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cation by retention time and iterative processing of the identified components, and combined strategies. By calling attention to this problem, it is hoped that further improvements in searching procedures will emerge.

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Determination of Heroin by Circular Dichroism

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A method of analysis for heroin is described in which separation of the drug is not a prerequisite to its quantitative determination. The normally encountered diluents and adulterants present in confiscated samples do not exhibit the phenomenon of circular dichroism and are noninterfering. Analysis is uncomplicated, quick, and easily reproducible. Correspondence with determinations which used gas chromatography is better than 1%.

Identification of illicit substances is a major effort of criminalistics laboratories and, consequently, very time-consuming. Any new technique, or modification of presently acceptable techniques, which has the potential to alleviate the load, is worthy of development. In this work we are making a case for the acceptance of circular dichroism (CD) spectropolarimetry as an important addition to the arsenal of analytical methods. We will show that the method is both quick and quantitative and refer to its general applicability in the identification of drugs of abuse.

In most states, legislation requires only the qualitative identification of a controlled substance, since mere possession alone is sufficient grounds for conviction. Even so, ratification of the presence of an anonymous compound in a complex and cleverly contrived mixture still can be difficult, particularly since the controlled substance is usually a very minor component. Confirmation of the presence of a suspected substance is a consequence of positive responses from a number of complementary tests.

In the case of heroin the standard accepted testing procedures include color spot-tests (*1*), microcrystalline tests (*2*), and a variety of instrumental methods (*3*) such as GC (*4, 5*), HPLC (*6*), IR and UV absorption spectrophotometry (*1, 7*), and mass spectrometry (*8, 9*) in its many forms such as CIMS, CEMS, MID, etc. Diluents, adulterants, and metabolites can mask the specificity of the tests for heroin, which is the motivation for their presence in the first place. Separation, therefore, is often a prerequisite to identification. In what might be one of the most complex procedures, there are instances where heroin is first extracted, then converted to morphine, and finally derivatized prior to analysis. Even then, positive identification requires that a comparison be made with a standard each and every time an analysis is performed, because of variations in instrument parameters.

Day to day variations in instrument parameters are not a factor in quantitation by UV absorption spectrophotometry. The method is not too specific, and not always preferred, because of the broad unstructured bands. To introduce specificity to the screening and analysis of drugs by UV absorption, we have measured the circular dichroism spectra of a number of opiates in anistropic (*10, 11*) and isotropic (*12, 13*) solvent media. It should be emphasized that a CD spectrum, in which ellipticity is plotted vs. wavelength, is really no more than a modified absorption spectrum. The ellipticity is directly proportional to the difference in absorption between left and right circularly polarized light.

The qualitative success of the technique has been adequately demonstrated for all three solvent systems (*10–13*). Of the three, the aqueous solvent system is most easily disposed to quantitative studies and the molar ellipticity coefficients are available for 10 opiates (*12*). The spectra are quite unique for the opiates which we have investigated and could be used as confirmation after separation from mixtures. We were more intrigued by the prospects of identification without separation. To this end we obtained four heroin confiscates which were analyzed for the drug before and after extraction and compared the results with an in-house quantitation by GC. The results from all three procedures are in excellent agreement.

EXPERIMENTAL SECTION

Standard samples of heroin as the hydrochloride were obtained from the National Institute for Drug Abuse via the Research Triangle Institute. Four samples of confiscated drugs were generously provided by the Criminalistics Laboratory of the Oklahoma State Bureau of Investigation. Three of these were typical of what is known as "brown" heroin, the fourth was a white specimen which proved to be very high in heroin composition. Each sample had been recovered from dead case files prior to incineration.

CD measurements were made on a Cary 61 spectropolarimeter over the wavelength range 220–350 nm. Sample sizes were on the order of 2–4 mg and were dissolved in 25 mL of either aqueous

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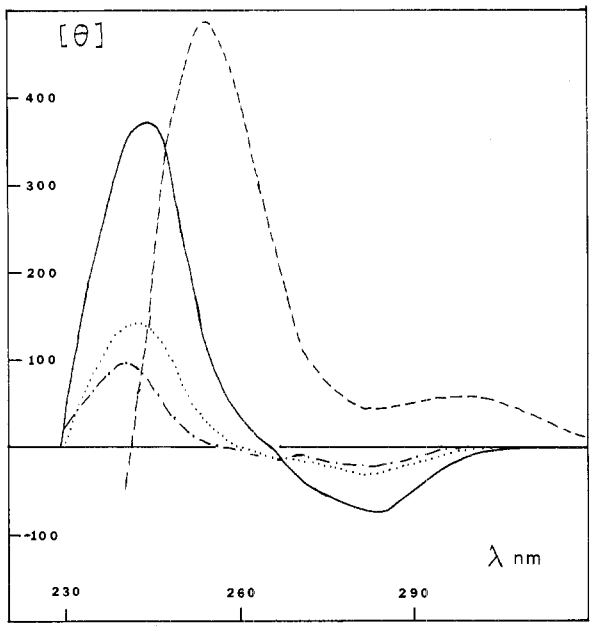

Figure 1. CD spectra of heroin (• – •), 6-MAM (• • •), and morphine (—) in water, and morphine (– – –) in excess sodium hydroxide.

0.1 M HCl or aqueous buffer of pH equal to 8.6. Insoluble materials were removed by centrifugation. Quantitative determinations were made either as heroin from data for the HCl solutions or as morphine after the complete hydrolysis of heroin on the addition of a pellet of sodium hydroxide. An alternative method used was to preferentially extract heroin from the 8.6 buffer solution into chloroform (*1*) and before quantitative determination to back-extract it into 0.1 M HCl. The heroin is now presumably free from all morphine and the extraction efficiency is in excess of 95%. A fourth method of determination was to add a pellet of sodium hydroxide to the acid extract and to measure the heroin composition after its immediate hydrolysis to morphine.

Extraction from the 8.6 buffer solution into chloroform was the sample preparation step taken prior to gas chromatographic determination. The instrument used was a Hewlett-Packard 7620-A; the column material was SP-2100 on 80/100 Supelcoport; and the oven temperature was 260 °C. For internal consistency the instrument was first calibrated using a number of dilutions of a stock heroin solution subjected to the same extraction procedure. Sample sizes for the confiscates were ca. 20 mg, and 1.2 ± 0.05 μL aliquots were introduced to the column.

RESULTS AND DISCUSSION

The CD spectra for morphine, 6-monoacetylmorphine (6-MAM), and heroin, dissolved in HCl, are shown in Figure 1. In excess NaOH, morphine is the only product and the CD spectrum is also shown in Figure 1. The ordinate, or ellipticity ψ_{exp}, is related to the difference between the molar absorption coefficients ϵ of the left and right circularly polarized components of the incident beam according to the equation

$$[\theta] = \frac{\psi_{exp}}{C(\text{mol/L})} = 3300(\epsilon_L - \epsilon_R) \qquad (1)$$

where $[\theta]$ is the molar ellipticity coefficient and C is the molar concentration. This definition of $[\theta]$ is a departure from that in the older literature, but in the context of CD being a modified absorption spectrum, we have preferred to define a quantity analogous to the ϵ or molar absorption coefficient of UV spectrophotometry.

Heroin would best be quantitated in HCl solutions at 238 nm, $[\theta] = +64$. The $[\theta]$ values for 6-MAM and morphine at the same wavelength are +270 and +311, respectively (*12*). In base, $[\theta]$ for morphine at the band maximum at 253 nm is +458. The results from the four analytical procedures which involve CD, and the GC results are given in Table I as percent

Table I. Quantitative Determinations of Heroin Confiscates[a]

OSBI no.[d]	as heroin[b] direct	as heroin[b] extracted	as morphine[c] direct	as morphine[c] extracted	as heroin GC	as heroin OSBI
1			0.7	0.6	0.83	
2	11.6	3.9	5.0	4.3	4.9	
3	4.7	2.6	1.8	1.3	1.1	
4	95.0	79.0	74.1	60.1	73.4	75

[a] Results presented as percent by weight of heroin free base. [b] 0.1 M HCl. [c] Excess NaOH. [d] OSBI is the Oklahoma State Bureau of Investigation.

heroin free base by weight in the confiscates. Variation in results from four to five independently weighed samples is less than ±0.2%. Only confiscate number four had been independently determined at the Oklahoma State Bureau of Investigation.

Heroin does not occur naturally but is prepared by the acetylation of morphine. Samples of heroin might ordinarily contain unreacted morphine and some of the monoacetyl intermediate 6-MAM. The results in Table I are consistent with this observation.

In column two of Table I the results from the direct determination as heroin are consistently high because of an enhanced experimental ellipticity caused by the presence of either dissolved 6-MAM and/or morphine. Results in column three would indicate that separation of heroin by chloroform extraction is incomplete, some 6-MAM being coextracted so that the percent compositions calculated from CD determinations are still high compared to GC determinations.

The best correspondence with GC is found in column four for the direct determination as morphine after hydrolysis. These figures are higher than the results in column five where any unreacted morphine would not have been extracted, or the extraction efficiency for 6-MAM is not as great as it is for heroin.

The results from the *direct* determination of heroin (as morphine) by CD spectropolarimetry are in excellent agreement with the standard and approved method of GC. The linear dynamic range and reproduciblity are excellent. We have not attempted to identify the other ingredients in the solid confiscates, but unless these are CD active they will not interfere with the determination. This is particularly true if there are no chromophores present in the additive molecules which would mean they are transparent to the incident light. The usually encountered ingredients for example are mono- and polysaccharides which, although optically active, do not absorb in the near-UV. In this case neither the qualitative identification nor the quantitative determination is affected. On the other hand, any achiral ingredient which absorbs in the wavelength range of the aromatic chromophore would reduce the intensity of the transmitted light and therefore the signal to noise ratio and the limit of detection. None of the heroin confiscates contained a strong UV absorber and with the present instrumentation the minimum detectable quantity is ca. 14 μg/mL, if determined as morphine.

Complications to qualitative and quantitative analysis will arise if more than one CD active substance is present. A case in point is opium which contains morphine and codeine among its principal ingredients. This problem will be addressed in detail in a future publication. Where one ingredient is considerably less CD active the problem is easily resolved. This has been demonstrated for the determination of tetracycline in human urine (*14*). Drugs which are achiral because of an inherent molecular symmetry, e.g., PCP, or because they occur as a racemic mixture, e.g., DL-methadone, cannot be determined directly by CD. Their presence is detrimental to an

analysis of a CD active drug only if they are present in excess and absorb in the UV spectral range, for the reason given above. It is conceivable that these achiral molecules can be quantitated if an extrinsic CD signal is induced (*15*). The remaining interference in this case is that other additives might also be susceptible to an induced CD signal, but complications would only rise if that ingredient absorbed radiant energy in the UV range of the drug of interest, namely, heroin in this case. Under these circumstances separation might be necessary.

A distinct advantage CD has over GC or mass spectrometry is the much shorter time needed to complete the quantitative determination. Separation and derivatization are not required and the instrument does not have to be calibrated once the molar ellipticity coefficient is known. The recommended procedure for heroin analysis would be to take the anonymous sample into a pH 8.6 buffer solution to first qualitatively identify the compound and then measure the ellipticity at 253 nm after the addition of sodium hydroxide. The actual time for quantitative analysis is 20 min, which includes weighing and centrifugation. CD spectropolarimetry is therefore a strong competitor as an analytical method for the quick and reliable determination of heroin in solid specimens.

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Enzyme-Linked Immunoassay of Human Immunoglobulin G with the Fluoride Ion Selective Electrode

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A fluoride-selective electrode has been used to detect immunoglobulin G (IgG) in human serum to a level of 0.9 μg/mL. The method is based on the selective detection of the horseradish peroxidase (HRP) label on the antibody after dissolving the precipitate resulting from the immunoreaction between IgG and HRP-labeled anti-IgG. The HRP label is quantitated by detecting the fluoride ion released as a result of the HRP-catalyzed oxidation of *p*-fluoroaniline by hydrogen peroxide. The fluoride ion concentration is shown to be proportional to the IgG concentration precipitated in the region of antibody excess, without interference from serum matrix components.

During the last 7 years applications of ion-selective electrodes (ISE) have been extended to include immunoassays. Methods of analysis of various immunoagents have been described in the literature as more new electrodes selective to organic compounds are developed and new uses for conventional electrodes are found for direct detection of the immunoagent (*1-5*) or of an appropriate label (*6-10*). Thus, the Ag_2S membrane electrode has been used in the immunoassay of human serum albumin in a semiautomated system (*1*) and the anti-benzoate antibody in a fully automated system (*2*).

A number of other electrode immunoassay methods are now available. Hepatitis-B surface antigen (Hb_sAg) was detected with an iodide membrane electrode using HRP-labeled anti-Hb_sAg (*3*). An ammonia electrode was used (*5*) for the detection of bovine serum albumin (BSA) and cyclic adenosine-3′,5′-monophosphoric acid (c-AMP) via detection of the urease label after separation with the double antibody solid phase (DASP) technique described previously by Van Weeman et al. (*4*).

A trimethylphenylammonium cation ($TMPA^+$) electrode (*6*) was used to detect the hemolysin antibody or complement which can cause the specific lysis of $TMPA^+$ loaded erythrocytes (*7*) by detection of the $TMPA^+$.

Novel specific "immunoelectrodes" have also been developed, e.g., the Concanavalin A (Con A) immunoelectrode selective to yeast mannan (*8*) and the anti-human chorionic gonadotropin (anti-HCG) immunoelectrode selective to HCG (*9, 10*). With the exception of the use of $TMPA^+$ marked erythrocytes for the detection of the hemolysin antibody or complement, all of the methods so far developed for immunoassay by ISE are heterogeneous.

In contrast, the voltammetric immunoassays developed (*11, 12*) were based on the homogeneous competitive binding technique. Weber and Purdy (*11*) applied the technique to a model system involving codeine and morphine where

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