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Journal of Chromatography A, 1073 (2005) 355–361
JOURNAL OF CHROMATOGRAPHY A
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Novel solid-phase extraction protocol for 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol from urine samples employing a polymeric mixed-mode cation-exchange resin, Strata-X-C, suitable for gas chromatography–mass spectrometry or liquid chromatography–mass spectrometry analysis

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Available online 6 November 2004

Abstract

A novel solid-phase extraction (SPE) method was developed for extraction and cleanup of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), the major metabolite of the active principle of marijuana, Δ^9-tetrahydrocannabinol, from urine samples. The protocol utilizes a polymeric mixed-mode cationic sorbent, Strata-X-C, which exhibits strong retention for the metabolite facilitating a more rigorous organic wash to eliminate matrix components/endogenous materials. Acetonitrile containing acetic acid was used as the elution solvent and is compatible with both LC–MS and GC–MS modes of analysis. The hydrophobic retention of Strata-X-C was demonstrated to be higher than a neutral polymeric sorbent, Strata-X, of the same backbone but devoid of the cation-exchange moiety (sulfonic acid), by LC studies employing homologous paraben probes. Simultaneously, the polar (non-ionic) interaction capability of Strata-X-C is also greater than that of Strata-X, as assessed through regioisomeric nitrophenol probes. These two features enable the metabolite to be retained strongly on Strata-X-C. Good linearity and precision was obtained for THC-COOH by GC–MS analysis of its trimethylsilyl derivative in the range 1–50 ng. A simplified room temperature instantaneous derivatization procedure was developed that is suitable for high-throughput screening of THC-COOH.

Keywords: Solid-phase extraction protocol; Polymeric strong cation exchange sorbent; LC–MS; GC–MS

1. Introduction

Of the total number of drug tests performed in North America encompassing workplace drug testing, driving under the influence of drugs and forensic cases, about 45% relate to 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in urine samples [1]. This carboxylic acid is one of the major metabolites of Δ^9-tetrahydrocannabinol (THC), the psychoactive component in Cannabis products (marijuana, hashish) [2]. The chemical structures of THC and THC-COOH are shown in Fig. 1. Notwithstanding the fact that THC is familiar to the public as an illicit drug, food products derived from or containing this substance have become popular during the last decade and these include nutritional supplements as well [3]. Moreover, pharmacological research on the therapeutic potential of cannabinoids also expanded with the elucidation of an endocannabinoid system [4].

The most popular (and widely accepted) method for establishing the uptake of THC by the human body is the quantitative determination of its main metabolite, THC-COOH, in urine by liquid–liquid or solid-phase extraction followed by derivatization and gas chromatographic analysis in tandem with mass spectrometric detection. Either electron impact (EI) or chemical ionization (CI) modes or tandem mass spectrometry (MS–MS) are the commonly employed modes of mass spectrometric detection [5–10]. Silylation or methylation are the two widely used protocols for converting THC-COOH into readily volatile silyl or methyl ester derivatives,

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doi:10.1016/j.chroma.2004.10.052

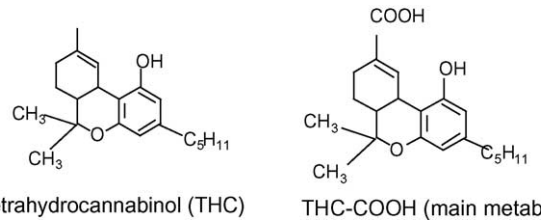

Fig. 1. Chemical structures of Δ^9-tetrahydrocannabinol and its major metabolite in urine.

respectively, for GC–MS analyses [11,12]. The phenolic hydroxyl of THC-COOH is also converted into trimethylsilyl ether or methyl ether simultaneously. Several recent publications have reported on the utilization of high-performance liquid chromatography (HPLC) in tandem with electrochemical [13] and mass spectrometric [14,15] detection modes for analyzing THC and metabolites in blood, hair, sweat, saliva, and meconium samples [16,17]. A few documented reports also utilized membrane based extraction [18] and immunoassay protocols [19].

Solid-phase extraction (SPE) has been the staple sample pretreatment technique for extraction and cleanup of THC-COOH or other metabolites from either urine or blood samples [20]. Silica-based C_{18} or mixed-mode alkyl/cation-exchange sorbents are frequently used during these SPE protocols, although the latter finds more universal acceptance. Surprisingly, there have been few published reports on the use of either neutral or cation-exchange polymeric sorbents, which are extremely popular for the sample preparation of small drug molecules from biological matrices [21]. Furthermore, Bogusz et al. [22] carried out an extensive study on the extraction of opiates using four different brands of mixed-mode silica-based cation-exchange sorbents and found considerable variations in extraction recoveries. Since polymeric cation-exchange sorbents are known to be more consistent with respect to reproducibility and cleanup efficiency for small drug samples from biological matrices [23], we carried out a detailed investigation of the extraction of THC-COOH from urine samples in the current work with Strata-X-C. This polymeric cation-exchange sorbent consists of a styrene-divinylbenzene backbone and carries a sulfonic acid functionality as well as other polar moieties capable of hydrogen bonding and dipolar interactions. Due to these structural features, Strata-X-C is expected to furnish cleaner extracts from the urine matrix, which contains a conglomeration of neutral, acidic, and basic organic matrix components together with proteins and inorganic salts [24].

2. Experimental

2.1. Materials, reagents, and solvents

THC, THC-COOH, and [$^2H_3$]THC-COOH (THC-COOH-d_3) were obtained from Sigma (St. Louis, MO, USA). Acetonitrile, methanol, and ethyl acetate were ACS reagent grade from Aldrich (Milwaukee, WI, USA). The reagents, potassium hydroxide, hydrochloric acid, acetic acid, imidazole, and *N*,*N*-bis(trimethylsilyl)trifluoroacetamide were obtained from Sigma. The aromatic probes used for hydrophobicity evaluation, viz. methyl, ethyl, propyl, and butyl parabens and 2/3/4-nitrophenols were also procured from Aldrich. Strata-X-C SPE cartridges (60 mg, 3 mL) and SPE vacuum manifold were from Phenomenex (Torrance, CA).

2.2. Alkaline hydrolysis of the glucuronide of THC-COOH in urine samples

The subject urine sample (2 mL, 1:1 diluted with deionized water) was spiked with the internal standard [THC-COOH-d_3, 100 μL of 0.1 μg/mL] and treated with potassium hydroxide (10 M aqueous, 100 μL). The mixture was vortexed and then heated at 60 °C for 20 min. The pH of the hydrolysate was adjusted to 3.5–4.0 by addition of glacial acetic acid (around 200 μL). This solution was used as the load for SPE. The reaction is summarized graphically in Fig. 2.

2.3. Solid-phase extraction of the glucuronide hydrolysate

The Strata-X-C cartridge is conditioned with methanol (2.0 mL), followed by hydrochloric acid (0.1 M aqueous, 2.0 mL). The above hydrolysis solution was loaded onto this conditioned cartridge under vacuum draining conditions. The sample loaded sorbent was then washed, first with hydrochloric acid (0.1 M aqueous, 2.0 mL) and then with acetonitrile–0.1 M hydrochloric acid (30:70, 2.0 mL). The cartridge was then vacuum dried for 4 min at 10 in.Hg pressure (1 in.Hg = 338.638 Pa). Glacial acetic acid (200 μL) was added and allowed to percolate through the sorbent under gravity. The sorbent was dried under vacuum for 1 min. The THC-COOH metabolite was then eluted with 2 mL of acetonitrile containing 2% glacial acetic acid.

2.4. Derivatization of THC-COOH from the SPE extraction for GC–MS analysis

The SPE extract was concentrated under nitrogen at 40 °C and reconstituted in 30 μL of 10 mM solution of

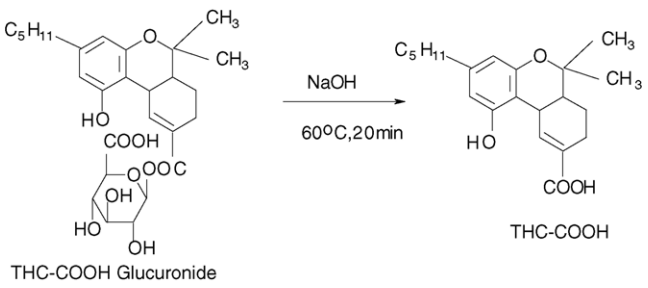

Fig. 2. Hydrolysis of THC-COOH glucuronide from urine.

imidazole in ethyl acetate and 30 μL of bis(trimethylsilyl)trifluoroacetamide containing 1.0% of trimethylchlorosilane. The mixture was stirred at room temperature for 5 min and the resulting solution used as such for GC–MS analysis.

2.5. GC–MS analysis

A Hewlett-Packard 6890 gas chromatograph, equipped with a 5973 mass-selective detector and chemstation software (Agilent Technologies), was used for the analysis of the derivatized THC-COOH metabolite and the internal standard present in the solution.

A Zebron ZB-5MS (Phenomenex) capillary column (30 m × 0.25 mm, 0.25 μm particle size) was used for gas chromatography under the following conditions: 3 μL splitless injection for 0.4 min at 270 °C injector temperature; carrier gas (helium) at a constant flow rate of 1.4 mL/min; oven temperature programmed from 120 to 235 °C at 20 °C/min and then to 250 °C at 3 °C/min and finally to 340 °C at 25 °C/min. Detection (MS) in the selected-ion monitoring (SIM) mode was used and the ions monitored are *m*/*z* 371, 473, and 488 for THC-COOH and *m*/*z* 374, 476, and 491 for the deuterated internal standard, at a source temperature of 240 °C and a transfer line temperature of 300 °C.

2.6. HPLC of underivatized SPE extract

The HPLC of the purified SPE extract of the metabolite was run on an Agilent 1100, using a Luna $C_{18}(2)$ column (150 mm × 4.6 mm, 5 μm particle size). A mobile phase of methanol–potassium monobasic phosphate (20 mM, pH 2.5) (60:40) was employed at a flow rate of 1.0 mL/min, with gradient from 1 to 6 min to 95% methanol. A detector wavelength

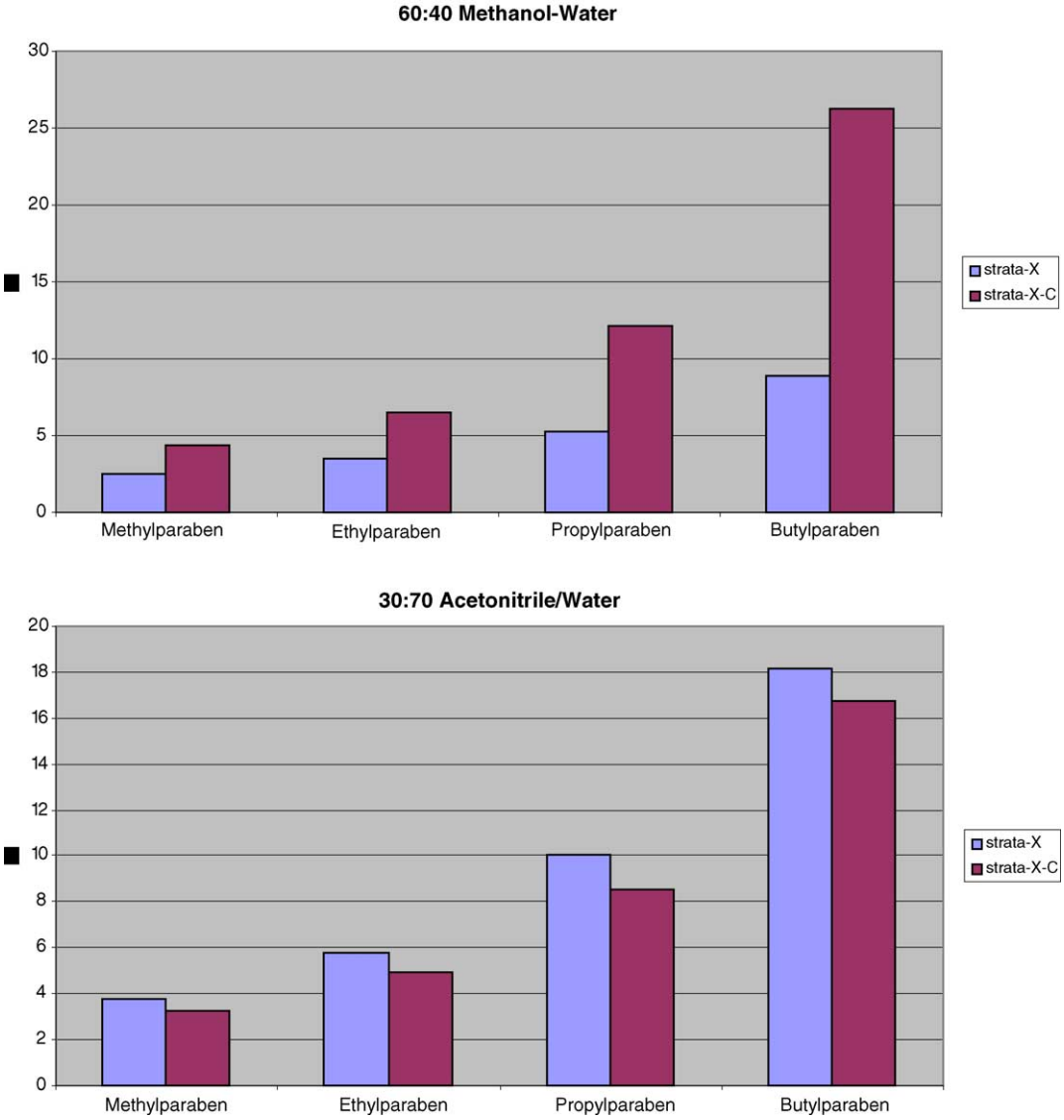

Fig. 3. Hydrophobic retention comparison between Strata-X-C and Strata-X in methanol and acetonitrile with homologous paraben probes.

of 230 nm and injection volume of 50 μL was used. Analyte concentration was 1.0 μg/mL.

3. Results

3.1. Utilization of a polymeric cation-exchange sorbent for SPE purification of THC metabolites

All the commercially available silica-based mixed-mode cation-exchange sorbents consist of an ion exchange moiety (sulfonic acid) and an alkyl chain (usually a C_8) on the silica surface. If one considers the structure of THC or THC-COOH, there are no cation-exchange sites available and the molecules must interact with these mixed-mode sorbents through hydrophobic interactions. There could possibly be some H-bond interactions between the surface silanols and the phenolic hydroxyl group on these drug/metabolites. In addition, the carboxylic group on THC-COOH can also contribute to such H-bond interactions. However, hydrophobic and H-bond interactions are not as strong as ionic interactions and therefore one would expect that these silica-based mixed-mode sorbents will not withstand strong organic wash for the cleanup of urine or plasma samples containing THC and/or its metabolites. On the other hand, anion exchange sorbents used for the SPE of THC-COOH do interact with this metabolite through ionic mechanism and hence are amenable for 100% organic wash, as has been demonstrated [25], and are expected to furnish very clean extracts. If the constituent endogenous molecules in urine are taken into account [24],

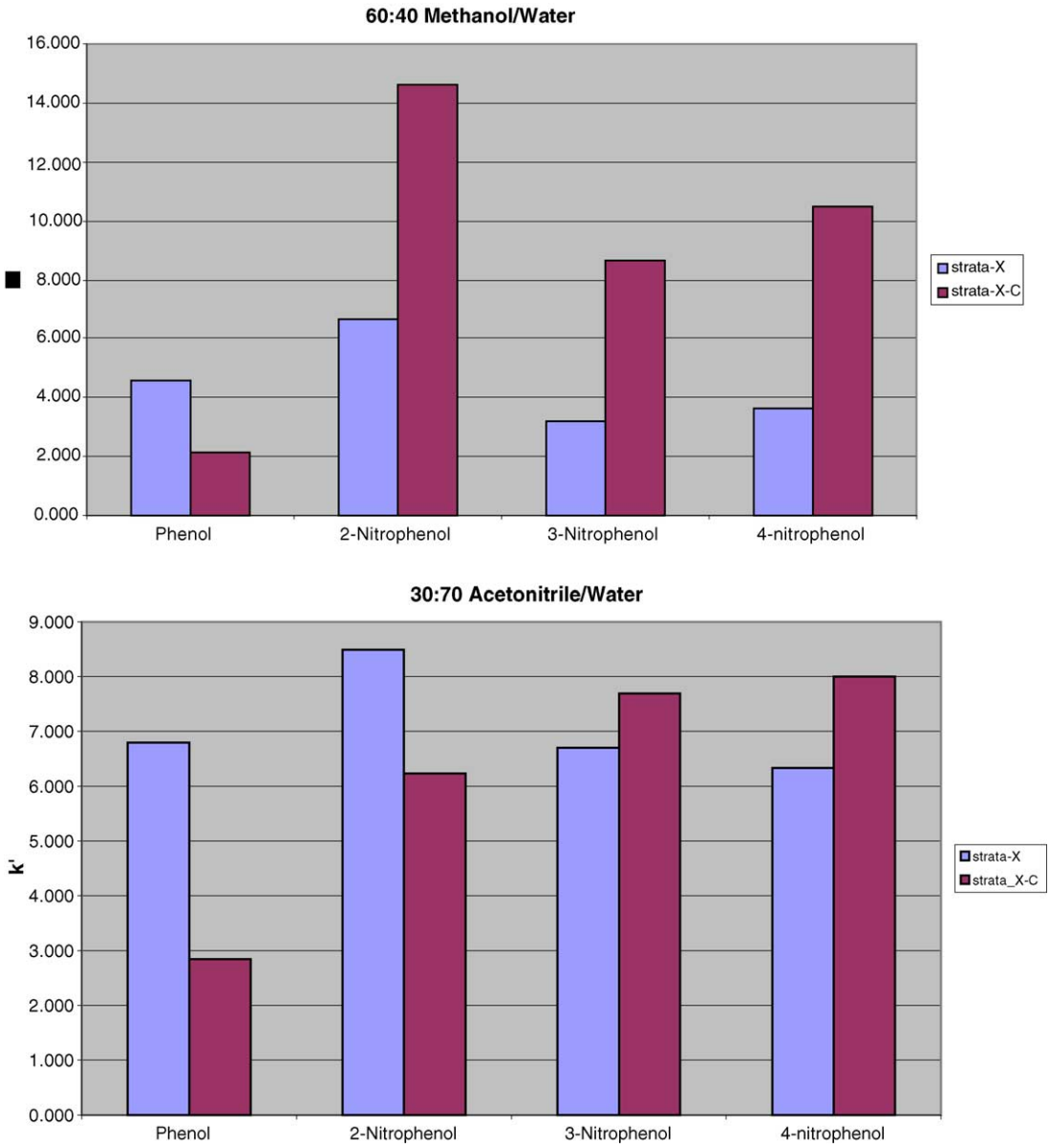

Fig. 4. Comparison of polar retention characteristics of Strata-X-C (cation-exchange resin) with Strata-X (neutral polymer with same backbone).

a significant number of them are organic acids (for example, oxalic acid, ascorbic acid, hippuric acid, homovanillic acid, and methylmalonic acid, to name a few), along with nucleosides, other amino acids and inorganic salts such as nitrates and nitrites, all of which are capable of interacting with anion-exchange sites. It is therefore possible that anion-exchange sorbents may exhibit coelution of some of these endogenous matrix constituents along with THC-COOH. As opposed to this, such endogenous materials will not react with cation-exchange sorbents through ionic mechanism and by virtue of their high polarity, can be readily washed out with aqueous organic solvents. An added bonus is that all basic endogenous constituents of the urine matrix will react with such cationic sites and hence will not elute with THC-COOH. All these aspects explain why mixed-mode cationic silica-based sorbents are popular in the toxicological and forensic fields.

Silica-based cation-exchange sorbents suffer from three major shortcomings, viz. significant variation in recovery yields from batch to batch and often even within the same batch itself, presence of leachable organic impurities, and low hydrophobic surface areas available for hydrophobic retention of THC and its metabolites. Polymeric mixed-mode sorbents address these problems effectively as they possess high surface areas, are obtainable in a reproducible manner with respect to chemical composition as well as particle sizes and do not carry any leachates. However, the question arises as to how hydrophobic these cation-exchange polymeric sorbents are in comparison with their neutral counterparts devoid of the sulfonic acid groups. To ascertain the answer to this question, we investigated the retention characteristics of the neutral polymeric sorbent Strata-X (Phenomenex) and its sulfonated analogue Strata-X-C (Phenomenex). These two polymers carry the same polystyrene–divinylbenzene backbone, along with polar entities that can display dipolar and H-bonding properties. Using HPLC columns packed with these sorbents, we determined the retention factors of a family of homolgous parabens in both methanol–water and acetonitrile–water mobile phases. Surprisingly, Strata-X-C showed much stronger retention for these probes in methanol and both Strata-X and -X-C showed similar retention features in acetonitrile (Fig. 3). We also examined the retention of the polar regioisomeric nitrophenols in both solvents and again, the results were comparable to the paraben probes (Fig. 4). It can then be concluded that the mixed-mode cation-exchange resin Strata-X-C shows better hydrophobic as well as polar retention characteristics compared to a neutral polymer with the same backbone structure, Strata-X. Therefore, for SPE based extraction and purification of urine samples of THC-COOH, Strata-X-C is the preferred sorbent.

The superior hydrophobic retention of Strata-X-C can be rationalized on the basis of the electron withdrawing nature of the sulfonic acid functionality, which induces a lot more electron polarization in the neighborhood of the aromatic ring attached to it. Thus, the two non-aromatic fused rings as well as the alkyl side chain of the THC-COOH structure are able to interact more strongly through dispersive and pi–pi interactions with the divinylbenzene nucleus of Strata-X-C in comparison with Strata-X. Furthermore, owing to the electron deficiency of the benzene ring attached to the sulfonic acid moiety, pi-stacking interactions with the aromatic ring of THC-COOH is also envisioned. Therefore, THC-COOH adsorbed to the Strata-X-C surface can withstand much stronger organic wash than any silica-based mixed sorbent and can furnish ultrapure extracts.

3.2. Solid-phase extraction of THC-COOH from urine

The urine sample was first treated with strong base (0.1 M KOH) to hydrolyze the glucuronide of THC-COOH to the free aglycone. The pH of the solution was adjusted to 3.5–4.0 to neutralize the potassium salts the carboxylic and phenolic moieties of THC-COOH can form under the basic conditions employed. After this neutralized hydrolysis solution was loaded onto the Strata-X-C cartridge, two wash steps were carried out. The initial acid wash with 0.1 M HCl removes all inorganic salts and strongly polar organic matrix constituents. The second wash step with acetonitrile–0.1 M HCl (30:70) gets rid of all the medium polar and non-polar organic contaminants from the urine matrix. It is interesting to note that in a majority of published work on SPE extraction of THC-COOH, utilizing silica-based C_{18} or cation-exchange mixed-mode sorbents, washing was done with either water and dilute acid [26] or 8% ethanol in water [10], methanol–water (50:50) [12,15] or 15–20% acetonitrile [13,20]. In fact, one of these [20] cautions against using more than 20% acetonitrile in order to prevent loss of analyte. In cases where higher (30%) acetonitrile was used for wash, lower recovery yields (75–85%) were registered. In the current protocol, employing 30% acetonitrile as wash solvent, near quantitative recovery was obtained. Our experimental observations also indicate that acetonitrile concentrations of up to 50% can be utilized for the wash step in SPE without any significant breakthrough of THC-COOH from the Strata-X-C surface.

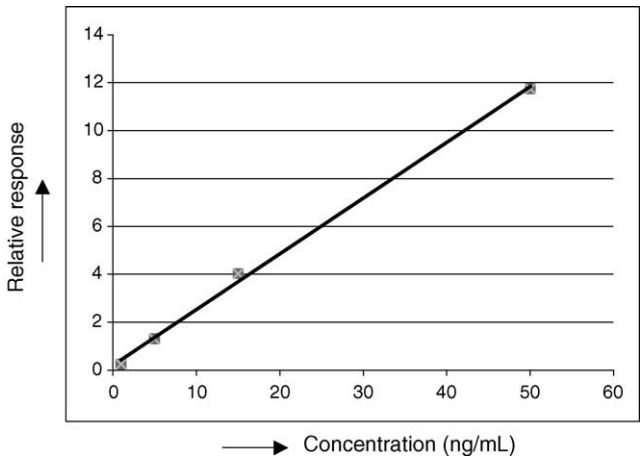

Fig. 5. Calibration curve and correlation coefficient over the range from 1.0 ng to 50.0 ng/mL (from GC–MS analysis).

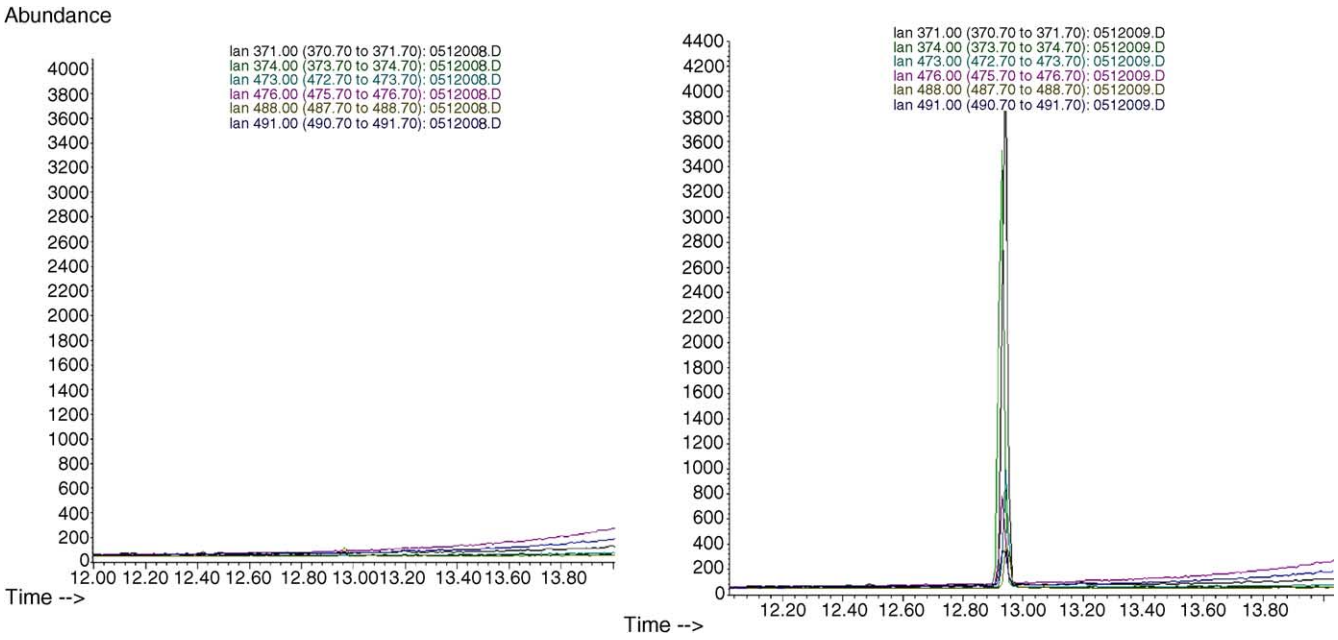

Fig. 6. Select ion chromatogram from GC–MS analysis of blank urine sample (left) and THC-COOH/Internal standard spiked urine sample (right).

The elution solvent used in the current method, viz. acetonitrile/acetic acid, is compatible with HPLC or LC–MS and can be used for THC-COOH in plasma, blood and tissue samples, where GC–MS is not the method of choice. On the other hand, for GC–MS analysis, the solvent may be easily evaporated and the residue dissolved in a GC compatible solvent such as ethyl acetate. The present method avoids the use of solvents such as hexane and dichloromethane, which are water immiscible and present problems with aqueous based SPE protocols. It is noteworthy that when Strata-X-C is used as SPE sorbent, solvents like methanol or acetone do not elute THC-COOH.

3.3. Derivatization prior to GC–MS analysis

In almost all the protocols documented to date, silylation reactions performed to generate trimethylsilyl derivatives of THC-COOH are carried out at elevated temperatures for about 15–20 min followed by a cool off period. Under these conditions, some loss of the silyl derivative can occur due to voltalization. In our present method, we have used imidazole as a catalyst to effect silylation at room temperature in 5 min. Thus, if high-throughput conditions are needed, the current method saves at least 30 min time during sample preparation.

3.4. Precision and linearity of the method

For the GC–MS analysis of the bis-trimethylsilyl derivative of THC-COOH, the molecular ion at *m*/*z* 488 (*m*/*z* 491 for the d_3 analogue used as internal standard) and the fragments generated by loss of a methyl radical (*m*/*z* 473 for THC-COOH and *m*/*z* 476 for the d_3 internal standard) and loss of the trimethylsilyl ester moiety (*m*/*z* 371 for THC-COOH and 374 for the internal standard) are monitored in the SIM mode. The ions at *m*/*z* 371 (and 374 for the standard) are the most intense and are used for computation of recovery yields and reproducibility, in analogy with Crockett et al. [27]. The other ions are used as cross-reference. Quantitation was done using the peak area ratios for the undeuterated and deuterated compounds. Fig. 5 shows the linearity of the method developed in the concentration range of 1–50 ng. A correlation coefficient (r^2) of 0.998 was observed for this dynamic range and demonstrates the reproducibility of response. In Fig. 6, the total ion chromatograms of a blank and spiked urine sample are presented for all the ions monitored, and for $n = 6$, a precision of around 0.55% was obtained for each concentration. These precision values for the concentration range 1–50 ng are much lower than those reported for silica-based C_{18} [15] and mixed-mode SPE sorbents, as well as for anion-exchange polymeric sorbents [27], indicative of the efficiency of the Strata-X-C SPE sorbent.

4. Conclusions

The present work demonstrates the applicability of the polymeric mixed-mode cation-exchange sorbent, Strata-X-C, for the solid-phase extraction of the major metabolite THC-COOH of THC, the active principle of marijuana, from urine samples. Strata-X-C is superior to silica-based mixed-mode cationic resins, not only from reproducibility point of view, but also for generating cleaner extracts. A simplified and rapid protocol for the preparation of trimethylsilyl derivatives for GC–MS analysis of THC-COOH is also presented. Excellent precision for extraction and linearity of the method

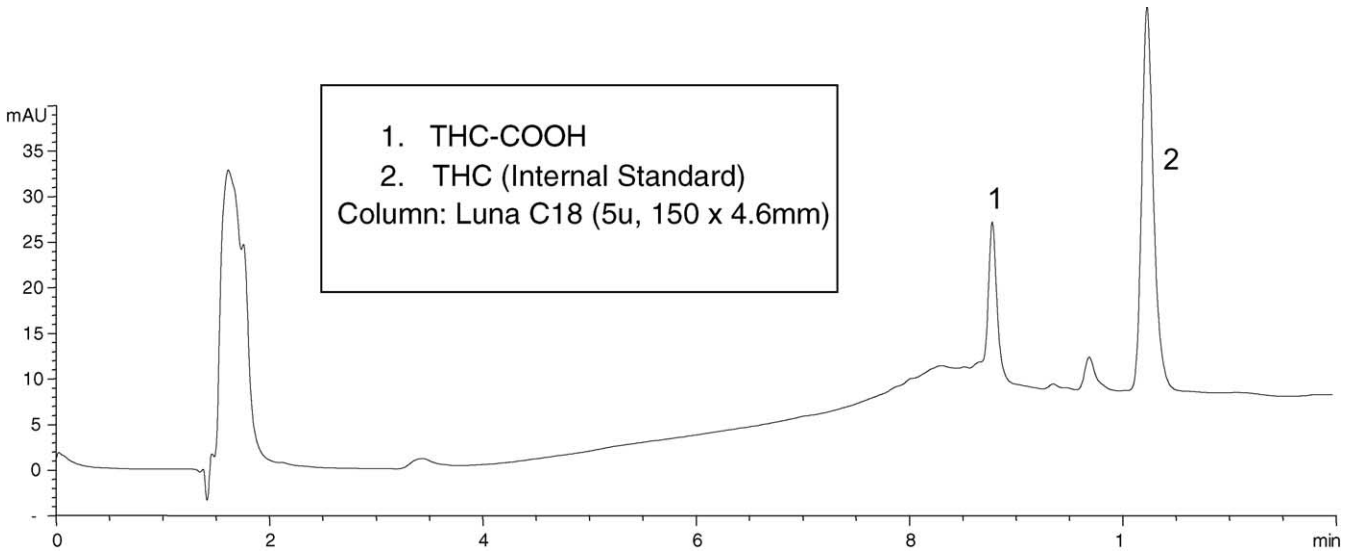

Fig. 7. HPLC chromatogram of underivatized SPE extract (see Section 2.6 for LC conditions).

developed were also obtained. The SPE method developed is applicable to either LC–MS (see Fig. 7 for a representative liquid chromatogram) or GC–MS analysis (Figs. 5 and 6), as desired.

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