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MICELLAR LIQUID CHROMATOGRAPHY, A GREEN TECHNIQUE FOR ANALYSIS OF DRUG FORMULATIONS; A REVIEW

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ABSTRACT: The most commonly used analytical method in drug research is reversed-phase high-performance liquid chromatography (RP-HPLC). RP-HPLC methods also use huge quantities of solvents and generate by-products that must be disposed of, posing environmental and operator health worries. Micellar Liquid Chromatography (MLC) is a green chromatographic process for pharmaceutical analysis in which surfactants are applied above the Critical Micellar Concentration in the mobile phase (CMC). The ability of micelles to solubilize compounds that are not soluble or sparingly soluble in water is one of their properties; this property is critical for advancing analytical methods. MLC is also compliant with current RP-HPLC systems. As a result, no changes to the current RP-HPLC instrument are needed. MLC (micellar liquid chromatography) is a green and long-term method for analyzing medication compositions. MLC is not only the more environmentally friendly option, but it is also the more cost-effective. It is believed to have a mobile phase of 90% or more water (v/v), which can be recycled if injection volumes are small and far apart. The present review work will focus on analyzing and quantifying antiretroviral, anti-diabetic, antitumor, and cardiac drug formulations adapting the MLC method.

INTRODUCTION: Micellar Liquid Chromatography (MLC) is a type of high-pressure liquid chromatography (HPLC) that uses micelles to separate molecules and in which the mobile phase contains an aqueous surfactant solution that is greater than the critical micellar concentration (CMC). Surfactants help modify internal interactions and decrease the quantity of organic solvent in the mobile phase that can be regenerated because of the high boiling point [1].

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Anionic Sodium dodecyl sulphate (SDS), cationic Cetyltrimethyl ammonium bromide (CTAB), and non-ionic Polyoxyethylene 23 lauryl ether are the most widely used surfactants for MLC. Surfactant concentrations should be kept low in MLC since a high concentration would result in a viscous solution, which would result in unwanted high device pressure and history in UV detectors [2].

A more advanced version of reversed-phase liquid chromatography (RPLC) offers higher resolution, peak performance, and time retention. A pure micellar solution is used as a mobile step in RPLC because it is inexpensive, non-toxic, and has a low environmental impact. Micelles are used in a number of methods, including electro kinetics, chromatography, ultra-filtration, and cloud point extraction [3-5].

The mobile phase's pH is also essential when developing MLC methods; the pH range for the mobile phase is 2.5–7.5. Two key factors determine pH values: the quality of the surfactant and the analytes used. Acidic buffer such as citric acid buffer or Phosphoric acid buffer is generally used to maintain the Micellar mobile phase's pH [6].

The two most important components of an RPLC system are the aqueous mobile phase, which is polar and non-polar stationary. There is an aggregation of amphiphilic micelle in surrounding bulk water or aqueous organic solvent containing surfactant monomers in a concentration approximately equal to the CMC are considered homogeneous hydro-organic mobile phases. When micellar solutions are made up of two different media are considered to be heterogeneous. The adsorption of surfactant monomers changes the stationary step, creating a structure similar to an open micelle and decreasing silanophilic interactions. In a micellar solution, the concentration of monomer surfactants must be constant and equal to CMC. As a result, varying the mobile phase's micelle concentration has no impact on the stationary phase's composition. This is in contrast to the behavior observed in RPLC, where the structure and conformation of the alkyl-bonded process are affected by the hydro-organic eluents [6]. MLC has the disadvantage of lowering column efficiency during the stationary process due to mass transfer limitation [7]. Three key approaches have been used to improve MLC efficiency: introducing small modifiers into the mobile phase, lowering the flow rate, and increasing the column temperature.

MLC has many benefits and is superior to RPLC in terms of cost and environmental impact. It can administer fluids directly. MLC is particularly useful for the study of serum, urine, and plasma since micelles can solubilize proteins [8]. MLC is very helpful in identifying the class of drugs known as b-antagonists, also known as beta-blockers, in urine samples [9]. MLC is also useful because it takes less time to prepare sample. MLC may also be used to analyze and separate inorganic compounds, such as simple ions. Ion-exchange or ion-pairing chromatography provides greater selectivity of inorganic ions than MLC [10]. Some researchers used the MLC technique to examine the stability of three new anti-HIV agents obtained by combining zidovudine with various amino acids, simulated gastric fluid and simulated intestinal fluid [11]. MLC technique is used to estimate toxic and hazardous substances in environmental samples and poisonous aromatic amines such as benzidine, 1-amino-2-methyl-benzene and 2-methoxy-5-methylaniline present in wastewater [12]. The present review focuses on how different drug formulations such as antiretroviral drugs, anti-diabetic drugs, antitumor drugs, and anti-cardiac drugs are analyzed and quantified in the MLC method.

Surfactants Used in MLC: A good MLC surfactant has a low CMC, aggregation number, and, in the case of charged surfactants, it should have a low Kraft point. It is the temperature when the solvation of ionic surfactants matches its CMC, which is much lower than room temperature. Surfactants with high CMC increase the viscosity of mobile phase and column pressure and create background noise in UV detectors. As UV detectors are commonly used for MLC, a surfactant with low molar absorptivity at the working wavelength is required. As micelles are only a few nanometers in size, light scattering by micelles should not be a concern.

Several surfactants of various types meet these requirements; however the amount of surfactants used in MLC has been reduced. More than 65 % of MLC analytical studies from 1997 to 2007 included sodium dodecyl sulphate (SDS) as a possible surfactant [6]. Other surfactants are cationic cetyltrimethylammonium bromide (CTAB) and non-ionic polyoxyethylene-(23)-dodecyl ether (Brij–35).

About 10.75% of other surfactants are used, including charged, uncharged, and zwitterionic surfactants. SDS is commonly chosen since it is commercially available in high purity and at low cost. SDS is widely utilized in detergent and cosmetics manufacturing and other scientific domains. Compared to RPLC with hydro-organic combinations, it allows for more cost-effective operations. SDS also dissolves proteins efficiently in physiological matrices (urine, plasma, serum, *etc*.), allowing for direct insertion of the samples into the chromatograph without any more treatment beyond filtration [13, 14]. Direct injection of samples is impossible in the case of cationic surfactants.

Hundreds of injections of pharmaceutical samples can be accommodated in typical SDS-modified octadecyl (C18) columns without causing back pressure to rise or column performance to deteriorate. Despite these factors, SDS is commonly employed since it has been used in hundreds of MLC experiments and its dynamics are more understood than those of other micellar systems. Brij-35, a non-ionic molecule, has also been used in clinical studies and QSAR tests [14, 15]. It was claimed that RPLC using Brij-35 micelle solutions as mobile phases may mimic the partitioning process in biomembranes *in-vitro*. As a result, MLC has been called "biopartitioning MLC" when used with Brij-35 micellar mobile phases.

MLC Mechanism: Hybrid micellar eluents with higher mobile phase strength are utilized to increase the resolution and intensity of the chromatograms. Three types of equilibriums, distribution, partition and direct transfer, have been observed at the time of retaining solutes in MLC between micelle, eluting medium and stationary phase. Distributing solutes between elution medium and micelles greatly influences solvent retention and selectivity. **Fig. 1** shows a model of phase equilibrium between three phases. The solute distribution between aqueous medium to micelles (P_{MW}) and from the aqueous mobile phase to the stationary phase (P_{SW}) as well as direct transfer from the micelles in the mobile phase into the stationary phase (P_{MS}) was reported to regulate retention behaviour in MLC [16].

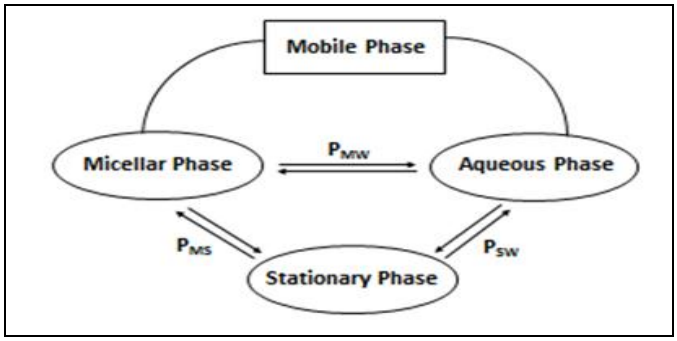

FIG. 1: EQUILIBRIUM BETWEEN MOBILE PHASE, STATIONARY PHASE AND MICELLAR PHASE IN MLC

The retention of high polar compounds is examined according to the partitioning behavior of polar compounds from the bulk aqueous phase into micelles and alkyl stationary phases; more hydrophobic compounds may be transported straight from micelles in the mobile phase to the stationary phase [17]. Micelles in the eluting medium of RPLC systems (whether solely aqueous or hydro-organic) significantly impact overall chromatographic features that differ significantly from those of classic aqueous-organic mediums [18]. The initial studies of solute retention models in MLC were done by Armstrong and Nome [19] and Arunyanart and Clin Love; later, it was modified by Zibas and Cline Love [20]. The two equations established the scenario that retention decreases as surfactant concentration rises. The following well-known equation is used to calculate the partition coefficients of solutes.

$$Vs / Ve - Vm = ({}^{P}MW - 1) \times VCm / {}^{P}sw + 1 / {}^{P}SW \ldots.(Eqn.\ 1)$$

Where $V_{s.}$, $V_{e,}$ and V_m are the volumes of the stationary phase, elution volume of a solute, and the mobile phase volume and P_{MW} and P_{SW} are the partition coefficients of a solute between a micellar and solution (aqueous) phase and between a stationary and solution phase, *V* is surfactant molar volume and *Cm* is the concentration of micelle surfactant.

An ion-exchange model can be used to quantify the mass action effect. The partition of anions to the stationary and micellar phases is reduced when salt concentration rises. For simplicity, monovalent anions were taken as an example, and the following two ion-exchange equilibria were used to characterize anions' retention in micellar chromatography [10].

$$Em + Cs \leftrightarrow ECsi\ k_1 \ldots\ldots\ldots \text{(Eqn. 2)}$$

$$Em + Cm \leftrightarrow ECmi\ k_2 \ldots\ldots\ldots \text{(Eqn. 3)}$$

Where E_m is the solute in the mobile phase, C_s is the concentration of the stationary phase, EC_s, EC_m is the complex formed between the solute in the mobile phase and stationary phase sites, k_1 and k_2 are equilibrium constants. Based on the above, another model was established, which helps to find the capacity factor of the analyte.

$$1 / k_{1=1/}\text{ø}\ Cs\ k_1 + k_2 / \text{ø}\ Cs\ k_1 \times Cm \ldots.(4)$$

ø Here is the phase ratio value given by V_s/V_m. Foley [21] presented the following equation to find the retention factor in micellar mobile solutions.

$$K = k_0 \times 1 / 1 + {}^{k}AM^{C}m \ldots\ldots .(5)$$

Where k_0 is the retention value without micelles and K_{AM} binding constants.

Various organic solvents interact with solutes and alter the polarity of the eluting medium, as well as the surfactant aggregation number and CMC value. The final effect on organic solvent retention is determined by the type of solutes and the amounts of organic modifier and surfactant. In the presence of an organic modifier, the solute equilibrium shifts away from micelles and stationary phase towards non-polar bulk solvent. In this case, the eluting ability of the mobile phase increases, and both solute binding constants reduce as before [22, 23]. This concept was used recently by some researchers for analysis of various medicines using MLC [24, 25].

Factors affecting MLC Separation:

Structure of Drugs: Micelles are diverse on a microscopic level. Solutes can be found in microenvironments with varying polarity in a particular micellar eluent. This is in stark contrast to a homogeneous aqueous-organic medium, in which all components have the same polarity in the mobile phase. Generally, hydrophobic selectivity (methylene selectivity, α CH_2) is inversely related to the mobile phase's microenvironment polarity. More polar eluents, for example, have higher methylene selective values in RPLC. This theory can be used to have a better understanding of MLC behaviour.

The smaller methylene selectivity values for long homologues in a series indicate that they are positioned in more hydrophobic micelle surroundings. Some researchers evaluated carbonyl selectivity between various homologues of substituted aromatic ketones between the micellar and aqueous-organic phases. Carbonyl selectivity is constant in the hydro-organic system, regardless of the hydrophobicity of the surrounding carbon chain. In the case of micellar mobile phase, this effect is not observed as selectivity is determined by the alkyl chain lengths of the homologous series. Similarly, fewer polar microenvironments exist for more hydrophobic series, such as alkyl benzenes, than alkyl phenyl ketones. The $–CH_2$ groups of alkyl phenyl ketones would be exposed to a different mobile phase environment than those of alkyl benzenes in MLC, whereas in hydro-organic RPLC, this is not the case.

Selectivity and Strength of Solvent: MLC separation capability will be completely revealed by investigating the simultaneous impacts of changing the micelle concentration and the amount of organic modifier on selectivity and eluent strength [24]. The concentration of charged surfactant adsorbed by alkyl-bonded silica stationary phases is nearly constant in MLC. Still, solutes are partitioning between the mobile phase and stationary phase and also between water and micelle within the mobile phase. As the concentration of micelles rises, the most common behaviour is a decrease in solute retention. The organic modifier enhances the hydrophobicity of the bulk liquid in the eluting medium, and the solute equilibrium shifts from the micelle to the water-modifier phase [26]. However, if the modifier concentration is too high, no micelles will form. There is a little restriction in MLC application compared to traditional RP-LC due to two major issues. The hydrophobic compound's retention increases due to the weak elution capacity of micellar mobile phases when utilized with conventional pore size LC stationary phases. Next is, the efficiency decreases due to higher analyte mass transfer resistance and anisotropical flow. When small concentrations (1–5%, v/v) of various alcohols are added to the micellar mobile phase and rise in column temperature improves MLC efficiency. Large pore size silica will be used for MLC due to the individual impact of alcohols on the micelles.

Gradient Elution: A fixed phase with a larger surface area in the mobile phase may be more vulnerable to surfactant absorption than one with a lower surface area, particularly if the surfactant concentration is low. The depth of the stationary phase's pore and the range of diffusion distance is critical in mass transfer. Finally, high values for all of the above variables may result in further band-broadening [16-18]. The utilization of more pore-size stationary phases increases the eluting capacity of micellar eluent and overcomes one of MLC's most significant limitations. When short alkyl chain bonded stationary phases, or a fluorooctyl chain is used, acceptable efficiencies might be achieved in MLC [27]. A comparable relation was also observed between elution strength improvement and methylene chain length for acids and alcohols.

As an alternative to alcohols, aliphatic carboxylic acids can be utilized as organic modifiers in MLC. The presence of aliphatic carboxylic acids in the micellar mobile phase boosts the mobile phase's elution strength slightly more than equivalent alcohols supplied at the same volume ratio [27, 28].

The best separation conditions for the least resolved peak pair are frequently found by maximizing a resolution descriptor such as selectivity or R_S values. Peak widths, asymmetries, and height ratios can all be considered when modifying the R_S parameter. Some resolution should be lost in such optimizations to balance other needs [29]. Gradient elution is significant in chromatography of mixtures with widely varying polarities, such as drug analysis and the analysis of highly polar metabolites. Gradient re-equilibration is quick in MLC because the micelles are constrained to the inter-particle space, which is one of the benefits. The inability to properly elute highly held species, such as non-polar hydrophobic chemicals, on long chain stationary phases has been a key challenge in gradient elution MLC (*e.g.* C18). It has been suggested that short-chain length stationary phases to be used to minimize analysis times, however, this decreases retention and peak resolution [30].

Selection of Organic Modifier: The values of CMC and the degree of counter-ion binding are mass action-based metrics. The value of SDS's counter-ion binding degree decreases with the use of alcohols, which lower CMC. The CMC values of SDS and the degree of counter-ion binding are reduced by small amounts of aliphatic alcohols and carboxylic acids (C4–5). The primary rule of the observed effects is that carboxylic acid's modifying action is nearly identical to that of regular alcohol with the same carbon atoms [31]. Retention factor in MLC is also depends upon electrostatic and hydrophobic interactions and is a function of structural characteristics and varies amongst substances. Due to conflicting equilibria and diverse interactions in MLC, any form of selectivity behaviour can be expected; nonetheless, partition into micelles and dynamically changed alkyl-bonded phase are closely associated for a broad range of chemicals (particularly non-ionics). Sluggish solute transfer from the aqueous to the micellar phase and slow transfer from the stationary phase to the aqueous phase and surfactant adsorption cause efficiency loss in MLC utilizing exclusively aqueous micellar mobile phases. MLC efficiency can be improved by lowering the mobile phase flow rate to get closer to the Knox plot's optimum, increasing the temperature, which lowers viscosities, raises rate constants, and lowers the amount of adsorbed surfactant, and especially by adding an organic modifier like alcohol, which lowers viscosities, raises rate constants, and lowers the amount of adsorbed surfactant. However, if the surfactant proportion is raised, more alcohol will be injected to fix the ratio of alcohol to surfactant for improvement in resolution factor [32]. When a more organic modifier is added, the micelles disintegrate and solubilize free monomers generating a concentrated sub-micellar solution. The mobile phases suitable for the micelles' persistence are: acetonitrile, methyl alcohol and ethyl alcohol 30%, 1-propanol, 22%; 1- butanol, 10% and 1-pentanol, 6%, and alcohol will be added with a certain ratio to give rise to hybrid mobile phases [33].

Advantages of MLC: MLC is a feasible substitute to standard RPLC with an aqueous-organic eluting medium due to growing attention toward green chemistry. The selectivity of the compounds varies due to different interactions between solutes, stationary phase, mobile phase, and micelles. The ionic and non-ionic solutes can be separated with the same eluting medium. An isocratic solvent system can be used to analyze samples containing a wide range of polarity compounds. Gradient elution is less favorable since equilibrium periods are faster with aqueous-organic mixtures. Micelles' excellent solubilizing potential allows them to dissolve many compounds quickly, saving time in sample preparation.

The possibility of direct injection of physiological samples increases due to high dissolution power. Micelles compartmentalize organic molecules, resulting in improved luminescence detection. Even for highly hydrophobic steroids, the amount of organic solvent required to form a hybrid micelle eluting medium is significantly less than in traditional RPLC with aqueous-organic mixtures. This decreases costs and toxicity and reduces the impacts of hazardous wastes on the environment. The micellar mobile phase effectively retains

organic solvents, reducing the possibility of evaporation. As a result, micellar phases remain stable for a long period. So high reproducibility is observed for retention. The method reliably predicts the variation in retention times by changing the amount of surfactants and volume of the organic modifier (mobile phase composition). This makes it easier to optimize the separation conditions. The same column, detector, injector, and pipes are used in MLC analysis as traditional RPLC. The properties of the surfactants actively used in MLC should be examined to prevent precipitation in the column. The hardware has prolonged longevity, and columns used for MLC analysis can be used multiple times. MLC has been considered a convenient procedure for bioanalytical studies due to its advantages in sample preparation and better chromatographic resolution. Indeed, it has been successfully used in recent years to determine various drugs and their biometabolites in physiological fluids. The separation of some medicinal compounds in pharmaceutical formulations by MLC is listed in **Table 1.**

TABLE 1: SUMMARIZATION OF MLC METHOD FOR ANALYSIS OF PHARMACEUTICAL FORMULATIONS

Class of Medicine	Sample Type	Micellar Mobile Phase	Stationary Phase	Elution Method	Reference
Anesthetics (Bupivacaine, lidocaine, Mepivacaine, Procaine, Propanocaine, Tetracaine) Anesthetics and muscle relaxants (Lidocaine, Tolperisone)	Diverse formulations	0.15M SDS–propanol (9:1)	Spherisorb ODS–2 (120 x 4 mm i.d.)	Isocratic	34
Anesthetics and muscle relaxants (Lidocaine,Tolperisone	Diverse formulations	0.075M SDS–7.5% propane	C_{18} column (125 x 4.6mm i.d.)	Isocratic	35
Antianginals (Diltiazem, Nadolol, Nifedipine, Propranolol, Verapamilo)	Diverse formulations	0.05M SDS–5% pentanol, pH7	Kromasil C18 (150 x 4.6mm i.d.)	Isocratic	36
Antibacterial agents (Sulfacetamide, Sulfadiazine, Sulfadimethoxine, Sulfaguanidine, Sulfamerazine, Sulfamethazine)	Diverse formulations	0.04M SDS–2% 2–propanol	Nucleosil C18 (150 x 4.6mm i.d.)	Isocratic	37
Antibacterial agents (Azithromycin)	Tablets and capsules	0.1M SDS–15% butanol, pH7	Hypersil C18 (200 x 4.6mm i.d.)	Isocratic	38
Antibacterial agents (Cefuroxime)	Tablets	0.02M SDS–8% acetonitrile	XTerra C18 (150 x 4.6mm i.d.)	Isocratic	39
Antibacterial agents (Sulfamethoxazole and Trimethoprim)	Diverse formulations	0.1M SDS–3% butanol	Hypersil ODS (125 x 4.6mm i.d.)	Isocratic	40
Antidiabetic drugs (Metformin, Glipizide, Glicazide)	Diverse formulations	2.5 mM disodium hydrogen phosphate, 50 mM SDS, 5% IPA, pH 7.2	Zorbax XDB C18	Isocratic	41
Anticonvulsant agents (Clorazepate, diazepam and diltiazem)	Diverse formulations	0.1M SDS–3% butanol, pH3	ODS–2	Isocratic	42
Anticonvulsant agents (Carbamazepine, Benzodiazepines Bentazepam, Halazepam, Oxazepam, Pinazepam and Tetrazepam)	Pills and capsules	0.1M SDS–3% butanol–0.1% triethylamine, pH3	C18	Isocratic	43
Anticonvulsant drugs (Acetazolamide, Carbamacepine, Chlordiazepoxide, Diazepam, Ethosuximide, Phenytoin, Phenobarbital, and Zopiclone)	Capsules, pills, tablets, injections, drops and suppositories	Solutions of cetyltrimethylammonium bromide (CTAB)	C18	Isocratic	44
Antihistamines (Azatadine, Carbinoxamine, Cyclizine, Cyproheptadine, Diphenhydramine, Doxylamine, and Tripelennamine)	Tablets, capsules, powders, solutions and syrups	0.15M SDS–6% pentanol	C18	Isocratic	45
Antihistamines (Brompheniramine, Chlorcyclizine, Chlorpheniramine,	Tablets, capsules,	0.02M CTAB–3% propanol, pH6;	Spherisorb C18 (250 x 4.6mm i.d.)	Isocratic	46

Diphenhydramine, Doxylamine, Flunarizine, Hydroxyzine, Promethazine, Terfenadine, Tripelennamine and Triprolidine)	suppositories, syrups and ointments	0.02M CTAB–3% propanol, pH7; 0.04M CTAB–3% butanol, pH3; 0.04M CTAB–3% butanol, pH5			
Antihistamines and phenethylamines (Carbinoxamine, chlorpheniramine, dexbrompheniramine, dexchlorpheniramine, diphenhydramine, doxylamine, pheniramine, phenyltoloxamine, tripolidine, azatadine and ephedrine, methoxyphenamine, phenylephrine, phenylpropanolamine, pseudoephedrine)	Diverse formulations	0.05M SDS–6% pentanol, pH7	Eclipse XDB C8 (150 x 4.6mm i.d.)	Isocratic	47
Phenethylamines (Amphetamine, Arterenol, Ephedrine, Phenylephrine, Phenylpropanolamine, Mephentermine, Methoxyphenamine, Pseudoephedrine, Tyramine)	Capsules, tablets, pills, powder, syrup and drops	0.15M SDS–5% pentanol, pH7	Spherisorb OD–2 (120 x 4.6mm i.d.)	Isocratic	48
Non-steroideal anti-inflammatory drugs (Acemetacin, Diclofenac, Indomethacin, Ketoprofen, Nabumetone, Naproxen, Tolmetin, Piketoprofen)	Diverse formulations	0.06M CTAB–10% butanol, pH7	Kromasil C18 (150 x 4.0mm i.d.)	Isocratic	49
Steroids (Beclomethasone, betamethasone, budesonide, danazol, dexamethasone, fludrocortisone, fluocinolone)	Creams, gels and ointments	0.1M SDS–4% butanol, pH7	C18 (12.5 x 4.6mm i.d.)	Isocratic	50
Steroids (Methyltestosterone)	Pills	0.04M SDS–10% propanol	Hypersil ODS (150 x 4.6mm i.d.)	Isocratic	51
Tricyclic antidepressants (Amitryptiline, clomipramine, doxepin, imipramine, maprotiline, nortriptyline, trimipramine)	Tablets and capsules	0.075M SDS–6% pentanol, pH3	Eclipse XDB C8 (150 x 4.6mm i.d.)	Isocratic	52
Vitamins (B3 (nicotinamide), B1 (thiamine), B2 (rivoflavin), B6 (pyridoxal, pyridoxine and pyridoxamine), B9 (folic acid), B12 (cyanocobalamin), C (ascorbic acid))	Multivitamin tablets	0.016M SDS–3.5 to 10% butanol, pH3.6	Particil ODS–2 (250 x 4.6mm i.d.)	Gradient	53
Vitamins (A and E)	Multivitamin syrup	0.077M SDS–12% butanol, pH7	Spherisorb ODS–2 (100 x 3.9mm i.d.)	Isocratic	54
Vitamins (B (nicotinamide), B1 (thiamine), B2 (riboflavin), B6 (pyridoxine and pyridoxamine))	Capsules, pills and syrups	0.1M SDS–4% pentanol, pH3	Kromasil C18 (120 x 4.6mm i.d.)	Isocratic	55

Limitations of MLC: Though MLC has several benefits, the technique's usefulness in analytical laboratories is limited due to the low elution capacity and effectiveness of a pure micellar eluting medium. Due to the nonreversible adsorption of certain surfactants during MLC, there is a risk of column damage. When compared to RP-HPPLC, some researchers believe it is a more complicated method. When multiple chromatographic conditions are considered, MLC is only marginally applicable. The MLC technique is not the best option for separating hydrophobic solutes. Micellar eluents are weaker than hydro-organic phases in general. Higher micelle concentrations combined with an organic co-solvent can fix the problem, but the mobile phase's higher viscosity leads to lower plate counts. Some studies looked at the retention behaviour of hydrophobic substances like polyaromatic hydrocarbons and used MLC to separate them. The

separation of ionic and non-ionic components or mixtures is one area where MLC should outperform other HPLC approaches. Ion pair chromatography (IPC) and ion-exchange chromatography (IEC) are powerful alternatives to MLC (IEC).

Analysis of Antiretroviral Drugs: Antiretroviral drugs are used to treat infections caused by retroviruses, the most common of which is HIV. Different antiretroviral drug groups are used at different points of the HIV life cycle. HAART (highly active antiretroviral therapy) is a medication that consists of a mixture of drugs that target various viral targets. HAART helps to preserve immune system activity, reduces the overall burden of HIV on patients, and cures opportunistic infections that can lead to death. Retroviruses belonging to the Retroviridae family are microscopic bacteria that attack cells. Retroviruses use RNA as their genetic material.

When a retrovirus infects a cell, it penetrates the DNA of the host cell and creates a DNA copy of its genome. Various forms of retroviruses cause human diseases such as cancer and HIV (Human Immunodeficiency Virus). These drugs only attack the virus and stop it from moving through its life cycle; they do not kill it. As a result, the virus is no longer able to reproduce itself. If antiretroviral therapy is continued for 6 months (generally, people will get the virus under control in this time), the virus population becomes untraceable [56]. If the treatment is not continued, the virus has the potential to regenerate and return to its original state in the body. Inconsistent dosing over time can lead to drug resistance and ultimately treatment failure [57].

Classification of Antiretroviral Drugs:

NRTIs (Nucleoside / Nucleotide Equivalent Reverse Transcriptase Inhibitors): These are the first ARV drugs approved by the Food and Drug Administration (FDA). They are used to treat HIV infection and AIDS. For example, zidovudine, adefovir and tenofovir.

NNRTIs (non-nucleoside reverse transcriptase inhibitors) bind to and block the HIV enzyme, preventing HIV replication. HIV uses reverse transcriptase to translate RNA into DNA. Efavirenz, Rilpivirine, and Nevirapine belong to this variety of drugs.

Protease Inhibitor (PI): This class of drugs aids in the reduction of HIV in the body. This delays the progression of HIV and aids in the treatment of its symptoms. Ritonavir and Darunavir are example of this type of drug.

Fusion Inhibitor: This type of drug prevents a virus from infiltrating a cell. For example, Enfuvirtide and Maraviroc.

Integrase Inhibitor: This class of drugs is designed to prevent integrase, a viral enzyme that inserts the viral genome into the DNA of the host cell. *e.g.*:- Raltegravir.

Quantification of Antiretroviral Drugs by MLC: The MLC approach is used to quantify Abacavir, Lamivudine, and Raltegravir in plasma. Raltegravir is an integrase inhibitor, while Abacavir and Lamivudine are reverse transcriptase inhibitors. Abacavir and Lamivudine interact with the HIV viral enzyme that produces another virus. Inhibiting this form of enzyme helps prevent the virus from completing this reproductive cycle [58]. Raltegravir prevents the viral genetic material from being integrated into human chromosomes [59]. The first reason for therapeutic failure is non-compliance with HAART, which should be calculated. Treatment compliance of approximately 95% is expected [60]. As a result, scientists use analytical methods for quantifying Abacavir, Lamivudine, and Raltegravir in plasma to improve the reaction of patients on this new HAART regimen. MLC method was used to pick ARV [61] and to quantify three HAART mixtures using SDS (Sodium Dodecyl Sulphate) as surfactants and three mobile phases [62]. MLC methods are used for the analysis of Abacavir, Lamivudine, and Raltegravir by direct injection of the sample after dilution in isocratic mobile phase using less toxic chemicals [63].

Stock solutions for Abacavir and Lamivudine have a concentration of 100 mg/L, while Raltegravir has a concentration of 250 mg/L. Kromasil C_{18} column is used in isocratic mode at 1mL/min flow rate at 25^{o}C with SDS concentration of 0.05M and pH 7. About 20 µL sample is injected, and absorbance is observed at wavelength of 260 nm.

The resolution time for analytes was less than 30 minutes using this mobile phase. These ARV drugs had a global resolution of 0.9997. Abacavir, Lamivudine, and Raltegravir had retention times of 28.2 minutes, 3.9 minutes and 21.4 minutes, respectively. The linear range of these ARV drugs should be 0.25-2.5 g/mL, linearity should be greater than 0.990, accuracy should be 92.3-102.4 percent, and robustness should be less than 7.1 percent, according to ICH guidelines.

Analysis of Lamivudine, Zidovudine and Efavirenz: Lamivudine, Zidovudine, and Efavirenz are reverse transcriptase inhibitors, and these drugs are analyzed in the serum of AIDS patients by MLC. Lamivudine and zidovudine are eluted in comparable retention times in different mobile phases. In 0.05M SDS and 0.15M SDS/2.5 % 1-propanol, these drugs do not overlap and show adequate separation; otherwise, they overlap in all the mobile phases. Lamivudine and Zidovudine show maximum separation between the analytes and are also eluted at a low retention time in 0.05M SDS/ 2.5% 1-propanol. Efavirenz show maximum efficiency in 0.05M SDS /1-pentanol (6%). The retention time of lamivudine, zidovudine, and efavirenz are approximately 2.05 min, 2.58 min, and 14.20 min, respectively [62].

Analysis of Lamivudine, Tenofovir and Efavirenz: Lamivudine, Zidovudine, and Efavirenz are reverse transcriptase inhibitors, and these drugs are analyzed in the serum of AIDS patients by MLC. Tenofovir cannot be eluted in aqueous mobile phases because it shows a very high retention time. Lamivudine and tenofovir can be analyzed only in 0.05M SDS/ 2.5% of 1-propanol and eluted at a high retention time approx 12.46 min with efficiency N=1889. Efavirenz and Tenofovir were quantified at 0.05M SDS/ 6% of 1-pentanol and eluted at a low retention time i.e., 4 min, with high efficiency (N= 2060). Lamivudine shows less retention time, approx 2.7min and adequate efficiency (N= 2009) by using aqueous 0.15M SDS without any organic modifier [62].

Analysis of Darunavir, Ritonavir, Emtricitabine and Tenofovir by MLC: AIDS is treated by a combination of drugs such as danuravir, ritonavir, emtricitabine, and tenofovir. The levels of these drugs in plasma were measured by using micellar liquid chromatography. Protease inhibitors, including ritonavir and Darunavir are used to treat HIV. By splitting the polyprotein of HIV into fragments, these drugs help to reduce the HIV virus in the body, delay the progression of the HIV reproductive cycle and treat the symptoms. Emtricitabine and Tenofovir are nucleoside and nucleotide reverse transcriptase inhibitors. These drugs prevent the enzyme that produces new viral DNA from HIV RNA and blocks the new virus's generation. SDS was used as a surfactant in MLC for simultaneous quantification of above-said drugs in plasma. MLC enables the analysis of these four drugs in the shortest possible period, with high sensitivity, efficacy, low cost, and is also an ecofriendly method [62]. The study of these drugs was performed at room temperature by using 0.060M SDS, 2.5 % 1-pentanol mobile phase with a pH of 7, and a 0.01M phosphate buffer using Kromasil C_{18} (150x4.6 mm; particle size 5 µm: pore size 100, working pH range 1.5–7.5) column with flow rate 1 mL/min. About 20 µL sample is injected and detected at a wavelength of 214nm. Tenofovir and ritonavir are highly retained on C_{18} columns, necessitating the use of a mobile phase with large elution potential. The maximum global resolution, 0.9998, was obtained in less time at the selected mobile phase. A plasma sample spiked with a mixture of these four ARV drugs at 5g/mL was analyzed under ideal conditions; the retention time and efficiency of these drugs are represented in **Table 2.**

TABLE 2: CHROMATOGRAPHIC PARAMETERS OF ANTIRETROVIRAL DRUGS ANALYZED IN MLC

Name of ARV drug	pK_a	Retention time (t_R)	Column Efficiency (N)	Asymmetry (B/A)
Darunavir	2.1	8.2	4000	0.97
Ritonavir	2.84	18.4	3200	1.07
Emtricitabine	2.65	3.6	4700	1.11
Tenofovir	3.75	5.5	4500	1.20

Quantification of Anti-diabetic Drugs by MLC: Anti-diabetic drugs are medications used to treat diabetes mellitus by lowering blood glucose levels. Basically, diabetes mellitus is a disorder that causes

hyperglycemia, ketoaemia and glycosuria. Diabetic mellitus are blurry vision, frequent urination, always hungry, wounds taking time to heal, sudden weight loss, *etc.*

Anti-diabetic Drugs are of Two Types: TYPE-1 diabetes and TYPE-2 diabetes. If you have type 1 diabetes, you must take insulin injections daily to stay healthy. To maintain a normal glucose level, a person with diabetes must eat properly. Type-2 diabetes mellitus can be controlled by exercise and proper regular diet to prevent the glucose level from going too low or too high. Anti-diabetes drugs work on the beta cell's surface by closing the potassium channel, which allows the entry of calcium ions into the cells, resulting from the flow of insulin outside the cellular storage vesicles. Rosiglitazone and Pioglitazone are the drugs that help decrease insulin resistance.

Classification of Anti-diabetic Drugs:

Biguanides: It belongs to type 2 diabetes group and is used to prevent glucose production in the liver and help improve the body's sensitivity towards insulin and reduce the amount of sugar absorbed by the intestines. With the help of biguanides, fats and amino acids are not converted into glucose by the liver and activate the enzyme to help the cells take in glucose from the blood. Metformin is a commonly used drug that belongs to biguanide class. *E.g.:-* Phenformin and Buformin.

Sulfonylureas: This group of medicines is used in the treatment of type2 diabetes. The person who is suffering from type2 diabetes their body is not able to use insulin properly, which changes the blood sugar level. *E.g.:-* Glipizide, Glimepiride, Chlorpropamide.

Meglitinides: This drug is used to cure type2 diabetes on the cell membrane of pancreatic beta cells; they bind the ATP- dependent potassium ion channels in the same manner as sulfonylurea but do not have the strong binding affinity and easily dissociation from sulfonylurea receptor binding site. *E.g.:-* Repaglinide, Nateglinide and Mitiglinide.

Thiazolidinediones: This is, also known as Glitazones, belongs to the class of the heterocyclic compound, which is used in the diabetes mellitus type 2 treatment for the promotion of adipogenesis; they help in binding the avidly to peroxisome proliferator-activated receptor gamma in adipocytes. *E.g.:-* Pioglitazone, Rosiglitazone.

Dipeptidyl Peptidase IV inhibitors: This class of drugs helps block dipeptidyl peptidase-4 and is used in the treatment of type2 diabetes. The peptide hormone, Glucagon, increases the blood sugar levels in the body and DPP-4 inhibitors help to reduce the glycogen level and blood sugar levels. E.g.:- Sitagliptin, Gemigliptin, Anagliptin.

α- glucosidase Inhibitor: These types of drugs in treating diabetes mellitus type 2. It helps prevent the digestion of carbohydrates such as starch and table sugar. By alpha-glucosidase enzymes, carbohydrates are easily converted into sugar present on cells' luminal surface of the intestine, enabling the sugar to be absorbed through the intestine. This inhibitor also reduces the effect of carbohydrates on blood sugar. *E.g.:-* Acarbose, Miglitol, Voglibose.

Analysis of Metformin, Nateglinide and Gliclazide by MLC: Metformin is classified as a biguanide, nateglinide as a meglitinide, and gliclazide as a sulfonylurea drug. The mobile phase used for MLC analysis was a mixture of 0.12M SDS, 10% 1-propanol, and 0.3 percent triethylamine at a pH of 5.6, adjusted with phosphoric acid. Nucleosil C_{18} column (150x4.6 mm; particle size 5 µm) was used in isocratic mode with a flow rate of 1.2 mL/min for 30 min [64]. The absorbance wavelength was set at 254nm. Metformin, nateglinide, and gliclazide stock solutions were prepared in methanol and stored at 40 ^{o}C, each containing 100 µg/mL. The working solution has diluted to a concentration range of 0.8-2.5 µg/mL. The separation of the Metformin, Nateglinide, and Gliclazide mixture was observed in a limited retention period of less than 10 minutes. Metformin has a 4.8 min retention period, nateglinide has a 7.1 min retention time, and gliclazide has an 8.99-minute retention time.

The retention factor of three anti-diabetic drugs decreased as the concentration of mobile phase containing SDS increased from 0.05M to 0.2M. NIDDM (Non-Insulin Dependent Diabetes Mellitus) is treated with a drug cocktail. The first mixture contains 500 mg metformin and 5 mg

glipizide, while the second contains 500 mg metformin and 80 mg gliclazide. Some researchers used MLC to examine these drug combinations [65]. Micellar mobile phase was used in the experiment, which included 0.0025M disodium hydrogen phosphate, 0.05M SDS, and 5% isopropyl alcohol with a pH of 7.2 adjusted by 10% orthophosphoric acid. Zorbax XDB C_{18} column (150x4.6 mm; particle size 5 µm) was used in isocratic mode with flow rate 1.2 mL/min at 300 ^{o}C with detection absorbance wavelength of 226nm. Metformin, glipizide and gliclazide stock solutions were prepared at concentration of 5 mg/mL, 1 mg/mL, and 0.8 mg/mL, respectively. The first mixture, which includes glipizide and metformin, showed peaks at approximately 3 minutes and 12 minutes, respectively. The second mixture, which includes gliclazide and metformin, has a retention time of approximately 6.5 and 12 minutes, respectively.

Quantification of Anti-tumor Drugs by MLC: Anti-tumor drugs, also known as antineoplastic antibiotics and anti-cancer antibiotics that function by interfering with DNA to stop cells from developing. Anti-tumor drug overdoses can result in permanent heart damage. Anti-tumor antibiotics alter the DNA of cancer cells, preventing them from growing and replicating. Anthracyclines are the most widely used anti-tumor antibiotics. It's used in conjunction with enzymes involved in DNA replication. These drugs are successful at all stages of the cell cycle and are widely used to treat a number of cancers. Daunorubicin, Doxorubicin, and Idarubicin are included under the anthracyclines group. Anti-cancer drugs consist of number of classes such as antimetabolites, an alkylating agent, natural products, and hormonal agents. TAMO **(**Tamoxifen) is a group of proteins found within cells that are widely used in treating breast cancer in humans and also used for treating other cancers. ENDO (Endoxifen) is a drug that has been developed to treat oestrogen receptor-positive breast cancer.

It is also prescribed as an antipsychotic for treating bipolar depression and other psychiatric disorders. ENDO has a stronger antitumor effect than TAMO. ENDO is the primary agent responsible for the production of TAMO therapy. The drugs were analyzed using 0.15M SDS, and 7% butanol buffered mobile phase with a pH of 3. The analysis is done using a C_{18} column in an isocratic mode with a flow rate of 1.5mL/min at 40°C [66]. The chromatograms were observed using plasma spiked with TAMO and ENDO at 10µg/mL concentrations and 3.6 µg/mL, respectively. The entire observation took less than 36 min. The antitumor drugs TAMO and ENDO have a retention period of 10.2 and 14.1 min, respectively [66].

Analysis of Cardiac Drugs by MLC: Cardio-vascular disease is caused by defects in the circulatory system, mainly in the heart and blood vessels. A variety of diseases included under this category are hypertension, heart failure, coronary artery disease, and stroke [67]. High blood pressure and diabetes are the major causes of cardiovascular disease. Antihypertensive and anti-diabetic drugs are used to treat these diseases. Anti-cardiac medications have three primary effects on heart function: inotropic, chronotropic, and rhythmic effects.

The inotropic effect will affect the force with which the heart muscle contracts. The chronotropic effect may affect the frequency of heartbeats and heart rate. The rhythmic effect can influence the regularity of the heartbeat. Aspirin, Atorvastatin, and Metformin are examples of anti-cardiac drugs.

Anti-cardiac Drugs Acting on Cardiovascular System: Antihypertensive drugs:- Hypertension is a condition in which the level of blood pressure rises above the maximum limit of the normal level. The normal blood pressure limit is 100-140 mm Hg, and the drugs used for the treatment of hypertension are called antihypertensive drugs.

It prevents stroke and heart attack. The classes of antihypertensive drugs used for the treatment in lowering the blood pressure are calcium channel blocker, beta blocker, thiazide diuretics, and ACE inhibitors. *E.g*. - Amlodipine, Felodipine, Nitedipine *etc*.

Anti-arrhythmic Drugs: These drugs are used in treating abnormal heartbeat and heart rhythm due to the disturbance in the heart's electrical system. Classes of anti-arrhythmic drugs are calcium channel blocker, beta-blocker, sodium channel blocker, and potassium channel blocker. *E.g.*- Dronedarone, Amiodarone, Sotalol *etc*.

Anti-anginal Drugs: Anti-anginal drugs are used to treat chest pain caused by a shortage of blood flow to the heart muscle, which shows the sign of coronary artery disease. Classes of anti-anginal drugs are beta-blockers, long-acting nitrates, and calcium channel blockers. *E.g.,* Propranolol, Atenolol, Metoprolol, *etc*.

Cardiotonic Drugs: The drugs that improve the heart muscle's efficiency and force of contraction, resulting in improved blood flow to all body tissues. Classification of cardiotonic drugs is cardiac glycoside, xanthines, and sympathetic drugs. E.g., Digoxime, Dopamine, Atropine *etc*.

Analysis of Aspirin, Atorvastatin, Metformin and Metoprolol: Metoprolol is a beta-1 receptor antagonist widely prescribed for elevated blood pressure, chest pain, and an abnormally quick heart rate. Aspirin is an anti-inflammatory and pain-relieving medicine. Patients who have had a heart attack or stroke should take aspirin in low doses as a preventative measure. Aspirin has also been used to prevent cancer, such as bowel cancer. Atorvastatin is a lipid-lowering medication used to treat elevated cholesterol and coronary heart disease and help patients with hyper-lipoproteinemia and other cardiovascular conditions normalize their blood pressure. Metformin is a diabetes medication that aims to lower the risk of cardiovascular problems. Metformin has been used when diet and exercise are not enough to prevent blood sugar levels. MLC has been widely used to quantify and study metformin, metoprolol, aspirin, and atorvastatin. Metoprolol standard stock solution has a concentration of 500µg/mL.

In contrast, Aspirin, Metformin, and Atorvastatin have a concentration of 1000µg/mL were prepared in double distilled water and ethanol with a ratio of 30:70 at 4°C. Every standard operating solution of aspirin, metformin and atorvastatin has a concentration of 5 µg/Ml and metoprolol has a concentration of 100 µg/mL, which was prepared by diluting stock solution in double-distilled water. The C_{18} TDS column (150x4.6mm, 5µm) was used with a flow rate of 1mL/min in isocratic mode using micellar mobile phase SDS containing 0.09M glacial acetic acid of pH 7.4 at 25°C. The volume of sample injection was 20 µLand detector wavelength was 225 nm. When 13.5% natural deep eutectic solvent was used with SDS, the retention time for aspirin, metoprolol, atorvastatin, and metformin was approximately 15 minutes, 24 min, 60 minutes and 65 min respectively. When 13.5 % butanol was used with SDS, the retention time of aspirin, a combination of atorvastatin, metoprolol, and metformin was approximately 6 minutes, 8 minutes and 9 minutes, respectively [68].

Analysis of Furosemide, Metoprolol and Verapamil by MLC: Furosemide is a diuretic that is used to treat heart failure, hypertension, and kidney disease. It is a diuretic that causes the body to produce more urine, removing excess water and salt from the body. Verapamil is a calcium antagonist that is used to treat chest pain, irregular heartbeat, high blood pressure, migraines, and neurological disorders. The mobile phase contains 0.15M SDS, 25mM disodium hydrogen phosphate, 1-butanol, and triethylamine in a ratio of 93/6/1 v/v/v with phosphoric acid to change the pH to 3. About 20µL of the sample was injected with a flow rate of 2mL/min at 40°C. Metoprolol and Verapamil were detected using a spectrofluorometer with excitation at 230nm and emission at 311nm [69] Furosemide was detected using a UV detector at 240nm and Metoprolol and Verapamil were detected using a spectrofluorometer with excitation at 230nm and emission at 311nm. Soltanil & Jouyban (2012) analyzed spiked plasma samples for separation of Furosemide, Metoprolol, and Verapamil and reported that Furosemide, Metoprolol, and Verapamil have roughly 3.5, 7, and 13-minute retention time respectively in the above MLC method [70].

CONCLUSION: Micellar liquid chromatography is an analytical technique used to separate pharmaceutical and environmental studies of different types of compounds. Analysis of different types of drugs such as antiretroviral drugs, anti-diabetic drugs, anti-tumor drugs, and anti-cardiac drugs by micellar liquid chromatography. Antiretroviral drugs are used to treat infections caused by retroviruses, the most common of which is HIV. For the analysis of antiretroviral drugs for AIDS patients, MLC is a suitable technique and the first technique to determine the three drugs simultaneously in plasma.

Anti-diabetic drugs are medications used to treat diabetes mellitus by lowering blood glucose levels. Quantification of metformin, nateglinide and gliclazide by MLC is simple and precise and analysis was done easily and in a short period. MLC can determine anti-tumor drugs by changing the surfactant concentration, which is used as a mobile phase and analysis done very quickly. MLC easily separates four cardiovascular drugs using a natural deep eutectic solvent containing ethylene glycol and choline chloride, which helps in chromatographic resolution. Separation of these drugs occurs less than 12min times by MLC. Henceforth, the MLC technique is greener than RPLC as micellar mobile phases are eco-friendly, cheap, less toxic, and non-flammable.

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