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Review Article In This Issue

Use of HPLC with Diode Array Spectrophotometric Detection in Forensic Drug Analysis

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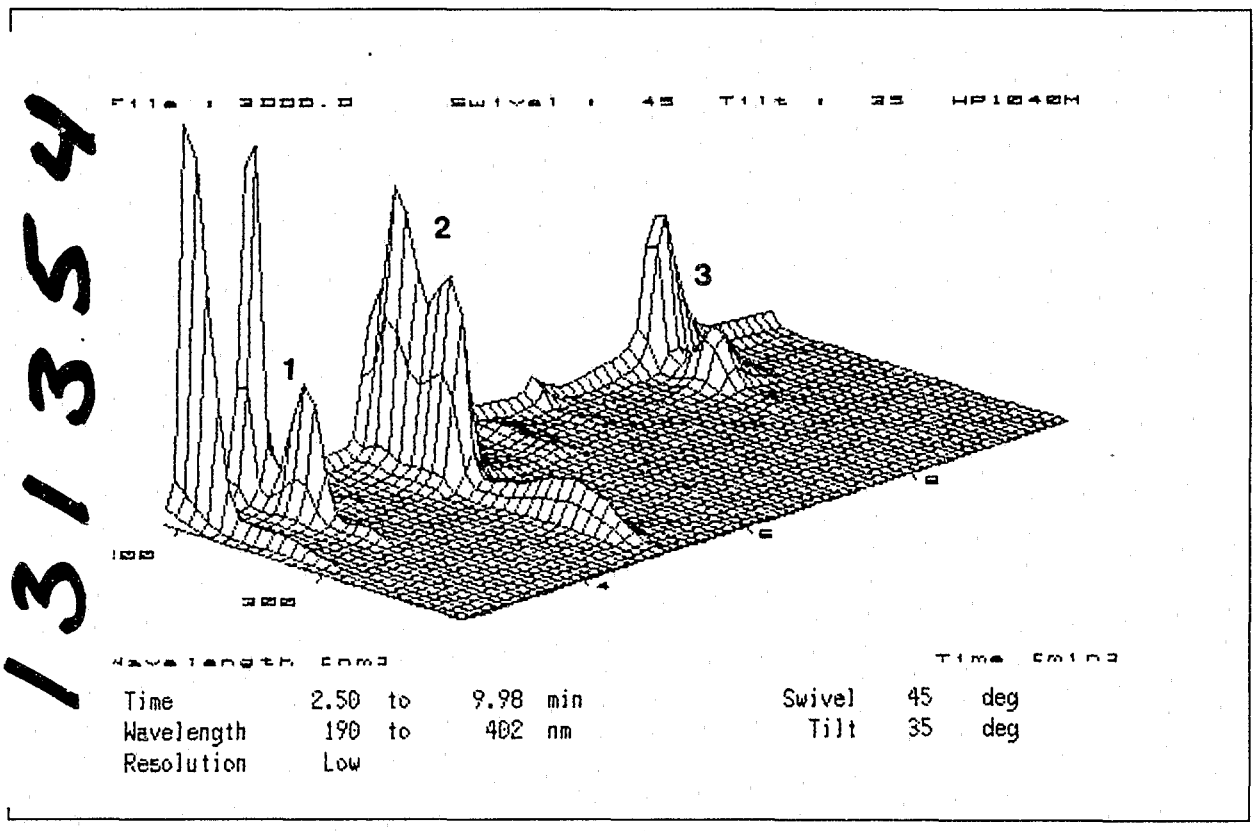

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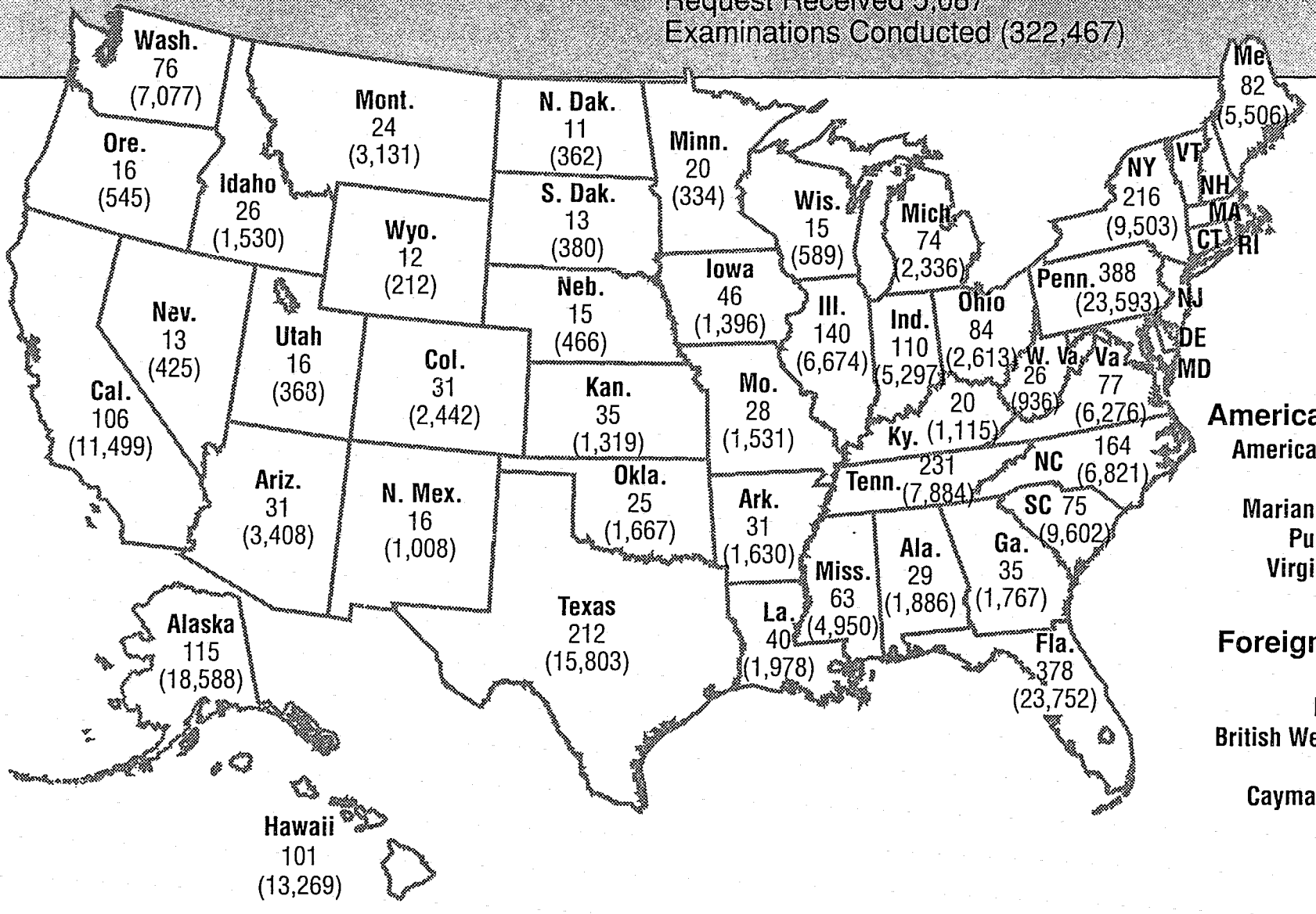
Fiscal Year **1989**
Request Received 5,087
Examinations Conducted (322,467)



VT	33	(488)
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RI	105	(12,218)
NJ	170	(9,331)
DE	139	(15,664)
MD	507	(24,642)
DC	521	(28,835)

American Possessions

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Guam	6	(234)
Mariana Islands	8	(624)
Puerto Rico	2	(81)
Virgin Islands	78	(5,773)

Foreign Governments

Australia	2	(297)
Bahamas	6	(87)
British West Indies	4	(903)
Canada	4	(420)
Cayman Islands	1	(95)
Jamaica	1	(12)

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Review Article

131353

The Use of HPLC with Diode Array Spectrophotometric Detection in Forensic Drug Analysis

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University of Tennessee
Memphis, Tennessee 38163

High performance liquid chromatography (HPLC) has been used in forensic laboratories for a number of years, with applications in drug analysis, toxicology and trace analysis (Saferstein 1982; 1988). Until recently, the detectors available for HPLC have provided only limited information about a chromatographic peak, namely its retention time and its response to a single measuring probe (for example, absorbance at a single wavelength, electrochemical activity at a given potential, etc.). This has restricted the role of HPLC, mostly to quantitative analysis (Gill 1986) and to profile comparisons for complex mixtures (Saner *et al.* 1976; Colwell and Karger 1977).

Two important developments in instrument technology, low cost diode array ultraviolet (UV)/visible spectrophotometric detection (Owen 1988; Tobias 1989) and more reliable interfaces for liquid chromatography/mass spectrometry (LC/MS) (Bowers 1989) now allow the measurement of multiple data points on any liquid chromatographic peak, markedly improving the discriminating power of HPLC. This article illustrates, with some authentic examples from our casework, the theoretical and practical advantages offered to forensic chemists by diode array UV/visible spectrophotometric detection.

THEORY

Measuring UV/visible absorbance at a single wavelength has been the favored detection method for HPLC for many years because of its sensitivity, general applicability and relatively low cost. In a variable wavelength detector, the monitoring wavelength is selected by the use of interchangeable filters or by using a prism or diffraction grating mounted on a turntable to diffract the light, then using a monochromator to select the desired wavelength (Figure 1a). The monochromated light beam is directed through a flow cell, where some of the light will be absorbed when a molecule with an appropriate chromophore passes through. The resultant light intensity is measured by a photocell and compared to the intensity of the unabsorbed beam. The difference in intensities is proportional to the amount of chromophore present in the flow cell, according to Beer's Law. A plot of this absorbance against time results in a chromatogram.

In the diode array detector, the components of the conventional spectrophotometer have been rearranged in the so-called reversed optics format (Figure 1b), such that undiffracted white light passes through the sample flow cell. Since all wavelengths from 190 to 600 nm are present in the white light, any compound with light absorbing properties in that range will be detected. After passing through the flow cell, the light is dispersed into a spectrum using a holographic grating and directed onto the diode array. The light sensitive unit of the diode array detector consists of a capacitor in parallel with a light sensitive diode. A large number (an array) of such photodiode/capacitor pairs arranged linearly, up to 200/cm in some cases, can be placed on a single silicon chip (Tobias 1989). Light falling on the photodiode allows the capacitor to discharge. The remaining charge in each capacitor, which is inversely proportional to the amount of light reaching the corresponding photodiode, is measured at regular intervals (timebase). As separate photodiodes are exposed to adjoining sections of the spectrum, a complete UV spectrum is obtained. Compared to conventional scanning spectrophotometers which can take several minutes to scan through 400 nm, the electronic scanning of the diode array can be accomplished in a matter of milliseconds, enabling the spectrum to be acquired instantaneously (Owen 1988).

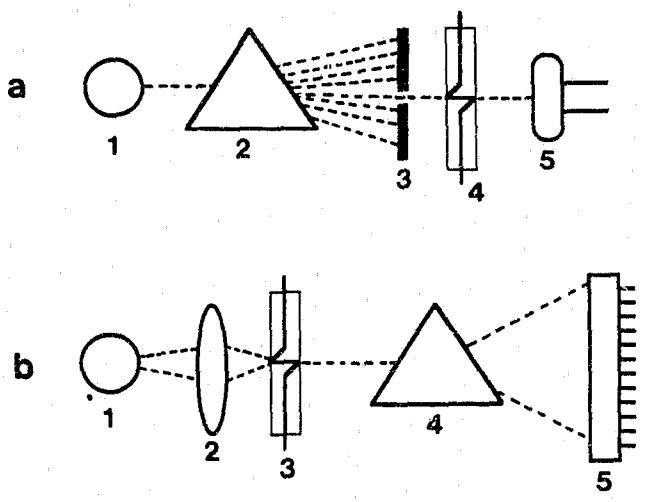


Figure 1. (a) Conventional UV optics for single wavelength HPLC detectors: 1) light source, 2) monochromator, diffraction grating or prism, 3) monochromator slit, 4) flow cell and 5) photo-cell; (b) Reversed optics format for diode array HPLC detectors: 1) light source, 2) lens system, 3) flow cell, 4) polychromator and 5) diode array.

The acquired data from a chromatographic run can be processed by plotting time versus absorbance (a chromatogram), absorbance versus wavelength (a UV/visible spectrum) or, with the appropriate software and computer memory, a three-dimensional plot of absorbance versus time versus wavelength (spectrochromatogram) (Figure 2a). A spectrochromatogram can also be represented as an isoabsorbance contour plot (Figure 2b). This representation of the three-dimensional data is often easier to interpret and more useful than the three-dimensional mountainscape plot.

The absolute sensitivity of the diode array detector is related to the electronics of the array. In earlier diode array detectors, the array was self-scanned, meaning that the electronics for switching the diodes in order for them to be read was performed by circuits on the diode array chip itself. This required diode bunching, or averaging of the signal to eliminate electronic noise (Sheehan *et al.* 1989). More recently, the development of externally scanned arrays which are less susceptible to electronic noise has allowed the development of intrinsically more sensitive detectors. In practical terms, however, other factors which influence sensitivity, notably the chromatographic and the photochemical properties of the analyte, are more significant than the configuration of the instrument's electronics.

PRACTICAL ASPECTS

The spectral resolution of the instrument is a function of the size and number of diodes, the range of the spectrum being measured, and the configuration of the instrument. Detectors currently on the market claim to offer resolution of between 1.2 and 4.0 nm.

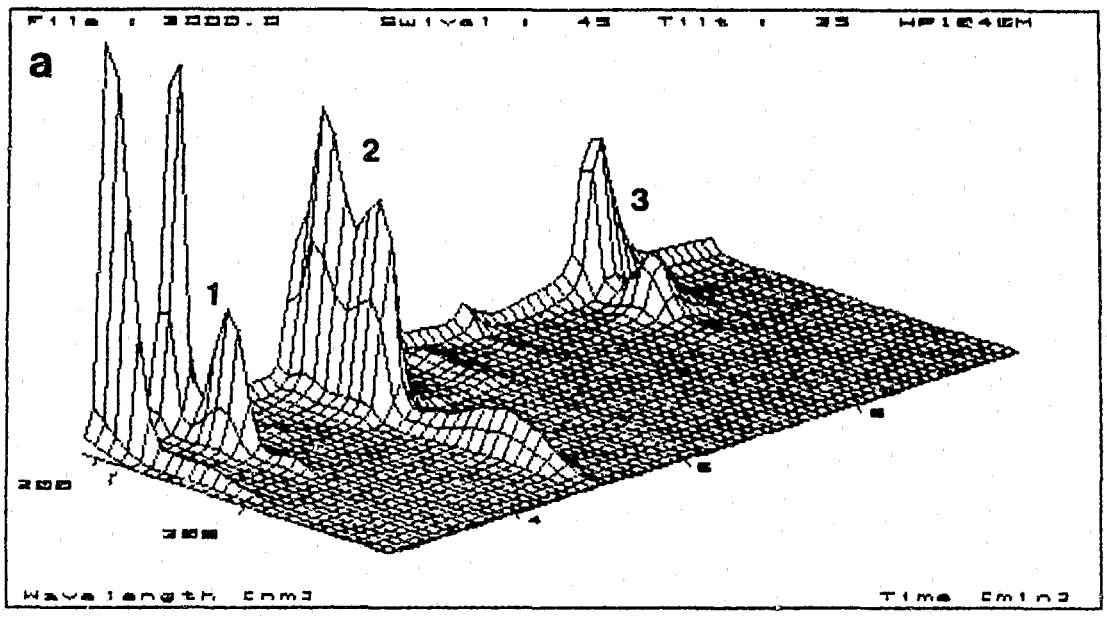
With higher resolution, the fine detail of many chromophores can be seen more accurately which can be of great importance in differentiating between structurally similar compounds. However, higher resolution can only be achieved through greater dispersion of light, and thus lower signal strength at the diode with correspondingly poorer sensitivity.

Therefore, when selecting an instrument for the laboratory, the particular application will determine whether sensitivity or spectral resolution is the prime consideration. For example, in street drug analysis, where sample size (milligrams or more), and thus sensitivity, is not a problem, an instrument with high resolution will yield greater benefits.

In contrast, toxicological analysis of biological fluids typically deals with nanogram quantities of material, and sensitivity is the primary concern. This is achieved at the cost of slightly poorer spectral resolution. Instruments with 4.0 nm resolution generally have a relative sensitivity cut-off (signal-to-noise ratio = 5) of five to eight times that of a single wavelength detector, the absolute sensitivity being a function of chromatographic conditions.

Absorption of UV/visible light occurs through the excitation of electrons between quantum energy states. Since the energy, and hence the wavelength, required for these transitions is dependent on the bond and relatively independent of substitutent groups on the bond atoms, UV/visible spectroscopy provides only limited structural information for individual compound identification compared to techniques such as nuclear magnetic resonance, infrared or MS. However, compounds within the same group or class (phenothiazines for example), often possess common structural features which result in similar UV/visible absorbance patterns in characteristic regions of the spectrum, allowing individual compounds which have not been characterized on the HPLC system to be tentatively assigned to a compound class. This assignment can be confirmed by subsequent analysis (Logan *et al.* 1990).

In order to store and process the large volume of information produced (for example, one UV spectrum every second for a 20-minute chromatographic run), computer based data systems



Time	2.50	to	9.98	min	Swivel	45 deg
Wavelength	190	to	402	nm	Tilt	35 deg
Resolution	Low					

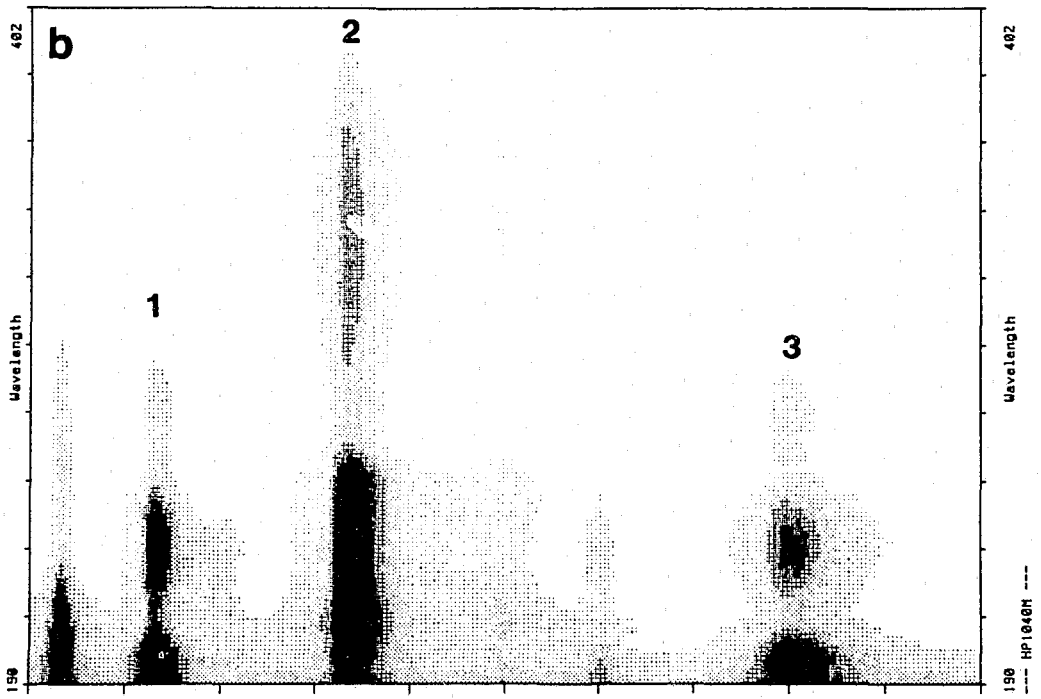


Figure 2. (a) 3-D spectrochromatogram and (b) Isoabsorbance contour plot of sample containing 1) benzoylecgonine, 2) quinine and 3) cocaine. Chromatography was performed on a 250 x 4.6 mm Lichrospher CH-8 HPLC column. The mobile phase was 30% acetonitrile in 0.01M KH2PO4 pH 2.3 buffer, flow rate was 1.5 ml/min. Plot obtained using Hewlett Packard 300 series data system with a 1040A diode array detector.

are required. These offer a wide range of accompanying software which enables the acquisition and searching of spectral libraries to assist in compound identification, derivative spectroscopy revealing absorption maxima and fine spectral detail with greater accuracy (Gill *et al.* 1982; Kirk and Fell 1989), three-dimensional spectrochromatograms and isoabsorbance plots, and mathematical manipulations including addition of spectra and subtraction of background absorbances. One notable feature is assessment of peak purity either through wavelength ratios or a purity parameter calculation. In MS, the use of ion ratios to determine the identity and homogeneity of a peak is a common practice. Diode array detection (DAD) also allows the use of wavelength ratios to determine the purity of a peak. In a homogeneous peak, the ratio at two carefully chosen characteristic wavelengths should be constant throughout the peak, independent of concentration and thus identical from run to run (Kirk and Fell 1989).

Many of these features of DAD can be used to provide additional information and more specific analyses, extending the usefulness of HPLC in forensic drug chemistry.

APPLICATIONS

The specificity of the diode array detector, coupled with the selectivity of an appropriate HPLC system, provides a valuable addition in preliminary screening of unknown samples and retains the advantages which have made HPLC a reliable quantitative procedure. One of the most valuable applications for DAD is in method development, where the identity of peaks in a mixture can be ascertained from a single run, rather than having to inject each component separately every time the mobile phase is adjusted. Thus, contaminants and spurious non-drug peaks can be identified, the elution of analyte in a solvent front peak can be diagnosed, and confusion of peak identities resulting from crossover between mobile phases of different strengths can be avoided. The coelution of two analytes can also be diagnosed.

The additional information provided by DAD has also been used with demonstrated efficiency as part of a comprehensive urinary basic drug screen method using solid phase extraction and gradient elution HPLC/DAD (Logan *et al.* 1990).

The complementary role which HPLC with DAD has played in identification and confirmatory analysis in our laboratory is illustrated through the following additional examples. Analyses were performed using Hewlett Packard 1040A and Varian Polychrome diode array detectors. HPLC conditions are given for each example discussed.

Case 1. Illicit Tablet

We recently received in our laboratory, a round, white, flat, half scored tablet, bearing markings (LEMMON 714) consistent with Quaalude (methaqualone). The orientation of the markings suggested, however, that this was an illicit tablet (Drug Enforcement Administration, personal communication), typical of a form popular in the early 1980's.

The resulting gas chromatographic (GC) and mass spectral data indicated the presence of diphenhydramine and a second, poorly chromatographed, unidentified peak (Figure 3).

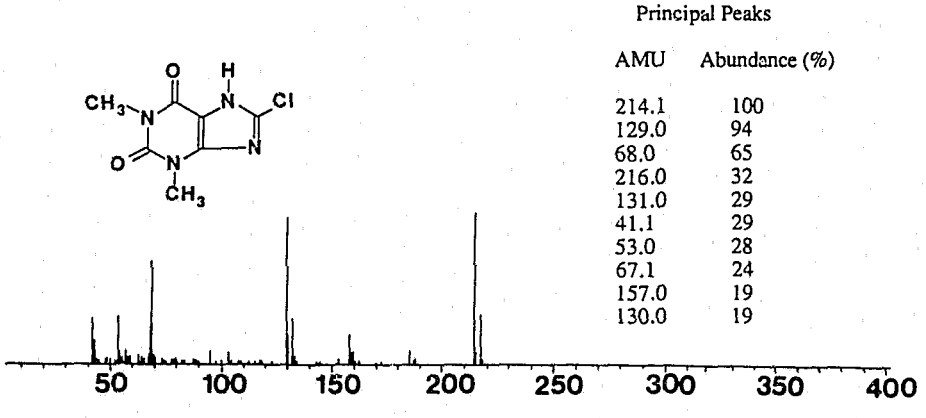


Figure 3. Mass spectrum for 8-chlorotheophylline. Acquired on 15 m DB-1, WCOT fused silica column, (J & W Scientific, Folsom, CA). Temperature program, 100 to 295 °C at 5 degrees/min. EI mode 40 to 475 AMU on 5970 Mass Selective Detector (Hewlett Packard, Palo Alto, CA).

Examination of base peak indexes in three extensive mass spectral data bases (Moffat 1986; Mills *et al.* 1982; Allen *et al.* 1982) gave no indication of the identity of this second peak. The tablet was analyzed by HPLC/DAD using a gradient drug screening method (Logan *et al.* 1990), and the analysis revealed the presence of two peaks (Figure 4), one whose identity was confirmed from retention time and UV spectrum as diphenhydramine, and a second peak whose UV spectrum was most similar to that of theophylline, but differed in retention time by 2.9 minutes.

An examination of prescription forms of diphenhydramine revealed the existence of dimenhydrinate (Dramamine®) (Searle, G. D. and Co., Skokie, IL), the 8-chlorotheophylline conjugate salt of diphenhydramine - a proprietary antiemetic/antinauseant.

The mass spectrum of this second peak was reexamined and found to be consistent with that for a monochloro substituted theophylline, and analysis of a dimenhydrinate standard confirmed its identification as 8-chlorotheophylline.

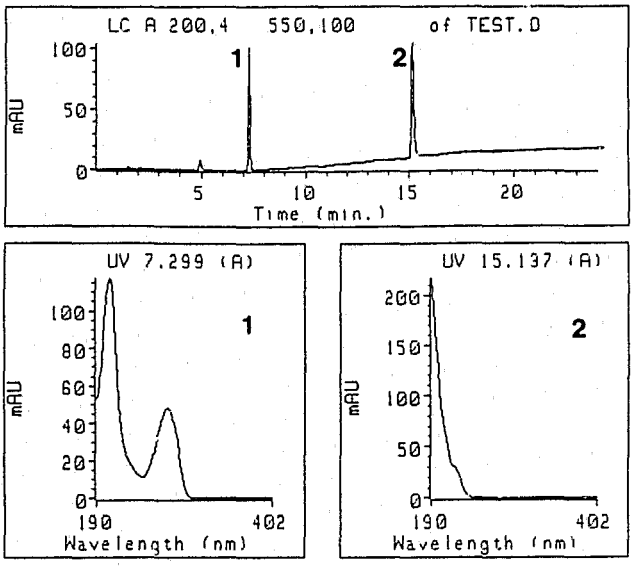


Figure 4. Chromatogram (top) from HPLC analysis of tablet containing 8-chlorotheophylline (1), RT = 7.30, and diphenhydramine (2), RT= 15.14, with their UV spectra (bottom, left and right). Chromatography was performed on a 250 × 4.6 mm Lichrospher CH-8 HPLC column. The mobile phase was a gradient from 10 to 50% acetonitrile in 0.05M KH2PO4 pH3 buffer. The flow rate was 1.5 ml/min. The eluent was monitored at 200 nm, and full spectra were acquired on all significant peaks from 190 to 400 nm.

Clarke (Moffat 1986) lists dimenhydrinate and also includes mass spectral data. The reported mass spectrum, however, is identical with that for diphenhydramine, with the exception of the presence of ions m/e 43 and 57. This suggests that the mass spectrum was obtained using direct insertion of the material on a probe or by incompletely resolved peaks by GC/MS.

The GC and LC analyses illustrated that these two components can readily be separated by GC and LC methods, and the mass spectrum of 8-chlorotheophylline is included for reference; 8-chlorotheophylline is not dispensed separately, but only as salt conjugates of diphenhydramine and promazine theoclate.

Case 2. Suspected Mushroom Material

A sample suspected of being a mushroom containing controlled substances was submitted for analysis. The sample was dried and crumbled, making any morphological examination inconclusive. Some material (200 mg) was triturated in methanol (2.5 ml) and analyzed directly by GC/MS and HPLC (Figure 5).

Psilocin (4-hydroxy-N,N-dimethyltryptamine) and psilocybin (4-phosphoryloxy-N, N-dimethyltryptamine) are the two major psychoactive indole alkaloids present in a number of species of mushroom in the genus *psilocybe.*

Psilocybin contains a phosphate ester linkage, a polar moiety which undergoes decomposition at 185 to 195 °C, severely limiting the GC conditions which can be used. In addition, its polarity makes it nonvolatile and difficult to analyze by GC.

The GC analysis showed only a single peak which had a mass spectrum consistent with psylocin. Both psilocin and psilocybin were identified by HPLC, by matching their retention times and corresponding UV spectra with those obtained for the authentic psilocin and psilocybin standards. The spectra obtained also matched the absorbance maxima characteristics reported in the literature (Christiansen and Rasmussen 1982). The peaks were symmetrical and eluted with retention times of 3.19 and 8.54 minutes for psilocybin and psilocin, respectively. A smaller peak with a similar UV spectrum to psilocybin was also noted (peak 3 in Figure 5). This may have been the related alkaloid, baeocystin (Christiansen and Rasmussen 1982). However, no authentic standard was available to confirm its identity.

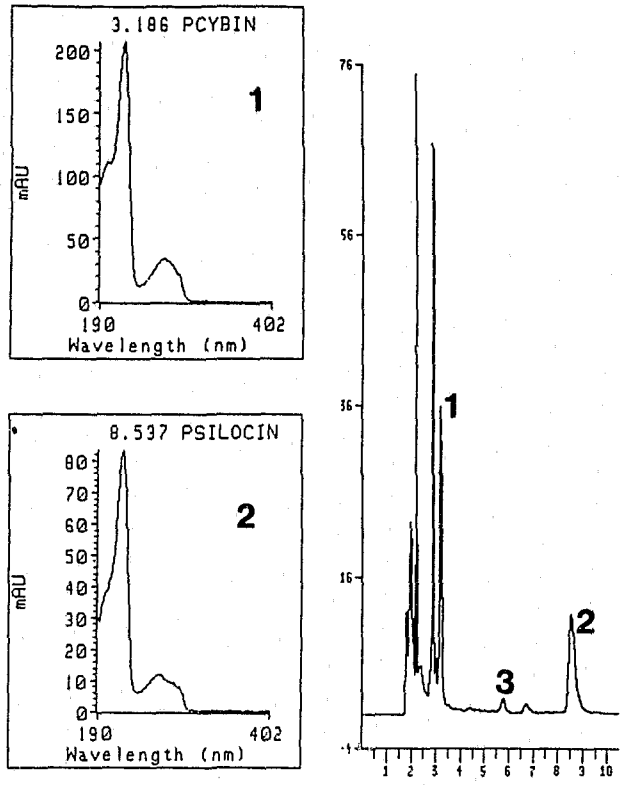


Figure 5. Chromatogram (right) from HPLC analysis of mushroom extract containing psilocybin (1), RT=3.19, and psylocin (2), RT=8.54, with their UV spectra (left, top and bottom). Chromatography was performed on a 250 × 4.6 mm Lichrospher CH-8 HPLC column. The mobile phase was 10% acetonitrile and 90% 0.05M KH2PO4 pH3 buffer. The flow rate was 1.5 ml/min. The eluent was monitored at 268 nm (absorbance maximum for psilocybin), and full spectra were acquired on all significant peaks from 190 to 400 nm.

Case 3. Colchicine in Blood

A fatality suspected of having resulted from excessive self-administration of the gout medication colchicine was investigated. The difficulties of chromatographing colchicine, a weakly basic compound (pKa = 1.7), on crosslinked methyl silicone GC columns have been documented (Caplan *et al.* 1980). This poor chromatography, coupled with the low levels of colchicine generally present due to its extremely short half life (1 hour), and the fact that death following a colchicine overdose is often delayed by as much as 48 hours, makes GC/GC/MS a less than ideal method for its determination.

In this case, the decedent had been hospitalized for 22 hours prior to death. A routine GC/MS drug screen (Anderson and Stafford 1983) had failed to show the presence of any drugs. Postmortem blood and bile samples were extracted, as described by Caplan *et al.* (1980), substituting a pH 6 citrate buffer for bicarbonate to improve recoveries. The same extracts were analyzed by HPLC/DAD using the conditions described in Figure 6, and a small peak was detected with a retention time identical to that of colchicine. The UV spectrum of the peak was compared to that of a standard run under the same conditions and was found to match. Extraction of standards from blood using pentobarbital as an internal standard allowed the quantitation of colchicine in the blood, which was found to be 0.026 μg/ml, within the range of reported toxic concentrations (Moffat 1986).

Since GC/MS, the normal confirmatory method required for absolute identification, was unable to identify the presence of the compound, the HPLC retention data and the UV spectrum, respectively, served as the only identification and confirmation methods in this case.

CONCLUSIONS

The introduction of DAD into HPLC has made it possible to identify and confirm the identity of compounds eluting from the HPLC column, thus eliminating the doubts that often accompany identification by retention time alone, a property which is subject to variations resulting from small changes in solvent composition, slight temperature changes, injection vehicle effects and system idiosyncrasies. As discussed earlier, its most important application is in the analysis of compounds with temperature labile or polar functional groups which are unsuitable for GC analysis.

The lack of specific structural information available from UV spectra is a limiting factor in absolute identification. However, as illustrated by the case of 8-chlorotheophylline, the similarities between UV spectra of compounds within a class can be extremely useful in the identification of unknowns, especially when considered in conjunction with other analytical data. This is an important feature of the technique and might be expected to assist in the identification of novel designer drugs.

Developments in microelectronics are likely to result in improvements in sensitivity and spectral resolution. New releases of computer

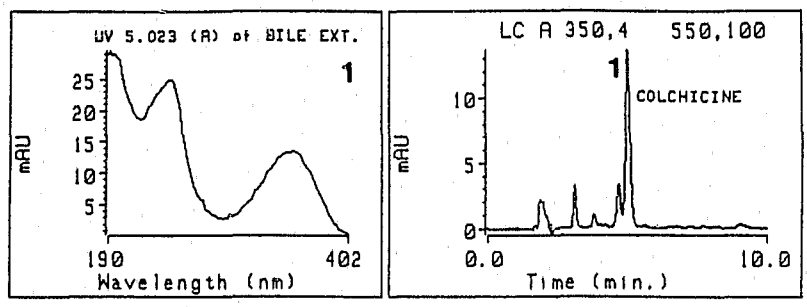


Figure 6. HPLC analysis of colchicine in bile. Chromatogram (left) and UV spectrum (right) of colchicine (1), RT = 5.02. Chromatography was performed on a 250 × 4.6 mm Lichrospher CH-8 HPLC column. The mobile phase was 25% acetonitrile in 0.05M KH2PO4 pH3 buffer. The eluent was monitored at 350 nm, and full spectra were acquired on all significant peaks from 190 to 400 nm. Other peaks have UV spectra similar to colchicine and are probably metabolites or conjugates.

software will undoubtedly improve the manipulation of the spectral data for the determination of derivative spectroscopy for more accurate determination of absorbance maxima, library comparisons, peak purity, baseline subtractions and spectral deconvolutions which are already a feature of many of the commercially available diode array detectors.

REFERENCES

Allen, A. R., Ardrey, R. E., Bal, T. S., Brown, C. and Moffat, A. C. (1982). An Eight Peak Index of Mass Spectra Compiled Specifically for Use in Forensic Science. HOCRE Report No. 448.

Anderson, W. H., and Stafford D. T. (1983). Applications of capillary gas chromatography in routine toxicological analysis, HRC 6:247-253.

Bowers, L. D., (1989). High performance liquid chromatography/mass spectrometry: state of the art for the drug analysis laboratory, Clin. Chem. 35:1282-1287.

Caplan, Y. H., Orloff, K. G. and Thompson, B. C. (1980). A fatal overdose with colchicine, J. Anal. Toxicol. 4:153-155.

Christiansen, A. L. and Rasmussen, K. E. (1982). Analysis of indole alkaloids in Norwegian Semilanceata using high performance liquid chromatography and mass spectrometry, J. Chromatogr. 244:357-364.

Colwell, L. F. and Karger, B. L. (1977). Ballpoint pen ink examination by high pressure liquid chromatography, J. Assoc. Off. Anal. Chem. 60:613-618.

Gill, R. (1986). High Performance Liquid Chromatography. In: Clarke's Isolation and Identification of Drugs. 2d ed. Edited by A. C. Moffat. Pharmaceutical Society, London, pp. 201-220.

Gill, R., Bal, T. S. and Moffat, A. C. (1982). The application of derivative UV-visible spectroscopy in forensic toxicology, J. Forensic Sci. Soc. 22:165-171.

Kirk, E. M. and Fell, A. F. (1989). Analysis of supplemented vitamin K1 (20) in serum microsamples by solid phase extraction and narrow bore HPLC with multichannel ultraviolet detection, Clin. Chem. 35:7:1288-1292.

Logan, B. K., Moore, C. M., Tebbett, I. R. and Stafford, D. T. (1990). Rapid screening for 100 basic drugs and metabolites in urine using cation exchange solid phase extraction and high performance liquid chromatography, J. Anal. Toxicol., in press.

Mills, T. A., III, Price, W. N., Price, P. T. and Roberson, J. C. (1982). Instrumental Data for Drug Analysis, Vol 1. Elsevier Science Publishing Co., Inc., New York.

Moffat, A. C., ed. (1986). Clarke's Isolation and Identification of Drugs. 2d ed. Pharmaceutical Society, London.

Owen, A. J. (1988). The Diode Array Advantage in UV/Visible Spectroscopy. Hewlett-Packard, Federal Republic of Germany.

Saferstein, R. D., ed. (1982). Handbook of Forensic Science. Vol. 1. Prentice-Hall, New Jersey.

Saferstein, R. D., ed. (1988). Handbook of Forensic Science. Vol. 2. Prentice-Hall, New Jersey.

Saner, W. A., Fitzgerald, G. E. and Welsh, J. P. (1976). Liquid chromatographic identification of oils by separation of the methanol extractable fraction, Anal. Chem., 48:1747-1754.

Sheehan, T. L., Adaskaveg, M. and Excoffier, J. L. (1989). Powerful implementation of spectral processing for photodiode array detection, Am. Lab. 21:66-70+.

Tobias, M. K. (1989). Diode array applications, Am. Lab. 21:102-108.