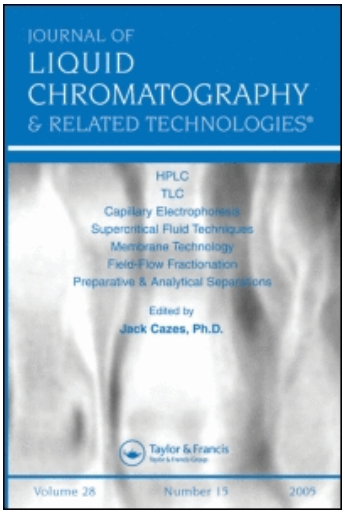

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Myers, R. D. , Garrison, J. L. and Critcher, E. C.(1983) 'Degradation Characteristics of Two Tetrahydroisoquinolines at Room and Body Temperatures: HPLC Determination with Electrochemical Detection', Journal of Liquid Chromatography & Related Technologies, 6: 2, 343 — 352
To link to this Article: DOI: 10.1080/01483918308066894
URL: http://dx.doi.org/10.1080/01483918308066894

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(2), 343-352 (1983)

DEGRADATION CHARACTERISTICS OF TWO TETRAHYDROISOQUINOLINES AT ROOM AND BODY TEMPERATURES: HPLC DETERMINATION WITH ELECTROCHEMICAL DETECTION

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ABSTRACT

Reversed-phase high-pressure liquid chromatography (HPLC) was utilized to determine the stability of tetrahydropapaveroline (THP) and salsolinol at two ambient temperatures and over varying time intervals of up to 27 hr. Although ascorbate, an antioxidant, was shown to retard the temporally contingent degradation of THP at both 22°C and 37°C, the breakdown of the tetrahydroisoquinoline product was more pronounced at 37°C. Salsolinol was virtually stable under all conditions. The formation of detectable by-products of THP was demonstrated by the presence of secondary peaks in the THP-water assay which were strikingly absent in the THP-ascorbate aliquots. Finally, the HPLC profiles of five THP samples obtained from four different sources revealed the presence of similar secondary peaks which varied considerably in shape and peak height from one sample to another. The implications of this lack of uniformity of THP for pharmacological studies of addictive processes is discussed.

INTRODUCTION

Certain of the amine-aldehyde condensation products, such as a tetrahydroisoquinoline (TIQ), exert potent pharmacological effects on the central nervous system (1). The catecholamine-aldehyde products can possess an addictive liability as evidenced

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from their pharmacological effects after direct administration into the brain (2, 3). Given systemically, they often exert opiate-like effects and analgesia (4).

Because of the relatively short half-life of the dopamine-dopaldehyde metabolite, tetrahydropapaveroline (THP) (5), a number of questions arise in terms of the potential instability of this class of compounds under various experimental conditions. Of paramount importance is the issue of whether a TIQ degrades at a more rapid rate at body temperature of ≃ 37°C than when the compound is maintained at a laboratory temperature of 22°C. Another question centers on the nature of the formation of secondary by-products during the breakdown of the alkaloid. Finally, does an antioxidant such as ascorbate, which also serves to lower pH, also alter the characteristics of the degradation of a TIQ amine-aldehyde product?

In the present experiments, HPLC procedures with electrochemical detection (6) were utilized to examine the rate of degradation of THP and salsolinol over time at two conditions of ambient temperature. The effect of the presence of ascorbate in the test solutions was also tested.

MATERIALS AND METHODS

Instrumentation

The HPLC system was comprised of a single pump (Altex Model 110, Solvent Metering Pump), with a pulse damper (Bioanalytical Systems [BAS]), 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 in the system. A glassy carbon electrochemical cell, TL-8A thin layer transducer (BAS), was coupled with a model LC4 amperometric detector (BAS). The electrode potential was set at +0.70 V using a silver-silver chloride electrode as a reference. Detector sensitivity was set at 10 nA/V. A strip chart recorder (Fisher Recordall Series 5000) connected in parallel with a

plotting integrator (Hewlett Packard Model 3390A) completed the system.

Mobile Phases

The mobile phase for the THP assay consisted of 0.15 M acetic acid, 14% v/v methanol, and 0.25 mM heptanesulfonic acid sodium salt (HSA) used as an ion-pairing agent. For salsolinol, the mobile phase contained 0.15 M acetic acid and 2.5% v/v acetonitrile. The pH of both phases was adjusted to 3.0-3.2 as necessary with dilute sodium hydroxide. Each mobile phase was passed under vacuum through a double filter (0.3 µm Gelman A/E glass fiber filter and 2-3 µm Whatman #5 filter) and degassed by sonication. A flow rate of 1.5 ml/min was maintained in both assays.

Sample Preparation

The THP and salsolinol assays were divided into two parts which were subjected to the same conditions. One sample line was dissolved in dilute ascorbic acid (0.1 mg/ml), an anti-oxidizing agent, while the other was dissolved in glass distilled water. All samples were readily solubilized with sonication. An initial sample concentration was chosen to maximize peak height while remaining on-scale on the recorder to allow accurate quantitation. After the column had been conditioned with mobile phase and the detector activated for 20-30 min, samples were injected onto the column in 10 µl volumes delivered from a 50 µl Hamilton syringe flushed repeatedly with water. The concentrations used consistently for the degradation analysis of THP and salsolinol were 50 ng/10 µl and 40 ng/10 µl, respectively.

HPLC Separation

Once properly diluted, the freshly prepared samples were injected directly onto the HPLC column. After three aliquots of each compound were dissolved in distilled water or ascorbic acid, they were kept at room temperature, placed in a water bath maintained at 37°C or kept in a -20°C freezer. Aliquots of THP kept at a room temperature of 22°C or at 37°C were tested at 2-4, 10-

12, or 24-27 hrs following the initial assay. Aliquots of salsolinol kept at room temperature were tested at 4 and 24 hrs, whereas water bath samples held at 37°C were injected after 1, 4 and 24 hrs. Samples of both THP and salsolinol kept at -20°C were tested after a 24 hr interval. For the analysis of different samples of THP obtained from different sources, a freshly prepared quantity of 1.0 µg/10.0 µl THP was injected onto the column without any intervening period of time.

Glassware used in preparation of samples was washed and rinsed, sonicated in chromic acid, then rinsed five times in deionized water and again in distilled water. After the glassware was drained, it was subsequently baked in an oven for two hrs at 185°C. Syringes were also flushed with dilute chromic acid cleaning solution at the end of each day. The chromatograph injector port was flushed with 800 µl distilled water prior to sample injections. These precautions were taken to reduce the possibility of contamination of the original samples in an actual injection.

Compounds were weighed accurately to 10^{-3} mg using a Cahn 21 Automatic Electrobalance. Dilutions were made in ratios no greater than 1:9 with graduated pipets.

Reagents

L-ascorbic acid, sodium hydroxide and methanol were obtained from Fisher Scientific, the latter two compounds being certified as HPLC grade. Glacial acetic acid was purchased from Mallinckrodt and the 1-Heptanesulfonic acid sodium salt was obtained from Eastman Kodak. Samples of THP were kindly provided by Hoffmann-LaRoche (RO6-1673), R. Deitrich of the University of Colorado, Z. Amit of Concordia University and Burroughs Wellcome. Salsolinol was obtained from Aldrich Chemical Company.

RESULTS

The marked degradation of THP in water as determined by the HPLC with EC detection is shown in Fig. 1A. The solvent peak,

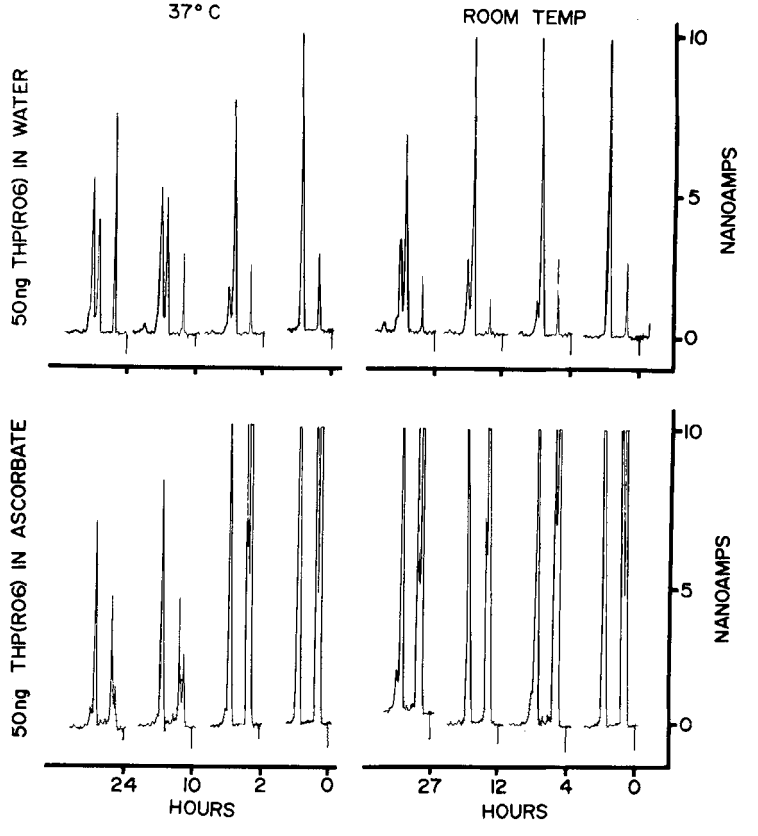

FIGURE 1: Degradation of THP in (A) water and (B) ascorbate at room temperature and at 37°C during 27 and 24 hrs, respectively.

with t_R = 3.1 min, was followed by the THP peak, t_R = 8.2 min. A second peak with t_R = 9.8 min became evident after four hours at both the ambient temperatures of 22°C and 37°C, and this peak increased over the duration of this experiment. As the second peak increased in size, a concomitant decline in the height of the actual THP peak also occurred. Though this trend was apparent both at room temperature and at 37°C, the higher ambient temperature caused a far greater rate of THP's degradation with

increase in this isoquinoline's by-products during the comparable period of time.

Figure 1B illustrates the decomposition of THP which was dissolved in ascorbic acid (0.1 mg/ml). The ascorbate peak itself was eluted at 2.0 min and the THP peak again followed thereafter at 8.2 min. Thus, the retention time of THP was unaffected by the presence of ascorbate. Further, in the test solution no clear-cut degradation of THP in the ascorbate medium occurred at a room temperature of 22°C. A small secondary peak was observed at four hours and at 27 hours, but the THP peak appeared to be unaffected. However, at 37°C evidence of degradation became apparent 10 hrs following preparation of the sample. A slight shoulder was registered after two hrs and stayed relatively constant throughout the remainder of the assay period. In contrast to the secondary peaks which dominated the THP chromatograms shown in Fig. 1A, no evidence of additional by-products was noted. A slight degradation of the ascorbate peak was observed at room temperature; however, its decomposition after 10 hrs in a 37°C environment was very pronounced.

In the second phase of this study, we obtained chromatograms of five different THP samples in order to compare their chemical profiles. The results of this analysis are shown in Fig. 2. The solvent eluted at 2.0 min followed by the primary THP peak at 8.0 min. Although each of the samples possessed four secondary peaks following the primary THP peak, large variations in the profile of these peaks as well as overall peak height were noted. The sample obtained from Deitrich exhibited the greatest amount of secondary activity whereas that supplied by Amit showed only minimal after-peaks.

Table 1 presents a composite comparison of the average percent degradation of THP and salsolinol under the various test conditions. Each calculated value is based on two-four replications of the assay for the given test condition. As shown in the Table, salsolinol retained its stability even at the 37°C temperature and after a 24-hr period.

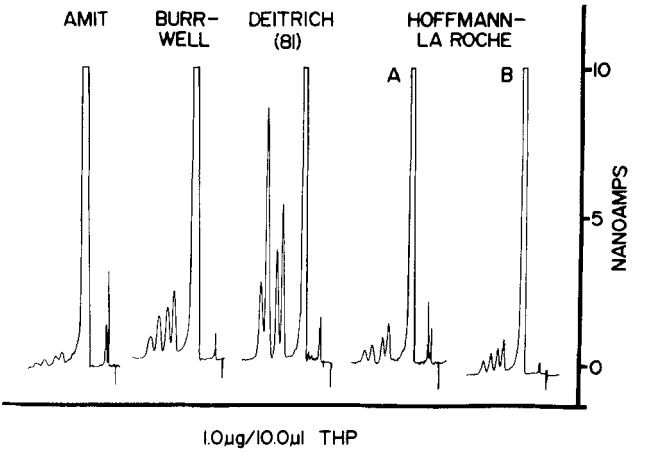

FIGURE 2: Profiles of five freshly prepared THP samples in a 1 µg quantity: two samples from Hoffmann-LaRoche (R06 1673) and one sample each from Deitrich, Amit and Burroughs-Wellcome (Burr-Well) as described in text.

TABLE 1

Percent Degradation Over Time of Tetrahydropapaveroline and Salsolinol at 37°C and 22°C in H_2O and Ascorbate Vehicles

THP			Salsolinol		
37°C			37°C		
Elapsed Time	H_2O	Asc	Elapsed Time	H_2O	Asc
2 hrs	22.5	5.6	1 hr	0.0	0.0
10 hrs	46.8	30.	4 hrs	1.5	0.0
24 hrs	78.3	48.7	24 hrs	3.2	0.0
22°C			22°C		
Elapsed Time	H_2O	Asc	Elapsed Time	H_2O	Asc
4 hrs	4.0	0.8			
12 hrs	16.2	13.1	4 hrs	0.0	0.0
27 hrs	45.0	26.1	24 hrs	2.8	0.0

DISCUSSION

TIQs are potent pharmacological agents which can cause analgesia (4) and a variety of physiological effects on pulse and respiratory rates, blood pressure and smooth muscle tension (1,7). More recently, certain of the TIQs have been implicated in the symptoms of physical dependence on alcohol (8) as well as the development of abnormal preference for alcohol (3). Recently, salsolinol has been found in CSF and brain substance of the human alcoholic (9) as well as rat treated with alcohol (10). Because of the potential clinical importance of these substances, it is necessary that the physico-chemical properties and stability of TIQs under laboratory conditions are understood if they are to be studied experimentally.

In our experiments, we found that temperature is a critical factor in the stability of THP in solution. Noticeable degradation with by-product formation occurs at 37°C in a sample unprotected by an anti-oxidant. Therefore, it is suggested that a mini-pump embedded under the skin of an experimental animal cannot be appropriately used for the chronic administration of this TIQ compound. Solutions that are used for repeated injections could be maintained at room temperature, but they necessarily would have to be changed every 24, if not 12 hrs, to ensure the stability of THP.

Further, our results indicate that ascorbate does help to protect the compounds from degradation at both ambient and body temperatures. The use of ascorbate or a similar agent which prevents decomposition of the alkaloids is essential to maximize stability. Even though ascorbate does not alter the retention time or HPLC profile of THP's activity, certain questions must be raised, however, concerning the pharmacological effects of ascorbate itself. For example, does ascorbic acid influence the pharmacological activity of a TIQ or does it exert its own pharmacological action on addictive behavior?

The differences observed among various samples of THP available to the investigators imply that a "standard" THP does not really exist. The secondary peaks presumably represent degradative by-products which in themselves could possibly alter or augment any of the pharmacological effects of THP. In any case, this difference in sample purity would lead to an explanation of the discrepancies observed in pharmacological studies of these compounds (11,12). Although some of the secondary peaks could be due to O-methylated products of THP, as identified by Meyerson et al. (13), to what extent each of these substances may be active biologically is not presently known.

ACKNOWLEDGEMENTS

This research was supported in part by the North Carolina Alcoholism Research Authority Grant #8102 and National Institute on Alcohol Abuse and Alcoholism Grant #AA 04200-02. E.C.C. was a Visiting Research Fellow from the University of Tennessee. The authors thank Dr. G. Frye for his generous advice and the staff of Bioanalytical Systems Inc. for kind assistance with HPLC technology.

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