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LC–MS method for the estimation of Δ^8-THC and 11-nor-Δ^8-THC-9-COOH in plasma

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Abstract

The aim of the present study was to develop a simple and sensitive LC–MS method for the estimation of Δ^8-tetrahydrocannabinol (Δ^8-THC) and its metabolite, 11-nor-Δ^8-tetrahydrocannabinol-9-carboxylic acid (11-nor-Δ^8-THC-9-COOH), in guinea pig plasma after topical drug application. The plasma samples were analyzed by LC–MS using negative-mode electrospray ionization detection and a simple liquid–liquid extraction technique. The mean recoveries for Δ^8-THC and its metabolite, 11-nor-Δ^8-THC-9-COOH, were 96.6 and 88.2%, respectively. The lower limits of quantification (LLOQ) for Δ^8-THC and 11-nor-Δ^8-THC-9-COOH were 3.97 and 7.26 nM, respectively. The topical treatment steady-state plasma concentrations of Δ^8-THC and 11-nor-Δ^8-THC-9-COOH were 8.24–27.63 and 19.66–23.17 nM, respectively, with a lag period of 0.3–2.2 h. This assay method is selective, sensitive, and reproducible for the determination of Δ^8-THC and 11-nor-Δ^8-THC-9-COOH at low concentrations in small volumes of plasma.

Keywords: Δ^8-THC; Δ^8-THC-9-COOH; Guinea pig plasma; Topical application; LC–MS

1. Introduction

Cannabinoids are useful in the treatment of nausea and vomiting associated with chemotherapy in cancer patients [1]. Additionally, cannabinoids are the most frequently used illicit drugs, and are therefore often encountered by the clinical laboratories involved in routine analysis of drugs of abuse. Δ^8-Tetrahydrocannabinol (Δ^8-THC) is an isomer of Δ^9-THC and is less psychotropic than Δ^9-THC [2]. Abrahamov et al. [3] reported that Δ^8-THC prevented vomiting completely when administered before anti-neoplastic therapy in cancer patients. Zero-order drug delivery of cannabinoids, like transdermal delivery, may help to reduce side effects associated with peak drug levels [4]. A sensitive and simple analytical method is necessary for the pharmacokinetic analysis of Δ^8-THC and its metabolite, 11-nor-Δ^8-tetrahydrocannabinol-9-carboxylic acid (Δ^8-THC-9-COOH), in plasma samples (Fig. 1). Several methods have been reported on the estimation of Δ^9-THC and its metabolites in plasma by gas chromatography–mass spectrometry (GC–MS) after liquid–liquid or solid-phase extraction and derivatization [5–8], by thin-layer chromatography (TLC), by high-performance liquid chromatography (HPLC) with UV or electrochemical detection, and by gas chromatography (GC) with electron capture, flame ionization, or nitrogen–phosphorus detection (ECD, FID, NPD). However, GC requires time-consuming sample preparation and the need to use various derivatization techniques for non-volatile and thermolabile compounds. The other previously published methods of Δ^9-THC quantitation lack either specificity or sensitivity. Recently, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) with LC–MS or LC–MS/MS were found to be suitable for the detection of THC-9-COOH and THC-9-COOH-β-glucuronide [9–16]. Two methods have been reported on the simultaneous determination of Δ^9-THC and its metabolites, one method used LC–MS/MS with 1 mL human plasma samples [15], and

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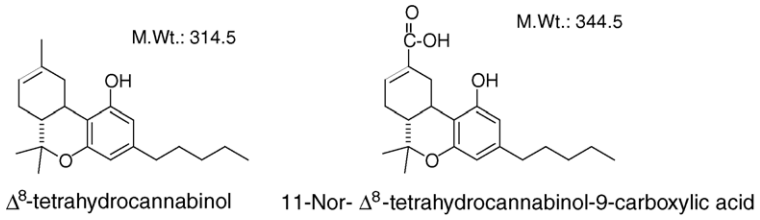

Fig. 1. Chemical structures of Δ^8-THC and Δ^8-THC-9-COOH.

the other method used LC–MS with 50 μL guinea pig plasma samples [16]. However, no reports have been published on the estimation of Δ^8-THC and its metabolite in plasma samples, except for one report [17] that utilized radioimmunoassay (RIA) for the quantification of Δ^8-THC and its metabolite Δ^8-THC-9-COOH after topical treatment in rats. Immunoassays may sometimes give overestimations of drug concentration because of antibody cross-reactivity with molecules of similar structure in the plasma or serum. No reports have been published on the LC–MS analysis of Δ^8-THC and its metabolite in small volumes of plasma containing low levels of drug, as observed in topical drug treatment pharmacokinetic studies. This manuscript describes a sensitive LC–MS method for estimation of Δ^8-THC and Δ^8-THC-9-COOH utilizing a small volume of guinea pig plasma (50 μL). The described method is more sensitive than the reported LC–MS method for Δ^9-THC and its metabolites [16]. This method was developed and validated specifically for the estimation of Δ^8-THC and Δ^8-THC-9-COOH in guinea pig plasma samples after topical drug application.

2. Experimental

2.1. Chemicals

Δ^8-THC and 11-nor-Δ^8-THC-9-COOH were obtained from Sigma (St. Louis, MO). Ammonium acetate, ethyl acetate, and acetonitrile (HPLC grade) were obtained from Fisher Scientific (Fairlawn, NJ). Water was purified by Millipore Elix 5 reverse osmosis and a Milli-Q® (Millipore) Gradient A10 polishing system (Millipore, Bedford, MA, USA).

2.2. Calibration standards and quality control samples

Validation of the method was performed using Δ^8-THC and the metabolite. Concentrations used in the preparation of standard samples were 3.97–635.93 nM for Δ^8-THC in acetonitrile and 7.26–580.72 nM for Δ^8-THC-9-COOH in acetonitrile. The blank (drug-free) guinea pig plasma was spiked with analytes to obtain final concentrations of 3.97–635.93 nM for Δ^8-THC and 7.26–580.72 nM for Δ^8-THC-9-COOH. Calibration curves were plotted in the range of 3.97–635.93 nM for Δ^8-THC and 7.26–580.72 nM for Δ^8-THC-9-COOH. The standard samples with concentrations of 3.97, 31.80, 158.98 and 635.93 nM for Δ^8-THC and 7.26, 29.04, 145.18 and 580.72 nM for Δ^8-THC-9-COOH were used to evaluate intra- and inter-assay precision and accuracy.

The extraction recoveries were determined in triplicate by extracting guinea pig plasma samples spiked with Δ^8-THC and its metabolite at 3.97–635.93 and 7.26–580.72 nM, respectively. The recoveries were calculated by comparison of the analyte's peak area from the extracted samples with those of standard samples (drug in acetonitrile).

The matrix effect was determined by extracting drug-free guinea pig plasma with acetonitrile containing a known amount of the analytes, analyzing the reconstituted extracts after evaporation, and then comparing the peak areas of the analytes with that of analytes in acetonitrile.

2.3. Sample preparation

The Δ^8-THC and metabolite stock solutions were made in acetonitrile and used immediately to spike the plasma. Fifty microliters of the plasma sample was placed into a siliconized microcentrifuge tube and extracted with 500 μL of acetonitrile–ethyl acetate (1:1, v/v). The mixture was vortexed for 30 s and centrifuged at 10,000 × *g* for 20 min. The supernatant was pipetted into a silanized 3 mL glass test tube and evaporated at 37 °C under nitrogen. The residue was reconstituted with 200 μL of acetonitrile and sonicated for 15 min. The samples were transferred into autosampler vials containing silanized low volume inserts and 20 μL was injected onto the HPLC column.

2.4. HPLC conditions

The liquid chromatograph was a Waters Alliance 2690 HPLC pump (Waters, Milford, MA, USA) with a Waters Alliance 2690 autosampler and column heater. The analytical column was a Waters Symmetry® C_{18} (2.1 mm × 150 mm, 5 μm) and the guard column used was a Waters Symmetry® C_{18} (2.1 mm × 10 mm, 3.5 μm). The mobile phase composition was: (A) 5% of 2 mM ammonium acetate in acetonitrile and (B) 2 mM ammonium acetate with 5% acetonitrile. The mobile phase gradient conditions were as follows: 60% A for 5.0 min followed by a linear gradient to 70% A in 1 min, then 70% A for 23 min, and a linear gradient to 60% A in 1 min. Between each run, the column was equilibrated for

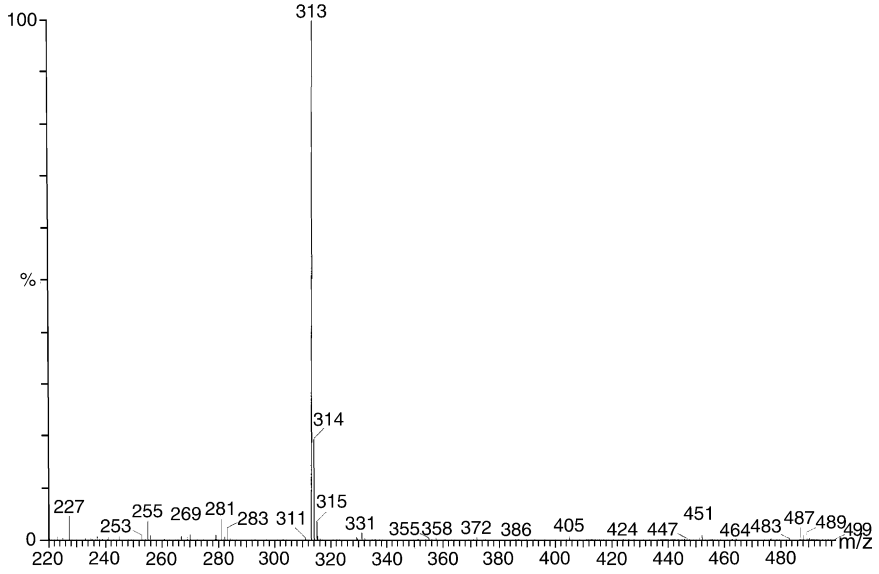

Fig. 2. Full scan mass spectrum of Δ^8-THC (*m*/*z* 313).

3 min at 60% A. The flow rate was 0.25 mL/min and the temperature of the column was maintained at 35 °C. The volume of injection was 20 μL.

2.5. Mass spectrometry conditions

The MS analyses were performed on a Micromass ZQ detector (Waters) equipped with an electrospray ionization probe. The MS was operated in the selected ion monitoring (SIM) mode for quantification of THC and the metabolite. The analysis was performed in negative mode for *m*/*z* 313 [Δ^8-THC − H]$^-$ and *m*/*z* 343 [Δ^8-THC-9-COOH − H]$^-$ (dwell time: 30 ms) (Figs. 2 and 3). The capillary voltage was 4500 V and the cone voltage was 40 V. The source block and desolvation temperatures were 120 and 250 °C, respectively. Nitrogen was used as a nebulization and drying gas at flow rates of 50 and 450 L/h, respectively.

2.6. Animal studies

IAF hairless guinea pigs (Charles River) weighing 430–458 g were used for this study. Catheters were surgically implanted into the jugular vein. Four empty Hill Top Chambers® were secured onto the dorsal region of the guinea pig with surgical glue the day of the surgery (1 day before the start of the study). To initiate the drug treatment, each chamber was loaded with 750 μL of Δ^8-THC drug formulation (F-I, 9.09 mg/mL in 1:9:1 (v/v/v) of ethanol, propylene glycol, and water; F-II, 4.42 mg/mL in 1:17:4 (v/v/v) of ethanol, propylene glycol and water; F-III, 1.41 mg/mL in

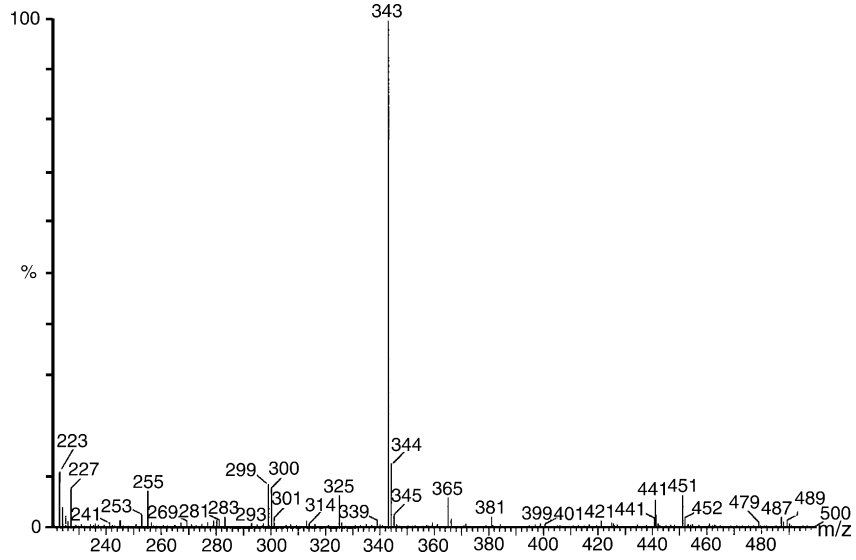

Fig. 3. Full scan mass spectrum of Δ^8-THC-9-COOH (*m*/*z* 343).

1:1.2 (v/v) of water and ethanol) and further secured with Tegaderm™ tape. The blood samples were obtained for 48 h or more throughout topical application. The blood samples were placed in siliconized microcentrifuge tubes containing heparin and immediately centrifuged at 10,000 × *g* for 3 min. Plasma was placed into silanized autosampler vials and stored at −70 °C until analysis by LC–MS. All animal studies were approved by the University of Kentucky IACUC.

2.7. Data analysis

The pharmacokinetic analysis of the data was carried out by non-compartmental analysis (WinNonlin Professional, version 4.0, Pharsight Corporation, Mountain View, CA) to determine peak concentration (C_{max}), time to peak concentration (t_{max}) and area under the curve from 0 to t (AUC_{0-t}).

3. Results and discussion

3.1. LC–MS method

Fig. 4 shows the typical ion chromatograms of 79.49 nM of Δ^8-THC and 72.59 nM of Δ^8-THC-9-COOH in guinea pig plasma. The retention times of Δ^8-THC and Δ^8-THC-9-COOH were 27.70–27.95 and 2.98–3.20 min, respectively. The total run time for each sample was about 30 min. No interfering peaks were observed at the retention times of both drug peaks. Standard curves prepared in plasma over a range of 3.97–635.93 nM for Δ^8-THC and 7.26–580.72 nM for Δ^8-THC-9-COOH were found to be linear. The mean ($n = 7$) calibration curves for Δ^8-THC and Δ^8-THC-9-COOH were $y = 111.93x + 276.88$, $R^2 = 0.999$ and $y = 21.10x + 69.14$, $R^2 = 0.998$, respectively, where y is the peak area and x is the concentration (nM). Typical ion chromatograms of processed blank plasma samples are shown in Fig. 5.

The mean absolute recoveries determined in triplicate in the concentration range of 3.97–635.93 nM for Δ^8-THC and 7.26–580.72 nM for Δ^8-THC-9-COOH were 96.60% (CV = 6.42%) and 88.20% (CV = 7.95%), respectively. The peak areas of the reconstituted samples had a less than 5% coefficient of variation, indicating that the extracts were "clean" with no co-eluting compounds influencing the ionization of the analytes. No significant matrix effect was observed for the analytes in the plasma samples.

The lower limits of quantification (LLOQ), defined as the concentration of Δ^8-THC and its metabolite Δ^8-THC-9-COOH which can still be determined with acceptable precision (CV < 10%) and accuracy, was found to be 3.97 nM for Δ^8-THC and 7.26 nM for Δ^8-THC-9-COOH. The LOD was 3.18 nM for Δ^8-THC and 3.63 nM for Δ^8-THC-9-COOH. Results of the intra- and inter-day validation assays presented in Tables 1 and 2 indicated that the accuracy of the assay was more than 95% and the CV was less than 5%. On-instrument stability was inferred from stability of samples which were prepared and included in the validation batch. No significant degradation was observed in the samples left in the autosampler at 12 °C for at least 48 h.

The present work was carried out without an internal standard, as the external standard assay had an accuracy of more

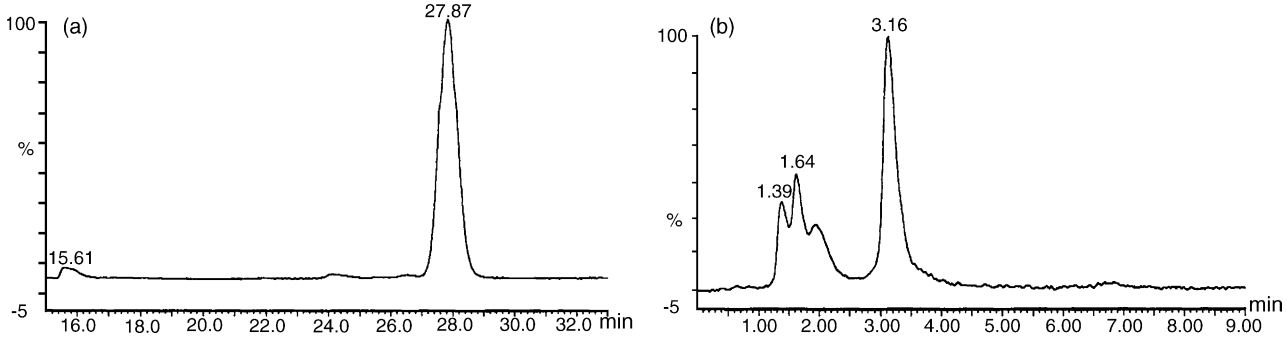

Fig. 4. Typical HPLC–MS ion chromatograms for Δ^8-THC and its metabolite in guinea pig plasma: (a) Δ^8-THC (27.87 min); (b) Δ^8-THC-9-COOH (3.16 min).

Table 1
Intra- and inter-day variability of LC–MS method for determination of Δ^8-THC

Intra-day variation				Inter-day variation			
Concentration (nM)	Observed mean concentration (nM)	CV (%)[a]	Accuracy (%)	Concentration (nM)	Observed mean concentration (nM)	CV (%)[a]	Accuracy (%)
3.97	3.82	2.25	98.00	3.97	3.79	1.36	99.20
31.80	31.64	3.7	99.50	31.80	31.83	1.36	100.10
158.98	158.54	2.36	99.72	158.98	158.98	2.98	100.00
635.93	636.03	1.36	100.01	635.93	634.53	3.01	99.78

[a] CV = coefficient of variation.

Table 2
Intra- and inter-day variability of LC–MS method for determination of Δ^8-THC-9-COOH

Intra-day variation				Inter-day variation			
Concentration (nM)	Observed mean concentration (nM)	CV (%)[a]	Accuracy (%)	Concentration (nM)	Observed mean concentration (nM)	CV (%)[a]	Accuracy (%)
7.26	7.23	2.03	99.60	7.26	7.26	3.05	100.00
29.04	29.01	3.62	99.90	29.04	28.60	2.65	98.50
145.18	143.15	1.98	98.60	145.18	144.08	3.98	99.24
580.72	580.14	2.30	99.90	580.72	578.75	1.38	99.66

[a] CV = coefficient of variation.

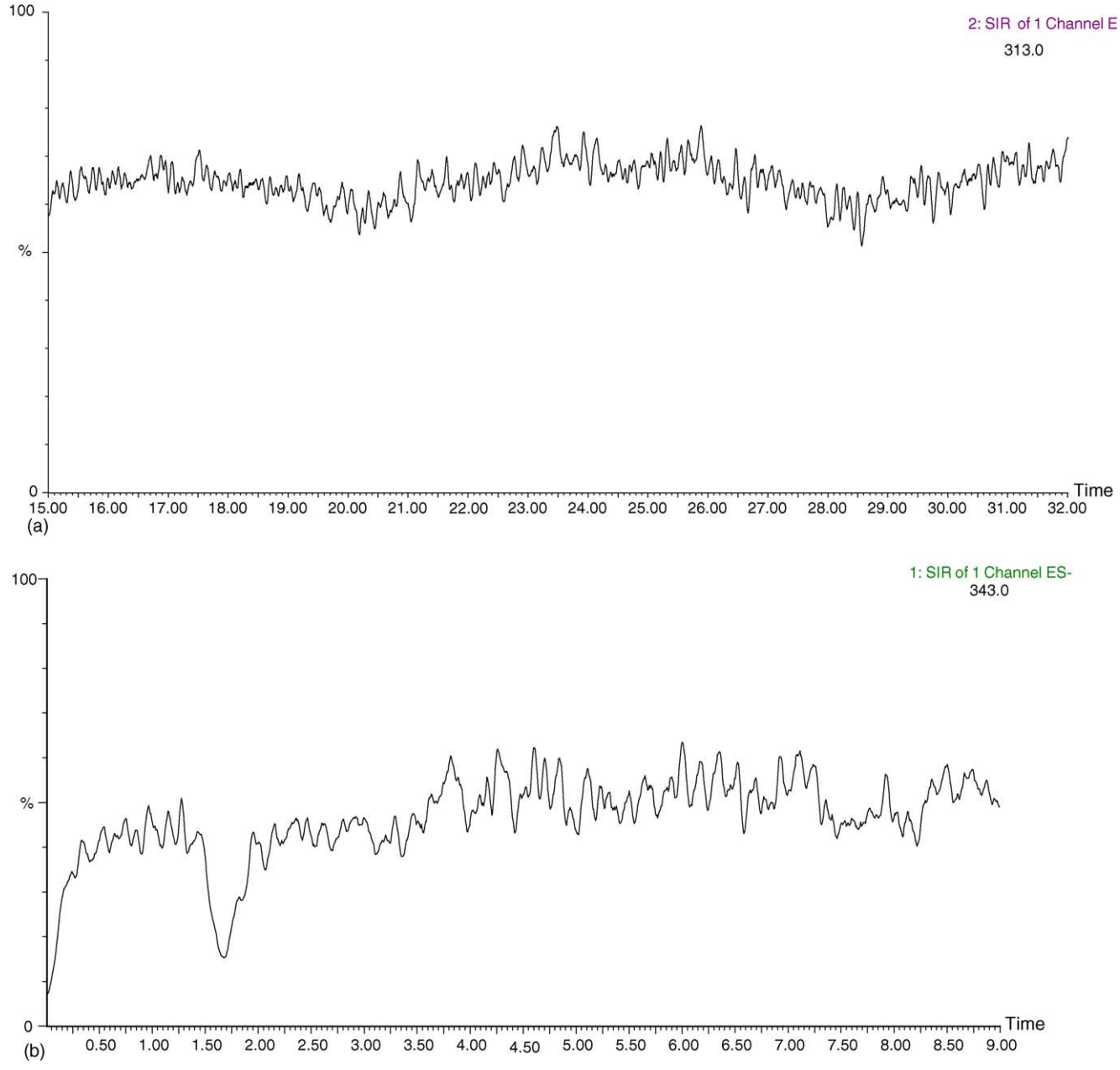

Fig. 5. The representative HPLC–MS ion chromatograms of processed blank plasma samples for Δ^8-THC (a) and its metabolite Δ^8-THC-9-COOH (b).

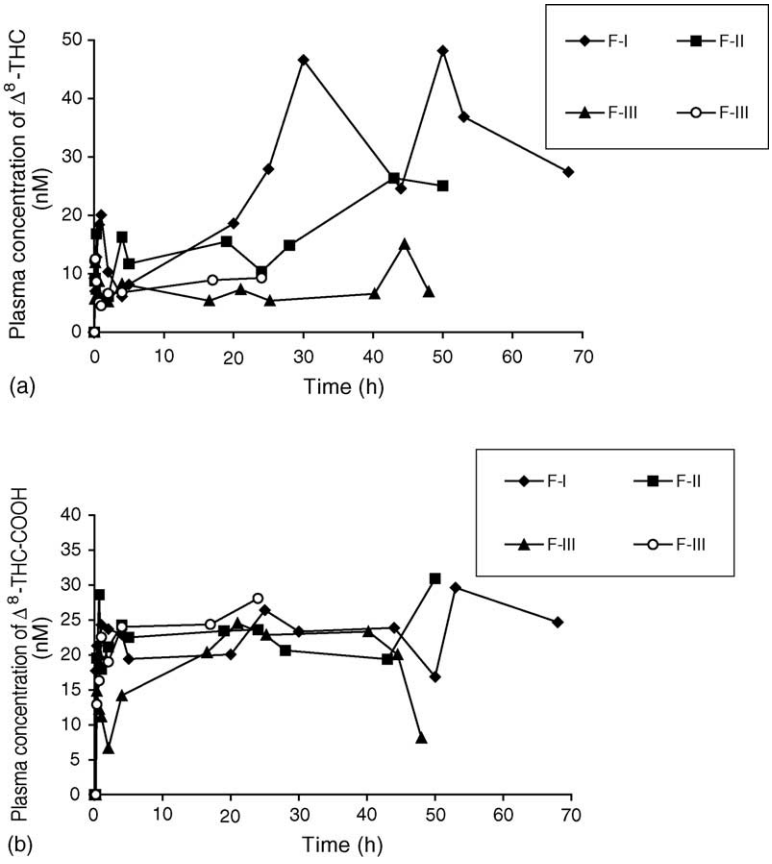

Fig. 6. Plasma concentration vs. time profiles of Δ^8-THC (a) and its metabolite Δ^8-THC-9-COOH (b) obtained after topical application in hairless guinea pigs.

than 95% and the percent coefficient variation did not exceed 10%. The extraction efficiencies were high and consistently reproducible. The ionization response monitored by injecting a system performance verification standard at the beginning and at the end of each batch indicated that the system response remained stable.

3.2. Application of the method in pharmacokinetics studies

The above successful LC–MS method was used for the pharmacokinetic studies of Δ^8-THC and its metabolite in guinea pigs after topical drug application. This is the first LC–MS method that has been described for the simultaneous analysis of Δ^8-THC and its metabolite in guinea pig plasma after topical application. Δ^8-THC in various solvent systems was applied topically by means of Hill Top Chambers® secured to the guinea pigs. The individual plasma concentration vs. time profiles of Δ^8-THC and its metabolite following the topical application of Δ^8-THC formulations are shown in Fig. 6a and b. The pharmacokinetic parameters of Δ^8-THC and its metabolite, including C_{max}, t_{max}, and AUC_{0-t} following topical application of the drug are given in Table 3. The plasma concentration of Δ^8-THC and its metabolite gradually increased and attained an average steady-state level of 27.63 and 23.17 nM for F-I, 16.85 and 19.66 nM for F-II,

Table 3
Pharmacokinetic parameters of Δ^8-THC and its metabolite after the topical application of the Δ^8-THC formulation in hairless guinea pigs

Parameter	Δ^8-THC			11-Nor-Δ^8-THC-9-COOH		
	F-I[a]	F-II[a]	F-III[b]	F-I[a]	F-II[a]	F-III[b]
AUC (nM h)	1880.89	845.25	260.60 ± 113.29	1576.42	944.83	829.38 ± 396.60
C_{max} (nM)	48.18	826.39	13.77 ± 1.84	29.70	24.51	27.44 ± 4.85
t_{max} (h)	50.0	43.00	15.62 ± 13.61	53.00	21.0	36.50 ± 24.35
C_{ss} (nM)	27.63	16.85	8.24 ± 0.99	23.17	19.66	22.44 ± 0.55
t_{lag} (h)	1	0.67	0.67 ± 0.0	1	0.33	2.21 ± 1.13

F-I: 9.09 mg/mL of Δ^8-THC in 1:9:1 (v/v/v) of ethanol, propylene glycol and water; F-II: 4.42 mg/mL of Δ^8-THC in 1:17:4 (v/v/v) of ethanol, propylene glycol and water; F-III: 1.41 mg/mL of Δ^8-THC in 1:1.2 (v/v) of water and ethanol.

[a] $n = 1$.

[b] $n = 2$.

and 8.24 ± 0.99 and 22.44 ± 0.55 nM for F-III. The lag times observed were 0.67–1 h for Δ^8-THC and 0.33–2.2 h for its metabolite. The steady-state levels were maintained for a period of 24–66 h for Δ^8-THC and its metabolite. The maximum plasma concentrations of 48.17 and 29.70 nM were observed for Δ^8-THC and its metabolite, respectively, after F-I application (drug 1:9:1 (v/v/v) of ethanol, propylene glycol and water).

4. Conclusion

An LC–MS method for the estimation of Δ^8-THC and its metabolite, Δ^8-THC-9-COOH, in guinea pig plasma was successfully developed and validated. The method is sensitive and simple with an LLOQ of 3.97 nM for Δ^8-THC and 7.26 nM for Δ^8-THC-9-COOH using a 50 μL plasma sample. It has been shown in pharmacokinetic studies with hairless guinea pigs that Δ^8-THC and its metabolite could be measured after topical application of Δ^8-THC. Thus, the method is appropriate for monitoring Δ^8-THC and its metabolite in pharmacokinetic studies.

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