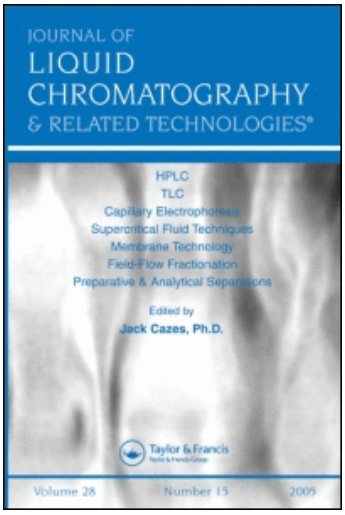

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DETERMINATION BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF STABILITY OF TETRAHYDRO-β-CARBOLINES AT DIFFERENT AMBIENT TEMPERATURES

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ABSTRACT

To determine the stability of tetrahydro-β-carboline compounds over time and at different temperatures, a reversed-phase high pressure liquid chromatography system with electrochemical detection was utilized. Noreleagnine (1,2,3,4-tetrahydro-β-carboline) and tetrahydroharman (1-methyl-1,2,3,4-tetrahydro-β-carboline) were dissolved in water or ascorbate (0.1 mg/ml) vehicle and stored at -20°C, 22°C, or 37°C for one, seven or 12 days. After each solution was injected in the column in a concentration of 400-600 ng/10 µl, peak height values were obtained for the compound under each condition. Analysis of percent recovery showed that the two β-carbolines were relatively stable with a maximal degradation of 14% occurring only at the 12-day assay interval. These results suggest that this class of compound can be used in pharmacological studies in which they can be dispensed from a mini-pump implanted in tissue. Further, an HPLC system with electrochemical detector provides a valid and reliable procedure for quantification of indoleamine-aldehyde condensation products.

INTRODUCTION

Tetrahydro-β-carboline (THBC) compounds belong to a class of tricyclic structures which can be formed in vivo as a result of a con-

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densation reaction between an indoleamine and an aldehyde (1,2). Certain of the THBCs have been found to exist in the rat's brain (3,4) and are now recognized as normal constituents of human plasma and platelets (5). Because a THBC reportedly can modify brain 5-hydroxytryptamine (6,7), these compounds have been the focus of numerous medical and biological investigations including those involving affective disorders, schizophrenia, alcoholism and neurotransmitter activity (8,9,10).

One of the THBCs, noreleagnine, has been implicated in the mechanism underlying alcohol drinking (11), but the general role of other β-carbolines in the excessive intake of this drug is not yet known. For example, the consumption of alcohol in the laboratory rat reportedly declines following noreleagnine (1,2,3,4-tetrahydro-β-carboline) administered peripherally (12) but increases after intracerebroventricular infusion of the product over 12 days. (13). This latter study was replicated with two β-carbolines, tetrahydroharman (1-methyl-1,2,3,4-tetrahydro-β-carboline) and noreleagnine which were infused continuously into the cerebral ventricle through the use of an osmotic mini-pump implanted in the rat's neck (14). Although mass spectrometry was used to verify the presence of the compound in a given pump, the actual stability of the β-carboline compounds over a prolonged period remains uncertain. To illustrate, we have found recently that other amine-aldehyde condensation products are unstable over time, particularly at a temperature analogous to a body temperature of 37°C (15).

A high pressure liquid chromatography (HPLC) assay has been developed to separate noreleagnine from brain tissue (16). The purpose of the present investigation, therefore, was to (1) develop a method using HPLC with electrochemical detection for the determination of β-carbolines in a fluid medium; (2) quantify the rates of degradation of the β-carboline com-

pounds; (3) ascertain the effect of different ambient temperatures on the decomposition of these compounds; and (4) determine whether an antioxidant such as ascorbic acid, which is typically added to solutions used for pharmacological studies, would influence the degradation of a β-carboline.

MATERIALS AND METHODS

The HPLC system was comprised of a single pump (Altex Model 110, Solvent Metering Pump) with a pulse damper (Bioanalytical Systems) and a syringe loading sample injector (Rheodyne Model 7120). A C_{18} reversed-phase column (3.9 mm i.d. x 300 mm μBondapak, Waters) protected by a pre-column filter (Rheodyne) was fitted into the system. A glassy carbon electrochemical detector cell, a TL-8A thin layer transducer (Bioanalytical Systems), was coupled to a model LC4 amperometric detector (Bioanalytical Systems). The electrode potential was set at +0.85V using a silver-silver chloride electrode as a reference, and the level of sensitivity of the detector was set at 10 nA/V. A strip chart recorder (Fisher Recordall, Series 500) connected in parallel with a plotting integrator (Hewlett Packard Model 3390A) completed the system.

Mobile Phase

The mobile phase consisted of 0.1 M sodium acetate, 0.15 M acetic acid and 20% (v/v) methanol with a pH of 4.4. The mobile phase was first passed through a double filter (0.3 μm Gelman A/E glass fiber filter and 2-3 μm Whatman #5 filter) and then degassed by sonication. The flow rate of the mobile phase through the column was maintained at 1.5 ml/min.

Sample Preparation

The β-carbolines tested were noreleagnine (1,2,3,4-tetrahydro-β-carboline) and tetrahydroharman (1-methyl-1,2,3,4-tetrahydro-β-carbo-

line). Each compound was weighed accurately to 10^{-3} mg using a Cahn 21 Automatic Electrobalance. Two samples of each compound were prepared: one was dissolved in glass-distilled water and the second in glass-distilled water containing 0.1 mg/ml of ascorbic acid.

The concentration of the β-carboline solutions was 400 or 600 ng/10 µl. Noreleagnine was readily solubilized in either medium by sonication; however, samples of tetrahydroharman required sonication and gentle warming. The solutions were tested immediately after their preparation and then multiple aliquots of each were maintained subsequently in sealed vials at one of three temperatures: 22°C (laboratory room); 37°C (water bath); and -20°C (freezer). Following their preparation, these aliquots were tested at intervals of one, seven and 12 days. As a control for daily variation in performance of the chromatography system, the aliquots maintained at -20°C were thawed briefly and tested also at these three intervals so that their peak values could serve as the respective baseline values. Each sample was injected from a 50 µl Hamilton syringe onto the column in a volume of 10.0 µl. An injection of each sample was then repeated at least three times in order to verify the reliability of the data collected. If a given peak response was not consistent, a further series of injections were made. Glassware and syringes were sonicated in chromic acid and rinsed repeatedly in glass-distilled water to avoid possible contamination of a sample.

Reagents

L-ascorbic acid and HPLC grade methanol were purchased from Fisher Scientific, whereas anhydrous sodium acetate and glacial acetic acid were obtained from Mallinckrodt Chemical. Noreleagnine was obtained from Sigma Chemical Company and tetrahydroharman was obtained from ICN Pharmaceuticals, Inc.

RESULTS

The percent recovery of the two β-carbolines was calculated from the integrated peak height values as compared with aliquots of the standard sample of each compound prepared for use on the respective test day. Each value is thus expressed as a mean ± standard error of the percent recovery for each sample tested against the standard for that given time period.

Table 1 presents a comparison of the percent recovery of both noreleagnine (NORL) and tetrahydroharman (THH) at a room temperature of 22°C as measured at one, seven and 12 days after their preparation. The results of the degradation of both compounds prepared in water and ascorbate are also shown in Table 1. It is evident that essentially no substantial cumulative degradation occurred over time under either condition even though by the 12th day the recovery of NORL, contrasted with the control value, was reduced to 93% and 89% in the water and ascorbate vehicles, respectively. The degradation of THH in water was virtually absent over the 12-day period; that is, THH in the ascorbate vehicle declined to the 91% level on day 7, but was quantified at 98% on day 12.

Essentially the same result was obtained when NORL and THH were stored at 37°C. Once again, NORL when prepared in the ascorbate vehicle, appeared to be the more labile of the two compounds, but yet exhibited a degradation of only 14% below the control value. However, THH remained relatively stable over the entire 12-day interval independent of the carrier vehicle in which it was dissolved.

Representative records of the HPLC tracings are shown in Fig. 1 for NORL tested in both of the carrier vehicles at 22°C and 37°C. Similarly, Fig. 2 illustrates typical HPLC tracings for the THH compound again analyzed under the same conditions as those of NORL. In all cases, the

TABLE 1

PERCENT RECOVERY OF NORELEAGNINE (NORL) AND TETRAHYDROHARMAN (THH) IN DISTILLED WATER OR ASCORBIC ACID VEHICLE AT ROOM TEMPERATURE OF 22°C

	WATER			ASCORBIC ACID		
	Time Elapsed (Days)			Time Elapsed (Days)		
COMPOUND	1	7	12	1	7	12
NORL	98.7±1.0 n=4	95.0±2.4 n=7	92.8±4.6 n=6	98.2±1.8 n=4	94.4±3.6 n=6	88.8±3.6 n=6
THH	94.1±2.8 n=5	98.8±0.6 n=6	95.8±1.9 n=6	93.8±1.1 n=5	91.3±3.2 n=6	97.8±1.0 n=6

TABLE 2

PERCENT RECOVERY OF NORELEAGNINE (NORL) AND TETRAHYDROHARMAN (THH) IN DISTILLED WATER OR ASCORBIC ACID VEHICLE AT 37°C

	WATER			ASCORBATE		
	Time Elapsed (Days)			Time Elapsed (Days)		
COMPOUND	1	7	12	1	7	12
NORL	97.6±0.8 n=4	96.6±1.9 n=6	94.1±3.7 n=6	99.0±0.5 n=4	95.8±2.4 n=6	86.3±3.6 n=5
THH	97.9±1.2 n=4	99.2±0.8 n=6	98.0±1.1 n=6	92.5±3.1 n=4	97.0±1.9 n=6	98.9±0.9 n=6

peak of the solvent front produced by ascorbate was always readily discernible from the respective β-carboline peak.

DISCUSSION

The results of this study show that a relatively straightforward analytical method employing HPLC with an electrochemical detector can be utilized for the assay of THBC compounds in terms of their stability under different physical conditions. The mobile phase established for this experiment provided reliable measures of the compounds over a two-

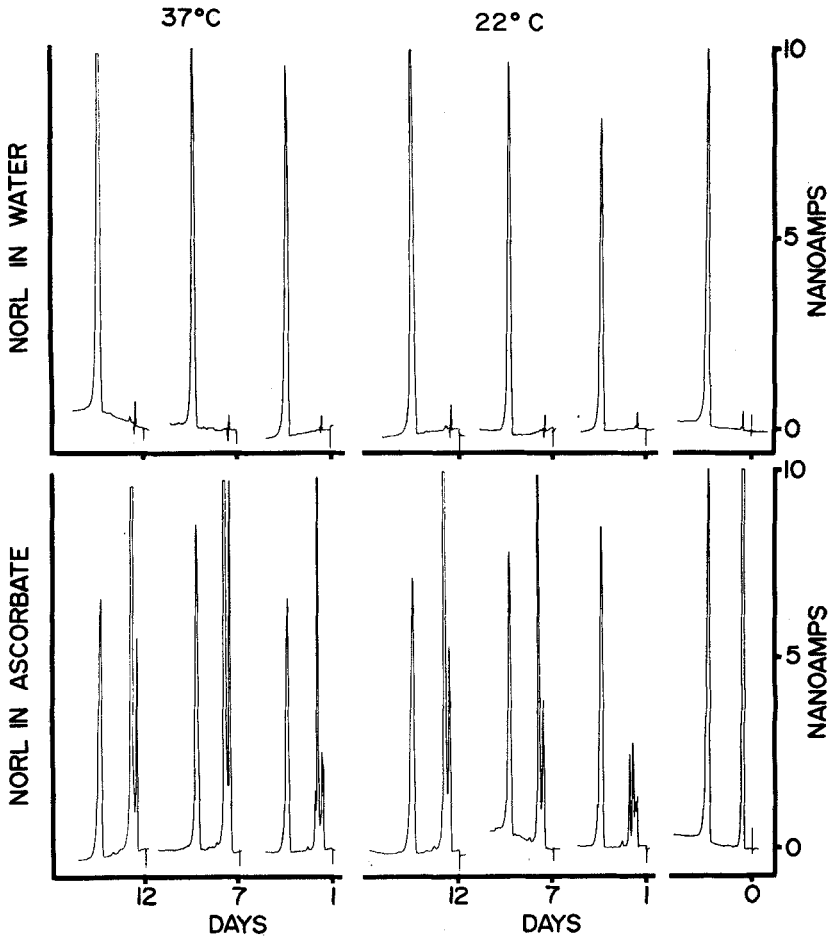

FIGURE 1: Chromatographic tracings of noreleagnine (NORL) dissolved in water or aqueous ascorbate (0.1 mg/ml). Samples were stored at either 37°C or 22°C and analyzed at one, seven and 12 days. The zero time represents the chromatograph of the freshly prepared sample. Peak height was measured in nanoamps.

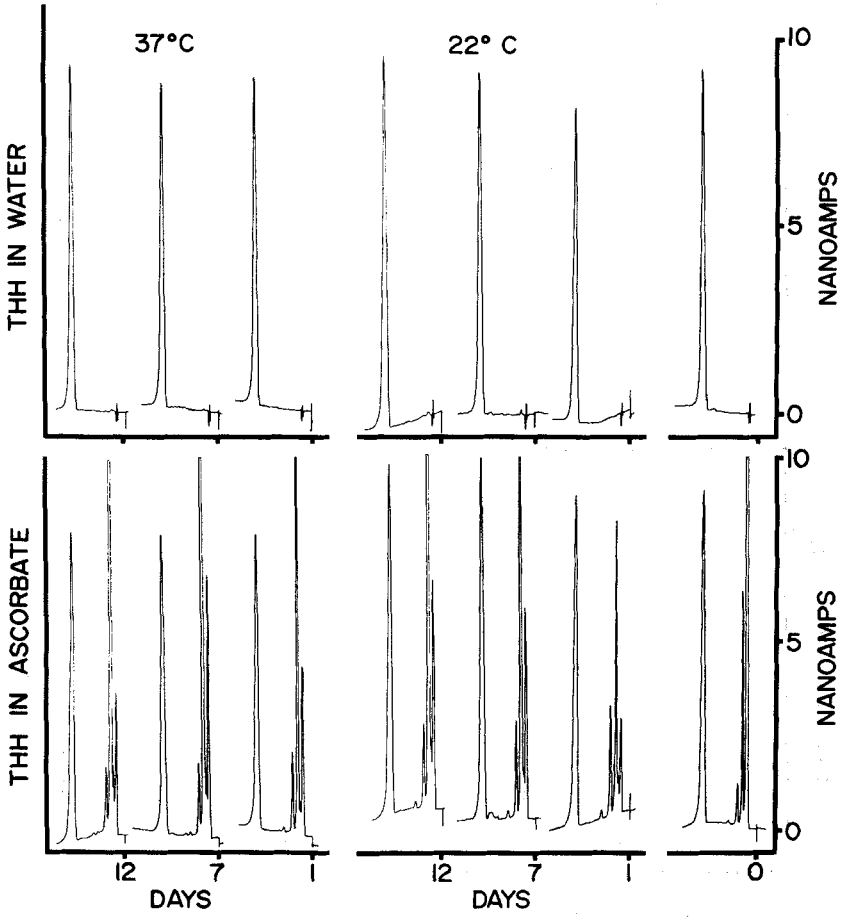

FIGURE 2: Chromatographic tracings of tetrahydroharman (THH) dissolved in water or aqueous ascorbate (0.1 mg/ml). Samples were stored at either 37°C or 22°C and analyzed at one, seven and 12 days. The zero time represents the chromatograph of the freshly prepared sample. Peak height was measured in nanoamps.

week period. Although a slight inconsistency was observed in the day-to-day column-detector sensitivity during this 12-day interval, quantification of a compound's activity was nevertheless readily attainable.

The relative stability of the two β-carbolines indicate that for pharmacological studies (10) the problem of degradation under physiological conditions is unlikely to be an experimental issue. Further, the absence or presence of ascorbate in the test medium also is not a factor in terms of the temporal integrity of these compounds, although the largest amount of decomposition occurred in the vehicle containing ascorbate. However, other condensation products, such as certain tetrahydroisoquinolines, do in fact require an antioxidant such as ascorbate to prevent their rapid degradation (15). Therefore, since the ascorbate does not appear to be necessary as an antioxidant, its deletion from a β-carboline solution used in an *in vivo* experiment is recommended, particularly since the acid may exert an effect on its own.

The results of this experiment also demonstrate that the two β-carbolines tested are equally stable at 22°C and 37°C. Since the elevated temperature did not compromise the stability of the compounds, the utilization of a mini-pump containing a β-carboline and implanted sub-dermally (14) in an animal with a body temperature of 37°C can be encouraged. According to our findings, the use of these compounds in a study in which repeated injections are required at either room (22°C) or body temperature over an interval such as two weeks would thus be a valid approach.

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