Stability of Cannabinoids in Urine in Three Storage Temperatures*

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ABSTRACT

Stability of cannabinoid compounds in urine samples were evaluated using several storage temperatures. Appreciable losses (>22.4 percent) were observed in some urine samples, after being stored at room temperature for 10 days. Lower losses (8.1 percent) were observed when the urine samples were refrigerated for 4 weeks. The behavior of urine samples depended on the analyzed urine. This could be due to the different stability of the cannabinoids present in each urine sample. Important losses of 8.0 ± 10.6, 15.8 ± 4.2, and 19.6 ± 6.7 percent were found when the urine samples were frozen during 40 days, 1 year, and 3 years, respectively. Average losses (>>5 percent) can be observed after one day which could mainly be due to the decrease of the solubility of 11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) or adsorption process of cannabinoid molecules to the plastic storage containers.

Introduction

Few papers have dedicated their attention to finding out the maximum storage time of illicit drugs and their metabolites in urine.¹ ² ³ ⁴ Cannabinoids in urine are relatively unstable among the illicit drugs. Decrease in the urine concentrations of cannabinoids is generally produced as a consequence of degradation processes of oxidation.² Some authors⁵ have indicated that the storage temperature is the main factor related with the degradation processes of cannabinoids. Also, problems associated with the surface adsorption on borosilicate⁶ or losses in concentrations owing to insolubility at the freezing temperatures³ of the 11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) have been described.

In this paper, the stability of cannabinoids present in urine, with respect to several storage temperatures, has been evaluated in order to find out the maximum times of storage to different temperatures without significant losses of concentration.

160
Experimental

Urine Samples

The 40 samples analyzed were obtained from urine specimens of patient consumers of Cannabis. A first analysis was carried out between 5 and 10 minutes after specimen collection. After initial analysis, the samples were stored in polypropylene containers at three temperatures: room temperature (20-25°C), refrigeration (4°C), and freezing temperature (-18°C). The cannabinoid concentrations were determined again after the time of storage considered.

Analytical Procedure

Analysis of all the cannabinoid compounds (expressed as THC-COOH) in urine was carried out using the technique of fluorescence polarization immuno assay (FPIA) on an Abbott ADx analyzer. Urine samples with high concentrations of THC-COOH were previously diluted with milli-Q water in order to decrease its concentration to the range of the calibration curve. The detection limit (calculated as the mean of the blank plus 3 standard deviations) was 7.5 ng/mL. The assay had a coefficient of variation of 4.2 percent when a urine sample of 93.5 ng/mL of concentration was repeated 10 fold.

Results and Discussion

Large average losses of cannabinoids (THC-COOH) (22.4 percent) were observed after 10 days at room temperature (20-25°C). However, these losses after four weeks at 0°C (8.1 percent) were lower than in the latter case. Stability of cannabinoids depends on implicit factors of the analyzed urine such as composition of urine or pH. So, storage of urine samples under basic conditions could cause oxidation of phenol to quinone. Also, different proportions and stability of the several cannabinoids present in urine could influence the Tdx readings.

Important losses of cannabinoids (THC-COOH) were observed in urine samples after 1 and 3 years of freezing, respectively (table I). Average concentrations of cannabinoids after 1 and 3 years were significantly (p < 0.05) lower than the average concentration initially found. Increase in losses of cannabinoids over time decreased when the storage time was higher. Thus, losses of cannabinoids changed from 5.1 to 15.8 percent during the first year of storage; 2 years later these losses increased only to 19.6 percent. A mathematic equation (r = 0.9997) that relates the average losses of cannabinoids (percent) and the time (months) could be established as:

$$\text{Average losses (percent)} = \frac{5.27 + 2.91 \cdot t(\text{months})}{1 + 0.13 \cdot t(\text{months})}$$

If the time of freezing is known, the approximate losses of cannabinoid compounds could be calculated.

If the urine samples with initial concentrations of cannabinoids lower than 100 ng of THC-COOH/mL were considered, the average decrease was of 23.7 ± 9.4 percent after 3 years of storage at freezing temperature. However, an average of 18.2 ± 5.2 percent was found in the urine samples with concentrations

<table>
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<th>TABLE I</th>
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<td><strong>Losses (%) of Cannabinoids (THC-COOH) of Urine Samples with the Time of Storage at Freezing Temperature</strong></td>
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<td><strong>Time of Storage</strong></td>
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<tr>
<td>n</td>
</tr>
<tr>
<td>X ± SD</td>
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<td>M - m</td>
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<td>C.V.</td>
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</table>

n = Number of data.

X ± SD = Standard deviation.

M - m = Maximum - minimum.

C.V. = Coefficient of variation.

The statistical data relative to the losses (%) of cannabinoids represent the specimen-to-specimen variation.
of these cannabinoids higher than 100 ng/mL. Thus, one can deduce that the losses of the cannabinoids were higher when the concentrations of the same were lower. Also, an average loss of 8.0 ± 10.6 (n = 7) was observed when the urine samples were maintained frozen for 40 days. These results agree with those found by Paul et al\(^3\) which present an average loss of 11 percent (0 to 34 percent) after 45 days. However, there are also contradictory results. Romberg and Post\(^8\) have shown losses of 19 percent in urine samples frozen after only 2 months. In contrast, other authors\(^2\) have observed an average decrease of the concentration of cannabinoids of 1 percent after 1 year of freezing.

Seven urine samples of positive cannabinoids were analyzed and frozen. These samples were defrosted and analyzed again the following day. Losses of cannabinoids ranged between +0.7 and 11.8 percent, with an average loss of 5.1 ± 3.9 percent observed. This suggests that other factors such as degradation processes influence the urinary concentrations of cannabinoids. In this sense, some authors\(^3\) indicate that the decrease in concentration of cannabinoids (THC–COOH) in urine is due to the decrease of the solubility of the main metabolite (THC–COOH) at freezing temperature. Also, these authors\(^3\) point out that the length of time the solutions are left at room temperature after defrosting could effectively change the concentrations in the solutions.

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References