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EFFECTIVE LIQUID CHROMATOGRAPHY FOR PHARMACEUTICAL EXAMINATIONS

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ABSTRACT

Key words: HPLC, stationary phase, enzyme separation, and breakdown products.

Over the past ten years, high performance liquid chromatography has supplanted many spectroscopic techniques and gas chromatography in the quantitative and qualitative analysis of pre-sale procedures, drug marketing, and their control. Although it was once believed that HPLC would be a complementary technique to gas chromatography, it has now almost entirely supplanted gas chromatography in pharmaceutical analysis. When compared to other methods, the use of a liquid mobile phase, which allows for the transformation of mobilized polarity during chromatography and other mobile phase modifications based on the properties of the substance being tested, is a significant advantage in the separation process.The next factor that makes good separation possible is the broader choice of stationary phase. The separation line is linked to particular and sensitive detection systems, such as spectrafluorimeters, diode detectors, and electrochemical detectors. Other hyphernated systems, such as HPLC-MS and HPLC-NMR, are the fundamental components that underpin the HPLC method's extensive and successful application. High performance liquid chromatography (HPLC) analysis is used to verify a drug's identity, provide quantitative data, and track the effectiveness of a disease's treatment.1) The chromatogram for the plasma of depressed individuals obtained 12 hours before to oral dexamethasone therapy is shown in Fig. 1.Through biological and therapeutic research conducted prior to medication registration, it may also be utilized to deepen our understanding of the human body's normal and pathological processes. One of the most difficult but often used applications of high performance liquid chromatography is the analysis of medicines and metabolites in biological fluids, especially plasma, serum, or urine. Numerous endogenous chemicals are frequently found in blood, plasma, or serum at concentrations far higher than those of the analyte. In the case of pharmaceuticals, the endogenous chemicals are sometimes structurally very similar to the drug to be evaluated, and analiyte concentrations are frequently low. The amount of free chemical that is detected may also be reduced by medication binding to the plasma protein.

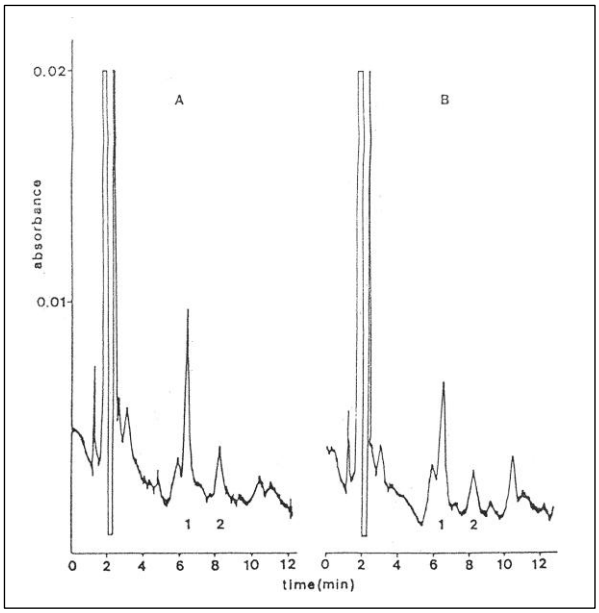


Figure 1. The plasma concentration of cortisol was calculated to be 108 ng ml^{-1} (A). The plasma. chro- matogram of the same subject 12 h after oral administration of 0. 5 mg of dexamethasone. The plasma concentration of cortisol was calculated to be 155 ng ml^{-1} (B). Both samples were spiked with 200 ng ml^{-1} of prednisone as internal standard. Key: (1) prednisone (internal standard), (2) cortisol. Conditions are as in Fig. 1.

The analyst has a number of challenges when conducting drug and metabolite tests in bodily fluids. The first issue stems from the complexity of bodily fluids; the pharmaceuticals must be separated using an extraction method that ideally yields a somewhat clean extract, and the separation system must be able to separate the desired drugs from co-extractives. As previously indicated, high-performance liquid chromatography necessitates careful detector selection, a suitable stationary phase, eluents, and an appropriate separation procedure. The most adaptable detector in high-performance liquid chromatography is the UV/VIS detector, however it's not necessarily the best because of its lack of selectivity, which may necessitate high analyte resolution. UV detection is preferred because it provides great linearity and allows for quick quantitative tests against a single drug standard.Although they are somewhat less sensitive than single wavelength detectors, diode arrays and rapid scanning detectors are helpful for peak identification and peak purity monitoring. Certain components in liquid chromatography may be entirely retained on the liquid chromatography column or have low UV chromophores if UV detection is being used.

For many compounds, fluorescence and electrochemical detectors are more selective than UV detectors in addition to being significantly more sensitive to the right analytes. Fluorescence detectors should ideally be sensitive, reliable, selective, and simple to use. In contrast to electrochemical detection, which is almost usually linked to a large frontal peak that tails significantly, selectivity manifests itself in the absence of frontal components seen in plasma extract.

Reductive electrochemical detection has shown to be the most sensitive technique to date, yielding good results in the study of some drug classes. For the analysis of medications and metabolites in bodily fluids, a number of high-performance liquid chromatography oxidative electrochemical techniques have been developed. Liquid chromatography-tandem mass spectrometry2,3,4,5, or mass spectrometer as a specific detector with all variations of ionization and interface (thermal spray, moving belt, etc.). Today, high performance liquid chromatography also uses NMR as a selective and

specific detector.When combined with an electrochemical detector, the creation of a non-aqueous eluent for ion-exchange separation on silica has produced an outstanding system that enables the analysis of a wide variety of medicines and metabolites, particularly basic ones.For some kinds of chemicals, such basic medicines, new packing materials like polymeric, base-deactivated silica, pyrolyzed carbon, and internal surface packing could provide better stability and higher efficiencies. Microbore columns should gain more traction since they provide both increased sensitivity and reduced solvent consumption, which reduces the need to discard hazardous solvents. The same technique of ion-exchange chromatography on unmodified silica columns with an eluent buffered to roughly pH 9 is still used for many tests of basic medications. While acidic pharmaceuticals like paracetamol and cannabis are separated using either ion suppression or ion-pair chromatography on a reversed-phase packing material, neutral or weakly acidic drugs like barbiturates can be chromatographed using a reversed phase system. Micelar liquid chromatography uses reversed-phase micelar mobile phases rather than traditional hydro-organic mobile phases. The solute in micelar liquid chromatography interacts with both stationary and mobile phases through complicated electrostatic hydrophobic and steric interactions. These make it possible to effectively separate samples of various kinds. The solvent's lower cost and toxicity, its biodegradability, and its ease of dissolving analytical samples are the primary benefits of using a micelar solution in reversed-phase liquid chromatography. This allows for the determination of drugs in physiological fluids without the need for prior separation of the proteins present in the samples.

Heptanes sulfonate is used as an ion pairing reagent and tetrabutylammonium phosphate as a competitive base in the study of sulphonamides. The term "ion pairing reagent" refers to improved retention that occurs when a big ion with a charge opposite that of the molecular ions to be separated is added to the mobile phase. Alkyl sulphates or sulfonates are typically used for molecular cations.

ISOMER SEPARATION ARE VERY IMPORTANT IN DRUG STUDIES AND USAGES OF HPLC

A compound is frequently a mixture of isomers when it exists in multiple isomeric forms. As an alternative, it could be necessary to keep an eye on samples of a single isomer to make sure they are pure. The ability to separate isomers using liquid chromatography is crucial in both situations. Since isomers will almost definitely have differing pharmacological and occasionally toxicological effects, this is particularly crucial for medicinal compounds. When one of the two enantiomers found in the drug ingredient was linked to the severe teratogenic consequences of thalidomide (children born to moms who had been taking thalidomide were born with shortened limbs), enantiomers—types of isomers—aroused a lot of curiosity. Enantiomers cannot be separated using traditional chromatography since they have the same physiochemical characteristics. Therefore, a "chiral selector" must be used in the liquid chromatography separation of enantiomers. This could be a chiral stationary phase, a chiral mobile phase additive, or a chiral derivation agent. Figure 2 illustrates typical common diastereomer forms and the kinds of derivation reagents that can be utilized. Diastereoisomers are the end products. Due to their distinct physicochemical characteristics, these compounds with two "chiral centers" can be separated using liquid chromatography on traditional "achiral" liquid chromatography columns. A chiral stationary phase6) is typically preferred over a chiral derivation agent for several reasons. Typically, a chiral mobile phase additive will precede a chiral stationary phase, in part because

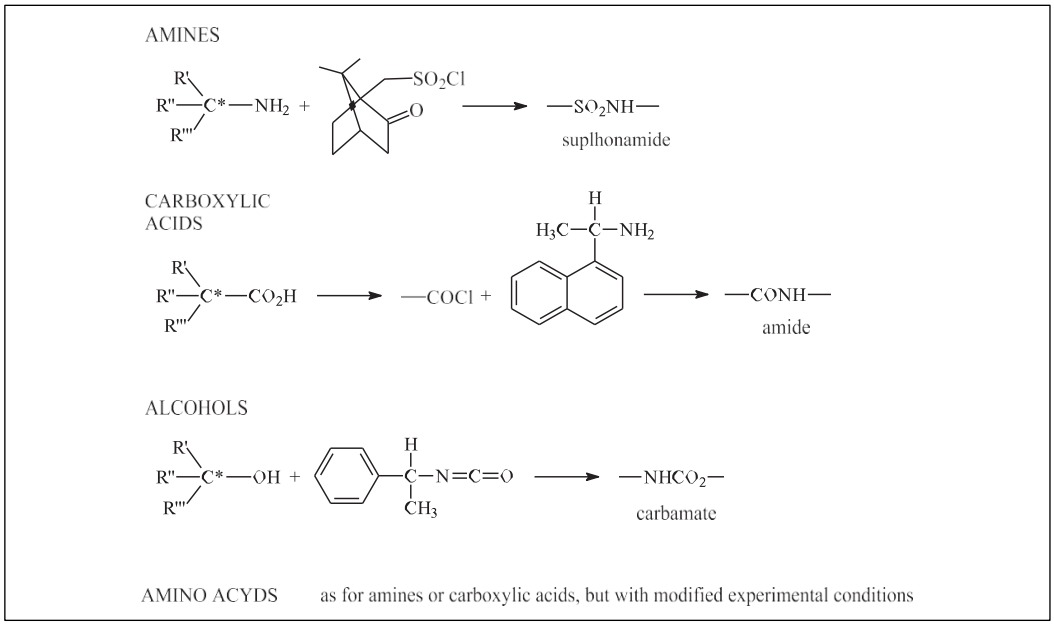


Figure 2. Typical common diastereomer formations.

The consumption of a frequently costly compound will increase significantly when a chiral selector is used as a mobile phase additive. Entatiomerically pure chiral chemicals can be found in nature and are reasonably priced. Thus, the majority of commercially available chiral stationary phases are based on these molecules. Proteins, cyclodextrins, derivatized polysaccharides, and derivatized amino acids are the most frequently utilized.

When studying the interactions between small and large molecules, especially drug-protein binding, liquid chromatography techniques are highly practical. Immobilized human serum albumin phase has been utilized by certain researchers to examine how medications including ibuprofen, warfarin, and benzodiazepines interact. It is possible to go one step further in using this phase as a model of interactions that occur in vivo. It is feasible to investigate how the presence of one drug affects the interaction of another with human serum albumin by adding a drug to the mobile phase. In drug dissolution studies on pharmaceutical formulations, liquid chromatography is also often employed to determine the potential availability of the medicinal substance from the formulation as it enters the stomach. The mixture is agitated in a dissolving bath that often contains an aqueous buffer intended to replicate stomach conditions. After that, samples of the aqueous buffer are taken over a predetermined amount of time, and the drug concentration is determined. Monitoring the excipient dissolution is also helpful since it may regulate the drug's release. This can be accomplished with liquid chromatography, but the analysis is more challenging because the excipients may be polymeric or have a weak chromophore. Naturally, it is far more difficult to determine the medication and excipient simultaneously in the dissolving samples.

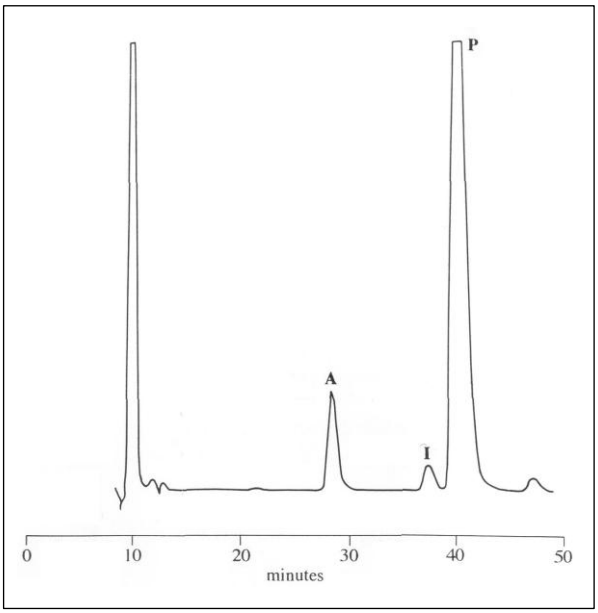
STABILITY STUDIES AS IMPORTANT PART IN QUALITY CON- TROL OF DRUGS

The study of stability in pharmaceuticals is vital because of the need to avoid the potentially toxic degradation products. In such studies it is necessary to demonstrate that the drug content of the formulation has not changed with time. Also if degradation does take place it will be necessary to identify and quantify the degradation prod- ucts. A good illustration of this is the liquid chromatog-

raphy conditions developed for the determination of pilo- carpine in ophthalmic solutions. The identical conditions are used for the determination of pilocarpine degradation products i. e. isopilocarpine and pilocarpic acid. [7]) Almost in all the laboratories for the quality control of drug the official methods for the assay of antibiotics are microbiological measurements. In order to provide it a separate laboratory for microbiological investigation has to be establishing with specialized staff (microbiologist). Nowa days high perfomance liquid chromatography technique is going to be used for the quantitative deter- mination of antibiotics. That is a great advantage in the field on quality control of drugs.Today in the Pharmacopoeias of USA, European, British and other high perfomance liquid chromatography is used instead of the chemical and meny instrumental methods for the control of drugs.

CONCLUSION

Figure 3. Sample chromatogram for separation of pilocarpine (P), isopilocarpine (I) and pilocarpic acid (A). A phenyl-bonded silica was used with mobile phase of acetonitrile-aqueous buffer (3:90 v/v), (pH 2. 5)The study of stability is not restricted to looking at degra- dation of the active. It is prudent to bear in mind that any degradation of a formulation excipient may lead to a change in the drug release characteristics of the formulation. For example lactose, a frequently used excipient, can undergo anomerisation in solution between its □ □ forms.



There are huge possibilities for further HPLC method development in several segments like creating new materials for preparing specific and more efficient stationary phases and related with that, discovering new combinations and modifications of mobile phases.

In scope of signal registration, new, more efficient hyphenated systems like HPLC-MS and HPLC-NMR are invented, as application of ^{1}H NMR and ^{13}C NMR tech- nique as well, which are more and more improving day after day, and whose application will open huge possibil- ities and assistance in medical diagnostics and in tracking the destiny of healing substances in body liquids.

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