Determination of drugs in pharmaceuticals and pesticides by micellar liquid chromatography

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Micellar liquid chromatography (MLC) is a reversed-phase liquid chromatography technique that uses surfactants as components in the mobile phase at a concentration higher than the critical micellar concentration. Chromatographic procedures using micellar mobile phases have been reported for the analysis of pharmaceutical formulations commercialised as tablets, pills, capsules, drops, solutions, syrups, gels, suspensions, enemas, sprays, oily injections, ointment and creams. The compounds studied in this work include benzodiazepines, phenethylamines, antidepressants, vitamins, and corticosteroids formulations, and carbamates pesticides. These compounds are usually determined by reversed-phase liquid chromatography (RPLC) with aqueous-organic mobile phases. MLC has the advantages of avoiding sample pretreatment, analysis time, accuracy, reproducibility, toxicity, environmental impact and low cost of the procedures respect to the classical RPLC. Some features of the analytical procedures are examined including modelling of the retention behaviour of solutes, selection of column, surfactant and alcohol, study of hydrophobicity, and screening analysis. Usually, optimum pH is fixed at 7, but pH 3 is used in the case of the most hydrophilic analytes. C18 columns are often used, but C8 columns allow the obtention of rapid procedures with the lowest analysis time. Pentanol, butanol or propanol are used in function of the hydrophobicity of the substrate. Finally, optimised procedures have been applied for the determination of the substances in pharmaceuticals and in biological samples, including serum and urine.

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Micellar liquid chromatography (MLC) can be considered as an offspring of ion-pair chromatography, which appeared in the sixties. MLC itself was introduced as a chromatography technique that uses surfactants in the mobile phase composition, at a concentration higher than the critical micellar concentration (cme), in the late seventies by Armstrong and developed and promoted in the eighties. Thus, MLC applications have been described in the fields of biological separations, hydrophobicity and quantitative structure retention relationships, in rapid analysis of untreated physiological fluids (urine, serum, etc.) or pharmaceuticals.

The separation in MLC is achieved due to the differential association of the solutes between the micelles in the mobile phase and to the stationary phase, which is modified by the adsorption of monomers of surfactant. Non-polar solutes only experience hydrophobic interactions, and positively or negatively charged solutes also have electrostatic attraction or repulsion, depending on the pH and the nature of the surfactant. Pure micellar solutions are often useless as mobile phases due to the long analysis time and low efficiencies achieved. To improve these two chromatographic parameters, a small amount of an organic modifier is added. In classical reversed-phase liquid chromatography (RPLC), the amount of organic solvent in the mobile phases usually exceeds 30-40% (v/v). In contrast, the percentage of alcohol is decreased in MLC with the addition of the surfactant; this point depends on the selected alcohol, as well as on the surfactant concentration used.

In many instances, micellar solutions can replace classical aqueous organic mixtures in the chromatographic control of pharmaceutical preparations with good results. Mixtures of drugs of different polarity can be resolved without the requirement of gradient elution. Moreover, organic modifiers in MLC (i.e., propanol, butanol and pentanol) are less toxic than those employed in RPLC (i.e., methanol and acetonitrile), and are highly retained in the micellar
solution, which reduces the risk of evaporation. Micellar mobile phases can therefore be kept stable for a long time and reduce the flammability, toxicity, environmental impact and cost of RPLC.

The stable and reproducible behaviour of solutes in micellar mobile phases allows an accurate prediction of their retention, using models, which can be fitted with data measured according to simple experimental designs. These models can be used to obtain the optimum mobile phase composition (pH and concentrations of surfactant and organic modifier) that separates the drugs.

Several chromatographic procedures using micellar mobile phases have been reported for the analysis of formulations commercialised as tablets, pills, capsules, drops, solutions, syrups, suspensions, enemas, sprays, oily injections, ointment, and creams. The compounds included in this work are: benzodiazepines, phenethylamines, antihistamines, vitamins, and corticosteroids formulations, and carbamates pesticides. There are different therapeutic actions of these drugs. Benzodiazepines are used as psychotherapeutic agents with hypnotic, antidepressive, anticonvulsant, and tranquillising properties. Phenethylamines are adrenegics and stimulants of the central nervous system. Antihistamines are used primarily for the symptomatic relief of hypersensitivity reactions, such as urticaria and angioedema, rhinitis and conjunctivitis, and in the control of pruritus associated with skin disorders. Therapeutic multivitamins are advisable for use in cases of deficiency in pathological conditions in which nutritional requirements are greatly increased or in conditions in which absorption, utilization, or excretion of vitamins is abnormal. The glucocorticosteroids can help to reduce inflammation, and are also exceptionally effective at reducing asthma, and the symptoms of allergic rhinitis.

In this review, C18 columns and hybrid mobile phases of alcohol and sodium dodecyl sulfate (SDS) were usually used. SDS is by far the most common surfactant employed in MLC. Some features of the analytical procedures are also examined, including mathematical treatment, selection of column type, surfactant and alcohol, correlation between retention and polarity, and screening analysis.

Developing a MLC method

To develop a MLC procedure several factors should be taken into account such as the nature and concentration of surfactant and modifier, and the pH of the mobile phase. Other less important factors are temperature and ionic strength. MLC is more complex than conventional RPLC due to the number of interactions of the compounds with mobile and stationary phases. Hydrophobic interactions inside lipophilic micelles, electrostatic interactions with the outside layer of anionic micelles and similar interactions are established in the stationary phase modified with adsorbed monomers of detergent. Finally, steric factor may be important.

**Modelling of the retention behaviour**

In liquid chromatography, the interpretative strategy to optimize the behaviour of compounds can be better than sequential approaches. The main requirement to achieve reliable results is the accurate description of the retention behaviour. Equations based on mechanistic models are usually used in MLC to describe the solute retention. The prediction errors obtained with the most adequate models and corresponding experimental designs are usually below 3-4%.

The mechanistic models proposed in MLC are based on the classical equation proposed for the capacity factor (k) using micellar mobile phases at a fixed volume fraction of organic modifier, which can be written as in Eqn (1).

$$k = \frac{K_{AS}}{1 + K_{AM}[S]}$$

where [S] is the concentration of surfactant, $K_{AS}$ the product of the solute-stationary phase partition coefficient by the phase ratio, and $K_{AM}$ the solute-micelle association constant.

Other model used for micellar mobile phases containing a modifier as expressed in Eqn (2).

$$k = \frac{1}{1 + K_{AM}[M]} \left( \frac{K_{AS} - 1}{1 + K_{MD}[S]} \right)$$

where [M] is the concentration of modifier, $K_{AS}$ and $K_{AM}$ correspond to the equilibria between solute in bulk water and stationary phase or micelle, respectively; $K_{AP}$, $K_{SP}$ and $K_{MP}$ measure the relative variation in the concentration of solute in bulk water, stationary phase and micelles due to the presence of modifier, referred to a pure micellar solution (without modifier).

Equation (3) is an example of a model that considers the simultaneous effect of three factors:
surfactant, modifier and pH, developed for solutes exhibiting a weak acid-base behaviour. 

\[ \frac{1}{K_{11M}} = \frac{1}{K_{11} [M]} + \frac{1}{K_{11D} [M]} \frac{1}{K_{11}[H]} \]

where

\[ \gamma_A = 1 + K_{AM} \frac{1}{1 + K_{AD} [M]} [S] \]

\[ \gamma_{HA} = 1 + K_{HAM} \frac{1}{1 + K_{HAD} [M]} [S] \]

\[ \gamma_{13} = 1 + \frac{K_{13} [M]}{1 + K_{13} [M]} \]

\[ \gamma_{23} = 1 + \frac{K_{23} [M]}{1 + K_{23} [M]} \]

\[ \gamma_{33} = 1 + \frac{K_{33} [M]}{1 + K_{33} [M]} \]

\[ \gamma_{12} = 1 + \frac{K_{12} [M]}{1 + K_{12} [M]} \]

\[ \gamma_{22} = 1 + \frac{K_{22} [M]}{1 + K_{22} [M]} \]

\[ \gamma_{32} = 1 + \frac{K_{32} [M]}{1 + K_{32} [M]} \]

\[ \gamma_{31} = 1 + \frac{K_{31} [M]}{1 + K_{31} [M]} \]

\[ \gamma_{1} = 1 + \frac{K_{1} [M]}{1 + K_{1} [M]} \]

\[ \gamma_{2} = 1 + \frac{K_{2} [M]}{1 + K_{2} [M]} \]

\[ \gamma_{3} = 1 + \frac{K_{3} [M]}{1 + K_{3} [M]} \]

\[ \gamma_{0} = 1 \]

\[ A = \frac{B}{A} + 1.25 \]

\[ N = \frac{1}{k} \left( \frac{1}{R + B} \right)^{2} \]

The efficiencies of the peaks were evaluated using Equation 7, suggested by Foley et al. 

The development of an analytical procedure for the analytical control of drugs in formulations is made through a sequential optimisation following the steps discussed above. First of all, a preliminary pH study should be made. To illustrate this, the example of benzodiazepines has been selected. In the literature two acid-base equilibria were found (log \( K_{1} \) 2-3 and log \( K_{2} \) 8-11) for benzodiazepines. These dissociation constants will increase in the presence of anionic SDS micelles, owing to stabilization of the positive charge of the protonated drugs. The working pH range of a column is from 2.5 to 7.5. Thus, it is necessary to select an adequate working pH to determine the drugs under study.

**Selection of column**

In MLC, the micelle formation property is linked to the mobile phase. Micelles act in the same way as the organic modifier in conventional RPLC. A significant number of surfactant molecules may be adsorbed on the stationary phase surface changing its properties; this quantity of adsorbed surfactant is related to the behaviour of the analytes.
Adsorption isotherms of SDS have demonstrated that the amount of adsorbed surfactant on C8 columns is lower than in C18 columns\(^1\). The adsorption curves increase rapidly and reach a plateau for an SDS concentration higher than the cmc. For C18 columns, the adsorbed amount is constant above the critical micellar concentration \((8 \times 10^{-3} \text{ M})\), but the plateau is only reached at 0.3 M SDS for C8 columns. The amount of anionic surfactant on the bonded phase is even lower for cyano columns and increases always with the concentration of surfactant in the mobile phase, at least up to 0.4 M SDS\(^2\).

Silica-based C8 and C18 columns are the most common stationary phases in RPLC. In these columns, selectivity and peak shape are influenced largely by the underlying silica rather than the bonded phase. Cyano columns are not frequently used. They show some hydrophilic properties due to the weakly polar cyano functionality but can be used for RPLC because of their short alkyl chain\(^2\).

To explain which column is better for a given analysis, the example of phenylethylamine-antihistamine combinations was chosen. Five phenylethylamines and ten antihistamines (see Figure 1 for identi-

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**Figure 1** — Retention factors (A), plate number (B), and asymmetry factor (C) for the phenylethylamines and antihistamines chromatographed with three stationary phases using 0.15 M SDS-2% pentanol at pH 7. Compounds: (1) phenylephrine, (2) phenylpropanolamine, (3) ephedrine, (4) pseudoephedrine, (5) methoxphenamine, (6) pheniramine, (7) carboxamine, (8) doxylamine, (9) chlorpheniramine-dexchlorpheniramine, (10) dextromethorphan, (11) diphenhydramine, (12) triprolidine, (13) azatadine, and (14) phenyltoloxamine. Reprinted with permission from Reference 15.
fication) were analysed in cough-cold pharmaceutical preparations using C8, C18 and cyano columns. The micellar mobile phases used for the analysis contained SDS and pentanol as a modifier, buffered at pH 7 using UV detection. Figure 1 compares the retention factors, efficiencies (N) and asymmetry factors (B/A) of the drugs for the three columns using a mobile phase of 0.15 M SDS-2% pentanol buffered at pH 7. The retention factor decreases at increasing concentration of SDS and pentanol for both C8 and C18 columns. For the C18 column the effect was more pronounced, being the solutes strongly retained in the absence of modifier. For the cyan column, the retention also decreases at increasing concentration of the surfactant, but increases when organic modifier was added. This column was not used for its low efficiencies (in the 100-1500 range) and high asymmetry factors (often >5). In a C18 column, the efficiencies and asymmetries of phenethylamines were usually in the 3000-5000 range and less than 1.3, respectively, while for antihistamines were in the 500-1500 range and less than 2.1, respectively. Better efficiencies and similar asymmetries were obtained using a C8 column, (N= 4000-6000 and B/A< 1.4 for the phenethylamines, and N=2000-2500 and B/A< 1.9 for the antihistamines). Because of these better values and also the lower retention times of the compounds, this column was finally chosen to separate the phenethylamine-antihistamine mixtures in the pharmaceuticals.

Selection of the type of surfactant and alcohol

An example of carbamates is chosen to illustrate the selection of the surfactant in pure micellar mobile phases. In that case, two surfactants were tested, SDS as anionic, and Brij-35 as non-ionic surfactant. The main difference between both is the presence of negatively charged micelles while using SDS and non-charged micelles when Brij-35 is used.

The usual behaviour of drugs when pure micellar mobile phases of SDS and Brij-35 are used is the diminution of retention times at increasing concentrations of surfactant. As can be seen in Figure 2, the graphics of k vs surfactant concentration usually draw a curve (Figure 2A for SDS), but in the case of Brij-35 a straight line was observed (Figure 2B) for poorly retained solutes like propoxur and carbofuran in the concentration range studied, and for carbaryl, methiocarb and desmedipham in the 0.05 to 0.1 M range.

The elution strength of Brij-35 is higher than SDS, that is in accordance with their aggregation numbers, and consequently the concentration of Brij-35 required for the separation of carbamates was less than the SDS. On the other hand, better separation was achieved using Brij-35. Desmedipham, the compound more retained, had a k value of 63.1 in 0.05 M SDS while decreased up to 21.3 using the same concentration of Brij-35. For this reason Brij-35 was selected to carry out the determination of carbaryl and other carbamates.

![Figure 2](image_url) — Effect of SDS (A) and Brij-35 (B) concentration on the retention of five carbamates, (○) carbaryl, (+) carbofuran, (△) desmedipham, (★) methiocarb, and (□) propoxur. Reprinted with permission from Reference 18.
The selection of alcohol is directly related to the polarity of the studied compounds. In the elution of hydrophobic substances, alcohols like butanol or pentanol are required due to their high elution strength that increases with the length of its carbon chain.

An indicative work to explain the selection of the modifier was made using phenethylamines. The association of the protonated phenethylamines to an SDS-modified C18 column was too strong as indicated by the long retention times of the drugs when were eluted with pure micellar eluents of the surfactant and also with mobile phases containing a weak modifier, such as propanol. Two alcohols, butanol and pentanol were selected to expedite the elution of the studied compounds. The concentration range studied for the surfactant and these modifiers was: 0.05-0.15 M for SDS, 3-6% for butanol and 2-5% for pentanol.

In MLC, the peak efficiencies decrease at increasing concentration of surfactant, but increase at higher concentrations of modifier. On the other hand, the retention factors decrease at increasing concentrations of SDS and modifier. It should be noted that the efficiencies obtained with the hybrid mobile phases of SDS-butanol and SDS-pentanol for the studied compounds are very high, mostly in the 3000-7000 range. The upper reported values of efficiency in MLC are frequently below 4000 (ref. 25).

The retention factors for the nine phenethylamines for experimental mobile phases of pentanol and butanol of similar elution strength (0.05 M SDS-2% pentanol vs 0.05 M SDS-3% butanol, 0.05 M SDS-5% pentanol vs 0.05 M SDS-8% butanol, and 0.15 M-2% pentanol vs 0.15 M SDS-3% butanol) are plotted in Figure 3. As can be seen, for the three pairs of mobile phases the points are approximately aligned along a straight-line with a slope close to the unity, which indicates that the relative interactions of all compounds with the micelles modified with both butanol and pentanol are similar. Butanol and pentanol show a distinctive behaviour when dissolved in a micellar medium of SDS, in comparison to other alcohols (i.e., methanol and propanol) or other organic solvents (i.e., acetonitrile and tetrahydrofuran). These alcohols are inserted into the micellar assembly, owing to their low solubility in water and also due to their particular structure that combines a polar group with a non-polar chain, similar to that of surfactant molecule. The alcohol and surfactant molecules align together in the micelle palisade, the polar hydroxyl group of the alcohol orientated towards the stern layer and the alkyl chain located in the non-polar micelle core. This gives rise finally to swollen mixed micelles.

Generally, HPLC procedures that use micellar mobile phases have the advantage of using small quantities of organic modifier. Furthermore, butanol and pentanol are less toxic than methanol or acetonitrile, used in conventional RPLC and moreover they are retained in the micellar solution of SDS, reducing the risk of evaporation.

Study of hydrophobicity of antihistamines

Good correlations have been observed in MLC between the retention data and the polarity of antihistamines (carbinoxamine, chlorpheniramine, cyclizine, cyproheptadine, dextromethorphan, diphenhydramine, doxylamine, pheniramine, phenyltoloxamine, pyrilamine, and tripelennamine). The structure of these molecules usually consists of 3 portions: a nucleus composed of aromatic or heterocyclic groups, a linkage such as nitrogen, oxygen or carbon, and an ethylamine group. Most of the studied antihistamines are highly hydrophobic as indicated by their log $P_{ow}$ values ranging from 2.02 to 4.92.
The retention data \((k\) or \(\log k\)) of the antihistamines eluted with experimental mobile phases of SDS-pentanol were plotted against the \(\log P_{ow}\) values to examine what kind of correlation exists between both parameters. It was found that the correlations were better for the \(\log k\) versus \(\log P_{ow}\) plots.

The retention behaviour of antihistamines was modelled using Eqn (2), employing only a few combinations of experimental mobile phases. Figure 4 depicts the regression coefficients of the \(\log k\) versus \(\log P_{ow}\) plots using calculated \(k\) values obtained with the aid of Eqn (2) for 99 mobile phases of SDS-pentanol homogeneously distributed in the selected factor space. The relatively high values of regression coefficients achieved in the \(\log k\) versus \(\log P_{ow}\) plots for a wide range of concentrations of the modifier indicate that the main factor that governs the retention is the hydrophobicity. Other effects, such as steric interactions or electrostatic attraction between positively charged antihistamines and negatively charged SDS micelles (or free silanol groups) on the chromatographic column are less important.

A parameter independent of the modifier concentration might also be useful in correlation studies. The solute-micelle association constant, \(K_{AM}\), shows a very good correlation with \(\log P_{ow}\) appearing as an interesting parameter to measure the hydrophobicity. In view of these results, the usefulness of micellar mobile phases in quantitative retention-structure relationship studies should be considered.

**Screening analysis**

MLC has been used to analyze physiological samples and is of an important use in pharmaceutical research, clinical chemistry, food quality control, doping and toxicology. Conventional methods raise lot of problems. Frequently, drugs are in very low concentration, strongly bound to proteins and in a complex matrix where interference from numerous endogenous compounds is expected, for this reason the direct use of conventional mobile phases is not feasible.

The results obtained in the study of nine phenylethylamines (see Figure 5 for identification) can be useful to examine the possible screening of these drugs in physiological fluids. In order to obtain the complete resolution of the nine phenethylamines, a reduced and selected number of mobile phases were used. Figure 5A shows the contour map for the separation of the drugs using mobile phases of SDS-pentanol. Maximum resolution was achieved with 0.115 \(M \) SDS-3.0\% pentanol \((R = 0.443)\). The chromatogram of the optimum mobile phase is given in Figure 5B.

The separation of seven phenethylamines (arterenol, phenylephrine, tyramine, pseudoephedrine, methoxymetamphetamine, phenylpropanolamine, and amphetamine) was also studied. Figure 5C shows the corresponding contour map where three narrow regions of maximum resolution were obtained for the compositions: 0.05 \(M \) SDS-2.6\% pentanol \((R = 0.969)\), 0.133 \(M \) SDS-3.6\% pentanol \((R = 0.948)\), and 0.15 \(M \) SDS-2.2\% pentanol \((R = 0.927)\). Figure 5D depicts the chromatogram of the mobile phase 0.133 \(M \) SDS-3.6\% pentanol.

Two important advantages of this technique are the direct injection of physiological samples (urine or serum), and the possibility to determine a mixture of different chemical structures from a single injection.
To develop a new procedure in MLC for the determination of drugs in formulations, it is necessary to make calibration graphs, and calculate limits of detection (LODs), repeatabilities and intermediate precision.

Usually, calibration graphs are constructed at least by triplicate injection of five solutions of the drugs at increasing concentrations (from 0.5 to 25 g mL⁻¹).

Calibration curves were obtained by measuring peak areas of each drug eluted with the optimum micellar mobile phase. Linear regression coefficients were usually $r > 0.999$.

LODs are calculated using the 3s criterion that corresponds to a signal equal to 3 times the standard deviation of the background noise, i.e., the signal-to-noise ratio is equal to 3. LODs are usually less than those required for the analysis of the pharmaceuticals.
Repeatabilities or intra-assay precision (average of ten measurements made the same day), and intermediate precision (average of ten measurements of repeatabilities taken on ten days over a three-months period and made by different analysts, equipments, etc) at three different drug concentrations in the range of calibration graph, are also calculated.

Analysis of formulations

Analytical procedures using micellar eluents are often considered inferior to the conventional ones that use hydro-organic mobile phases. Drugs are easily extracted when the samples are treated with micellar solutions, but the excipients are frequently not soluble in the micelles. The addition of a small amount of alcohol before micellar solution can improve the solubility. The drug solution can be injected into the chromatograph without any other treatment than filtration which reduces the time in the sample preparation.

The sample preparation is very simple and depends on the type of formulation studied. For example, capsules, tablets and pills (solid form); drops, solutions, suspensions, sprays, oily injections and syrups (liquid form); and ointment and creams29.

The recommended treatment for different type of drug formulation samples are as follows:

Solid: Ten tablets are weighed, ground to fine powder and homogenized and then several portions of this powder are taken, weighed and dissolved with an adequate mobile phase. For capsules, 10 units are weighed and after that they are carefully emptied and cleaned to obtain an accurate weight of the capsule contents.

Liquid: Three aliquots of the homogenized samples are mixed separately with a small amount of alcohol and diluted with a solution of SDS.

Ointments and creams: The analysis are performed by mixing 2 g with a hybrid micellar solution. When the sample contains fat-soluble excipients in large concentrations, an emulsion is formed. In this case, more volume of hybrid micellar solution is added to further dilute the sample and to obtain a clear solution for analysis, using also the aid of a mechanical stirring and an ultrasonic bath.

Following this sample preparation in a MLC procedure and selecting an adequate micellar mobile phase, the recoveries usually agreed with the compositions declared by the manufacturers within the tolerance limits, and with those obtained using methods that employ aqueous-organic mobile phases. The required times for sample preparation are shorter than those obtained when a recommended conventional procedure is used, which often need long and tedious extraction steps.

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