerts analytical interference for quantification of active substances by direct or ordinary spectrophotometric method (Anchisi et al., 2001). The DS method (first-derivative at 388.0 nm) has revealed to be specific and selective for the reason that excipients/matrix did not interfere the active substance quantification during the determination of the samples (Fig. 4) (Kazemipour et al., 2002).

The absorption spectrum of O/W emulsion analyte-free in the 300.0–450.0 nm wavelength region, reported in Fig. 3, produced a large overlap of spectral bands of matrix and sample preventing determination of total flavonoids by ordinary absorbance measurements (Trabelsi et al., 2002). DS method, zero-to-peak for first-derivative, has eliminated the interferent response from polyacrylamide (and) C13-14 isoparaffin (and) laureth-7, allowing the development of a new DS method, analytical validation process and the quantification of total flavonoids (expressed in rutin) present in O/W emulsion containing *T. catigua* (and) *P. olacoides* commercial extract. An analytical method is specific when it is capable to provide truthful measurements of the compound in study in the presence of other components, impurities, degradation products and components of the matrix (Guide for Validation of Analytical and Bioanalytical Methods, 2003; US Pharmacopeia XXVII, 2004).

Recovery experiment was conduct to corroborate with accuracy of the proposed DS method for first-derivative. The mean recovery value ($n = 9$) for spiked O/W emulsion with standard rutin was 108.78% indicating acceptable accuracy.

Intra-run ($n = 9$) and inter-run ($n = 9$) assays for precision, calculated as R.S.D. (%), and for accuracy generated values that are reported in Table 4. Relative small amount of R.S.D. (%), not exceeding the limit of 5.0%, with mean values for intra- and inter-run assays were 2.23 and 4.13%, respectively, confirmed the precision, reproducibility and repeatability of the first-derivative method at 388.0 nm for total flavonoids (expressed in rutin) present in O/W emulsion containing the commercial extract of *T. catigua* (and) *P. olacoides*. Accuracy mean values were 106.91 and 110.66%, correspondingly, for intra- and inter-run assays. Satisfactory accuracy values indicated no considerable differences between the amounts of total flavonoids present in O/W emulsion obtained by cold process containing the extract and the theoretical concentration values of samples dilutions (analytical concentrations of total flavonoids, expressed in rutin content, ranging from 20.0 to 40.0 μg ml^{-1}) (Analytical Procedures and Methods Validation, 2000; Jenke, 1996; Karnes and March, 1993; Polesello, 1997).

LOD and LOQ were estimated as 0.62 and 1.86 μg ml^{-1}, respectively. According to those values, DS method is capable to detect concentrations of total flavonoids (expressed in rutin), differentiating from equipment noise, concentrations above 0.62 μg ml^{-1}, but not precise and accurately. Concentrations values higher than 1.86 μg ml^{-1} (LOQ) of total flavonoids

Table 4
Intra- and inter-run assays for determination of precision and accuracy of DS method for first-derivative (λ = 388.0 nm), zero-to-peak ($n = 9$)

Analytical concentrations of samples (μg ml^{-1})	Intra-run			Inter-run		
	S.D.[a]	R.S.D.[b] (%)	E[c] (%)	S.D.[a]	R.S.D.[b] (%)	E[c] (%)
20.0	0.0005	1.30	112.19	0.0013	3.48	118.44
30.0	0.0009	1.75	108.54	0.0025	4.68	112.29
40.0	0.0023	3.65	100.00	0.0028	4.24	101.25

Intra-run assay was evaluated in 1 day and inter-run, in three consecutive days.
[a] Standard deviation.
[b] Precision: relative standard deviation (%).
[c] Accuracy (%).

Table 5
Detection (LOD) and quantification (LOQ) limits for total flavonoids (expressed in rutin) determined by mean values of *y*-intercepts ($n = 6$) and slopes of calibration curve for standard rutin (first-derivative, $\lambda = 388.0$ nm)

y-Intercepts	σ[a] ($n = 6$)	Slope	LOD[b] ($\mu g\,ml^{-1}$)	LOQ[c] ($\mu g\,ml^{-1}$)
0.00009				
0.0009				
0.00008	0.0003	0.0016	0.62	1.86
0.0004				
0.0003				
0.0003				

[a] Standard deviation of *y*-intercepts from calibration curve of standard rutin for first-derivative ($n = 6$).
[b] Estimated detection limit.
[c] Estimated quantification limit.

in ethanol 99.5% may be precise and accurate quantified by the proposed DS method for first-derivative, zero-to-peak, at 388.0 nm (Polesello, 1997; Bioanalitical Method Validation, 2000). Data for LOD and LOQ are reported in Table 5.

4. Conclusions

So far, no derivative procedures have been described for assay of total flavonoids, expressed in rutin, present in topical formulation. As a result, a new derivative spectrophotometric method was developed for routine determination of total flavonoids. Method advantages are the lack of sample extraction process, rapidity, specificity and selectivity, equipment convenience and relative low cost of reagents (Baranowska and Raróg, 2001; Lastra et al., 2000; Sözgen and Tüttem, 2004). Total flavonoids solutions were prepared with ethanol, nontoxic, low environmental impact and cheaper instead of methanol, a solvent habitually employed to flavonoids quantification (Tatar and Saglik, 2002).

The proposal of a first-derivative spectrophotometric method was found to be suitable for total flavonoids (expressed in rutin) quantification present in O/W emulsion obtained by cold process containing the commercial extract of Catuaba (*Trichilia catigua* Adr. Juss, Meliaceae) (and) Marapuama (*Ptychopetalum olacoides* Bentham, Olacaceae), standardized in total flavonoids content (1.02% w/w). The experimental results have provided that analytical parameters and validation process were precise and accurate according to adequate linearity, specificity, recovery, repeatability, reproducibility, LOD and LOQ.

This described method has eliminated the interferents from excipients, allowing the quantification of total flavonoids (expressed in rutin) in O/W emulsion or a corresponding matrix based on polyacrylamide (and) C13-14 isoparaffin (and) laureth-7.

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