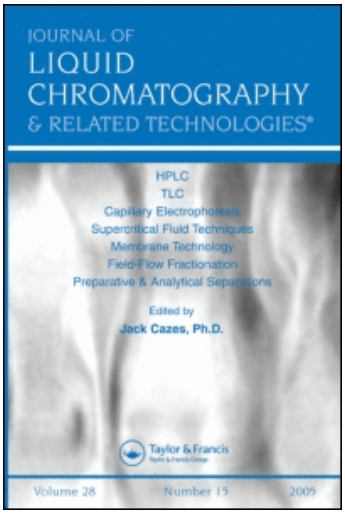

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HPLC Photodiode Array UV Detection for Toxicological Drug Analysis

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HPLC PHOTODIODE ARRAY UV DETECTION FOR TOXICOLOGICAL DRUG ANALYSIS

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ABSTRACT

An HPLC/UV system for the analysis of drugs and toxic compounds is presented. Retention data for 157 compounds in an acid HPLC system and 144 compounds in a basic HPLC system are listed. UV spectral data for 25 compounds from each system are listed. The usefulness of a computer search routine, utilizing UV and HPLC retention data, is discussed.

INTRODUCTION

The analysis of biological tissues and fluids for drugs and other toxic substances is a complicated process. There are currently in excess of seven million recognized compounds that have been reported in the literature (1). All of these compounds must be considered to be possible candidates when analyzing a sample for the presence and identification of a foreign substance. In order for a toxin to be identified, it must first be detected in the sample, which in the case of biological

systems, is a complex matrix of endogenous compounds. Once the suspect compound has been recognized and isolated from the other substances in the sample, identification methods can be employed. When the data collected uniquely distinguishes a compound from all other existing compounds, the absolute identity of that compound can be determined. Historically this process has been left to the discretion and experience of the individual analyst. Recently however, attention has been given to developing a standardized method of deciding when the accumulated data is sufficient for absolute identity. The concept of the number of distinguishable regions (DR) in an analytical system, as an evaluation of data uniqueness, has been introduced by Deur et al (2). "A minimum necessary mathematical condition for identification of a compound is that the number of distinguishable regions must be greater than or equal to the relevant population of compounds in order for absolute identification to be possible." Each analytical method, i.e. chromatographic, spectrometric, etc., produces a defined number of DR. Combining different types of analyses increases the number of distinguishable regions for a given analysis.

Chromatographic methods are primarily useful for separation of unknown substances from endogenous compounds in biological matrices. Performing a chromatographic technique on a sample results in retention data which reflects its partition coefficient and as such offers the analyst one physiochemical property of the compound in question. This data can be used as

one piece of evidence toward compound identity, but since many compounds exhibit the same retention characteristics, the information is not unique. Different types of chromatography have been shown to generate retention data that is correlated (2). Performing additional chromatographic analyses will add little to the identity profile of a compound.

Spectrometric analysis generally results in a greater number of distinguishable regions than chromatography. The combination of chromatography and spectral analyses can produce the quality of data needed to enhance confidence in the identity of an unknown compound.

Thin-layer chromatography (TLC) is generally economical and provides faster analysis than gas chromatography (GC) or high performance liquid chromatography (HPLC). It is an efficient method for handling large quantities of samples, and easily allows the use of different chemical reactions to detect the presence of functional groups. The method is best used to screen extracts of biological samples for drugs and toxic compounds. TLC is more limited than gas chromatography or high performance liquid chromatography in the range of compounds capable of being analyzed in a single system and it generally provides lower resolution than gas chromatography or high performance liquid chromatography.

Gas chromatography is most efficiently used for the analysis of volatile compounds, and results in higher resolution of compounds than TLC and most HPLC methods. Gas chromatography

interfaces well with a wide variety of detectors, especially spectrometric types. When used with temperature programming, a group of compounds with diverse polarities can be analyzed in a single GC system. Non-volatile compounds cannot be analyzed by GC unless they are made volatile by derivatization. The chemical data obtained from GC on a derivative is useful for confirming a compound's identity if GC data on the parent compound can also be obtained. The usefulness of GC data on derivatives alone is limited. A major disadvantage of GC is poor chromatography of polar compounds. Gas chromatographic analysis of highly polar compounds results in broad asymmetrical peaks and in some instances, adsorption prevents detectable elution of the compound. Retention times of polar compounds at low concentrations on GC are dependent on the amount of the compound applied to the column, (3) which is not controllable with unknown samples, and are therefore unreliable for comparison with reference data.

High performance liquid chromatography is a versatile chromatographic technique in which compound volatility is not a requirement. It provides higher resolution than TLC and when solvent programming is used, a large polar range of compounds can be analyzed in a single system. The greatest disadvantage to HPLC is the difficulty with which it is interfaced with some spectrometric detectors, particularly mass spectrometers and fourier transform infrared spectrometers. Mass spectrometric detection with HPLC puts some restrictions on the mobile phase

composition that can be used. Non-volital modifiers have a tendancy to restrict the orifice of the interface, requiring frequent cleaning. The use of thermospay or direct introduction of HPLC effluent necessitates the use of chemical ionization modes. The mass spectra will then depend on the reagent gas used, which in this case is the gaseous mobile phase. The mass spectra will therefore depend on the mobile phase composition. The restrictions placed on the use of mobile phases for mass spectral determination of compounds eluted by HPLC diminishes its flexibility.

The use of most mobile phases with infrared (IR) detection also poses difficulties. Mobile phases with limited infrared transparency will effect detection and quality of IR spectra, therefore mobile phases must be restricted to those with few absorption bands in the IR region. Since any mobile phase will generate some absorption bands in the IR region, the useful regions of the spectrum will be limited and will vary with the mobile phase that is used.

High performance liquid chromatography does however, easily interface with ultraviolet and visible detectors to provide useful data toward the identity of compounds. Although mobile phases are restricted to those which do not absorb in the ultraviolet spectral range, these are readily available, and compositions which allow the efficient separation of a wide range of polar compounds meet these requirements for ultraviolet spectral analysis.

Evaluation of the data obtained from these various analytical methods can be interpretive or comparative. Interpretation of some physiochemical data can lead to structural information. Mass spectra, infrared spectra and nuclear magnetic resonance spectra produce data that can be helpful in structure determination. Ultraviolet spectra, however will yield little in the way of interpretive data. Identity of compounds by data matching depends on generating a set of data for reference compounds for each of the spectrometric methods. Data obtained from unknown compounds can then be directly compared to data generated by reference compounds. Success of this technique depends on the size of the data base, efficiency of the algorithm used to identify identical data profiles and to differentiate between data profiles of different compounds, and the reproducibility of the data collected.

EXPERIMENTAL

Materials

Reference compounds were obtained from Applied Science, United States Pharmacopea or directly from the manufacturer and used without further purification. Phosphoric acid (HPLC grade) and ammonium hydroxide were obtained from Fisher Chemical Co. High performance liquid chromatography grade methanol and acetonitrile (Omnisolv) were obtained from E. M. Merck Co. and reagent grade water was generated by a Millipore MILLI-Q Water Purification System.

Instrumentation

High performance liquid chromatography was performed on a Waters HPLC system consisting of two Model 6000A solvent delivery systems, a 660 solvent programmer and a U6K injector. The compounds separated on this system were detected by a Hewlett-packard 1040A spectrophotometric detector at 230 nm. Ultraviolet data generated by the detector was collected by a Hewlett-Packard 85 controller and stored on a Hewlett-Packard 9135A disk drive. Retention data was collected by a HP3352D data system.

For analysis of acidic and neutral drugs, a Zorbax C_8 column maintained at 31C was used. Solvent A was 0.1% (v/v) H_3PO_4 and solvent B was 0.1% (v/v) H_3PO_4, 10% H_2O in CH_3CN. At a flow rate of 2.0 mL/min, compounds were eluted using a linear solvent program of 0 to 100% B/A in 30 minutes.

For analysis of basic and neutral compounds, a PRP-1 (Hamilton) column at ambient temperature was used. Solvent A was 1.0% (v/v) NH_4OH and solvent B was 1.0% (v/v) NH_4OH in CH_3CN. At a flow rate of 2.0 ml/min, compounds were eluted in a linear solvent program of 0 to 100% B/A in 30 minutes.

Methods

Reference compounds were dissolved in methanol at a concentration of 500 ug/mL. Ten to 40 uL of this solution was analyzed on the appropriate HPLC system. The retention data was recorded and the UV spectra (200 - 402 nm at 2 nm steps) at the apex of the peak was normalized (Equation 1) and stored in a reference library.

Calculations

The absorbance at each wavelength was normalized to the area under the spectral curve by Equation 1 (4).

Equation 1

$$N_i = A_i \Big/ \left(\sum_{j=200}^{402} A_j\right)$$

Where: N_i = FTA (Fraction of Total Absorbance) at wavelength i

A_i = Absorbance at wavelength i

i = Individual wavelengths

j = Wavelength in spectral profile

The comparison of the unknown spectral profile to that of a reference spectral profile was performed by Equation 2 (4).

Equation 2

$$M = \frac{2 - \sum \left| S_i - R_i \right|}{2} .1000$$

Where: M = goodness of fit value (FIT)

S = response in the sample spectra at λ_i

R = response in reference spectra at λ_i

RESULTS AND DISCUSSION

Combining HPLC separation of drugs with UV detection and computer matching of spectra can be an effective analytical tool. Tables I and II list the retention times of drugs separated in the acid and basic HPLC system respectively. The data is

TABLE I

RETENTION TIMES OF DRUGS SEPARATED IN THE BASIC HPLC SYSTEM

Drug Name	Retention Time (Min.)
Acepromazine	—
Acetaminophen	2.7
Acetophenazine	19.9
Acetophenetidine	13.8
Allylcyclopenterylbarbital	7.0
Allylisobarbituric acid	7.2
Alphaprodine	15.5
Alphenal	7.4
Atropine	15.7
Aminoantipyrine	11.1
Amitriptyline	29.0
Amobarbital	7.9
Amylocaine	24.4
Antipyrine	10.7
Aprobarbital	6.2
Barbital	5.6
Benzocaine	16.6
Benzoylegonine	9.3
Benzphetamine	26.0
Brucine	14.6
Butabarbital	6.7
Butacaine	25.5
Butethal	6.9
Caffeine	9.2
Cannabinol	30.0
Carvacrole	21.5
Chloramphenicol	13.6
Chlordiazepoxide	16.6
Chloroquine	25.0
8-Chlorotheophylline	6.0
Chlorphenesin carbamate	14.2
Chlorpheniramine	21.4
Chlorpropamide	9.2
Chlorpromazine	32.1
Cinchonidine	18.5
Cinchonine	19.6

(continued)

TABLE I (continued)

RETENTION TIMES OF DRUGS SEPARATED IN THE BASIC HPLC SYSTEM

Drug Name	Retention Time (Min.)
Clonazepam	14.1
Cocaine	21.7
Codeine	13.6
Colchicine	13.5
Cortisone	14.1
Coumarin	17.0
Creatinine	1.8
Danthron	14.4
Dapsone	13.9
Despropanylfentanyl	26.5
Dexamethasone	15.2
Diallylbarbituric acid	6.0
Diazepam	21.5
Dibucaine	25.1
Didrate	13.4
Diethylstilbestrol	18.2
Diethyltryptamine	1.1
Dilaudid	6.7
1-3-Dimethylbarbituric acid	2.2
2,3-Dimethyl-1-(4-Methyl-phenyl) -3-pyrazolin-5-one	10.2
Diphenoxylate	24.5
Diphenyhdramine	23.5
Diphenylhydantoin	9.1
Dipropyltryptamine	25.9
Doxapram	20.2
Dyphylline	7.6
Estrone	20.0
Ethonitazene	25.2
Ethosuximide	1.2
Ethylmorphine	14.9
N-Ethylnornicotine	14.4
Etonitazene	25.2
Eugenol	25.2

TABLE I (continued)

RETENTION TIMES OF DRUGS SEPARATED IN THE BASIC HPLC SYSTEM

Drug Name	Retention Time (Min.)
Fenfluramine	31.2
Fentanyl	24.0
Flurazepam	18.8
Gentisic acid	1.2
Glutethimide	17.4
Guaiacol	9.8
Hexabarbital	8.2
Hydrocortisone	17.8
Hydroxyethyltheophylline	8.3
4-Hydroxyguinoline	7.2
2-Hydroxyguinoline	10.8
5-Hydroxyisoquinol	5.8
8-Hydroxyquinoline	12.6
Imipramine	28.7
Indol-3-carboxaldehyde	13.7
Indomethacin	12.4
3-Isobutyl-1-methylxanthine	7.8
Isoquinoline-N-oxide	8.7
Levorphanol	15.8
Lidocaine	19.6
Meclizine	33.9
Mefenamic acid	12.7
Meperidine	19.1
Mephensin	12.8
Mephobarbital	8.6
Mepivacaine	17.3

(continued)

TABLE I (continued)

RETENTION TIMES OF DRUGS SEPARATED IN THE BASIC HPLC SYSTEM

Drug Name	Retention Time (Min.)
Mescaline	11.6
Methocarbamol	11.9
Methoxamine	13.4
Methylphenidate	18.7
3-methylxanthine	1.8
Morphine	7.9
N-acetylprocainamide	11.9
Nalorphine	8.8
Naloxone	11.9
6-B-Naltrexol	11.8
Naphthalene	27.4
Naproxen	8.7
Nicotine	12.9
Nikethamide	10.1
N-Normorphine	6.5
Oxazepam	15.0
Papaverine	18.5
Paraxanthine	2.9
PCP N-ethyl analog	24.3
Pemoline	8.2
Pentazocine	20.2
Pentobarbital	8.6
Phencyclidine	28.9
Phenobarbital	7.1
Phentermine	15.5
Physostigmine	15.1
Prednisolone	13.6
Prednisone	14.2
Procaine	16.5
Propiomazine	27.8
Pyrilamine	22.4

TABLE I (continued)

RETENTION TIMES OF DRUGS SEPARATED IN THE BASIC HPLC SYSTEM

Drug Name	Retention Time (Min.)
Quinoline-N-oxide	9.0
Reserpine	25.2
Secobarbital	8.9
Sulfacetamide	1.3
Sulfadimethoxine	7.7
Sulfamerazine	5.9
Sulfamethazine	7.0
Sulfamethizole	7.3
Sulfamethoxazole	6.5
Sulfanilamide	6.3
Sulfapyridine	6.9
Tetracaine	21.1
Thebaine	18.3
Theophylline	2.9
Thiamylal	10.2
Thienylcyclohexylpiperidine	28.1
2-Thiobarbituric acid	1.2
Thiosalicylic acid	6.0
Warfarin	11.6

TABLE II

RETENTION TIMES OF DRUGS SEPARATED IN THE ACID HPLC SYSTEM

Drug Name	Retention Time (Min)
Acetaminophen	8.2
Acetophenetidine	14.6
Acetylsalicylic acid	13.6
Aflatoxin B_1	17.0
Allylcyclopentenyl barbaturic acid	13.9
Allylisobarbituric acid	13.9
Alpha-Naphthol	17.8
m-Aminobenzamide	2.9
2-Aminobenzamide (Anthranilamide)	6.0
m-Aminobenzoic acid	5.3
o-Aminobenzoic acid	10.5
p-Aminobenzoic acid	7.2
Aminophylline	8.8
Amobarbital	15.5
o-Anisic acid	12.9
Antipyrine	12.9
Aprobarbital	12.3
Barbital	9.4
Benzoic acid	13.2
Benzoylecgonine	21.7
Butabarbital	13.1
Butethal	14.2
Butylparaben Dyphylline	16.7
Caffeine	10.2
Cannabichromene	27.1
Cannabinol	26.0
Carvacrole	21.1
Chloramphenicol	14.4
7-2-Chloroethyltheophylline	12.8
p-Chlorophenol	16.8
Chlorothiazide	9.1
Chlorphenesin carbamate	15.4
Chlorpropamide	17.7
8-Chlorotheophylline	10.3
Colchicine	15.3
Cortisone	15.0
Coumarin	15.1
Cyclothiazide	18.6
Cyheptamide	17.8

TABLE II (continued)

RETENTION TIMES OF DRUGS SEPARATED IN THE ACID HPLC SYSTEM

Drug Name	Retention Time (Min)
Danazol	19.3
Danthron	22.3
Dapsone	13.1
Diallylbarbituric acid	14.0
Dichlorophene	21.9
Diethylstilbestrol	21.5
3-4-Dihydroxyphenethyl alcohol	7.8
3-4 Dihydroxyphenylacetic acid	8.2
3-4-Dihydroxyphenylglycol	3.5
6-6-Dithiodinicotinic acid	14.7
β-Estradiol	19.4
Estriol	15.0
Estrone	20.3
Ethosuximide	9.8
2-Ethyl-2-phenylmalonamide	9.7
2-Ethyl-2(p-tolyl)malonamide	11.9
Eugenol	18.8
Fenoprofen	19.2
5-Fluorouracil	3.0
Fluoxymesterone	15.0
Gentisic acid	9.5
Gitoxigenin	16.0
Glutethimide	16.3
Guaiacol	11.4
Hexabarbital	15.1
Hexahydrocannabinol	26.8
4-Hexylresorcinol	20.8
Hippuric acid	9.6
Homovanillic acid	10.2
Hydrocortisone	14.4
Hydroquinone	3.3

(continued)

TABLE II (continued)

RETENTION TIMES OF DRUGS SEPARATED IN THE ACID HPLC SYSTEM

Drug Name	Retention Time (Min)
B-Hydroxyethyltheophylline	9.2
5-Hydroxyindole-3-acetic acid	9.4
5-Hydroxyisoquinoline	7.5
11α Hydroxy-17a-methyltestosterone	14.5
6-Hydroxynicotinic acid	6.5
p-Hydroxyphenobarbital	10.6
p-Hydroxyphenylpyruvic acid	11.1
2-Hydroxyquinoline	11.6
4-Hydroxyquinoline	9.3
5-Hydroxyquinidine	7.5
8-Hydroxyquinoline	8.6
Ibuprofen	22.4
3-Indole acetic acid	13.8
Indole-3-carboxyaldehyde	12.2
Indomethacin	22.0
3-Isobutyl-1-methylxanthine	12.7
Isocarbostyril	11.8
Isoquinoline-N-oxide	11.2
Lasix	17.0
Lysergic acid	12.1
Lysergic acid hydroxyethylamide	11.0
Mefenamic acid	23.7
Methocarbamal	12.6
2-Methoxy-3-6-dichlorobenzoic acid	11.6
αMethyl-L-dopa	6.4
3-O-Methyldopamine	7.2
Methylparaben	12.8
4-Methylprimidone	13.2
Methylsalicylate	19.3

TABLE II (continued)

RETENTION TIMES OF DRUGS SEPARATED IN THE ACID HPLC SYSTEM

Drug Name	Retention Time (Min)
Methyltestosterone	19.6
17-Methyltestosterone	17.3
17α-Methyltestosterone	17.2
3-Methylxanthine	6.8
2-Naphthoyacetic acid	17.3
Nicotinic acid	3.9
Nitrofurantoin	11.2
m-Nitrophenol	15.2
Normethsuximide	14.5
Oxyphenbutazone	19.8
Paraxanthine	8.7
Pentobarbital	15.2
p-Phenetidine	8.7
Phenylbutazone	23.4
Piperonyl butoxide	26.1
Prednisolone	15.2
Prednisone	15.5
Primidone	11.3
Progesterone	21.8
Probenecid	20.5
Propylparaben	18.3
Pyrithydione	11.2
Pyrocatechol	4.6
Quinoline-N-oxide	11.2

(continued)

TABLE II (continued)

RETENTION TIMES OF DRUGS SEPARATED IN THE ACID HPLC SYSTEM

Drug Name	Retention Time (Min)
Reserpine	22.5
Resorcinol	4.3
Saccharin	8.0
Salicylamide	11.0
Salicylic acid	15.0
Secobarbital	15.7
Stanozolol	19.2
Sulfacetamide	8.4
Sulfadimethoxine	7.7
Sulfaethidole	13.4
Sulfamerazine	10.1
Sulfamethazine	7.0
Sulfamethizole	11.5
Sulfamethoxazole	13.6
Sulfanilamide	4.0
Sulfapyridine	9.6
Sulfasoxazole	14.2
Sulindac	19.1
Testosterone acetate	20.5
Testosterone enanthate	25.0
Testosterone propionate	24.1
Testosterone-17β-cypionate	25.2
Δ-8-tetrahydrocannabinol	26.3
Theobromine	8.3
Thiamylal	18.2
2-Thiobarbituric acid	2.4
Thiosalicylic acid	15.4
Tolbutamide	18.8
Tolmetin	18.7
Triamcinolone	13.8
Triamcinolone acetonide	17.8
m-Trifluromethylbenzoic acid	17.5

TABLE II (continued)

RETENTION TIMES OF DRUGS SEPARATED IN THE ACID HPLC SYSTEM

Drug Name	Retention Time (Min)
Vanillylmandelic acid	5.9
Warfarin	20.0

presented here to 1) demonstrate the variety of drugs that can be analyzed by this system and 2) to give a general idea of the position of elution in the two systems. The relative order of elution of compounds with retention times within a minute of each other cannot be predicted from this information since retention times over a long period of time are not reproducible.

The reproducibility of retention characteristics can be improved by normalizing the data relative to reference compounds. Reproducibility of retention indices is quite good. HPLC retention data is generally recorded as retention time (R_t), relative retention time (RR_t) or capacity ratio (K'). Retention time and K' data cannot be reproduced from run to run between columns of the same manufacturer or between laboratories. As such, this data is not suitable reference material. Relating retention times of unknown compounds to the R_t of a co-analyzed reference standards (RR_t) is reproducible within a single laboratory but has a tendancy to vary between instruments and

laboratories. Co-analyzing unknown compounds with a pair of compounds that elute before and after the unknown compound and calculating the retention relative to both reference standards results in reasonably reproducible data. The most commonly used reference standards for this purpose in HPLC have been the homologous series of alkylphenones. This approach to improving reproducibility of HPLC data is not however, without certain limitations.

Smith (5) et al showed that using an isocratic reverse-phase (RP) system in analyzing partially ionized barbiturates, a change in the H_2O/MeOH ratio, the pH, the ionic strength or the temperature caused changes in the retention index. The retention index of non-ionizable compounds was not affected by pH, ionic strength or temperature changes. Changes in the H_2O/MeOH ratio, however, did affect the retention index of non-ionized compounds. Repeated analyses of partially ionized barbiturates and non-ionic compounds resulted in reproducible retention indices when the mobile phase composition and temperature was the same and analyses were performed on the same brand of column from the same or different production batch. Switching brands of RP columns of the same type (i.e. octacecyl) resulted in retention indices that were quite different (6). This seems to indicate that the bonding chemistry of columns varies between manufacturers.

Other work done using retention indices have shown reproducibility between columns of the same brand. Hill et al (7) analyzed a group of mycotoxins in an HPLC solvent gradient

system using an alkylphenone retention index scale. This work demonstrated a method whereby retention of the alkylphenones in the homologous series could be calculated relative to two reference compounds on a daily basis. Only the two reference compounds are co-analyzed with the sample, reducing the possibility of an unknown compound in a mixture co-eluting with one of the sample components as might occur if the entire homologous series is co-analyzed. Using a solvent program system, Equation 3 would be used if the relationship of the retention of an alkylphenone (T_p) and the number of carbons in the alkylphenone (n) is assumed to be linear between two reference compounds that vary by only one unit in the homologous series.

Equation 3

$$RI_B = \frac{(T_x - T_{p1})\ \Delta z100}{T_{p2} - T_{p1}} + z100$$

Where: RI_B = the bracketed retention index

T_x = the retention time of test compound

T_{p1} = the retention time of the alklyphenone that elutes before the test compound

T_{p2} = the retention time of the alkylphenone that elutes after the test compound

z = number of carbons in alkylphenone that elutes before the test compound

However, the true relation of alkylphenone retention in the reverse-phase solvent gradient system appears to be a function of

the log of the number of variable units in the homologous series, therefore Equation 4 should be used to calculate the retention index for compounds eluted from this type of HPLC system.

Equation 4

$$RI_G = \left(e^{\frac{T_x - T_1}{T_2 - T_1}(\ln z_2 - \ln z_1) + \ln z_1} \right) .100$$

Where: RI_G = retention index in solvent gradient system

T_x = retention time of test compound

T_1 = retention time of alkylphenone eluting before the test compound

T_2 = retention time of alkylphenone eluting after the test compound

z_1 = number of carbons in the alkylphenone eluting before the test compound

z_2 = number of carbons in the alkylphenone eluting after the test compound

Generally, the following criteria are needed for a HPLC/UV system: 1) a mobile phase which has low absorbance in the UV spectral range of 200-402 nm, 2) the ratio of absorbance between the two mobile phases in a binary solvent program system should not change significantly over the 200-402 nm range, 3) in a single HPLC system, the changes in the mobile phase across the solvent gradient should not significantly affect the UV spectral profile of eluting compounds and 4) the compounds should elute in narrow non-tailing peaks. The HPLC system used

in this study met these criteria. Water and acetonitrile possess little UV absorption between 200-402 nm. Changes in H_2O/CH_3CN ratios do not cause differences in absorbances that cannot be corrected by baseline subtraction.

Most compounds will generate different UV spectral profiles when analyzed at different hydrogen ion concentrations. In the developed solvent gradient system the hydrogen ion concentrations and ionic strengths in both solvents of the binary system were the same. This resulted in a constant hydrogen ion concentration across the solvent gradient.

Basic drugs usually elute with broad asymmetrical peaks in acid mobile phases on silica based reverse-phase columns, probably due to hydrogen bonding of the proteinated amines with the free silol groups on the silica columns. This situation has been circumvented by tying up the charged group with a counter-ion and performing ion-pair chromatography. Another approach to eleviate this problem has been to deactivate the silol groups by addition of an organic amine to the mobile phase. Adding modifiers such as these to the mobile phase will result in unacceptable UV absorption at lower wavelengths.

It is desirable to introduce a basic modifier to HPLC systems for the separation of basic compounds, which supresses the ionization of the basic constituent. However, silica based reverse phase materials are unstable at high pH and the column life cannot be effectively maintained. Carbon based reverse-phase columns are now available, however, and they are not susceptible to degradation in basic pH.

While reproducibility of chromatographic data is limited without the use of normalization techniques, the reproducibility of spectral data is usually quite good under reasonably controlled conditions and defined parameters (4). Spectral data generated on a variety of instruments at different laboratories is sufficiently reproducible to make accurate comparisons. This lends itself well to combining data from different locations for developing a spectral data base.

Table III and Table IV present representative UV spectral data obtained for drugs eluted in the acid mobile phase and basic mobile phase, respectively. Spectral comparisons have been accomplished using a computer search routine developed by Hill, et al. (4). In this system, the fraction of total absorbance (FTA) at each wavelength in the spectral profile is calculated as the ratio of the absorbance to the total absorbance under the spectral curve. (Equation 1). The unknown spectrum is first compared to reference library spectra by matching wavelengths of maximum absorbance and FTA values at these wavelengths within a given range of variation. The unknown spectrum is compared to the reference spectra meeting these presearch criteria. Using the FTA value as a comparative parameter, in addition to the traditional comparison of wavelengths of maximum absorbance, narrows the number of possible compounds that have spectral profiles close to the spectrum of an unknown. Whether this manipulation is accomplished manually or by computer, combining these two parameters enhances the efficiency of comparing spectral data.

TABLE III

UV SPECTRAL DATA OF DRUGS SEPARATED

IN THE ACID HPLC SYSTEM

DRUG	λMAX	FTA
Acetaminophen	245	.0327
Acetophenetidine	249	.0357
Acetylsalicylic Acid	227	.0398
	277	.0057
Benzoylecgonine	233	.0544
	275	.0042
Caffeine	205	.0583
	273	.0238
Cannabinol	221	.0356
	283	.0195
Cyclothiazide	225	.0488
	271	.0294
	315	.0042
Dexamethasone	241	.0399
Dyphylline	207	.0578
	273	.0226
Ibuprofen	221	.0580
	263	.0022
Lasix	235	.0432
	275	.0222
	343	.0064

(continued)

TABLE III (continued)

UV SPECTRAL DATA OF DRUGS SEPARATED IN THE ACID HPLC SYSTEM

DRUG	λ MAX	FTA
Lysergic Acid	225	.0280
	311	.0104
Mefenamic Acid	221	.0352
	279	.0101
	351	.0082
Naproxen	230	.0721
	264	.0057
	272	.0058
	318	.0015
	330	.0018
Phenylbutazone	239	.0299
Prednisone	243	.0395
Probenecid	227	.0261
	251	.0269
Salicylic Acid	205	.0867
	237	.0236
	303	.0110
Salicymide	203	.0825
	237	.0212
	299	.0102
Sulfamethazine	243	.0201
	261	.0198

TABLE III (continued)

UV SPECTRAL DATA OF DRUGS SEPARATED

IN THE ACID HPLC SYSTEM

DRUG	λMAX	FTA
Sulindac	227	.0151
	259	.0113
	285	.0119
	329	.0107
Testosterone	245	.0532
Thiosalicylic Acid	221	.0588
	249	.0187
	311	.0068
Tolmetin	257	.0123
	313	.0247
Warfarin	205	.0511
	273	.0152
	281	.0165
	305	.0139

Figure 1 illustrates a basic HPLC separation of compounds isolated from a urine sample. The UV spectra at the apex of the peak was normalized and searched through a reference file of 350 spectra. The result of the search is shown in Figure 2. The search gave a best fit for matching the unknown spectra to that of oxazepam. An analysis of authentic oxazepam in the basic

TABLE IV

UV SPECTRAL DATA OF DRUGS SEPARATED IN THE BASIC HPLC SYSTEM

DRUG	λ_{MAX}	FTA
Acepromazine	211	.0150
	243	.0240
	279	.0233
Acetaminophen	257	.0262
Acetophenetidine	249	.0378
n-Acetylprocainamide	205	.0379
	267	.0328
Amitriptyline	211	.0585
	239	.0245
Amobarbital	239	.0472
Brucine	207	.0367
	265	.0182
	303	.0131
Butacaine	221	.0172
	289	.0353
Caffeine	205	.0612
	273	.0235
Chlorpheniramine	225	.0411
	261	.0156

TABLE IV (continued)

UV SPECTRAL DATA OF DRUGS SEPARATED

IN THE BASIC HPLC SYSTEM

DRUG	λMAX	FTA
Cocaine	229	.0649
	259	.0039
	273	.0044
Codeine	211	.0690
	283	.0043
Dexamethasone	241	.0408
Dyphylline	207	.0600
	273	.0225
Hydrocortisone	207	.0737
	281	.0114
Mephensin	215	.0565
	271	.0120
Methagualone	203	.0439
	225	.0429
	265	.0116
	305	.0045
	315	.0036
Morphine	217	.0486
	297	.0057
Phenobarbital	241	.0316
Predisone	243	.0403

(continued)

TABLE IV (continued)

UV SPECTRAL DATA OF DRUGS SEPARATED IN THE BASIC HPLC SYSTEM

DRUG	λ_{MAX}	FTA
Procaine	221	.0154
	291	.0347
Promazine	207	.0334
	255	.0475
	309	.0063
Pyrilamine	227	.0234
	251	.0350
	313	.0088
Secobarbital	241	.0422
Theophylline	207	.0443
	275	.0266

HPLC system indicated a good match of the UV spectra as well as the retention time (Figure 1).

It is possible to analyze for a broad range of compounds using an HPLC/UV computer search system. It can provide a high degree of efficiency and reproducibility, and accurate results have been obtained both experimentally and in actual sample analysis. Analyzing biological samples for drugs or toxins using HPLC/UV can contribute significantly to the level of confidence

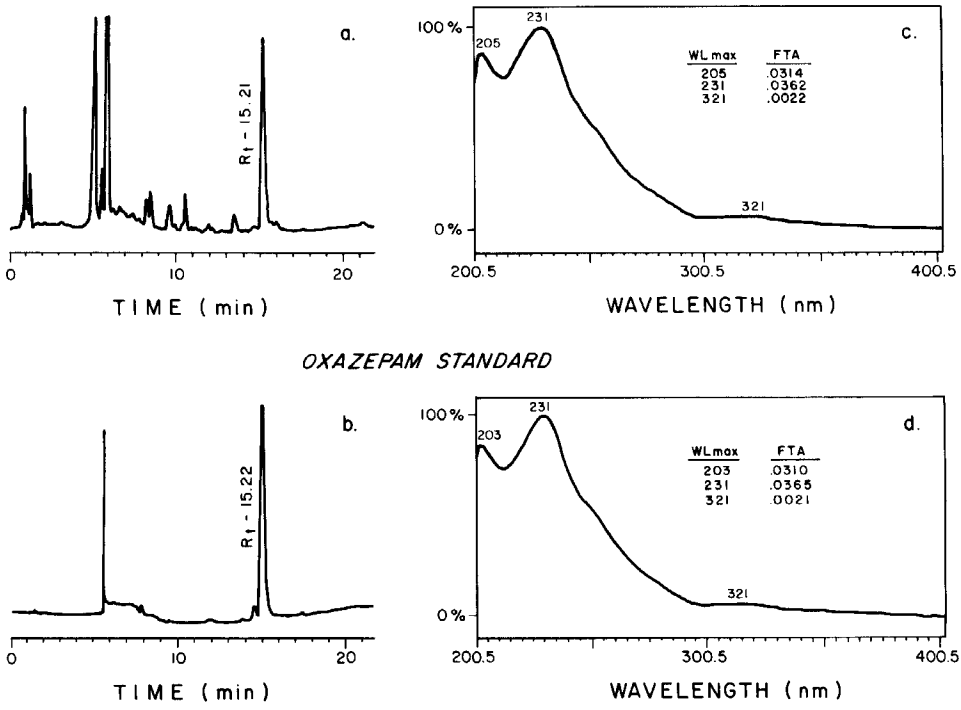

Figure 1 HPLC/UV data for human urine extract analyzed on basic HPLC system (see text). a) chromatogram of urine extract b) chromatogram of oxazepam standard c) UV spectra at apex of peak at R_t - 15.21 in urine extract chromatogram d) UV spectra at apex of peak at R_t - 15.22 in oxazepam standard chromatogram.

in compound identification. Combining a HPLC retention index data base with the UV spectral data base would provide a higher degree of confidence in results obtained from a library search routine.

```
                    UV LIBRARY SEARCH

ENTER FTA SEARCH WINDOW (±) AND # OF FTA MATCHES
BEFORE ABORT (MAX 50)

?

.002,25

ENTER λ WINDOW FOR SEARCH (± 0,2,4 or 6).

?

2

—

PRE-SEARCH MATCHES ARE:  21

        DRUG LIBRARY SEARCH RESULTS

REC#          FIT          NAME

298           986          OXAZEPAM
276           948          DIAZEPAM
291           938          FLURAZEPAM
270           830          METHAQUALONE
 16           806          PAPAVERINE
```

Figure 2 Reproduction of computer printout of library search routine performed on spectra obtained from chromatogram of urine extract (Figure 1c). The first two queries establish the pre-search parameters for FTA and wavelength maximum deviation, respectively.

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