



## 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  $\beta$ -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  $\mu$ g/mL in plasma, and 0.060 to 6.0  $\mu$ g/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 [1,4–10], 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  $\beta$ -diketone structures and

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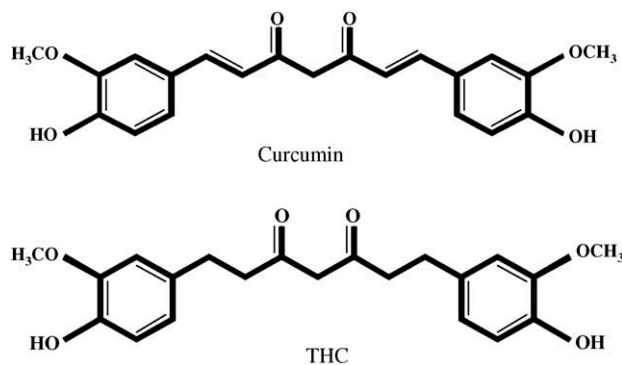


Fig. 1. Chemical structures of curcumin and its major metabolite, tetrahydrocurcumin (THC). Curcumin and THC have similar  $\beta$ -diketone structures and phenolic groups. Circle shows the major difference of their H-bonding side, which may make THC less polar than curcumin.

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  $\beta$ -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 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  $\times$  150 mm, 5  $\mu$ m C18 Column (Waters, Milford, MA, USA). The column was coupled with an Alltech Prevail Amide 30 mm  $\times$  4.6 mm, C18 guard column (Alltech Associates, Deerfield, IL, USA).

### 2.3. Mobile phase reagent preparation

#### 2.3.1 Reagent A: acetonitrile

#### 2.3.2 Reagent B: ammonium sulfate

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- $\mu$ m filter (Millipore, Bedford, MA, USA) and then degassed by a solvent de-gas system (Fisher Scientific, Pittsburg, PA, USA).

#### 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- $\mu$ m filter (Millipore, Bedford, MA, USA) and degassed (Fisher Scientific, Pittsburg, PA, USA). This reagent was stable at room temperature for at least 4 weeks.

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 also have 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:quinone 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 radiolabeling 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

#### 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 in urine. The plasma was obtained from whole blood collected using heparinized vacutainer tubes, with samples stored at  $-80^{\circ}\text{C}$ . 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],  $\beta$ -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  $-80^{\circ}\text{C}$ , 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)  $\beta$ -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 quantitation 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. The extrac-

Table 1  
Summary of results for recovery of added tetrahydrocurcumin

Sample type	Calculated concentration (µg/mL)	Number of assays	Recovered concentration (mean and (S.D.)) (µg/mL)	Coefficient of variation (%)	Recovery (%)
Plasma, low	0.500	5	0.500 (0.000)	0.00	100.0
Plasma, medium	2.000	5	1.970 (0.176)	8.90	98.5
Plasma, high	6.000	5	6.001 (0.080)	1.40	100.0
Urine, low	0.500	5	0.505 (0.005)	0.90	101.0
Urine, medium	2.000	5	2.034 (0.059)	2.90	101.7
Urine, high	4.000	5	4.055 (0.165)	4.10	101.4

tion 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 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 matri-

ces from different analysis were within 95% of their actual concentrations.

### 3.4. Precision around the standard curve

The precision around the standard curve was calculated as a ratio of the area count of the THC divided by the area count of the internal standard. For the plasma standard curve, six different concentration points were prepared covering the range 0.25–6.0 µg/mL. Each standard was analyzed once during a four-day period. The precision between replicates was determined to be between 3.7 and 9.8%. Linear regression analysis for day one was  $R^2 = 0.999$ , slope 0.0108 and intercept 0.0188. For succeeding days, linear regression results were  $R^2 = 0.995$  and  $0.999$ , with slopes of 0.0116 and 0.0112, and intercepts of  $-0.0544$  and  $-0.0159$ , respectively.

For the urine standard curve, six different concentration points were prepared, covering the range 0.25–6.0 µg/mL. Each standard was analyzed once during a three-day period.

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

Sample type	Calculated concentration (µg/mL)	Number of assays	Concentration (mean and (S.D.)) (µg/mL)	Coefficient of variation (%)	Day of analysis
Plasma, low	0.500	5	0.520 (0.045)	8.6	1
	0.500	5	0.480 (0.022)	4.5	2
	0.500	5	0.498 (0.008)	3.0	3
	0.5000	5	0.502 (0.031)	6.2	4
Plasma, medium	3.000	5	2.980 (0.010)	2.8	1
	3.000	5	2.990 (0.192)	6.4	2
	3.000	5	3.068 (0.005)	1.2	3
	3.000	5	3.004 (0.130)	4.3	4
Plasma, high	6.000	5	6.000 (0.158)	2.6	1
	6.000	5	6.333 (0.194)	3.9	2
	6.000	5	5.974 (0.101)	1.7	3
	6.000	5	5.668 (0.180)	3.2	4
Urine, low	0.500	5	0.493 (0.018)	3.8	1
	0.500	5	0.511 (0.010)	2.0	2
	0.500	5	0.492 (0.018)	3.6	3
Urine, medium	1.5000	5	1.472 (0.041)	2.8	1
	1.500	5	1.525 (0.018)	1.2	2
	1.500	5	1.508 (0.012)	0.8	3
Urine, high	5.000	5	5.122 (0.137)	2.7	1
	5.000	5	5.063 (0.093)	1.8	2
	5.000	5	5.000 (0.157)	3.1	3

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 number one, and subsequently, the balance of each pool sample was frozen at  $-80^\circ\text{C}$  for plasma, and at  $-20^\circ\text{C}$  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  $\mu\text{L}$  was removed and assayed. The remainder of the pooled samples were re-frozen at  $-80^\circ\text{C}$  and at  $-20^\circ\text{C}$ , 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  $\mu\text{g/mL}$ . 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  $\mu\text{g/mL}$ . This value was within 5% of the original calculated plasma concentration. The lower limit of quantitation obtained for the urine the urine matrix was 0.060  $\mu\text{g/mL}$ . This value was within 2% of the original calculated urine concentration.

### 3.7. In vivo assays

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

Table 3  
Summary of plasma and urine freeze/thaw cycle results

Sample type	Number of assays	Freeze/thaw cycle number	Baseline concentration ( $\mu\text{g/mL}$ )	Concentration (mean [S.D.]) ( $\mu\text{g/mL}$ )	Coefficient of variation (%)
Pool 1, plasma	5	0	0.600	0.601 (0.015)	2.5
	5	1	0.600	0.610 (0.029)	4.7
	5	2	0.600	0.586 (0.008)	1.3
	5	3	0.600	0.594 (0.034)	5.8
Pool 2, plasma	5	0	1.500	1.492 (0.005)	3.7
	5	1	1.500	1.436 (0.052)	3.6
	5	2	1.500	1.490 (0.014)	0.9
	5	3	1.500	1.367 (0.094)	6.9
Pool 3, plasma	5	0	3.000	3.200 (0.076)	2.3
	5	1	3.000	2.934 (0.007)	2.4
	5	2	3.000	3.038 (0.065)	2.1
	5	3	3.000	3.0313 (0.038)	1.3
Pool 1, urine	5	0	0.600	0.604 (0.015)	2.6
	5	1	0.600	0.597 (0.015)	2.5
	5	2	0.600	0.604 (0.013)	2.2
	5	3	0.600	0.572 (0.027)	4.7
Pool 2, urine	5	0	1.500	1.498 (0.048)	3.2
	5	1	1.500	1.500 (0.035)	2.3
	5	2	1.500	1.472 (0.155)	10.5
	5	3	1.500	1.391 (0.047)	3.3
Pool 3, urine	5	0	3.000	3.073 (0.109)	3.6
	5	1	3.000	3.050 (0.073)	2.4
	5	2	3.000	2.987 (0.033)	1.1
	5	3	3.000	3.070 (0.090)	2.9

316 two hours. After another 9-hour fast, mice were fed the second  
317 oral dose of THC in the small volume of rodent chow paste,  
318 and then were sacrificed 5 h later. Each dose was comprised  
319 of 74.4  $\mu\text{g}$  (0.2  $\mu\text{moles}$ ) of THC, mixed into a paste with  
320 2.5 g rodent chow, which is 30 mg/kg body weight per day  
321 THC administration. THC solution was prepared to add to  
322 the rodent chow paste as THC working solution. The stock  
323 solution was made by dissolving THC in 0.5 molar sodium  
324 hydroxide solution (74.4 mg/mL), and the working solution  
325 was made by diluting the stock solution, 1:100 in phosphate  
326 buffered saline (pH 7.4). Following the 9 h fast, mice were  
327 observed to ravenously eat all of the 100  $\mu\text{l}$  of THC paste,  
328 leaving no trace of paste. Mice were first anesthetized with  
329 phenobarbital (40 mg/kg body weight i.p.) for blood sample  
330 collection. Blood was drawn from the abdominal aorta into  
331 EDTA-containing tubes, centrifuged at  $1600 \times g$  for 20 min  
332 at  $4^\circ\text{C}$ , and then stored immediately at  $-80^\circ\text{C}$  until further  
333 analysis.

334 Fig. 2A and B show the chromatographic results; THC was  
335 recovered from pooled plasma of the THC fed mice ( $n = 3$ ,  
336 Fig. 2B), yielding plasma concentration of 0.139  $\mu\text{g/mL}$ ,  
337 while plasma of the control diet-fed mice (THC free stan-  
338 dard diet; PMI 5015, PMI International LabDiet, St.Lois,  
339 MO) showed no THC (Fig. 2A). No plasma and urine matrix  
340 interference with the peaks of interest (THC and internal stan-  
341 dard) was observed in the chromatograms following spiking  
342 of human plasma and urine.

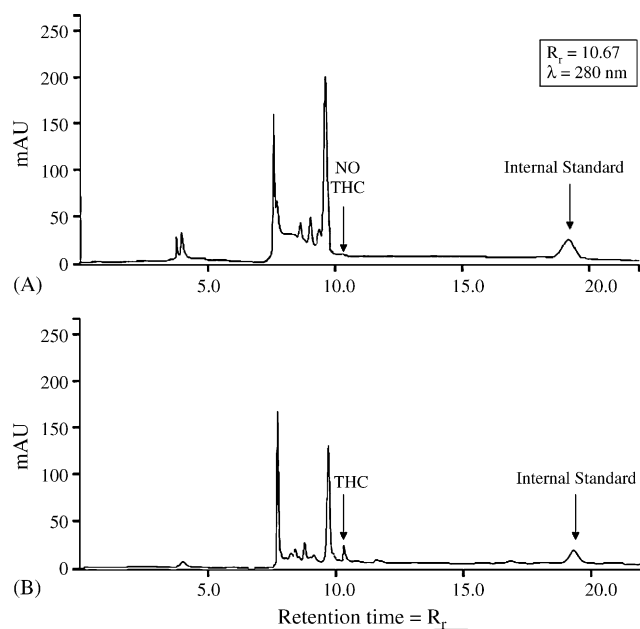


Fig. 2. High performance liquid chromatogram of extracted plasma and internal standard from mice ( $n = 3$ ). A. Control group, in which mice were fed regular chow without THC and B. The THC group in which mice fasting for 9 h were fed THC (74.4  $\mu\text{g}$  or 30 mg/kg body weight) in a paste of 100  $\mu\text{L}$  volume (mixed with chow). Waters Symmetryshield C18 column (150 mm  $\times$  3.9 mm, 5  $\mu\text{m}$  particle size), ultraviolet detection at 280 nm, flow rate 1.0 mL/min, and mobile phase reagent B (100%) for 6 min, then reagent C (100%) for 14 min, and reagent A 100% for 8 min. The retention times for THC and B17-estradiol acetate were 10.6 and 19.9 min, respectively.

#### 4. Discussion

343  
344 Several advantages of this new methodology described in  
345 this manuscript include shorter analytical run times (28 min  
346 compared to 50 min), an easily-obtainable internal standard,  
347 a single extraction step, and higher sensitivity, when com-  
348 pared with the methods described by Ireson et al. [8]. Our  
349 method had higher sensitivity in plasma and urine than pre-  
350 vious methods. In our hands, other HPLC methods descri-  
351 bing curcumin/tetrahydrocurcumin detections, including those  
352 reported by Pan et al. [6], Sugiyama et al. [5], Naito et al.  
353 [10], and Inano et al. [22], were not sufficiently reproducible  
354 or adaptable to measuring compounds in biological fluids.

355 Other advantages of this new methodology are that both  
356 recovery and extraction of added THC from plasma and urine  
357 matrices were high with this method, reducing error and  
358 improving efficiency of detection. This is necessary to com-  
359 pensate for the protein removal and sample loss and other  
360 extraction variations, such as efficiency and errors. Further-  
361 more, we successfully recovered THC from pooled blood  
362 plasma from mice fed THC, demonstrating sensitivity to  
363 detecting THC in plasma after intestinal absorption of THC in  
364 chow. Additionally, chromatographic data from human urine  
365 and plasma analysis demonstrate first that the spiking of these  
366 controls (negative for THC) did not interfere with the peaks of  
367 interest (THC and internal standard), indicating the utility of  
368 the assay for measuring metabolites in body fluids collected  
369 from humans in curcumin clinical trials. Also, the THC could  
370 be totally recovered from spiked human urine and plasma,  
371 demonstrating the lack of interference of compounds in bio-  
372 logical fluids with the assay. The freeze-thaw cycle did not  
373 reflect a variation of the THC concentration, and the coeffi-  
374 cient of variation confers stability of the compound during  
375 analysis and experimentation. Therefore, application of this  
376 method should permit an improved evaluation of the biolog-  
377 ical activities and role of this potentially important curcumin  
378 metabolite in future animal and human studies, with accurate,  
379 stable and reproducible results.

380 Because our analytical samples were not pre-treated prior  
381 to the extraction step, it is reasonable to assume that in our  
382 extracted chromatograms we are detecting total THC concen-  
383 tration, both bound and unbound, and including the uncon-  
384 jugated form of THC. Additional peaks that were detected  
385 may represent the conjugated forms of THC or curcumin,  
386 but this cannot be resolved without developing methods to  
387 produce and precisely detect the sulfated and glucuronidated  
388 standards. Because glucuronidated curcuminoids may also  
389 be bioactive, future directions may incorporate measuring  
390 metabolites both with and without added glucuronidase.

#### 5. Conclusions

391  
392 We have developed an HPLC method for the analysis of  
393 THC that has the advantages of being both relatively simple  
394 and practical for ease of use. This method has several advan-  
395 tages when compared with previously described methods

because it is more rapid, accurate, precise, easily standardized, and is capable of handling large batches. It has high sensitivity and relatively short analysis time. The method presented 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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