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Co-Quantification of Curcuminoids and Piperine by RP-HPLC/DAD from Spray-Dried Microparticles for Routine Quality Control

Marcia Viviane Marcon[1*]
https://orcid.org/0000-0003-1657-9152

Vinícius Monteiro Schafka[2]
https://orcid.org/0000-0001-7780-1728

Barbara Justus[1*]
https://orcid.org/0000-0003-3943-9304

Jessica Mendes Nadal[3]
https://orcid.org/0000-0002-2419-2110

Cyntia Maria Schnekenberg[4]
https://orcid.org/0000-0002-8812-1952

Jaqueline de Bortoli Shirabayashi[5]
https://orcid.org/0000-0002-4775-4585

Francislaine Aparecida dos Reis Lívero[6]
https://orcid.org/0000-0001-6533-0850

Paulo Vitor Farago[3]
https://orcid.org/0000-0002-9934-4027

[1]Universidade Estadual de Ponta Grossa, Departamento de Análises Clínicas e Toxicológicas, Ponta Grossa, Paraná, Brasil; [2]Universidade Estadual de Ponta Grossa, Departamento de Química, Ponta Grossa, Paraná, Brasil; [3]Universidade Estadual de Ponta Grossa, Departamento de Ciências Farmacêuticas, Ponta Grossa, Paraná, Brasil; [4]Universidade Estadual de Ponta Grossa, Departamento de Odontologia, Ponta Grossa, Paraná, Brasil; [5]Universidade Paranaense, Umuarama, Paraná, Brasil; [6]Universidade Federal do Paraná, Universidade do Paraná, Curitiba, Brasil.

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*Correspondence: barbara.justus@hotmail.com; Tel.:+55 42 9903-3709

HIGHLIGHTS

- Microparticles were successfully produced using the solid dispersion technique by spray-drying.
- A fast and simple RP-HPLC/DAD method was validated for curcuminoids and piperine quantification.
- Curcuminoid and piperine-loaded microparticles showed high encapsulation efficiency (~100%)

Abstract: Curcuminoids (CURs) are frequently co-administered with piperine (PIP) to enhance their pharmacological potential. Curcumin (CUR), the principal phenolic compound in the rhizome of *Curcuma longa* L. (turmeric), has limited oral bioavailability, while PIP can act as a bioenhancer. In this context, reliable simultaneous quantification of CURs-PIP is essential for routine quality control in herbal medicine industry. This study aimed to develop and validate a simple, precise, and robust RP-HPLC/DAD method for the simultaneous determination of CURs and PIP in spray-dried microparticles. Microparticles were produced by spray-drying using Eudragit® S100, and the analytical method was validated according to ICH Q2(R1) and ANVISA RDC 166/2017. Specificity, linearity, precision, accuracy, robustness, and encapsulation efficiency were assessed. Linearity was demonstrated within 200–500 $\mu g \cdot mL^{-1}$ (CURs expressed as curcumin, CUReq) and 8–20 $\mu g \cdot mL^{-1}$ (PIP) with $r>0.996$. Recoveries were ~100% (100.18% for CUReq; 99.56% for PIP) with CV<5%. Encapsulation efficiency was ≥99% for both analytes. The method proved accurate, precise, robust, and suitable for routine quality control of microparticulate formulations containing both CURs and PIP.

Keywords: drug delivery; encapsulation efficiency; functional foods; methacrylic polymers; nutraceuticals; phytochemicals.

INTRODUCTION

Curcuminoids, including curcumin (≈75% of total), demethoxycurcumin, and bisdemethoxycurcumin, are the main phenolic compounds in the rhizome of *Curcuma longa* L. (turmeric). These diarylheptanoid pigments are largely responsible for the broad spectrum of biological activities from turmeric, interacting with multiple cellular signaling pathways and exhibiting antioxidant, anti-inflammatory, antidiabetic, antibacterial, antifungal, antiproliferative, anticancer, and hepatoprotective effects [1,2]. Despite encouraging in vitro and in vivo findings, clinical trials have not conclusively demonstrated therapeutic efficacy, largely because of the poor oral bioavailability of curcuminoids, especially curcumin [3–5]. To overcome this limitation, encapsulation in nano- or microparticulate systems has been proposed to improve curcuminoid stability and absorption [6]. Co-administration with piperine, an alkaloid from *Piper nigrum* L., is another promising strategy, as piperine markedly enhances the bioavailability of curcumin [7,8]. Robust analytical methods are needed to accurately quantify the bioactive compounds in such combined curcuminoid–piperine formulations and to support regulatory compliance, reproducibility, and quality assurance [9].

Although numerous methods quantify curcumin and piperine individually, reports of their simultaneous HPLC quantification remain relatively scarce, particularly in solid formulations such as spray-dried microparticles, which are gaining increasing relevance in pharmaceutical development, nutraceuticals, functional foods, and biotechnology. Existing approaches are mostly limited to an isocratic HPLC/UV-DAD method validated to ICH standards in curcumin–piperine microparticles [9], RP-HPLC procedures applied to nanoparticles [10–12], and a classical RP-HPLC/PDA application in Eudragit® nanoparticles [13]. However, none of these studies has systematically addressed the simultaneous quantification of both analytes in spray-dried formulations. By filling this gap, the present study introduces a transferable analytical strategy specifically designed for routine quality control of microparticulate formulations containing both curcuminoids and piperine in herbal medicine industries, thus extending applicability beyond research settings and into translational contexts.

In this study, we aimed to develop and validate, in accordance with the International Conference on Harmonisation (ICH) [14] and Brazilian ANVISA Resolution RDC 166/2017, an isocratic RP-HPLC/DAD method for the simultaneous quantification of curcuminoids and piperine in spray-dried microparticles. In addition, we evaluated process yield, encapsulation efficiency, mean particle diameter, zeta potential, and morphology, as these complementary techniques are indispensable for a comprehensive characterization of dispersed systems. By providing a reproducible and time-efficient analytical tool, this study supports the quality control of pharmaceutical and nutraceutical formulations and establishes a translational foundation for broader biotechnological applications in health, environmental sustainability, and functional foods.

MATERIAL AND METHODS

Reagents and Solvents

A curcumin-rich curcuminoid extract (95.2% total curcuminoids expressed as curcumin; consisting of 74.2% curcumin, 15.5% demethoxycurcumin, 5.5% bisdemethoxycurcumin) was supplied by Tianxu Natural Pigment (China). Piperine (95.69% purity) was obtained from Xi'an Tian Ben Bio-Engineering (China). Eudragit® S100 was donated by Evonik (Brazil). The analytical standard of curcumin (CUR, ≥98% pure) was obtained from Sigma-Aldrich (USA). HPLC-grade methanol and acetonitrile (Merck, Germany) were used, and ultrapure water was prepared using a Millipore® system (USA). For clarity purposes, throughout this paper all curcuminoid contents are expressed as curcumin equivalents (CUReq), since the analytical calibration was performed using such CUR analytical standard.

Preparation of Polymeric Microparticles Containing Curcuminoids and Piperine

Microparticles containing curcuminoids (CURs) and piperine (PIP) were prepared by spray-drying with Eudragit® S100 (MSCP). The theoretical formulation (2.6 g) comprised 500 mg CURs and 20 mg PIP (20% w/w active loading). The solvent system consisted of ethanol:water (60:40, v/v). Table 1 summarizes the composition of the loaded and the non-loaded formulations. CURs and PIP were dissolved in ethanol under magnetic stirring (800 rpm, Fisatom, Brazil). Eudragit® S100 was then added under the same stirring conditions. Distilled water was gradually added, and the mixture was maintained under continuous stirring for 12 h. The resulting dispersion was dried by spray-drying (Labmaq MSD 0.5, Brazil) using the following

parameters: atomizer diameter 0.82 mm, atomization pressure 3 kgf·cm^{-2}, drying air flow 25 L·min^{-1}, feed rate 0.40 L·h^{-1}, inlet temperature 135 ± 5 °C, and outlet temperature 60 ± 5 °C. The formulations were prepared in triplicate (n = 3) from three independent batches. The dry microparticles were collected and stored in amber glass containers.

Table 1. Composition of polymeric microparticle formulations

Formulation	CURs (g)	PIP (g)	Eudragit® S100 (g)	Water (mL)	Ethanol (mL)
MPES100 (non-loaded)*	—	—	10.0	480	720
MPCP (loaded)*	1.923	0.077	8.0	480	720

*MPES100: Eudragit® S100 microparticles containing no active; MPCP: Eudragit® S100 microparticles containing curcuminoids and piperine, n = 3 from independent batches

Particle Size and Zeta Potential

The particle size and zeta potential of the microparticles were measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS90 (Malvern Instruments, UK). Samples of each formulation were dispersed in ultrapure water (1:500, v/v), sonicated for 15 min, and analyzed in triplicate at 25 °C. The mean hydrodynamic diameter (Z-average) and polydispersity index (PDI) were recorded. Zeta potential was measured by electrophoretic light scattering on the same instrument. All measurements were performed in amber vials to minimize light exposure of CURs, and the samples were handled at controlled room temperature to prevent any thermal degradation of curcuminoids during analysis.

Morphology

Microparticles and raw materials were examined by field-emission scanning electron microscopy (FE-SEM, Tescan Mira 3, Czech Republic). The spray-dried microparticles, pure curcuminoid extract, pure PIP, the polymer, and their physical mixture were mounted on metallic stubs using double-sided carbon tape, gold-sputtered (Shimadzu IC-50, Japan), and observed at an accelerating voltage of 15 kV.

Chromatographic Conditions

Chromatographic analyses were conducted on a Shimadzu Nexera-i LC-2040C HPLC system (Japan) equipped with a diode array detector (DAD). Separations were achieved on a Shim-pack C18 reversed-phase column (150 × 4.6 mm, 5 µm particle size) maintained at 25 °C. The mobile phase consisted of acetonitrile: methanol:water + 0.1% formic acid (55:5:40, v/v/v) delivered isocratically at a flow rate of 1.0 mL·min^{-1}. The injection volume was 10 µL. Detection wavelengths were set at 410 nm (for CURs, measured as CUR equivalents) and 341 nm (for PIP). The total run time was 15 min.

Standard Solutions

Stock solutions of the curcumin analytical standard (CUR) and PIP (each 500 µg·mL^{-1}) were prepared in methanol. These were appropriately diluted with methanol to obtain working standard solutions corresponding to 200–500 µg·mL^{-1} CUReq (total curcuminoids expressed as curcumin) and 8–20 µg·mL^{-1} PIP. All solutions were filtered through 0.22 µm PTFE membranes (Macherey-Nagel, Germany) prior to injection.

Method Validation for Simultaneous Quantification of Curcuminoids and Piperine

Method validation was performed in accordance with ICH Q2(R1) [14] and ANVISA RDC 166/2017 [15] guidelines. The parameters evaluated included specificity, linearity, limits of detection (LOD) and quantification (LOQ), precision, accuracy, and robustness, following standard validation procedures.

Specificity was confirmed by comparing chromatograms of loaded microparticles (MPCP, containing CURs and PIP) with those of non-loaded microparticles (MPES100). The absence of any peaks interfering with the CURs or PIP signals demonstrated that the method meets the specificity criterion. Linearity was assessed using three independent calibration curves, each constructed with six concentrations for CUR (200, 250, 300, 350, 400, 500 µg·mL^{-1}) and for PIP (8, 10, 12, 14, 16, 20 µg·mL^{-1}). The regression equations and correlation coefficients (r) were obtained from the mean calibration data.

Sensitivity was determined by calculating the LOD and LOQ according to ICH Q2 (R1), recommendations. The calculations used the standard deviation of the response (σ) and the slope of the calibration curve (S) for each analyte, according to the equations 1 and 2.

$$LOD = \frac{3.3xSD}{S} \qquad \text{(Equação 1)}$$

$$LOQ = \frac{10\ x\ SD}{S} \qquad \text{(Equação 2)}$$

Precision was evaluated at two levels, repeatability (intra-day) and intermediate precision (inter-day, including analyses on different days by different analysts). For each level, triplicate analyses (n = 3) were performed at three concentration levels for each analyte. Results were expressed as the coefficient of variation (%CV).

Accuracy was determined by recovery tests using the standard addition method. Known amounts of CUR standard and PIP were spiked into a non-loaded formulation (MPES100 microparticle solution), and the mixtures were analyzed. Three concentration levels were tested for each analyte (low, medium, high within the linear range) with three replicates each (n = 9 per analyte in total). For CURs, the spike levels were 200, 300, 500 μg·mL^{-1}; for PIP: 8.0, 12.0, 20.0 μg·mL^{-1}. Acceptable recoveries were defined between 95% and 105% (Equation 3).

$$\%\ accuracy = \frac{experimental}{theoritical}\ x\ 100 \qquad \text{(Equation 3)}$$

Robustness was examined at a mid-range concentration (300 μg·mL^{-1} CURs and 12 μg·mL^{-1} PIP) under small deliberate variations of chromatographic conditions. The tested variations included altering the mobile phase composition by ±1% (testing 54:5:41 and 56:5:39 ratios instead of 55:5:40), column temperature by ±3 °C (22 °C and 28 °C instead of 25 °C), flow rate by ±0.1 mL·min^{-1} (0.9 and 1.1 mL·min^{-1}), and detection wavelength by ±5 nm (405 nm and 415 nm for curcuminoids; 336 nm and 346 nm for PIP). Additionally, the method was tested on a different C18 column batch of the same specifications to evaluate column-to-column consistency. Results were analyzed via ANOVA (with Tukey's post hoc test for multiple comparisons). System suitability criteria were also consistently met in all cases, tailing factor < 1.5 for both peaks, and resolution between CURs and PIP peaks ≥ 1.5 under all conditions.

Encapsulation Efficiency (EE%)

The EE% was determined as described in Equation 4 [16]. Microparticles were weighed into 10 mL volumetric flasks, dispersed in 8 mL methanol, stirred at 1,000 rpm for 12 h, diluted to the final volume, and filtered through PTFE membranes (0.22 μm).

$$EE(\%) = \frac{drug\ mass\ in\ microparticle}{initial\ theoritical\ drug\ mass} \qquad \text{(Equation 4)}$$

Theoretical drug concentrations were 288.48 μg·mL^{-1} for CUR and 11.52 μg·mL^{-1} for PIP. All assays were performed in triplicate using the validated method, with detection at 410 nm (CURs) and 341 nm (PIP).

Statistical Analysis

Results are expressed as mean ± standard deviation. Statistical significance between groups was evaluated using one-way ANOVA, with α = 0.05.

RESULTS

Preparation of Polymeric Microparticles Containing Curcuminoids and Piperine and Yield

Spray-drying of the Eudragit® S100 dispersions yielded free-flowing micropowders with distinct coloration. Non-loaded microparticles (MPES100) were white, whereas CURs–PIP microparticles (MPCP) were bright orange, consistent with the characteristic yellow-orange hue of CURs. The process yields, which represent the mass of dried products recovered relative to total solids, were 56.27% ± 0.85% for MPES100 and 53.34% ± 0.71% for MPCP. These yields are typical for lab-scale spray-drying.

Particle Size and Zeta Potential

Loaded microparticles (MPCP) showed a mean hydrodynamic diameter of 680 ± 18 nm with a unimodal size distribution and a polydispersity index PDI ≈ 0.14 ± 0.01. In contrast, the non-loaded MPES100 microparticles exhibited a larger mean diameter of 1506 ± 198 nm under the same conditions and a polydispersity index PDI ≈ 0.27 ± 0.01. The zeta potential of the drug-loaded microparticles was – 40.53 mV, reflecting the anionic nature of the Eudragit® S100 polymer in neutral to basic media due to deprotonated carboxylate groups. The non-loaded MPES100 microparticles showed a similarly high negative zeta potential of approximately – 42 mV as expected. The sub-micron size of MPCP suggests that the presence of CURs and PIP influenced the particle formation, potentially leading to smaller and more uniform particles.

Morphology

FE-SEM analysis revealed distinct morphological features for the raw materials and formulated microparticles. The curcuminoid extract displayed a crystalline appearance, and pure PIP exhibited a heterogeneous crystalline structure. The Eudragit® S100 polymer showed roughly spherical particles with low size variability (Figure 1). After encapsulation, the CURs–PIP loaded microparticles (MPCP) were predominantly spherical with smooth surfaces and slight roughness, and no crystalline drug residues were observed on the surface (Figure 2). The absence of surface drug deposits and the consistency of SEM observed particle sizes with DLS measurements both support the conclusion of efficient encapsulation of the active compounds within the polymer matrix.

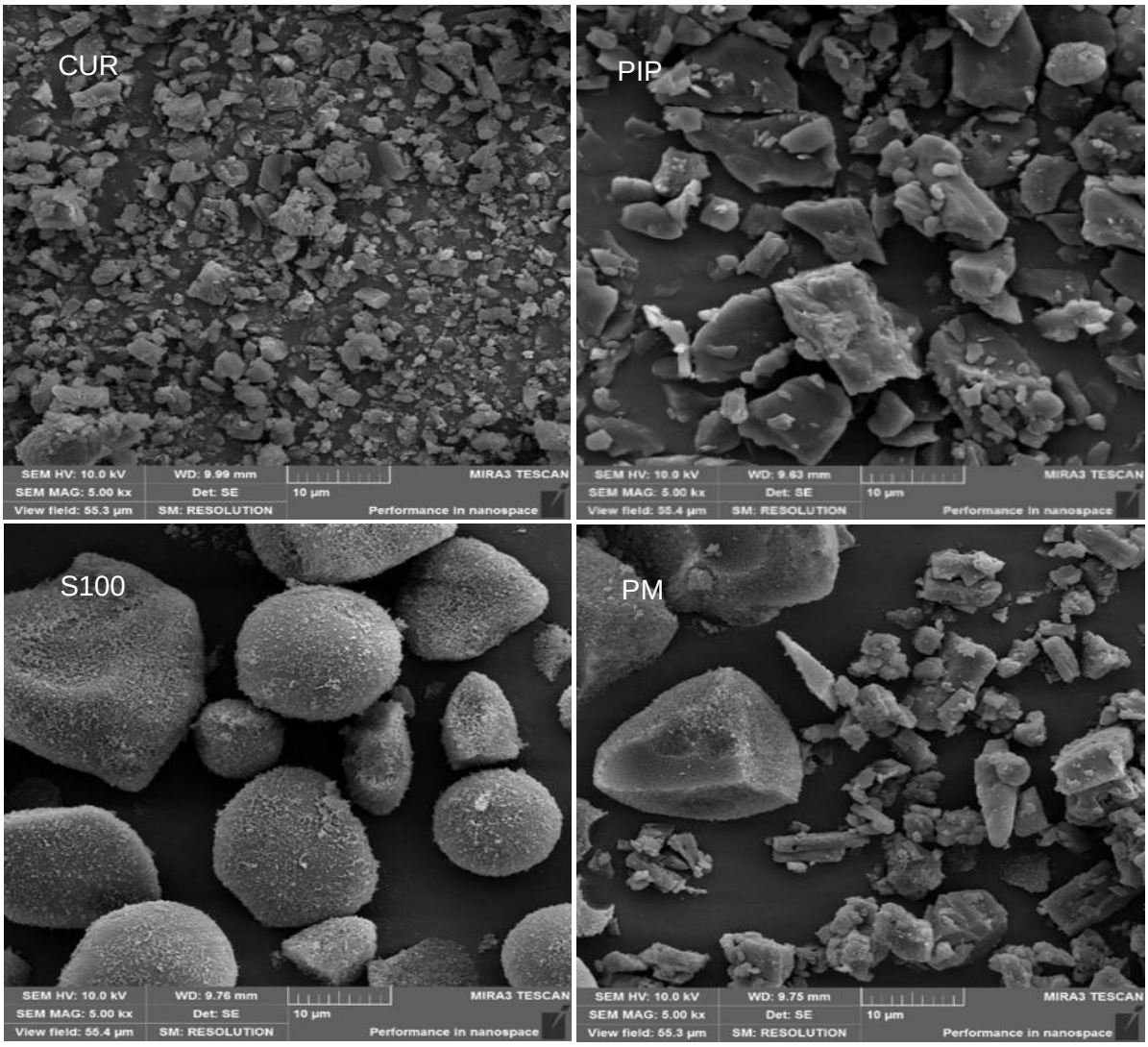

Figure 1. FE-SEM micrographs of raw materials: curcuminoid extract (CUR) and piperine (PIP); Eudragit® S100 polymer (S100); and their physical mixture (PM). Images were obtained at 5,000× magnification (scale bars = 10 µm)

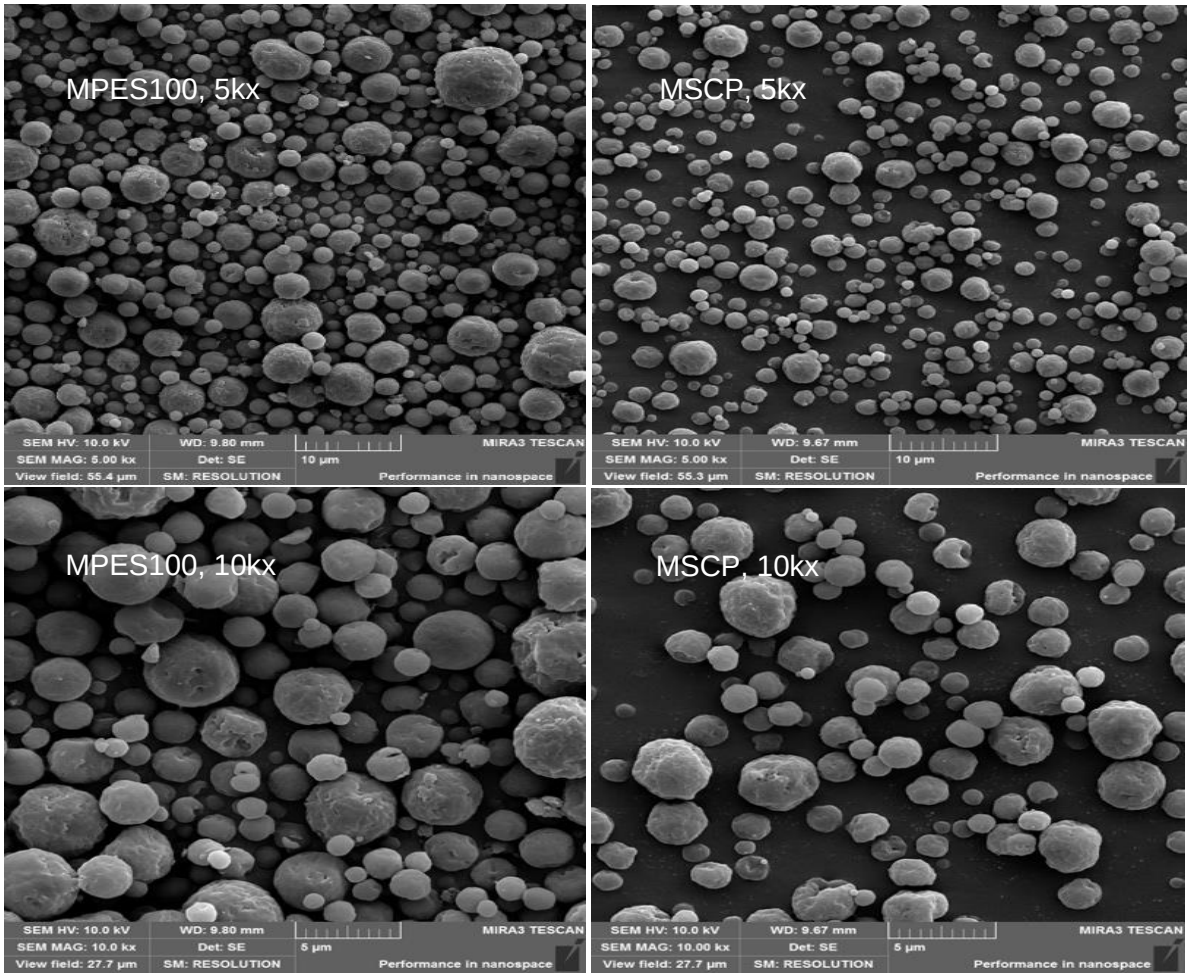

Figure 2. FE-SEM micrographs of spray-dried microparticles with no active (MPES100, non-loaded) and with CURs + PIP (MPCP, loaded). Images at 5,000× and 10,000× magnifications (*scale bars = 5 µm and 2 µm, respectively*) show spherical morphology and smooth surfaces for loaded particles with no crystalline drug on the surface

Chromatographic Performance and Specificity

Under the optimized isocratic conditions, the CURs and PIP were suitably resolved as indicated in Figure 3B. The loaded microparticles MPCP revealed the retention times of bisdemethoxycurcumin (BDMC), demethoxycurcumin (DMC), and curcumin (CUR) as described in the curcuminoid extract used. PIP peak was eluted at 4.88 min. Chromatograms of the non-loaded microparticles (MPES100) showed no peaks at the retention times of CURs and PIP, confirming that no excipient or impurity interfered with analyte detection (Figure 3). Thus, the method demonstrated specificity for the simultaneous analysis of CURs and PIP in the spray-dried microparticles. The use of separate detection wavelengths (410 nm for CURs and 341 nm for PIP) provided selective monitoring of these compounds with no cross-interference.

Linearity, Range, and Sensitivity

The calibration curves for CUR and PIP demonstrated excellent linearity over the evaluated ranges of 200–500 $\mu g \cdot mL^{-1}$ and 8–20 $\mu g \cdot mL^{-1}$, respectively. Regression coefficients were high ($r = 0.9965$ for CUR and $r = 0.9971$ for PIP), and analysis of variance (ANOVA) confirmed that the linear models were statistically significant with no evidence of lack of fit (Figure 4, Table 2). Each calibration point was prepared in triplicate across three independent runs, supporting the robustness of the regression analysis. These ranges adequately cover the expected concentrations of curcuminoids and piperine in microparticle assay preparations, and confirm that detector response remains proportional to analyte concentration within these limits. The resulting detection limits were 21.24 $\mu g \cdot mL^{-1}$ for CUR and 0.90 $\mu g \cdot mL^{-1}$ for PIP, while the quantification limits were 64.37 $\mu g \cdot mL^{-1}$ for CUR and 2.73 $\mu g \cdot mL^{-1}$ for PIP.

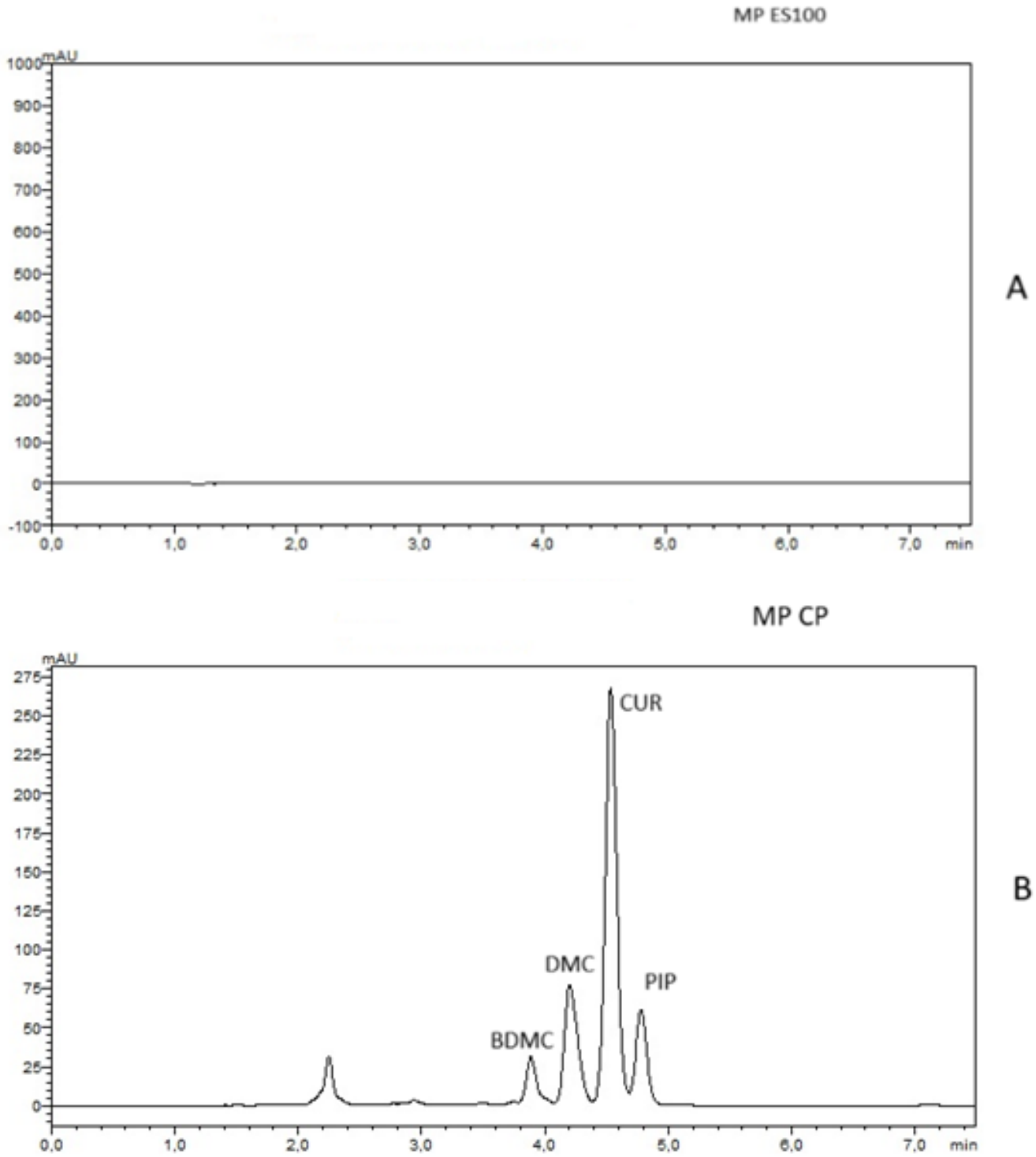

Figure 3. Chromatograms of spray-dried formulations of non-loaded microparticles (MPES100) (A) and loaded microparticles (MPCP) (B) containing CUR and PIP obtained by the proposed RP-HPLC/DAD

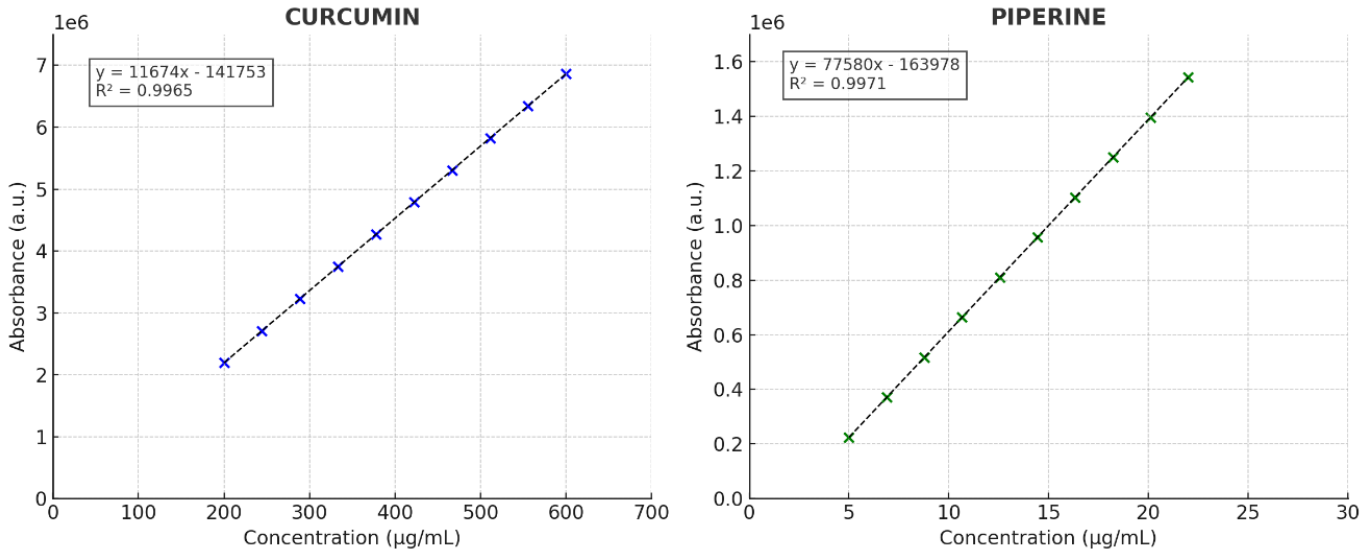

Figure 4. Average calibration curves for CUR and PIP using acetonitrile:methanol:water + 0.1% formic acid (55:5:40, v/v/v)

Table 2. Summary of ANOVA results* for linearity of the analytical method for CUR and PIP

Anova results	SS	DF	MS	*F*	*F* tab
CUR					
Model	2.1012×10^{13}	1	2.1012×10^{13}	15216.8	4.49
Residual	2.2099×10^{10}	16	1.3812×10^{9}	Linear	
Lack of fit	9.4160×10^{9}	4	2.3540×10^{9}	2.227	3.26
Pure error	1.2683×10^{10}	12	2.3540×10^{9}	No lack of fit	
			PIP		
Model	8.994396×10^{9}	1	8.9943963×10^{9}	2997.81	4.49
Residual	8.994396×10^{9}	16		Linear	
Lack of fit	4.8732098×10^{9}	4	1.218302×10^{9}	3.25	3.26
Pure error	4.1211866×10^{9}	12	343.43×10^{6}	No lack of fit	

*Note: Where SS = sum of squares; DF = degrees of freedom; MS = mean square; F = F test value; F tab = tabulated F value

Precision, Accuracy, and Robustness

The developed HPLC method showed appropriate precision. Intra-day and inter-day %CV values for both CURs expressed as curcumin (CUReq) and PIP were all below 5% (Table 3), indicating that the method yields consistent results upon repeated analysis. Accuracy was within 99–104% across low, medium, and high levels for both analytes (Table 4), meeting the typical acceptance criteria of 95–105%. These results confirm that the method can accurately recover known amounts of CURs and piperine from the loaded microparticle formulation. The robustness against small variations in chromatographic conditions was evidenced by minimal changes in results under the altered conditions (Table 5). The assay remained within acceptable precision and accuracy bounds when the mobile phase ratio, temperature, flow, or detection wavelength were slightly varied, and when a different batch of the column was used. No statistically significant differences were observed, demonstrating the reliability of the method for routine use in quality control.

During the robustness tests, system suitability was verified by replicate standard injections. The relative standard deviation (%RSD, n = 6) of peak areas and retention times remained below 2% for both analytes, confirming instrumental precision. Column efficiency was high (>5000 theoretical plates), and tailing factors were close to unity (1.1 for CURs and 1.2 for PIP), indicating adequate peak symmetry. In addition,

chromatographic selectivity was demonstrated by a resolution of 1.50 between CUR and PIP, while peak purity indices determined by DAD were 1.00 for CURs and 0.99 for PIP, exceeding acceptance thresholds and confirming the absence of co-eluting impurities (Figure 5). The consistent system performance, together with adequate resolution and purity, further supports the method suitability for routine analysis.

Table 3. Intra-day and inter-day precision for CURs and PIP

Set	Nominal CURs (µg/mL)	Found CURs (µg/mL)	CV% (CURs)	Nominal PIP (µg/mL)	Found PIP (µg/mL)	CV% (PIP)
Intra-day #1	500	504.11	0.405	20	20.17	0.072
Intra-day #2	300	305.51	0.078	12	11.99	0.433
Intra-day #3	200	202.46	0.313	8	8.31	0.054
Inter-day #1	500	474.95	2.397	20	18.38	1.978
Inter-day #2	300	274.71	2.895	12	12.30	2.111
Inter-day #3	200	177.30	1.212	8	6.55	4.909

Table 4. Accuracy at three concentration levels for CURs and PIP

Analyte	Theoretical (µg/mL)	Experimental (µg/mL)	Recovery (%)	CV (%)
CURs	200	202.46	101.23	0.51
CURs	300	305.51	101.84	0.51
CURs	500	504.11	100.82	0.51
PIP	8	8.31	103.92	2.06
PIP	12	11.99	99.91	2.06
PIP	20	20.17	100.86	2.06

Table 5. Robustness at 300 µg/mL CURs and 12 µg/mL PIP under temperature and mobile phase variations.

Analyte – Parameter	Condition	CV (%)	Recovery (%)
CURs – Mobile phase	54/5/41 (v/v/v)	0.11	95.29
CURs – Mobile phase	56/5/39 (v/v/v)	1.46	95.33
CURs – Temperature	22°C	0.35	95.02
CURs – Temperature	28°C	1.79	95.06
PIP – Mobile phase	54/5/41 (v/v/v)	0.77	101.49
PIP – Mobile phase	56/5/39 (v/v/v)	0.65	103.56
PIP – Temperature	22°C	0.48	99.02
PIP – Temperature	28°C	0.04	102.43

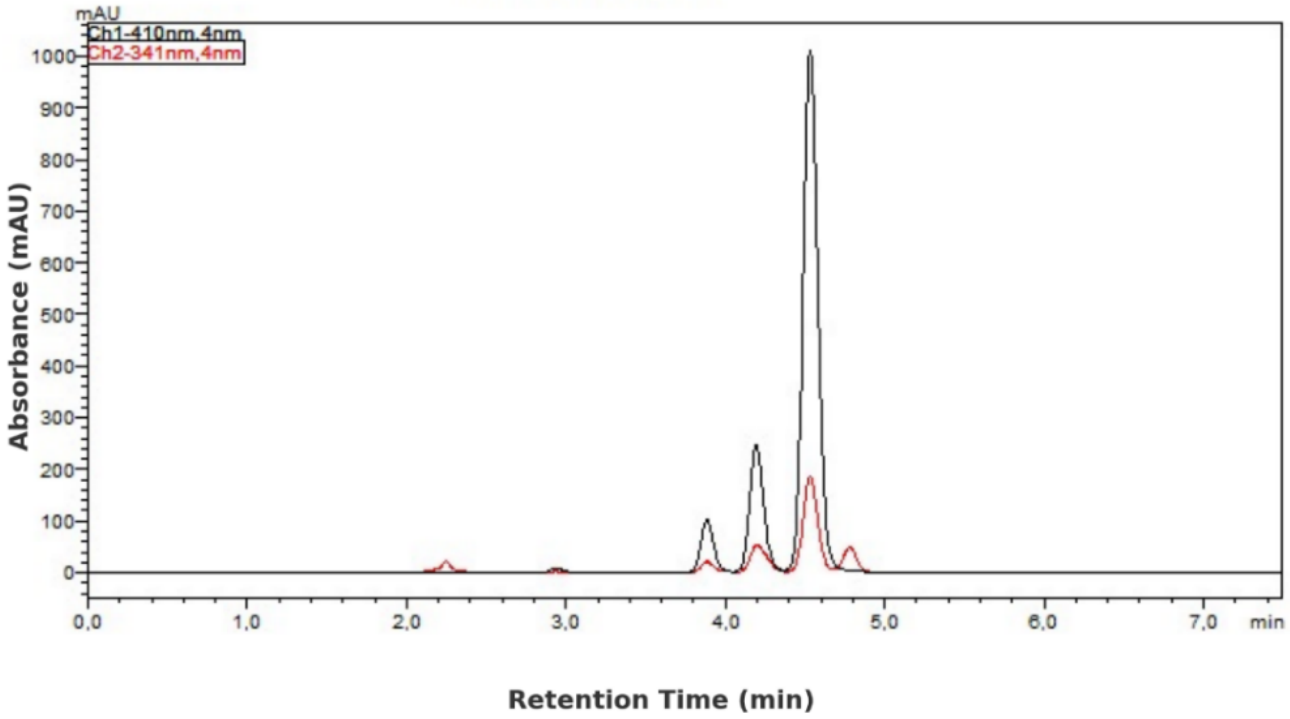

Figure 5. Chromatography in 410 nm e 341 nm with acetonitrile:methanol:water + 0.1% formic acid (55:5:40, v/v/v)

Encapsulation Efficiency

The spray-dried Eudragit® S100 microparticles containing CURs and PIP exhibited high encapsulation efficiency. Encapsulation efficiency (%EE), calculated by the indirect method was 100.18% for CUReq and 99.56% for PIP (Table 6). Essentially ~100% of the added CURs and PIP were recovered in the spray-dried microparticles, indicating complete entrapment with negligible loss of compounds during processing. Triplicate batches yielded consistent %EE values and standard deviations < 3%, underscoring the reproducibility of the formulation process.

Table 6. Encapsulation efficiency (%EE) of CURs as CUReq and PIP in spray-dried Eudragit® S100 microparticles

Formulation	Theoretical CURs (µg/mL)	Theoretical PIP (µg/mL)	Encapsulated CUReq (µg/mL±SD)	Encapsulated PIP (µg/mL±SD)	%EE (CUReq)	%EE (PIP)
MPCP (loaded)	288.48	11.52	289.01 ± 6.97	11.47 ± 0.24	100.18	99.56

DISCUSSION

Curcuminoid–piperine microparticles produced by spray-drying were successfully obtained, with yields of approximately 53 to 56%. These yields fall within the range commonly reported for spray-dried polymeric microparticles. Losses during drying likely arose from inherent process inefficiencies, such as powder adhesion to the dryer chamber walls and cyclone, which are typical for lab-scale spray-drying. Comparable outcomes have been reported for similar systems. For example, Eudragit-based microparticles loaded with piperine achieved yields of 40–57% and encapsulation efficiencies of 66–90% in another study, where stickiness and polymer migration to the droplet surface limited recovery [17,20]. Recent spray-drying studies have reported a wide range of yields depending on formulation components, with Yupanqui and coauthors [18] obtaining 31.7% for chitosan/mannitol spray-dried turmeric extract microparticles, and Tabaré and coauthors [19] observing yields from 45% up to 90% by modifying Eudragit® FS content and adding drying adjuvants. Mankan and coauthors (2025) [21] reported 70–86% yields for curcumin–lecithin spray-dried complexes using maltodextrin, along with improved powder flowability and stability. In light of these comparisons, our yield results are within expectations for a Eudragit® S100-based formulation with no auxiliary matrix formers.

Dynamic light scattering revealed submicron particle sizes for the loaded formulation. The CUR–PIP microparticles MPCP had an average diameter of ~680 nm, significantly smaller than the ~1500 nm size of the non-loaded microparticles MPES100. This size reduction upon drug loading suggests that the presence of CURs and PIP promoted the formation of smaller and more uniform particles, possibly by affecting the

drying dynamics or reducing droplet viscosity. Notably, according to ISO/TS 80004-2, nanoparticles are defined as having dimensions 1–100 nm and microparticles 1–1000 μm; thus, our particles are in the submicrometer regime [24]. Such submicron sizes are advantageous for oral delivery of hydrophobic compounds, as they can improve dissolution rates and bioavailability. The size range observed here is consistent with other spray-dried Eudragit® S100 systems intended for intestinal release [22,25], and in fact is smaller than the particle sizes commonly reported for similar spray-dried formulations [22,26,27]. The substantial size difference between loaded and unloaded particles in our study indicates that inclusion of CURs and PIP influences the spray-drying outcome, potentially by altering surface tension or polymer coalescence during solvent evaporation.

The zeta potential of approximately –40.5 mV for the drug-loaded microparticles reflects the anionic character conferred by the carboxylate groups of Eudragit® S100, a methacrylic acid copolymer. At the neutral pH, these carboxylic groups are deprotonated, yielding a negatively charged particle surface. Zeta potentials with absolute values exceeding ±30 mV generally indicate a well-stabilized colloid due to electrostatic repulsion [28]. In our case, the high negative zeta potential suggests suitable electrostatic stabilization of the microparticles, which would minimize aggregation in suspension and is favorable for uniform redispersion and handling of the powder. The non-loaded microparticles MPES100 exhibited a similarly high negative zeta potential, indicating that the surface charge dominates the zeta potential in both formulations.

FE-SEM imaging further supported the effective incorporation of CURs and PIP [29]. The loaded microparticles MPCP were smooth and spherical, lacking any visible crystalline residues on their surfaces, in contrast to the raw CURs and PIP powders which showed crystalline structures. The absence of drug crystals or phase separation on MPCP surfaces implies that CURs and PIP were largely embedded or molecularly dispersed within the polymer matrix and provided a true solid dispersion [30]. This observation correlates with the high encapsulation efficiency measured. The FE-SEM-estimated particle diameters matched well with the DLS-measured hydrodynamic diameters, indicating that the observed particles were individual microspheres rather than large aggregates, and that the DLS values are reliable for the primary particle size. Overall, the morphology and size data agree with previous studies using similar spray-drying methods and polymers [17], showing that Eudragit® S100 can produce smooth, spherical microcarriers for hydrophobic nutraceutical compounds.

Considering the analytical method, the RP-HPLC/DAD method was the main focus of this study and it successfully met all ICH and ANVISA validation criteria. The method demonstrated high specificity, as no interfering peaks from the polymer or other matrix components were present at the retention times of CURs and PIP. The linear dynamic range for CUR could potentially be extended to lower concentrations if needed for applications like bioavailability or pharmacokinetic studies. However, the current range is appropriate and fit-for-purpose for quality control assays of formulations, where analyte concentrations are typically in the high $\mu g \cdot mL^{-1}$ range [31,32]. Indeed, assays of finished nutraceutical or pharmaceutical products often operate in similar concentration ranges focusing on content uniformity and stability, as exemplified by prior simultaneous CUR–PIP determinations in formulations [9], multi-laboratory studies of curcuminoid supplements [30], and stability-indicating analyses of CURs-containing tablets and capsules [34]. In our case, choosing the 200–500 $\mu g \cdot mL^{-1}$ range for CURs aligns with a target dose of approximately 500 mg CURs per serving, which is a clinically relevant dose commonly used in combination with 5–10 mg PIP in human studies [35,36,37,38,39]. By aligning the analytical range with such practical dose levels, our method strengthens its translational applicability, bridging the gap between bench-top analysis and real-world therapeutic contexts.

In comparing the chromatographic performance of our method with literature, our isocratic separation achieved very short retention times for both CURs and PIP while maintaining baseline resolution and using analyte-specific detection wavelengths (410 nm for CURs, 341 nm for PIP). Many published methods show longer retention times and different detection choices, owing to variations in columns, mobile phase composition, and wavelength selection. For instance, single-wavelength UV detection around 350–360 nm yielded retention times of 7.2 min for CUR and 8.5 min for PIP in one method [11]. Ultra-fast methods have been reported [10], but those often detect at 282 nm, a wavelength far from the absorbance maximum of CURs, which can sacrifice specificity or sensitivity for the sake of speed. Our chosen dual-wavelength approach (410 nm and 341 nm) is close to the respective λ max of CURs and PIP, ensuring high sensitivity and selectivity for each. With a total run time of 15 min, our method is comparable to or only slightly longer than some reports [9–11], but this includes a comfortable window to ensure complete separation and robust performance. The accuracy of our method is on par with reference methods. Khismatrao and coauthors [11] reported CUR recovery between 98.7–102.9% and PIP recovery between 97.8–100.9%, while Kuber and coauthors [10] found 98.0–100.6% for CUR and 99.4–100.0% for PIP.

The encapsulation efficiency results were notably high. Such efficiency indicates that essentially all of the CURs and PIP content initially added to the feed was retained in the spray-dried microparticles. These values are consistent with or higher than those in similar systems. Yupanqui and coauthors [18] reported 88% encapsulation of CURs in spray-dried turmeric extract microparticles, and our earlier reference [17] on PIP microparticles with methacrylate polymers showed 66–90% depending on conditions. The nearly 100% encapsulation in our formulation likely reflects a strong affinity between the hydrophobic drug molecules (CURs and PIP) and the hydrophobic domains of Eudragit® S100, as well as the optimized solvent system (ethanol–water 60:40) which may promote intimate mixing and fast solidification that traps the actives efficiently. The slight value above 100% for CUReq (100.18%) is within experimental error but suggests a minor systematic bias, possibly due to the conversion of CURs into CUR equivalents. It could also be influenced by matrix effects that enhance the UV response of CUR in the sample solution compared to the standard solution, given strong absorbance and fluorescent properties for CUR. However, we acknowledge this as a point where further confirmation by an independent method as UHPLC or LC–MS/MS analysis of individual CURs could be useful to rule out any analytical bias [31,39].

Another limitation to note is that our quantification method expresses total CURs as CUR equivalents (CUReq), assuming that all three major curcuminoids have a similar detector response as curcumin. In reality, demethoxycurcumin and bisdemethoxycurcumin have slightly different molar absorptivities at 410 nm compared to CUR. In our formulation, CUR is the predominant component (~74% of total CURs), so using a single calibration curve for CUR is a reasonable approximation of the total curcuminoid content. However, if the relative proportions of CURs were to change significantly or if one needed to track each curcuminoid individually, the single-wavelength CUReq method could introduce a small bias or mask individual behaviors. For the purposes of routine quality control, where the goal is typically to assay total active content, the CUReq approach is convenient and effective. Nevertheless, for more advanced applications such as detailed stability-indicating analysis or pharmacokinetic studies, a more resolutive analytical technique is recommended.

Overall, the formulation and method developed in this paper demonstrate a successful integration of CURs and PIP into a spray-dried delivery system and a reliable means of quantifying them simultaneously. The high encapsulation, appropriate particle size, and apparent stability indicated by lack of aggregation and the absence of crystalline drug on particle surfaces suggest that these microparticles could be an effective oral delivery system for CURs with enhanced bioavailability provided by the PIP presence and the polymer protection. The validated RP-HPLC/DAD method ensures that such formulations can be quantitatively monitored for drug content and uniformity, which is crucial for quality control during product development and manufacturing.

CONCLUSION

The proposed isocratic RP-HPLC/DAD method demonstrated suitable specificity, linearity, accuracy, precision, and robustness for the simultaneous quantification of CURs and PIP within the tested ranges. With a short analysis time, conventional solvents, and simple isocratic conditions, the method reduces analytical complexity and is cost-effective for routine use. Its reproducibility across different days and analysts indicates it can be confidently implemented for batch release testing, formulation screening, and process monitoring. In brief, the method is simple, sensitive, and transferable, providing a practical quality control tool for nutraceutical/phytochemical products.

Author Contributions

The authors would like to express their sincere appreciation for the valuable contributions of all collaborators involved in this research. Conceptualization, validation and methodology was carried out by MARCON, M.V. and FARAGO, P.V. Formal analysis and investigation activities was performed by MARCON, M.V., SCHENEKENBERG, C.M., and SHIRABAYASHI, J.B. Data curation was managed by MARCON, M.V., SCHAFKA, V.M. and JUSTUS, B.J. The original draft, the review and editing were completed by MARCON, M.V., JUSTUS, B.J. and NADAL, J.M. Supervision and project administration were overseen by FARAGO, P.V. All authors have read and approved the final version of the manuscript.

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REFERENCES

1. Soleimani V, Sahebkar A, Hosseinzadeh H. Turmeric (Curcuma longa) and its major constituent (curcumin) as nontoxic and safe substances: review. Phytother. Res. 2018;32(6):985-95. doi:10.1002/ptr.6054.
2. Tagde P, Tagde P, Islam F, Tagde S, Shah M, Hussain ZD, *et al.* The multifaceted role of curcumin in advanced nanocurcumin form in the treatment and management of chronic disorders. *Molecules* [Internet]. 2021 [cited 2021 Dec 10];26(23):7109. Available from: https://doi.org/10.3390/molecules26237109
3. Gupta T, Singh J, Kaur S, Sandhu S, Singh G, Kaur IP. Enhancing bioavailability and stability of curcumin using solid lipid nanoparticles (CLEN): a covenant for its effectiveness. *Front Bioeng Biotechnol* [Internet]. 2020 [cited 2020 Jul 15];8:879. Available from: https://doi.org/10.3389/fbioe.2020.00879
4. Wang YJ, Pan MH, Cheng AL, Lin LI, Ho YS, Hsieh CY, et al. Stability of curcumin in buffer solutions and characterization of its degradation products. J Pharm Biomed Anal. 1997 Aug;15(12):1867-76. doi: 10.1016/s0731-7085(96)02024-9. PMID: 9278892.
5. Hegde M, Girisa S, Chetty BB, Vishwa R, Kunnumakkara AB. Curcumin formulations for better bioavailability: what we learned from clinical trials. *Ther Adv Drug Saf* [Internet]. 2023 [cited 2023 Mar 20];14:20420986231155500. Available from: https://doi.org/10.1177/20420986231155500
6. Evans AC, Martin KA, Saxena M, Bicher S, Wheeler E, Cordova EJ, et al. Curcumin nanodiscs improve solubility and serve as radiological protectants against ionizing radiation exposures in a cell-cycle dependent manner. *Nanomaterials* [Internet]. 2022 [cited 2022 Oct 25];12(20):3619. Available from: https://doi.org/10.3390/nano12203619
7. Baspinar Y, Üstündas M, Bayraktar O, Sezgin C. Curcumin and piperine loaded zein-chitosan nanoparticles: development and in-vitro characterisation. Saudi Pharm J. 2018;26(3):323-34.
8. Mhaske DB, Sreedharan S, Mahadik KR. Role of piperine as an effective bioenhancer in drug absorption. *Pharm Anal Acta* [Internet]. 2018 [cited 2018 Jan 10];9:1–4. Available from: https://doi.org/10.4172/2153-2435.1000591
9. Setyaningsih D, Santoso YA, Hartini YS, Murti YB, Hinrichs WLJ, Patramurti C. Isocratic high-performance liquid chromatography (HPLC) for simultaneous quantification of curcumin and piperine in a microparticle formulation containing *Curcuma longa* and *Piper nigrum*. *Heliyon* [Internet]. 2021 [cited 2021 Mar 15];7(3):e06541. Available from: https://doi.org/10.1016/j.heliyon.2021.e06541
10. Kuber RB. Method development and validation for estimation of curcumin and piperine by RP-HPLC. Int J Appl Pharm. 2019;11(1):1-12.
11. Khismatrao A, Bhairy S, Hirlekar R. Development and validation of RP-HPLC method for simultaneous estimation of curcumin and piperine. Int J Appl Pharm. 2018;10(5):1-15.
12. Shaikh S, Jain V. Development and validation of an RP-HPLC method for the simultaneous determination of curcumin, piperine and camphor in an Ayurvedic formulation. Int J Pharm Pharm Sci. 2018;10(4):1-10.
13. Moorthi C, Senthil Kumar C, Mohan S, Krishnan K, Kathiresan K. Application of validated RP-HPLC-PDA method for the simultaneous estimation of curcumin and piperine in Eudragit E100 nanoparticles. *J Pharm Res* [Internet]. 2013 [cited 2013 Apr 4];7(4):299-303. Available from: http://dx.doi.org/10.1016/j.jopr.2013.03.006
14. International Conference on Harmonisation (ICH). Validation of analytical procedures: text and methodology Q2(R1). Geneva: ICH; 2005.
15. Brazilian Health Regulatory Agency (ANVISA). Resolution RDC No. 166, July 24, 2017. Criteria for validation of analytical methods.
16. Li M, Rouaud O, Poncelet D. Microencapsulation by solvent evaporation: state of the art for process engineering approaches. Int J Pharm. 2008;363(1-2):26-39.
17. Biscaia PB, et al. Meth(acrylic) microparticles containing piperine: RP-UHPLC-DAD method for drug-loading and in vitro release. Lat Am J Pharm. 2023;42(9).
18. Yupanqui CT, Suksanga A, Ardhanwanich S, Ungphaiboon S. Spray-dried microparticles of turmeric extract for improved delivery and low toxicity. Pharmacia. 2024;71:e126108. doi:10.3897/pharmacia.71.e126108.
19. Tabare E, Dauchot T, Cochez C, Glonti T, Antoine C, Laforêt F, et al. Eudragit® FS microparticles containing bacteriophages, prepared by spray-drying for oral administration. Pharmaceutics. 2023;15(6):1602. doi:10.3390/pharmaceutics15061602.
20. Chassot JM, Ribas D, Silveira EF, Grünspan LD, Pires CC, Farago PV, et al. Beclomethasone dipropionate-loaded polymeric nanocapsules: development, in vitro cytotoxicity, and in vivo evaluation of acute lung injury. J Nanosci Nanotechnol. 2015;15(1):855-64.
21. Mankan E, Sagdic O, Karadag A. Development and characterization of spray-dried curcumin–lecithin complexes with improved solubility and in vitro digestive and thermal stability. Foods. 2025;14(18):3157. doi:10.3390/foods14183157.
22. Schneider AC, Brener CES, Daudt NF, Cruz L, Silva CB. Development of microparticles and microparticulated tablets containing piperine. Braz J Pharm Sci. 2023. doi:10.1590/s2175-97902023e21265.
23. Umerska A, Paluch KJ, Inkielewicz-Stepniak I, Tajber L. Exploring the release mechanisms of curcumin from polymeric carriers—a Weibull model approach. Eur J Pharm Biopharm. 2018;132:180-90. doi:10.1016/j.ejpb.2018.09.001.
24. Al Khatib AO, Yousfan A, Abed A, El-Tanani M, Al-Obaidi H. Development of pimozide spray-dried lipid nanoparticles with enhanced targeting of non-small cell lung cancer. J Drug Deliv Sci Technol. 2025;106969:1-25.

25. Hassan AS, El-Mahdy MM, El-Badry M, El-Gindy GE-DA. Different approaches for enhancement of curcumin aqueous solubility and dissolution rate. J Adv Biomed Pharm Sci. 2019;2:152-361. Available from: http://jabps.journals.ekb.eg
26. Mohylyuk V, Simões S, Veiga F, Cabral-Marques H. Wurster fluidized bed coating of microparticles: towards scalable production of oral sustained-release liquid medicines for patients with swallowing difficulties. AAPS PharmSciTech. 2020;21(3).
27. Mithu SH, Rani R, Dhingra D, Kumar S, Dilbaghi N. Evaluation of the surface chemistry and drug–polymer interaction of semi-crystalline microparticles for the development of controlled release formulations. Mater Sci Eng C. 2017;76:559-67.
28. Neves AR, Lúcio M, Martins S, Lima JL, Reis S. Novel resveratrol nanodelivery systems based on lipid nanoparticles to enhance its oral bioavailability. *Int J Nanomedicine* [Internet]. 2013 [cited 2013 Jan 15];8:177–87. Available from: https://doi.org/10.2147/IJN.S37840
29. Bobek VB, Heiden G, de Oliveira CF, de Almeida VP, de Paula JP, Farago PV, et al. Comparative analytical micrographs of “vassouras” (Baccharis, Asteraceae). Rev Bras Farmacogn. 2016;26(6):665-72.
30. Maluf DF, Gonçalves MM, D’Angelo RWO, Girassol AB, Tulio AP, Pupo YM, et al. Cytoprotection of antioxidant biocompounds from grape pomace: further exfoliant phytoactive ingredients for cosmetic products. Cosmetics. 2018;5(3):46.
31. Peters FT, Drummer OH, Musshoff F. Validation of new methods. Forensic Sci Int. 2007;165(2-3):216-24.
32. Heath DD, Pruitt MA, Brenner DE, Rock CL. Curcumin in plasma and urine: quantitation by high-performance liquid chromatography. J Chromatogr B. 2003;783(1):287-95.
33. Mudge EM, Brown PN, Rimmer CA, Phillips MM. Determination of curcuminoids in turmeric dietary supplements by HPLC-DAD: multi-laboratory study through the NIH-ODS/NIST quality assurance program. J AOAC Int. 2020;103(6):1625-32. doi:10.1093/jaoacint/qsaa069.
34. Mohammed HA, Alsahabi DS, Hegazy AM, Khan RA, Ahmed AM. Analytical purity determinations of universal food-spice *Curcuma longa* through a QbD validated HPLC approach with critical parametric predictors and operable-design’s Monte Carlo simulations: analysis of extracts, forced-degradants, and capsules and tablets-based pharmaceutical dosage forms. *Foods* [Internet]. 2023 [cited 2023 Mar 5];12(5):1010. Available from: https://doi.org/10.3390/foods12051010
35. Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PS. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta Med. 1998;64(4):353-56.
36. Lao CD, Ruffin MT, Normolle D, Heath DD, Murray SI, Bailey JM, *et al.* Dose escalation of a curcuminoid formulation. *BMC Complement Altern Med* [Internet]. 2006 [cited 2006 Mar 15];6:10. Available from: https://doi.org/10.1186/1472-6882-6-10
37. Vilela AP, Rezende M, Terra RMO, da Silva KL, Sutil E, Calixto AL, et al. Effect of topical application of nanoencapsulated eugenol on dental sensitivity reduction after in-office dental bleaching: a randomized, triple-blind clinical trial. J Esthet Restor Dent. 2021;33(4):660-67.
38. Oberg C, Pochapski MT, Farago PV, Granado CJ, Pilatti GL, Santos FA. Evaluation of desensitizing agents on dentin permeability and dentinal tubule occlusion: an in vitro study. Gen Dent. 2009;57(5):496-501.
39. Rezende M, da Silva KL, Miguel TC, Farago PV, Loguercio AD, Martins LD, et al. Prior application of 10% potassium nitrate to reduce post-bleaching sensitivity: a randomized triple-blind clinical trial. J Evid Based Dent Pract. 2020;20(2):101406.
40. EURACHEM/CITAC. Quantifying uncertainty in analytical measurement. 3rd ed. 2012.
41. Niessen WMA. Liquid Chromatography–Mass Spectrometry. 3rd ed. Boca Raton: CRC Press; 2006.
42. González O, Blanco ME, Iriarte G, Bartolomé L, Maguregui MI, Alonso RM. Bioanalytical chromatographic method validation according to current regulations, with a special focus on LOQ, robustness and matrix effect. J Chromatogr A. 2014;1353:10-27.