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Rapid Analysis of Curcumin and Curcumin Metabolites in Rat Biomatrices Using a Novel Ultraperformance Liquid Chromatography (UPLC) Method

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The bioavailability of the putative cancer chemopreventive agent curcumin is limited, making measurement either in target tissues or in biofluids difficult and variable between studies. The purposes of these investigations were to develop validated methods of extraction of curcumin from biomatrices and of detection of curcumin and its conjugated metabolites using ultraperformance liquid chromatography (UPLC) and to identify metabolites of curcumin using online tandem mass spectrometry (MS/MS). The limit of detection for curcumin after solid-phase extraction from plasma or urine was 2.5 ng/mL. Extraction efficiencies were 62 and 64% for urine and plasma. Intra- and interday variabilities (RSD) for extraction of curcumin from biofluids were less than 10 and 15%, respectively, and accuracies were 92 ± 10% for plasma and 95 ± 6% for urine. Curcumin was extracted from tissues using protein precipitation with quercetin as internal standard. Curcumin extraction from intestinal mucosa spiked with 0.2, 1, and 5 μg/g curcumin was validated. Extraction efficiency was 65−84%, accuracy was 94−106%, limit of detection was 12.5 ng/g, and intra- and interday variabilitiies (RSD) were 0.7−4.9 and 4.9−5.5%, respectively. The methods were applied to measure curcumin in tissues from rats that had received oral curcumin (340 mg/kg). Curcumin was found in plasma (16.1 ng/mL), urine (2.0 ng/mL), intestinal mucosa (1.4 mg/g), liver (3671.8 ng/g), and, for the first time, kidney (206.8 ng/g) and heart (807.6 ng/g). Curcumin metabolites identified by UPLC-MS/MS in plasma and urine were phenolic glucuronides and, probably, alcoholic glucuronides. Products of reduction of curcumin and their metabolites were found in the liver. The methods described here represent improvements on existing analytical methods for curcuminoids and metabolites in terms of sensitivity, speed, and separation.

KEYWORDS: Curcumin; UPLC; analysis; rat; plasma; urine

INTRODUCTION

The incidence of cancer and other degenerative disorders continues to rise in an increasingly aging population. Early diagnosis of individuals at risk of cancer has rendered chemopreventive intervention strategies possible. The diet is considered to be a major source of novel and safe potential cancer chemopreventive agents (*1*). Curcumin [1, 7-bis(4-hydroxyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], the primary constituent of turmeric, a spice extracted from the root of *Curcuma longa* Linn., has undergone extensive preclinical and some early clinical evaluation as a putative cancer chemopreventive agent. In vitro, curcumin acts as a powerful antioxidant, suppresses activation of the transcription factor NFκB and other signaling molecules involved in carcinogenesis (*2−5*), and inhibits cancer cell proliferation (*1−7*). In rodents, curcumin can delay carcinogenesis in the colon, skin, stomach, duodenum, soft palate, tongue, sebaceous glands, and breast (*8−10*), whereas in clinical pilot studies curcumin caused some incidences of regression of premalignant lesions in small studies of bladder, soft palate, stomach, cervix, and skin (*11, 12*). During these investigations only low concentrations of curcumin were detected in plasma and target tissues, probably as a consequence of rapid metabolism and clearance (*13−17*). Curcumin is metabolized via conjugation (glucuronidation and sulfation) and reduction (*13, 14, 17*) and (*18*). Two glucuronide metabolites have been identified following incubation with rat and human microsomes and recombinant human UDP-glucuronyltransferases (*19−21*): a predominant phenolic and a minor alcoholic glucuronide. In addition, glutathione conjugation has been demonstrated in vitro

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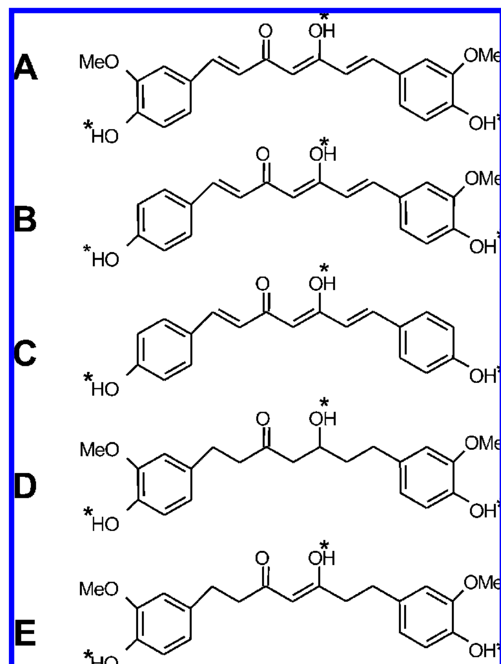

Figure 1. Structures of curcumin (**A**), desmethoxycurcumin (**B**), bisdesmethoxycurcumin (**C**), hexahydrocurcumin (**D**), and tetrahydrocurcumin (**E**). Asterisks indicate potential sites for conjugation.

(*22*). Several strategies have been employed to improve on the limited bioavailability of curcumin including coadministration with piperine, an inhibitor of glucuronidation (*23*), or formulation with phosphatidylcholine (*17*). In the latter study in rats, a phosphatidylcholine formulation increased plasma C_{max} and AUC for curcumin and curcumin glucuronide 5- and 20-fold, respectively, compared with unformulated curcumin (*17*).

Low levels of curcumin in human biomatrices and extensive metabolism following oral dosing (*11, 15, 16*) suggest that further clinical development of curcumin would benefit from highly sensitive analytical methods for measurement of curcumin and its metabolites. Ultraperformance liquid chromatography (UPLC) represents an innovation over standard HPLC whereby the ability to withstand high back pressures enables the use of sub 2 μ stationary phase, which increases separating power and sensitivity and decreases run time. We report here the development and validation of a novel sensitive UPLC method for the quantitation of curcumin in plasma, urine, and intestinal mucosa. The method was applied to detect and quantitate levels of parent curcuminoids and their metabolites in biomatrices of rats after administration of Meriva, a curcumin formulation with phosphatidylcholine. Furthermore, the chemical identity of the curcuminoid species detected was confirmed by mass spectrometry.

MATERIALS AND METHODS

Chemicals and Reagents. Curcumin (CAS Registry No. 458-37-7) and Meriva, a curcumin formulation with EpiKuron 130 P (a deoiled, powdered soybean lecithin enriched with 30% phosphatidylcholine), were supplied by Indena Spa (Milan, Italy). Meriva contained 16.89% curcuminoids, of which 93.82% was curcumin, and the ratio of curcumin to Epikuron 130 P was 1:4. Curcumin obtained by extraction from *Curcuma* spp. contained 94% curcuminoids, of which 77% was curcumin, 17% desmethoxycurcumin, and 6% bisdesmethoxycurcumin as determined by HPLC (for structures see **Figure 1**). β-Glucuronidase type HP-2 from *Helix pomatia* (111,400 units/mL) was purchased from Sigma-Aldrich (Poole, U.K.).

UPLC and UPLC-MS/MS Analyses. Measurement of curcumin and separation of curcumin metabolites was achieved using UPLC (Acquity, Waters Corp., Elstree, U.K.) with an Acquity UPLC BEH RP18 (2.1 × 100 mm, 1.7 μm) column at 35 °C. Mobile phases comprised (A) 5% aqueous acetonitrile adjusted to pH 3.5 with acetic acid and (B) acetonitrile. Resolution of curcuminoids and their metabolites was achieved with a gradient system comprising 0−0.2 min, 15% B, flow rate = 0.54 mL/min; at 10 min, 45% B at 0.6 mL/min; at 14 min, 52% B at 0.6 mL/min. All gradient changes in composition and flow rate were linear. The column was washed with 100% B for 2 min at 0.85 mL/min before re-equilibration. Eluant was monitored at 426 nm with an Acquity TUV detector (Waters Corp.).

Table 1. Transitions for Curcuminoids and Their Metabolites Investigated in Rat Biofluids and Tissues

curcuminoid	MRM transition (*m*/*z*, amu)
curcumin	367> 134
desmethoxycurcumin	337> 119
bisdesmethoxycurcumin	307> 119
curcumin glucuronide	543> 134
desmethoxycurcumin glucuronide	513> 119
bisdesmethoxycurcumin glucuronide	483> 119
curcumin sulfate	447> 134
desmethoxycurcumin sulfate	417> 119
bisdesmethoxycurcumin sulfate	387> 119
curcumin glucuronide sulfate	623> 134
curcumin diglucuronide	719> 134
curcumin disulfate	527> 134
curcumin glutathione	676>367,676>134
dihydrocurcumin	369> 135
tetrahydrocurcumin	371> 135
hexahydrocurcumin	373> 179
octahydrocurcumin	375> 179
hexahydrocurcuminol	374> 134
dihydrocurcumin glucuronide	545> 135
tetrahydrocurcumin glucuronide	547> 135
hexahydrocurcumin glucuronide	549> 179
octahydrocurcumin glucuronide	551> 179
dihydrocurcumin sulfate	449> 135
tetrahydrocurcumin sulfate	451> 135
hexahydrocurcumin sulfate	453> 179
octahydrocurcumin sulfate	455> 179

Curcuminoids were identified by negative ion electrospray tandem mass spectrometry. Analysis was performed by incorporating an API 2000 MS/MS (Applied Biosystems MDS Sciex, Warringon, U.K.) in line with the Acquity UPLC, bypassing the UV detector. Separation of curcumin and metabolites was achieved as described above, MS/MS conditions were as previously published (*17*). Identification of curcuminoids was by multiple reaction monitoring (MRM) using suitable transitions (**Table 1**).

Validation. Measurement of curcumin by UPLC was validated according to FDA guidelines (*24*). Specificity was determined by comparing analyses in authentic biomatrices or those spiked with 0.5 μg/mL curcumin. Range and linearity were determined for curcumin extracted from urine, plasma, and intestinal mucosa. Reproducibility for curcumin retention time was calculated following 15 injections of 50 ng on column. Efficiency of extraction was determined for five samples of plasma and urine spiked with 50 ng/mL curcumin and for three concentrations of curcumin-spiked intestinal mucosa ($n = 3$, 200, 1000, and 5000 ng/g) on three separate days. Accuracy was determined using similarly spiked samples by comparing observed with expected concentrations. Limits of quantitation and detection were calculated for plasma, urine, and mucosa and were defined as the lowest concentrations producing signal/noise ratios of at least 7 and 3, respectively.

In Vitro Curcumin Glucuronide Synthesis and Deconjugation. Curcumin (10 μg) was incubated at 37 °C for 30 min with a rat hepatic microsomal preparation (0.42 mg of protein), UDPGA (4.79 mM), sodium phosphate buffer (0.2 M, pH 7.6), $MgCl_2$ (40 mM), and 5 μL Triton X-100 (0.2%). Following incubation, the reaction mixture was divided into 10 μL aliquots, and the glucuronidation reaction was stopped by protein precipitation with ice-cold acetone (100 μL). Protein was removed by centrifugation (6000g, 5 min) following sample chilling at −20 °C for 20 min. Supernatant was filtered through a 47 mm, 0.2

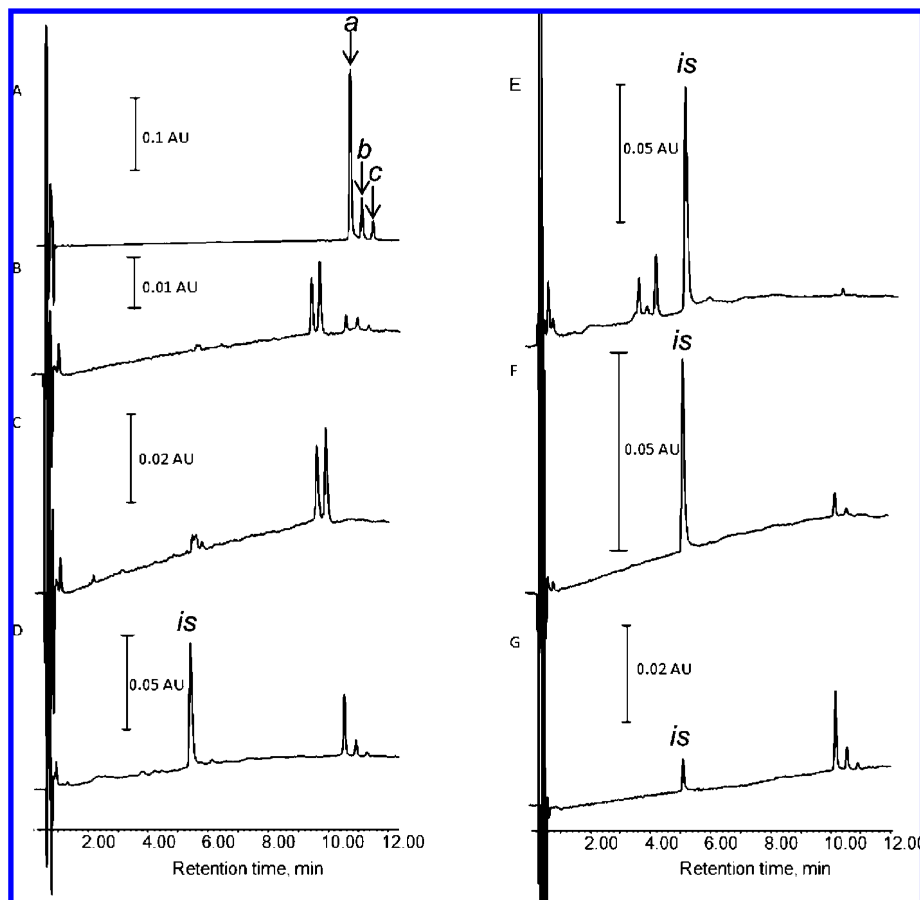

Figure 2. UPLC chromatograms of curcuminoids in authentic Meriva (**A**) and extracts of plasma (**B**), urine (**C**), liver (**D**), kidney (**E**), heart (**F**), and intestinal mucosa (**G**) obtained from rats that had received Meriva (340 mg/kg). Authentic curcuminoids are curcumin (*a*), desmethoxycurcumin (*b*), and bisdesmethoxycurcumin (*c*). For tissue extractions, quercetin was incorporated as an internal standard (*is*).

μm microdisk filter (Waters Corp.) and then dried under a constant stream of nitrogen. Samples were resuspended in 12 μL of methanol/glacial acetic acid (95:5) and analyzed by UPLC. Alternatively, aliquots (10 μL) of reaction mixture were incubated with β-glucuronidase (220 units) or water for 20 min at 37 °C followed by protein precipitation with 20 μL of acetone, centrifugation (16000*g*, 5 min), drying of supernatant under nitrogen, and resuspension in 12 μL of methanol/glacial acetic acid (95:5).

Animal Treatment and Sample Collection. Dosing suspensions were prepared in 1% methylcellulose at 17 mg of curcumin/mL. Experiments were carried out under animal project license PPL 40/2496, granted to Leicester University by the U.K. Home Office. The experimental design was vetted by the Leicester University Local Ethical Committee for Animal Experimentation and met the standards required by the UKCCCR for animal welfare (*25*). Male Wistar albino rats (250 g, Harlan U.K. Ltd., Bicester, U.K.) were fasted overnight and received either Meriva at 340 mg/kg (in terms of curcumin) or vehicle alone by oral gavage ($n = 3$). At 20 min postdose, animals were exsanguinated under terminal anesthesia. Whole blood was collected by cardiac puncture into heparinized tubes and centrifuged at 6000*g* for 15 min. Plasma was decanted and stored at −80 °C until analysis. Liver, kidneys, heart, and gastrointestinal tract from stomach to anus were removed. The intestinal tract was flushed with ice-cold phosphate-buffered saline, dissected longitudinally, and then washed a second time to remove residual gut contents. Mucosa was collected from the small intestine and colon by scraping gently with a spatula. Liver, heart, kidney, and intestinal mucosa were flash-frozen in liquid nitrogen and stored at −80 °C for no more than 4 weeks prior to analysis.

Curcumin and curcumin metabolites were extracted from plasma and urine by solid phase extraction (SPE). Plasma (0.5 mL) or urine (0.3 mL) was diluted to 1 mL with water, acidified with orthophosphoric acid (0.5% v/v final concentration), and loaded onto a preconditioned 1 cm^3 Oasis HLB cartridge (Waters), which was washed with 25:25:1 methanol/water/glacial acetic acid (1 mL) and eluted in 1 mL of methanol containing 2% glacial acetic acid. Eluant was evaporated to dryness at 45 °C under a stream of nitrogen, and the residue was resuspended in 12 μL of methanol/glacial acetic acid (95:5).

To allow quantitation, standard solutions of curcumin (2.5−20 ng/mL) were prepared in 1 mL of human plasma (obtained from the National Blood Transfusion Centre, Sheffield, U.K.) or urine from a healthy male volunteer and extracted as described above.

The limited availability of tissue samples for generating external standard curves for quantitation of curcumin in tissues led us to develop an internal standard method of quantitation using quercetin. Mucosa (10 mg) was suspended in 1.15% KCl (0.1 mL) and centrifuged (16000*g*, 60 s). The pellet was resuspended in fresh 1.15% KCl (0.1 mL), and 20 μg of quercetin was added as internal standard prior to homogenization with a blade homogenizer. Protein was precipitated with 0.2 mL of acetone/formic acid (9:1), and the mixture was vortexed and kept at −20 °C for 20 min prior to centrifugation (16000*g*, 10 min). The supernatant was decanted and evaporated to dryness at 45 °C under a stream of nitrogen. The residue was resuspended in 500 μL of methanol/glacial acetic acid (95:5) prior to injection (10 μL). Liver, kidneys, and heart were homogenized 1:1 in PBS. An aliquot (0.2 mL) of homogenate was mixed with 40 μg of quercetin followed by 0.4 mL of acetone/formic acid (9:1), and the mixture was immediately vortexed. Samples were kept at −20 °C for 20 min prior to centrifugation (16000*g*, 10 min). Supernatant was evaporated to dryness at 45 °C under a stream of nitrogen, and the residue was resuspended in 25 μL of methanol/glacial acetic acid (95:5) prior to analysis.

All sample processing was conducted, when possible, on ice and using amber Eppendorf tubes to prevent light-dependent curcumin degradation. Standard curves were generated by plotting curcumin

Table 2. Validation Parameters for the Extraction and Measurement of Curcumin in Plasma, Urine, and Intestinal Mucosa

parameter	value
Plasma	
extraction efficiency	64%
intraday variability (RSD[a])	1.9%
interday variability (RSD)	9.0%
limit of detection	2.5 ng/mL
limit of quantification	5.0 ng/mL
range	5−350 ng/mL
linearity (5−200 ng/mL)	R^2 = 0.994
accuracy	92 ± 10%
Urine	
extraction efficiency	62%
intraday variability (RSD)	8.6%
interday variability (RSD)	14.7%
limit of detection	1.25 ng (2.5 ng/mL)
limit of quantification	2.50 ng (5.0 ng/mL)
range	5−700 ng/mL
linearity (5−200 ng/mL)	R^2 = 0.999
accuracy	95 ± 6%
Mucosa	
extraction efficiency	
200 ng/g	65%
1000 ng/g	80%
5000 ng/g	84%
intraday variability (RSD)	
200 ng/g	4.9%
1000 ng/g	4.9%
5000 ng/g	0.7%
interday variability (RSD)	
200 ng/g	4.9%
1000 ng/g	5.2%
5000 ng/g	5.5%
limit of detection	1.25 ng (125 ng/g)
limit of quantification	5.0 ng (500 ng/g)
range	500−5000 ng/g
linearity (5−5000 ng/mL)	R^2 = 0.999
accuracy	
200 ng/g	94 ± 5%
1000 ng/g	108 ± 8%
5000 ng/g	106 ± 5%

[a] RSD, relative standard deviation (coefficient of variation × 100%).

concentration against peak response (peak area curcumin/[peak area quercetin × quercetin concentration]).

RESULTS

Validation of UPLC Analysis of Curcumin in Rat Plasma, Urine, and Intestinal Mucosa. The UPLC method described above achieved near-baseline separation of the three curcuminoids found in commercially available preparations, curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin (**Figure 2A**). Its specificity is reflected by the absence of peaks coeluting with curcuminoids in control biomatrices (data not shown). The method was reproducible with 15 injections giving a mean curcumin retention time of 10.09 min and a RSD of 0.05%. The limits of detection (LOD) and quantitation (LOQ) for authentic, nonextracted curcumin were 0.5 and 1.0 ng on column, respectively.

Validation parameters for the extraction of curcumin from plasma and urine using SPE are presented in **Table 2** and demonstrate acceptable levels of extraction efficiency, reproducibility, and accuracy (*24*). Overall, the relative standard deviations (RSD%) for the extraction and measurement of curcumin from plasma and urine over 3 days were 9 and 15%, respectively, which comply with the guidelines of the FDA (*24*). Likewise, the protein precipitation method employed for extraction of curcumin from tissue was accurate and robust, meeting FDA guidelines (*24*) with RSDs of 4.9, 5.2, and 5.5% for interday extraction variability of 200, 1000, and 5000 ng/g spiked curcumin, respectively.

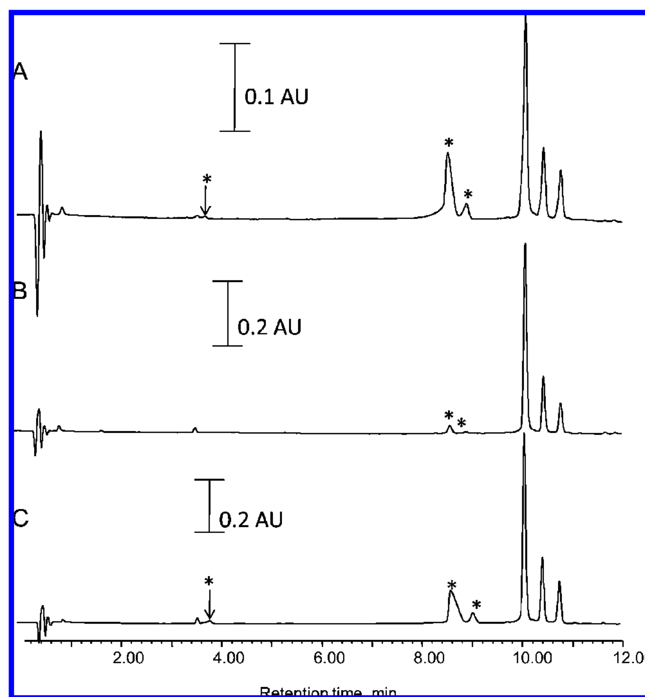

Figure 3. In vitro identification of glucuronide metabolites. Curcuminoids were incubated with microsomes and then analyzed for metabolites by UPLC (**A**). Incubates were also analyzed by UPLC following further incubation either with (**B**) or without (**C**) β-glucuronidase. Potential monoglucuronides of curcuminoids are indicated with an asterisk, and a solid arrow points to a more hydrophilic metabolite, which is also decreased by β-glucuronidase treatment.

Identification of Curcuminoids in Rat Biomatrices. In an orientation experiment conducted using rat hepatic microsomes in vitro, putative metabolite peaks were found by UPLC-UV (**Figure 3A**). On incubation of microsomal samples with β-glucuronidase, peak heights were reduced compared to incubates omitting enzyme (**Figure 3B**,**C**), suggesting that most metabolites were glucuronides, in accordance with past investigations (*13, 14, 17, 18*). The pattern of metabolites observed by UPLC-UV in the plasma and urine of rats that had received Meriva (**Figure 2B**,**C**) was similar to that in microsomal incubates. Analysis of plasma and tissues by UPLC-MS/MS clearly identified parent curcuminoids (**Figure 4A−E**). In plasma, in addition to parent curcuminoids, monoglucuronides of curcumin (*m/z* 543 > 134) at retention times predicted by the studies described above, desmethoxycurcumin glucuronide (*m/z* 513 > 119), bisdesmethoxycurcumin glucuronide (*m/z* 483 > 119), and desmethoxycurcumin monosulfate (*m/z* 417 > 119) were identified (**Figure 4A**). Metabolites identified in both liver and kidney were hexahydrocurcumin (*m/z* 373 > 179) and its sulfate (*m/z* 453 > 135), and liver also contained dihydrocurcumin (*m/z* 369 > 135), tetrahydrocurcumin (*m/z* 371 > 135), and tetrahydrocurcumin sulfate (*m/z* 451 > 135) (**Figure 4B**,**C**). No metabolites were identified in either heart or mucosa (**Figure 4D**,**E**).

Concentrations of Curcumin and Curcumin Metabolites in Rat Biomatrices. Curcumin levels in plasma, urine, and tissues from rats that had received an oral dose of Meriva were measured using the UPLC method described above, and the results are presented in **Table 3**. Plasma was collected after 20 min, a time previously shown to be a compromise between the

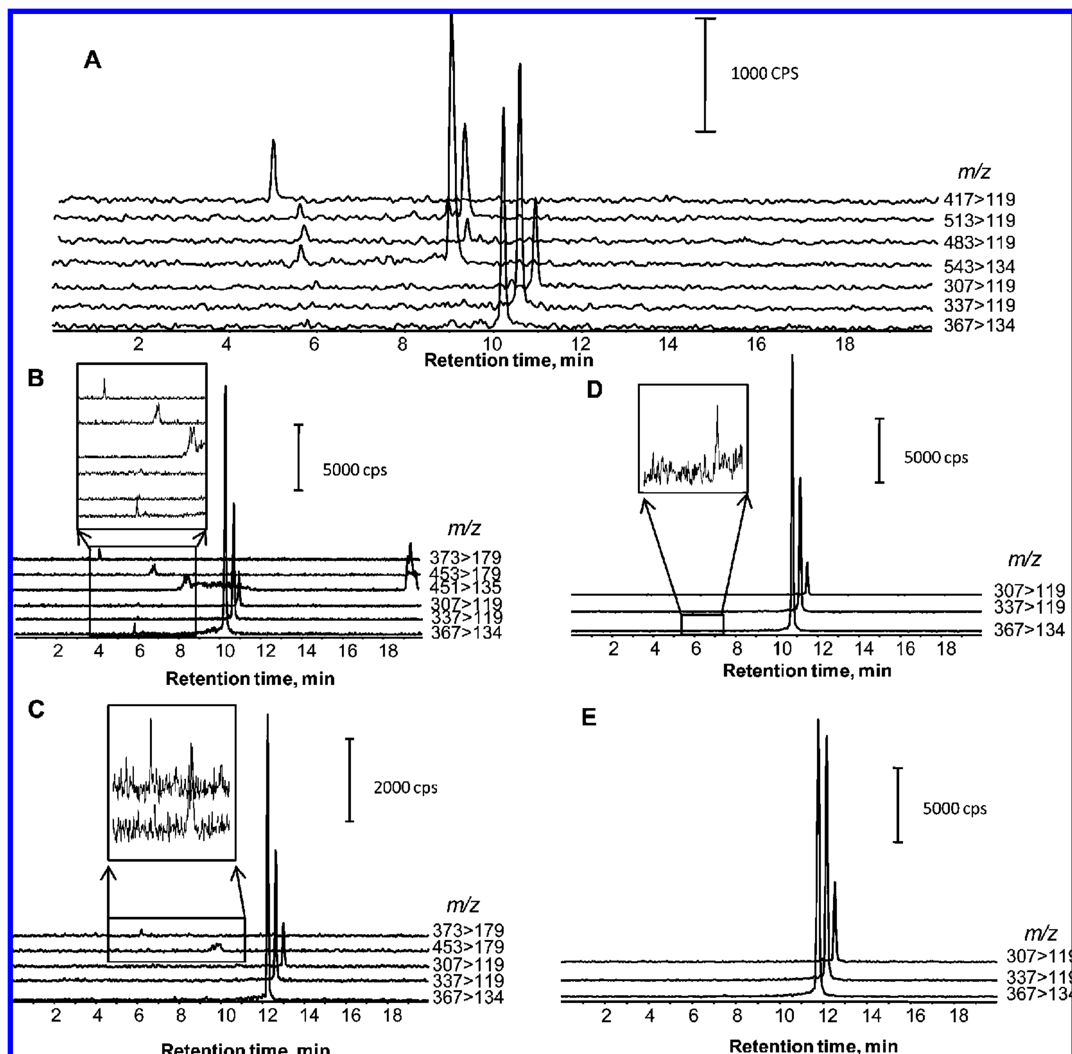

Figure 4. Identification of curcuminoids and curcuminoid metabolites in plasma (**A**), liver (**B**), kidney (**C**), heart (**D**), and intestinal mucosa (**E**) from rats receiving an oral dose of Meriva (340 mg/kg) by UPLC-MS/MS. Spectrograms were obtained in multiple reaction monitoring mode (MRM) as described under Materials and Methods. Each panel (**A**−**E**) presents spectrograms for every MRM transition that yielded a peak of signal/noise > 3. MRM transitions displayed represent curcumin (367 > 134), desmethoxycurcumin (337 > 119), bisdesmethoxycurcumin (307 > 119), curcumin monoglucuronide (543 > 134), desmethoxycurcumin monoglucuronide (513 > 119), bisdesmethoxycurcumin monoglucuronide (483 > 119), desmethoxycurcumin monosulfate (417 > 119), tetrahydrocurcumin monosulfate (451 > 135), hexahydrocurcumin monosulfate (453 > 179), and hexahydrocurcumin (373 > 179). These results are representative of three separate extractions of each tissue.

Table 3. Curcumin Concentrations in Plasma, Urine, and Tissues from Rats That Had Received 340 mg/kg Oral Curcumin[a]

tissue	curcumin concn
plasma	16.1 ± 11.4 ng/mL
urine	2.0 ng/mL
intestinal mucosa	1.4 ± 0.2 mg/g
liver	3671.8 ng/g
kidney	206.8 ± 51.5 ng/g
heart	807.6 ± 170.4 ng/g

[a] Results are presented as mean ± standard deviation of triplicate extractions from three rats. Urine collection was limited, and the result presented here represents the mean of triplicate extractions from a pooled urine sample.

plasma t_{max} values for curcumin (15 min) and curcumin glucuronide (30 min) (*17*). Curcumin concentration was 16.1 ng/mL (**Table 3**). The minor curcumin monoglucuronide peak with retention time at 5.3 min was below the limit of quantitation (**Figure 2B**), whereas concentrations of desmethoxycurcumin, the major curcumin glucuronide, and desmethoxycurcumin glucuronide were estimated at 13.6 ± 10.7, 61.3 ± 40.2, and 96.1 ± 55.5 ng/mL, assuming molecular absorbance characteristics similar to those of curcumin.

In urine, curcumin concentrations were at or below the LOQ (5 ng/mL) (**Figure 2C**). Metabolite peaks present in urine were consistent with those found in plasma. Estimated concentrations for the glucuronides of curcumin and desmethoxycurcumin were 426 and 532 ng/mL, respectively.

All three parent curcuminoids were detected in mucosa, liver, kidney, and heart from animals receiving Meriva (**Figure 2D**−**F**). A number of unidentified peaks were observed in kidney with retention times around 3.5−4.0 min (**Figure 2E**), but these were present in control kidney, implying that they are unrelated to curcumin (data not shown). No metabolites were observed in mucosal samples (**Figure 2G**). Curcumin concentrations in tissues were significantly higher than those observed in plasma (**Table 1**) and quantitatively are in the order intestinal mucosa > liver > heart > kidney.

DISCUSSION

The analytical methods described above separate curcuminoids and their major glucuronide metabolites in a total run time

of 16 min, which represents a marked improvement over many previously published methods (*13, 17, 18, 20, 26−32*). Vareed et al. (*33*) also demonstrated recently the separation of curcumin conjugates and curcuminoids within 15 min. The advantage of UPLC over conventional HPLC is that the sub 2 μ stationary phase generates analyte peaks with short base widths and consequently greater sensitivity with correspondingly improved LOD and LOQ. Here we observe LOD and LOQ in plasma and urine of 2.5 and 5 ng/mL, which are an improvement over previously published methods (*17, 18, 26, 28, 30*) and comparable with those of Pak et al. (*31*), although the latter method measured only curcumin and did not demonstrate separation of curcumin from other curcuminoids or metabolites. The UPLC method used here was reproducible and demonstrated suitable linearity over the expected physiological range (5−200 ng/mL). The recovery of curcumin from plasma and urine using SPE (64 and 62%, respectively) was lower than previously published for solvent extraction methods (70−100%) (*13, 17, 26, 30, 32, 33*). Nevertheless, the RSD values (9 and 15%, respectively) were comparable to those in previously published methods (*13, 17, 26−33*) and are within the limits given by the FDA (*24*). Although SPE gave a lower recovery than published solvent extraction techniques, we continued with SPE as the improved sample cleanup associated with SPE equates to longer column lifetime, decreased baseline noise, and decreased matrix effects when samples are analyzed by MS/MS.

We chose intestinal mucosa for validation as the colorectal tract is a target of curcumin, which is under consideration as a putative gastrointestinal malignancy chemopreventive agent (*8, 16, 17*). The method was characterized by good accuracy, linearity, extraction efficiency, reproducibility, and an LOD (125 ng/g) that is orders of magnitude lower than curcumin concentrations observed in many experimental animals (*8, 17, 34*) and levels observed in patients receiving oral curcumin (*16*).

Curcumin was measured here in plasma (16 ng/mL, 43.5 nM), urine (2.0 ng/mL, 5.4 nM), intestinal mucosa (1.4 mg/g), liver (3.7 μg/g), heart (0.8 μg/g), and kidney (0.2 μg/g) of rats following a single oral dose of Meriva (**Table 3**). Plasma concentrations observed here are consistent with results from a previous investigation of Meriva (*17*). The low concentrations of curcumin in urine are probably a consequence of the early sampling time. Levels of curcumin in tissues are high compared to plasma as described before (*17*) and are within the viable range for the assay (**Tables 2** and **3**). This is the first observation of levels of curcumin in heart and kidney. The highest concentrations were found in intestinal mucosa (**Table 3**), consistent with previous observations (*17*). The observed concentrations in liver are, however, significantly higher in this current study compared with our previous observations (*17*). It is conceivable that improved accuracy as a consequence of the inclusion of an internal standard here for tissue extractions has contributed to this increase in observed hepatic curcumin concentration.

The major curcuminoid metabolites were glucuronide conjugates (**Figures 2−4**), in particular the phenolic glucuronides of curcumin and desmethoxycurcumin. UPLC-MS/MS analyses of biomatrices demonstrated minor glucuronide metabolites with earlier retention times consistent with the alcoholic glucuronides as reported recently in vitro (*19−21*). Alternative identities of these peaks are curcumin diglucuronide or curcumin glucuronide sulfate, even though the search for species characterized by transitions specific for these metabolites (*m/z* 719 > 134, 623 > 134) did not yield peaks (data not shown). Only limited evidence for sulfation was observed in plasma, where UPLC-MS/MS identified desmethoxycurcumin sulfate, consistent with the previous investigations of curcumin metabolism in rat following oral Meriva (*17*). No evidence for glutathione conjugates of curcuminoids was found using the parent ion masses described by Usta et al. (*22*). In agreement with previous studies, curcuminoids underwent reductive metabolism predominantly in the liver (*13, 20*), yielding hexahydrocurcumin and sulfate conjugates of both hexa- and tetrahydrocurcumin (**Figure 4**).

In conclusion, the novel UPLC methods described herein possess the sensitivity, accuracy, and reproducibility suitable for analysis of curcumin in clinical investigations. Furthermore, UPLC enables the separation of curcuminoid metabolites within a short run time and, when coupled with tandem mass spectrometry, provides a useful tool for curcumin metabolism studies.

ABBREVIATIONS USED

UPLC, ultraperformance liquid chromatography; MS/MS tandem mass spectrometry; C_{max}, maximum concentration; AUC, area under the concentration versus time curve; SPE, solid-phase extraction; LOD, limit of detection; LOQ, limit of quantitation; RSD, relative standard deviation.

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