Stable Solutions for Marijuana Analysis

Keyphrases □ Marijuana—GLC analysis, stability of cannabinoids in chloroform and ethanol solutions □ Cannabinoids—GLC analysis, stability in chloroform and ethanol solutions □ GLC—analysis, marijuana, stability of cannabinoids in chloroform and ethanol solutions

To the Editor:

Research on the stability of synthetic and naturally occurring cannabinoids in chloroform solution resulted in conflicting reports by Parker *et al.* (1) and Turner and Henry (2). Greater decomposition of synthetic cannabidiol stored in spectrograde chloroform over an 8-day period was reported relative to its stability in ethanol (1). However, in a subsequent 3-month stability study (2), chloroform solutions of synthetic cannabidiol were stable for 21 days, despite daily exposure to both ambient temperature and a combination of natural and artificial light under normal laboratory conditions.

This discrepancy was of interest because of our current involvement with marijuana analyses; our past experience with such analyses seemed to be midway between these two conflicting reports. Observations made during the development of our methods led us to the conclusion that chloroform solutions of cannabinoids should be refrigerated at all times (3).

Since the authors of the cited articles had used differing commercial brands of chloroform, we repeated the Parker *et al.* (1) study, replacing their brand of spectrograde chloroform[1] with the one[2] used by Turner and Henry (2). The GLC analysis was performed using a gas chromatograph[3] equipped with a hydrogen flame-ionization detector and a glass column packed with 3% OV-17 on 100–120-mesh Supelcoport[4]. The experimental conditions were: column temperature, 210°; injector temperature, 250°; detector temperature, 250°; and internal standard, *dl*-methadone hydrochloride.

Our results were nearly identical to those reported by Parker *et al.* (1); the ratio of the areas of the cannabidiol[5] *versus* internal standard peaks decreased an average of 16%/day for the chloroform solutions as compared to an average decrease of only 0.6%/day for the ethanol solutions. Solutions were stored in darkness at room temperature in well-sealed vials. We also tried 4-androsten-3,17-dione[6] as an internal standard since it was used by the previous researchers. No quantitative differences were found between 4-androsten-3,17-dione and *dl*-methadone hydrochloride. Methadone hydrochloride was preferred for our studies since it gave a sharper, more easily quantified GLC peak than did 4-androsten-3,17-dione.

Dr. Turner informed us that the cannabidiol used for analysis in his laboratories is first purified using column chromatography. In addition, the solute concentration used was 10 times greater than that used by Parker. Since either of these factors might affect solution stability, we tried to attain a stable solution of the column-purified material at both concentrations. Again, the solutions were unstable overnight and strongly discolored by the 3rd day. Thus, although the chloroform solutions of synthetic cannabidiol stored in darkness showed less than a 1% decrease on the day they were prepared, significant decomposition occurred by the 2nd day and continued steadily thereafter (Table I).

Table I—Decomposition of Synthetic Cannabidiol in Chloroform Solution

Conditions	Ratio of Cannabidiol to Methadone Peak Areas		
	Initially	After 3 hr	After 24 hr
Constant exposure to ambient temperature and fluorescent light	1.2	1.1	0.62
Constant exposure to ambient temperature and darkness	1.4	1.4	1.3

The stability of cannabinoid constituents in various solvents during analysis of actual plant material is of forensic importance. For this reason, we studied extracts from plant material, continuing to investigate cannabidiol stability but giving primary consideration to the stability of the active constituent, tetrahydrocannabinol. Turner and Henry (2) also included plant material in their study.

Our procedure was as follows. For the chloroform solution, 1 g of plant material was placed in a stoppered flask containing 20 ml of chloroform and 17.4 mg of methadone hydrochloride. The flask was placed in the refrigerator and shaken at 10-min intervals for 1 hr; then the plant material was filtered off and discarded. This chloroform solution was used directly for injection. To prepare the ethanol solutions, 1 g of plant material was extracted with pure chloroform as already described. After filtration, the chloroform was evaporated and the residue was redissolved in 20 ml of ethanol containing 17.4 mg of methadone hydrochloride.

Standard chloroform and ethanol extraction solutions were refrigerated at all times, and peak area ratios for cannabidiol to methadone and tetrahydrocannabinol to methadone were obtained daily. Both solutions were stable for several weeks under these conditions.

Preliminary degradation studies were done to determine the effect of sunlight, fluorescent light, and temperature on cannabinoid stability. The standard ethanol and chlo-

Table II—Effect of Sunlight and Laboratory Conditions on the Stability of Chloroform and Ethanol Solutions of Plant Material

Conditions	Total Exposure Time	Ratio of Δ^9-Tetrahydrocannabinol[a] to Methadone Peak Areas	Ratio of Cannabidiol[a] to Methadone Peak Areas
	Chloroform Solution		
Sunlight	30 min	0.63	0.20
Ambient laboratory	Overnight	0.68	0.12
Control	—	1.00	0.27
	Ethanol Solution		
Sunlight	30 min	0.63	0.26
Ambient laboratory	Overnight	0.81	0.26
Control	—	0.81	0.28

[a] The identities of the Δ^9-tetrahydrocannabinol and cannabidiol peaks were confirmed by mass spectrometry.

[1] Fisher Scientific.
[2] Mallinckrodt nanograde.
[3] Perkin-Elmer model 900.
[4] Supelco.
[5] Applied Science Laboratories (purity of 93%).
[6] Applied Science Laboratories.

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Table III—Effect of Sunlight on Chloroform Solutions with and without Added Stabilizers

Parameter	Total Exposure Time, min	Ratio of Δ^9-Tetrahydrocannabinol to Methadone Peak Areas	Ratio of Cannabidiol to Methadone Peak Areas
With stabilizers	30	1.1	0.32
Control	—	1.1	0.31
Without stabilizers	30	0.63	0.20
Control	—	1.0	0.27

roform extraction solutions (100-μl portions) were exposed to these conditions for controlled periods. Stability was tested by comparison of ratios from the exposed solutions to daily ratios from the standard solutions (Table II).

Thirty minutes of exposure to sunlight caused rapid decomposition and decolorization in the chloroform solution and a significant, although lesser, amount of decomposition in the ethanol solution. Overnight exposure to a combination of fluorescent light and room temperature, an approximation of normal ambient laboratory conditions, caused a marked decomposition in the chloroform solution; the ethanol solution remained stable within experimental error after more than 18 hr of exposure to these conditions.

Decomposition in chloroform solution is not significant for our current method, which limits solution exposure to ambient laboratory conditions to 30–45 min. For other laboratories, handling and sampling procedures may make lengthy exposure to normal ambient laboratory conditions unavoidable. In such cases, the demonstrated cannabinoid stability in ethanol suggests that the most reliable procedure for analysis would be extraction with chloroform, flash evaporation of the solvent, and redissolution in ethanol. This procedure is still inconvenient, however, for any analyst who does not do such work on a routine basis or who has a large number of samples to analyze. The convenience of maintaining the cannabinoids in the extracting solvent is often desirable, so a means of stabilizing the chloroform solution would be useful.

Since cannabinoid degradation by chloroform is thought to be a free radical reaction (4), a combination of free radical inhibitors was added to portions of the chloroform solutions immediately subsequent to the extraction procedure. To each 5-ml aliquot, 2.6 μl of mercaptoethanol and enough sodium diethyldithiocarbamate to saturate the solution were added. Portions of this solution (100 μl) were removed for timed exposures to sunlight or normal laboratory conditions. Control solutions were refrigerated routinely, and stability was measured by comparison of peak area ratios from these 100-μl portions with those from the control solutions.

After 30 min of exposure to sunlight, there was no evidence of decomposition in the stabilized solution, as compared to 25–35% decreases in the cannabinoid–methadone ratios for the solution without added stabilizers (Table III). In addition, the solution containing the stabilizers remained stable for up to 10 hr of exposure to normal ambient laboratory conditions. Therefore, we suggest that chloroform solutions of naturally occurring cannabinoids either be refrigerated at all times or, if substantial exposure to fluorescent or natural light cannot be avoided, that the combination of stabilizers, sodium diethyldithiocarbamate and mercaptoethanol, be added as a protective measure.

(1) M. J. Parker, M. L. Borke, L. M. Block, and T. G. Cochran, *J. Pharm. Sci.*, **63**, 970 (1974).
(2) C. E. Turner and J. T. Henry, *ibid.*, **64**, 357 (1975).
(3) P. Lerner, *Bull. Narcotics*, **21**, 39 (1969).
(4) E. R. Garrett, A. J. Gouyette, and H. Roseboom, *J. Pharm. Sci.*, **67**, 27 (1978).

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Selectivity of Silicone Rubber toward Prostaglandin Permeability

Keyphrases □ Silicone rubber membranes—selectivity toward transport of E and F prostaglandins □ Prostaglandins, E and F—transport selectivity of silicone rubber membranes □ Delivery systems, potential—silicone rubber membranes, selectivity toward transport of E and F prostaglandins

To the Editor:

Considerable interest is being focused on the design of controlled-release drug delivery systems (1, 2). Their rational development requires a basic understanding of the physical–chemical parameters that control drug transport through the delivery module. The type of polymer matrix and the chemical form of the drug must provide a delivery rate in phase with the pharmacological potency of the therapeutic agent.

In the field of fertility regulation, several prostaglandins in various vaginal dosage forms have been studied to optimize bioavailability, reduce side effects, eliminate mul-

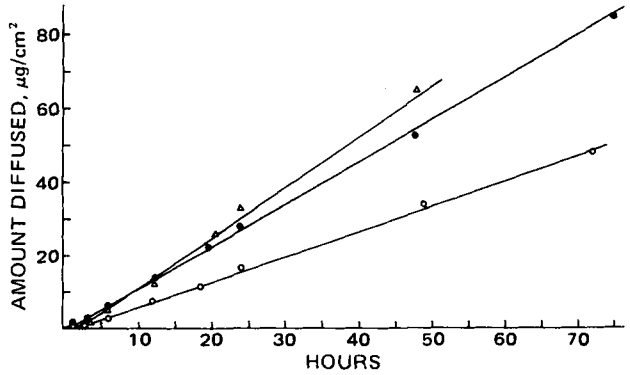

Figure 1—*Amount of prostaglandin transported across a silicone rubber membrane as a function of time. The donor compartment was maintained saturated with drug, and the membrane thickness was 0.05 cm. Key:* △, *dinoprost isopropyl ester;* ●, *dinoprost ethyl ester; and* ○, *dinoprost methyl ester.*

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