



Original article

Bioanalytical method by HPLC-FLD for curcumin analysis in supplemented athletes



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ABSTRACT

In sports, curcumin, a substance derived from the rhizome of *Curcuma longa* (turmeric) plant with antioxidant effect 8 times greater than vitamin E, has attracted the attention of scientists because of its potent antioxidant action, since in athletes subjected to intense exercise the—endogenous mechanisms of neutralization of reactive species are saturated. However, the pharmacokinetic characteristics of curcumin do not favor its medicinal use due to its low absorption, accelerated metabolism and rapid systemic elimination. Thus, the determination of plasma levels in supplemented patients is a crucial step in their pharmacodynamic evaluation. Therefore, the objective of this work was to develop and validate an analytical method by HPLC-FLD for curcumin evaluation in plasma of supplemented athletes. Luna column (C18; 150 × 4 mm; 3 μm), acetonitrile: acetic acid pH 3.2 (45:55 to 60:40) as mobile phase, flow rate of 1 mL min⁻¹, excitation at 429/285 nm and emission at 529 nm and injection of 10 μL were the chromatographic conditions used. Plasma samples were extracted using ethylacetate and methanol (95: 5, 500 μL) and estradiol (30 μg mL⁻¹) as internal standard, with subsequent stirring (3 min) and centrifugation (8 min) (triple extraction). The organic fraction was evaporated under N₂ (20 min) and the dried residue reconstituted in acetonitrile. The method was linear between 44 and 261 ng mL⁻¹, showing intra-day (2.05.6%) and inter-day (4.0–5.1%) precision with accuracy and selectiveness (curcumin t_R = 8.7 min and internal standard t_R = 13.9 min with relative recovery of 83.2%). So, it can be successfully used for curcumin evaluation in plasma samples from supplemented athletes, as well as being an alternative and advantageous method to UV–Vis and MS/MS in bioavailability studies.

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1. Introduction

Curcumin (CUR, Fig. 1) is the major yellow pigment present in turmeric which is derived from the rhizome of the *Curcuma longa* plant (Ireson et al., 2002). Preclinical studies have shown high potential against chronic inflammatory diseases (Aggarwal and

Harikumar, 2009). Specifically, in sports its powerful antioxidant activity has been receiving great attention (Singh et al., 2011). Evidences point to their usage on attenuating muscles oxidative stress contributing on reducing its damage and fatigue (Takahashi et al., 2010; Ferreira and Reid, 2008; McGinley et al., 2009).

However, CUR pharmacokinetic characteristics were not favorable for medical use due to its low absorption, rapid metabolism, and rapid systemic clearance (Anand et al., 2007). The determination of plasma levels in athletes after its administration is a critical step of pharmacodynamics evaluation. So, an important stage in the bioavailability study is the validation of analytical methodologies that ensure results with high quality data (Kollipara et al., 2011).

Techniques such as liquid chromatography (LC) coupled with mass spectrometry (MS/MS) have been commonly used to quantify compounds in biological matrices due to its sensitivity, specificity, and accuracy (Ramalingam and To, 2014). There are many sensitive

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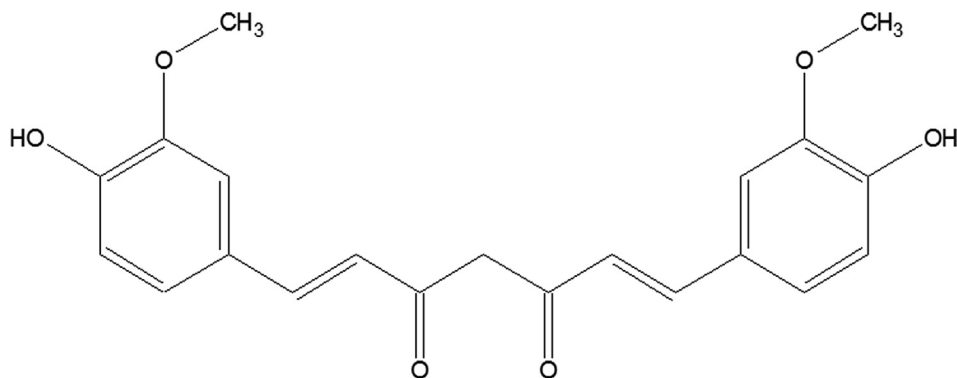


Fig. 1. Chemical structure of curcumin (CAS 458-37-7).

LC-MS methods for the determination of CUR plasma levels that have been published (Liu et al, 2006; Ramalingam and To, 2014). However, even with its advantages, the high cost of buying and maintenance still barriers to their use (Martinez and Garrido, 2005).

So that, the fluorimetric detectors appear as an alternative when good sensibility at affordable costs are desired. Typically, fluorescence detectors exhibit sensitivity between 10 and 1000 folds higher than the UV–VIS detector (Ramni et al, 2011). However, this has not yet been observed for CUR determination. For example, Setyaningshi and co-authors (2016) using UV/VIS found 6 ng mL⁻¹ for the lower limit of quantitation (LLOQ) while Schiborr and colleagues (2010) using fluorescence detection obtained 20 ng mL⁻¹ for LLOQ. A very few fluorimetric methods are up to date described for curcumin (Kotra et al, 2019) predominating LC-MS/MS analysis for biological samples (Kotha and Luthria, 2019).

For fluorimetric methods, selectivity is one of the advantages when compared with photometric methods, since there are fewer fluorescing molecules compared to those that absorb light (Bright, 1988). This feature, combined with the use of selective extraction methods such as binary mixture and, enrichment of samples after the extraction process from the biological matrix, can significantly increase sensitivity. This is particularly interesting when, a low interference from endogenous and exogenous compounds on the instrument's signal is observed (Schiborr et al, 2010; Dou et al, 2017a; Dou et al, 2017b; Jiang et al, 2019).

Therefore, the objective of this study was to develop and validate an affordable sensitive and selective method in HPLC-FLD for analysis of CUR after enzymatic treatment of athletes over continuous supplementation of *Curcuma longa*.

2. Experimental part

2.1. Material, chemicals and reagents

All reagents such as curcumin (CUR), β -estradiol-17-acetate (IS), β -Glucuronidase from *Helix pomatia* were of analytical grade and purchased from Sigma Aldrich (Germany). The sample to be analyzed was plasma from 15 athletes. Acetic acid was purchased from Vetec Quimica Ltd. (Brazil). Acetonitrile (ACN), ethylacetate (EtOAc) and methanol (MeOH) used were of HPLC analytical grade (Merck Millipore, Germany). Purified water was obtained from Elga Purelab Option Q, United Kingdom.

2.2. Physicochemical properties

The physicochemical properties of the analyte play an important role in chromatographic separations, especially the partition coefficient (Log P) and pKa. It allows the selection of an optimum pH for

mobile phases to be adjusted favoring the non-ionized form of molecules, leading for a higher retention of the analyte in the reverse stationary phase, increasing the selectivity. On the other hand, the Log P represents the partition coefficient of the molecules. In this sense, the nonpolar property of reverse stationary phase, for those compounds which present higher Log P values, they usually show higher retention time. The physicochemical properties of the CUR and β -estradiol-17-acetate are presented in Table 1.

2.3. Chromatographic conditions

The HPLC system Infinity 1260 (Agilent Technologies, USA) was comprised of a quaternary pump, automatic injector, and fluorescence detector. The chromatographic separations were achieved using a C18 Luna[®] column (150 × 4.0 mm, 3 μ m) coupled with a Security Guard[®] Gemini C18 column (4 × 3 mm) (Phenomenex, USA). The mobile phase was composed of gradient elution of ACN and a mixture aqueous acetic acid pH 3.2. The following linear gradient was programmed: zero – 8 min: 45% ACN, 8.05–15 min 60% ACN; 15.05–20: 45% ACN. Detector was initially set up (10 min) for excitation wavelengths of internal standard (285 nm) and automatically changed at 10.1 min for CUR (430 nm). For CUR/IS emission wavelength, detector was set at 523 nm (Weinreb and Werner, 1969; Weinreb and Werner, 1974; Wang et al., 2006; Neale et al., 2009; Zhang et al., 2009; Chan et al., 2013).

2.4. Preparation of stock solutions and calibration standards

Stock solutions (SS) of CUR (43.0 μ g mL⁻¹) and IS (1060.0 μ g mL⁻¹) were prepared in acetonitrile and ethylacetate, respectively. Subsequently, other seven intermediate solutions (220.0; 232.0; 310.0; 413.0; 550.0; 979.0; 1305.0 ng mL⁻¹) were prepared from serial dilutions in acetonitrile.

Calibration curve (CC) samples were spiked by adding equal volumes of those intermediate solutions to human plasma to yield eight CUR concentrations (8.5; 8.9; 11.9; 15.9; 21.2; 28.2; 37.7; 50.2 ng mL⁻¹) in order to keep organic solvent constant in plasma samples (<4.0%). Aliquots of organic solvent in plasma samples were kept constant (3.8%). Four levels of quality control (QC) standards (Lowest – 8.5; Low – 11.9; Medium – 37.7; High –

Table 1
Physicochemical properties of CUR and estradiol.

Compound	LogP	pKa	Water solubility (mg mL ⁻¹)
CUR ^a	4.12	8.5	Insoluble
β -estradiol-17-acetate ^b	3.75	10.33	0.0213

^a ChemSpider (<http://www.chemspider.com>).

^b DrugBank (<http://www.drugbank.ca>).

50.2 ng mL⁻¹) were also prepared in the same way. Subsequently, CC and QC spiked plasma samples were aliquoted (500 µL) and frozen (-80 °C) prior to the extraction.

2.5. Enzymatic reaction and extraction procedure

Acetate buffer 0.1 M pH 5.0 was used to maintain the constant pH during the entire incubation time (30 min). To break the glucuronide, β-Glucuronidase aliquots (50 µL) type HP-2 from *Helix pomatia* were activated by pre-incubation at 37 °C for 5 min with acetate buffer 0.1 M pH 5.0 (500 µL) in a neutral borosilicate tube. Next, plasma aliquot (500 µL) was added and samples were incubated for 30 min. After this time, the reaction was quenched by adding a mixture of EtOAc: MeOH (95:5), the same solvent used in the extraction process. CUR was extracted from the plasma samples using the following described method (Fig. 2).

Solvent extraction mixture (3 mL; EtOAc: MeOH 95:5) containing the IS (3.0 µg) were added to the samples, vortexed at 2000 rpm/3 min, centrifuged (2.575 × g/8 min) and supernatant aliquot (90%) transferred to clean borosilicate tubes. The extraction procedure was repeated twice and the final supernatant volume (8.1 mL) was evaporated under N₂. Finally, the residue was reconstituted with ACN (100 µL) and injected (10 µL) into the HPLC-FLD system. Thus, the original samples were 5 times concentrated for analysis (Schiborr et al., 2010).

2.6. Bioanalytical method validation

2.6.1. Selectivity

The selectivity was evaluated by the direct comparison of the blank chromatogram with the chromatogram containing CUR and IS (β-estradiol-17-acetate) in plasma as previously described by Araújo and co-authors (2009). Additionally, blank plasma from

six different male volunteers was evaluated for co-eluting peaks with the analytes (EMA, 2011).

2.6.2. Linearity and limit of quantification

The linearity of the method was determined in triplicate by means of seven calibration standards over the concentration range of 44–261 ng mL⁻¹. The results were expressed by calculating the coefficient of determination (R²), slope and linear coefficient (Tiwari and Tiwari, 2010) as directed by ICH (1996).

The lowest limit of quantification (LLQ) was determined using the dilution method. Additionally, the theoretical concentration was determined based on the baseline threshold (Eq. (1)) and compared with those obtained by the dilution method.

$$LLQ = \frac{DP_a \times 10}{IC} \quad (1)$$

DPa: SD of the intercept
IC: calibration curve slope.

2.6.3. Recovery

The efficiency of the liquid-liquid extraction (LLE) method was evaluated by comparing the extracted (ES) with the non-extracted (NS) samples to get relative recovery. Respectively, this corresponds to spiked plasma submitted to extraction process and, the blank plasma submitted to extraction process spiked at the reconstitution time (FDA, 2013). Then, the relative recovery was calculated based on ratio of the peak areas of each sample using the equation below (Eq. (2), Kadian et al., 2016).

$$\text{Recovery (\%)} = \frac{\text{Peak area of drug in extracted sample (ES)}}{\text{Peak area of drug in non-extracted sample (NS)}} \times 100 \quad (2)$$

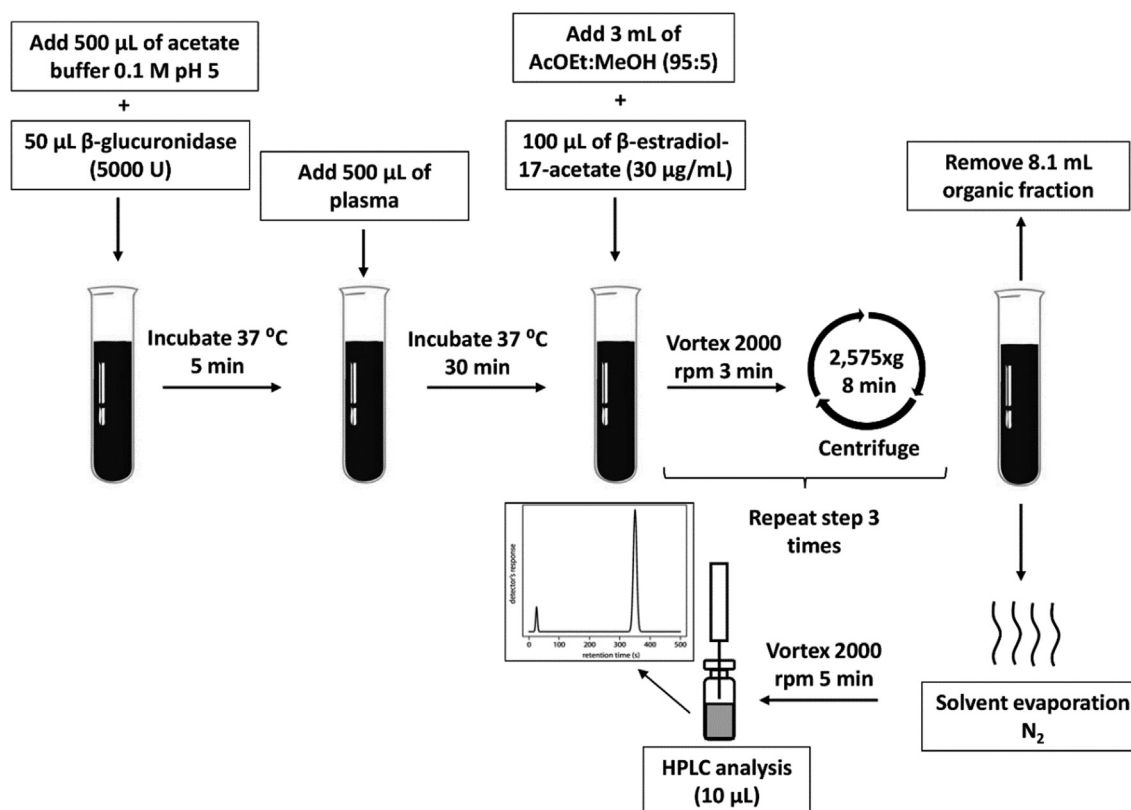


Fig. 2. Enzymatic hydrolysis reaction and liquid-liquid extraction procedure used for CUR and IS (β-estradiol-17-acetate).

2.6.4. Precision and accuracy

Precision and accuracy were determined by intra and interday samples at four different concentration levels: high (HQC, 261.0 ng mL⁻¹), medium (MQC 147 ng mL⁻¹) and low (LQC 63 ng mL⁻¹) quality controls (QC) and also at lowest limit of quantification (LLQ 44 ng mL⁻¹).

Precision was expressed by means of relative standard deviation (RSD) from intraday (n = 5) and interday (n = 20) sample replicates. Accuracy was calculated as follows (Eq. (3)) and expressed by the relative error (RE, Kadian et al., 2016).

$$\text{RE (\%)} = \frac{\text{Measured concentration} - \text{nominal concentration}}{\text{nominal concentration}} \times 100 \quad (3)$$

For both parameters, results were considered acceptable when RE values were lower than $\pm 20\%$ for LLQ and $\pm 15\%$ for QC samples, and RSD values were $\leq 20\%$ for LLQ and $\leq 15\%$ for QC samples.

Additionally, the analysis of variance test (ANOVA) was applied the interdays precision, *i.e.*, whether there was a statistical difference between groups for mean comparison ($\alpha = 0.95$). Finally, for calculating statistical parameters the Microsoft Excel software version 2010, BioEstat (2005) and STATISTICA 7 (2008) were used.

2.7. Pharmacokinetic application

The applicability of the LC-FLD method developed for plasma monitoring studies was conducted in half marathon (21 km) male athletes' volunteers (n = 28, 23 to 50 years old) receiving turmeric capsules twice a day (1.5 g per day). The CUR content in turmeric was determined by HPLC-FLD. Total curcuminoids were tentatively estimated by back-calculation from curcumin calibration curve equation.

Prior to the administration, blood sampling was performed for *time zero* (T₀). The volunteers were divided in two groups: (G1, n = 15) treated with CUR capsules for 30 days and (G2; n = 13) set as control group taking placebo capsules. Blood samples were taken during the half marathon of Goiânia (Goiânia, Goiás, Brazil) at three different moments: Time 1 (T₁) at fasting state, before the competition. After that, at 7 a.m., the athletes received two capsules and, a balanced breakfast. Time 2 (T₂) was set at the end of the half-marathon race (21 km; 111.6 \pm 25 min). Finally, the third sampling time (T₃) was performed 2 h after finishing the marathon (around 240 min after administration). Blood samples were kept under refrigeration, transported to the College of Nutrition (FANUT-UFG) and centrifuged after approximately 40 min. The plasma obtained was then transferred to borosilicate tubes and stored in ultrafreezer (-80 °C) until the moment of analysis.

3. Results and discussion

3.1. System suitability

The validated analytical method showed adequate separation of CUR (8.7 min) and estradiol (13.9 min) peaks. Also, chromatographic column showed to be efficient in the separation of compounds, expressed as plate numbers (N), 6330 and 54674, for CUR and IS, respectively, as well as appropriate peak asymmetry (A) of 1.11 and 1.10, as recommended by the FDA (1994). The capacity factor (K') was higher than 8.87, showing excellent interaction of the analyte with the stationary phase as well as suitable relative retention time (RT) compared to the column dead time.

3.2. Bioanalytical method validation

3.2.1. Selectivity

Selectivity was evaluated through blank samples analysis and its chromatograms visual inspection compared to the analyte and IS retention times and possible co-eluted interferences peaks. During the validation process no peaks were found at CUR and IS retention time in plasma from three different individuals, as shown in Fig. 3, its ability to measure the analyte of interest.

3.2.2. Recovery

The LLE method allowed high relative recovery (83.2%) for CUR in two different concentration levels, low (63 ng mL⁻¹) and high (261 ng mL⁻¹) and IS at a concentration of 30.0 ng mL⁻¹ (94.3%). The average and standard deviation of recovery results are shown in Table 2.

Therefore, the extraction of CUR in plasma used a binary mixture of ethylacetate and methanol (95:5) as described by Schiborr and coworkers (2010), triple extraction, resulting in proper recovery to CUR (82.3%) and estradiol (94.3%), maintaining excellent selectivity. In an attempt to maximize the time of preparation of the samples, a process using double extraction was performed, but this showed low efficiency, *e.g.*, recovery presented below 60% for CUR, which affect its detection and quantification.

3.2.3. Linearity

The method showed to be linear over the concentration range of 44 to 261 ng mL⁻¹, with a linear correlation coefficient (R²) greater than 0.98 (Table 3).

The Brazilian official guidance (Brazil, 2012; Kadian et al., 2016) which regulates the validation of bioanalytical methods in the country recommends using a simple regression model, if possible. However, it does not clarify which parameters to evaluate or a limit for the acceptance criteria. Similar actions are seen for FDA (1994) and EMA (2011) guidance. Herein validated method had its linearity reported by the coefficient of determination, slope, linear coefficient and residuals as described by Tiwari and Tiwari (2010). Furthermore, the analysis of variance by means of test F was also performed in order to strengthen the indicative of a positive correlation between the analyte/IS ratio and concentration (Brito et al., 2003).

Worthy mentioning that linearity at high analyte concentrations is unlikely to happen, as there is absorption of the excited light before it reaches the nucleus of the cell where the emission is produced and accepted by the optical detector, or distortions in the emission lead to a nonlinear relationship with the concentration. Consequently, the linearity between absorbed light and the emitted fluorescence is valid only at low absorptions (Elmer, 2000). Thus, applying an adequate lower concentration range allowed a precise and accurate quantification method since there is greater quantum yield of fluorescence detection (Penzkofer and Lu, 1986). On the other hand, at high concentrations, quenching occurs due to the formation of dimers or complex that behave as flexible molecules in solvents with low viscosity, relaxing by rapid internal conversion (Bartrop and Coyle, 1978). Frequently, the complexes formed are known as non-fluorescent (Selanger et al., 1977) or fluorescence shifted in wavelength (Packer, 1968).

3.2.4. Lowest limit of quantification

Quantifying CUR in supplemented athlete's plasma samples required a high sensitivity method and thus, the lowest limit of quantification was an essential parameter. As a result, the photomultiplier tube factor (PMT) was set at value of 15 without compromising precision (5.8%) and accuracy (-9.0 to 12.7%) at LLQ (44 ng mL⁻¹), with excellent signal/noise relation. Thus, at such

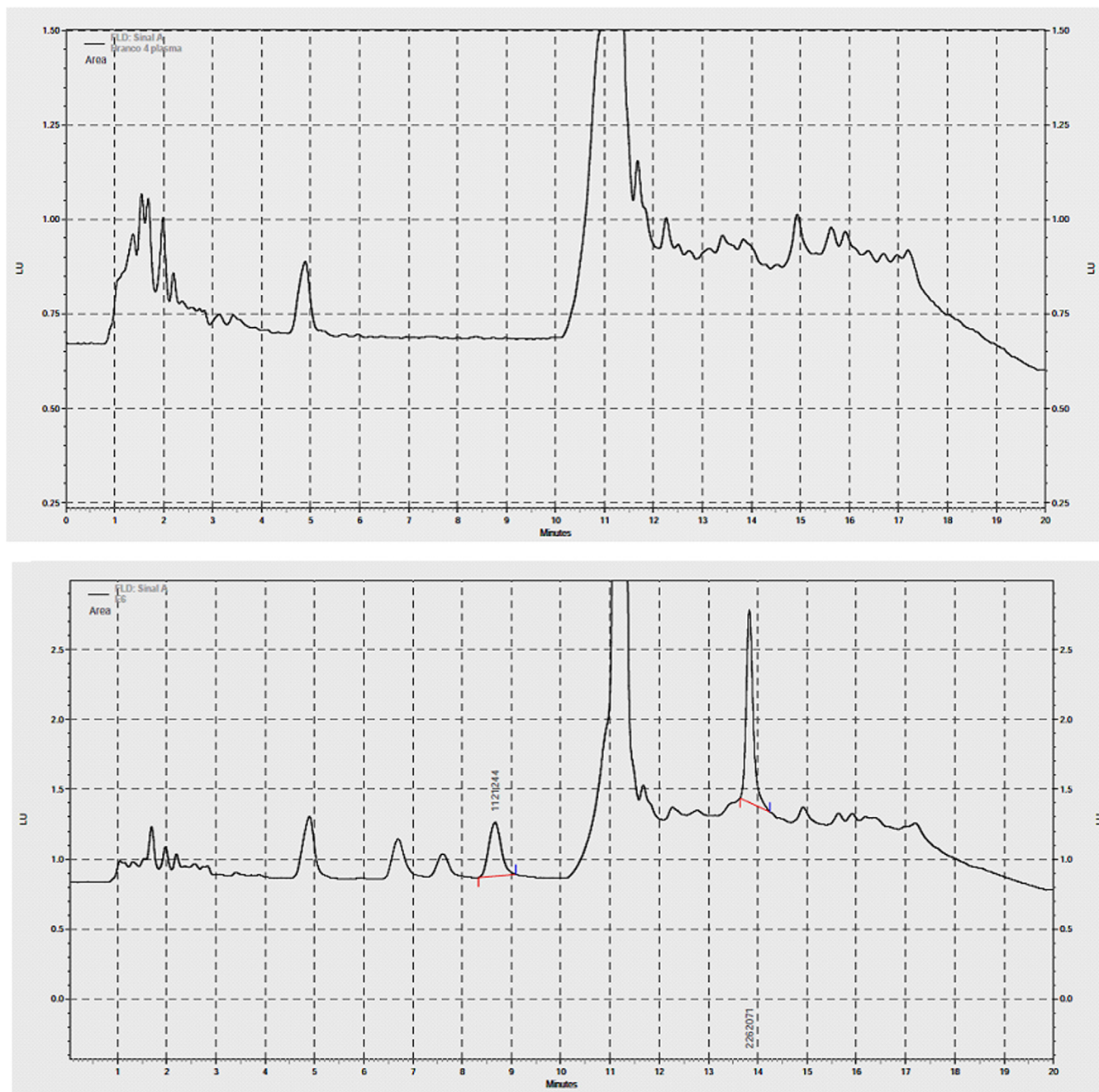


Fig. 3. Representative chromatograms of samples **a**) blank plasma (top) **b**) plasma spiked with curcumin (8.7 min; 178.0 ng/mL) and estradiol (13.7 min; 27 ng/mL) at bottom. Chromatographic conditions: Luna C18 150 × 4 mm, 3 μm; ACN: acidified water pH 3.2 (45:55 to 60:40); 1 mL min⁻¹; 10 μL; FLD (nm) excitation at 430 for CUR (up to 10 min) and 285 for IS (10.1–20 min). Emission was full time set at 523, for CUR/IS.

Table 2

Efficiency of the LLE method for CUR (analyte) and β-estradiol-17-acetate (PI) in human plasma.

Drug	C nominal (ng mL ⁻¹)	Recovery (n = 6) Mean (%) ± RSD (%)
CUR	62.0	83.7 ± 9.5
	261.0	82.7 ± 12.3
β-estradiol-17-acetate	30.0	95.0 ± 9.2
		93.3 ± 10.1

Table 3

Regression parameters of the calibration line obtained on each day.

Day	a	b	R ²
1	0.0037	0.0002	0.9951
2	0.0037	0.003	0.9881

LLOQ level, plasma samples originally measuring 8.8 ng mL⁻¹ were read as 5 × concentrated (44.0 ng mL⁻¹), after analytical treatment.

3.2.5. Precision and accuracy

The results of precision and accuracy for two different analysis performed at seven-day intervals are shown in [Table 4](#).

Table 4
Intra and inter-day precision and accuracy results for CUR extracted from the plasma samples.

Nominal (ng mL ⁻¹)	Intra-day			Accuracy (%)	Inter-day		
	Measured (ng mL ⁻¹)	Mean ± SD	RSD (%)		Measured (ng mL ⁻¹)	Mean ± SD	RSD (%)
44	45.1 ± 2.3		5.1	2.5	44.0 ± 1.9		4.3
63	67.6 ± 3.8		5.6	7.3	68.0 ± 3.3		4.9
147	161.0 ± 5.0		3.1	9.5	157.1 ± 8.0		5.1
261	273.3 ± 5.5		2.0	4.7	268.5 ± 10.6		4.0

The relative standard deviation (RSD %) indicated that the analysis performed on different days had variation values according to the recommended ($\leq 15\%$) (FDA, 2013).

3.3. Pharmacokinetic application

HPLC-FLD analysis of *Curcuma* capsules (3 capsules, 1.5 g) administered to athletes showed a content of $2.2\% \pm 16.0$ (% w/w) meaning that 33.0 mg of CUR was averaged found in the daily dose. When the amount of three major curcuminoids were inferred by back-calculating from curcumin calibration curve equation, all 3 capsules of *Curcuma longa* provided a total curcuminoids dose of 64.5 mg (4.3%) i.e. 33.0 mg of curcumin; 16.5 mg of demethoxycurcumin and, 15 mg of bisdesmethoxycurcumin (Fig. 4).

LC-FLD analysis of athletes' plasma showed quantifiable CUR concentrations for 24 (53.3%) out of 45 samples ($n = 15$) (Table 5). At the sampling time T_1 (before supplementation), no quantifiable levels of CUR (5.4 ± 2.6 ; $n = 15$) were mostly seen, except for subject #3 and #11. Accordingly, assayed samples are representing the fasted state after 30 days of supplementation (3 capsules daily).

Additionally, at the sampling T_2 (immediately after ending half-marathon; 111.6 ± 25 min), mean plasma concentrations (ng mL⁻¹) were higher (15.6 ± 5.3 ; $n = 14$) compared to the T_1 , since athletes were supplemented with two capsules of 500 mg of the morning dose of turmeric powder, equivalent to 22.0 mg of CUR.

Finally, two hours after the end of the half-marathon race (T_3), CUR plasma concentration (ng mL⁻¹) showed to be already declined (15.8 ± 6.1 ; $n = 15$) compared to T_2 , but still greater than concentrations in T_1 .

At the control group (placebo), although non-quantifiable, three of thirteen athletes showed detectable plasma levels of CUR.

Table 5
Plasma concentrations of CUR in the samples of 15 supplemented athletes.

Volunteer	Plasma concentration (ng mL ⁻¹)*		
	Time 1 (T_1)	Time 2 (T_2)	Time 3 (T_3)
1	N.Q.	18.3	13.9
2	N.Q.	18.5	N.Q.
3	10.4	26.0	21.3
4	N.Q.	19.5	12.5
5	N.Q.	13.8	13.6
6	N.Q.	9.0	N.Q.
7	N.Q.	11.3	N.Q.
8	N.Q.	24.1	9.4
9	N.Q.	N.Q.	N.Q.
10	N.Q.	14.8	10.1
11	12.7	14.2	12.0
12	N.D.	9.8	N.Q.
13	N.Q.	23.7	16.6
14	N.D.	12.3	N.Q.
15	N.Q.	17.1	N.Q.

* Plasma concentration before LC-analysis (sample treatment enhanced 5xCUR value).

The low plasma concentrations at steady state, especially in T_2 , can be tentatively justified by the low water solubility (~ 11 ng mL⁻¹) due to the hydrophobic nature of CUR, representing a major barrier to absorption (Aggarwal et al., 2003). Furthermore, studies have shown low apparent permeability ($P_{app} > 0.1 \times 10^{-6}$ cm s⁻¹) indicating reduced intestinal absorption rate (0–20%) (Artursson and Karlsson, 1991; Dempe et al., 2013).

Chemical instability in neutral and alkaline conditions has also been mentioned as a limiting factor for bioavailability. Wang and co-authors (1997) showed a degradation by about 90% of CUR when incubated in serum-free medium and 0.1 M phosphate buffer pH 7.2 at 37° C for 30 min. However, when in human blood, less

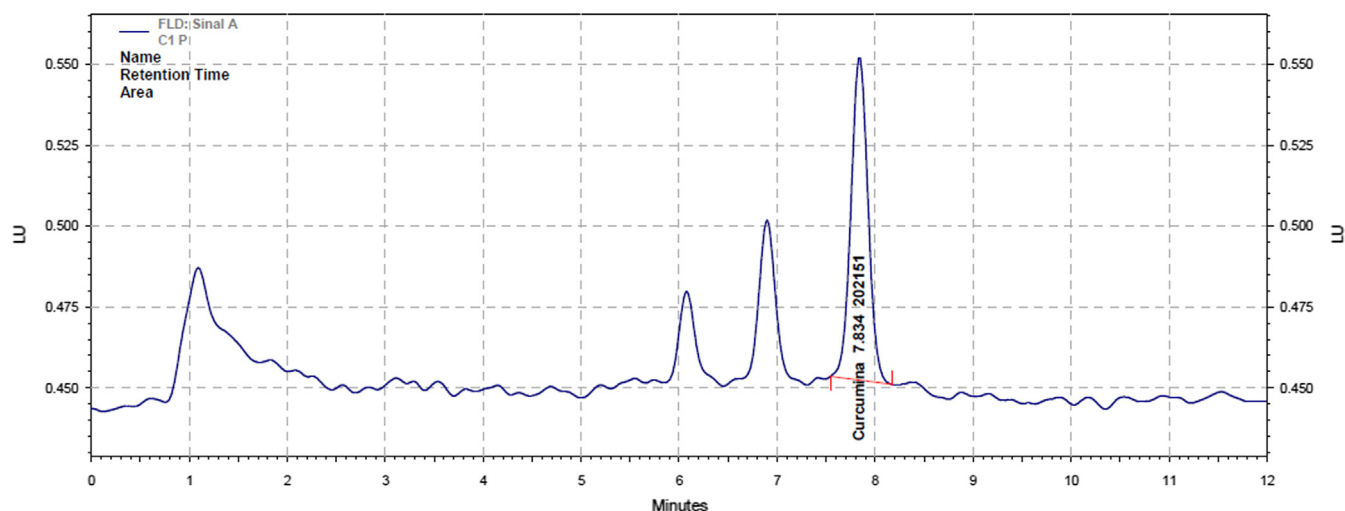


Fig. 4. Chromatogram of *Curcuma longa* capsules administered to athletes. LC-FLD analysis conditions: Luna C18 column (150 × 4 mm, 3 μm); mobile phase (ACN: acidified water pH 3.2 45:55 60:40) at 1 mL min⁻¹ and FLD (nm) excitation at 430 for CUR (up to 10 min) and 285 for IS (10.1–20 min). Emission was full time set at 523, for CUR and IS.

than 20% decomposed in 1 h, and after 8 h about 55% was still intact. In our study, blood samples were collected and immediately ice cooled (40 min) before centrifugation. After that, obtained plasma was kept in ultrafreezer until LC-analysis. After thaw, all samples remained in plasma at room temperature for only a short period of time, always protected from light exposure, thus avoiding significant degradation.

Additionally, low plasma concentrations of CUR were found by Ringman and co-authors (2012) in the verification of the efficacy and tolerability in humans with Alzheimer's disease. In this assay, supplementation with approximately 22 mg of CUR for 24 weeks resulted in concentrations equivalent to $15.8 \pm 6.1 \text{ ng mL}^{-1}$. In other words, in a higher supplementation dose was observed lower plasma levels of CUR compared to the levels observed in the plasma of athletes at T₁, highlighting the variation in bioavailability of CUR between studies.

Carroll and co-authors (2011), in a clinical trial (Phase II) with CUR for colorectal cancer prevention, observed low plasma concentrations ($15.8 \pm 14.8 \text{ ng mL}^{-1}$) even after 30 days supplementation with high dosage (2 g) of the curcuminoids. Among the factors mentioned, low bioavailability is also justified by the degree of intestinal first pass metabolism and with formation of metabolites and conjugates reduced heptatriene chain (Kostalová et al., 2013).

In T₂, the concentrations increased slightly after approximately 2 h of the administration of morning dose of CUR (22 mg). Besides previously mentioned low bioavailability, its limiting pharmacokinetic shows high tissue distribution of CUR contributing to plasma low circulating levels. Matabudul and colleagues (2012) using dogs as experimental model, observed higher distribution of CUR in the lungs compared to other tissues. The explanation for such accumulation is due to lipophilic nature of CUR and less reducing metabolic rate of tetrahydrocurcumin. Also, organs such as liver and spleen showed significant accumulated amounts. Another explanation is the down regulation of efflux transporters in the tissue, progressively increasing cellular uptake resulting in low plasma levels at steady state (Matabudul et al., 2012; Shukla et al., 2009).

4. Conclusion

The developed and validated bioanalytical method by HPLC-FLD was selective, sensitive, precise, accurate and adequate to quantify the samples from athletes supplemented during 30 days with *Curcuma longa* capsules. From our knowledge, this the first fluorimetric method using enzymatic treatment for plasma samples after *Curcuma longa* administration. Thus, it is an alternative and advantageous method to UV-Vis and MS/MS in bioavailability studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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