Tetrahydrocurcumin in plasma and urine: Quantitation by high performance liquid chromatography

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Abstract

Tetrahydrocurcumin (THC), one of the major metabolites of curcumin, exhibits many of the same physiologic and pharmacological activities as curcumin and in some systems may exert greater antioxidant activity than curcumin. However, evaluation of clinical efficacy is limited by lack of sensitive methods for quantifying intake/absorption in blood or urine. We have developed a sensitive high performance liquid chromatography (HPLC) analytical method for detection of THC in plasma and urine. The method involves extracting the THC from 0.2 mL samples with 95% ethyl acetate/5% methanol, and 17-estradiol acetate as an internal standard. Analysis with a reversed-phase C18 column and UV detection at 280 nm demonstrates linear performance from 0.050 to 6.0 μg/mL in plasma, and 0.060 to 6.0 μg/mL in urine. The coefficients of variation for intra- and inter-assays were each <8.6%. The average recovery of THC from plasma and urine was greater than 98.5%. These data demonstrate a rapid, sensitive and accurate method for HPLC quantification of THC in plasma and urine.

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1. Introduction

Curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is the major yellow pigment extracted from turmeric, a commonly used spice, derived from the rhizome of the herb Curcuma longa Linn [1]. It is a naturally-occurring polyphenolic phytochemical currently being examined in preclinical trials for cancer chemoprotective drug development, with pharmacological actions that include anti-inflammatory [2] and anti-amyloidogenic neuroprotection [3]. Tetrahydrocurcumin (THC) is one of the major metabolites of curcumin, with potential potent bioactivity. This metabolite was identified in intestinal and hepatic cytosol from humans and rats [4–10], and similar to curcumin, THC possesses hydroxyl groups that make it a typical substrate for glucuronide conjugation. The reduction of curcumin to THC seems to occur primarily in a cytosolic compartment (intestinal or hepatic, possibly via a reductase enzyme) [1]. Final reduction of THC to hexahydrocurcuminol may occur in microsomes (possibly by cytochrome P450 reductase) [1].

Recently, attention has focused on THC, as one of the major metabolites of curcumin, because this compound appears to exert greater antioxidant activity in both in vitro and in vivo systems [9,11]. Structurally, THC and curcumin (Fig. 1) have identical β-diketone structures and

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phenolic groups, but differ in that THC lacks the double bonds [5,9]. Sugiyama et al. [5] demonstrated that THC exhibited similar physiological and pharmacological properties as the active form of curcumin in vivo. Naito et al. [10] showed clear involvement of THC in biochemical and molecular actions at the cellular level in ameliorating oxidative stress in cholesterol-fed rabbits [10]. Some researchers have also focused on the neuroprotective role of curcumin in amyloid neurotoxicity and amyloid fibril formation in Alzheimer’s models and other possible neurodegenerative diseases [12–14]. Furthermore, Okada et al. [9] have claimed that THC has more potent antioxidant activity than curcumin. Curcuminoids induce antioxidant enzymes, such as glutathione peroxidase, glutathione S-transferase and NADPH-quione reductase, but THC was found to be more active than curcumin and scavenged Fe-NTA-induced free radicals more effectively than curcumin in vitro.

A role for curcumin in the prevention of cancer and other chronic diseases, due to various biological activities, has also been implicated [15–19]. However, whether curcumin or its metabolites, such as THC, are responsible for the reported effects is still to be determined. Some studies involving radio labeling in the measurement of plasma levels suggest that curcumin is poorly absorbed from the gut [6]. The fact that dietary curcumin may have widespread beneficial effects, yet is poorly absorbed, has created debate and controversy. Until better methods are developed for measuring metabolites, it remains unclear whether very low plasma levels of curcumin are adequate for efficacy or whether unidentified metabolites are responsible for benefits.

If indeed THC is the critical metabolite for efficacy, it is known that it can be directly administered. But it is not clear whether THC is absorbed better than curcumin. It is controversial whether it is more polar [6] or less polar [5]. Even reduced polarity alone does not determine whether THC would be better absorbed than curcumin, because absorption is influenced by solubility, permeability and efflux transport. In addition to understanding intestinal absorption differences, it is also important to consider differences in transfer across various body pools. However, elucidation of all of these controversies and questions await better established methods for detecting THC in biological samples. Therefore, our goal was to develop sensitive and accurate methods for its detection in biological fluids.

2. Experimental methods

2.1. Chemicals

THC was provided by Sabinsa Corporation (Piscataway, NJ, USA). Ammonium sulfate and β-17-estradiol acetate were obtained from Sigma (St. Louis, MO, USA). Acetonitrile, methanol, acetic acid, ethyl acetate, sodium hydroxide and de-ionized water were all HPLC grade (Fisher Scientific, Pittsburg, PA, USA).

2.2. Instrumentation and equipment

The HPLC system consisted of a 9100 auto-sampler with a refrigeration unit, a 9050 UV visible detector and a 9010 solvent delivery system, with Star 5.52 chromatography software (Varian Inc., Walnut Creek, CA, USA). Chromatographic separation was accomplished using a Waters SymmetryShield 3.9 mm × 150 mm, 5 μm C18 Column (Waters, Milford, MA, USA). The column was coupled with an Altech Preval Amide 30 mm × 4.6 mm, C18 guard column (Altech Associates, Deerfield, IL, USA).

2.3. Mobile phase reagent preparation

2.3.1 Reagent A: acetonitrile

The use of phosphate buffer at pH 7.8 was unsuitable as it resulted in severe salt precipitation and damage to the HPLC system. We therefore explored different buffers. Although ammonium sulfate is not an ideal buffer, we determined that ammonium sulfate solution at pH 7.8, gave superior HPLC separation. We dissolved 0.01% ammonium sulfate (w/v) in de-ionized water, and brought the pH to 7.8 with 1.0 molar sodium hydroxide. The mixed reagent, prepared fresh daily in order to prevent bacterial growth, was filtered under vacuum, through a 0.45-μm filter (Millipore, Bedford, MA, USA) and then degassed by a solvent de-gas system (Fisher Scientific, Pittsburg, PA, USA).

2.3.2 Reagent B: ammonium sulfate

This reagent was prepared by mixing 41% acetonitrile, 35% de-ionized water, 23% methanol and 1% acetic acid (v/v/v/v). The mixed reagent was vacuum filtered 0.45-μm filter (Millipore, Bedford, MA, USA) and degassed (Fisher Scientific, Pittsburg, PA, USA). This reagent was stable at room temperature for at least 4 weeks.

2.3.3 Reagent C: THC reagent

This reagent was prepared by mixing 41% acetonitrile, 35% de-ionized water, 23% methanol and 1% acetic acid (v/v/v/v). The mixed reagent was vacuum filtered 0.45-μm filter (Millipore, Bedford, MA, USA) and degassed (Fisher Scientific, Pittsburg, PA, USA). This reagent was stable at room temperature for at least 4 weeks.
2.4. Extracting reagent preparation

The extracting reagent was prepared by mixing 95% ethyl acetate and 5% methanol (v/v). Stored at room temperature, this was stable for at least 4 weeks.

2.5. Standard and quality control samples preparation

5.0 mg of powdered THC were dissolved in 2.5 mL methanol, making final volume of 5.0 mL with THC reagent C, to achieve the desired concentration 1000 μg/mL (stock standard). From this stock solution, three working standard solutions of THC were prepared:

(i) Working standard "a" = 100.0 μg/mL,
(ii) Working standard "b" = 10.0 μg/mL,
(iii) Working standard "c" = 1.0 μg/mL.

The standard solutions were prepared fresh as required.

Calibration was performed, using the established spiking technique of addition to spike human plasma or urine with known amounts of the standards. Plasma and urine samples were collected from human volunteers. Sample pools were screened, and THC was not detected in the plasma or urine. The plasma was obtained from whole blood collected using heparinized vacutainer tubes, with samples stored at −80 °C. The urine samples were collected as freshly voided random specimens free of preservatives.

Six THC standards were prepared using spiked human plasma and urine samples for quality control as follows. A total of six independent plasma matrices were prepared and each plasma matrix was spiked with varying amounts of THC from the previously prepared stock and working solutions.

2.6. Internal standard preparation

As previously described by Heath et al. and Snyder et al [20,21], β-17-estradiol acetate is a suitable internal standard because it conforms to chemical requirements for compounds used as internal standards, and it is well-resolved from the compound of interest and other peaks. The internal standard solutions were prepared with mobile phase reagent and the final concentration of internal standard was 1000 μg/mL. This reagent, when stored at ~80 °C, was stable for at least three months [21], based on chromatographic evidence indicating no apparent deterioration during that time period. The working internal standard (20 μg/mL) solution of estradiol acetate was prepared by further diluting (1:50) in mobile phase reagent C.

2.7. Analytical procedure and sample preparation

Two-hundred microlitres of standard, unknown and quality control samples were diluted 1:1 in de-ionized water and then vortexed for 20 seconds. 60 μL of internal standard, (20 μg/mL) β-17-estradiol acetate was then added, and sample was re-vortexed and then the samples were extracted with 700 μL extracting reagent. After centrifugation at 11,500 rpm for 2 min, the upper organic layer was removed into a clean micro-centrifuge tube and dried under a stream of nitrogen gas using a low heat setting. The sample was then re-suspended in 120 μL prepared mobile phase reagent C, vortexed for 30 s and left at room temperature in the dark for at least 10 min. Samples were vortexed again, and then transferred to an injection sample vial (about 100 μL) for HPLC quantification.

2.8. HPLC analytical run

THC in plasma and urine was separated and quantified by HPLC using ultraviolet (UV) detection at a wavelength of 280 nm (attenuation 0.200 AU). An aliquot (75 μL) was injected onto a reversed-phase column and eluted with a mobile phase containing a gradient mixture of reagent B (100%) for six minutes, and reagent C (100%) for 14 min, followed by reagent A (100%) column wash for 8 min. Flow rate was 1.0 mL/minute. The quantification of THC was accomplished by peak area ratio (THC to internal standard) and was based on a standard curve in a plasma or urine matrix and was generated by using an external standard to spike plasma or urine. A linear curve was generated from a single analysis of six different standard concentrations.

3. Validation results

3.1. Recovery of added THC

The amount of added THC recovered from plasma and urine pool samples was estimated in the high, medium and low concentration ranges. These concentrations were 0.500, 2.000 and 6.000 μg/mL for plasma and 0.500, 2.000 and 4.000 μg/mL for urine, respectively. These sample pools were prepared by the standard addition technique. Five estimations were made on each sample pool. The results are summarized in Table 1 and indicated that recovery of THC was >98.5% in the plasma and urine matrices.

3.2. Extraction efficiency

To determine the extraction efficiency of the method, the neat solution of THC containing the internal standard was analyzed at the low (1.0 μg/mL) and high (4.0 μg/mL) levels of concentration. Additionally, the corresponding low and high levels of THC in urine and plasma matrices were extracted with internal standard. All samples were assayed in the same analytical run.

The results show that the extraction efficiency of plasma and urine at the low concentration level was 95.0 and 106.6%, respectively. At the high concentration level, the extraction efficiency was 103.0% for both plasma and urine.
Table 1
Summary of results for recovery of added tetrahydrocurcumin

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Calculated concentration (µg/mL)</th>
<th>Number of assays</th>
<th>Recovered concentration (mean and [S.D.]) (µg/mL)</th>
<th>Coefficient of variation (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma, low</td>
<td>0.500</td>
<td>5</td>
<td>0.500 (0.000)</td>
<td>0.00</td>
<td>100.0</td>
</tr>
<tr>
<td>Plasma, med</td>
<td>2.000</td>
<td>5</td>
<td>1.970 (0.176)</td>
<td>9.0</td>
<td>98.5</td>
</tr>
<tr>
<td>Plasma, high</td>
<td>6.000</td>
<td>5</td>
<td>6.001 (0.080)</td>
<td>1.4</td>
<td>100.0</td>
</tr>
<tr>
<td>Urine, low</td>
<td>0.500</td>
<td>5</td>
<td>0.505 (0.005)</td>
<td>0.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Urine, med</td>
<td>2.000</td>
<td>5</td>
<td>2.034 (0.059)</td>
<td>2.9</td>
<td>101.7</td>
</tr>
<tr>
<td>Urine, high</td>
<td>4.000</td>
<td>5</td>
<td>4.055 (0.165)</td>
<td>4.1</td>
<td>101.4</td>
</tr>
</tbody>
</table>

A recovery extraction efficiency for the internal standard was between 95.4 and 104.1%. Note that the extraction efficiency results that exceed 100% are merely a reflection of analytical and or technical errors. This percentage error is within one standard deviation for our method, so the amount is small and technically not significant.

3.3. Estimation of accuracy and precision

Accuracy and precision was assessed from the results of replicate assays on three different sample pools in the high, medium and low concentration range. These samples were prepared in plasma and urine matrices by the standard addition technique. Five estimations were made on each sample pool during a 4-day period for plasma and a 3-day period for urine. The pool samples were prepared as fresh aliquots throughout the period of the experiment. The results are summarized in Table 2. The mean values (accuracy) of the prepared samples in plasma and urine matrices from different analysis were within 95% of their actual concentrations.

Table 2
Summary of within-run and run-to-run variation results: plasma

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Calculated concentration (µg/mL)</th>
<th>Number of assays</th>
<th>Concentration (mean and [S.D.]) (µg/mL)</th>
<th>Coefficient of variation (%)</th>
<th>Day of analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma, low</td>
<td>0.500</td>
<td>5</td>
<td>0.520 (0.005)</td>
<td>8.6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0.500</td>
<td>5</td>
<td>0.480 (0.022)</td>
<td>4.5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>0.500</td>
<td>5</td>
<td>0.498 (0.008)</td>
<td>3.0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>0.500</td>
<td>5</td>
<td>0.502 (0.031)</td>
<td>6.2</td>
<td>4</td>
</tr>
<tr>
<td>Plasma, med</td>
<td>3.000</td>
<td>5</td>
<td>2.990 (0.010)</td>
<td>2.8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3.000</td>
<td>5</td>
<td>2.990 (0.192)</td>
<td>6.4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3.000</td>
<td>5</td>
<td>3.068 (0.005)</td>
<td>1.2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3.000</td>
<td>5</td>
<td>3.009 (0.130)</td>
<td>4.3</td>
<td>4</td>
</tr>
<tr>
<td>Plasma, high</td>
<td>6.000</td>
<td>5</td>
<td>6.000 (0.158)</td>
<td>2.6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>6.000</td>
<td>5</td>
<td>6.333 (0.194)</td>
<td>3.9</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>6.000</td>
<td>5</td>
<td>5.974 (0.010)</td>
<td>1.7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>6.000</td>
<td>5</td>
<td>5.668 (0.180)</td>
<td>3.2</td>
<td>4</td>
</tr>
<tr>
<td>Urine, low</td>
<td>0.500</td>
<td>5</td>
<td>0.499 (0.018)</td>
<td>3.8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0.500</td>
<td>5</td>
<td>0.513 (0.010)</td>
<td>2.0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>0.500</td>
<td>5</td>
<td>0.492 (0.018)</td>
<td>3.6</td>
<td>3</td>
</tr>
<tr>
<td>Urine, med</td>
<td>1.500</td>
<td>5</td>
<td>1.472 (0.041)</td>
<td>2.8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1.500</td>
<td>5</td>
<td>1.325 (0.018)</td>
<td>1.2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1.500</td>
<td>5</td>
<td>1.508 (0.012)</td>
<td>0.8</td>
<td>3</td>
</tr>
<tr>
<td>Urine, high</td>
<td>5.000</td>
<td>5</td>
<td>5.122 (0.137)</td>
<td>2.7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5.000</td>
<td>5</td>
<td>5.063 (0.093)</td>
<td>1.8</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>5.000</td>
<td>5</td>
<td>5.060 (0.157)</td>
<td>3.1</td>
<td>3</td>
</tr>
</tbody>
</table>
The precision between replicates was determined to be between 1.1 and 10.5%. Linear regression analysis for day one was $R^2 = 0.997$, slope 0.0043 and intercept $-0.0363$. For succeeding days, linear regression results were $R^2 = 0.998$ and 0.991, with slopes of 0.0049 and 0.0106, and intercepts of 0.03829 and $-0.1351$, respectively.

3.5. Assessment of stability under freeze/thaw conditions

Stability of the THC in plasma and urine matrices was assessed from the results of replicate assays on three different sample pools. These sample pools were prepared by the standard addition technique. No preservative was added to any of the pooled samples.

Five estimations were made on each pool during three freeze/thaw cycles. Each pool sample was assayed on day one number, and subsequently, the balance of each pool sample was frozen at $-80^\circ$C for plasma, and at $-20^\circ$C for urine. On each succeeding day (cycle), the sample pool was removed from the freezer, thawed at room temperature in the dark, and then vortexed very gently. Next, an aliquot of 200 μL was removed and assayed. The remainder of the pooled samples were re-frozen at $-80^\circ$C and at $-20^\circ$C, respectively. This procedure was repeated for three freeze/thaw cycles.

The results are summarized in Table 3. The mean values of each sample pool in the plasma matrix were between 96% and 106% of the original prepared baseline values. For the urine matrix, after three freeze/thaw cycles, the mean values were between 92% and 102% of their original prepared baseline values.

3.6. Assessment of the limit of quantitation

The lower limit of quantitation was determined using four different sample pools. These were prepared using standard addition technique in plasma and urine matrices. The concentration of each pool was calculated to reflect values below 0.2 μg/mL. Five independent assays were done on each sample pool.

The lower limit of quantitation, the lowest concentration on the THC standard curve that was measured with acceptable accuracy, precision and variability, was determined to be 0.050 μg/mL. This value was within 5% of the original calculated plasma concentration. The lower limit of quantification obtained for the urine the urine matrix was 0.060 μg/mL. This value was within 2% of the original calculated urine concentration.

3.7. In vivo assay

C57Bl male and female mice (3–4 months of age) were administered the first oral dose of THC in a small-volume rodent chow paste after a 9-h fast, so that the experimenter could ensure that the mice ate all of the required dose. After allowing 4 h for absorption, mice were fed normal chow for 4 h before they were killed. The samples were collected and assayed as described in the previous sections.

### Table 3

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Number of assays</th>
<th>Freeze/thaw cycle number</th>
<th>Baseline concentration (μg/mL)</th>
<th>Concentration (mean [S.D.]) (μg/mL)</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool 1, plasma</td>
<td>5</td>
<td>0</td>
<td>0.600</td>
<td>0.601 (0.015)</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1</td>
<td>0.600</td>
<td>0.610 (0.029)</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
<td>0.600</td>
<td>0.586 (0.008)</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3</td>
<td>0.600</td>
<td>0.598 (0.014)</td>
<td>5.8</td>
</tr>
<tr>
<td>Pool 2, plasma</td>
<td>5</td>
<td>0</td>
<td>1.500</td>
<td>1.492 (0.005)</td>
<td>3.7</td>
</tr>
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<td>1.500</td>
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<td>1.490 (0.014)</td>
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</tr>
<tr>
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<td>5</td>
<td>3</td>
<td>1.500</td>
<td>1.367 (0.004)</td>
<td>6.9</td>
</tr>
<tr>
<td>Pool 3, plasma</td>
<td>5</td>
<td>0</td>
<td>3.000</td>
<td>3.200 (0.076)</td>
<td>2.3</td>
</tr>
<tr>
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<td>5</td>
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</tr>
<tr>
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<td>5</td>
<td>2</td>
<td>3.000</td>
<td>3.033 (0.065)</td>
<td>2.1</td>
</tr>
<tr>
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<td>5</td>
<td>3</td>
<td>3.000</td>
<td>3.0313 (0.030)</td>
<td>1.3</td>
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<tr>
<td>Pool 1, urine</td>
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<td>0.600</td>
<td>0.604 (0.015)</td>
<td>2.6</td>
</tr>
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<td>0.597 (0.015)</td>
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<td>0.604 (0.013)</td>
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<td>0.600</td>
<td>0.572 (0.027)</td>
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<td>Pool 2, urine</td>
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<td>1.498 (0.048)</td>
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<td>5</td>
<td>1</td>
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<td>1.500 (0.035)</td>
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<td>1.472 (0.155)</td>
<td>10.5</td>
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<tr>
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<td>1.500</td>
<td>1.391 (0.047)</td>
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</tbody>
</table>
Two hours after another 9-hour fast, mice were fed the second oral dose of THC in the small volume of rodent chow paste, and then were sacrificed 5 h later. Each dose was comprised of 74.4 μg (0.2 μmoles) of THC, mixed into a paste with 2.5 g rodent chow, which is 30 mg/kg body weight per day THC administration. THC solution was prepared to add to the rodent chow paste as THC working solution. The stock solution was made by dissolving THC in 0.5 Molar sodium hydroxide solution (74.4 mg/mL), and the working solution was made by diluting the stock solution, 1:100 in phosphate buffered saline (pH 7.4). Following the 9th fast, mice were observed to ravenously eat all of the 100 g of THC paste, leaving no trace of paste. Mice were first anesthetized with phenobarbital (40 mg/kg body weight i.p.) for blood sample collection. Blood was drawn from the abdominal aorta into EDTA-containing tubes, centrifuged at 1600 × g for 20 min at 4 °C, and then stored immediately at −80 °C until further analysis.

Fig. 2A and B show the chromatographic results. THC was recovered from pooled plasma of the THC fed mice (n = 3), Fig. 2B, yielding plasma concentration of 0.139 μg/mL while plasma of the control diet-fed mice (THC free standard diet; PMI 5015, PMI International LabDiet, St.Louis, MO) showed no THC (Fig. 2A). No plasma and urine matrix interference with the peaks of interest (THC and internal standard) was observed in the chromatograms following spiking of human plasma and urine.

4. Discussion

Several advantages of this new methodology described in this manuscript include shorter analytical run times (28 min compared to 50 min), an easily-obtainable internal standard, a single extraction step, and higher sensitivity, when compared with the methods described by Ireson et al. [8]. Our method had higher sensitivity in plasma and urine than previous methods. In our hands, other HPLC methods describing curcumin/tetrahydrocurcumin detections, including those reported by Pan et al. [6], Sugiyama et al. [5], Naito et al. [10], and Inano et al. [22], were not sufficiently reproducible or adaptable to measuring compounds in biological fluids.

Other advantages of this new methodology are that both recovery and extraction of added THC from plasma and urine matrices were high with this method, reducing error and improving efficiency of detection. This is necessary to compensate for the protein removal and sample loss and other extraction variations, such as efficiency and errors. Furthermore, we successfully recovered THC from pooled blood plasma from mice fed THC, demonstrating sensitivity to detecting THC in plasma after intestinal absorption of THC in chow. Additionally, chromatographic data from human urine and plasma analysis demonstrate first that the spiking of these controls (negative for THC) did not interfere with the peaks of interest (THC and internal standard), indicating the utility of the assay for measuring metabolites in body fluids collected from humans in curcumin clinical trials. Also, the THC could be totally recovered from spiked human urine and plasma, demonstrating the lack of interference of compounds in biological fluids with the assay. The freeze-thaw cycle did not reflect a variation of the THC concentration, and the coefficient of variation confirms stability of the compound during analysis and experimentation. Therefore, application of this method should permit an improved evaluation of the biological activities and role of this potentially important curcumin metabolite in future animal and human studies, with accurate, stable and reproducible results.

Because our analytical samples were not pre-treated prior to the extraction step, it is reasonable to assume that in our extracted chromatograms we are detecting total THC concentration, both bound and unbound, and including the unconjugated form of THC. Additional peaks that were detected may represent the conjugated forms of THC or curcumin, but this cannot be resolved without developing methods to produce and precisely detect the sulfated and glucuronidated standards. Because glucuronidated curcuminoins may also be bioactive, future directions may incorporate measuring metabolites both with and without added glucuronylase.

5. Conclusions

We have developed an HPLC method for the analysis of THC that has the advantages of being both relatively simple and practical for ease of use. This method has several advantages when compared with previously described methods.
because it is more rapid, accurate, precise, easily standard-
ized, and is capable of handling large batches. It has high
sensitivity and relatively short analysis time. The method pre-
sented here will be a useful analytical tool in the future for
the determination of THC in plasma and urine for wide range
of clinical research applications.

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