Long - term Storage and Cannabis Oil Stability

IRENNE GABRIELA TROFIN[1]*, GABRIEL DABIJA[1], DANUT–IONEL VAIREANU[2], LAURENTIU FILIPESCU[3]

[1]General Inspectorate of Romanian Police, Central Laboratory for Drug Analysis and Profiling, Bucharest, Romania.

[2]University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, Department of Applied Physical Chemistry and Electrochemistry, 313 Splaiul Independentei, 060042, Bucharest, Romania

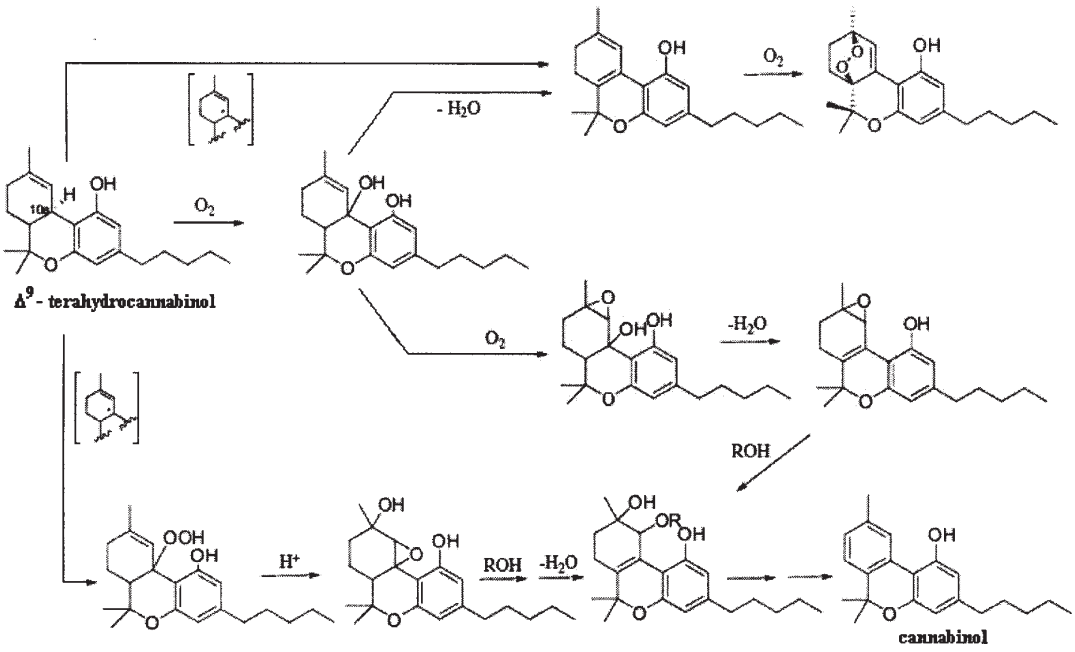
[3]University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, Department Technology of Inorganic Substances and Environmental Protection, 313 Splaiul Independentei, 060042, Bucharest, Romania

The paper presents the results of an experimental study regarding the stability of cannabis oil during its long-term storage in different conditions. The content of major cannabinoids, namely tetrahydrocannabinol (Δ^9–THC), cannabinol (CBN), and cannabidiol (CBD) contained in two batch samples of cannabis oil seizures made by criminal prosecution authorities from Romania was measured during their storage over a period of four years in darkness at 4°C and in laboratory light at 22°C. The results revealed a steadily decay of Δ^9–THC over the entire storage period from a very high initial content up to a relatively low final content. A slight difference regarding the degree of decay of Δ^9–THC between the two storage conditions was recorded, meaning that this is more pronounced when the samples were exposed to light at 22°C. The same trend was recorded for CBD. As expected, the content of CBN increases during storage and the increase is higher when the samples were exposed to light at 22°C.

Keywords: cannabis, oil, decay, cannabinoid

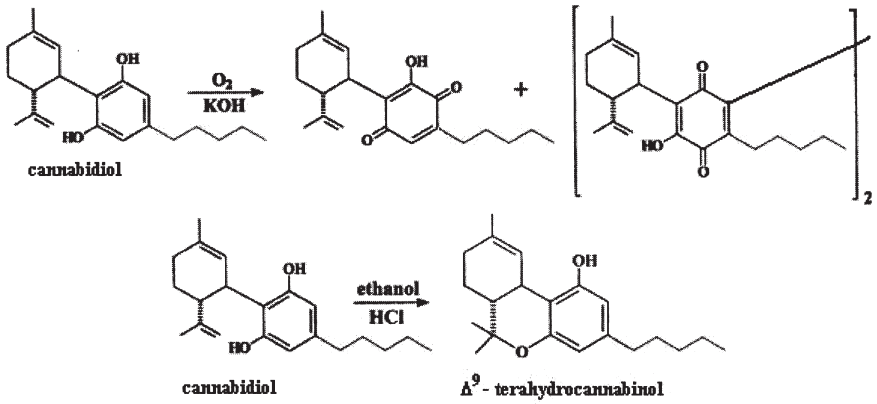
Cannabis oil, often called hashish oil, is a liquid cannabis product with a high content of tetrahydrocannabinol (Δ^9–THC) obtained by extraction from either herbal cannabis or from cannabis resin. Although such a product requires more complex preparation methods than other cannabis products, it is often preferred by drug dealers because they may traffic more psychoactive material in a smaller quantity of cannabis product [1].

As with other cannabis products, cannabis oil has different stability in various environmental conditions [2-4]. The psychoactive component, namely Δ^9–THC, has a relatively high instability when the cannabis products are exposed to air, light or acidic environments [5-7]. Its instability to heat has been also demonstrated [8]. It is widely accepted that the main pathway of cannabis products deactivation is the conversion of Δ^9–THC to cannabinol (CBN). After five years of storage in an ethanol/propylene glycol solution of a Δ^9–THC sample, it was proposed a correlation between the oxidative derivatives of this cannabinoid formed during its degradation to the less psychoactive component CBN [9].



* email: irennetrofin@yahoo.com

Other major cannabinoid present in cannabis products, namely cannabidiol may also suffer changes depending on storage conditions. In this respect, under acidic conditions it may transform into Δ^9–THC by acid-catalyzed cyclization and, in the presence of oxygen, is oxidized to monomeric and dimeric hydroxyquinones [10].



Although the cannabinoids stability was intensively studied previously, most of the researches were done on pure solutions, which can have a different behaviour towards actual cannabis products. Therefore, the objective of this paper is to explore experimentally the influence of storage conditions such as temperature and light on the stability of the major cannabinoids in the cannabis oil.

Experimental part

Chemicals and reagents

All chemicals and reagents used for samples preparation and analysis were of analytical grade from Merck (Darmstadt, Germany). The etalons of Δ^9–tetrahydrocannabinol (Δ^9–THC), cannabidiol (CBD), and cannabinol (CBN) were purchased from Switzerland. The ultrapure water used in HPLC analyses was prepared in-house using a Millipore system, model Milli-Q Integra 3.

Cannabis oil samples

Cannabis oil from two different seizures (marked with symbols U1 and U2) made by criminal prosecution authorities from Romania and provided by Central Laboratory for Drug Analysis and Profiling were subject to experimental investigation. The samples of cannabis oil were supplied in small bottles and have a black-brown colour and a high consistency comparable to that of a paste. The samples were stored in the darkness at 4°C and in the laboratory light at 22°C for four years. At regular intervals, namely at every three months, samples were taken for analysis in order to determine the content of their major cannabinoids (Δ^9–THC, CBD, and CBN).

Methods

The procedure that led to the sample preparation for analysis consisted of extracting 0.05 g of cannabis oil in 20 mL of a methanol-chloroform (9:1, v/v) mixture. Thus, the samples were shaken for 30 min and then placed in an ultrasonic bath at ambient temperature for 15 min in order to increase the cannabinoids extraction rates. The extracts were filtered and some aliquots (0.6 mL) of the filtrates were transferred to a 4 mL vials and then evaporated to dryness by oven evaporation, only up to 80°C for prevention of any decomposition reactions. Then, the vials were put into a heating unit at 220°C for 12 min when the traces of tetrahydrocannabinolic acid (THCA) are decarboxylated. Decarboxylation is particularly required for the determination of the entire content of Δ^9–THC of the sample. Before analyses, the residues were extracted in 1.5 mL extraction solvent (methanol-chloroform 9:1, v/v). After this, the samples were subject to analyses of the major cannabinoids content (Δ^9–THC, CBD, and CBN) [1].

Analytical protocol

Extracts obtained by procedure described above have been subject to analytical investigations through instrumental methods (GC–FID – Gas Chromatography–Flame Ionization Detector and HPLC – High Performance Liquid Chromatography) in order to find out the content in major cannabinoids (Δ^9–THC, CBD, and CBN).

GC–FID analyses were carried out on a 7890A gas chromatograph with a flame ionization detector. Separation was achieved on a fused silica capillary column (HP-5MS, 30 m×0.32 mm i.d., 0.25μm film thickness, J&W Scientific, Folsom, CA, USA). Temperature program: 150°C hold for 1 min, 10°C/min to 280°C, hold for 5 min. The injection port and interface temperature were 250°C and 300°C, respectively. Split injection mode was used (20:1) and hydrogen, with a flow rate of 30 mL per min, was used as carrier gas [11].

HPLC analyses were carried out on an Agilent 1100 Series HPLC chromatograph equipped with a quaternary pump, autosampler, column oven and diode-array detector (DAD) UV Lamp ON (223 nm). Chromatography was achieved on a 250 mm × 4.6 mm i.d., 5 μm Hypersil ODS column. The HPLC operates with constant flow at 1 mL mobile phase (acetonitrile 37.5% and ultrapure water) per minute.

Results and discussions

GC–FID and HPLC chromatograms revealed a very high content of Δ^9–THC in the cannabis oil samples and subsequently, a very high potency of these type of cannabis product compared to other cannabis products such as herbal cannabis and cannabis resin. The difference between the two samples regarding their potency is significant and comes probably from the use of different procedures for their obtaining. The difference could be due to the type of solvent used for extraction, number of extractions, conditions of extractions etc. These results indicate, on the one hand, that the samples were prepared in different clandestine laboratories, improvised by different drug traffickers and, on the other hand, that the samples went through different routes of trafficking originating from different geographical areas. Following a detailed analysis of such data, the prosecution authorities could identify trafficking routes, and finally the places where this type of drugs were made.

Table 1
THE INITIAL CONTENT OF MAJOR CANNABINOIDS IN CANNABIS OIL

Cannabinoid, %	Seizure	
	U1	*U2*
Δ^9–THC	37.85	45.82
CBN	1.83	2.23
CBD	16.15	14.82

The experimental results concerning the stability of the major cannabinoids indicate a small but constant difference between cannabinoids content of the cannabis oil depending on storage conditions. Figures 1 and 2 show the variation of the major cannabinoids content in the two samples of cannabis oil as a function of time and storage conditions. As one can see, in both samples the Δ^9–THC content decreases during storage and is always higher in the samples stored in the darkness at 4°C than in the samples stored in the laboratory light at 22°C. The same trend can be also observed for CBD content. As it was expected, the CBN content increases during storage period and is always higher in the samples stored in laboratory light at 22°C than in the samples stored in darkness at 4°C. The results obtained for the two samples derived from the two different seizures regarding the evolution of the major cannabinoids content during storage in different conditions are presented in table 2. The results revealed a steadily decay of Δ^9–THC over the entire storage period. Moreover, the decay of Δ^9–THC in the samples exposed to light at 22°C is higher than in the samples stored in the darkness at 4°C. In this respect, when the samples from seizure U1 were stored in the darkness at 4°C, 21.6% of Δ^9–THC (fig. 1 a) was lost in the first year with an average loss of 4.4% every tree months, 21.83% in the second year with an average loss of 5.46%, 21.64% in the third year with an average loss of 5.41%, and 18.69% in the fourth year with an average loss of 4.67%. When the samples from the same seizure were stored in the laboratory light at 22°C, 23.16% of Δ^9–THC was lost in the first year with an average loss of 5.79% every tree months, 23.25% in the second year with an average loss of 5.81%, 22.99% in the third year with an average loss of 5.75%, and 20.45% in the fourth year with an average loss of 5.11%. Finally, after four years of storage, the samples stored in the darkness at 4°C lost 83.75% of Δ^9–THC and the samples stored in the laboratory light at 22°C lost 89.85% of Δ^9–THC (6.1% higher).

Table 2
EVOLUTION OF THE MAJOR CANNABINOIDS CONTENT IN CANNABIS OIL DURING STORAGE IN DIFFERENT CONDITIONS

Cannabinoid %	Time years	Seizure			
		U1		U2	
		darkness, 4°C	*light, 22°C*	*darkness, 4°C*	*light, 22°C*
Δ^9–THC	1	29.69	29.10	35.76	35.20
	2	21.42	20.30	25.63	24.57
	3	13.23	11.59	15.57	14.01
	4	6.15	3.84	6.46	4.21
CBN	1	4.47	5.27	4.97	5.69
	2	7.47	8.91	8.09	9.18
	3	9.28	10.89	10.22	11.59
	4	10.95	14.24	11.55	14.56
CBD	1	14.37	13.98	13.39	12.91
	2	12.41	12.04	11.68	11.12
	3	11.20	10.94	10.69	9.86
	4	9.66	9.56	9.20	8.15

The variation of CBN (fig. 1 b) of the same samples over the storage period indicates that when these were stored in the darkness at 4°C, 59.03% of CBN was formed in the first year with an average gain of 14.76% every three months, 16.49% in the second year with an average gain of 4.12%, 4.76% in the third year with an average gain of 1.19%, and 3.01% in the fourth year with an average gain of 0.75%. When the samples from the same seizure were stored in the laboratory light at 22°C, 65.29% of CBN was formed in the first year with an average gain of 16.32% every three months, 14.18% in the second year with an average gain of 3.54%, 3.72% in the third year with an

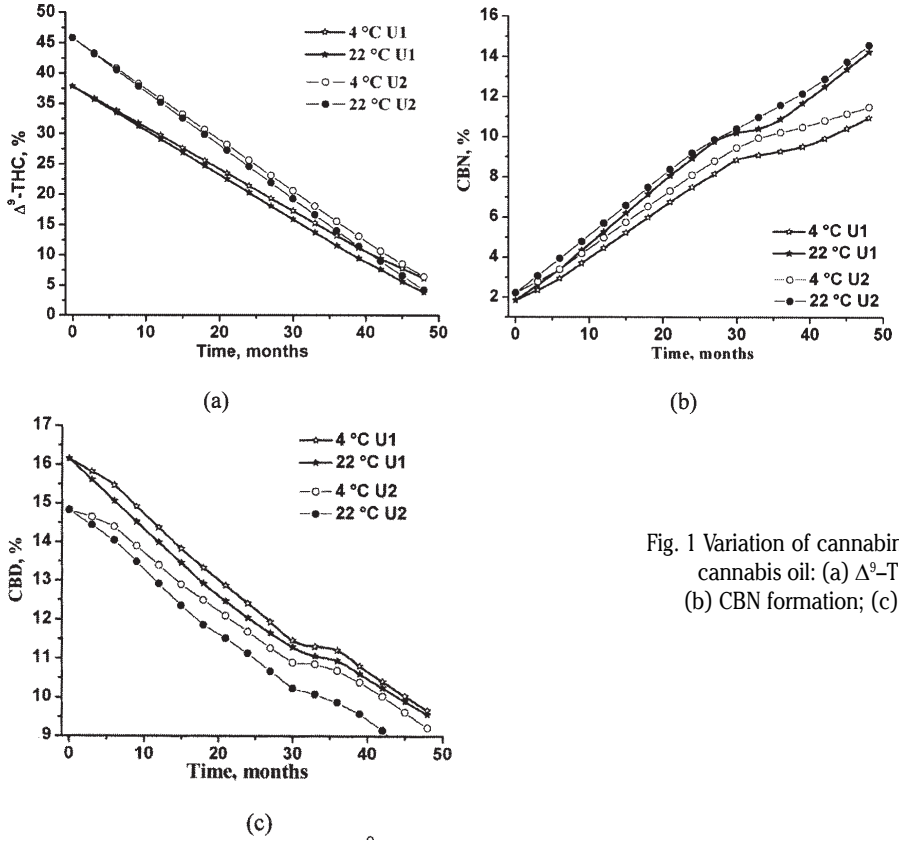


Fig. 1 Variation of cannabinoids content in cannabis oil: (a) Δ^9–THC decay; (b) CBN formation; (c) CBD decay

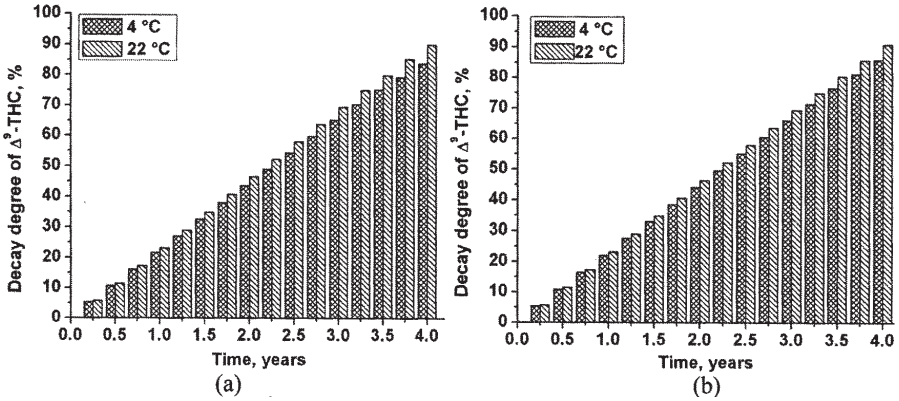


Fig. 2 Decay degree of Δ^9–THC in cannabis oil derived from: (a) seizure U1; (b) seizure U2

average gain of 0.93%, and 3.95% in the fourth year with an average gain of 0.99%. Finally, after four years, the samples stored in the darkness at 4°C gained 83.29% of CBN and the samples stored in the laboratory light at 22°C gained 87.15% of CBN (3.86% higher). The same trend was also recorded for the second cannabis oil sample.

Comparing the decay degree of Δ^9–THC in the first year of storage period of cannabis oil with the formation degree of CBN in the same year, it can be seen that the latter yield is with about 35% higher than the first yield. In this respect, the changes regarding the content of CBN during the storage period can not be entirely correlated with the chemical and/or biochemical decay processes of Δ^9–THC to CBN. Some other unknown variables beyond the control, such as the origin place, degradation already started during the trafficking transports play also an important role. Also, other degrading routes of other cannabinolic compounds must be considered as contributors to the overall increase of CBN content upon long-term storage.

The variation of CBD (fig. 1 c) in the same samples over the entire storage period indicates that in the case of samples from seizure U1 stored in the darkness at 4°C, 11.03% of CBD was lost in the first year with an average loss of 2.76% every tree months, 12.15% in the second year with an average loss of 3.04%, 7.47% in the third year with an average loss of 1.87%, and 9.54% in the fourth year with an average loss of 2.39%. When the samples from the same seizure were stored in the laboratory light at 22°C, 13.45% of CBD was lost in the first year with an average loss of 3.35% every three months, 12.05% in the second year with an average loss of 3.01%, 6.81% in the third year with an average loss of 1.7%, and 8.5% in the fourth year with an average loss of 2.12%. Finally, after four years of storage, the samples stored in the darkness at 4°C lost 40.18% of CBD and the samples stored in the laboratory light at 22°C lost 44.8% of CBD (with less 0.62%). The same trend was recorded for all cannabis oil samples.

Analyzing these results it can be seen that the decay degree of CBD in the first year of storage period of cannabis oil is about 10% (approximately quarter the difference of 35% between the decay degree of Δ^9–THC and respectively, the formation degree of CBN) in the case of the samples stored in the darkness at 4°C and about 19% (approximately half the difference of 40% between the decay degree of Δ^9–THC and respectively the formation degree of CBN) was observed in the case of the samples stored in the laboratory light at 22°C. These results suggest a different degrading rout of CBD, probably dependent on the storage conditions. When the samples were stored in the darkness one of the degradative route could be the biochemical cyclization of CBD to Δ^9–THC, followed by the oxidative decay of Δ^9–THC to CBN. In addition, when the samples were exposed to light, CBD might achieve photo-reactive properties and transforms into Δ^9–THC [10].

A pseudo zero-order kinetic was used (fig. 3) in order to calculate the kinetic parameters of the Δ^9–THC decay such as the rate constant (k), the half-time ($t_{1/2}$), and the decay rate (v). The linear regression parameters and the correlation coefficients are presented in the table 3. As can be seen from the table 4, both rate constant and decay rate are higher in the samples stored in the laboratory light at 22°C than those stored in the darkness at 4°C. The values of the half-time corresponding to the samples stored in the laboratory light at 22°C are smaller than those stored in the darkness at 4°C. These results suggest a higher rate of Δ^9–THC decay in the cannabis oil stored in normal conditions (natural light and ambiental temperature) than in the case of special storage conditions (darkness and low temperature).

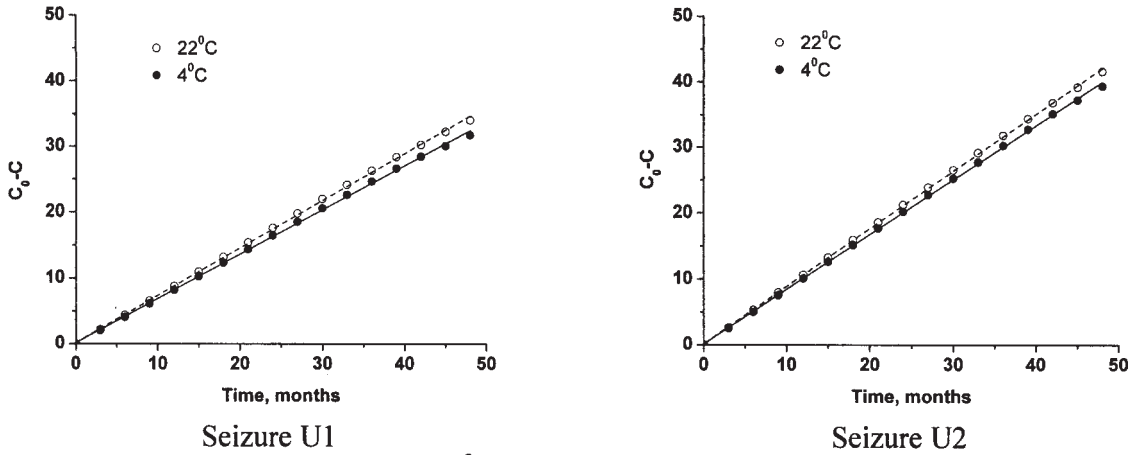


Fig. 3 Pseudo zero-order kinetic of Δ^9–THC decay in the cannabis oil; the solid line represents the linear regression of data corresponding to 4°C and darkness storage conditions and, the dashed line represents the linear regression of data corresponding to 22°C and laboratory light storage conditions

Seizure	Storage conditions					
	4°C, darkness			*22°C, laboratory light*		
	y=a + bx					
	a	b	R^2	a	b	R^2
U1	0.1679	0.6717	0.9993	0.1957	0.7174	0.9995
U2	0.1388	0.8302	0.9997	0.1598	0.8734	0.9998

Table 3
LINEAR REGRESSION PARAMETERS AND CORRELATION COEFFICIENTS

Seizure	Storage conditions					
	4°C, darkness			*22°C, laboratory light*		
	k', $months^{-1}$	$t_{1/2}$, months	v, %/month	k', $months^{-1}$	$t_{1/2}$, months	v, %/month
U1	0.67	28.26	25.37	0.72	26.03	27.27
U2	0.83	27.60	38.03	0.87	26.33	39.86

Table 4
KINETIC PARAMETERS OF Δ^9-THC DECAY CALCULATED FROM A PSEUDO-ZERO ORDER KINETIC

Conclusions

Chemical characterization of the cannabis oil samples derived from two different seizures revealed a very high content of Δ^9–THC compared with other cannabis products such as the herbal cannabis and cannabis resin. The difference between the potency of the two samples suggests a different preparation method or a different route of trafficking, which could finally indicate the places where the samples were prepared.

The experimental results regarding the stability of the major cannabinoids species revealed differences that should be taken into consideration between cannabinoids content of the cannabis oil are related to the storage conditions. Thus, the results revealed a steady decay of Δ^9–THC over the entire storage period. Moreover, the decay of Δ^9–THC in the samples exposed to light at 22°C is more pronounced than in the samples stored in the darkness at 4°C. The content of CBN increases during storage, and increase is more pronounced for the samples exposed to light at 22°C than those stored in the darkness at 4°C. These results are in part consistent with those obtained for Δ^9–THC. The CBD content decreases during storage especially for samples exposed to light at 22°C. This evolution could be explained by considering the biochemical cyclization of CBD to Δ^9–THC, followed by the decay of Δ^9–THC to CBN as a degrading route for the samples stored in the darkness at 4°C and, both biochemical and photochemical cyclization of CBD to Δ^9–THC followed by decay of Δ^9–THC to CBN as a degrading routes for the samples exposed to light at 22°C.

The decay of Δ^9-THC takes place up on a pseudo zero-order kinetic and the calculated values of the kinetic parameters suggest a higher rate of Δ^9–THC decay in normal storage conditions such as light and ambiental temperature than in special ones such as darkness and low temperature.

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