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Article

Micellar Thin Layer Chromatography and Computer-Aided Analysis of Empagliflozin, Linagliptin and Metformin HCl Ternary Mixture

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Abstract

The present work was performed in order to study the mechanism of micellar thin layer chromatography (MTLC) and to develop a new simple and sensitive simultaneous MTLC method for separation of empagliflozin, Linagliptin and metformin hydrochloride ternary mixture. The study was done using three different surfactants; sodium dodecyl sulphate (SDS), benzalkonium chloride (BAC) and polysorbate 80 (tween 80). Chromatographic procedure was performed using micellar mobile phase that composed of aqueous solution of each surfactant and methanol (6: 4 v/v) and micellar TLC determination at λ_{max} 237 nm. Separation using SDS (anionic surfactant) and BAC (cationic surfactant) depends on ionization potential (AMI-IP), partition coefficient (logP (o/w)) and hydrogen bond donor atoms (a-don), whereas separation using tween 80 depends mainly on the lipophilicity (R_{M0}), solvation energy (E-sol) and Van der Waals energy (E-vdw). Quantitative structure–retention relationships study was carried out, modeled, evaluated and validated using molecular operating environment software.

Introduction

Diabetes is a fast-growing global problem with huge social, health and economic consequences (1). Therefore, combination of empagliflozin (EMPA), linagliptin (LING) and metformin (MET) in tablet formulation could provide a valuable treatment option mainly for patients with Type 2 diabetes (T2D) who are inadequately controlled with empagliflozin either alone or in combination with metformin HCl and improving glycemic control with a low-risk of hypoglycemia (2). The chemical structures of the investigated drugs were shown in Figure 1. Micellar liquid chromatography (MLC) was defined as a reversed-phase liquid chromatography (RPLC) mode with an aqueous solution of surfactant above its critical micellar concentration (CMC) as a mobile phase that was reported by Kawczak and Bączek (3). In this system, the mechanism of analyte retention depends on three different equilibria: distribution of the analyte between micelles and a bulk mobile phase in between, partition of solute molecules between a stationary phase and a bulk mobile phase and direct transfer of solute between micelles and surfactant-modified surface (4). In literature review, there were some examples of application of micellar TLC in order to predict lipophilicity; correlation between chromatographic constants and lipophilicity of triazole derivatives and N5 phenyltrichloro acetamide derivatives reported by Janicka *et al.* (5). The multivariate analysis and principal component analysis (PCA) revealed that the typical RP conditions outperform the parameters obtained by micellar chromatography (6). Micellar thin layer chromatography (MTLC) has been also used for separation and quantification of 16 metal cations (7), five water-soluble vitamins (8) and gamma aminobutyric acid (GABA) in pharmaceutical formulation (9). The utility of MTLC as potential tool for assessment of lipophilicity is up-to-date not clear. For this reason, we decided to measure the practical chromatographic lipophilicity, other two and three-dimension descriptors or parameters to investigate the mechanism of MTLC. On the other hand, this study was to provide information about the effect of surfactants of different chemical properties, modifiers effects of mobile phase in MTLC and to illustrate the molecular mechanism of retention for the three drugs; EMPA, LING and MET based on quantitative structure–retention relationships (QSRR) approach using three different chromatographic systems. In order to fulfill those purposes, different mathematical calculations were performed as one parameter liner regression methods (OPLR), multiple linear regression (MLR), multidimensional analysis (MDA) and other statistical functions were carried out. The last and important objective was to develop a new MTLC method for simultaneous separation of the three investigated drugs.

Experimental

Instrumentation and reagents

A CAMAG TLC scanner III (Muttenz, Switzerland) provided with linomat 5 sample automatic applicator (Muttenz,

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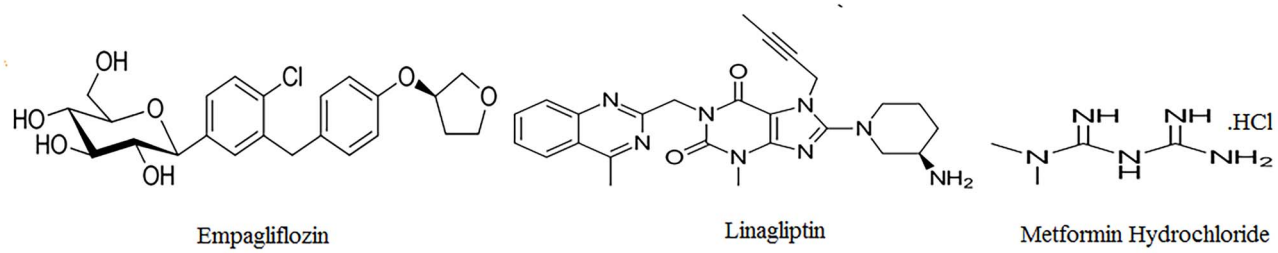

Figure 1. Chemical structures of the investigated drugs.

Switzerland) and CAMAG 100 μL sample syringe (Hamilton, Bonaduz, Switzerland) were used. EMPA, LING and MET were obtained as a gift from the National Organization for Drug Control and Research, Cairo, Egypt, purity 99.5, 98.5 and 99%, respectively, their purity was farther confirmed by the appearance of a single spot when tested on TLC plates. Sodium dodecyl sulfate (SDS), benzalkonium chloride (BAC) and polysorbate 80 (Tween 80) were brought from Sigma-Aldrich (Steinheim, Germany). Methanol HPLC grade (Fisher, Loughborough, UK) and distilled water with high purity were used. All chemicals and reagents were of analytical grade. A pre-coated TLC plates (20 × 20 cm) with silica gel F_{254} were purchased from E. Merck, (Darmstadt, Germany, Catalogue number 1.05554.0001). QSRR studies were carried out on Dell Precision™ T3600 Workstation [Intel Xeon E5–1660 3.3 GHz, 16 GB 1600 MHz DDR3, ECC RDIMM 1 TB (7200 RPM), 1 GB NVIDIA Quadro 2000, Windows 7 Professional (64 bit)]. Molecular operating environment (MOE) package version 2011.10 was used.

Preparation of standard and working solutions

Stock solutions of 1.0 mg mL^{-1} of EMPA, LING and MET were prepared in methanol in ratio 1 : 1 : 1. Solutions containing (100 ng mL^{-1}) of each drug were prepared by diluting the stock solution with methanol in a 10.0-mL volumetric flask.

Preparation of aqueous solutions of the surfactants

Different aqueous concentrations of each surfactant sodium dodecyl sulphate (SDS; an anionic surfactant), BAC (cationic surfactant) and polysorbate 80 (tween 80; nonionic surfactant) were prepared and tested in the range of 0.05–0.25 M.

Chromatographic procedure

Firstly, the TLC plates were cut into 20 cm × 7 cm, prewashed with methanol and dried. Then samples were spotted in the form of bands of width 4 mm with a Camag 100-μL sample syringe by sample automatic applicator. A constant application rate of 150 nL s^{-1} was used and spots were spaced 10 mm from the bottom of the plate edge. For the sample application; the application volume used was 5.0 μL after optimization. The chosen slit dimension was 3 × 0.45 mm and scanning speed was 20 mm/s. The mobile phase (Methanol: aq.surfactant solution (4: 6, v/v)) was introduced into TLC tank, which was lined with a thick filter paper to accelerate chamber saturation. After that, the tank was covered and pre-saturated with the mobile system for at least 20 min at room temperature (25 ± 2°C). The development was done over a distance of 6.5 cm of the plate in a closed container; the plates were completely dried and scanned using Camag TLC scanner III in the reflectance/absorbance mode at λ_{max} 237 nm. The data obtained were treated with win CATS software version1.4.4.6337.

Results

Spectral analysis

The ultraviolet-absorption spectra of the three investigated drugs showed that they have maximum absorbance at λ 226, λ 228 and λ 237 nm for EMPA, LING and MET, respectively. A wavelength of 237 nm has been chosen as co-absorptive wavelength for their simultaneous determination in their ternary mixture.

The relationship between R_M factor (RM) values and the concentration of different surfactants in the mobile phase

The effect of the surfactant concentration on R_M factor was studied in the range of 0.05–0.25 M of each surfactant. The R_M values were calculated according to Bate-Smith and Westall equation (1) and plotted against surfactants concentration. The R_M values, which characterize the retention in TLC, were defined by Bate-Smith and Westall by the following equation (10):

$$R_M = \log [(1/R_F) - 1] \quad (1)$$

Retention behavior of solutes in case of RP-TLC is described by the Soczewiński–Wachtmeister equation. This equation describes the linear relationship between the R_M values and concentrations of organic solvent in the mobile phase (11):

$$R_M = R_{M0} + mC \quad (2)$$

where C is the concentration of the surfactant, R_{M0} represents R_M values extrapolated to zero organic modifier and it is the most commonly used lipophilic TLC parameter, whereas the m value represents specific hydrophilic surface area of a compound (11). The R_M values were plotted against surfactants concentration and the relationship as shown in Figure 2.

Thin layer chromatographic behavior under the effect of volume fraction of the organic modifier

Mobile phase with different ratios 3:7, 4:6, 5:5, 6:4 and 7:3 and 8:2 of aq. 0.20 M solution of surfactant and methanol were tested. The R_M values of each drug were calculated and plotted against volume fraction of the organic modifier (methanol) in the mobile phase as shown in Figure 3.

Discussion

The relationship between the R_M values and molar concentration of the studied surfactants is illustrated in Figure 2 and indicates the same adsorption mechanism of all the investigated drugs. From the data obtained by applying equation (2), it is apparent that the relationships are linear and each

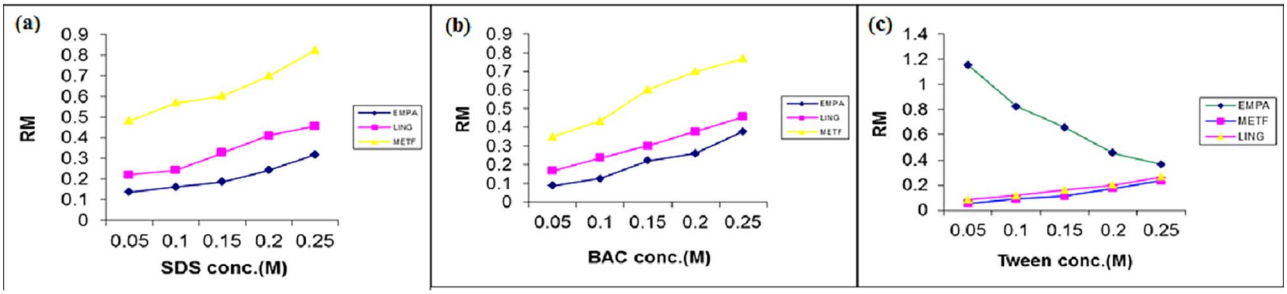

Figure 2. The relationship between R_M values and (a) SDS (b) BAC and (c) Tween 80 conc. (M) for the investigated drugs.

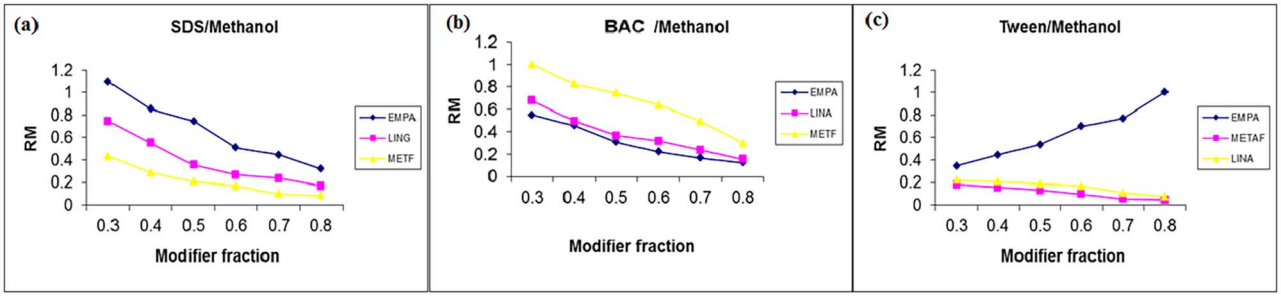

Figure 3. Effect of volume fraction of organic modifier (methanol) on R_M values of the investigated drugs using (a) SDS, (b) BAC and (c) Tween 80 as a mobile phase in the MTLC method.

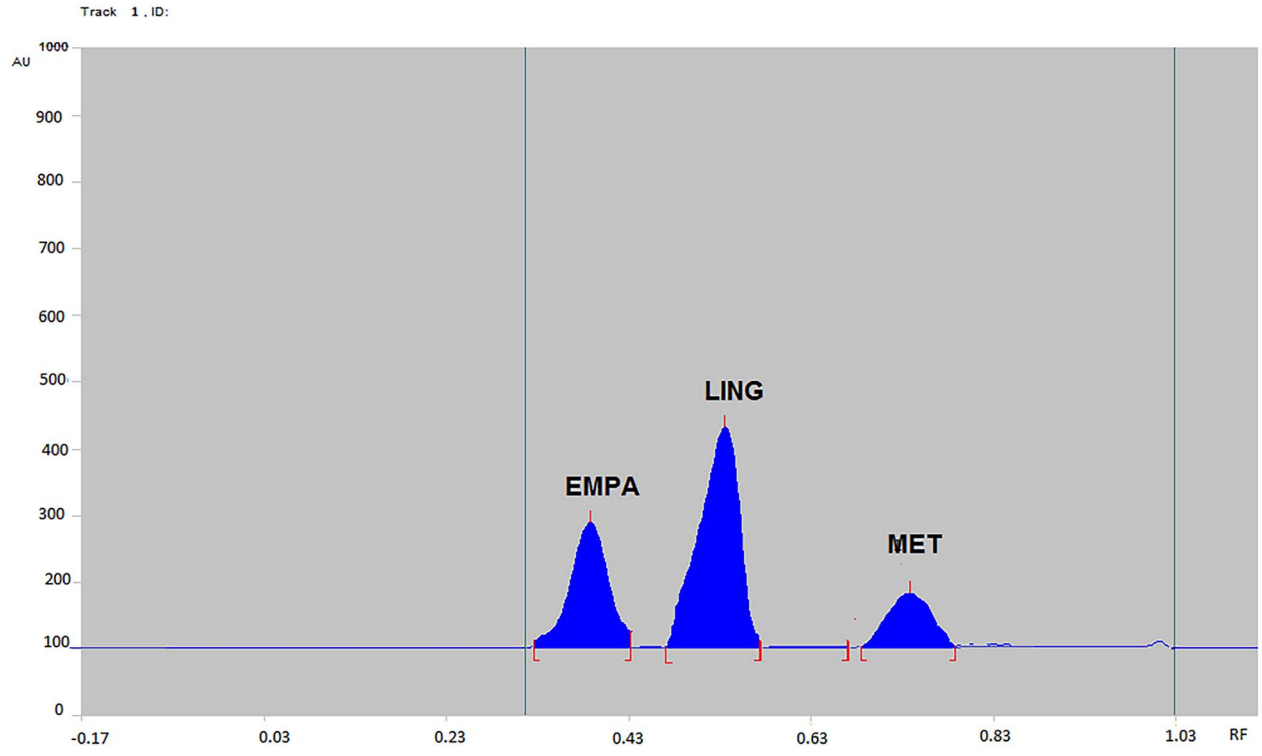

Figure 4. 2D spectrodensitogram for separation of MET, LING and EMPA at R_F 0.85, 0.64 and 0.48, respectively using methanol: aq.0.15 M SDS with ratio of (4: 6 v/v) as mobile phase obtained by Camag TLC scanner III.

surfactant can be used for separation of the investigated drugs in their ternary mixture as represented in Figures 4–6. Data of linear regression analysis LR for chosen surfactant and the studied drugs are summarized in Table I. The small variation of results in case of using tween 80 is due to reasons that will be explained in this discussion according to the structural variety between the studied drugs and physical properties. The effect of volume fraction of the organic modifier revealed by the almost parallel curves in Figure 3, which means a strong correlation between the R_M values and volume fraction of the organic modifier (methanol) in the mobile phase, which indicates the same adsorption mechanism of all the investigated drugs except in the case of tween 80 with EMPA. The obtained results also indicate marked improvement in the selectivity

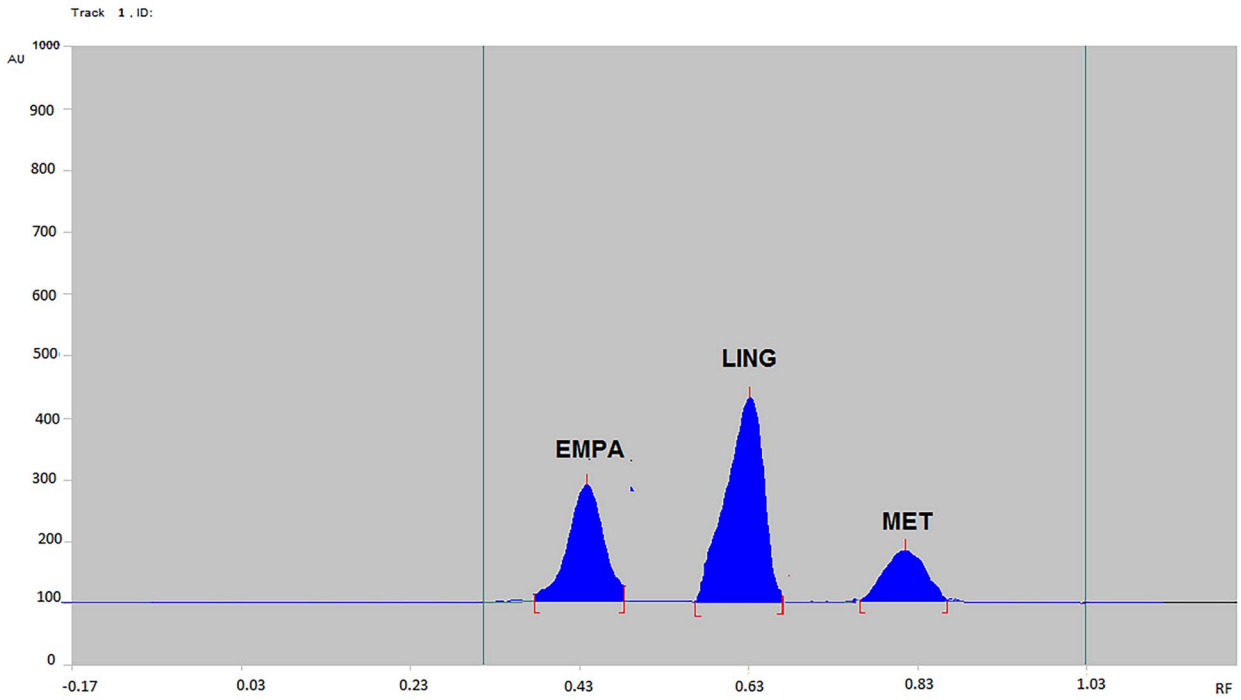

Figure 5. 2D spectrodensitogram for separation of EMPA, LING and MET at R_F 0.45, 0.58 and 0.80, respectively using methanol: aq.0.20 M BAC with ratio of (4: 6 v/v) as mobile phase obtained by Camag TLC scanner III.

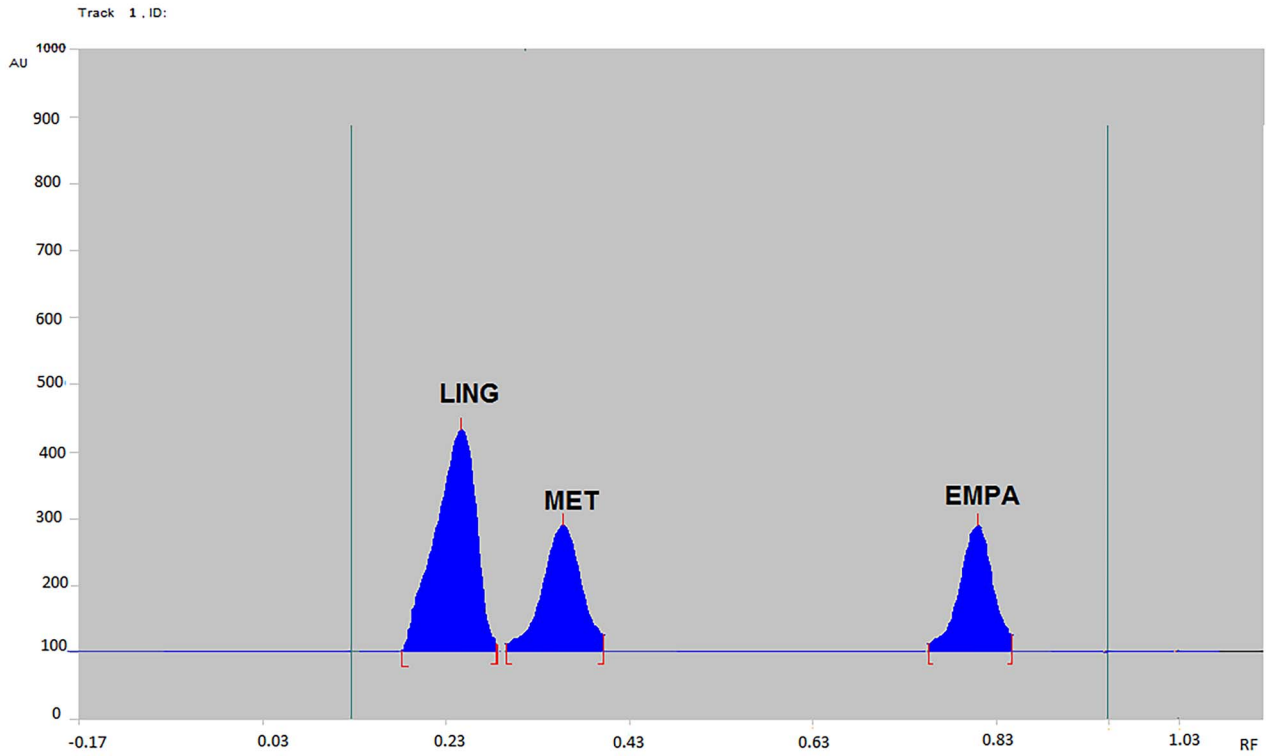

Figure 6. 2D spectrodensitogram for separation of EMPA, LING and MET at R_F 0.78, 0.23 and 0.31, respectively using methanol: aq.0.15 M Tween 80 with ratio of (4: 6 v/v) as mobile phase obtained by Camag TLC scanner III.

achieved by changing the volume fraction of organic modifier and aqueous surfactant in the mobile phase system (Figure 7), thus retention can be increased by decreasing the volume of the organic modifier.

QSRR analysis

Regression was used for derivatization of the QSRR analysis equations, models, evaluation and validation depending

Table I. Regression Equations Relating R_M Values of the Studied Drugs and SDS, BAC and Tween 80 Concentrations in the Range (0.05–0.25 M)

		MET			LING		EMPA		
	R	*m*	R_{M0}	*r*	*m*	R_{M0}	*R*	*m*	R_{M0}
SDS	0.969	0.894	0.075	0.987	1.266	0.142	0.982	1.630	0.390
BAC	0.984	1.432	−0.001	0.999	1.434	0.092	0.989	2.225	0.236
Tween 80	−0.979	−3.889	1.275	0.982	0.889	0.001	0.991	0.889	0.034

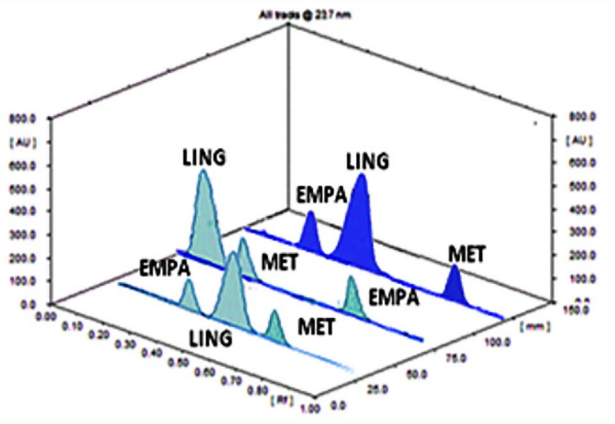

Figure 7. 3D Chromatograms of the proposed MTLC separation of EMPA, LING and MET using (a) SDS, (b) BAC and (c) Tween 80 as micellar mobile phases (MMP).

on retention data as the dependent variable and structural parameters (descriptors) as the independent ones. Computer-aided using molecular operating environment MOE software (for QSRR modeling, linear regression LR, MRA and multidimensional analysis MDA analysis) and Excel 2003 (for multiple regression analysis) were used for relating three-dimension (3D) structure and molecular descriptors of the drug under screening with chromatographic retention and represented PCA. Four two dimension (2D) descriptors; number of rings, octanol–water partition coefficient [logP (o/w)], number of hydrogen bond donor atoms (a-don) and the molar refractivity (MR) and three 3D descriptors; ionization potential (AMI-IP), Van der Waals energy (E_vdw) and solvation energy (E-sol) were chosen in this study, then calculated. The correlation matrix between retention data (R_{M0}) of the three micelles and the chosen descriptors revealed some important correlation coefficients (Table II). The highest relationship ($r = 1.000$) was obtained between (mr & ring), (E-vdw & E-sol), (tween 80 R_{M0} & E-sol) and (tween 80 R_{M0} & E-vdw). Significant relationship was also obtained between SDS RM_0 and both (a-don and AM-IP), (BAC R_{M0} and AM-IP) also between (tween R_{M0} and (logP (o/w)) in the range (0.61–0.84). Most interesting point that there is indirect relationship between R_{M0} for both SDS and BAC against logP(o/w) (−0.96 to −1.0) in contrary in case of tween 80, which is directly proportional to logP(o/w). Regression using one-parameter equations for the three compounds led to the correlation coefficients matrix as shown in Table II. Three statistically significant equations (3)–(5) relating lipophilicity R_{M0} to highly correlated descriptors:

Estimated linear models

$$\text{Tween 80 } R_{M0} = 0.03499 + 0.00004 * \text{E\_sol} - 0.00001 * \text{E\_vdw } (F = 0.62, r^2 = 1.000) \quad (3)$$

$$\text{BAC } R_{M0} = -0.22513 - 0.06307 * \text{logP(o/w)} + 0.04831 * \text{AM1\_IP } (F = 2.97, r^2 = 1.000) \quad (4)$$

$$\text{SDS } R_{M0} = -0.14504 + 0.07335 * \text{a\_don} + 0.02537 * \text{AM1\_IP } (F = 2.57, r^2 = 1.000) \quad (5)$$

where r^2 is the square multiple correlation coefficient and F the ratio of the mean square regression to the mean squared residual. logP (o/w), E-sol and E-vdw are seems to be the most important factors in the micellar TLC using tween 80, so EMPA has high R_F value, then MET and the least value with LING. Separation by SDS and BAC depends on ionization potential and a-don but related indirectly with logP(o/w). Separation by MTLC using BAC and SDS depends on ionization of the compounds to be separated, since the compound that have high ionization potential reacts more with the BAC micelles and travel with micelles so have high R_F value, thus MET have high R_F value then LING and EMPA that have least value. MTLC separation using SDS depends also on the ionization potential of the separated compounds, since EMPA and LING not ionized easily and repelled by these micelles and tends to interact with the stationary phase

Table II. Correlation Matrix of Lipophilicity (R_{M0}) Obtained Experimentally and Calculated Molecular Descriptors Using MOE Software

	Ring	E-sol	a-don	log P(o/w)	mr	AM1-IP	E-vdw	SDS R_{M0}	BAC R_{M0}	Tween 80 R_{M0}
Ring	1.00	0.33	−0.87	0.78	1.00	−0.43	0.34	−0.92	−0.83	0.31
E-sol		1.00	0.19	0.85	0.36	−0.99	1.00	−0.67	−0.80	1.00
a-don			1.00	−0.36	−0.85	−0.80	0.18	0.61	0.44	0.21
log P(o/w)				1.00	0.80	−0.90	0.86	−0.96	−1.00	0.84
Mr					1.00	−0.46	0.37	−0.94	−0.85	0.34
AM1-IP						1.00	−1.00	0.74	0.86	−0.99
E-vdw							1.00	−0.67	−0.81	1.00
SDS R_{M0}								1.00	0.98	−0.65
BAC R_{M0}									1.00	−0.78
Tween8$_0$ R_{M0}										1.00

Table III. Database of the Investigated Compounds Showing the Lipophilicity, QSRR Model Evaluation and Validation

Drug	SDS				Tween80				BAC			
	RM0	$PRED	$RES	$ZSCORE	RM0	$PRED	$RES	$ZSCORE	RM_0	$PRED	$RES	$ZSCORE
EMPA	0.075	0.075	−0.000	0.000	1.275	1.275	−0.000	0.000	−0.001	−0.396	0.395	−0.396
LINA	0.142	0.142	0.000	0.000	0.001	0.001	−0.000	0.000	0.092	0.092	0.000	0.000
METF	0.390	0.390	0.000	0.000	0.034	0.034	0.000	0.000	0.236	0.236	0.000	0.000

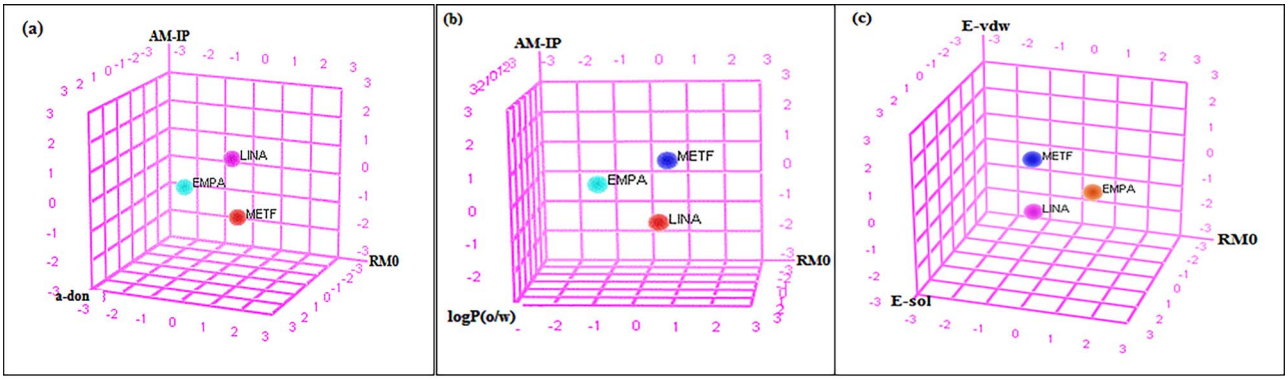

Figure 8. 3D plot of R_{M0} of (a)SDS, (b) BAC and tween 80 against AM-IP, E-vdw, logP (o/w), a-don and E-sol obtained by MOE software.

group and have low R_F value in opposite to MET that ionized easily and reacts with these micelles so move with the mobile phase with long time and have high R_F. There is a good agreement between the three-dimension structures of the drugs according to the 3D plots of the models with the most important molecular descriptors. QSRR models evaluation shows that the obtained values between the R_{M0} (practical value) and the predicted values (calculated by the software) are <0.0, − 0.396 and 0, 1.275 for using SDS, BAC and tween 80, respectively, which represented an evidence that the model is accepted for evaluating the relationship (Table III and Figure 8). The three models also were validated regarding $Z-SCORE values (the absolute difference between the predicted values and the practical lipophilicity R_{M0}, divided by the square root of the mean square error of the dataset), which were ≤ − 0.396 (Table III), indicating that there were no outliers in the data sets (12).

Conclusion

This study represents the first research, which revealed the mechanism of MTLC separation using three different types of surfactants (anionic, cationic and nonionic) and relating the practical data with computational analysis of this type of separation. The study concluded the most effective 2D and 3D descriptors that are logP(o/w), AMI-IP, E-sol, E-vdw, mr and a-don. For cationic and anionic micelles, the most effective factors are ionization potential and a-donor while for nonionic micelles; the most effective factors are E-vdw, E-sol and logP(o/w). The method can be applied for determination of the three mentioned drugs simultaneously and simply.

Conflict of interest statement

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

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