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Recent theoretical and practical applications of micellar liquid chromatography (MLC) in pharmaceutical and biomedical analysis

Review Article

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Abstract: Micellar liquid chromatography (MLC) is an analytical technique belonging to the wide range of reversed-phase liquid chromatographic (RP-LC) separation techniques. MLC with the use of surfactant solutions above its critical micellar concentration (CMC) and the addition of organic modifiers is currently an important analytical tool with still growing theoretical considerations and practical applications in pharmaceutical analysis of drugs and other biologically active compounds. The use of MLC as an alternative, relatively much faster in comparison to conventional chromatographic separation techniques has several advantages, especially as being suitable for screening pharmaceutical analysis. The analytical data received from MLC analysis are considered a useful source of information to predict passive drug absorption, drug transport and other pharmacokinetics and physicochemical measures of pharmaceutical substances.

In the review several MLC assays for determination of drugs and other active compounds in biological samples were compared and critically discussed. The presented overview provides information on recent applications and achievements connected with the practical use of MLC. The review covers fields of interest related to theory and mechanism of MLC separation, direct applications of MLC in pharmaceutical analysis, including optimization and efficiency of separation with the use of modification of stationary phase and mobile phase compositions as well as the determination of physicochemical characteristics of drugs by MLC.

Keywords: *Micellar liquid chromatography (MLC) • Reversed-phase liquid chromatography (RP-LC) • Prediction of retention • Hydrophobicity • Applications of MLC*

1. Introduction

MLC technique has received attention due to its advantages and capabilities such as simultaneous separation of charged and uncharged solutes, direct on-column injection of physiological fluids, rapid gradient capability, unique separation selectivity, robustness, enhanced luminescence detection, high reproducibility and low cost and safety of analysis. Over the past more than twenty five years the use of micelles in liquid chromatography (LC) and capillary electrophoresis (CE) has been developed. Unique advantages such as speed of analysis, high efficiency, method development, feasibility of incorporating different sets of chemical compounds to influence retention and selectivity are particularly of interest in various disciplines such as pharmaceutical, clinical, biotechnological and environmental sciences. The focus of this paper is to provide a short overview in recent theoretical and practical applications of MLC in pharmaceutical and related analysis.

2. The basis of micellar liquid chromatography (MLC)

In RP-LC micellar solutions applied above their CMC value possess undoubtedly serious of advantages like possibility of direct injection of biological fluids, shorter equilibration times for gradient elution or compatibility of mobile phases with salts and water-insoluble compounds but there are also some disadvantages and the primary could be the insufficient efficiency. To overcome the

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poor efficiency limited of the MLC application the initial conditions and chromatographic parameters modifications could be apply like reducing of flow rate, increasinig temperature also adding small percentage of short-chain alcohol organic modifiers to create a hybrid mobile phases [1,2].

2.1. Retention mechanisms in MLC

To improve resolution and shape of the chromatographic peaks hybrid micellar mobile phases which increased the eluent strength are used. There are observed three types of equilibriums during the retention of solutes in MLC between micelle, bulk water and stationary phase respectively: distribution, partition and the direct transfer [3].

For the first time Armstrong and Nome [4] and Arunyanart and Clin Love [5] studied in MLC on solute retention modeling. The both performed models of equations establish situation that when surfactant concentration increases the retention will decrease. In the first on of above models the solute can partitioning followed by equation:

$$\frac{V_s}{V_e - V_m} = \frac{1}{P_{sw}} + \frac{P_{mw} - 1}{P_{sw}} v[M_m] \quad (1)$$

between micelles and aqueous phase, P_{mw}, the stationary phase modified by adsorption of surfactant monomers and the aqueous phase, P_{sw}, and the stationary phase and the micellar pseudophase, P_{sm},V_s is the stationary phase volume, V_e the elution volume of the solute, V_m the mobile phase volume, v the surfactant molar volume, and $[M_m]$ the micellized surfactant concentration. The second obtained model relates to the capacity factor:

$$\frac{1}{k'} = \frac{1}{\Phi[L_s]k_1} + \frac{k_2}{\Phi[L_s]k_1}[M_m] \quad (2)$$

$$E_m + L_s \leftrightarrow EL_s;\ k_1 \quad (3)$$

$$E_m + M_m \leftrightarrow EM_m;\ k_2 \quad (4)$$

Φ is in presented model the phase ratio value (V_s/V_m), $[L_s]$ the concentration of the stationary phase sites, Em is the solute in the mobile phase and EL_s EM_m are the complex formed between the solute in the mobile phase and the stationary phase sites, k_1 and k_2 are the constants of the two equilibriums. The retention factor in micellar mobile solutions can be presented also follow by Foley [6] as:

$$k = k_0 \frac{1}{1 + K_{AM}[M]} \quad (5)$$

where (k_0) is the retention value in the absence of micelles and KAM binding constant [2,3].

Different type of organic modifiers can interact with the solutes and change polarity of mobile phase also aggregation number of surfactant and the CMC value. The finally effect on retention of organic solvent depends on the kind of the solutes but also on the concentrations of organic modifier and surfactant The solute equilibrium in the presence of organic modifier is shifted away from the micelles and stationary phase towards non-polar the bulk of solvent in that situation power of elution of mobile phase increases and both solutes binding constants decrease [7,8].

There are obvious principal similarities in the retention mechanism controlling aqueous-organic process in RP-LC, MLC and MEKC. Linear solvation energy relationships (LSERs) have been suggested for characterization of solvent-related properties of analytes (*i.e.*, the logarithm of the retention factor, *log k*) by linear combination of the solvatochromic parameters: molar volume of the analyte, dipolarity/polarisability interactions with the solvent and the analyte's basicity and acidity [9].

LSER methodology has been extensively applied in MLC studies. The interpretation of MLC LSERs is complicated due to the fact that the system is commonly described using a three-phase model with three accompanying partition coefficients (mobile to stationary phase, mobile to micelle phase, and stationary to micelle phase transfers). Regarding the interpretations of the role of stationary phase in determining changes in the LSER coefficients as a function of surfactant concentration there should be an assumption that the stationary phase does not change with the surfactant concentration and the stationary phase environment in MLC is independent of micelle concentration in the mobile phase, also the amount of surfactant adsorbed by the stationary phase remains constant above the CMC [10].

It has been suggested that thermodynamic approach by Armstrong and Nome may be suitable for the chemical reactions to the micelle formation, and the micelle formation equilibrium can be described by mass action law (MAL) [11,12].

Among physicochemical retention models discussed earlier only conceptual models can be used for hybrid mobile phases. However, a number of empirical and mechanistic retention models in MLC have also been proposed [13].

The potential advantages of using ANNs instead of classical statistical methods for modeling the retention behavior of different solutes in MLC as a function

of surfactant and alcohol concentrations (SDS or cetyltrimethylammonium bromide – CTAB are used as surfactants and *n*-propanol or *n*-butanol as organic modifiers) has been presented as well [14].

2.2. Influence of experimental conditions on MLC separation

Optimization of separation in MLC could be successfully achieved only by careful optimization of mobile phase composition which is the most important factor of selectivity modification. An investigation of the simultaneous effects of variation of the micelle concentration and amount of organic modifier influenced on selectivity and eluent strength will fully disclose separation capability of the method [13].

In MLC the amount of ionic surfactant adsorbed by alkyl-bonded silica stationary phases is almost constant also the solutes partition not only between water and the stationary phase but also, within the mobile phase, between water and the micelle. The most usual behavior is a decrease in the retention of the solutes as the concentration of micelles increases. The organic modifier increases the hydrophobic character of the bulk liquid in the mobile phase, which shifts the solute equilibrium from the micelle and the stationary phase to the water-modifier phase but if the concentration of modifier becomes too large no micelles will form [15].

MLC has been limited in its applicability due to two main problems compared to conventional RP-LC. The first is the excessive retention observed for hydrophobic compounds due to the weak eluting power of micellar mobile phases when used with conventional pore size LC stationary phases. The second is the reduced efficiency due to greater resistance to analyte mass transfer and/or increased flow anisotropy. To enhance efficiency in MLC the two main approaches that have been used are: addition of small concentrations (1–5%, *v/v*) of various alcohols to the micellar mobile phase, and increases in the column temperature; also the use of large pore size silica must be considered in addition to the individual impact of alcohols on the micelles. In MLC the total band-broadening can be expressed as:

$$\sigma^2_{tot} = \sigma^2_{inj} + \sigma^2_{det} + \sigma^2_{eddy} + \sigma^2_{diff,mp} + \sigma^2_{diff,sp} + \\ + \sigma^2_{mt,sp} + \sigma^2_{mt(interstitial)} + \sigma^2_{mt(intraparticle)} \quad (6)$$

where (σ^2_{tot}) is the total peak variance which describe the sum of the variances due respectively to sample injection, detection, eddy diffusion, diffusion of the solute in the mobile phase, diffusion of the solute in the stationary phase, stationary phase mass transfer, mobile phase mass transfer and stagnant mobile phase mass transfer [16].

The stationary phase with higher surface area could be more open for influence of surfactant absorption than a stationary phase with a lower surface area in the mobile phase particularly with the use of low surfactant concentration. With the respect to mass transfer also the depth of pore of stationary phase and range of diffusion distance are very important. Finally all of the above variables in large values could become the sources of increased band-broadening [16-18].

The use of wide-pore stationary phases is helpful in overcoming one of the important limitations in MLC, that of the apparently weak eluting power of micellar eluents. Acceptable efficiencies could be reached in MLC using stationary phases bonded with short alkyl chain or a fluorooctyl chain [19].

A similar relationship between elution strength enhancement and alkyl chain length was found for acids and alcohols. Aliphatic carboxylic acids can also be used as useful alternatives to alcohols as organic modifiers in MLC. Their presence in the micellar mobile phase enhances the elution strength of the mobile phase slightly more that the corresponding alcohols added at the same volume ratio [19,20].

The best separation conditions are often searched by maximising a resolution descriptor such as selectivity or R_S values for the least resolved peak pair. Modifications of the R_S parameter which consider peak widths, asymmetries and height ratios can be incorporated. In such optimizations, some resolution should be sacrificed eventually to balance other requirements [21].

Gradient elution is important in chromatography of mixtures of widely differing polarity such as the analysis of drugs and more polar metabolites. In MLC, gradient re-equilibration is rapid because the micelles are restricted to the inter-particle space, which is one of its more attractive features. A major difficulty encountered in gradient elution MLC has been the inability to effectively elute highly retained species, such as nonpolar hydrophobic compounds, on long chain stationary phases (*e.g.* C_{18}). The use of shorter chain length stationary phases has been recommended to reduce analysis times; this effectively reduces retention although it decreases resolution [22].

The values of CMC and degree of counter-ion binding (β) are parameters based on the concept of mass action. In the presence of alcohols, which reduce CMC, the value of counter-ion binding degree of SDS is also decreased. Small additions of aliphatic alcohols and carboxylic acids (C_4–C_5) reduce the CMC values of SDS and the degree of counter-ion binding. The main

rule of the observed effects is that modifying action of carboxylic acid practically does not differ from that of normal alcohol with the same number of carbon atoms [23].

In MLC the contribution of electrostatic and hydrophobic interactions to retention is a function of structural properties and would not be the same for different compounds. Due to the competing equilibria and different types of interactions in MLC, one can expect any form of selectivity behavior, however, for a large group of compounds (especially non-ionics), partition into micelles and into dynamically modified alkyl-bonded phase is directly related [13].

The loss of efficiency observed in MLC using purely aqueous micellar mobile phases is due to a slow solute transfer from the aqueous to the micellar phase and to a slow transfer from the stationary phase to the aqueous phase and surfactant adsorption that changes the porosity and surface of the stationary phase. MLC efficiency can be enhanced by reducing the mobile phase flow-rate to work closer to the optimum of the Knox plot, by increasing the temperature which decreases the viscosities, increases the rate constants and decreases the amount of adsorbed surfactant, specially by adding an organic modifier such as an alcohol. However, in order to improve the resolution factor the amount of alcohol added should be increased if the surfactant concentration is increased to keep the alcohol to surfactant ratio constant [24].

3. The application of MLC in physicochemical studies

The micellar media are easy to prepare, but their physicochemical properties should be known in order to be able to use them correctly in MLC. The two important factors are surfactant associations and surfactant adsorption at any interface which are process dynamic and they form and break more or less rapidly [25].

3.1. Partitioning behavior of analytes measured by MLC

The MLC technique has also been named as pseudo-phase liquid chromatography; this term was coined to describe separations where significant partitioning of a solute occurs to discrete aggregates dissolved in the mobile phase, rather than to bulk solvent [25].

In MLC the surfactant is associated to both mobile and stationary phases, by formation of micelles and by adsorption on the stationary phase. Adsorption of the monomers of an ionic surfactant on a C_{18} column will mainly be produced by hydrophobic interactions between the tail of the surfactant and the hydrocarbon chains of the bonded-stationary phase. The conditional constants, K'_{AM} and K'_{SW} for the partition of the solute between micelle and water, and stationary phase and water can be expressed as:

$$K'_{AM} = \frac{AM'}{A'M} = \frac{AM + \Delta AM}{(A+\Delta A)M} \quad (7)$$

$$K'_{SW} = \frac{AS'}{A'} = \frac{AS + \Delta AS}{A+\Delta A} \quad (8)$$

where *A', AM'* and *AS'* are the total concentrations of free solute in bulk water and solute associated to the micelle and stationary phase, respectively; *A, AM* and *AS* are the equilibrium concentrations in the pure micellar solution, and *A, ΔAM* and *ΔAS* are the changes in the concentrations produced by the presence of the modifier; *M* is the concentration of monomers of surfactant forming micelles in the presence of modifier. If the following is assumed:

$$\frac{\Delta A}{A} = K_{AD}\varphi \quad (9)$$

$$\frac{\Delta AM}{AM} = K_{MD}\varphi \quad (10)$$

$$\frac{\Delta AS}{S} = K_{SD}\varphi \quad (11)$$

then Eqs. 7 and 8 will yield the following:

$$K'_{SW} = K_{SW}\frac{1+K_{SD}\varphi}{1+K_{AD}\varphi} \quad (12)$$

From Eqs. 8-10 it may be deduced that K_{MD} depends inversely on the concentration of micelles, since the effects produced by the modifier in the micellar pseudo-phase are attenuated by an increased micelle concentration, whereas K_{MD} and K_{AD} account for the displacement of the micelle-water equilibrium. K_{SD} and K_{AD} describe the modification of the stationary phase-water equilibrium. The use of K_{SD} is necessary for strong hydrophobic solutes having a high affinity for the non-polar stationary phase [26].

To explain the distribution mechanism in MLC in further detail, it is necessary to investigate the effect of molecular structure on the retention value of the solutes. The linear free-energy relationship (LFER). of the solute can be expressed by the solvatochromic parameters of the following equation:

$$X = X_0 + mV_w/100 + s\pi^* + b\beta_m + a\alpha_m n \quad (13)$$

where $mV_w/100$ is the cavity term, which measures the endoergic process of separating the solvent molecules to provide a suitable sized enclosure for the solute, $s\pi^*$ measures the exoergic effects of the solute-solvent

dipole-dipole and dipole-induced dipole dielectric interactions, $b\beta_m$ and $a\alpha_m$, measure the exoergic effects of hydrogen bonding involving the solvent as a hydrogen bond donor (HBD) acid and the solute as a hydrogen bond acceptor (HBA) base, the solvent as an HBA base and the solute as an HBD acid, respectively [27].

Solvation parameter model based on a LFER can be written also as equation:

$$SP = c + eE + sS + aA + bB + vV \quad (14)$$

where *SP* is the dependent solute property (*e.g.* log *P*, log *k*, where *k* is the LC retention factor) and *E*, *S*, *A*, *B* and *V* are the Abraham solute descriptors. *E* is an excess molar refraction, *S* the solute dipolarity/polarizability, *A* and *B* are parameters characterizing the effective hydrogen-bond acidity and hydrogen-bond basicity, respectively, and *V* is McGowan's characteristic volume. The values of the coefficients of the correlation (*e*, *s*, *a*, *b* and *v*) are related to the chemical nature of the chromatographic system, mobile and stationary phase. These coefficients reflect differences in the two phases between which the compound is transferred. Thus, *e* depends on the difference in capability of the mobile and stationary phases to interact with solute *n*- or π-electrons; *s* is a measure of the difference in dipolarity/polarizability between the two phases, *a* and *b* are measures of the difference in hydrogen-bond acidity and basicity between the two bulk phases, and *v* is a measure of forming a cavity for the solute in the phases [28].

In MLC, the uncertainties in binding constant of solute to micelles are linked to errors in CMC and the stationary phase-related partition coefficient. The experimental conditions are easily controlled for calculation of binding constant with respect to practical pH ranges [29].

The capacity factors at zero micellar concentration and the solute-micelle association constants could be used as the hydrophobicity index of compounds and are important in QSAR studies [30]. In studies related to structure-retention (QSRR) and activity-retention (QRAR) quantitative relationships, it may be necessary to obtain both values for a series of compounds with different hydrophobicities in the same chromatographic conditions. It is common for compounds that are weakly retained to coincide with others that are strongly retained [30-33].

The use of MLC to ascertain solute-micelle association constants and distribution coefficients has advantages. This determination can be made for all compounds experiencing a chromatographic retention in the system which varies when the surfactant concentration in the mobile phase is modified. Distribution coefficients and solute-micelle association constants can simultaneously be determined for numerous compounds since a mixture of them can often be injected in the chromatographic system enabling the simultaneous determination of many capacity factors under the experimental conditions [34].

MLC due to its micellar properties has also attracted considerable attention as an *in vitro* model for protein-drug binding studies to predict the pharmacological and pharmacokinetic properties of drugs in the early stage of the drug discovery phase [35]. In drug absorption studies where not only are physiological factors important but also are factors such as solubility, particle size, chemical form, and other physicochemical characteristics of the drug itself MLC systems reflect adequately the relative importance of the hydrophobic and hydrophilic interactions of drugs that occurs in the naturals biomembranes. The *log* k_{MLC} values can be used to identify drugs which may be poorly absorbed at an early stage of the drug discovery process. The MLC could also be applicable in the prediction of passive drug transport across other epithelial barriers, such as the blood–brain barrier [36].

3.2. MLC determination of hydrophobicity

Hydrophobicity is commonly understood as a measure of the relative tendency of a solute to prefer a nonaqueous rather than an aqueous environment, or as a measure of the tendency of two (or more) solute molecules to aggregate in aqueous solution. The hydrophobicity of solutes is a relative property and depends mostly on the environment, which is why when comparing the behavior of various solutes in the same environment, a quantitative scale can be established to demonstrate the abilities of individual solutes to participate in hydrophobic interactions. MLC has shown to be an interesting chromatographic technique to measure the hydrophobicity of solutes [25].

The retention of a compound on classic RP-LC is governed by hydrophobic interactions. In that kind of measurements estimations depend on the mobile phase composition and nature of the stationary phase, and for alkyl-bonded stationary phases, distinct relationships are observed for different groups of congeneric compounds. The advantage of the use MLC is that the stationary phase environment in MLC is independent of the micelle concentration in the mobile phase and is quite similar to a purely aqueous eluent. The adsorption of surfactant monomers on the alkyl-bonded stationary phase reduces silanophilic interactions and increases the hydrophobicity of stationary phase, also the behavior of compounds (apolar, polar or ionic) chromatographed with anionic, cationic and non ionic surfactants could

be accurately modelled. The micelles are structurally more similar to biomembranes than *n*-octanol or RP-LC stationary phases, and form amphiphilic aggregates with anisotropic microenvironments that provide both hydrophobic and electrostatic sites of interaction [30].

The unique characteristics of MLC may be advantageous in quantitation of hydrophobic properties of bioactive molecules in QSAR studies. Molecular size and shape are significant factors in the partitioning of solutes in anisotropic environments while they are not determinants of the partition process in an isotropic solvent such as *n*-octanol. Despite the existence of certain differences in partitioning behavior in micelles as compared to that in octanol, there is a correlation between micelle-water and *n*-octanol-water partition coefficients for a group of compounds with a similar partitioning behavior in these systems. Satisfactory correlation between *log* P_{mw} *vs*. *log* P_{ow} can be achieved through proper adjustment of the lipophilic-hydrophilic balance of the chromatographic system (including H-bonding interactions) to mimic octanol-water environments, reducing the role of mobile phase selectivity and eliminating or reducing silanophilic interactions [37]. In RP-LC with hydroorganic mobile phases the relationship between retention and *log* P_{ow} is often expressed in the logarithmic form as:

$$logk = alogP_{ow} + b \quad (15)$$

The correlation between retention in micellar RP-LC and *log* P_{ow} depends upon the type of solute, mobile, and stationary phases. For the micellar eluents a better linear relationship between *k* not *log k* and *log* P_{ow} is observed.

MLC is an example of the use of secondary chemical equilibrium in liquid chromatography, where the retention is influenced by two competing equilibria of solute interactions with micelles in the mobile phase (controlled by K_{AM}) and their partitioning into the stationary phase (controlled by k_m). Both partitioning processes depend on the hydrophobicity, among the size and shape of the solute. The relationship between *k* or log *k* and log *P* can be modeled by determining log k_m and log K_{AM} of the compounds and their fitting parameter with the corresponding log *P* values. Such a model permits the prediction of log *P* values from *k* or log *k* data, and also the estimation of the retention of congeneric compounds from their *log P* values [38].

In micellar equilibrium inside the MLC systems the adsorption on the stationary phase and the ionic surfactant is observed. The environment of the stationary phase becomes independent of the mobile phase micelle concentration above the CMC value in most of the used surfactants and chromatographic colums possessed then both anisotropic and amphiphilic properties. The retention behaviour of all type of compounds can be precise modelled because the influence of surfactant absorption on the stationary phase which reduces the concentration of residual silanol groups and makes it more hydrophobic [39].

The RP-LC and MLC retention scales differ from each other, but both are similarly correlated to the commonly applied *n*-octanol/water reference lipophilicity scale. It should be remarked that the correlation quality does not only depend on the RP-LC and MLC results but also on the reliability of the *clog P* (*clog D*) values. In comparison to RP-LC, MLC is a faster technique to estimate the lipophilicity of xenobiotics. MLC tends to increase the retention of the more polar compounds and reduces the more apolar, lipophilic ones making their retention measurable with one mobile phase, which is why MLC on monolithic columns could be recommended as a fast experimental alternative for lipophilicity estimations in early discovery stages of drugs, where many compounds need to be screened [40].

4. The application of MLC in pharmaceutical and biomedical studies

The research on MLC has been largely dedicated to the retention mechanism inside the chromatographic system and the relationships between the retention behavior and hydrophobicity of solutes. Indeed, micellar mobile phases are quite interesting from a mechanistic standpoint, and some practical analytical utility had to be demonstrated before they could be considered as an alternative to the more traditional aqueous-organic mobile phases [25].

4.1. Pharmaceuticals

According to the literature, MLC is a suitable technique for the screening of pharmaceutical compounds. Details regarding the application of MLC in pharmaceutical studies are summarized in Table 1.

In the study performed by Ruiz-Angel *et al.* [41] this possibility and the mobile phase characteristics have been discussed critically. Experimental work is reported in the MLC literature for amino acids, β-blockers, diuretics, polycyclic aromatic hydrocarbons (PAHs), phenethylamines, phenols and sulfonamides. They were used, along with new results for sulfonamides and steroids, separated with SDS mobile phases containing acetonitrile and 1-pentanol, respectively, and compared with classical acetonitrile-water and methanol-water

mixtures. The results of this work showed that the peculiar behaviour of MLC with regard to the selectivity and elution strength should be taken into account for screening purposes. Direct injection and resolution of complex was possible using a conventional alkyl-bonded stationary phase that has given rise to fast and economical procedures.

A chromatographic procedure for the determination of sulfonamides in pharmaceutical preparations after precolumn derivatization from NED azodyes has been reported [42], wherein a micellar eluent of SDS and pentanol was used. The precolumn derivatization improved the resolution of the chromatograms and increased the selectivity in the determination of mixtures of sulfonamides and in preparations where other drugs were present. The derivatization reaction was readily performed in a micellar medium of SDS at pH 1, leading to a rapid and simple procedure to control these compounds in pharmaceutical preparations. Sulfonamide azodyes were found to be very stable, with no degradation being observed for several days. In all cases, the mixtures were well resolved and good agreement was found between taken and observed values.

In another study [43] the possibility of using micellar mobile phases in the analysis of pharmaceuticals containing β-blockers (acebutolol, atenolol, carteolol, celiprolol, labetalol, metoprolol, nadolol, oxprenolol, propranolol and timolol) was analyzed. It was demonstrated that these determinations can be performed in less than 15 min with a unique mobile phase of SDS and propanol. The results were compared with those obtained with hydro-organic eluents of methanolphosphate buffer. The proposed procedure for the determination of β-blockers in pharmaceuticals with a micellar mobile phase of SDS and propanol was rapid and the variation coefficients below 2.5% and the values correlated well with observed results.

Rukhadze *et al.* [44] selected a group of seven barbiturates as the model mixture. It contained compounds of different polarity, *e.g.* polar barbital and nonpolar benzonal. The selection of these barbiturates was also influenced by their wide medical application. Some of them are often used simultaneously, some are metabolized in the organism and transformed into each other. An empirical regression equation was deduced which describes the influence of micelle concentration, amount of organic modifier and mobile phase pH (as principal factors) on the selectivity and resolution of the chromatographic separation. A strong interaction between principal factors was revealed. This indicated that the use of higher order mathematical equations found *via* second order design methods is essential for the precise description of the quantitative relationships. With this mathematical model the respective hypersurfaces can be created and explored. The approach allowed for prediction of experimental conditions for successful separation of pairs of compounds which are difficult to resolve.

The other scientific team [45] examined the experimental conditions to prevent the degradation of furosemide in the analytical laboratory. A simple chromatographic procedure with micellar mobile phases of SDS was developed, which was applied to the control of numerous pharmaceuticals in several dosage forms. The procedure also resolved furosemide jnto its photodegradation products or usual impurities, which was useful to check the purity of the solutions. The studies have shown that furosemide solutions, protected from light, are stable at pH 3–5. Exposed to light the degradation was complex giving rise to several products. Furosemide can be completely resolved from its degradation products and usual impurities using a mobile phase of 0.04 M SDS-2% propanol at pH 3. The chromatographic procedure can be applied to control the decomposition degree of the solutions of standards and pharmaceuticals during analysis, or the quality of the pharmaceuticals. Good results were also obtained for determination of furosemide in diverse dosage forms. Since no interference from common additives, excipients or drugs that might be found in commercial preparations was noticed, a previous extraction of the drug or the use of an internal standard are unnecessary.

The development of a rapid and simple RP-LC procedure for the determination of pharmaceutical preparations containing combinations of chlorpheniramine and the phenethylamines, which utilized a micellar mobile phase of SDS and pentanol was showed [46]. It demonstrated MLC as a useful technique to control diverse groups of drugs in a combination of 14 pharmaceuticals, which contained either chlorpheniramine or dexchlorpheniramine, mixtures of chlorpheniramine and phenylephrine, phenylpropanolamine or pseudoephedrine, and a mixture of dexchlorpheniramine and pseudoephedrine. The retention behavior of compounds could be predicted with high accuracy, which in fact simplified the optimization of mobile phase composition. The use of other alcohols than propanol such as butanol and pentanol can be more convenient to decrease the retention times of highly retained compounds. Also, pentanol is less toxic than methanol and is highly retained in the SDS micellar solution, which reduces the risk of evaporation.

Capella-Peiró *et al.* [47] developed an MLC procedure for the determination of corticosteroids in creams and ointments, with direct injection and without pre-treatment

Table 1. Summarized details for the application of MLC in pharmaceutical studies.

Determined substances	Mobile phase	Detection UV-Vis (nm)	LOD	Refs.
amino acids **phenols** **β-blockers** **diuretics** **PAHs** **sulfonamides** **phenethylamines** **steroids**	0.055 *M* SDS–0.8% 1-propanol, pH 3 0.10 *M* CTAB–10% 2-propanol, pH 2.5 0.10 *M* SDS–15% 1-propanol, pH 3 0.05 *M* SDS–6% 1-propanol, pH 3 0.15 *M* SDS–15% 2-propanol, neutral pH 0.07 *M* SDS 6% 1-propanol, pH 3 0.065 *M* SDS 15% 2-propanol, 6% 1-butanol and 0.12 *M* SDS–3% 1-pentanol, pH 3 SDS, 1-pentanol	335 254 225 274 254 254 256 274 246	-	[41]
sulfonamides	0.1 *M* SDS, 4% propanol, pH 7 and 3	490, 550	0.02-0.2 μg mL^{-1}	[42]
β -blockers	0.075-0.15 *M* SDS, propanol 15%, pH 3	225	-	[43]
barbiturates	SDS, pentanol–heptanol 3:1 pH 3.5-6.5	220	-	[44]
furosemide	0.04 *M* SDS-2% propanol, pH 3, 0.06 *M* SDS-8% propanol, pH 3	222-278	7 ng mL^{-1}	[45]
chlorpheniramine and phenethylamines	0.15 *M* SDS–6% pentanol, pH 7	260	0.01-0.2 μg mL^{-1}	[46]
corticosteroids	0.10 *M* SDS–4% butanol, pH 7	240	0.86-4.7 ng mL^{-1}	[47]
danazol	40 *mM* SDS-2% pentanol	280	3.0 ng g^{-1}	[48]
anticonvulsants	SDS and Brij-35 with propanol and pentanol	240	-	[49]
non-steroidal anti-inflammatory drugs	0,04 *M* CTAB and 0,15 *M* SDS at pH 3 with 10% 1-propanol or 5% 1-butanol	234-322	0.001-0.5 μg mL^{-1}	[50]
vitamins A, E	11.7% 1-butanol, 76.9 *mM* SDS, pH 6.73	285	1.71 μg mL^{-1} and 4.52 μg mL^{-1}	[51]
antianginals	0.05 *M* SDS–5% pentanol, pH 7.	220	0.028-0.130 μg mL^{-1}	[52]
antihistamines	0.02 *M* CTAB, pH 3 with 3% 1-propanol or 1, 3 % 1-butanol	225-273	0.03-0.65 μg mL^{-1}	[53]
selenium (VI)	10% 1-butanol in 0.05 *M* SDS	378	0.1 μg mL^{-1}	[54]
β -blockers	0.075-0.15 *M* SDS, 20% methanol, 15% ethanol, 15% acetonitrile, 15% propanol, pH 3	225	-	[55]

of the samples. They proved that MLC in determination of corticosteroids in creams and ointments has the following advantages: speed, simplicity, direct sample injection without sample pre-treatment (it avoids extraction losses during the evaporation and reconstitution steps), enhanced reproducibility, reduced risk of contamination, and no contact with toxic solvents.

A simple, rapid, sensitive, accurate, precise, reproducible and robust MLC method was developed and validated for danazol determination in samples prepared from Danatrol capsules using UV absorbance detection [48]. This method can be considered as an alternative to those methods reported by the most important pharmacopoeias for quality control purposes.

Moreover, the robustness test indicated that different working conditions were possible because small variations in the main variables of the methods did not significantly affect the results. These methods achieved the established pharmacopoeias requirements to be used as routine methods for quality control and stability studies of danazol in capsules.

The separation of a group of eight anticonvulsants with different polarity by using MLC technique was performed [49]. The selection of these compounds was also based on their wide medical application. In order to optimize the time of the chromatographic analysis and the resolution, chemometric methods of experimental design, multivariate analysis, and multi-criteria decision-making were employed. Subsequently, the method of stepwise multiple linear regression (MLR) was used to select the most important effects and to calculate the coefficients relating the effects to retention time.

Martínez-Algaba *et al.* [50] studied the retention behaviour of non-steroidal anti-inflammatory drugs (NSAIDs) using mobile phases of SDS and compared that with values obtained using mobile phases of CTAB. A new MLC procedure for the determination of six non-steroidal anti-inflammatory drugs (acemetacin, diclofenac, indomethacin, ketoprofen, naproxen and tolmetin) in pharmaceutical preparations was proposed. In spite of NSAIDs are highly hydrophobic compounds, the use of SDS hybrid micellar mobile phases allowed the rapid elution of analytes using a C_{18} column due to the existence of repulsive electrostatic interactions between analytes and the modified stationary phase. The proposed procedures for the determination of NSAIDs in pharmaceuticals was fast (between 6 and 10 min per sample), reliable, free from interferences, and sample preparation was simple.

A method for separation and determination of fat-soluble vitamins A and E using MLC has been reported [51]. This method is rapid, facile and more environmentally benign. The effects of the dependent parameters including SDS concentration, pH, 1-butanol percent and flow rate of the mobile phase were optimized by the SMS optimization method. The optimized method was applied to the analysis of these vitamins in a multivitamin syrup sample. In order to evaluate the applicability of the proposed method, multivitamin syrup was analyzed and results were compared with those reported by manufacturer. There was good agreement between the results obtained by the proposed method and reported values. Application of the SMS optimization procedure together with advantages of MLC resulted in successful separation and determination of Vitamins A and E with good sensitivity, and less toxicity in a reasonable time.

In another study [52] optimization of the experimental conditions for determination of combinations of calcium channel blockers (diltiazem, nifedipine and verapamil) and β-blockers (nadolol and propranolol), generally associated in pharmaceutical formulations was proposed, to achieve a procedure in which the analysis time and resolution of five antianginals will be the best using a C_{18} column and UV detection. The presented method proved the feasibility of using hybrid micellar mobile phases. The five drugs that were studied were analysed directly using a fast, simple procedure that required only one injection. Good resolution and low analysis time (9 min) were achieved due to the optimization of the composition of the mobile phase using only five experimental mobile phases. The selected mobile phase contained a surfactant and a low amount of pentanol which was retained in the micellar solution and less toxic than others used in conventional RP-LC in aqua-organic solutions.

Simple and rapid methods for the analysis of pharmaceutical preparations containing the most used antihistamines (brompheniramine, chlorcyclizine, chlorpheniramine, diphenhydramine, doxylamine, flunarizine, hydroxyzine, promethazine, terfenadine, tripelennamine and triprolidine) and other active components such as caffeine, dextromethorphan, guaifenesin, paracetamol and pyridoxine in several pharmaceutical preparations (tablets, capsules, syrups and creams) have also been developed [53]. In order to adjust the eluent strength of the micellar mobile phase and reduce the analysis time CTAB was used. The limits of detection and relative standard deviations values were sufficiently good for the applicability of this method in the quality control of pharmaceutical formulations. Due to the versatility of the interactions in MLC, it was possible to determine a great variety of compounds including those with high hydrophobicity in adequate times of analysis.

Kulikov [54] developed and validated a MLC method with UV detection for quantitative analysis of selenium (IV) in pharmaceutical preparations (multi-vitamin syrups, tablets, capsules) and premixes after pre-column derivatization with 2,3-diaminonaphthalene. The method was rapid, facile with the use of low amounts of toxic organic solvents and can be an alternative to other methods: ICP-MS, AAS. On the basis of the successful validation according to USP and ICH recommendations it can be stated that the method was specific, linear, accurate and precise within the established range.

Ruiz-Ángel *et al.* [55] studied and compared the peak shape behavior for set of β-blockers obtained using acetonitrile with that for other organic solvents

(methanol, ethanol and propanol) used as co-modifiers in mobile phases containing SDS. They considered the external peak broadening contribution to the global variance, which becomes less significant as the retention time increases. The usefulness of peak half-width plots versus retention times for this kind of study was demonstrated. The peak half-width plots can be considered as a simple tool to reveal the kinetics of the interactions of the basic drugs with the stationary phase.

4.2. Biomedical studies

MLC allows biological samples to be analyzed without needing to eliminate proteins and other interfering substances, thus considerably reducing the cost and analysis time. In addition, one of the main applications of MLC is the possibility of direct sample injection of biological material into the column due to the ability of micellar aggregates to dissolve sample proteins and other compounds. Details regarding the application of MLC in biomedical studies are summarized in Table 2.

Carda-Broch *et al.* [56] described and applied a simple, rapid and selective chromatographic procedure that used a micellar mobile phase of SDS and butanol to the control of some pharmaceutical formulations and urine samples containing trazodone. The procedure resolved the drug from its major metabolites. The chromatographic procedure proposed for the determination of trazodone in pharmaceuticals and in urine with a hybrid micellar mobile phase was rapid and simple, with analysis times below 10 min. The procedure was sensitive enough for the screening and routine analysis of trazodone at therapeutic urine levels, with limit of quantification (LOQ) value of 9.5 ng mL^{-1}. This value was obtained when the urine samples were injected without any previous treatment in order to separate or concentrate the analyte. Good sensitivity and linearity were also obtained. No interferences were found from the endogenous compounds of urine or the trazodone metabolites when analyzing real urine samples from a patient who had ingested the antidepressant.

Carretero *et al.* [57] evaluated MLC for 21 banned drugs: stimulants, anabolic steroids and diuretics. Retention parameters in the presence of variable amounts of pentan-1-ol at different temperatures were studied. The range of drugs amenable to direct sample injection was restricted to compounds with retention times longer than those provided by endogenous urine components. Analytical figures of merit for drugs excreted free in urine have been established. The detection limits obtained with UV detection at 260 nm are low enough for detection of the illegal use of drugs.

MLC has also been evaluated and compared with conventional RP-LC in order to develop a suitable chromatographic method for the separation and determination of desferrioxamine (DFO) [58] and their chelated forms, AlDFO and FeDFO in serum of haemodialysis patients. Direct injection of uremic serum using MLC, avoiding any sample preparation, for the determination of free DFO was possible but proved to be impractical in terms of sensitivity. Reversed phase conventional LC, however, allows the separation and determination of DFO, FeDFO and AlDFO in real uremic serum samples provided that a previous simple ultrafiltration step was performed. The presented technique was adequate to study removal of aluminium in renal failure patients treated with DFO.

In MLC, untreated physiological fluids directly injected into the LC system have been reported [59] to be simple allowing repetitive serial injections with no increase in system pressure, no noticeable clogging of the injection valve or analytical column, no changes in retention factors, or system contamination evident. These advantages make MLC a particularly useful technique. The use of MLC can help avoid the sample preparation step entirely, by making direct injection of the physiological sample in the chromatographic system furthermore important feature of direct injection is that the same column can be used for all assays in a series of specimens

A MLC procedure for screening and determination of four barbiturates (amobarbital, barbital, hexobarbital and secobarbital) in serum [60] has been reported, which uses a mobile phase of SDS and a modifier with UV detection and direct injection of the samples, which largely simplifies the determination of these compounds. This method can be useful for monitoring these barbiturates in the fields of forensic toxicology. The results obtained indicate that the MLC procedure can be used easily for determination of several barbiturates in serum samples with analysis time below 8 min using a mobile phase of 0.10 M SDS–4% butanol at pH 7 and showed the good recoveries obtained for each barbiturate for the two different concentrations. The procedure was sensitive enough for monitoring barbiturates in serum at therapeutic and toxicological levels, taking into account that the serum sample was injected without any pretreatment.

Izquierdo-Homillos *et al.* [61] examined micellar mobile phases containing SDS and different organic modifiers to evaluate cortisol and cortisone retention and separation characteristics using a RP Hypersil C_{18} (150×3.2-mm, i.d. 5 µm) column with a flow rate of 0.5 mL min^{-1}. Based on this study, a separation method

Table 2. Summarized details for the application of MLC in biomedical studies.

Determined substances	Matrix	Mobile phase	Detection	LOD	Refs.
trazodone	urine	0.2 *M* SDS and 8% 1-butanol, pH 3	fluorescence em. 440 nm; exc. 325 nm	9.5 ng mL^{-1}	[56]
banned drugs in sport	urine	0.1 *M* SDS, 10% methanol	UV-Vis 260 nm	0.07-10.95 $\mu g\ mL^{-1}$	[57]
desferrioxoamine and their chelated forms	uremic serum	0.02 *M* SDS pH 6, 5% acetonitrile/ phosphate buffer pH 3.5-7.5 or 0,003 M Brij-35, pH 7.4	UV-Vis 210, 220 nm	0.1 $\mu g\ mL^{-1}$	[58]
barbiturates	serum	0.1 M SDS–4% butanol, pH 7,	UV-Vis 230 nm	30-70 ng mL^{-1}	[60]
cortisol, cortisone	urine	0.18 *mM* SDS 8.3 % tetrahydrofuran,	UV-Vis 245 nm	3.7 ng mL^{-1} and 3.3 ng mL^{-1}	[61]
ciprofloxacin, enoxacin	serum	75 *mM* SDS—10 *mM* phosphate buffer—18 *mM* tetrabutylammonium bromide 3% 1-propanol at pH 3.0.	UV-Vis 281 nm	0.024 $\mu g\ mL^{-1}$ and 0.025 $\mu g\ mL^{-1}$	[62]
amitryptyline, nortryptyline	serum	0.15 *M* SDS-6% pentanol, pH 7,	electrochemical at 650 mV	0.25 ng mL^{-1} and 0.31 ng mL^{-1}	[63]
verapamil	urine, serum	0.15 *M* SDS 5% pentanol, pH 7.	fluorescence em. 312 nm exc. 230 nm	11 ng mL^{-1} and 20.2 ng mL^{-1}	[64]
sulfonamides	milk	80 *mM* SDS-8.5% propanol, pH 7	UV-Vis 490 nm	0.72 ng mL^{-1} and 2.4 ng mL^{-1}	[65]
antiretrovirals and antivirals	serum	SDS 50 *mM*, SDS 120 *mM*/4.5% propanol and SDS 150 *mM*/5% pentanol	UV-Vis 214, 260 nm	6-30 ng mL^{-1}	[67]
omeprazol and metabolites	urine, serum	0.08 *M* SDS–10% propanol–pH 7	UV-Vis 305 nm	6 ng mL^{-1} and 25 ng mL^{-1}	[68]
nicotine	biological fluids	0.15*M*–6% , pentanol, pH 6	electrochemical at 0.8 V	4 ng mL^{-1}	[69]
tamoxifen and endoxifen	plasma	0.15 *M* SDS–7% n-butanol, pH 3	fluorescence em. 380 nm exc. 260 nm	50 ng mL^{-1} and 75 ng mL^{-1}	[70]
anti-HIV agents	simulated gastric and intestinal fluids	0.05 *M* SDS–1% 1-butanol, pH 3	UV-Vis 267 nm	7-15 $\mu g\ mL^{-1}$	[71]

that could be used for the simultaneous determination of cortisol and cortisone using tetrahydrofuran (THF) as organic modifier, that provides good resolution with short elution time, has been developed and applied to urine samples of rugby players before and after stress for doping control purposes. Different separations with different selectivities were obtained depending on the nature of organic modifier and SDS concentration. Satisfactory results were obtained using THF, which was adequate for urine sample analysis.

A MLC method for the simultaneous determination of bactericide fluoroquinolones ciprofloxacin and enoxacin in human serum samples [62] has been reported. The MLC technique was easy to handle and showed good reproducibility. Low amounts of required sample, simple sample treatment, good recovery rates and relative short analysis times were the main advantages of this method, making it a useful method for clinical and medical researchers.

A simple and easy to use MLC method for the determination of amitriptyline and its active metabolite, nortriptyline, in serum samples was developed [63]. Direct injection of the samples simplified the method and electrochemical (ED) detection increased the sensitivity and selectivity of the determination in the biological matrix. The proposed procedure could be applied to determine these drugs in routine monitoring analyses. ED detection was useful to determine the drugs at low concentrations, taking into account that the biological samples were injected after diluting them 10 times with the micellar mobile phase. For final verification, a conventional aqueous-organic method that uses previous extractions steps was selected from bibliography to be compared with the developed micellar procedure. Authors obtained similar recoveries as those using micellar procedure.

Verapamil content in biological fluids was determined using a new simple and easy to use MLC method [64].

The method was partially validated according to FDA guidelines, and applied to determine verapamil in real urine samples of a volunteer under treatment for hypertension. The LOQ achieved using the fluorescence detector was sensitive enough to check levels of toxicity in serum and can be used in the area of clinical or forensic sciences. The use of a mobile phase consisting of 0.15M SDS-5% pentanol at pH 7 allowed the elution of verapamil in 12.5 min. Results of calibration curves, repeatability, reproducibility and accuracy granted the method to be useful for quality control in the determination of verapamil in pharmaceutical formulations. To validate the method in biological fluids, it was applied to serum and urine. Results of spiked samples were in accordance with the injected quantity, and recoveries were close to the concentration that was added.

A procedure for simplified sample preparation for simultaneous determination of six sulfonamides in milk was described [65]. The strategy of forming chromogen sulfonamides together with the use of MLC increased selectiveness in biological matrices. Derivatisation of sulfonamides coupled with the Bratton–Marshall reagent [*N*-1(naphythyl)ethylene-diamine dihydrochloride (NED) was simple, fast and reproducible, with absorptivities at visible wavelengths that improve the signal-to-noise ratio when dealing with complex samples (*e.g.* milk). This method does not require complex procedures such as sample extraction and/or sample cleaning, and there is no need for large volumes of samples and solvents. Analysis times are below 16 min, with LODs and LOQs that were smaller than or similar to the values reported in the literature. Results obtained indicate that the procedure described is useful for screening and quantification.

The development of in vitro tools for estimation and prediction of pharmacokinetic and pharmacodynamic properties of drug candidates is an alternative to the traditional studies. Wang *et al.* [66] presented an approach (*i.e.*, QRAR models) that could reduce experimental efforts and costs and facilitate screening of drug candidates for their pharmacokinetic and pharmacodynamic properties. The retention of compounds could predict *in vitro* human bioactivity parameters of HMG-CoA reductase inhibitors. This approach could be helpful in the development of new HMG-CoA reductase inhibitors in the early stages of studies.

Studies aimed to develop and validate a fast, simple method for monitoring 13 antiretrovirals and antivirals used in the treatment of patients with AIDS has been reported [67]. This study employed three mobile phases that contained SDS alone or with propanol, butanol or pentanol along with direct injection of human serum. Thanks to the absence of interferences, the methods were specific and reliable. The method does not require complex procedures such as sample extraction and/or sample cleaning. Chromatographic analyses required only 9, 9 and 18 min for the three recommended mobile phases, respectively, making this method suitable for rapid monitoring and quantifying of antiretrovirals and antivirals.

A sensitive method for the simultaneous determination of omeprazol and its two main metabolites (omeprazole sulphone and 5-hydroxyomeprazole) in biological fluids without any previous pretreatment was developed and validated [68]. It was also applied to the analysis of omeprazol in pharmaceutical formulations. The method was validated according to Food and Drug Analysis (FDA) guidelines, with an analysis time below 11 min. Validation according to FDA guidelines was performed efficiently with satisfactory results for selectivity, precision and accuracy. The limits of detection and quantification were good enough to monitor these compounds in physiological matrices. This procedure also allows pharmacokinetic studies to be conducted. Pharmaceutical formulations containing omeprazole were analyzed and good claim percentages (around 100%) were obtained. The results showed that the procedure was sensitive enough for routine analyses of the drug in both biological and pharmaceutical applications.

Chin-Chen *et al.* [69] developed a MLC procedure for rapid screening and determination of nicotine in cigarette, pharmaceuticals and serum samples using a hybrid SDS-modifier mobile phase with electrochemical detection and direct injection of the samples. The method was validated according to the ICH harmonized tripartite guideline. This method can be useful to analyze compounds tobacco products and pharmaceutical quality control as well as in clinical monitoring in the de-addiction and forensic cases. A major advantage of the method developed is the capability to perform direct injection of biological as well as pharmaceutical or commercial samples by solublizing the component in desired solvent. The variety of possible interactions gives a large versatility to this technique as an alternative to conventional LC and makes it appropriate for a wide range of solute analysis.

Aranda *et al.* [70] performed a fast, easy, accurate and reliable methodology using micellar mobile phases for the simultaneous quantification of tamoxifen and endoxifen in the plasma of breast cancer patient samples. This methodology has been validated following the ICH guidelines in terms of linearity, limits of detection and quantification, intra- and inter-day precisions, selectivity, recovery and robustness. MLC has proved

to be a suitable technique. One advantage of this procedure is the possibility of injecting diluted sample into the chromatographic system, thus avoiding long, tedious extractions. After sample irradiation to improve sensitivity, the analyte was satisfactorily resolved using a mobile phase of 0.15 mol/L SDS–7% n-butanol at pH 3 from the matrix in an analysis time of under 20 min. The LOD and the lineal range were sufficient to detect the usual amount of tamoxifen and endoxifen in patients' plasma.

According to the guidelines for the Industry, it is necessary to employ an appropriate analytical method to evaluate correctly the intact drug molecules in the presence of their degradation products. Raviolo *et al.* [71] evaluated the stability behaviour of three new anti-HIV agents, which were obtained by the association of zidovudine (AZT) with different amino acid derivatives: acid group (AZT-Ac), leucine (AZT-Leu) and valine (AZT-Val). The agents were first analyzed in aqueous matrices and later in SGF and SIF using MLC, and then the results were compared with those obtained with LC to establish MLC method as a stability indicating LC method. The significant degradation (>5%) of AZT-Ac, AZT-Leu and AZT-Val evaluated in this study may indicate potential drug instability in the gastrointestinal tract. Based on these results, zidovudine derivatives were considered unstable in SGF and SIF at 37°C for reaction times of 1 h and 3 h, respectively, in accordance with the FDA/CDER's BCS guidelines. The studies have demonstrated that they must be formulated in a form that differs from an oral one. These analytical methods have been validated and were in accordance with all the necessary analytical stability requirements.

5. Concluding remarks

Micellar Liquid Chromatography is a powerful separation technique that has been applied to different biomedical and environmental studies of complex and single compounds. Micellar eluents can be used to dissolve samples or extract analytes. Surfactants can cosolubilize nonpolar and polar compounds, derivatization reagents and products. They can also induce favorable shifts in the equilibrium constants and spectral properties, inhibit undesirable reactions, stabilize reaction intermediates, and expedite the reactions. MLC allows for direct injection of the samples thus facilitating faster analysis procedure compared to conventional methods of analysis. Finally MLC analysis meets the requirements of "green chemistry" conception by using environment-friendly reagents; micellar mobile phases are less toxic and flammable and have lower environmental impact compared to conventional LC methods.

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