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Original Article

Development and validation of an RP-HPLC method for simultaneous determination of curcumin and metronidazole in combined dosage form

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ABSTRACT

Background and Aims: The present study aimed to develop and validate a simple reverse phase-high pressure liquid chromatography (RP-HPLC) method for simultaneous determination of natural compound curcumin and metronidazole in bulk and its combined dosage form.

Methods: *In situ* gel formulation containing curcumin and metronidazole was prepared as a model combined system. The chromatographic separation was accomplished isocratically on Eclipse XDB-C18 (150 mm x 4.6 mm, 5 µm particle size) column using UV-detection at 254 nm. The optimized mobile phase contained a mixture of Phosphate Buffer pH4.5-Acetonitrile (50:50, v/v), and the flow rate was set to 1.0 mL/min with 10 µL injection volume. The method was validated in compliance with International Council for Harmonisation (ICH) standards, and it was successfully used for quality control assays for their combined drug product

Results: The results for retention times were 8.60 and 1.40 min for curcumin and metronidazole, respectively. The method indicated linear responses within the concentration ranges of 3.0-80 and 4.8-128 µg/mL with LOD values of 0.62; 1.03 µg/mL and LOQ values of 1.88; 3.13 µg/mL for curcumin and metronidazole, respectively. Precision results were within acceptable limits (RSD<2%), and the determination of the two active substances was not interfered with by any formulation components.

Conclusion: The proposed validated RP-HPLC method was successfully applied to determine the total contents of curcumin and metronidazole *in situ* gel formulation. The validation results showed that the proposed method was simple, specific, and precise, and that it could be used for routine quality control for their combined pharmaceutical application.

Keywords: Curcumin, Metronidazole, Simultaneous-quantification, HPLC method development

INTRODUCTION

Curcumin (CUR) [(E,E)-1,7-bis(4-hydroxy-3-METhoxy-phenyl)-1,6-heptadiene-3,5-ione] (Figure 1 (A)) is the main active ingredient of *Curcuma longa* (turmeric rhizome). Therapeutic use of this herbal drug has been recorded in Asian traditional medicine for over thousand years. Even at very high doses CUR is safe and has not been associated with any toxicities or adverse side effects that have been documented or studied at the population level. (Basnet & Skalko-Basnet, 2011; Berginc, Škalko-Basnet, Basnet, & Kristl, 2012; Wachter et al., 2014). However, due to its low aqueous solubility, photosensitivity, rapid hydrolysis at alkaline pH, and rapid systemic elimination, its clinical use has been limited. Novel drug delivery technologies or combinations with other drugs are commonly used in order to improve the potency of CUR (Yuan et al. 2012).

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Moreover, CUR has emerged as an appealing natural ingredient in combination therapy with potential to enhance the clinical outcomes of many antibiotics (Chanda & Rakholiya, 2011; Ejim et al., 2011; Lakshmi et al., 2016; Sasidharan et al. 2014). Combination treatment is one of the most effective ways to avoid defense mechanisms and dose-related adverse reactions. Synergistic combinations of two or more therapeutically relevant compounds that operate *via* distinct pathways increase therapeutic efficacy by allowing for a multi-target therapy strategy (Jain et al., 2016). CUR's anti-parasitic action has lately been thoroughly researched, revealing that it has a great potential to serve as an effective medication alone or in combination against a variety of parasites (Cheraghipour et al. 2018; Rangel-Castañeda et al. 2018).

Metronidazole (MET; Figure 1-B) is a highly effective broad-spectrum antibiotic used to treat gastrointestinal infections as well as sexually transmitted diseases (STDs) such as *trichomoniasis*, *giardiasis,* parasitic infections, and bacterial *vaginosis*. Topical delivery of MET has been favored due to the circumvention of its numerous drawbacks caused by the systemic administration of drug (Held, 1987; Ibrahim et al., 2012; Topal et al., 2015). Therefore, finding novel treatments that are more effective and have fewer adverse effects is critical. One of these innovative therapies is combination therapy, which employs antibiotic resistance inhibitors. Some edible natural and dietary ingredients have recently been found to improve the antiparasitic action of certain drugs such as metronidazole and artemisinin (Isacchi et al. 2012; Rangel-Castañeda et al. 2018). Moreover, CUR has been shown to protect DNA against damage and oxidative stress produced by some drugs and environmental mutagens, including MET (Singh & Giri 2013). As previously mentioned, several studies on the biological activities of CUR have been conducted, but the effects of this natural substance in combination with various antibiotics have yet to be thoroughly investigated (Teow & Ali 2015; Mun et al. 2013; Sasidharan et al. 2014).

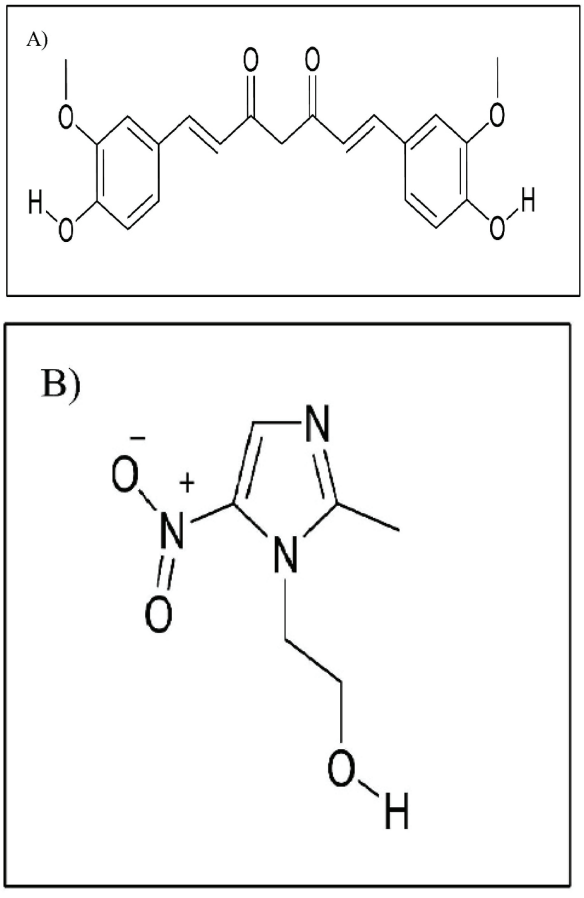

Figure 1. Chemical structures of CUR (A) and MET (B).

A variety of different HPLC determination methods have been proposed individually for CUR and MET in the literature (Chaudhary et al. 2012; Ji et al. 2009; USP 2015; Venkateshwaran and Stewart 1995). Since there is not any product on the market for this combination, there is yet to be a validated analytical method for the simultaneous evaluation of these drugs. The current study aimed to develop and validate a simple sensitive, precise, and reproducible RP-HPLC method for determining both active substances in bulk and in its dosage form. In this analysis, single *in situ* gel system containing CUR and MET was prepared and used as a representative formulation for aforementioned combined model system.

MATERIAL AND METHODS

CUR was purchased from Merck (Darmstadt, Germany). MET was obtained from Ibrahim Etem Ulagay Menarini (Istanbul, Turkey). Poloxamer 188 (PLX 188), Poloxamer 407 (PLX407) and potassium dihydrogen phosphate were provided from Sigma-Aldrich (Saint-Quentin Fallavier, France). Pharmasolve® (PHR) was obtained from Ashland (Oregon, USA). Phosphate buffer pH 4.5 (PBS pH 4.5) and Acetonitrile (ACN) were HPLC grade and purchased from Merck (Darmstadt, Germany). Cellulose sterile acetate syringe filters (Pore size: 0,45µm) were obtained from ISOLAB (Eshau, Germany). Double distilled water was used for all experiments.

Instrumentation and chromatographic conditions

This research was conducted using the Agilent 1260 Infinity HPLC system (Wilmington, DE, USA) fitted with a solvent degasser, a quaternary pump, an auto sampler, a column oven, and a diode array detector. The Agilent ChemStation software was used for the management and data processing of instrument operations. The column Eclipse XDB-C18 (5 µm, 150 mm x 4.6 mm) was used for separation. The optimized mobile phase contained mixture of PBS pH 4.5:ACN (50:50, v/v), and flow rate was set to 1.0 mL/min with 10 µL injection volume. The column oven was conditioned at 37 °C, and analysis run time took 15 minutes. 254 nm wavelength (λ max) was determined by standard scanning for CUR and MET with DAD detector between 200-400 nm. Before each injection, column was equilibrated until the UV signal, and back pressure were stabilized with the mobile phase flowing through the system.

Standard solutions and preparation of the samples

A standard stock solution was prepared by dissolving 0.2 $mg.mL^{-1}$ CUR and 0.32 $mg.mL^{-1}$ MET in ACN. The solution was placed in an ultrasonic bath (Selecta Ultrason HD, Spain) for 30 min, aiming complete dissolution of the combination.

Preparation and analysis of *in situ* gels with CUR and MET

In situ gel system was prepared with a cold method technique (Baloglu et al., 2011b; Garala et al., 2013); PLX 407 (20%); PLX 188 (5%), PHR (15%) and, 0.7% CUR and 0.7% MET (w/w) combination were gradually added to cooled and distilled water placed in an ice bath (4 °C) with constant stirring. The final gel system (CUR-MET-Gel) was left at 4 °C for 24 h in order to ensure a full wetting and elimination of the air bubbles. The same protocol was also adopted in order to prepare a drug free *in situ* gel sample.

Following organoleptic and physical examination of the CUR-MET-Gel, critical parameters such as *sol-gel* transition temperature, gelation time, and viscosity characterized with *in situ* gel systems were also analyzed using the HR-1 Discovery Hybrid Rheometer (TA Instruments, England). This evaluation was conducted using a steel probe with a diameter of 40 mm and a set interval of 500 µm with a fixed frequency of 0.01 Hz. The change in the viscosity (Pa.s) of the samples was monitored by heating the samples at a rate of 2 °C/minute within the range of 15-50 °C. The region where viscosity changes significantly was taken as basis for determination of the *sol-gel* transition temperature and gelation time (Baloglu, Karavana, Senyigit, & Guneri, 2011a; Edsman, Carlfors, & Petersson, 1998). The pH of the CUR-MET-Gel was examined by pH–meter (Ohaus Starter 3100, USA).

Analytical method validation

The method was validated in scope of system suitability, linearity, limits of detection (LOD) and quantitation (LOQ), precision, accuracy, specificity, selectivity and stability in compliance with ICH guidelines (ICH 2005).

Linearity

Linear calibration curves of the proposed method were obtained by diluting stock solutions with mobile phase (PBS pH 4.5:ACN) (50:50) (v/v) for both of the drugs with concentrations values of 3, 5, 10, 20, 40, 60 and 80 µg/mL for CUR and 4.8, 8, 16, 32, 64, 96 and 128 µg/mL for MET. Linearity was evaluated by fitting least-squares regression analysis.

Specificity

The specificity was determined by assessing chromatograms of the interference of excipient(s) with CUR and MET determination. To accomplish this, chromatograms of drug free *in situ* gel solution, bulk solution with concentration of 10 µg/mL from CUR and 20 µg/mL of MET and mobile phase were injected into the chromatographic system.

Accuracy

The accuracy of the analytical method was confirmed by comparing the experimental results to the theoretical findings. For this objective, three sets of CUR (4 µg/mL, 12 µg/mL, and 30 µg/mL) and MET (6.4 µg/mL, 19.2 µg/mL, and 48 µg/mL) concentrations were added to the matrix samples of pH 4.5 phosphate buffer and acetonitrile (50:50) medium. The results were reported as percent recovery.

Precision

Precision of the system was validated in terms of repeatability, intermediate accuracy, and reproducibility. For repeatability, six individual samples at a concentration of 30 µg/mL for CUR and 48 µg/mL for MET were prepared and injected to HPLC system. Furthermore, intermediate precision was tested by preparing six solutions of the same concentration (CUR: 30 µg/mL and MET: 48 µg/mL) and checking them on two consecutive days by two different analysts. All results were evaluated in terms of standard deviation (SD) and relative standard deviation (RSD).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limits of detection and quantification value were determined based on the standard deviation (SD) of the responses and the slope (S). The equations (Eq.1 and Eq.2) were used to calculate LOD and LOQ values:

$$LOD = 3.3\ SD/S \qquad \text{(Eq.1)}$$

$$LOQ = 10\ SD/S \qquad \text{(Eq.2)}$$

Short-term Stability of Curcumin and Metronidazole Solution

A solution containing 70 µg/mL CUR and MET was prepared and tested for short-term solution stability. For 48 hours, the prepared solution was maintained at 37 °C. Samples were collected and analyzed after 0, 24, and 48 hours (n=3).

Assay procedure for in situ gel formulations

Drug contents of the CUR and MET in gel formulations were determined by dissolving an accurately weighed quantity of gel (about 500 mg) in ACN. This solution was transferred to 50 mL volumetric flask, and appropriate dilutions were made with the mobile phase (PBS pH 4.5:ACN (50:50, v/v). The solution was sonicated for 20 minutes to achieve complete dissolution of the active pharmaceutical ingredients (API) with yield concentrations 70 µg/mL for CUR and 70 µg/mL for MET. The resulting solution was then filtered through 0.45 µm syringe membrane filters (ISOLAB, Eshau, Germany) and proceed for HPLC analysis.

RESULTS AND DISCUSSION

Preparation and analysis of *in situ* gels with CUR and MET

In situ gel system was successfully prepared with cold method. Obtained gel had a light orange color, elegant appearance, homogeneous texture, and it was free of gritty particles. Findings for *sol-gel* transition temperature and gelation time, viscosity and pH are given in Figure 2. The *sol-gel* transition temperature of stimulus sensitive gels is the temperature at which the rheological properties of the system shift its rheological behavior from *Newtonian/elasto-viscous* to *viscoelastic*. As can be seen from Figure 2 (A), the viscosity profile of the formulation decreased as the sliding speed increased at 37 °C. The gelation temperature for mucosal formulations preferred to be in range of 30-36 °C. Gelation temperatures below 30°C facilitate the forming of gel at room temperature, creating difficulties in manufacturing, handling and administration, whereas gelation temperatures above 37 °C cause gel to remain in liquid state, resulting in rapid elimination after administration (Giuliano et al., 2018). Rheological performance of topical gel formulations plays an important role in achieving maximum clinical efficacy by affecting both ease of application and re-

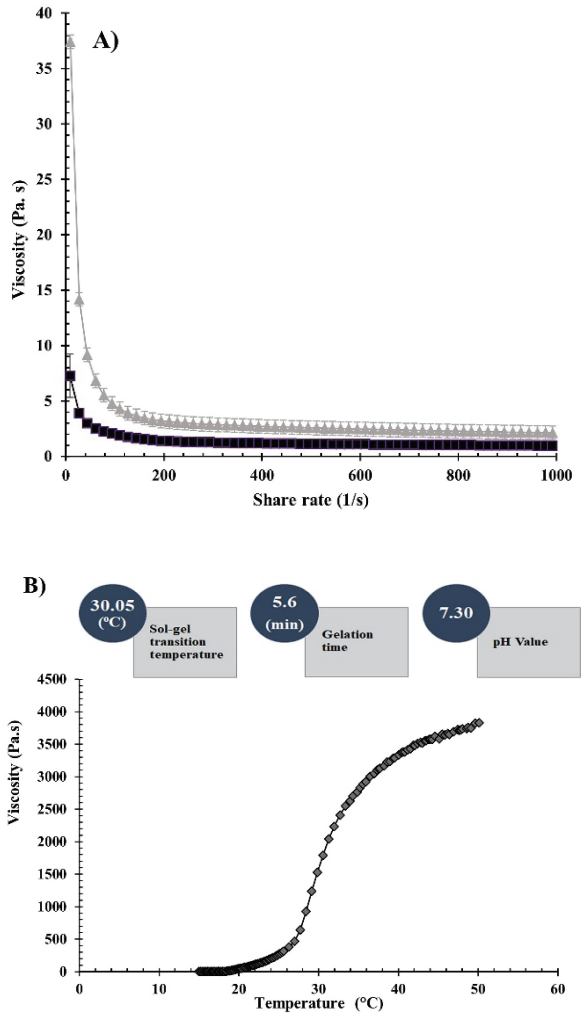

Figure 2. Flow rheograms measured at 37 °C (-▲-) and 25 °C (-■-) (A); Viscosity versus temperature graph, *Sol-gel* transition temperature, gelation time and pH value for CUR-MET-GEL formulation (B).

tention on vaginal surface. Prepared gel sample demonstrated suitable features for mucosal administration in terms of flow property, optimum *sol-gel* transition temperature and gelation time (30.05 °C; 5.6 min) and, pH (7.30 ± 0.08) in scope of physiological limitations (Baloglu, et al., 2011b; Yu et al., 2011).

HPLC method development and optimization

There are various HPLC methods available in the literature for both active substances separately; based on these studies, numerous trials were conducted to develop an optimal chromatographic method for the simultaneous estimation of CUR and MET in combination. For this aim, combinations of different solvents (tetrahydrofuran: water; 0.1% ortho phosphoric acid: ACN; 0.01 M monobasic potassium phosphate buffer pH 4.5: methanol) as a mobile phase were varied to optimize the separation conditions. Knowing that for isocratic elution, a mixture of buffered solution and water-miscible organic solvents approach is frequently utilized for drug combinations. When compared to tetrahydrofuran or methanol, ACN was chosen as an organic phase since it has the lowest viscosity, strongest eluting power, and the highest selectivity for the separation of curcuminoids (Chaudhary et al., 2012; Galmier et al., 1998; Jangle and Thorat, 2013; Jayaprakasha et al., 2002; Ji et al., 2009; Venkateshwaran & Stewart, 1995). From all trailed combinations PBS pH 4.5 and ACN (90:10, 80:20, 70:30, 60:40, 50:50, 40:60, and 30:70 v/v) showed the optimum separation. Due to the form and symmetry of the peaks, the best result was achieved with PBS pH 4.5 and ACN with a ratio of 50:50. Under these conditions, MET and CUR were eluted at 1.40 min and 8.60 min, respectively, and the peaks for both APIs were specifically defined. The current approach has offered the advantage of having a relatively short run time, which allows increased production (Chaudhary et al. 2012; Jayaprakasha et al. 2002). Additionally, the lowered acid content in the mobile phase, the use of only two solvents, and the lower flow rate (1.0 mL/min) insured that the column and system would survive longer (Jangle and Thorat, 2013). Furthermore, the use of isocratic elution rather than gradient elution for this research provided low cost, simplicity, and consistency over the entire testing timeframe. The summary of the HPLC conditions, retention time and symmetry factor are presented in Table 1.

Table 1. Data for optimized HPLC method.

Parameters	
Mobile phase:	Isocratic mixture: Phosphate buffer pH 4.5: acetonitrile (50:50, v/v)
Flow rate:	1.0 mL/s
Injection volume:	10 µL
Wavelengths:	254 nm
Dilution solvent:	Mobile phase
Retention time for MET:	1.40 min
Retention time for CUR:	8.60 min
Symmetry factor for MET:	0.75
Symmetry factor CUR:	0.80

Analytical method validation

The Method was validated with respect to system suitability, linearity, limits of detection (LOD) and quantitation (LOQ), precision, accuracy, specificity and selectivity in accordance with ICH guidelines (ICH 2005) with additionally short-term solution stability analysis.

Specificity

Methodology for specificity was found to be specific as there was no interference between the chromatograms of active substances within the gel formulation excipients or mobile phase constituents (Figure 3).

Linearity

Standard lines were plotted within 3-80 and 4.8-128 µg/mL concentration ranges, with the linear regression equation y=21.946x+0.738 (R^2=0.999) and y=12.975x+9.434 (R^2=0.999) for

CUR and MET, respectively. The calculated coefficients of determination of both CUR and MET were close to 1, and standard deviation was low, which indicates that the equipment response is in proportional relationship to the drugs concentrations in the analyze. The analyses of calibration are shown in Figure 4.

Accuracy and recovery

The percentage of recovery results for CUR and MET are shown in Table 2. Obtained 95% confidence interval, and RSD values illustrated good precision and accuracy of the method for both APIs.

Precision

Neither of the peak areas changed by more than 2% for CUR and MET, suggesting that the method was highly repeatable. Intermediate precision checked by two analysts, and during the two consecutive days was evaluated by six analyses. All RSD results were lower than 2% in all assays, which meets the criteria for precision (Çelebier et al. 2010; Chaudhary et al. 2012). Tables 3 and 4 summarize the key results.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

In order to assess the sensitivity of the method, the LOD and LOQ values were determined using Eq. 1 and 2. The obtained findings are shown in Table 5 indicating that the method is sensible enough to evaluate both CUR and MET in combination.

Short-term stability of curcumin and metronidazole solution

The CUR and MET solution short-term stability test findings demonstrated no change in retention time or deterioration in peak characteristics for the detected HPLC peaks. Both drugs were stable at 37 °C for 48 hours with an RSD of less than 2%.

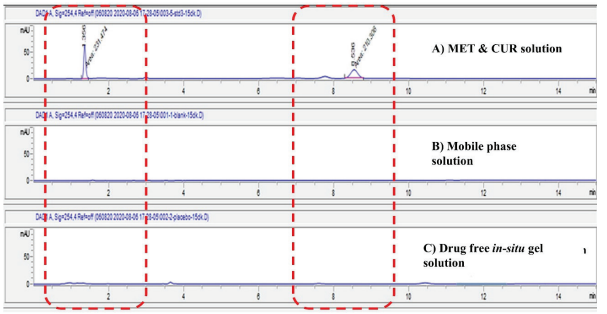

Figure 3. Chromatogram of (A) CUR and MET injection, (B) mobile phase solution-placebo and C) drug free *in situ* gel solution.

Application of the Method for Assay of APIs Within the *in situ* Gel Formulation

The proposed validated method was successfully applied to determine the total drug content of CUR and MET for prepared *in situ* gel formulation (CUR-MET-Gel). The obtained results were similar with matching labelled amounts (Table 6).

CONCLUSION

It is well recognized that the validation process is a crucial part of the development of the analytical method. The developed

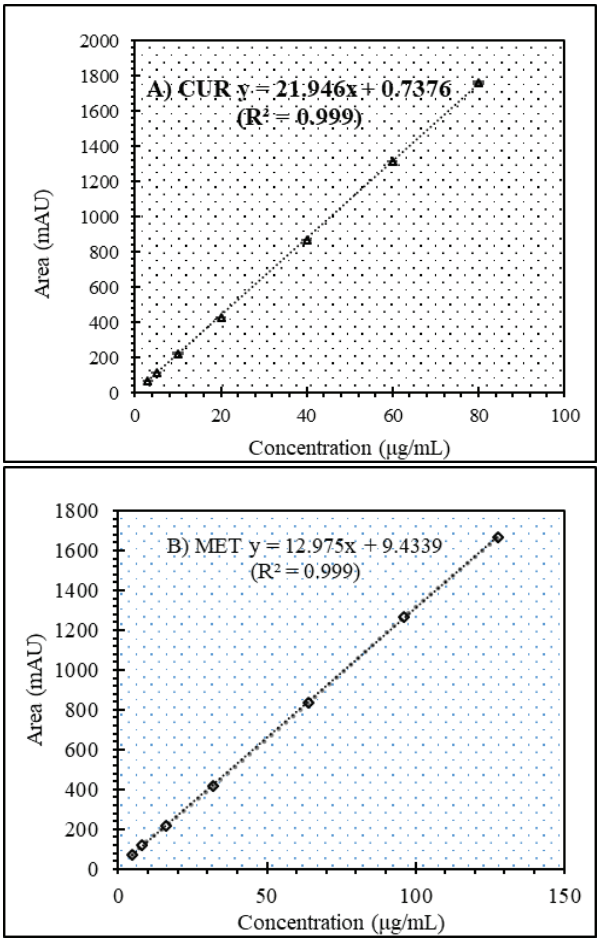

Figure 4. Calibration curves of CUR (A) and MET (B).

Table 2. Recovery results for CUR and MET.

API	n	Theoretical Concentration (µg/mL)	Practical Concentration (µg/mL)	Recovery (%)	SD	RSD (%)	CI (95%)
CUR	6	4.00	3.95	98.70	0.42	0.17	98.22-99.18
	6	12.00	12.09	100.78	2.58	1.05	98.37-104.29
	6	30.00	29.71	99.04	0.95	0.39	98.27-100.27
MET	6	6.40	6.81	106.42	1.10	0.45	105.15-107.68
	6	19.20	19.28	100.46	1.68	0.68	98.53-102.39
	6	48.00	49.21	102.52	1.07	0.44	101.29-103.75

SD: Standard Deviation; RSD: Relative standard deviation; CI: Confidence Interval (95%, Lower and Upper Limit)

Table 3. Precision test results of CUR and MET in pH 4.5 phosphate buffer and acetonitrile (50:50) medium.

Sample number	AUC for CUR (30 µg /mL)	AUC for MET (48 µg /mL)
1	649.00	654.20
2	650.40	650.00
3	648.90	642.20
4	652.80	640.30
5	645.90	646.40
6	660.70	650.00
AVR	651.28	647.35
SD	4.68	5.05
RSD (%)	1.91	1.96
CI (95%)	645.89-656.67	641.54-653.15

SD: Standard Deviation; RSD: Relative standard deviation; CI: Confidence Interval (95%, Lower and Upper Limit)

Table 4. Intermediate precision checked by two analysts and on two different days.

API		1. Analyst	2. Analyst	1. Day	2. Day
CUR (%)	Concentration: 30 µg/mL (n=6)	99.31	98.90	99.86	100.01
	SD	0.62	0.77	0.59	0.45
	RSD (%)	0.25	0.31	0.24	0.18
	CI (95%)	98.65-99.96	98.09-99.70	99.24-100.48	99.55-100.48
MET (%)	Concentration: 48 µg/mL (n=6)	104.74	103.27	102.84	101.88
	SD	1.98	0.51	0.51	1.34
	RSD (%)	0.81	0.21	0.21	0.55
	CI (95%)	103.48-106.57	102.79-103.75	102.25-103.44	100.64-103.12

SD: Standard Deviation; RSD: Relative standard deviation; CI: Confidence Interval (95%, Lower and Upper Limit)

Table 5. Limits of detection (LOD) and quantitation (LOQ) for CUR and MET.

	CUR (µg/mL)	MET (µg/mL)
Limits of detection - LOD	0.62	1.03
Limits of quantitation - LOQ	1.88	3.13

Table 6. *CUR and MET*assay for prepared *in situ* gel dosage form.

Prepared *in-situ* gel formulation	n	Recovery for CUR (%) ± RSD (%)	Recovery for MET (%) ± RSD (%)
CUR-MET-GEL	6	94.00 ±0.16	98.00±1.25

RSD: Relative standard deviation

method was tested in accordance with the ICH guidelines. Regarding the validation results, the proposed method was found to be simple, specific, accurate, and precise and could be applied to the quantitative analysis of CUR along with a MET combination in a bulk solution and as well in a formulation as *in situ* gel. Furthermore, the method is suitable for regular analysis and quantitative testing of CUR and MET combinations in pharmaceutical dosage forms.

Peer-review: Externally peer-reviewed.

Informed Consent: Written consent was obtained from the participants.

Author Contributions: Conception/Design of Study- L.B.P., E.B.; Data Acquisition- L.B.P., E.V.B.; Data Analysis/Interpretation- L.B.P., E.V.B., E.B.; Drafting Manuscript- L.B.P., E.V.B.; Critical Revision of Manuscript- L.B.P., E.B.; Final Approval and Accountability- L.B.P., E.V.B., E.B

Conflict of Interest: The authors have no conflict of interest to declare.

REFERENCES

- Baloglu, E., Karavana, S. Y., Senyigit, Z. A., & Guneri, T. (2011a). Rheological and mechanical properties of poloxamer mixtures as a mucoadhesive gel base. *Pharmaceutical development and technology*, *16*(6), 627-636. doi: 10.3109/10837450.2010.508074.
- Baloglu, E., Karavana, S. Y., Senyigit, Z. A., Hilmioglu-Polat, S., Metin, D. Y., Zekioglu, O., ... & Jones, D. S. (2011b). In-situ gel formulations of econazole nitrate: preparation and in-vitro and in-vivo evaluation. *Journal of Pharmacy and Pharmacology*, *63*(10), 1274-1282. doi: 10.1111/j.2042-7158.2011.01315.x.
- Basnet, P., & Skalko-Basnet, N. (2011). Curcumin: an anti-inflammatory molecule from a curry spice on the path to cancer treatment. *Molecules*, *16*(6), 4567-4598. doi: 10.3390/molecules16064567.
- Berginc, K., Škalko-Basnet, N., Basnet, P., & Kristl, A. (2012). Development and evaluation of an in vitro vaginal model for assessment of drug's biopharmaceutical properties: Curcumin. *AAPS PharmSciTech*, *13*(4), 1045-1053. doi: 10.1208/s12249-012-9837-9.
- Çelebier, M., Kaynak, M. S., Altınöz, S., & Sahin, S. (2010). HPLC method development for the simultaneous analysis of amlodipine and valsartan in combined dosage forms and in vitro dissolution studies. *Brazilian Journal of Pharmaceutical Sciences*, *46*, 761-768. doi: 10.1590/S1984-82502010000400018.
- Chanda, S., & Rakholiya, K. (2011). Combination therapy: Synergism between natural plant extracts and antibiotics against infectious diseases. In A. Méndez-Vilas (Ed.), *Science against microbial pathogens: communicating current research and technological advances* (pp. 520-529). Formatex, Spain.
- Chaudhary, H., Kohli, K., Amin, S., Arora, S., Kumar, V., Rathee, S., & Rathee, P. (2012). Development and validation of RP-HPLC method for simultaneous estimation of diclofenac diethylamine and curcumin in transdermal gels. *Journal of Liquid Chromatography & Related Technologies*, *35*(1), 174-187. doi: 10.1080/10826076.2011.597068.
- Cheraghipour, K., Marzban, A., Ezatpour, B., Khanizadeh, S., & Koshki, J. (2018). Antiparasitic properties of curcumin: A review. *AIMS Agriculture and Food*, *3*(4), 561–578. doi: 10.3934/AGRFOOD.2018.4.561.
- Edsman, K., Carlfors, J., & Petersson, R. (1998). Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. *European Journal of Pharmaceutical Sciences*, *6*(2), 105-112. doi: 10.1016/S0928-0987(97)00075-4.
- Ejim, L., Farha, M. A., Falconer, S. B., Wildenhain, J., Coombes, B. K., Tyers, M., Brown, E.D., & Wright, G. D. (2011). Combinations of antibiotics and nonantibiotic drugs enhance antimicrobial efficacy. *Nature Chemical Biology*, *7*(6), 348-350. doi: 10.1038/nchembio.559.
- Galmier, M. J., Frasey, A. M., Bastide, M., Beyssac, E., Petit, J., Aiache, J. M., & Lartigue-Mattei, C. (1998). Simple and sensitive method for determination of metronidazole in human serum by high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications*, *720*(1-2), 239-243. doi: 10.1016/S0378-4347(98)00443-5.
- Garala, K., Joshi, P., Shah, M., Ramkishan, A., & Patel, J. (2013). Formulation and evaluation of periodontal in situ gel. *International journal of pharmaceutical investigation*, *3*(1), 29. doi: 10.4103/2230-973x.108961.
- Giuliano, E., Paolino, D., Fresta, M., & Cosco, D. (2018). Mucosal applications of poloxamer 407-based hydrogels: An overview. *Pharmaceutics*, *10*(3), 159. doi: 10.3390/pharmaceutics10030159.
- Held, R. B. (1987). Rural resource management. In Cloke, P.J., & Park, C.C. London: Croom Helm, 1985. 473.
- Ibrahim, S.A., Ismail, S., Fetih, G., Shaaban, O., Hassanein, K., & Abdellah, N. H. (2012). Development and characterization of thermosensitive pluronic-based metronidazole in situ gelling formulations for vaginal application. *Acta pharmaceutica*, *62*(1), 59-70. doi: 10.2478/v10007-012-0009-y.
- ICH. (2005). ICH Topic Q2 (R1) Validation of Analytical Procedures: Text and Methodology. *International Conference on Harmonization* 1994 (p.17).
- Isacchi, B., Bergonzi, M. C., Grazioso, M., Righeschi, C., Pietretti, A., Severini, C., & Bilia, A. R. (2012). Artemisinin and artemisinin plus curcumin liposomal formulations: enhanced antimalarial efficacy against Plasmodium berghei-infected mice. *European Journal of Pharmaceutics and Biopharmaceutics*, *80*(3), 528-534. doi: 10.1016/j.ejpb.2011.11.015.
- Jain, A., Doppalapudi, S., Domb, A. J., & Khan, W. (2016). Tacrolimus and curcumin co-loaded liposphere gel: Synergistic combination towards management of psoriasis. *Journal of Controlled Release*, *243*, 132-145. doi: 10.1016/j.jconrel.2016.10.004.
- Jangle, R. D., & Thorat, B. N. (2013). Reversed-phase high-performance liquid chromatography method for analysis of curcuminoids and curcuminoid-loaded liposome formulation. *Indian Journal of Pharmaceutical Sciences*, *75*(1), 60-66. doi: 10.4103/0250-474X.113555.
- Jayaprakasha, G. K., Jagan Mohan Rao, L., & Sakariah, K. K. (2002). Improved HPLC method for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. *Journal of Agricultural and Food Chemistry*, *50*(13), 3668-3672. doi: 10.1021/jf025506a.
- Ji, L., Jiang, Y., Wen, J., Fan, G., Wu, Y., & Zhang, C. (2009). A rapid and simple HPLC method for the determination of curcumin in rat plasma: assay development, validation and application to a pharmacokinetic study of curcumin liposome. *Biomedical Chromatography*, *23*(11), 1201-1207. doi: 10.1002/bmc.1244.
- Lakshmi, Y. S., Kumar, P., Kishore, G., Bhaskar, C., & Kondapi, A. K. (2016). Triple combination MPT vaginal microbicide using curcumin and efavirenz loaded lactoferrin nanoparticles. *Scientific reports*, *6*(1), 1-13. doi: 10.1038/srep25479.
- Mun, S. H., Joung, D. K., Kim, Y. S., Kang, O. H., Kim, S. B., Seo, Y. S., ... & Kwon, D. Y. (2013). Synergistic antibacterial effect of curcumin against methicillin-resistant Staphylococcus aureus. *Phytomedicine*, *20*(8-9), 714-718. doi: 10.1016/J.PHYMED.2013.02.006.
- Rangel-Castañeda, I. A., Hernández-Hernández, J. M., Pérez-Rangel, A., González-Pozos, S., Carranza-Rosales, P., Charles-Niño, C. L., ... & Castillo-Romero, A. (2018). Amoebicidal activity of curcumin on Entamoeba histolytica trophozoites. *Journal of Pharmacy and Pharmacology*, *70*(3), 426-433. doi: 10.1111/jphp.12867.
- Sasidharan, N. K., Sreekala, S. R., Jacob, J., & Nambisan, B. (2014). In vitro synergistic effect of curcumin in combination with third generation cephalosporins against bacteria associated with infectious diarrhea. *BioMed Research International*, *2014*. doi: 10.1155/2014/561456.
- Singh, S., & Giri, S. (2013). Curcumin protects oxidative stress and DNA damage induced by metronidazole and gamma radiation in vivo. Paper presented at the: Environmental Mutagen Society of India and national conference on current perspectives on environmental mutagenesis and human health, Mumbai (India), Abstract retrieved from https://www.osti.gov/etdeweb/biblio/22082419
- Teow, S. Y., & Ali, S. A. (2015). Synergistic antibacterial activity of Curcumin with antibiotics against Staphylococcus aureus. *Pakistan Journal of Pharmaceutical Sciences*, *28*(6), 2109-2114.
- Topal, M., Şenel, G. U., Arslan Topal, E. I., & Öbek, E. (2015). Antibiyo-

tikler ve kullanım alanları. *Erciyes Üniversitesi Fen Bilimleri Enstitüsü Fen Bilimleri Dergisi*, *31*(3), 121-127.

- USP, (2015). U.S. Pharmacopoeia-national formulary [USP 38 NF 33]. *Rockville, Md United States Pharmacopeial Convention*. volume 1.
- Venkateshwaran, T. G., & Stewart, J. T. (1995). Determination of metronidazole in vaginal tissue by high-performance liquid chromatography using solid-phase extraction. *Journal of Chromatography B: Biomedical Sciences and Applications*, *672*(2), 300-304. doi: 10.1016/0378-4347(95)00217-7.
- Wachter, B., Syrowatka, M., Obwaller, A., & Walochnik, J. (2014). In vitro efficacy of curcumin on Trichomonas vaginalis. *Wiener klinische Wochenschrift*, *126*(1), 32-36. doi: 10.1007/s00508-014-0522-8.
- Yu, T., Malcolm, K., Woolfson, D., Jones, D. S., & Andrews, G. P. (2011). Vaginal gel drug delivery systems: understanding rheological characteristics and performance. *Expert Opinion on Drug Delivery*, *8*(10), 1309-1322. doi: 10.1517/17425247.2011.600119.
- Yuan, Y., Cui, Y., Zhang, L., Zhu, H. P., Guo, Y. S., Zhong, B., ... & Chen, L. (2012). Thermosensitive and mucoadhesive in situ gel based on poloxamer as new carrier for rectal administration of nimesulide. *International Journal of Pharmaceutics*, *430*(1-2), 114-119. doi: 10.1016/j.ijpharm.2012.03.054.