Stability of Brompton Mixtures: Determination of Heroin (Diacetylmorphine) and Cocaine in Presence of Their Hydrolysis Products

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Abstract □ The application of a rapid, selective, and sensitive reversed-phase high-performance liquid chromatographic method to the separation of the hydrochloride salts of heroin (diacetylmorphine) and cocaine and their hydrolysis products is described. The method was used to study the stability of heroin and cocaine in Brompton mixtures in pharmaceutically useful pH range and vehicles at different temperatures. The pH range of optimal stability for both heroin and cocaine was 3.0–3.5. The disappearance of heroin and cocaine in Brompton mixtures followed pseudo-first-order kinetics in buffered solutions. Increased alcohol and decreased syrup concentrations diminished heroin hydrolysis but did not influence cocaine stability. Substitution of morphine for heroin in Brompton mixtures markedly increased the rate of cocaine hydrolysis.

Keyphrases □ Cocaine—high-performance liquid chromatographic analysis with heroin and hydrolysis products, Brompton mixtures □ Heroin—high-performance liquid chromatographic analysis with cocaine and hydrolysis products, Brompton mixtures □ High-performance liquid chromatography—analysis, cocaine and heroin with hydrolysis products, Brompton mixtures □ Brompton mixtures—stability, high-performance liquid chromatographic analysis of cocaine and heroin with hydrolysis products □ Diacetylmorphine—high-performance liquid chromatographic analysis with cocaine and hydrolysis products, Brompton mixtures

The treatment of patients with chronic severe pain has triggered interest in the use of morphine and heroin (diacetylmorphine) in popular oral formulations, namely Haustus elixir and diacetylmorphine and cocaine elixir, respectively (1). The latter formulation, referred to as a Brompton mixture, has been in use in Great Britain and Canada. Since heroin is a Schedule I narcotic in the United States, morphine frequently is substituted for this agent. There is no set composition for Brompton mixture. However, most variations contain salts of morphine or heroin combined with cocaine hydrochloride (hereafter referred to as their respective bases), alcohol, and a sweetening agent in an aqueous medium. Varying degrees of effectiveness have been reported for these analgesic mixtures (2–4).

The hydrolysis of diacetylmorphine to morphine *via* 6-monoacetylmorphine has been confirmed qualitatively (5, 6) and quantitatively (7). Furthermore, numerous methods have been published for the stability determination of cocaine (8–10). Several methods include the determination of one or more of its hydrolysis products, mainly benzoylecgonine, ecgonine, and benzoic acid (10–12). However, no single system has been used for both of these substances in the presence of their decomposition products.

Separation and quantitation of these components in such a complex mixture by TLC (12–14) is laborious and quantitatively imprecise. GLC requires extraction and derivatization and suffers from thermal degradation limitations (8, 9, 12). On the other hand, the reversed-phase high-performance liquid chromatographic (HPLC) method described here allows the simultaneous quantitation of heroin and cocaine in the presence of their hydrolytic products, an internal standard, and "inert" components by a simple, direct isocratic elution on a single analytical column. The separation was carried out in aqueous solution at a pH that showed minimal degradation of heroin and cocaine and eliminated preparatory procedures. The HPLC–UV method was applied to evaluate the stability of morphine or heroin plus cocaine at various temperatures and pH levels. The influence of alcohol and sucrose concentrations on heroin and cocaine stability was studied also.

EXPERIMENTAL

Reagents—Hydrochloride salts of heroin, 6-monoacetylmorphine, morphine, cocaine, and benzoylecgonine were used as received[1]. Benzoic acid, 2-chloro-3-nitropyridine, and the internal standard, 4-chloropyridine hydrochloride, were obtained commercially[2]. Acetonitrile[3] (HPLC grade) and distilled water were filtered through 0.45-μm solvent-resistant filters[4]. All other chemicals were reagent grade and were used without further purification.

Equipment—A modular high-pressure liquid chromatograph equipped with a constant-flow pump[5] was used to deliver the eluent to a stainless steel column packed with fully porous 10-μm silica particles bonded with a monomolecular layer of octadecylsilane[6]. Injections were made with a rotary valve injector equipped with a 10-μl injection loop[7]. No attempt was made to control the column temperature. A variable-wavelength UV detector[8] set at 235 nm was operated at an attenuation of 0.02 aufs, and the integrator output to the recorder was attenuated ×4 to detect the eluted compounds. The output signals were recorded with a strip-chart recorder[9].

Peak retention times and peak areas were monitored and quantitated automatically relative to the internal standard by area measurements using a computing integrator[10].

Chromatographic Conditions—The chromatographic mobile phase consisted of one volume of acetonitrile and three volumes of 0.015 *M* monobasic potassium phosphate adjusted to pH 3.0 with 2 *N* phosphoric acid[11]. The apparent pH of the eluent mixture was 3.30. The column pressure at a flow rate of 0.8 ml/min was ~620 psi. Separations were effected isocratically at ambient temperature. The chromatographic parameters, *i.e.*, capacity factors (k'), separation factors (relative retention, α), and resolution (R_s), of various solutes were calculated from the adjusted retention times (15).

Preparation of Solutions—Heroin and cocaine were dissolved in appropriate media (Table I) to yield concentrations of 1 and 0.5 mg/ml, respectively. Morphine–cocaine solutions at respective concentrations of 1 and 0.5 mg/ml were prepared only in the "standard vehicle" (II).

The solutions were filtered[4] through solvent-resistant filters and dispensed promptly (1.0 ml) in 2-ml flint ampuls. These ampuls were sealed

[1] National Institute on Drug Abuse, Rockville, Md.
[2] Aldrich Chemical Co., Milwaukee, Wis.
[3] Fisher Scientific Co., Fair Lawn, N.J.
[4] Millipore, Bedford, Mass.
[5] Model 3500B, Spectra-Physics, Santa Clara, Calif.
[6] μBondapak C_{18}, Waters Associates, Milford, Mass.
[7] Valco Instruments Co., Houston, Tex.
[8] Model SP 770, Spectra-Physics, Santa Clara, Calif.
[9] OmniScribe, Houston Instruments, Austin, Tex.
[10] System I, Spectra-Physics, Santa Clara, Calif.
[11] Beckman Zeromatic pH meter, Beckman Instruments, Irvine, Calif.

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Table I—Composition of Various Vehicles

Vehicle	Alcohol, % (v/v)	Syrup (16), % (v/v)	Distilled Water, % (v/v)	0.05 *M* Citrate Buffer, % (v/v)
I	0	25	75	—
II	12.5	25	62.5	—
III	25	25	50	—
IV	40	25	35	—
V	100	—	—	—
VI	12.5	12.5	75	—
VII	12.5	40	47.5	—
VIII	25	75	—	—
IX	12.5	25	—	62.5 (2.30)[a]
X	12.5	25	—	62.5 (3.00)
XI	12.5	25	—	62.5 (3.50)
XII	12.5	25	—	62.5 (4.50)
XIII	12.5	25	—	62.5 (5.25)

[a] The pH values are given in parentheses.

and stored at 5 ± 0.1, 25 ± 0.1, and 50 ± 0.1°. Periodically, 300-μl samples were removed and diluted to 2.0 ml with the mobile phase containing 85 μg/ml of the internal standard, 4-chloropyridine hydrochloride. The stability of heroin, morphine, and cocaine in these mixtures was monitored over a period not exceeding 65 days.

RESULTS AND DISCUSSION

Chromatographic Behavior—The hydrochlorides of morphine, 6-monoacetylmorphine, benzoylecgonine, 4-chloropyridine, heroin, and cocaine were eluted in that order. Figure 1 illustrates the separation of a synthetic mixture of these substances and benzoic acid. The chromatographic retention data for each substance are listed in Table II. At a flow rate of 0.8 ml/min, the analysis time was ~22 min, and baseline resolution was achieved in nearly every case. The retention times (T_R in seconds), k', α, and R_s for all of these substances in hydrolyzed samples were identical to those determined for the synthetic mixture. During the 2-month study, there was no noticeable loss in column performance.

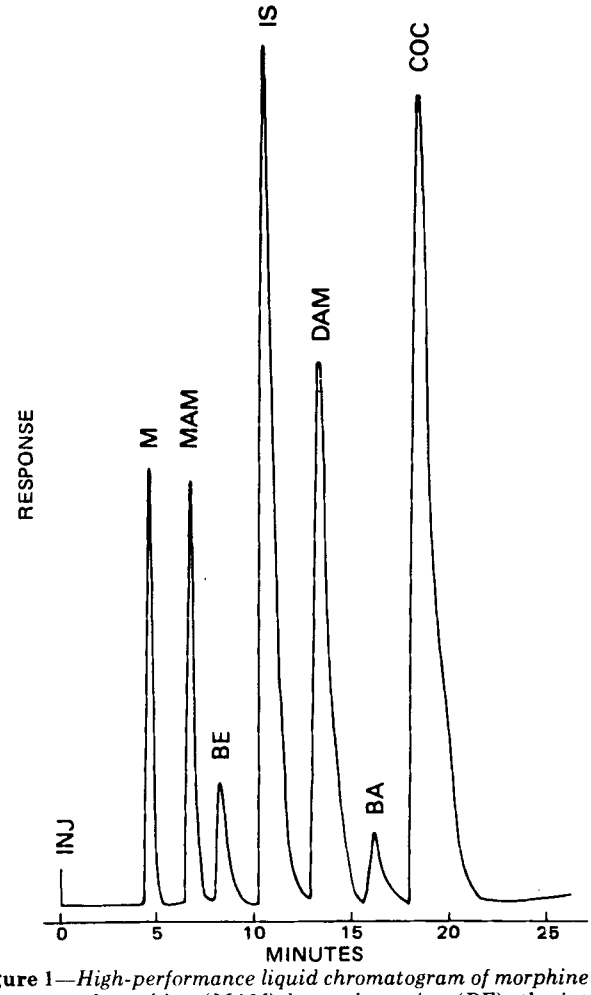

Figure 1—*High-performance liquid chromatogram of morphine (M), 6-monoacetylmorphine (MAM) benzoylecgonine (BE), the internal standard (IS), diacetylmorphine (DAM), benzoic acid (BA), and cocaine (COC).*

Table II—Retention Times (T_R), Capacity Factors (k'), Separation Factors (α), and Resolution (R_s) of Various Solutes[a]

Compound	T_R, sec	k'	α	R_s
Morphine	270	0.42	2.88	4.89
6-Monoacetylmorphine	420	1.21	1.35	2.19
Benzoylecgonine	500	1.63	1.48	3.12
4-Chloropyridine	648	2.41	1.38	2.87
Diacetylmorphine	820	3.32	1.24	2.14
Benzoic acid	972	4.12	1.22	2.02
Cocaine	1140	5.00	1.26	2.46
2-Chloro-3-nitropyridine	1390	6.32		

[a] Retention data of various solutes from a 300 × 4.6-mm i.d. μBondapak C_{18} (10 μm) column. The mobile phase was acetonitrile–monobasic potassium phosphate (0.015 *M*) (pH 3.0), the flow rate was 0.8 ml/min, and UV detection was at 235 nm.

Table III—Effect of Alcohol Concentration on the Stability of Heroin and Cocaine in the Brompton Mixture at Various Temperatures

Alcohol, % (v/v)	t_{90} at 5 ± 0.1°, days		t_{90} at 25 ± 0.1°, days		t_{90} at 50 ± 0.1°, days	
	Heroin	Cocaine	Heroin	Cocaine	Heroin	Cocaine
0 (I)	16	>60	6	>60	1.5	5
12.5 (II)	25	>60	10	>60	2	5
25 (III)	40	>60	15	>60	4	5
40 (IV)	75	>60	30	>60	8	7
100[a] (V)	—	—	—	—	50	48

[a] Contains heroin (1 mg/ml) and cocaine (0.5 mg/ml).

Table IV—Effect of Syrup Concentration on Heroin and Cocaine Stability in the Brompton Mixture at 50 ± 0.1°

Simple Syrup, % (v/v)	t_{90}, days	
	Heroin	Cocaine
12.5 (VI)	4	5
25 (II)	2	5
40 (VII)	1.5	5

The HPLC system may be used to quantitate every component in solution when desired. In this study, only the disappearance of heroin and cocaine was followed. The chromatographic responses for these compounds were linear ($r > 0.999$) in the working concentration range (20–200 μg/ml). The sensitivity limits were 10 ng for heroin and 3 ng for cocaine with a signal-to-noise ratio of better than five. To reduce the duration of each analysis, 4-chloropyridine hydrochloride was selected as the internal standard on the basis of its shorter retention time. However, 2-chloro-3-nitropyridine may be used instead (Table II).

Effects of Alcohol and Syrup Concentrations—The disappearance rates of heroin and cocaine as a function of the alcohol content in the mixture containing a fixed concentration of simple syrup were evaluated at various temperatures (5 ± 0.1, 25 ± 0.1, and 50 ± 0.1°) (Table III). Cocaine was stable at 25 and 5° and did not approach the t_{90} values (time for 10% disappearance of original concentration) during the 2-month observation period. However, heroin was markedly less stable at all temperatures studied. Thus, recommendations are based on the stability of heroin in these mixtures.

Hydrolysis of heroin was diminished with increasing proportions of alcohol, while the disappearance rate of cocaine was unaffected. Under the usual alcohol concentrations found in Brompton mixtures (12.5–25%), these solutions had a useful life of 10–14 days at 25°. However, the stability of diacetylmorphine was markedly enhanced in alcohol. The t_{90} of either heroin or cocaine in ethanol was >45 days at 50°. When stored at 5°, such a mixture may be used as a stock solution to facilitate preparation of Brompton mixtures at the time of dispensing.

Unlike alcohol, decreasing the syrup content at a fixed alcohol concentration in the mixture had a less pronounced effect on diacetylmorphine and no effect on cocaine stability (Table IV).

Effects of pH and Temperature—The influence of the Brompton mixture pH on the stability of heroin and cocaine was of interest since

Table V—Disappearance Rates of Heroin and Cocaine in Various Brompton Mixtures

Vehicle	k_1 at 5 ± 0.1°, day^{-1}		k_1 at 50 ± 0.1°, day^{-1}	
	Heroin	Cocaine	Heroin	Cocaine
I	6.56×10^{-3}	0.42×10^{-3}	—	—
II	4.20×10^{-3}	0.42×10^{-3}	—	—
III	2.62×10^{-3}	0.35×10^{-3}	—	—
IV	1.40×10^{-3}	0.31×10^{-3}	—	—
V	—	—	2.10×10^{-3} (50)[a]	2.20×10^{-3} (48)
IX	—	—	146×10^{-3} (0.7)	1.75×10^{-3} (60)
X	—	—	70.0×10^{-3} (1.5)	2.15×10^{-3} (49)
XI	—	—	39.5×10^{-3} (2.7)	4.25×10^{-3} (24)
XII	—	—	36.2×10^{-3} (2.9)	16.15×10^{-3} (6.5)
XIII	—	—	105×10^{-3} (1)	70.0×10^{-3} (1.5)

[a] Period (days) for 10% disappearance of original concentration is given in parentheses.

Table VI—Effects of Morphine and Heroin on Cocaine in Brompton Mixture

Vehicle	t_{90} at 25 ± 0.1°, days		t_{90} at 50 ± 0.1°, days	
	Heroin	Cocaine	Heroin	Cocaine
II	10	>60	2	5
IIa[a]	—	—	1.75	—
IIb[b]	—	10	—	1.5
XII	—	—	2.9	6.5
XIIa[c]	—	>60	—	11

[a] Contains only heroin (1 mg/ml) in standard vehicle II. [b] Contains morphine (1 mg/ml) and cocaine (0.5 mg/ml) in vehicle II. [c] Contains only cocaine (0.5 mg/ml) in vehicle XII.

the optimal pH values for minimal degradation of diacetylmorphine (7) and cocaine (11, 17) were shown separately to be 4.0–4.5 and 2.5–3.0, respectively. Hence, the pH range of 2.30–5.25 was chosen. The results (Table V) indicate that the optimal pH is between 3.0 and 3.5 for the overall minimal hydrolysis of heroin and cocaine in such a mixture.

The available data show the predominant influence of temperature on the rate of diacetylmorphine and, particularly, of cocaine hydrolysis in the Brompton mixture (Tables III and VI). A 10-fold decrease in temperature of the Brompton mixture diminished the rate of hydrolysis of heroin by ~10-fold and that of cocaine by at least 50-fold.

Kinetic Evaluations—Semilog plots of heroin or cocaine concentrations in the Brompton mixture *versus* time exhibited excellent linearity ($r > 0.99$) in 95% ethanol or buffered solutions (V and IX–XIII). Thus, the disappearance of heroin and cocaine followed pseudo-first-order kinetics under the conditions studied. Table V presents the calculated pseudo-first-order rate constants and t_{90} values at 50 ± 0.1° for heroin (2.36×10^{-3} *M*) and cocaine (1.47×10^{-3} *M*) in 0.05 *M* citrate buffers (18) or in 95% ethanol.

However, the disappearance rates of both diacetylmorphine and cocaine in unbuffered solutions (common in Brompton mixtures) at 25 ± 0.1 and 50 ± 0.1° were biphasic. A rapid change was followed by a more gradual one, with the final phase being linear on a semilogarithmic plot ($r > 0.99$). The extent of the initial change and the terminal phase slope of each solution were temperature dependent and were more dominant at high temperature. The t_{90} values of heroin and cocaine in these solutions were determined from the respective plots of concentration *versus* time. At 5 ± 0.1°, the hydrolysis rates of heroin and cocaine in unbuffered Brompton mixtures followed pseudo-first-order kinetics over the period studied (Table V). However, the amount of hydrolysis was minimal and did not approach t_{90} values.

Effects of Heroin, Cocaine, and Morphine—The results (Table VI) indicated that although cocaine had no effect on the heroin decomposition rate in the Brompton mixture (II *versus* IIa), the presence of diacetylmorphine increased the cocaine decomposition rate in the mixture (XII *versus* XIIa).

The frequent substitution of morphine for heroin in the Brompton mixture in the United States warranted a study to determine the effect of this substitution on the stability of cocaine and morphine in 12.5% alcohol and 25% simple syrup USP. Morphine appeared to be stable, but such a replacement adversely affected the cocaine hydrolysis rate at all temperatures studied (II *versus* IIb). In fact, the t_{90} value (10 days) was essentially the same as that observed in the diacetylmorphine–cocaine mixture at 25°. This acceleration of cocaine hydrolysis is of special concern. Cocaine hydrolyzes to pharmacologically inactive substances (19), whereas the diacetylmorphine decomposition products, 6-monoacetylmorphine and morphine, retain analgesic activity. The chemical stability of both cocaine and diacetylmorphine was enhanced by adjustment to pH 3.0–3.5. However, the biological effects of this pH adjustment have not been evaluated.

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