Table I—Comparison of Deproteinization Methods [a]

Deproteinization Method	Recovery, %	CV, %
Protein-coated ODS column	100.1	0.9
5% Acetonitrile	101.0	3.2
5% Trichloroacetic acid	25.9	4.0

[a] Conditions: 300 ng of propranolol/mL of plasma; injection of 100 μL of plasma; $n = 5$.

metabolite; therefore, an internal standard was not required. By using a flow direction switching valve, the deterioration of the analytical column was minimized. At least 150 successive analyses with almost no column deterioration were carried out by the back-flush method.

Table I shows the comparison of the methods of deproteinization. Note that in the present method the deproteinization was performed at the initial stage after the injection of sample onto the column. In comparison with common HPLC analyses that include pretreatment, *i.e.*, solvent extraction or deproteinization, the proposed method proved superior in simplicity and accuracy.

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Determination of "Brompton's Cocktails" by Circular Dichroism

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Abstract □ Circular dichroism spectropolarimetry has been applied to the simultaneous determination of chiral compounds in binary mixtures without separation or sample preparation steps. A strategy which uses data measured at equivalent wavelength pairs simplifies the calculations. Correspondence is within ±2% of the compositions of prepared standard mixtures.

Keyphrases □ Circular dichroism—spectropolarimetry, UV absorption, Brompton's solutions, *R*-(−)-cocaine hydrochloride, morphine sulfate, (±)-methadone, (−)-fructose, β-cyclodextrin □ Brompton's solutions—circular dichroism, spectropolarimetry, UV absorption

A medically approved practice used to relieve the physical distress from chronic pain, such as is experienced by some terminally ill cancer patients, is to administer a mixture of controlled, addictive substances in an oral preparation commonly referred to as "Brompton's cocktails." Cocaine is common to all preparations. Other components differ, but are most often either morphine or (±)-methadone. The mixture is served in a fruit-flavored alcohol base.

Although documented evidence for the abuse of this prescription is limited at this time, the potential exists. We have developed a simple protocol for a rapid direct determination of the drugs by circular dichroism (CD) spectropolarimetry which requires no separation or sample prepreparation. We have previously reported data for opium alkaloids (1, 2), *R*-(−)-cocaine (3, 4), (+)-lysergide (D-LSD) (5), and tetracycline (6). The CD technique focuses only on those components which absorb light and which are simultaneously chiral. Chirality may be either intrinsic to the molecule (1–6) or induced by its complexation with a chiral substrate molecule (4).

By being able to focus on only the CD-active components, the problem of deconvoluting the cumulative UV spectra of the mixtures is considerably simplified. Absorption by the other CD-inactive components only affects the signal-to-noise ratio of the detector.

EXPERIMENTAL SECTION

Standard samples of *R*-(−)-cocaine hydrochloride[1], morphine sulfate[1], and (±)-methadone[2] were used without further purification. The fruit-flavored syrups commonly used contain (−)-fructose. Standard (−)-fructose[3] was obtained commercially. In-house standard mixtures were prepared by weight. Samples of unknown composition were either blind in-house preparations, or were provided[4]. Chirality is induced into (±)-methadone by complexation with the chiral cyclic oligosaccharide β-cyclodextrin[5] present in large excess in aqueous solution, $\sim 10^{-2}$ M.

CD measurements were made on an automatic recording spectropolarimeter[6], and data analyses were made on the ancillary data processor[7]. Daily calibration of the ellipticity scale was made against a standard solution of androsterone in dioxane, as recommended. Samples were diluted with the appropriate volume of dilute hydrochloric acid or water, usually 1:10.

[1] Mallinckrodt Inc.
[2] Drug Enforcement Administration.
[3] Fisher Scientific.
[4] Arizona Department of Public Safety and the local hospital pharmacy.
[5] Kodak.
[6] Model 500A; JASCO.
[7] Model DP-500N.

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Table I—Circular Dichroism Spectral Characteristics [a]

Compound	Characteristic[b]	Wavelength, nm	[θ], degree/M-cm
Morphine Sulfate	λ^-_{max}	286	−66
	λ^o	263	0
	λ^+_{max}	244	372
Cocaine hydrochloride	λ^+_{max}	278	11.5
	λ^o	262	0
	λ^-_{max}	245	−55
(−)-Fructose	λ^+_{max}	273	0.3[d]
	λ^o	235	0
(±)-Methadone[c]	λ^+_{max}	290	9.6[e]
	λ^+_{max}	273	6.4[e]
	λ^+_{max}	266	5.6[e]

[a] For 0.1 M HCl aqueous solutions. [b] λ^+_{max} and λ^-_{max} are wavelengths of the maximum positive or negative signals. λ^o is the wavelength of zero ellipticity. [c] In β-cyclodextrin solution only. [d] Value is calculated for total sugar although only the open-chain form is CD active in the 220–350-nm region. [e] Values for 1:1 complex with β-cyclodextrin.

Table II—Determinations of Binary Mixtures [a]

Sample[b]	Cocaine	Morphine	(−)-Fructose	(±)-Methadone	λ Pairs
S1	1.06 (1.05)	—	—	(0.98)	—
S2	2.31 (2.32)	—	—	(1.55)	—
S3	1.72 (1.74)		9.65 (9.87)	—	7
S4	2.61 (2.55)	—	15.94 (16.23)	—	4
S5	4.15 (4.18)	—	15.04 (14.23)	—	6
S6	2.22 (2.24)	3.44 (3.28)	—	—	6
S7	5.78 (5.81)	1.59 (1.56)	—	—	6
S8	2.95 (2.99)	1.06 (1.05)	—	—	6
B1	0.95 (1)[c]	1.53 (1.5)[c]	—	—	10
B2	0.98 (1)[c]	1.55 (1.5)[c]	—	—	7
B3	1.08 (1)[c]	—	133[d]	present	7
B4	1.01 (1)[c]	—	137[d]	present	6
B5	0.95 (1)[c]	1.46 (1.5)[c]	119[d]	—	6

[a] In mg/mL. Data in parentheses are calculated from known weights. [b] S represents an in-house preparation and B represents an externally supplied Brompton's solution. [c] Figures correspond with pharmacist's recipe. [d] Results are for (−)-fructose in fruit-flavored additives; no composition given.

(−)-Fructose solutions have low ellipticities (7, 8), so solution concentrations were as high as 0.2 M.

RESULTS AND DISCUSSION

CD spectral parameters for the four compounds are collected in Table I. Molar ellipticity [θ] values calculated from the slopes of the respective Beer's law calibration curves at the reported wavelength maxima are also presented. Significant spectral overlap is observed for mixtures of *R*-(−)-cocaine and morphine and *R*-(−)-cocaine and (−)-fructose. The compositions of the in-house preparation of these mixtures were made to be consistent with the pharmacy prepared prescriptions which are on the order of 1.5 mg/mL of morphine sulfate and 1 mg/mL *R*-(−)-cocaine hydrochloride or (±)-methadone. If the binary mixture consists of *R*-(−)-cocaine and (±)-methadone, the problem reduces to the determination of only *R*-(−)-cocaine by direct measurement of the aqueous solution which is conveniently done at the 278 nm positive maximum for the analyte.

For binary mixtures of CD-active components, the observed ellipticity at any wavelength for a 1-cm cell is given by the expression:

$$\psi_{exp} = [\theta]_1 C_1 + [\theta]_2 C_2$$

where the subscripted [θ] and *C* terms refer to the molar ellipticities and molar concentrations of the two components, respectively. The resolution of broad UV absorption bands into positive and negative components in CD spectra offers a simpler option to the simultaneous solution of the equation which is fairly general. The equation reduces to a single unknown if data are measured at preselected wavelength pairs on the standard spectrum of either component at which the absolute values of [θ] are equal in magnitude. Experience with the blind in-house mixtures shows us that the best correspondence in determinations is obtained if the pairs are chosen from the standard spectrum of the component which has the lower [θ]; namely cocaine in mixtures with morphine, and fructose in mixtures with either cocaine or morphine. Results from the calculations and the number of wavelength pairs used are given in Table II for several binary mixtures.

Compared with the direct determination of complex mixtures by UV absorption spectrophotometry, which is commonly done using computer simulation methods, CD effectively reduces the number of analytes contributing to the overall spectrum by focusing on only those which are CD active. The number of curve-fitting parameters is reduced accordingly, and the precision in the determination of each component is improved. Matrix distortions of the CD spectra of the analyte(s) by the simultaneous absorption of energy by achiral components are not observed.

Determinations made on the unknowns obtained from external sources indicated that the preparations were within the recommended limits prescribed for administration to patients (Table II). One ternary mixture was investigated in which tampering with the prescription was suspected. The mixture was reported to consist of morphine, *R*-(−)-cocaine, and (−)-fructose in alcohol. The CD spectrum was a close replica of the mixture prepared according to the prescription. Measurements of data at wavelength pairs for (−)-fructose and the simultaneous solution of the equation for the remaining constituents yielded 1.46 mg/mL for morphine and 0.95 mg/mL for *R*-(−)-cocaine.

At its present state of development, CD spectropolarimetry offers a rapid and quantitative procedure for drug assay and as such has a useful application to product quality control. As the spectral library is extended to include more and more standards, anonymous compound recognition and identification can be anticipated. With the appropriate computer software the same simulation techniques applied to UV absorption determination of multiple-component mixtures can be applied equally well.

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