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Stability of cocaine hydrochloride solutions at various pH values as determined by high-pressure liquid chromatography

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Summary

A stability-indicating assay method based on high-pressure liquid chromatography for the quantitation of cocaine hydrochloride has been developed. The excipients, chlorobutanol, green color and phenol did not interfere with the assay procedure. The developed method was also used to study the stability of cocaine hydrochloride solutions in water and some buffer solutions (phosphate and carbonate). The cocaine solutions in water below pH 4 did not hydrolyze when stored at 24°C for up to 45 days, while the hydrolysis was very fast above pH 5.5. The hydrolysis was catalyzed by OH^- and HPO_4^{2-} and the increase in the ionic strength decreased the rat of hydrolysis.

Introduction

Cocaine hydrochloride solutions (2–10% in water with or without excipients) are used topically as local anesthetic. Many hospital pharmacists have shown interest in the stability of aqueous solutions of cocaine hydrochloride (I).

There is very little information available in the literature about the stability of I in water. In one study (Sadlin, 1928) it was found that a 1% solution of I in water lost approximately 10% of potency when stored at 20°C for one month. After one month no further hydrolysis was noticed during one year. The author used two methods of analysis, gravimetric and titrimetric whose results varied widely. For example, one solution was found to be 96% potent with the gravimetric method versus 83% with the titrimetric method.

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In another study (Murray and Al-Shoura, 1976), the authors reported that BPC 1973 method is not stability-indicating. They recommended an extraction procedure before UV measurements. The optimum pH of stability at 80°C was reported (Murray and Al-Shoura, 1976) to be approximately 2.2. Another report (Murray and Al-Shoura, 1978) indicated that cocaine degraded only to benzoylecgonine.

The purpose of this paper is to report: (i) a reliable stability-indicating assay method for the determination of I in aqueous solutions; and (ii) stability of I in some aqueous solutions.

Materials and methods

Reagents and chemicals. All reagents and chemicals were ACS, USP or NF quality and used without further purification.

Apparatus. The high-pressure liquid chromatograph * was connected to a multiple-wavelength detector ** and a recorder ***.

Column. The semipolar column † (30 cm × 4 mm i.d.) consisted of a monomolecular layer of cyanopropylsilane permanently bonded by silicone-carbon bonds.

Chromatographic conditions. The mobile phase contained 25% v/v of methanol in 0.02 M aqueous solution of ammonium acetate (pH ~ 7). The flow rate was 2.0 ml/min. The temperature was ambient. The detector sensitivity was 0.04 AUFS (275 nm) and the chart speed was 30.5 cm/h.

Solution preparation. All the solutions were prepared using a simple solution method and are listed in Table I. All pH values were measured using a pH meter ††.

After the initial assays, the majority of the solutions (see Table I) were stored at room temperature (24 ± 1°C) and some were stored in the refrigerator (5 ± 1°C). The solutions were re-assayed at appropriate intervals using high-pressure liquid chromatography (HPLC) as described below.

Standard solutions. The standard solutions containing 100–400 μg/ml of I with 5.0 μg/ml of ethyl-*p*-aminobenzoate (internal standard) in water were prepared as needed.

Assay solutions. The solutions were diluted to contain 125.0–250.0 μg/ml of I. Before bringing to volume, an appropriate quantity of the internal standard was added.

Assay procedure. A 20.0-μl aliquot of the assay solution was injected into the chromatograph using the described conditions. An identical volume of the appropriate standard solution was injected for comparison after the assay solution had been eluted. The standard solution contained exactly the same quantity of I as the label claim of the assay solution.

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* Waters ALC 202 equipped with a U6K universal injector, Waters Associates, Milford MA.

** Spectroflow monitor SF770, Schoeffel Instruments, Westwood, NJ.

*** Omniscribe 5213-12, Houston Instruments, Austin, TX.

† μBondapak CN, Waters Associates, Milford, MA.

†† Model 4500 digital pH meter, Beckman Instruments, Irvine, CA.

TABLE I

LIST OF AQUEOUS SOLUTIONS PREPARED

Solution No.	Conc. of I (g/100 ml)	Buffer conc. (M)	Type of buffer	Ionic strength [a]	Initial pH [b] (±0.05)	Temp. of storage [c] (°C)
1	0.5	0.05	Phosphate	0.3	1.6	24 and 5
2	0.5	0.05	Phosphate	0.3	2.5	24 and 5
3	0.5	0.05	Phosphate	0.3	4.5	24 and 5
4	0.5	0.05	Phosphate	0.3	5.8	24 and 5
5	0.5	0.05	Phosphate	0.3	6.0	24 and 5
6	0.5	0.05	Phosphate	0.3	6.6	24 and 5
7	0.5	0.05	Carbonate	0.3	7.5	24 and 5
8	0.5	0.05	Carbonate	0.3	8.0	24 and 5
9	0.2	0.2	Carbonate	0.3	8.1	24
10	0.2	0.3	Carbonate	0.3	8.1	24
11	0.2	0.3	Carbonate	0.6	8.1	24
12	0.025	0.1	Phosphate	0.4	6.35	24
13	0.025	0.2	Phosphate	0.4	6.35	24
14	0.025	0.3	Phosphate	0.4	6.35	24
15	0.025	0.1	Phosphate	0.4	5.9	24
16	0.025	0.2	Phosphate	0.4	5.9	24
17	0.025	0.3	Phosphate	0.4	5.9	24
18	0.025	0.1	Phosphate	0.2	6.8	24
19	0.025	0.1	Phosphate	0.4	6.8	24
20	0.025	0.1	Phosphate	0.6	6.8	24
21	0.025	0.025	Phosphate	0.8	6.8	24
22	1.0	(solution prepared in water)	None		3.6	24
23	2.0 [d]	(solution prepared in water)	None		3.4	24
24	10.0 [d]	(solution prepared in water)	None		2.75	24

[a] All ionic strengths were adjusted with potassium chloride.

[b] Final pH values were also similar.

[c] All solutions were stored in 3-oz. amber-colored dispensing bottles except 2.0 and 10.0% solutions in water which were stored in 100 ml volumetric flasks (Pyrex).

[d] Commonly used concentrations in the hospitals.

Calculations. Since preliminary investigations indicated that ratios of peak heights of I and internal standard were directly related to concentrations of I, the results were calculated using:

$$\frac{(Ph)_a}{(Ph)_s} \times 100 = \text{percent contained in the assay solution} \quad (1)$$

where $(Ph)_a$ is the ratio of peak heights of the analyte (cocaine) to the internal standard (ethyl-*p*-aminobenzoate) in the assay solution and $(Ph)_s$ is the ratio of

TABLE 2

ASSAY RESULTS OF FRESHLY PREPARED SOLUTIONS IN WATER

Solution No.	Conc. of I (g/100 ml)	Per cent of the label claim	Per cent standard deviation [a]
1	1	100.3	1.4
2	2	100.7	1.4
3	3	100.0	1.3
4	4 [b]	100.8	1.5
5	5	100.3	1.4
6	10	99.7	1.5

[a] Based on 6 assay results.

[b] As received from a local hospital. The pH of this sample was 2.8. The sample also contained per 250 ml, 20 drops of liquid phenol, 25 drops of chlorobutanol (50% aqueous solution) and 10 drops of green color (chemical name was not available).

cocaine peak to the internal standard in the standard solution.

If the standard solution does not contain the identical concentration of cocaine, the results can be corrected by multiplying the per cent found by:

$$\text{Factor} = \frac{\text{Actual concentration of cocaine (mg/ml) in the standard solution}}{\text{Label claim of cocaine (mg/ml) in the assay solution}}$$

When the cocaine hydrochloride peak was too small (less than 25% of the standard) to quantify accurately, a new assay solution containing a higher concentration of I was injected. Appropriate calculations were then made to determine the exact concentration of I in the assay solution. The results which are the average of 6 assays, are presented in Tables 2, 3 and Figs. 1–5.

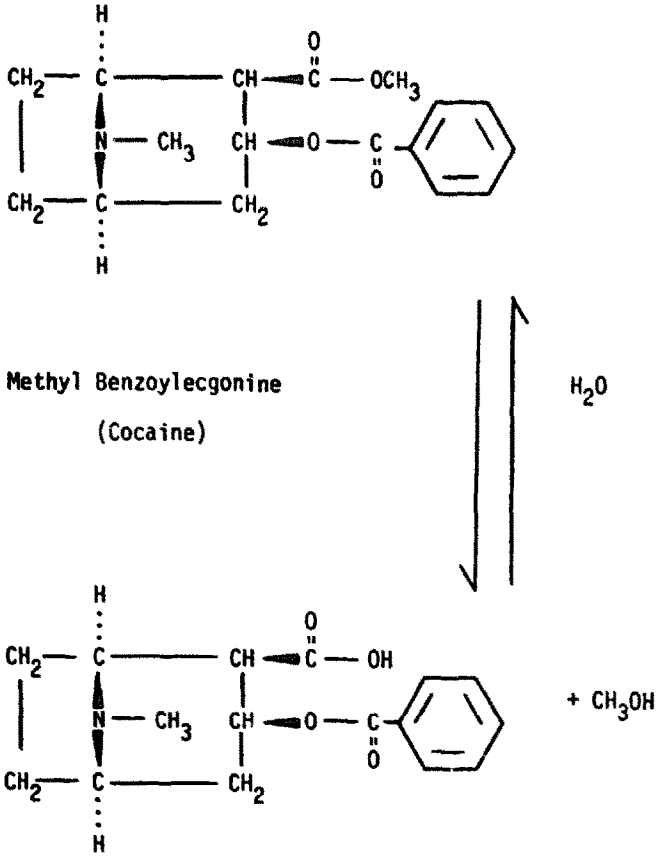
Spectrophotometric assays. Some of the samples were assayed using UV spectroscopy at two wavelengths (275 and 234 nm) of maximum absorption. All standards and assay solutions were diluted with 0.1 M HCl. The concentrations of I were 10 and 100 g/ml for measurements at 234 and 275 nm, respectively. Results were calculated by a simple comparison of absorbance values of the standard and assay solutions since Beer's law was followed.

Results and discussion

Assay method

The high-pressure liquid chromatography (HPLC) assay method is reproducible and accurate (Table 2). The excipients (chlorobutanol, green color and phenol) being added by a local hospital did not interfere with the assay procedure (Table 2). In decomposed samples, it was possible to separate benzoylecgonine (peak 1 in Fig. 1B), the product of hydrolysis (Scheme I), using HPLC. The other product of

hydrolysis (I), methanol, did not interfere in the assay procedure.



The increase in the height of benzoylecgonine peak was directly related to decrease in the peak height of I. In fact, the assay results could also be determined from the peak height of benzoylecgonine (II). Moreover, the results from peak heights of benzoylecgonine and cocaine always added up to the original concentration of I.

A sample in pH 6.9 phosphate buffer was completely hydrolyzed with the aid of heat. The peak height of this sample indicated that for each unit loss in the peak height of I, there was a gain of approximately 2.3 units in the peak height of II. This information was used to determine the results from peak height of benzoylecgonine. The results so obtained were in excellent agreement with results determined using Eqn. 1.

The UV methods did not prove reliable. For example, after 13 days of storage, one sample showed a potency of 0% (Table 3) using HPLC method. The same sample gave 99.1 and 97.5% results using UV methods at 275 and 234 nm, respectively. Apparently, the molar absorption of benzoylecgonine at 275 and 234 nm is about the same as that of methyl benzoylecgonine (cocaine).

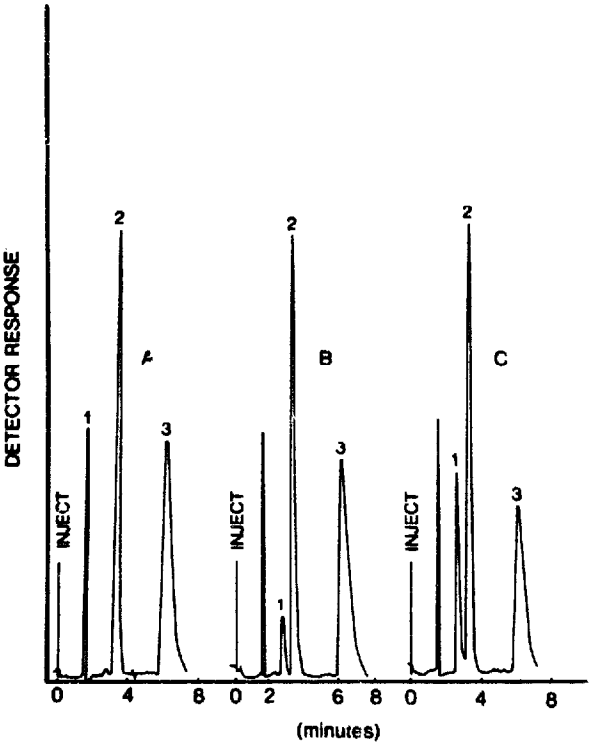


Fig. 1. Sample chromatograms. Peak 1 is from benzoylecgonine, peak 2 from the internal standard and peak 3 from cocaine. Chromatogram A is from a standard solution containing 250 μg/ml of cocaine HCl; chromatograms B and C are from solutions 7 and 8, respectively (see Table I) after 13 days of storage at 5°C. The elution was carried to 13 min without seeing another peak.

TABLE 3

RESULTS OF SELECTED SAMPLES [a] USING HPLC WHEN STORED AT 24°/5°

Solution no.	Buffer pH (see Table I)	Percent retained after:				
		13 days at:		24 days at:		45 days at:
		24°C	5°C	24°C	5°C	24°C
1	Phos. 1.6	98.9	99.8	99.1	99.5	–
2	Phos. 2.5	98.8	99.5	98.7	99.3	–
3	Phos. 4.5	99.3	99.5	98.6	99.5	–
4	Phos. 5.8	89.7	99.8	83.8	98.9	–
5	Phos. 6.0	81.7	99.3	71.8	97.2	–
6	Phos. 6.0	42.5	99.3	24.2	96.1	–
7	Carb. 7.5	16.8	92.5	4.3	87.2	–
8	Carb. 8.0	0	70.1	–	53.9	–
21	Phos. 6.8	99.9	100.0	100.3	101.1	99.3
22	Water 3.6	99.7	99.5	100.3	100.3	99.8
23	Water 3.4	100.2	100.0	99.8	99.8	99.3
24	Water 2.8	100.5	100.5	100.1	100.5	100.0

[a] The data from other solutions are plotted in Figs. 2–5.

Effect of pH

The effect of pH becomes prominent only if the value is above about 5.5 (Fig. 2). At lower pH values (below 4) especially without buffering agents, no decomposition of I was found (Table 3) even after 45 days of storage at room temperature. In an earlier study (Murray and Al-Shoura, 1976) conducted at 80°C, the optimum pH of stability was reported to be approximately 2.2

Based on our studies, the effect of solvent and H^+ on the decomposition of cocaine can be assumed as 0 (Solutions 22–24, Table 3). The hydrolysis of cocaine at higher pH values was catalyzed not only by OH^- but also by the buffering agent phosphate (Solutions 12–17, Table 1). The increase in the concentration of carbonate buffer did not increase the hydrolysis of cocaine (Solutions 9–10, Table 1).

The hydrolysis of cocaine may be represented by:

$$k_{obs} = k_1(OH^-) + k_2(H_2PO_4^-) + k_3(HPO_4^{2-}) \quad (2)$$

At a constant pH value, $k_1(OH^-)$ is a constant and the equation may be rewritten as:

$$k_{obs} = k + k_2(H_2PO_4^-) + k_3(HPO_4^{2-}) \quad (3)$$

where $k = k_1(OH^-)$.

Since the decomposition followed first-order laws (Fig. 3), the k_{obs} values were determined using first-order plots. Also, Eq. 3 may be rearranged as follows:

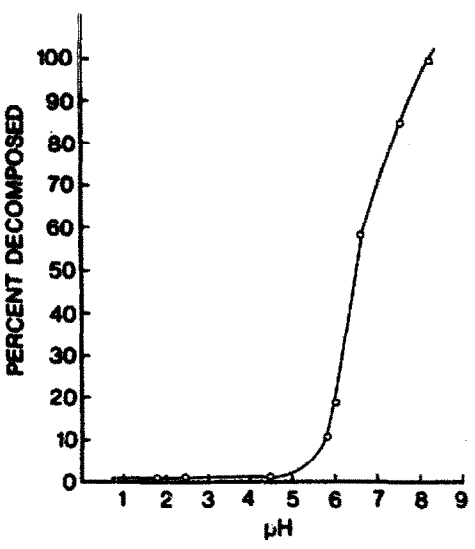
$$k_{obs} = k + (H_2PO_4^-)\left(k_2 + \frac{k_3}{q}\right) \quad (4)$$

where $q = \dfrac{(H_2PO_4^-)}{(HPO_4^{2-})}$.

On plotting, k_{obs} values versus concentrations of $(H_2PO_4^-)$ straight lines were obtained (Fig. 4). From the slopes and using q values of 7.08 and 19.96 at pH values of 6.35 and 5.9, respectively, the two simultaneous equations were solved for k_2 and k_3. The k_2 and k_3 were determined to be 0.0062 and 0.1109 $M^{-1} \cdot day^{-1}$. Furthermore, by substituting these values in Eqn. 2, the k_1 was estimated to be approximately 5.38×10^5 $M^{-1} \cdot day^{-1}$.

Effect of ionic strength

From Fig. 5, it is obvious that k_{obs} value decreases with increase in ionic strength. Therefore, the reacting ions are probably the protonated form of cocaine and OH^-/HPO_4^{2-}. However, the slope of the line was approximately 0.336 versus the expected value of 1.02. This may be due to high ionic strengths since the equation $\log k = \log k_0 + 1.02\,(z_A z_B)\sqrt{\mu}$ was deduced by assuming a value of 0.01 or less. In these studies, it was necessary to keep the concentration of buffering agent high in



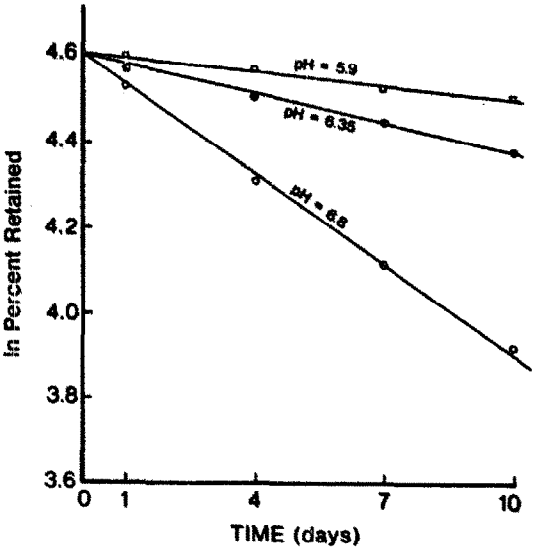
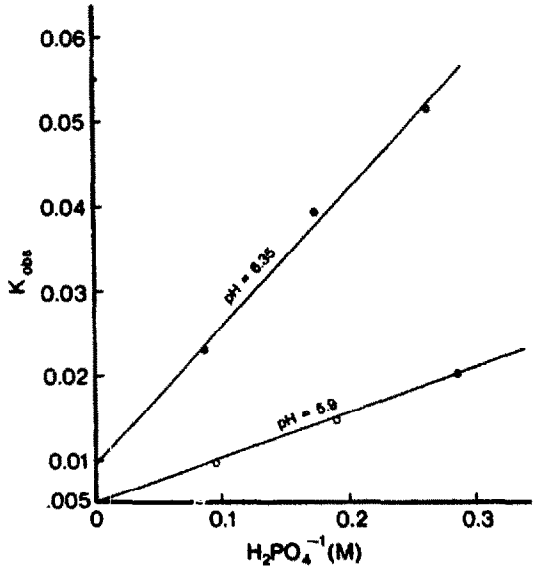


Fig. 2. A pH–rate profile curve from data of solutions 1–8 (Table 3) after 13 days of storage at 24°C.

Fig. 3. First-order plots of solutions 12, 15 and 19 (see Table 1).

order to maintain constant pH values. Therefore, the ionic strengths were high. The equation cannot be expected to hold (Moore, 1972) at salt concentrations much beyond the range of validity of the Debye-Huckel theory.

The other explanation may be that there are two parallel routes of hydrolysis. The



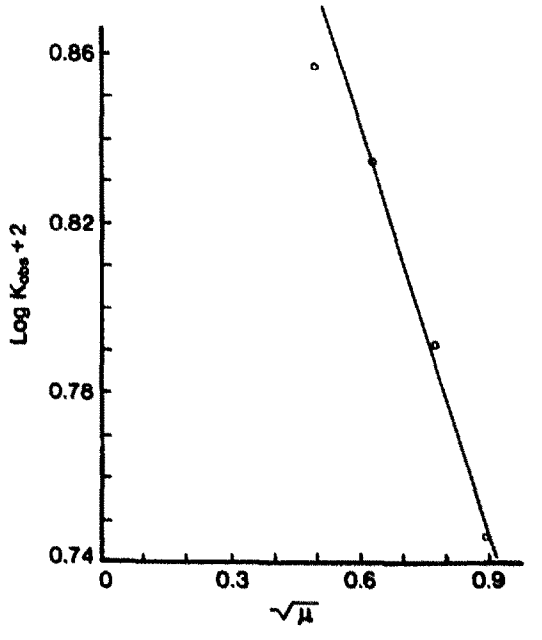


Fig. 4. Plots of k_{obs} (Solutions 12–17, Table 1) vs molar concentration of $H_2PO_4^-$.

Fig. 5. Plot of log k_{obs} vs square-root of ionic strength (Solutions 18–21, Table 1).

second route involves reaction between the undissociated cocaine molecule and OH^-/HPO_4^{2-}. If so, this route will not be affected by ionic strength, and hence reduce the slope value.

Effect of temperature

As expected, the solutions were far more stable when stored at 5°C versus at 24°C (Table 3). Since the solutions at lower pH values (1.7–4.5) did not hydrolyze even at room temperature (especially if prepared in water), there is no advantage in storing these solutions in the refrigerator.

From the estimated k values for solution 7 (pH 7.5), the stability at 5°C appears to be approximately 23 times better than at 24°C.

Finally, it should be pointed out that a 1.0, 2.0 and 10.0% solution in water (pH range 2.75–3.6) did not hydrolyze even in 45 days (Table 3) when stored at 24°C. This is contrary to observations made by Sadlin (1928) who reported up to 10% loss in potency within 30 days at 20°C.

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