Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 760-769 | ISSN:2251-6727

Development of New, Rapid and Sensitive Validated High Performance Thin Layer Chromatographic Method for Estimation of Berberine Hcl and Thymoquinone in Bulk and Its Combination Containing Microemulgel

[1] Pranali Badhe, [2] Jeeja Pananchery *, [3] Monika Jadhav, [4] Dr. Ashish Jain

[1] M. Pharm student Department of Quality Assurance Shri D. D. Vispute College of Pharmacy & Research Center, New Panvel, Navi Mumbai, Maharashtra. India-410206.

[2] Assistant Professor Department of Pharmacognosy D. Y. Patil Deemed to be University School of Pharmacy, Sector 7, Nerul, Navi Mumbai, Maharashtra. India-400706.

[3] Research Scholar Department of Pharmaceutical Chemistry C. U. Shah College of Pharmacy, SNDT Women's University, Juhu campus, Mumbai 400049

[4] Associate Professor Department of Pharmacognosy, Shri D. D. Vispute College of Pharmacy & Research Center, New Panvel, Navi Mumbai, Maharashtra, India-410206.

(Received: 27 October 2023 Revised: 22 November Accepted: 26 December)

KEYWORDS

Berberine HCl, Thymoquinone, HPTLC, Method development and Validation, ICH guideline

ABSTRACT:

According to ICH Q2 (R1) criteria, two different, simple and sensitive densitometric High Performance Thin Layer Chromatography methodologies for assessment of Berberine HCl (BER) and Thymoquinone (TQ) in bulk individually and in established microemulgel formulation has been developed and validated. The analysis was conducted separately for BER and TQ on Merck HPTLC aluminum sheets of silica gel 60 F254 by using a mixture of Ethanol: Glacial acetic acid: Water (16:2:2 v/v/v) for BER and Toluene: Acetone: Formic acid (5:4:0.2 v/v/v) for TQ as a mobile phase and densitometric determination was carried out by TLC scanner (CAMAG) at 366 nm and 254 nm respectively for BER and TQ. The approach produced well-defined peaks for BER and TQ with Rf values of 0.468 and 0.817, respectively. The calibration plots for BER and TQ exhibited a satisfactory linear relationship with regression coefficient (r2) of 0.9991 and 0.9953, respectively, in the range of concentrations of 50-350 ng/band and 50-300 ng/band. The limit of detection and quantification were 10.76 and 32.65 ng/band respectively for BER, 3.77 and 11.43 ng/band respectively for TQ. Further, the study provides additional evidence that accuracy, precision and repeatability were all within the acceptable range. The method has been successfully applied to the identification and quantitative determination of BER and TQ in bulk and its combination containing formulation.

1. Introduction

Because of its sensitivity, affordability, reliability in quantifying analytes at micro and even nanogram levels, accuracy, suitability for high-throughput screening, and ease of use, high-performance liquid chromatography (HPTLC) is the most preferred analytical method for the identification and quantification of marker compounds in herbal medications.[1] As compared with High Performance Liquid Chromatography (HPLC), it has considerable benefits, including the ability to comprehend a sufficient amount of data at once by employing a small amount of the mobile phase and ability to consistently detect (or scan) chromatogram by utilizing the same or different parameters, which cuts down on both the amount of time and energy spent on analysis.[2]

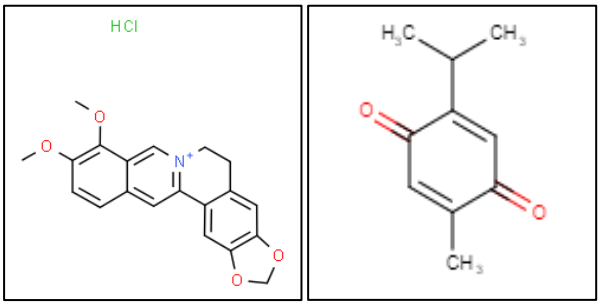
Berberine is yellow bioactive component found in many members of the Berberis genus (Berberidaceae, Hindi; Daruharida).[3] It belongs to the protoberberine alkaloids family, with a tetracyclic ring based on the dibenzoquinolizidine system.[4] Berberine has a broad spectrum of physiological effects and its conventional application comprises antimicrobial activity, antidiarrheal and antiprotozoal application.[4] Berberine HCl damage the integrity of bacterial membrane and affect the metabolic activity, inhibiting protein and DNA

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(1), 760-769 | ISSN:2251-6727

synthesis, which causing bacteria to die. Thus, antibacterial activity demonstrated. Berberine was evaluated in *Tinospora cordifolia* stems when Satija S. et. al., developed and validated HPTLC method for the quantitative estimation of berberine in *Tinospora cordifolia* utilizing mobile phase consisting of methanol, acetic acid, water (8:1:1 v/v/v), berberine was assessed in *T. cordifolia* stems.[5]

Nigella sativa (NS) is a the member of *Ranunculaceae* botanical family, while its seeds are known as a black seed, kalonji, black caraway or black cumin .[6] NS seeds have undergone considerable phytochemistry and pharmacological research. Thymoquinone is one of the major bioactive essential component of NS, and it belongs to the monocyclic monoterpene family.[7] Thymoquinone, which was extracted from the volatile oil of NS, was discovered to have high action against gram-positive bacteria such as *Staphylococcus aureus*.[8] Taleuzzaman M. et. al., reported HPTLC method for the measurement of thymoquinone (TQ) by employing n-hexane: ethyl acetate: methanol (7:2:1 v/v/v) as mobile phase.[2]

Although there is a literature on the estimation of berberine HCl and thymoquinone, there are no methodologies for their estimation in the formulation of microemulsion gels. This work's goal is to create, improve and validate an HPTLC method for quantifying both the drug i.e., BER and TQ in its bulk (standard) and in microemulgel formulation.



(a) (b)

Figure 1: Chemical structure of (a) Berberine HCl, (b) Thymoquinone

2. Materials and Methods

2.1 Materials:

Standard BER and TQ were supplied by Yucca Enterprises, Mumbai. Analytical grade ethanol, glacial acetic acid, acetonitrile, formic acid, toluene and acetone were acquired from Research lab chemicals. The details of High-Performance Thin Layer Chromatography (HPTLC) instrument are listed in table 1.

Table 1: Details of HPTLC instrument

SR.NO.	HPTLC SYSTEM	MODEL NUMBER	APPLICATION
1.	CAMAG Automatic TLC Sampler 4	250243	Application of samples
2.	CAMAG Twin-trough chamber	NA	Development of plate
3.	CAMAG TLC Visualizer 2	290203	Scanning at R white, 254, 366 nm
4.	TLC Scanner 4	170422	Scanning at 254, 366 nm

2.2 Standard solution preparation:

BER and TQ stock solutions were prepared by weighing exactly 50 mg of standard BER and 10 mg of standard TQ, which were dissolved in acetonitrile in a 10 ml volumetric flask to yield concentrations of 5000 µg/ml and 1000 µg/ml, respectively. These solutions were considered as a reference solution or stock solution. 0.1 ml of the BER stock solution was diluted to 10 ml. Similarly, 1 ml of TQ stock solution was diluted to 10 ml. As a working standard solution, the final concentration of the standard solution was 50 µg/ml BER and 100 µg/ml TQ.

2.3 Sample solution preparation:

1 gm of microemulgel (contains equivalent to 2.5 mg of BER and 1.25 mg of TQ respectively) was weighed exactly and transferred it to volumetric flask of capacity 10 ml, containing diluent (acetonitrile). It was then sonicated for 30 minutes with intermittent shaking after every 5 minutes. The supernatant was removed after centrifuging the sample for 10 minutes at 2000 rpm. 1 ml of supernatant was pipette out, which was further diluted

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(1), 760-769 | ISSN:2251-6727

with acetonitrile to provide a BER and TQ working solution for analysis.

2.4 Optimization of chromatographic conditions:

Merck, HPTLC silica gel 60 F_{254} plates were employed for HPTLC densitometric analysis. The prepared sample solutions were applied to TLC plate as 8 mm bands using a Camag microliter syringe and Camag Automatic TLC Sampler 4 (ATS 4) applicator with the rate of application of 150 nl/s. The plates were developed in a linear ascending manner up to a distance of 70 mm with mobile phase of Ethanol: Glacial acetic acid: Water (16:2:2 v/v/v) for BER and mobile phase of Toluene: Acetone: Formic acid (5:3:0.2 v/v/v) for TQ in a Camag glass twin-trough chambers which were saturated with mobile phase vapour for 20 minutes at room temperature. Following development, the plates were dried and scanned using vision CATS software at 366 nm and 254 nm, respectively, for BER and TQ. The slit size was 6 × 0.45 mm, and the scanning speed was 20 mm/s.

2.5 Method validation:

The validation of established analytical method was performed to ensure that it was appropriate for the purpose indicated in the International Conference on Harmonization (ICH) Q2 (R1) guidelines. Specificity, linearity, system suitability, precision, accuracy, limit of quantification, limit of detection and robustness have all been thoroughly validated for the method presented.[9]

2.5.1 System suitability:

Aanalysis of the system suitability was done to confirm the analytical system's accuracy. The %RSD and Rf value were calculated using six replicates of the standard solution.

2.5.2 Specificity:

Blank and placebo interference was checked at the main drug peaks. The method's specificity was determined by observing the spectra of mobile phase, solvent, standard drug and sample used. By comparing R_f and spectra of the band to that of standards, the bands for BER and TQ in the sample were confirmed. The interference was investigated in the analysis.

2.5.3 Linearity:

Linearity was assessed by establishing standard solutions at six different drug concentration levels. A calibration curve was assembled between concentration and peak area using the average peak area. Linearity was determined by applying 2-12 µl/band (2.0, 4.0, 6.0, 8.0, 10.0, 12.0) of standard solution of BER and 0.5- 3.5 µl/band (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5) of standard solution of TQ on the TLC plate from 50 µg/ml and 100 µg/ml working solution. Plates were developed, dried and scanned densitometrically at 366 nm and 254 nm respectively for BER and TQ. For each concentration level, the drug peak area and graph of sample concentration versus peak area was displayed.

2.5.4 Accuracy (% Recovery):

The accuracy of the developed method was proven by performing multiple level recovery tests by replicate analysis (n=3) in accordance with ICH recommendations at three different concentration levels (80%, 100%, 120%) as shown in table 2.

Table 2: Accuracy data

Concentration Level (%)	**BER**			**TQ**		
	Amount of standard added (µl/band)	Amount of sample added (µl/band)	Final concentration (ng/band)	Amount of standard added (µl/band)	Amount of sample added (µl/band)	Final concentration (ng/band)
80%	0.8	5.0	280	0.8	1.0	180
100%	1.0	5.0	300	1.0	1.0	200
120%	1.2	5.0	330	1.2	1.0	220

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 760-769 | ISSN:2251-6727

2.5.5 Precision:

Reproducibility (intraday) was evaluated by applying 1.0 µl/band of BER and TQ solution for six times on a TLC plate, then developing the plate and quantifying the peak area for six bands. This method was assessed by examining standard solution at 100 ng/band for BER and TQ for 3 times on the same day and interday precision was determined by analyzing similar set of standards for next 3 days.

2.5.6 Limit of detection (LOD) and limit of quantification (LOQ):

LOD and LOQ were calculated using the following calculation based on the slope of the calibration curve (s) and the standard deviation of the blank sample:

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

2.5.7 Robustness:

Analysis of the effect of moderate changes of parameters such mobile phase composition, wavelength, and saturation time was performed for the determination of robustness. At 200 ng/band, both BER and TQ were evaluated, and the impact of changes on peak areas and Rf value was examined.

2.5.8 Analysis of BER and TQ from developed microemulgel formulation:

The quantification of BER and TQ from an internally designed BER and TQ loaded microemulgel was done using the validated established method. In terms of ensuring full drug extraction, 1 gm of microemulgel was extracted with 10 ml of acetonitrile and centrifuge at 3000 rpm for 10 minutes. Sample's supernatant was appropriately diluted to achieve the desired concentration (25 ng/band for BER and 100 ng/band for TQ). This was applied on a TLC plate, and plate was developed as per the established protocol. Regression equation was utilized to quantify samples based on the peak area result that corresponded to BER and TQ. The outcome of the three-sets analysis was presented as the average BER and TQ in percent w/w.

3. Result and Discussion:

3.1 Mobile phase optimization:

An effective and precise densitometric HPTLC method for the estimation of BER and TQ was devised by the optimization of the mobile phase composition. For the separation, many solvent systems have been tested. Ethanol: Glacial acetic acid: Water (16:2:2 v/v/v) and Toluene: Acetone: Formic acid (5:3:0.2 v/v/v) were determined to be the optimal mobile phase systems for BER and TQ, with R_f values of 0.468 and 0.817 ±0.010, respectively, (figure 2 and 4). It was discovered that 20 minutes was enough time to completely fill the development chamber with the mobile phase vapour and achieve compound separation. The mobile phase was developed across a 70 mm distance using a 20 ml aliquot.

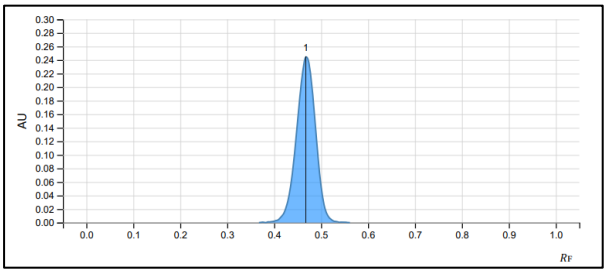


Figure 2: HPTLC chromatogram of BER, at 366 nm

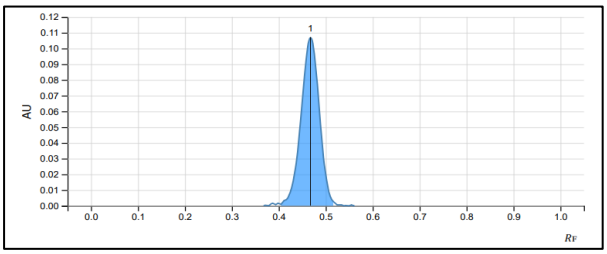


Figure 3: HPTLC chromatogram of formulation containing BER

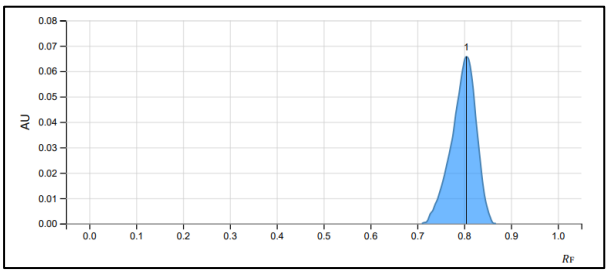


Figure 4: HPTLC chromatogram of TQ, at 254 nm

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(1), 760-769 | ISSN:2251-6727

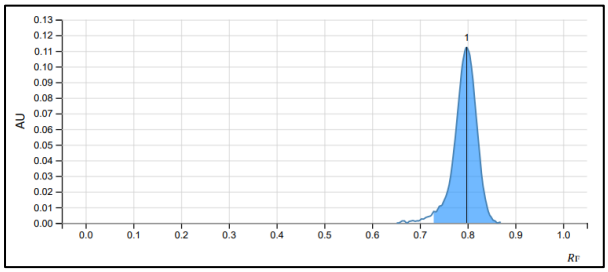


Figure 5: HPTLC chromatogram of formulation containing TQ

3.2 Method validation:

3.2.1 System suitability:

The peak area and R_f values for six bands of BER and TQ were recorded as shown in table 3. The relative standard deviation (%RSD) for the peak area was 1.416 and 1.130 respectively for BER and TQ, while for retention factor it was 0.826 and 0.689 (less than 2). This indicates the system suitability and adequate reproducibility of the equipment.

Table 3: System suitability of the method

BER				**TQ**			
Track no.	Concentration (ng/band)	Peak area	R_f	Track no.	Concentration (ng/band)	Peak area	R_f
1	100 ng/band	0.00327	0.439	1	100 ng/band	0.00342	0.905
2	100 ng/band	0.00320	0.435	2	100 ng/band	0.00347	0.898
3	100 ng/band	0.00315	0.439	3	100 ng/band	0.00340	0.897
4	100 ng/band	0.00322	0.431	4	100 ng/band	0.00348	0.892
5	100 ng/band	0.00327	0.440	5	100 ng/band	0.00350	0.890
6	100 ng/band	0.00321	0.440	6	100 ng/band	0.00343	0.885
Mean		0.00332	0.437	Mean		0.00345	0.894
Standard deviation		4.5607E-05	0.0036	Standard deviation		3.89872E-05	0.0061
%RSD		1.416	0.826	%RSD		1.130	0.689

3.2.2 Specificity:

With regard to the mobile phase, solvent and blank in relation to the standard and sample, there was no interference as shown in fig 6. The bands for BER and TQ in the sample were verified by comparing the R_f and spectra of the band to those of standards.

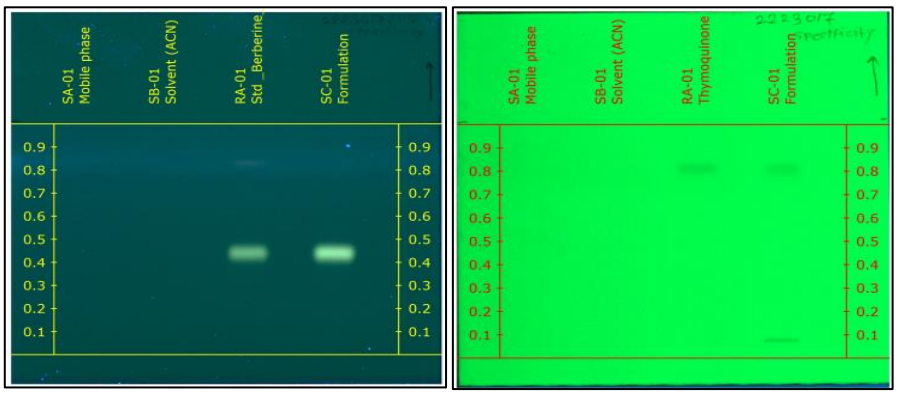


Figure 6: TCL plate images captured in TLC visualizer at R 366 nm (for BER) and at R 254 nm (for TQ) for specificity study

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(1), 760-769 | ISSN:2251-6727

3.2.3 Linearity:

For BER and TQ, the calibration was linear in the concentration ranges of and 50-350ng/band and 50-300 ng/band, respectively as shown in fig. 7 and 8. The plot's linear regression data provided confirmation of the strong linear relationship (fig 9 and 10). The correlation coefficient (R^2) was 0.9953 for TQ and 0.9991 for BER. For BER, the linear regression equation was Y= 0.0001x + 0.0011, while for TQ it was Y= 2E-05x + 0.0021, where Y is the response and x denote the amount of standard BER and TQ.

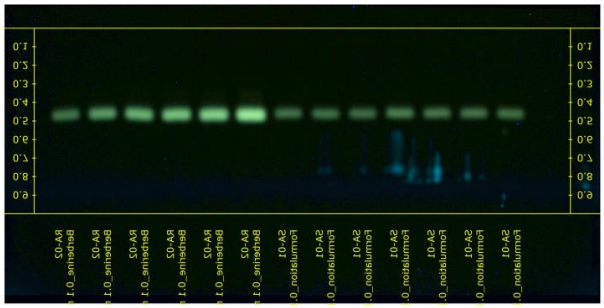


Figure 7: TLC plate image captured in TLC visualizer at R 366 nm (BER) for linearity study

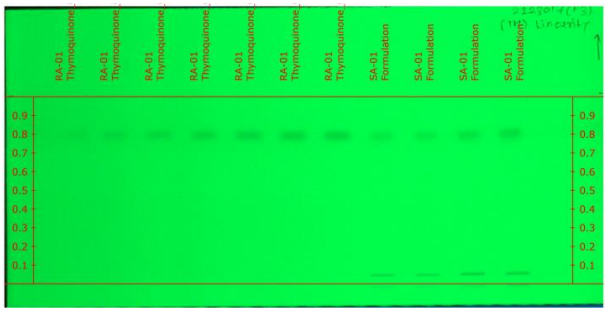


Figure 8: TLC plate image captured in TLC visualizer at R 254 nm (TQ) for linearity study

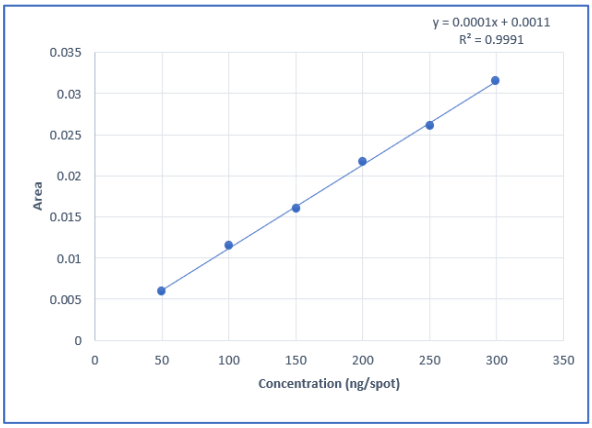


Figure 9: Graph showing linearity of BER

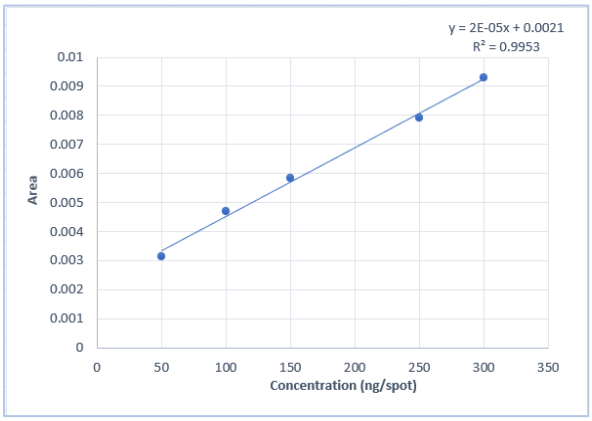


Figure 10: Graph showing linearity of TQ

3.2.4 Accuracy (% Recovery):

Recovery after spiking with 80, 100 and 120 % was used to measure accuracy of the procedure. The investigation was conducted using the conventional addition procedure in three sets as shown in table 4 and fig. 11 and 12. For BER and TQ, the mean percentages were found to be 99.72 % and 97.00% respectively.

Table 4: Accuracy data of BER and TQ

Drug	Concentration level (%)	Amount of standard added (µl/band)	Amount of sample added (µl/band)	Final concentration (ng/band)	Area	Amount recovered (ng/band)	Mean Amount recovered (ng/band)	Recovery (%)	Mean (%)
	80%	0.8	5.0	280	0.03002	278.4	278.26	99.70%	99.69%
					0.02998	277.6			
					0.03004	278.8			
					0.03103	298.6			

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(1), 760-769 | ISSN:2251-6727

BER	100%	1.0	5.0	300	0.03108	299.6	299.4	99.90%	
					0.03110	300			
	120%	1.2	5.0	330	0.03210	328	328	99.39%	
					0.03180	329			
					0.03200	326			
TQ	80%	0.8	1.0	180	0.00568	327	176.06	97.81%	97.00%
					0.00555	172.7			
					0.00563	176.5			
	100%	1.0	1.0	200	0.00596	193	191.83	95.91%	
					0.00594	192			
					0.00591	190.5			
	120%	1.2	1.0	220	0.00639	214.5	214	97.27%	
					0.00643	216.5			
					0.00632	211			

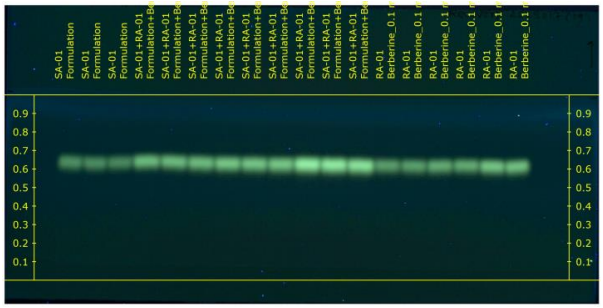


Figure 11: TLC plate image captured in TLC visualizer at R 366 nm (BER) for % recovery study

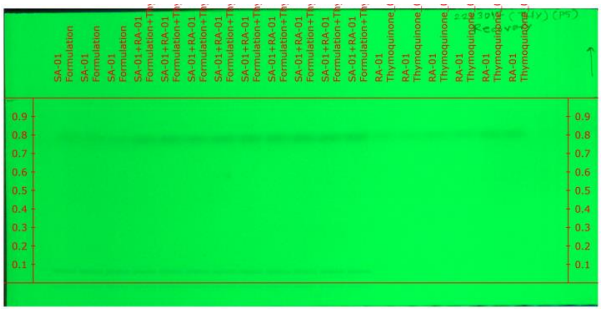


Figure 12: TLC plate image captured in TLC visualizer at R 254 nm (TQ) for % recovery study

3.2.5 Precision (reproducibility):

Intra-day precision (reproducibility) of BER and TQ were expressed as percent relative standard deviation (%RSD). The %RSD value was found to be less than 2% in all cases (Table 5) i.e., 1.653 and 1.137 respectively for BER and TQ, the low value of %RSD indicated the high precision of the method.

Table 5: Precision data (Intra-day) for BER and TQ

Berberine HCl				TQ			
Track no.	Area (Scan 1)	Area (Scan 2)	Area (Scan 3)	Track no.	Area (Scan 1)	Area (Scan 2)	Area (Scan 3)
1	0.00334	0.00320	0.00318	1	0.00426	0.00324	0.00298
2	0.00345	0.00319	0.00315	2	0.00427	0.00334	0.00295

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(1), 760-769 | ISSN:2251-6727

3	0.00341	0.00311	0.00308	3	0.00423	0.00339	0.00290
4	0.00340	0.00329	0.00318	4	0.00420	0.00331	0.00289
5	0.00343	0.00326	0.00324	5	0.00423	0.00335	0.00293
6	0.00335	0.00321	0.00321	6	0.00428	0.00329	0.00294
Average	0.00339	0.00321	0.00317	Average	0.00424	0.00332	0.00293
Standard deviation	4.36654E-05	6.22896E-05	5.50151E-05	Standard deviation	3.01662E-05	5.21536E-05	3.3116E-05
% RSD	1.285	1.940	1.733	%RSD	0.710	1.570	1.129
Mean %RSD	1.653			1.137			

3.2.6 Limit of detection (LOD) and Limit of quantification (LOQ):

LOD and LOQ were calculated as per ICH guidelines as shown in table 6.

Table 6: Result of LOD and LOQ

	Berberine HCl	**TQ**
LOD	10.76 ng/band	3.77 ng/band
LOQ	32.65 ng/band	11.43 ng/band

3.2.7 Robustness:

Table 7 and 8 shows SD and %RSD data calculated for the parameter change in wavelength at a concentration 200 ng/band (in triplicate). The study's findings demonstrated that the area and Rf value are not significantly affected by a slight, intentional modification to the chromatographic setting. The developed method's robustness was indicated by the low %RSD values (less than 2).

Table 7: Robustness of the developed HPTLC method of BER

Duration of saturation (20 ± 5)							
Concentration (ng/band)	Time (minutes)	Area (mean)	Standard deviation	%RSD	R_f (mean)	R_f (SD)	R_f %RSD
200 ng/band	15	0.00318	2.51661E-05	0.7905	0.464	0.0032	0.6922
	20	0.00348	3.51188E-05	1.0072	0.471	0.0025	0.5339
	25	0.00372	4E-05	1.0752	0.473	0.0026	0.5593
Wavelength (366 ± 2 nm)							
Concentration (ng/band)	Wavelength (nm)	Area (mean)	Standard deviation	%RSD	R_f (mean)	R_f (SD)	R_f %RSD
200 ng/band	364	0.00802	5.1316E-05	0.6395	0.476	0.0025	0.5301
	366	0.00766	4E-05	0.5221	0.471	0.0020	0.4416
	368	0.00780	2.3094E-05	0.2958	0.467	0.0037	0.8101

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(1), 760-769 | ISSN:2251-6727

Table 8: Robustness of the developed HPTLC method of TQ

Duration of saturation (20 ± 5)							
Concentration (ng/band)	Time (minutes)	Area (mean)	Standard deviation	%RSD	R_f	R_f (SD)	R_f %RSD
200 ng/band	15	0.00294	4.04145E-05	1.3730	0.809	0.0026	0.3270
	20	0.00335	4E-05	1.1940	0.805	0.0035	0.4358
	25	0.00582	2.88675E-05	0.4957	0.813	0.0045	0.5636
Wavelength (254 ± 2 nm)							
Concentration (ng/band)	Wavelength (nm)	Area (mean)	Standard deviation	%RSD	R_f	R_f (SD)	R_f %RSD
200 ng/band	252 nm	0.00740	1.52753E-05	0.2062	0.820	0.0015	0.1861
	254 nm	0.00777	1.52753E-05	0.1964	0.822	0.0036	0.4386
	256 nm	0.00830	1.52753E-05	0.1838	0.812	0.0036	0.4440

3.2.8 Estimation of BER and TQ in microemulgel formulation:

The extracted BER and TQ from the developed formulation, along with other ingredients, showed up on the chromatogram as a single band at R_f = 0.468 for BER (fig 3) and 0.817 for TQ (fig 5). For the microemulgel formulation of BER and TQ, the drug content was determined to be 98.8 % w/w and 96.80 % w/w respectively, with %RSD 0.3287 and 1.710 for three replicates of BER and TQ respectively. The low percent RSD result (table 9) demonstrated the method's appropriateness for routine analysis of BER and TQ in pharmaceutical dosage form.

Table 9: Estimation of BER and TQ in microemulgel formulation

Microemulgel formulation contains	**Label claim**	**Amount found (mean ± SD)**	**%RSD**
BER	2.5 mg/gm	2.47 ± 0.008	0.3287
TQ	1.25 mg/gm	1.21 ± 0.020	1.710

4. Conclusion

The developed HPTLC method for estimation of BER and TQ, which was validated as per the ICH guidelines can be employed for routine analysis of BER and TQ in bulk drug as well as formulations.

Acknowledgement:

Authors are thankful to Anchrom Enterprises for guidance and support for the project and for providing the necessary instrumentation and advanced techniques.

Conflict of interest:

No conflict of interest.

References

1. Velho-Pereira, R., Barhate, C., Kulkarni, S., Jagtap, A., (2011). Validated High-performance Thin-layer Chromatographic Method for the Quantification of Thymoquinone in Nigella sativa Extracts and Formulations, Phytochemical Analysis, 22: 367–373.
2. Taleuzzaman, M., Imam, S., Gilani, S., (2017). Quantitative Determination of thymoquinone in Nigella Sativa and its nano formulation using validated stability indicating HPTLC densiometric method, International Current Pharmaceutical Journal, 6(10): 53-60.
3. Doğru, M., Baysal, Z., Aytekin, C., (2006). Dyeing of wool fibres with natural dyes: effect of proteolytic enzymes. Preparative Biochemistry and Biotechnology, 36(3): 215–221.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 760-769 | ISSN:2251-6727

4. Raju, M., Kulkarni, Y.A., Wairkar, S., (2019). Therapeutic potential and recent delivery systems of berberine: A wonder molecule, Journal of Functional Foods, 61, 1-14.
5. Satija, S., Malik, S., Garg, M., (2016). Development of a New, Rapid, and Sensitive Validated High-Performance Thin-Layer Chromatographic Method for the Estimation of Berberine in Tinospora cordifolia, Journal of Planar Chromatography, 29(3): 209-215.
6. Darakhshana, S., Pour, A., Colagar, A., Sisakhtnezhad, S., (2015). Thymoquinone and its therapeutic potentials, Pharmacological Research 95–96: 138–158.
7. Salih, H., Aljabre, O., Alakloby, Randhawa M., (2015). Dermatological effects of Nigella sativa, Journal of Dermatology & Dermatologic Surgery, 1-12.
8. FataryEl-, (1975). Isolation and structure assignment of an antimicrobial principle from the volatile oil of Nigella sativa L. seeds, Pharmazie 30 (2): 100-111.
9. ICH, Validation of Analytical Procedures: text and methodology, Q2 R1, Geneva, 2005.