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A Green RP-HPTLC-Densitometry Method for the Determination of Diosmin in Pharmaceutical Formulations

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Abstract: Green analytical technologies for the determination of a bioactive compound diosmin (DIOM) in the real samples of pharmaceutical formulations and biological fluids are scarce in literature. Therefore, the present investigation was carried out to develop a novel, rapid, simple, and economical green "reversed phase high-performance thin-layer chromatography (RP-HPTLC)" method for the determination of DIOM in commercial tablets and in-house developed spray-dried microparticles (MPs). The quantification of DIOM was conducted via "RP-18 silica gel 60 F254S HPTLC plates". The binary combination of green solvents, i.e., ethanol:water (5.5:4.5 *v/v*) was proposed as a green mobile phase. The analysis of DIOM was conducted in absorbance/reflectance mode of densitometry at λ_{max} = 348 nm. The densitograms of DIOM from the commercial tablets and in-house developed spray-dried MPs were verified by recording their single band at R_f = 0.80 ± 0.02 compared to standard DIOM. Green RP-HPTLC method was observed as linear in the range of 100–700 ng/band with R^2 = 0.9995. The proposed method was found as "accurate, precise, robust, and sensitive" for the determination of DIOM in the real samples of commercial tablets and in-house developed spray-dried MPs. The % content of DIOM in the real samples of commercial tablets and in-house developed spray-dried MPs was obtained as 99.06 and 101.30%, respectively. The recorded results of this research suggested that the green RP-HPTLC method can be effectively used for the routine analysis of DIOM in pharmaceutical products.

Keywords: bioactive compound; diosmin; green RP-HPTLC; microparticles

1. Introduction

Flavonoids are reported as an important class of bioactive compounds which are obtained from various medicinal plants [1]. Such bioactive compounds have been identified as secondary metabolites which have phenolic compounds [2]. These compounds have been reported to found in the foods of plant origin such as vegetables, beverages, and citrus fruits [2,3]. In literature, bioflavonoids have been reported to have various therapeutic activities due to the presence of phenolic compounds as the biomarkers [2]. Diosmin (DIOM) is a bioflavonoid (chemical name: 5,7,3-trihydroxy-4′-methoxyflavone 7-rutinoside) which was first isolated from *Scrophularia nodosa* [4]. It can now be isolated from several medicinal plants. It was found as a prodrug which metabolizes to an active

metabolite diosmetin after enzymatic hydrolysis into the body [5]. DIOM had several therapeutic activities which includes antioxidant [6], anti-inflammatory [7], antimutagenic [6], antidiabetic [8,9], and anticancer [10–12]. It has also been found clinically effective in improving various vein disorders [13–15]. Various ultra-violet (UV) spectrometry methods have been reported for the determination of DIOM in pharmaceutical preparations either alone or in combination with other bioflavonoids [16–18]. Several "high-performance liquid chromatography (HPLC)" methods coupled with UV detector have also been reported for the determination of DIOM in pharmaceutical preparations either alone or in combination with other bioflavonoids [1,19–22]. A HPLC method using ionic liquids as the mobile phase modifiers had also been reported for the quantification of DIOM in pharmaceuticals [23]. The related substances and impurities of DIOM were also identified using HPLC and liquid chromatography mass-spectrometry (LC-MS) methods [4]. A HPLC method has also been reported for the determination of active metabolite of DIOM, i.e., diosmetin in human plasma [5]. The utility of silver nanoparticles was also tried in the analysis of DIOM in plant extracts and pharmaceutical dosage forms [24]. Some other techniques such as colorimetry [25], fluorimetry [26], Fourier transforms infra-red spectrometry [27], and voltammetry [28] methods were also found suitable for the determination of DIOM in plant extracts and pharmaceutical dosage forms. A single "high-performance thin layer chromatography (HPTLC)" method has also been reported for the determination of DIOM in different citrus fruit extracts and pharmaceutical dosage forms [29]. Although, different techniques have been reported for the determination of DIOM in plant extracts and pharmaceutical dosage forms, but none of them are green analytical technique. In addition, reported HPTLC method had also utilized most of the toxic solvents in the mobile phase [29]. In the recent years, the analytical techniques associated with "green analytical chemistry or environmentally-benign analytical techniques" have been increased significantly in literature [30–32].

Green HPTLC method has not been reported for the analysis of DIOM in pharmaceutical dosage forms. In addition, reversed phase HPTLC (RP-HPTLC) techniques have several merits compared with conventional HPTLC techniques which include "avoidance of the non-polar fractions from the sample, avoidance of the interference of the impurities, formation of compact spot, detection clarity, and non-toxic to the environment" [33–35]. Therefore, this study was carried out to develop and validate a green, simple, rapid, and sensitive RP-HPTLC technique for the determination of DIOM. The green RP-HPTLC methodology was validated in terms of linearity, accuracy, precision, robustness, sensitivity, and specificity as per "International Conference on Harmonization (ICH)" Q2 (R1) recommendations [36]. The utility of the proposed RP-HPTLC method was also verified in the analysis of DIOM in the real samples of commercial tablets and in-house developed spray-dried microparticles (MPs).

2. Materials and Methods

2.1. Materials

DIOM (purity 99%) and sodium hydroxide (NaOH) were procured from "Sigma Aldrich (St. Louis, MO, USA)". Soluplus® was obtained from BASF (Ludwigshafen, Germany). Chromatography grades methanol, ethanol and dimethyl sulfoxide (DMSO) were acquired from "E-Merck (Darmstadt, Germany)". Chromatography grade water was obtained from "Milli-Q unit". Commercial tablets of DIOM (containing 500 mg of DIOM) were purchased from pharmacy shop in "Riyadh, Saudi Arabia". Other chemicals and reagents were of analytical grade.

2.2. Instrumentation and Analytical Procedures

RP-HPTLC-densitometry analysis of DIOM in standard and the real samples of commercial tablets and spray-dried MPs was performed using "HPTLC CAMAG TLC system (Muttenz, Switzerland)". The quantification was performed on "10 × 20 cm glass backed plates pre-coated with RP silica gel 60 F254S plates (E-Merck, Darmstadt, Germany)". The samples to RP silica gel plates were applied

as a 6 mm bands using a "CAMAG Automatic TLC Sampler 4 (ATS4) Sample Applicator (Geneva, Switzerland)". The sample applicator was fitted with "CAMAG microliter Syringe (Hamilton, Bonaduz, Switzerland)". The application rate for the analysis of DIOM was kept constant at 150 nL/s. Linear ascending development of the plates at a distance of 80 mm was carried out using a mixture of ethanol:water (5.5:4.5 *v/v*) as the green mobile phase in a "CAMAG automatic developing chamber 2 (ADC2)." The developing camber was saturated previously with the green mobile phase for 30 min at 22 °C. The densitometry scanning was performed in absorbance/reflectance mode at λ_{max} = 348 nm. The slit dimensions and scanning rate were 4 × 0.45 mm and 20 mm/s, respectively. Each analysis was performed at least for three times and baseline was monitored. The software used for data processing was "WinCAT's (version 1.4.3.6336)".

2.3. Calibration Curve of DIOM

The stock solution (SS) of DIOM was prepared by dissolving an accurately weighed 10 mg of DIOM in 3 mL of DMSO and volume was made 10 mL using methanol. About 1 mL of SS of DIOM was diluted further with mobile phase in order to obtain the final SS of 100 µg/mL. Serial dilutions of SS of DIOM were made by taking different volumes of DIOM SS and diluting with mobile phase in order to obtain the concentrations in the range of 100–700 ng/band of DIOM. About 200 µL of each concentration of DIOM was applied on TLC plates and the peak area was recorded. The calibration curve (CC) of DIOM was obtained by plotting the concentrations against measured area of DIOM.

2.4. Sample Preparation for the Determination of DIOM in Commercial Tablets

Five commercial tablets (each tablet containing 500 mg of DIOM) were weighed and an average weight was calculated. The DIOM tablets were then crushed and powdered finely using glass pestle and mortar. A weight of powder containing 500 mg of DIOM was dissolved in 20 mL of DMSO and volume was made 100 mL by diluting with methanol. This solution was filtered to eliminate any insoluble excipient of tablet and sonicated for about 30 min. About 1.0 mL of this solution was taken and diluted using mobile phase to get 50 mL solution. The diluted sample was subjected for the determination of DIOM in commercial tablets by applying the proposed RP-HPTLC method.

2.5. Preparation and Characterization of DIOM-Loaded Spray-Dried MPs

DIOM-loaded in-house developed spray-dried MPs were obtained using a spray-drying method [37]. The binary mixture of DIOM and a carrier (Soluplus®) was prepared by dissolving 1:2 (*w/w*) mass ratio of DIOM: Soluplus® in a binary mixture of ethanol: 0.1 M NaOH (80:20 *v/v*). The prepared solution was then sonicated using ultrasonicator bath for about 30 min. The solution was then spray-dried using a "Spray Dryer (Büchi Mini Spray Dryer, B-290, Flawil, Switzerland)" apparatus using the inlet temperature of 70 °C, outlet temperature of 70 °C, solution feed rate of 20% and aspiration rate of 100%. The spray-dried MPs were collected in an airtight container. DIOM-loaded spray-dried MPs were characterized for particle size, polydispersity index (PDI), zeta potential (ZP), drug content and % yield.

2.6. Sample Preparation for Determination of DIOM in Spray-Dried MPs

Approximately 750 mg of spray-dried MPs (containing 250 mg of DIOM) were dissolved in 20 mL of DMSO and volume was made 100 mL using methanol. This solution of DIOM was filtered and sonicated for about 30 min. About 1.0 mL of this solution was taken and diluted further with mobile phase in order to obtain 50 mL solution. The diluted solution was applied for the determination of DIOM in spray-dried MPs using the proposed RP-HPTLC technique.

2.7. Method Validation

Green RP-HPTLC-densitometry method for DIOM analysis was validated for "linearity, precision, accuracy, robustness, sensitivity, and specificity" using ICH Q2 (R1) guidelines [36]. The linearity of DIOM was determined by plotting the concentration of DIOM against its HPTLC response. It was evaluated in the range of 100–700 ng/band. The method accuracy was measured as the percent of recovery (% recovery) using "standard addition method". The previously analyzed concentration of DIOM (200 ng/band) was spiked with extra 0, 50, 100, and 150% amounts of DIOM. The resultant concentration of 200, 300, 400, and 500 ng/band (n = 6) were re-analyzed using the proposed green RP-HPTLC method. The % recovery was obtained at each concentration of DIOM.

Method precision was found out as "repeatability and intermediate precision". Repeatability (intra-day precision) was obtained by the analysis of samples on the same day at 200, 300, 400, and 500 ng/band concentrations (n = 6). However, intermediate (inter-day precision) was obtained by the analysis of samples on three consecutive days at 200, 300, 400, and 500 ng/band concentrations (n = 6).

Method robustness was obtained by making some minor modifications in the composition of the mobile phase during DIOM analysis. The original mobile phase composition of ethanol:water (5.5:4.5) was modified into ethanol:water (5.7:4.3) and ethanol:water (5.3:4.7) for the positive and negative level, respectively.

Method sensitivity was obtained as the "limit of detection (LOD) and limit of quantification (LOQ)" using "standard deviation (SD)" technique. The "LOD and LOQ" values for DIOM were calculated using the following equations:

$$\text{LOD} = 3.3 \times \frac{\text{SD}}{\text{S}} \quad (1)$$

$$\text{LOQ} = 10 \times \frac{\text{SD}}{\text{S}} \quad (2)$$

in which, S = slope of the CC of DIOM.

Method specificity was obtained by comparing the retardation factor (R_f) values and UV-absorption curve of DIOM in the commercial tablets and in-house developed spray-dried MPs with that of standard DIOM.

2.8. Application of a Green RP-HPTLC Method in Pharmaceutical Assay of DIOM in Commercial Tablets and Spray-Dried MPs

The obtained samples of commercial tablets and in-house developed spray-dried MPs were applied on TLC plates and their densitograms were recorded under the same experimental procedures as described for the determination of standard DIOM. The HPTLC area of DIOM in commercial tablets and in-house developed spray-dried MPs was recorded. The amount and % assay of DIOM in both formulations was estimated using the CC of DIOM.

2.9. Statistical Evaluation

The experimental values are presented as mean ± standard deviation (SD) of three or six replicates. The statistical evaluation was performed by applying Dunnett's test using "GraphPad Prism software (version 6, GraphPad, San Diego, CA, USA)". The statistical parameters were evaluated at 5% level of significance.

3. Results and Discussion

3.1. Preparation and Characterization of DIOM-Loaded Spray-Dried MPs

DIOM-loaded spray-dried MPs were prepared using a spray drying technique [37] and characterized physicochemically. The formulation composition of spray-dried MPs and their physicochemical characterization data are listed in Table 1.

Table 1. The composition and characterization of in-house developed spray-dried microparticles (MPs) of diosmin (DIOM) (mean ± SD; n = 3).

Formulation Composition		Characterization Parameter	
DIOM (mg)	250	Particle size ± SD (µm)	2.80 ± 0.26
Soluplus (mg)	500	Polydispersity index	0.25
Total (mg)	750	Zeta potential ± SD (mV)	−12.50 ± 3.50
DIOM: Soluplus ratio (*w/w*)	1:2	Drug content ± SD (%)	82.00 ± 7.80
		Yield ± SD (%)	73.00 ± 6.60

The particle size and PDI of spray-dried MPs were recorded as 2.80 ± 0.26 µm and 0.25, respectively. The ZP of MPs was determined as −12.50 ± 3.50 mV. The drug content and yield of spray-dried MPs were found as 82.00 ± 7.80 and 73.00 ± 6.60%, respectively. The particle size and PDIs of different bosentan hydrate-loaded spray-dried MPs have been reported as 2.20–2.70 µm and 0.60–0.80, respectively [38]. The ZP of different bosentan hydrate-loaded spray-dried MPs was found as −16.70 to −27.2 mV in literature [38]. The recorded values of particle size and ZP for in-house developed spray-dried MPs were in accordance with those reported for bosentan hydrate-loaded spray-dried MPs in literature. However, the PDI of in-house developed MPs (0.25) was much lower than reported PDI for bosentan hydrate-loaded spray-dried MPs [38]. This observation suggested that the size uniformity of in-house developed spray-dried MPs was much better than those reported for bosentan hydrate-loaded spray-dried MPs [38]. In addition, the drug content and % yield value recorded in this work were also found in acceptable limits. Overall, these results suggested that the spray-dried MPs of DIOM were well prepared in the laboratory.

3.2. Method Development

Not a single green RP-HPTLC method was found in literature for the determination of DIOM in the real samples of pharmaceutical dosage forms. Therefore, the proposed research was carried out to develop a suitable green RP-HPTLC method for the determination of DIOM in the real samples of commercial tablets and in-house developed spray-dried MPs. For this, the green mobile phase was prepared by the simple mixture of ethanol and water (green solvents) compared to conventional HPTLC technique. The application of RP analytical methods presents many advantages over conventional/normal phase techniques. The main advantages are "avoidance of the non-polar fractions from the sample, avoidance of the interference of the impurities, formation of compact spot and detection clarity" [35,39]. Green RP-HPTLC method for the determination of DIOM could also reduce the chances of toxicity to the environment [33,34].

In the present study, various compositions of ethanol and water such as 6:4 (%, *v/v*), 7:3 (%, *v/v*), 8:2 (%, *v/v*) and 6.5:3.5 (%, *v/v*) were studied as green mobile phase compositions in order to develop a suitable band for RP-HPTLC-densitometry analysis of DIOM. The green mobile phase was developed under chamber saturation condition and resulting spots of DIOM are presented in Figure 1. The HPTLC spots of DIOM were found clear, well spotted and in accordance with those reported for other drugs such as rivaroxaban, valerenic acid and delafloxacin using a green RP-HPTLC-densitometry method [31,32,35]. These results suggested that HPTLC spots of DIOM were developed well under the chamber saturation condition using a green mobile phase.

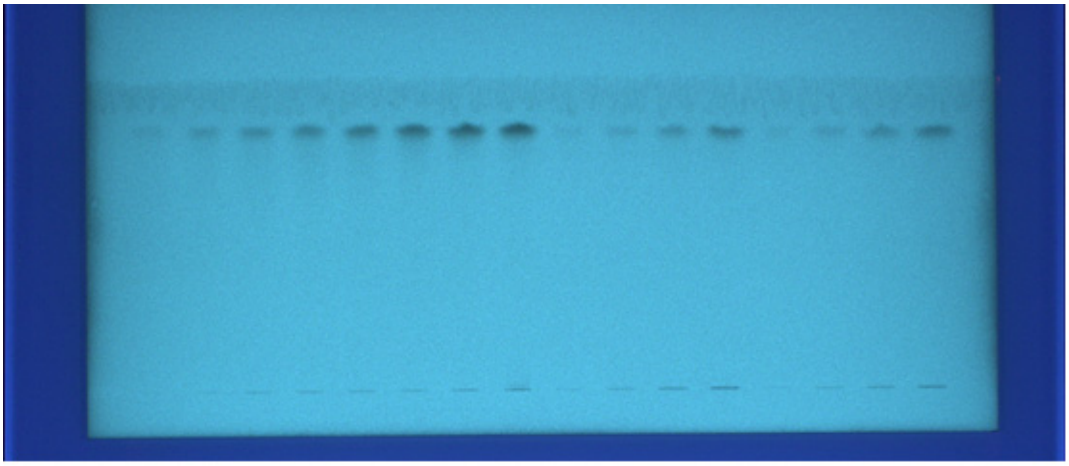

Figure 1. Pictorial diagram for the developed high-performance thin layer chromatography (HPTLC) plate of diosmin (DIOM).

The binary composition of ethanol and water such as 6:4 (%, *v*/*v*), 7:3 (%, *v*/*v*) and 8:2 (%, *v*/*v*) were found to give poor densitometry peak with poor symmetry. However, the binary mixture of ethanol and water such as 6.5:3.5 (%, *v*/*v*) gave a well-resolved, symmetrical, and compact densitometry peak of DIOM at R_f = 0.80 ± 0.02 (Figure 2).

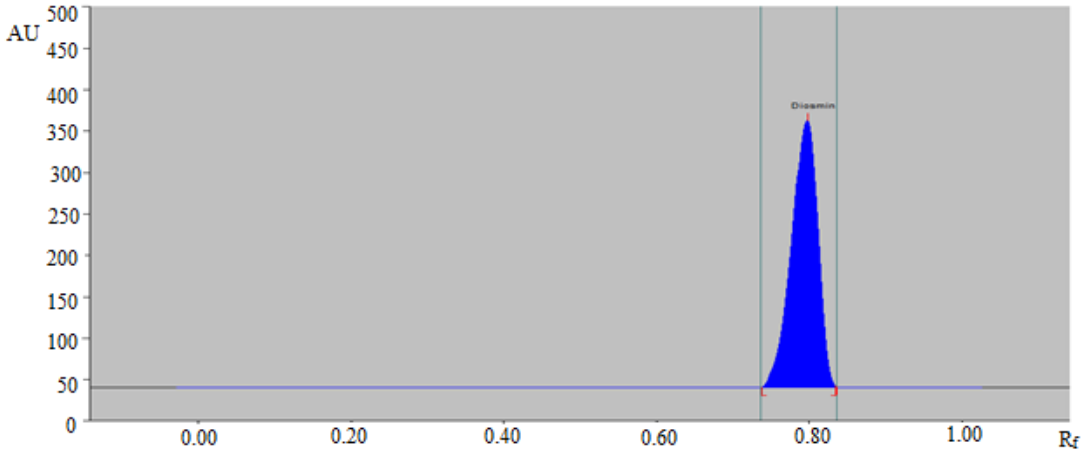

Figure 2. HPTLC-densitogram of standard DIOM.

Based on these observations, the binary mixture of ethanol:water 6.5:3.5 (%, *v*/*v*) was optimized as the green mobile phase for the determination of DIOM in the real samples of commercial tablets and spray-dried MPs. The bands spectra were recorded in densitometry mode and maximum HPTLC response under reflectance/absorbance mode was observed at λ_{max} = 348 nm. Hence, all analysis of DIOM were carried out at 348 nm.

3.3. Method Validation

Various validation parameters for DIOM analysis were determined according to ICH guidelines. The results for linear regression analysis of CC of DIOM are listed in Table 2.

Table 2. Linear regression data for the calibration curve of DIOM (mean ± SD; n = 6).

Linearity Range (ng/Band)	100–700
Regression equation	Y = 21.43x − 274.88
Determination coefficient	0.9995
Slope ± SD	21.43 ± 1.14
Intercept ± SD	274.88 ± 7.53
Standard error of slope	0.46
Standard error of intercept	3.07
95% confidence interval of slope	19.42–23.43
95% confidence interval of intercept	261.65–288.10
LOD ± SD (ng/band)	33.72 ± 2.28
LOQ ± SD (ng/band)	101.16 ± 6.84

The CC of DIOM was recorded as linear in the range of 100–700 ng/band. The data suggested good linear relationship between the concentration and response of DIOM. The determination coefficient (R^2) for DIOM was regressed as 0.9995 which was significant ($p < 0.05$). The regression equation for linearity was obtained as Y = 21.43x − 274.88, in which Y is the measured HPTLC response and x is DIOM concentration. The slope and intercept values for CC of DIOM were computed as 21.43 ± 1.14 and 274.88 ± 7.53, respectively. The 95% confidence intervals of slope and intercept were computed as 19.42–23.43 and 261.65–288.10, respectively. The linearity range of DIOM was recorded as 100–3000 ng/band in literature [29]. The recorded linearity range of DIOM (100–700 ng/band) in the present work was much narrow compared with reported one. However, the lower concentrations were found in accordance with literature.

Method accuracy was obtained as % recovery and results are tabulated in Table 3.

Table 3. Accuracy data of HPTLC method (mean ± SD; n = 6).

Excess Drug Added to Analyte (%)	Theoretical Content (ng)	Conc. Found (ng) ± SD	Recovery (%)	RSD (%)
0	200	196.21 ± 2.98	98.10	1.51
50	300	294.76 ± 3.81	98.25	1.29
100	400	401.56 ± 5.10	100.39	1.27
150	500	505.76 ± 6.08	101.15	1.20

The % recovery of DIOM was recorded as 98.10–101.15% using the proposed method. The % RSD in the accuracy of DIOM was observed as 1.20–1.51%. Using a reported HPTLC method, the % recovery of DIOM was obtained as 96.64–103.21% [29]. The recorded recoveries of the proposed green RP-HPTLC method (98.10–101.15%) were better than reported HPTLC method. In addition, the recorded % recovery within the range of 100 ± 2% suggested that the proposed RP-HPTLC method was accurate for the determination of DIOM.

Method precision was obtained as % RSD and results are tabulated in Table 4.

Table 4. Precision data of HPTLC method (mean ± SD; n=6).

Conc. (ng/Band)	Repeatability (Intraday Precision)			Intermediate Precision (Interday)		
	Area ± SD (n = 6)	Standard Error	% RSD	Area ± SD (n = 6)	Standard Error	% RSD
200	3912.41 ± 77.26	31.54	1.97	3831.29 ± 75.87	30.97	1.98
300	6321.52 ± 97.98	40.00	1.54	6165.84 ± 99.42	40.58	1.61
400	8288.41 ± 124.12	50.67	1.49	8423.62 ± 131.81	53.81	1.56
500	10,256.13 ± 167.28	68.29	1.63	10,412.39 ± 171.29	69.92	1.64

The % RSD values for the repeatability/intraday precision were recorded as 1.49–1.97%. However, the % RSD values for intermediate/interday precision were recorded as 1.56–1.98%. Using a reported

HPTLC method, the % RSD values were obtained as 1.43–2.84 and 0.97–2.80% for intraday and interday precision, respectively [29]. The recorded % RSD values of the proposed green RP-HPTLC method were better than reported HPTLC method. In addition, the recorded values of % RSD within the limit of ± 2% showed that the green RP-HPTLC method was precise for the determination of DIOM.

Results of robustness are tabulated in Table 5. The errors in terms of % RSD after making minor changes in the composition of mobile phase were recorded as 1.43–1.57%. The R_f value for DIOM was obtained in the range of 0.79–0.81. The minor variations in R_f values and lower % RSD values indicated that green RP-HPTLC method was robust for the determination of DIOM.

Table 5. Robustness of HPTLC method (mean ± SD; n = 6).

Conc. (ng/Band)	Mobile Phase Composition (Ethanol:Water)			Results		
	Original	Used		Area ± SD (n = 6)	% RSD	R_f
300	5.5:4.5	5.7:4.3	+0.2	6442.31 ± 101.26	1.57	0.79
		5.5:4.5	0.0	6312.74 ± 94.25	1.49	0.80
		5.3:4.7	−0.2	6251.87 ± 89.64	1.43	0.81

The method sensitivity was recorded as "LOD and LOQ" and results are tabulated in Table 2. The "LOD and LOQ" of green RP-HPTLC method were recorded as 33.72 ± 2.28 and 101.16 ± 6.84 ng/band, respectively for DIOM. The recorded values of "LOD and LOQ" showed that green RP-HPTLC method was sensitive enough for the determination of DIOM.

Method specificity and the peak purity of DIOM were obtained by comparing the overlaid UV-absorption spectra of DIOM in commercial tablets and spray-dried MPs with that of standard. The overlaid spectra of standard DIOM and DIOM in commercial tablets and spray-dried MPs are presented in Figure 3.

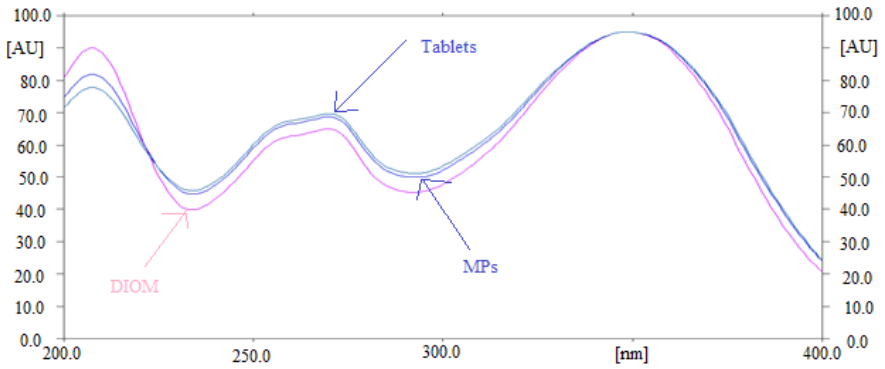

Figure 3. Overlaid ultra-violet (UV) absorption spectra of standard DIOM, commercial tablets and in-house developed spray-dried microparticles (MPs).

The maximum densitometry response of DIOM in standard, commercial tablets and spray-dried MPs were obtained at λ_{max} = 348 nm under reflectance/absorbance mode. The similar overlaid UV-absorption spectra, R_f values and λ_{max} of DIOM in standard, commercial tablets and spray-dried MPs showed the method specificity and its peak purity.

3.4. Application of Green RP-HPTLC Method in Pharmaceutical Assay of DIOM in Commercial Tablets and Spray-Dried MPs

Not a single green HPTLC method has been reported for the determination of DIOM in the real samples of pharmaceutical formulations. Hence, a green RP-HPTLC method could be an alternative approach for the determination of DIOM in the real samples of pharmaceutical formulations. The proposed RP-HPTLC method was applied for the analysis of DIOM in the real samples of

commercial tablets and in-house developed spray-dried MPs. There was no connection of commercial tablets with in-house developed spray-dried MPs. However, in order to enhance the application of the proposed analytical methodology, the DIOM was analyzed in the real samples of both commercial tablets and in-house developed spray-dried MPs. The densitometry peaks of DIOM from commercial tablets and spray-dried MPs were verified by comparing their single TLC spot at $R_f = 0.80 \pm 0.02$ with that of standard DIOM. The representative densitogram of DIOM in commercial tablets is shown in Figure 4 which was similar to that of standard DIOM.

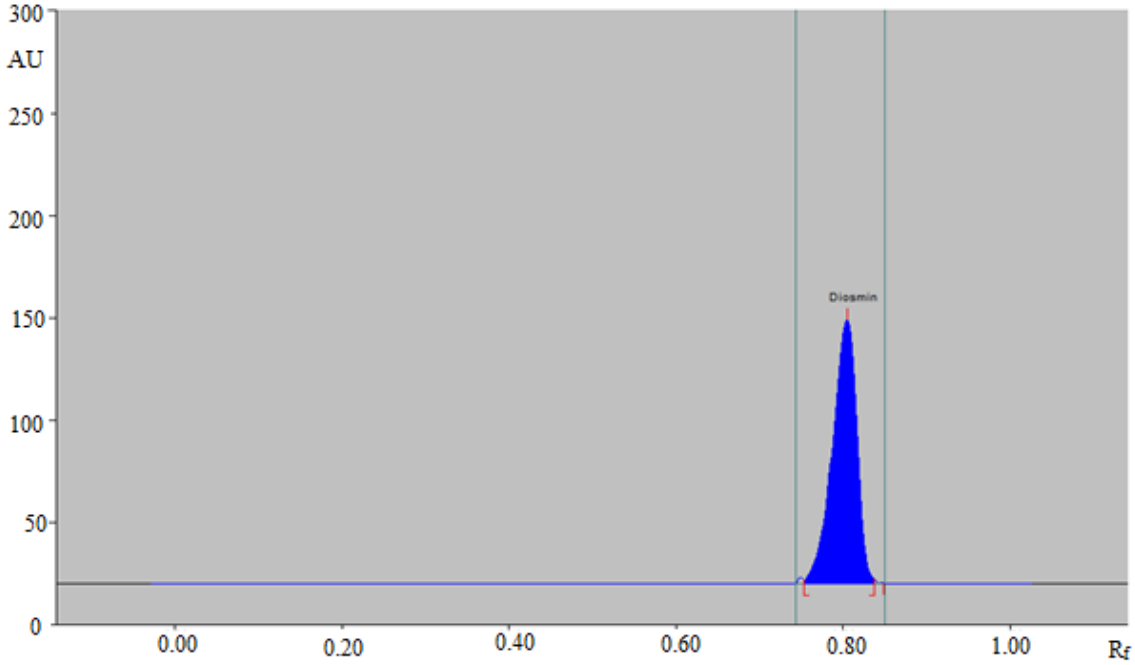

Figure 4. HPTLC densitogram of DIOM in commercial tablets.

The representative densitogram of DIOM in spray-dried MPs is shown in Figure 5 which was also similar to that of standard DIOM.

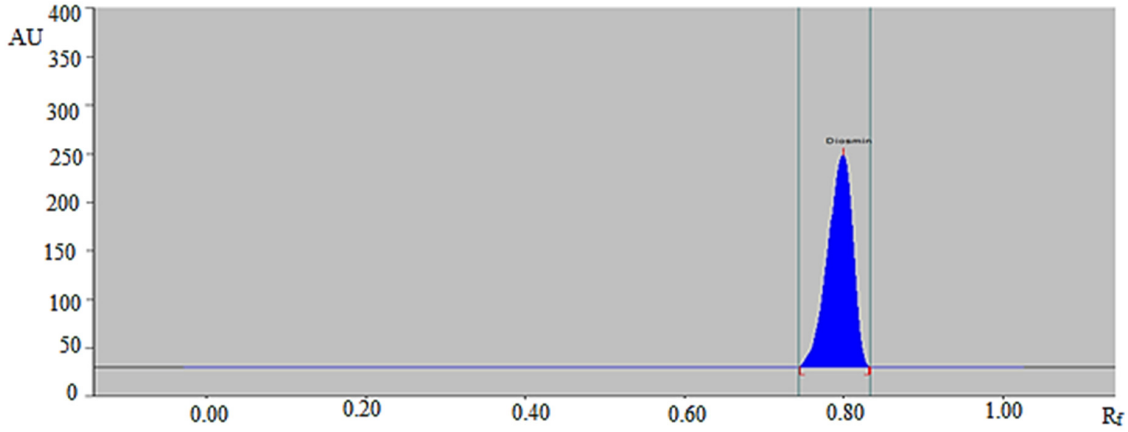

Figure 5. HPTLC densitogram of DIOM in in-house developed spray-dried MPs.

The amount of DIOM in commercial tablets and spray-dried MPs was obtained by the CC of DIOM. The results of pharmaceutical assay of DIOM in commercial tablets and spray-dried MPs are tabulated in Table 6.

Table 6. Pharmaceutical assay of DIOM in commercial tablets and in-house developed MPs formulations (mean ± SD; n = 3).

Samples	Theoretical Content (mg)	Content Found (ng) ± SD	Assay (%)
Commercial tablets	500	495.31 ± 2.89	99.06
MPs	250	253.26 ± 2.58	101.30

The amount of DIOM in commercial tablets was recorded as 495.31 ± 2.89 mg out of 500 mg of claimed DIOM. However, the amount of DIOM in spray-dried MPs was estimated as 253.26 ± 2.58 mg out of 250 mg of claimed DIOM. The % assay of DIOM in commercial tablets and spray-dried MPs was recorded as 99.06 and 101.30%, respectively. The pharmaceutical assay of DIOM within the range of ±2% showed that green RP-HPTLC method can be successfully utilized in the pharmaceutical assay of DIOM in pharmaceutical formulations containing DIOM as an active constituent.

4. Conclusions

A green RP-HPTLC method is developed and validated for the determination of DIOM in the real samples of commercial tablets and spray-dried MPs. DIOM-loaded spray-dried MPs were prepared using a spray drying method and characterized well for particle size, PDI, ZP, drug content and yield. The proposed method is "simple, accurate, precise, robust, sensitive, and specific" for the determination of DIOM. The method is successfully applied in pharmaceutical assay of DIOM in the real samples of commercial tablets and spray-dried MPs. The proposed technology can be utilized in the pharmaceutical assay of DIOM in the real samples of pharmaceutical formulations containing DIOM as an active constituent.

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Conflicts of Interest: The authors declare that they have no conflict of interests associated with this manuscript.

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