TECHNICAL NOTE

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Problems in Using High Performance Liquid Chromatography for Drug Analysis

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ABSTRACT: Some problems encountered in using high performance liquid chromatography (HPLC) for drug analysis are discussed. These include high procurement and operational costs, lengthy training required, excessive downtimes, lower precision in HPLC versus gas chromatography (GC), and lack of a universal sensitive detector. Some solutions to these difficulties are presented.

KEYWORDS: toxicology, chromatographic analysis, comparative analyses

Commercial high performance liquid chromatography (HPLC) equipment has been available since the late 1960s. With the pumps, columns, and detectors now available, HPLC can provide resolving power, speed, and sensitivity comparable to gas chromatography (GC). The technique is used widely in government and private laboratories that analyze licit pharmaceuticals. However, based on informal discussions, it appears that HPLC is relatively unavailable or unused in forensic science laboratories that perform analyses of controlled substances. The objective of this presentation is to examine problems, real or perceived, that have restricted use of HPLC, and offer some comments and suggestions that may minimize or eliminate these problems.

Costs and Benefits

Costs of HPLC equipment are roughly comparable to costs of GC equipment. A basic HPLC unit can be obtained for under $10 000 while a computer-coupled model, with fraction collector, autosampler, variable wavelength detector, and other expensive accessories can cost over $90 000. As with GCs most laboratories will find that models that meet their requirements will have costs between these extremes. If GCs and HPLCs had comparable capabilities there would be no justification for a laboratory already equipped with GCs to acquire an HPLC. However, compounds that have low volatility, are highly polar, or are heat labile are difficult to analyze by GC. It has been estimated that only about 20% of all known

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organic compounds can be chromatographed without chemical modification [*1*]. Lysergic acid diethylamide (LSD), psilocybin, phenethylamines, barbiturates, and benzodiazepines are examples of compounds seen with some regularity in forensic science laboratories whose analysis by GC is complicated by one or more of the factors mentioned previously, but which can be analyzed directly by HPLC.

It is a common misperception that operating costs of HPLC are much higher than GC. However, the actual experience of a Drug Enforcement Administration (DEA) laboratory that uses both HPLC and GC for routine analyses is that costs can be comparable depending on the chromatographic mode. Figure 1 compares the comparative costs of operating two chromatographs for GC and HPLC. Using recently developed technology for high-speed HPLC the illustrated costs could be cut approximately in half. Identical, if not better separations can be obtained in less time with reduced solvent consumption by using shorter columns packed with 3- or 5-μm diameter packing materials.

Training

It has been estimated that it takes six months for the average analyst to become sufficiently proficient in HPLC to perform independent method development [*2*]. Workloads in most laboratories preclude an uninterrupted investment of this duration. However, instrument manufacturers, the FBI, and other sources can provide basic courses in HPLC which include hands-on training. With such training, methods available in the literature can be adapted and experience can be gained gradually while productive work is accomplished.

Delays and Downtime

One of the major problems encountered in the use of HPLC for forensic drug analysis is long start-up times. Start-up times can be shortened to little more than those needed for GC if some common errors are avoided and if analytical procedures are standardized.

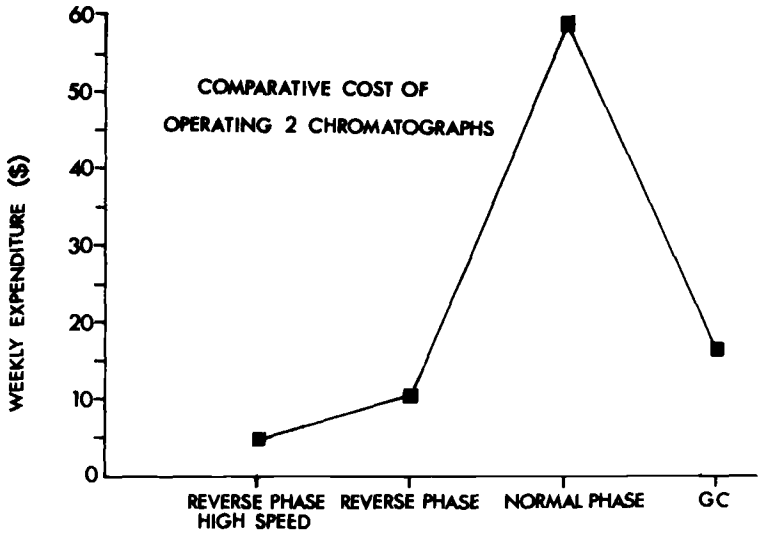

FIG. 1—*Comparison of the cost of operating two chromatographs. The comparison is based on the cost of carrier gases in GC and mobile phases in HPLC.*

1. Filter all samples and aqueous mobile phases. Small particles can cause failures that will be time-consuming and costly.

2. Degas all solvents. Gas bubbles in HPLC equipment cause erratic retention times.

3. Record the last eluant used in an HPLC system. Using mobile phases immiscible or insoluble with the existing eluant in the instrument will increase the start-up time.

4. Use as few mobile phases as possible [*3*]. One approach is to use two clearly labeled mobile phases common to two instruments, both equipped with reverse phase C-18 columns. One mobile phase is used for the analysis of cocaine, LSD, phencyclidine (PCP), barbiturates, and methaqualone, and the other is used for the analysis of phenethylamines and some opium alkaloids [*4*]. The mobile phases are made up in 4- to 8-L batches which generally last several weeks.

Low Precision

In general retention times have not been as reproducible for HPLC as they are with GC. It has been reported in a study involving over 75 drugs of forensic science interest that 45% could be distinguished on the basis of GC retention time while only 9% on the basis of HPLC retention time [*5*]. One solution to this problem is to arrange detectors to monitor the ultraviolet (UV) response at two different wavelengths and ratio the absorbance. When this was done in the study cited it was shown that 95% of the drugs could be distinguished by HPLC.

Precision comparable to GC can be obtained on the newer liquid chromatographs having microprocessor-controlled pumps and constant temperature ovens.

Detector Problems

One perceived disadvantage of HPLC relative to GC is the lack of a universal sensitive HPLC detector equivalent to the flame ionization detector for GC. Almost all drugs of forensic science interest exhibit absorbance using the most commonly employed detector in HPLC, the fixed wavelength UV detector at 254 nm. However, phenethylamines, barbiturates, some cannabinoids, and certain opium alkaloids are weak UV absorbers at this wavelength in most mobile phases used in HPLC. The absorbances of these compounds increase significantly at lower wavelengths. For example, the absorbance of heroin increases fiftyfold at 214 nm. Therefore, the variable UV detector can be used as an almost universal sensitive detector for drugs of forensic science interest provided the mobile phase has negligible absorbance at low wavelengths.

Conclusion

Although various problems, real and perceived, do exist in the analysis of drugs of forensic science interest by HPLC, the technique, if properly used, can be of use as a routine instrument in a forensic science laboratory.

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