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Determination of urinary metabolites of the emerging UV filter Octocrylene by online-SPE-LC-MS/MS

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ABSTRACT: Octocrylene (OC) is an emerging UV filter, which is used in the majority of sunscreens as well as other personal care products (PCP) and consumer products. Its presence in various environmental matrices has been reported. However, information on the internal OC exposure in humans is not available, due to the lack of appropriate biomarkers of exposure and analytical methods. Here, we describe a rugged, precise, and accurate analytical method for the determination of three OC metabolites (ester hydrolysis and alkyl chain oxidation products) in human urine by stable isotope dilution analysis. Urine samples are incubated with β-glucuronidase (*E. coli* K12) and then analyzed by liquid chromatography-electrospray ionization-triple quadrupole-tandem mass spectrometry with online turbulent flow chromatography for sample cleanup and analyte enrichment. Syntheses of analytical standards, including deuterium-labeled internal standards, are also described. In a pilot study, we investigated the applicability of the metabolites as biomarkers of exposure in urine samples from the general population (n = 35). OC metabolites were detected in 91% of the samples, with highest concentrations for three individuals having used sunscreen within five days prior to sample collection. We will apply the method in future human biomonitoring studies for OC exposure and risk assessment.

2-Ethylhexyl 2-cyano-3,3-diphenylacrylate (octocrylene, octocrilene, OC; CAS registry no. 6197-30-4; EC no. 228-250-8) is an emerging UV filter, absorbing mainly in the UVB region.[1] It is used in sunscreen formulations worldwide,[1] typically serving the additional purpose of stabilizing the photosensitive UVA filter avobenzone.[2,1] It is also used in other personal care products (PCPs) such as creams, lip care products, and make-up.[3,4] OC has been increasingly used in sunscreens since the early 2000s and is present in the majority of products today, based on samples from the USA, United Kingdom, Switzerland and Germany.[5,3,4,6,7] The maximum permitted concentration in PCPs is 10%, both in the European Union and the USA.[8,9] Apart from being used in PCPs, OC is also implemented as an additive in the production of plastic materials with a specific migration limit of 0.05 mg/kg for food contact materials.[10] Further reported applications are perfumes, fragrances, laboratory chemicals, pharmaceuticals, photo-chemicals, fillers, putties, plasters, modelling clay, and coating products.[11]

Although no photosensitization potential was found in guinea pig studies,[12] several photopatch tests in human studies showed skin reactions indicative for photocontact allergy against octocrylene, mainly after sensitization against ketoprofen.[13-15] Contact allergy against OC, mainly in children, has also been reported, although less frequent and animal studies in guinea pigs do not support a skin sensitizing potential of OC itself.[16,14,15,17] Results from *in vitro* tests suggest antiandrogenic and antiestrogenic effects of OC, but disagree on androgenic effects.[18,19] Another *in vitro* study suggests effects of OC on steroidogenesis.[20] However, these *in vitro* effects could not be confirmed in reproductive and developmental toxicity studies in rats.[16] Currently, a hazard assessment regarding PBT (persistent, bioaccumulative and toxic) properties of OC is in progress on the EU level.[21]

Environmental monitoring data are available for octocrylene and revealed its presence in wastewater treatment plant effluents, sewage sludge and landfill leachate.[22-26] Due to its application in sunscreen products, OC has also been found in a large number of environmental matrices, including surface freshwater and seawater,[22,27,23,26,28,29] sediments,[30,23,24,31–34] freshwater fish,[22,35] and various marine wildlife organisms.[36-38,24,39-41] Furthermore, OC has been detected in the indoor environment.[42,43]

Given the widespread use of OC in PCPs, involving long contact times and high concentrations in consumer products, and further considering its release into the environment, the determination of OC body burdens is desirable. OC exposure assessment, focusing on dermal application, has been performed by Manova et al.[44] by probabilistic modelling based on reported PCP use. Schlumpf et al.[45,46] analyzed OC in human milk samples including exposure assessment for infants. They also found a correlation between OC in milk samples and

maternal use of PCPs, containing OC. However, the internal exposure of OC of the general population has yet to be described. Accordingly, OC has been selected as a substance of interest within the large scale 10 year cooperation project between the German Federal Ministry for the Environment (BMUB) and the German Chemical Industry Association (VCI), which has the aim to establish new human biomonitoring (HBM) methods to perform exposure and risk assessments for new or emerging chemicals.[47] The aim of this study was to develop a fast and rugged analytical method for the determination of urinary octocrylene metabolites, including syntheses of the analytical standards. Three target metabolites have been postulated and identified as urinary OC metabolites within the scope of a human metabolism study after oral dosage (publication in preparation) (figure 1): 2-cyano-3,3-diphenylacrylic acid (CPAA), 2-ethyl-5-hydroxyhexyl 2-cyano-3,3-diphenylacrylate (5OH-OC), and 2-(carboxymethyl)butyl 2-cyano-3,3-diphenylacrylate ("dinor OC carboxylic acid"; DOCCA). The method presented here shall be applied in future HBM studies, and results shall be used for future exposure and risk assessments.

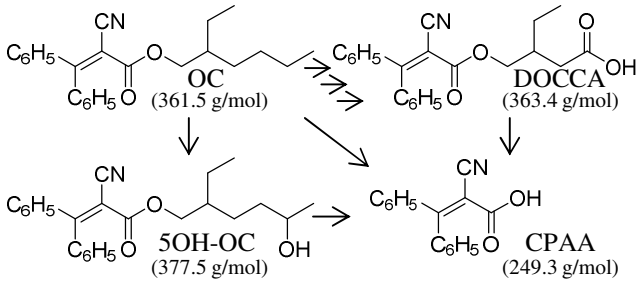


Figure 1. Metabolism pathway of OC leading to the three urinary metabolites under investigation. Phase II metabolites (e.g. glucuronides) are not shown (see figure S-1 for structure of glucuronides).

EXPERIMENTAL SECTION

For reagents, materials (including abbreviations) and syntheses of analytical standards see Supporting Information.

High and Ultrahigh Resolution Mass Spectra

High resolution (HR)-MS full scan and product ion spectra of all standards were recorded with LIT-Orbitrap-MS and Q-Orbitrap-MS, respectively. Ultrahigh resolution (UHR)-MS product ion spectra of CPAA and CPAA-d_{10} were recorded with FT-ICR-MS (for details see Supporting Information).

Standard Solutions

Seven calibration solutions (0.2 to 100 µg/L CPAA; 10 ng/L to 5 µg/L DOCCA and 5OH-OC) were prepared in water. In addition, an internal standard mix was prepared in acetonitrile (200 µg/L CPAA-d_{10}; 5 µg/L DOCCA-d_{10}; 1 µg/L 5OH-OC-d_{10}). For a more detailed description, see Supporting Information.

Sample Collection and Preparation

Urine samples were collected in 250 mL polyethylene (PE) containers, aliquoted in 15 mL PE tubes and everything stored at -20 °C. Urinary creatinine was determined as contract analyses by L.u.P. GmbH Labor- und Praxisservice (Bochum, Germany). For OC metabolite analysis, 300 µL of homogenized (by inverting several times) urine were transferred into an HPLC vial and 30 µL internal standard mix, 100 µL 1 M ammonium acetate pH 6.0-6.4 (prepared in ultrapure water), and 6 µL of β-glucuronidase, premixed with ammonium acetate buffer 1:1 (v/v) (~0.4 u), were added. Samples were mixed by inverting several times and incubated in a water bath at 37 °C for 3 h. After incubation, 30 µL formic acid were added and samples were homogenized and then frozen at -20 °C overnight to precipitate cryophobic proteins. After thawing and centrifugation (1900 g; 10 min) the supernatant was transferred into a new HPLC vial and used for analysis. Calibration solutions underwent the same sample preparation as urine samples.

Chromatographic Conditions

For online sample cleanup and chromatographic separation a 1260 Infinity HPLC (Agilent Technologies, Waldbronn, Germany) was used, consisting of a G1312B binary high pressure gradient pump with G4225A degasser, a G1311B quaternary low pressure gradient pump, a G1367E autosampler equipped with G1330B thermostat, and a G1316A thermostatted column compartment with 6-port switching valve. For a detailed description of the hardware setup see Supporting Information and Modick et al. 2013[48]. A TurboFlow® Phenyl (50 x 0.5 mm; Thermo Scientific, Franklin, MA, USA) turbulent flow chromatography (TFC) column was applied as enrichment column for online sample cleanup and analyte enrichment. For chromatographic separation a Kinetex® C18 HPLC column (150 x 3 mm, particle size 2.6 µm; with corresponding SecurityGuard™ ULTRA guard column; Phenomenex, Aschaffenburg, Germany) was used. Water (eluent A) and acetonitrile (eluent B), each containing 0.05% (v/v) acetic acid, were used as eluents on both pumps. For further details see Supporting Information.

Mass Spectrometric Conditions

For detection of OC metabolites a 4500 triple quadrupole mass spectrometer (Sciex, Darmstadt, Germany) with ESI ionization in Scheduled MRM™ detection mode was used. Instrument gases (nitrogen) were set as follows: curtain gas 20 psi, nebulizer gas 40 psi, heater gas 50 psi, collision gas 6 arbitrary units. Source heater temperature was set to 450 °C. Electrospray voltages were 5.5 kV (+ESI) and 4.5 kV (-ESI). MRM detection window was 60 s and target scan time 0.125 s for both polarities. Entrance potential was 10 V/-10 V (+ESI/-ESI) and collision cell exit potential was 12 V/-12 V (+ESI/-ESI). Further Scheduled MRM™ conditions are shown in table 1. Declustering potentials and collision energies were optimized manually. Analyst 1.6 (Sciex, Darmstadt, Germany) was used for instrument control and quantitative data analysis.

Calibration, Validation and Quality Control

The calibration functions were calculated by weighted (concentration^{-1}) linear regression. Two quality control samples of low (Q_{low}) and high (Q_{high}) metabolite concentrations (for exact concentrations see table 2) were prepared by mixing different native urine samples (five individual spot urines for Q_{low} and seven for Q_{high}), followed by freezing, thawing and filtrating three times. In order to obtain metabolite concentrations

Table 1. Time-programmed MRM conditions

	t_R [min]	ESI polarity	MRM transition		DP [V]	CE[a] [eV]
			m/z precursor ion	*m/z* product ion[a]		
CPAA	9.72	negative	248	202 (203)	-30	-25 (-25)
CPAA-d_{10}	9.65	negative	258	214 (212)	-35	-10 (-20)
DOCCA	14.84	positive	364	250 (115)	30	10 (14)
DOCCA-d_{10}	14.70	positive	374	260 (115)	25	10 (15)
5OH-OC	18.60	positive	378	232 (176)	30	20 (68)
5OH-OC-d_{10}	18.36	positive	388	242 (260)	30	19 (15)

t_R: retention time, DP: declustering potential, CE: collision energy; [a]: quantifier (qualifier)

covering the full calibration range, without CPAA exceeding the calibration range, the other two metabolites present at lower concentrations (5OH-OC and DOCCA) were spiked using the respective stock solutions (Supporting Information). The material obtained in that way was aliquoted in HPLC vials and stored at -20 °C. Method precision was determined by analyzing both materials eight times in one series (intraday) and on eight different days (interday). After validation, this material was used for quality control (quality control chart). For the determination of the method's accuracy eight different urine samples (0.28 to 2.31 g/L creatinine) were analyzed spiked at three different levels prior to sample preparation (see table 3) and without spiking.

Pilot Population

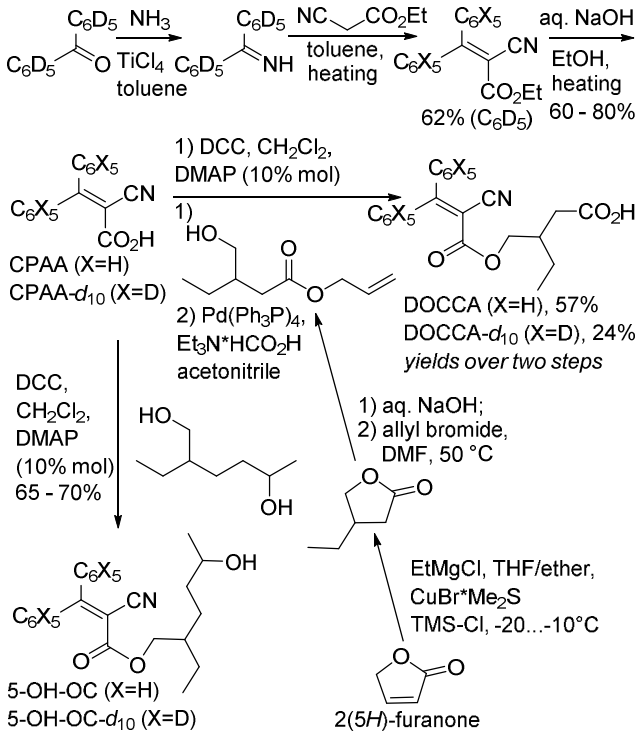
Spot urine samples (convenience samples) of 35 German volunteers (11 males and 24 females, age 23 to 59 (median 42)), not occupationally exposed to OC, were analyzed. Urinary creatinine ranged from 0.09 to 2.31 g/L. The samples were collected in April 2017. Volunteers provided information about their use of sunscreens, lipsticks with sun protection factor (SPF), and day creams with SPF within four weeks preceding sample collection. The study was approved by the ethical board of the Ruhr University Bochum (IRB Reg. No.: 3867-10 and 4288-12). All volunteers provided written informed consent.

RESULTS AND DISCUSSION

Syntheses of OC Metabolite Standards

All reference compounds and their deuterated analogs were prepared from CPAA[49] and CPAA-d_{10}. CPAA and CPAA-d_{10} were obtained by saponification of ethyl 2-cyano-3,3-diphenylacrylate and ethyl 2-cyano-(d_{10}-3,3-diphenyl)acrylate (see Scheme 1). Ethyl 2-cyano-(d_{10}-3,3-diphenyl)acrylate with high deuterium content cannot be obtained straightforwardly, by heating d_{10}-benzophenone and ethyl cyanoacetate in benzene or toluene with a catalyst and removal of water, because this reaction is accompanied by D-H exchange. We discovered that if the reaction between d_{10}-benzophenone imine and ethyl cyanoacetate is carried out according to the method disclosed for the unlabeled compound,[50] ethyl 2-cyano-(d_{10}-3,3-diphenyl)acrylate is formed cleanly and without any D-H exchange. d_{10}-Benzophenone imine, in turn, was synthesized also without D-H exchange from d_{10}-benzophenone and ammonia in toluene, in the presence of $TiCl_4$, as described for the

Scheme 1. Synthesis of reference compounds and their deuterated analogs (see text for details).



unlabeled compound.[51] 5OH-OC and 5OH-OC-d_{10} were prepared from CPAA, CPAA-d_{10} and 2-ethylhexan-1,5-diol (1:1 mixture of 2 diastereomers).[52] Under mild conditions, the primary hydroxyl group is acylated more readily than the secondary one (DCC, DMAP, CH_2Cl_2), and the protection is not required. DOCCA and DOCCA-d_{10} were obtained from CPAA, CPAA-d_{10} and allyl 3-(hydroxymethyl)-pentanoate (see Scheme 1). The copper(I) catalyzed 1,4-addition of ethylmagnesium chloride to 2(5*H*)-furanone was carried out as described for an *n*-propyl analog[53] and afforded dihydro-4-ethyl-2(3*H*)-furanone[54] which was converted to the sodium salt of 3-(hydroxymethyl)pentanoic acid (1 M aq. NaOH) and then to allyl 3-(hydroxymethyl)pentanoate (allyl bromide, DMF, 50 °C, overnight). Esterifications of CPAA and CPAA-d_{10} with 2-ethylhexane-1,5-diol and allyl 3-(hydroxymethyl)pentanoate were achieved by using *N,N'*-dicyclohexylcarbodiimide in DCM in the presence of DMAP (10 % mol). Deprotection of the intermediates – 2-

[(allyloxycarbonyl)methyl]butyl 2-cyano-3,3-diphenylacrylate and its d_{10}-counterpart – was accomplished by using $Pd(Ph_3P)_4$ (5-7 % mol) in acetonitrile in the presence of triethylammonium formate (5 – 10 eq.) and afforded DOCCA and DOCCA-d_{10}.

Mass Spectrometry

For all analytical standards, including deuterium-labeled standards, postulated structures of fragments observed in QqQ-MS are shown in figure 2. For product ion spectra see Supporting Information figure S-12. Fragments described below were verified by their accurate mass with FT-ICR-MS (below 0.2 ppm) or Q-Orbitrap-MS (below 5.0 ppm) (see Supporting Information for details), with exception of CPAA-d_{10} fragments *m/z* 210 and 208 corresponding to non-labelled CPAA fragments *m/z* 202 and 201, respectively. These fragments were not observed in FT-ICR-MS and mass resolution in Q-Orbitrap-MS was too low for separation of isobaric fragments at *m/z* 210 and 208.

CPAA and CPAA-d_{10} were detected by tandem-MS. Interestingly, the isotope-labeled analog showed some unexpected signals, which gave reason to a detailed investigation of the fragmentation pattern. CPAA and CPAA-d_{10} were well ionized both in negative and positive ion mode. In negative ion mode, only three fragments were observed. The fragment of highest mass is explained by elimination of CO_2 from $[M-H]^-$. The two fragments of lower masses can be explained by subsequent elimination of hydrogen (H•/D• or H_2/D_2). In positive ion mode, the observed mass signals for CPAA can be explained by the following fragmentations and rearrangements: First, $[M+H]^+$ eliminates water to yield *m/z* 232. Further elimination of CO yields a fragment at *m/z* 204, which can gradually eliminate hydrogen to yield *m/z* 203, 202, and 201. Elimination of CN•, HCN, or CN• and H_2 from *m/z* 204 yields *m/z* 178, 177, and 176 (formation of *m/z* 177 and 176 from *m/z* 178 and 177 can also be assumed and fragments at *m/z* 203 and 202 might also contribute to their formation). The fragment at *m/z* 105 can be explained by rearrangement of *m/z* 232 (migration of one phenyl group from position 3 to 1) and subsequent elimination of nitrile of 3-phenylpropynoic acid.

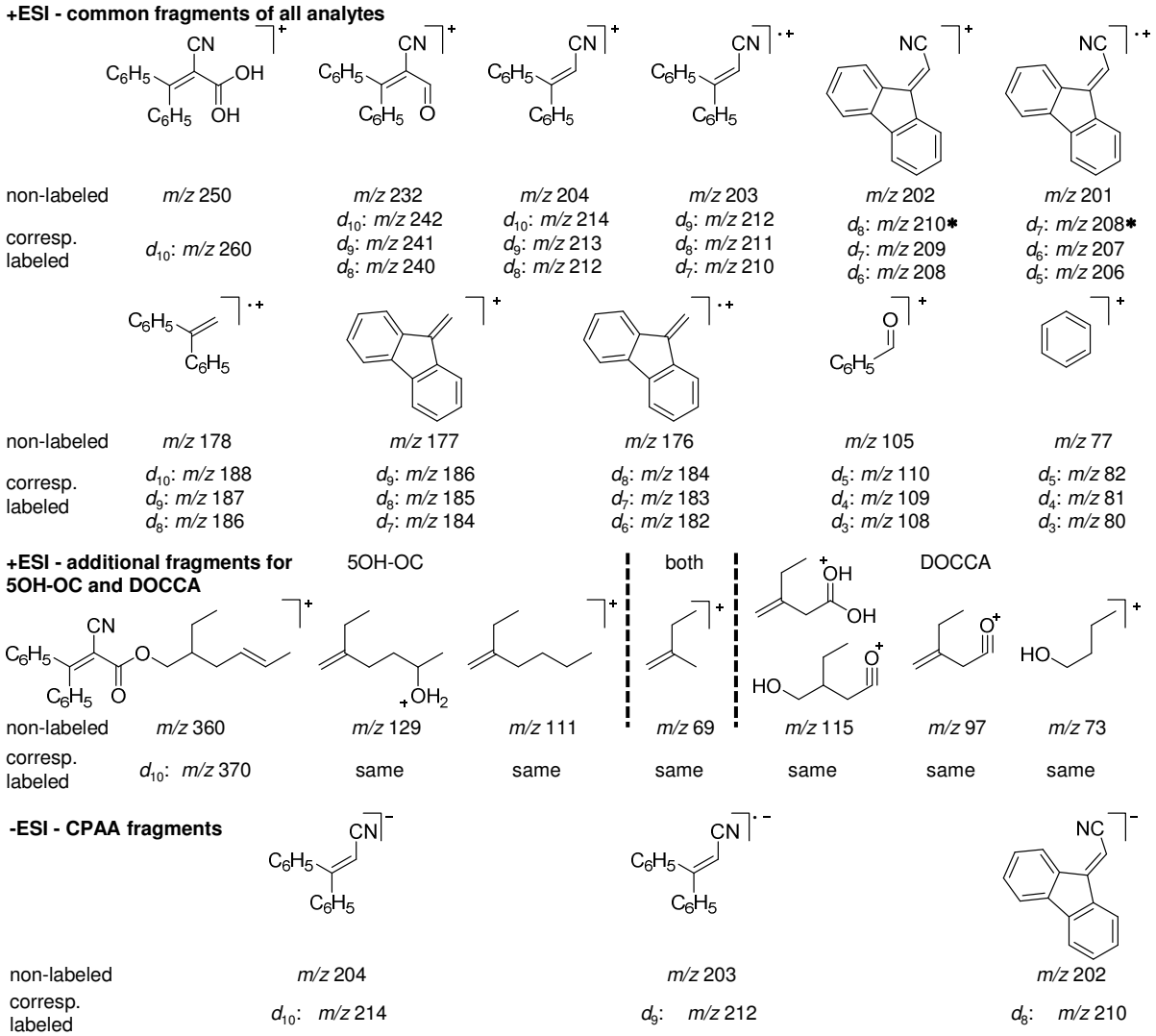


Figure 2. MS/MS fragments for all analytical standards. Postulated fragment structures of the non-labeled standards are shown, corresponding fragments of the deuterium-labeled standards are listed below. Fragments marked with an asterisk could not be verified by (U)HRMS (see text).

Table 2. Method precision.

	CPAA		DOCCA		5OH-OC	
	Q_{low}	Q_{high}	Q_{low}	Q_{high}	Q_{low}	Q_{high}
Intraday imprecision (n = 8)						
Mean concentration [µg/L]	3.13	64.2	0.140	2.37	0.045	2.74
Coefficient of variation	2.8%	1.0%	3.5%	2.4%	7.4%	3.6%
Interday imprecision (n = 8)						
Mean concentration [µg/L]	3.02	64.1	0.143	2.41	0.048	2.91
Coefficient of variation	3.2%	3.2%	3.8%	3.3%	2.9%	2.5%

The formation of *m/z* 77 can be explained by elimination of CO from *m/z* 105. The QqQ product ion spectrum of CPAA-d_{10}, turned out to be more complex than expected (e.g. *m/z* 182, 183, 184, 185, 186, 187, and 188, instead of expected signals *m/z* 184, 186 and 188). To clarify this behavior, fragmentation of CPAA-d_{10} was scrutinized with FT-ICR-MS. It turned out, that all observed fragments can be explained by an initial protium-deuterium exchange between the deuterium-labeled phenyl rings and the twice protonated carboxylic acid group in $[M+H]^+$, which can occur up to two times. The subsequent loss of H_2O, HDO, or D_2O results in three series of fragments, each corresponding to those of non-labeled CPAA. The FT-ICR-MS product ion spectra of CPAA-d_{10} were further complicated by the presence of the $^{13}C_1$ isotopomer of CPAA-d_9 (impurity), which is isobaric to CPAA-d_{10} (see Supporting Information table S-5).

For the other two analytes, fragments containing or derived from the sidechain were observed in addition to fragments already known from CPAA and CPAA-d_{10}. DOCCA and DOCCA-d_{10} were ionized much better in positive ion mode, than in negative ion mode (accordingly not reported). For both analytes a fragment at *m/z* 115 was detected, which can be explained by a McLafferty-type rearrangement of $[M+H]^+$ (upper structure in figure 2). Fragment *m/z* 73 however, is derived from *m/z* 115 (Q-Orbitrap-MS product ion spectrum of *m/z* 115, formed by in-source CID) and cannot be explained by fragmentation of the McLafferty-type product. On the other hand, the lower structure in figure 2 could yield *m/z* 73 by elimination of ethenone (ketene). The formation of this alternative structure for *m/z* 115 would most likely involve charge-remote fragmentation (cleavage of the C-O bond between the side chain alcohol and the carboxylic C=O group and cleavage of a C-H bond in position 2 of one of the phenyl rings), resulting in 3-phenylindenone 2-carbonitrile as neutral loss. Regardless of its structure, fragment *m/z* 115 can eliminate water to yield fragment *m/z* 97, which can further eliminate CO to yield *m/z* 69. As expected, 5OH-OC and 5OH-OC-d_{10} could only be ionized in positive ion mode. For 5OH-OC and 5OH-OC-d_{10} mass signals corresponding to $[M+H-H_2O]^+$ were observed. In addition, the McLafferty-type rearrangement product corresponding to DOCCA's *m/z* 115 was found at *m/z* 129. Elimination of water yields *m/z* 111. The fragment at *m/z* 69 can either be formed by elimination of propene from *m/z* 111 or by simultaneous elimination of propene and water from *m/z* 129.

Quantifier and qualifier MRM transitions were chosen based on signal-to-noise ratio in matrix and absence of interfering matrix peaks. For CPAA, sensitivity in matrix was higher in ESI negative ion mode than in positive ion mode. Linearity was low (up to <50 µg/L) in both ion modes with MS/MS conditions optimized for highest signal-to-noise ratio. Reduction of injection volume to increase linearity was not an option due to the necessary sensitivity for the other two analytes. However, in negative ion mode, increase of collision energies above optimal values allowed to increase linearity to 100 µg/L, maintaining an appropriate sensitivity (LOQ 0.5 µg/L).

Method Performance

As in several other HBM methods,[55–58] choosing a pure β-glucuronidase without arylic sulfatase activity for enzymatic deconjugation of glucuronides (figure S-1) was necessary to avoid cleavage of the analytes' ester moieties (for data on ratios free/conjugated metabolite and ruggedness of enzymatic hydrolysis see Supporting Information). It has to be mentioned, that accordingly only the sum of free and glucuronidated metabolites are captured. Sulfate metabolites on the other hand, possibly formed as well, are not captured by this approach. Exemplary chromatograms of a urine sample with native OC metabolite concentrations and a calibration standard are shown in figure 3. Calibrations were linear (with r ≥

Table 3. Relative recoveries in spiked urine samples (n = 8; 0.28 to 2.31 g/L creatinine).

	CPAA			DOCCA			5OH-OC		
Native concentrations [µg/L]	0.50-9.13			<LOQ-0.131			<LOQ		
Spiked concentration [µg/L]	2	10	40	0.1	0.5	2	0.1	0.5	2
Accuracy (range) [%]	104 (91-124)	100 (95-105)	97 (90-102)	95 (81-116)	89 (77-98)	91 (77-104)	110 (103-129)	116 (110-127)	110 (100-124)

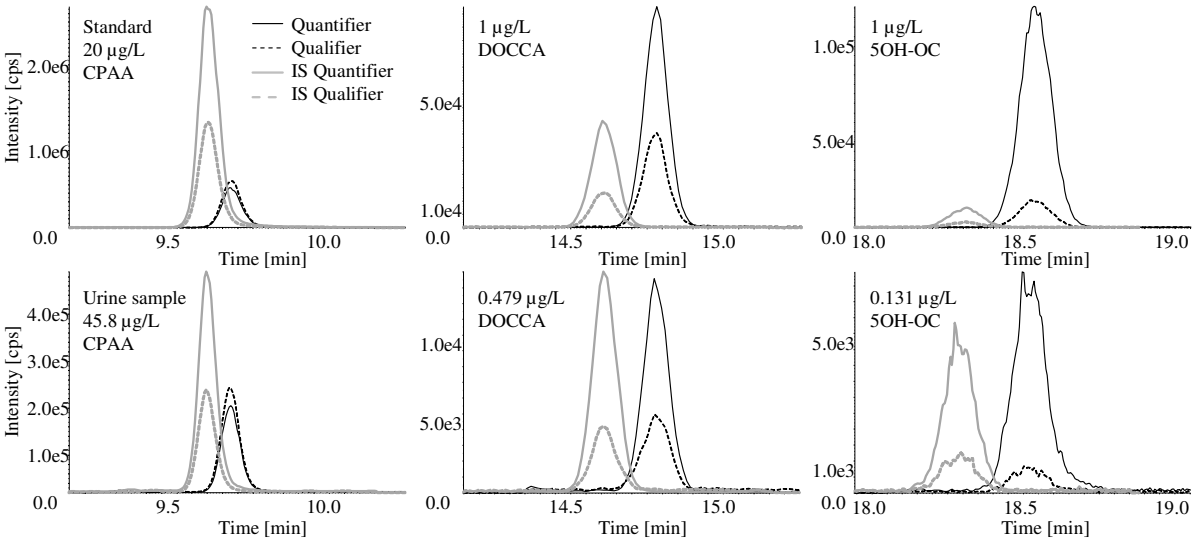


Figure 3. Chromatograms of a calibration standard solution (top) and an exemplary urine sample (0.86 g/L creatinine) with native metabolite concentrations (bottom). From left to right: CPAA, DOCCA, 5OH-OC (non-labeled analytes and internal standards in black and gray; quantifier and qualifier transitions as continuous and dashed lines).

0.9994; n = 10) over the whole calibration range (for exemplary calibration curves see figures S-13 to 15). Limits of quantification (LOQ) based on a signal-to-noise ratio of 10 in matrix (procedure further described in Supporting Information) were estimated as 0.5 µg/L (CPAA), 0.05 µg/L (DOCCA), and 0.015 µg/L (5OH-OC). Precision data, obtained from analyses of quality control samples Q_{low} and Q_{high}, is shown in table 2. Method imprecision was ≤ 7.4% for all analytes, both intraday and interday. Concentrations of Q_{low} and Q_{high} were chosen to reflect background concentrations of the general population (see below) as well as concentrations observed after single oral administration (human metabolism study – publication in preparation). Results on method accuracy are shown in table 3. Relative recoveries were calculated after subtraction of native metabolite concentrations. Mean relative recoveries were between 89% and 116% over all analytes and spiking levels with single values ranging from 77% to 129%.

Pilot Human Biomonitoring Study

The relevance of the analyzed OC metabolites for human biomonitoring of the general population was verified in a pilot study. Results are shown in table 4. CPAA was detected above LOQ in 91% of the analyzed 35 spot urine samples, randomly collected from volunteers, not occupationally exposed to OC. DOCCA was found above LOQ in 37% of the samples and 5OH-OC in 17%, with 5OH-OC not being present >LOQ in samples with CPAA or DOCCA <LOQ and DOCCA not being present in samples with CPAA <LOQ. Interestingly, three samples collected from volunteers, who had stated to have used sunscreen within the last five days before sample collection, had relatively high concentrations of all three metabolites, compared to the majority of the remaining 32 samples (no sunscreen application within last 5 days). The results of this pilot study show that all three OC metabolites might be suitable biomarkers of OC exposure, with CPAA being the most sensitive biomarker and the other two providing coverage for high CPAA concentrations. Only a small population, not necessarily representative for the general population, was investigated. Also, samples were collected in springtime and higher metabolite levels can be expected during summer. Accordingly, all three metabolites need to be analyzed in larger sample collectives to confirm their applicability for

Table 4. Pilot human biomonitoring study

	Total (n = 35)		Sunscreen users (previous 5 days) (n = 3)		Sunscreen non-users (previous 5 days) (n = 32)	
	Median concentration (range) [µg/L]	n > LOQ (percentage)	Median concentration (range) [µg/L]	n > LOQ (percentage)	Median concentration (range) [µg/L]	n > LOQ (percentage)
CPAA	12.5 (<LOQ-95.5)	32 (91%)	59.0 (38.4-95.5)	3 (100%)	3.18 (<LOQ-35.9)	29 (91%)
DOCCA	n.a. (<LOQ-0.805)	13 (37%)	0.663 (0.651-0.805)	3 (100%)	n.a. (<LOQ-0.569)	10 (31%)
5OH-OC	n.a. (<LOQ-0.093)	6 (17%)	0.044 (0.030-0.093)	3 (100%)	n.a. (<LOQ-0.038)	3 (9%)

Median concentration not reported ("n.a.") if less than 50% of the samples were >LOQ.

exposure biomonitoring.

Conclusion

The method described here is rugged, precise, accurate, and little labor-intensive. Results from the pilot population, give reason to expect that OC metabolites can be determined in the vast majority of urine samples from the general population, even in case of background exposures (i.e. no recent use of sunscreens). Urinary excretion factors of the three metabolites in conjunction with the method described here, will allow for a precise internal OC exposure and risk assessment, independent of uptake routes and exposure sources.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Supporting Information includes: structural formulae of glucuronides; commercial reagents and materials (with abbreviations); syntheses and characterization of products; further details on HR-MS and UHR-MS conditions, preparation of standard solutions, chromatographic conditions, estimation of LOQs, and discussion of enzymatic hydrolysis and MS/MS fragmentation behavior of analytical standards; exemplary calibration curves.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Shaath, N. A. *Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology* **2010**, *9* (4): 464–469.

(2) Forestier, S. *Journal of the American Academy of Dermatology* **2008**, *58* (5 Suppl 2): S133-8.

(3) Manová, E.; Goetz, N. von; Hauri, U.; Bogdal, C.; Hungerbühler, K. *International journal of hygiene and environmental health* **2013**, *216* (4): 508–514.

(4) Uter, W.; Gonçalo, M.; Yazar, K.; Kratz, E.-M.; Mildau, G.; Lidén, C. *Contact dermatitis* **2014**, *71* (3): 162–169.

(5) Kerr, A. C. *Clinical and experimental dermatology* **2011**, *36* (5): 541–543.

(6) Wahie, S.; Lloyd, J. J.; Farr, P. M. *Clinical and experimental dermatology* **2007**, *32* (4): 359–364.

(7) Wang, S. Q.; Tanner, P. R.; Lim, H. W.; Nash, J. F. *Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology* **2013**, *12* (1): 197–202.

(8) *21 Code of Federal Regulations § 352.10.*

(9) European Parliament and the Council. *Regulation (EC) No 1223/2009 of the European Parliament and the Council of 30 November 2009 on cosmetic products*, 22.12.2009.

(10) European Commission. *Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food*, 14.01.2011.

(11) *Octocrilene - Substance Information - ECHA,* https://echa.europa.eu/de/substance-information/-/substanceinfo/100.025.683.

(12) BASF. *unpublished study: Phototoxic and Photoallergenic Potential by Cutaneous Route in Guinea Pigs. 48H0495/009044.* **2001**.

(13) *The British journal of dermatology* **2012**, *166* (5): 1002–1009.

(14) Groot, A. C. de; Roberts, D. W. *Contact dermatitis* **2014**, *70* (4): 193–204.

(15) Karlsson, I.; Vanden Broecke, K.; Mårtensson, J.; Goossens, A.; Börje, A. *Contact dermatitis* **2011**, *64* (6): 343–352.

(16) *ECHA disseminated dossier,* https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14858/7/5/2; https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14858/7/9/2; https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14858/7/9/2/?documentUUID=38b5fcf8-c7aa-4eb9-89f5-d89e8a1c26bb; https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14858/7/9/3.

(17) Uter, W.; Lessmann, H.; Geier, J. *Contact dermatitis* **2017**, *77* (2): 127–128.

(18) Balázs, A.; Krifaton, C.; Orosz, I.; Szoboszlay, S.; Kovács, R.; Csenki, Z.; Urbányi, B.; Kriszt, B. *Ecotoxicology and environmental safety* **2016**, *131*: 45–53.

(19) Kunz, P. Y.; Fent, K. *Aquatic toxicology (Amsterdam, Netherlands)* **2006**, *79* (4): 305–324.

(20) Strajhar, P.; Tonoli, D.; Jeanneret, F.; Imhof, R. M.; Malagnino, V.; Patt, M.; Kratschmar, D. V.; Boccard, J.; Rudaz, S.; Odermatt, A. *Toxicology* **2017**, *381*: 51–63.

(21) *PACT – RMOA and hazard assessment activities - Octocrylene - ECHA,* https://echa.europa.eu/de/addressing-chemicals-of-concern/substances-of-potential-concern/pact/-/substance-rev/8118/del/200/col/synonymDynamicField_3413/type/desc/pre/2/view.

(22) Balmer, M. E.; Buser, H.-R.; Müller, M. D.; Poiger, T. *Environ. Sci. Technol.* **2005**, *39* (4): 953–962.

(23) Kameda, Y.; Kimura, K.; Miyazaki, M. *Environmental pollution (Barking, Essex : 1987)* **2011**, *159* (6): 1570–1576.

(24) Langford, K. H.; Reid, M. J.; Fjeld, E.; Øxnevad, S.; Thomas, K. V. *Environment international* **2015**, *80*: 1–7.

(25) Plagellat, C.; Kupper, T.; Furrer, R.; Alencastro, L. F. de; Grandjean, D.; Tarradellas, J. *Chemosphere* **2006**, *62* (6): 915–925.

(26) Rodil, R.; Schrader, S.; Moeder, M. *Journal of chromatography. A* **2009**, *1216* (24): 4887–4894.

(27) Fisch, K.; Waniek, J. J.; Schulz-Bull, D. E. *Marine pollution bulletin* **2017**, *124* (1): 388–399.

(28) Sánchez Rodríguez, A.; Rodrigo Sanz, M.; Betancort Rodríguez, J. R. *Chemosphere* **2015**, *131*: 85–90.

(29) Tsui, M. M. P.; Leung, H. W.; Wai, T.-C.; Yamashita, N.; Taniyasu, S.; Liu, W.; Lam, P. K. S.; Murphy, M. B. *Water research* **2014**, *67*: 55–65.

(30) Gago-Ferrero, P.; Díaz-Cruz, M. S.; Barceló, D. *Analytical and bioanalytical chemistry* **2011**, *400* (7): 2195–2204.

(31) Mandaric, L.; Diamantini, E.; Stella, E.; Cano-Paoli, K.; Valle-Sistac, J.; Molins-Delgado, D.; Bellin, A.; Chiogna, G.; Majone, B.; Diaz-Cruz, M. S.; Sabater, S.; Barcelo, D.; Petrovic, M. *The Science of the total environment* **2017**, *590-591*: 484–494.

(32) Mizukawa, A.; Molins-Delgado, D.; Azevedo, J. C. R. de; Fernandes, C. V. S.; Díaz-Cruz, S.; Barceló, D. *Environmental sci-*

ence and pollution research international **2017**, *24* (22): 18284–18294.

(33) Pintado-Herrera, M. G.; Combi, T.; Corada-Fernández, C.; González-Mazo, E.; Lara-Martín, P. A. *The Science of the total environment* **2017**, *605-606*: 980–994.

(34) Tsui, M. M. P.; Leung, H. W.; Kwan, B. K. Y.; Ng, K.-Y.; Yamashita, N.; Taniyasu, S.; Lam, P. K. S.; Murphy, M. B. *Journal of hazardous materials* **2015**, *292*: 180–187.

(35) Buser, H.-R.; Balmer, M. E.; Schmid, P.; Kohler, M. *Environ. Sci. Technol.* **2006**, *40* (5): 1427–1431.

(36) Bachelot, M.; Li, Z.; Munaron, D.; Le Gall, P.; Casellas, C.; Fenet, H.; Gomez, E. *The Science of the total environment* **2012**, *420*: 273–279.

(37) Gago-Ferrero, P.; Alonso, M. B.; Bertozzi, C. P.; Marigo, J.; Barbosa, L.; Cremer, M.; Secchi, E. R.; Domit, C.; Azevedo, A.; Lailson-Brito, J.; Torres, J. P. M.; Malm, O.; Eljarrat, E.; Díaz-Cruz, M. S.; Barceló, D. *Environ. Sci. Technol.* **2013**, *47* (11): 5619–5625.

(38) He, K.; Timm, A.; Blaney, L. *Journal of chromatography. A* **2017**, *1509*: 91–101.

(39) Peng, X.; Fan, Y.; Jin, J.; Xiong, S.; Liu, J.; Tang, C. *Environmental pollution (Barking, Essex : 1987)* **2017**, *225*: 55–65.

(40) Picot Groz, M.; Martinez Bueno, M. J.; Rosain, D.; Fenet, H.; Casellas, C.; Pereira, C.; Maria, V.; Bebianno, M. J.; Gomez, E. *The Science of the total environment* **2014**, *493*: 162–169.

(41) Tsui, M. M. P.; Lam, J. C. W.; Ng, T. Y.; Ang, P. O.; Murphy, M. B.; Lam, P. K. S. *Environ. Sci. Technol.* **2017**, *51* (8): 4182–4190.

(42) Ao, J.; Yuan, T.; Ma, Y.; Gao, L.; Ni, N.; Li, D. *Chemosphere* **2017**, *184*: 575–583.

(43) Negreira, N.; Rodríguez, I.; Rubí, E.; Cela, R. *Journal of chromatography. A* **2009**, *1216* (31): 5895–5902.

(44) Manová, E.; Goetz, N. von; Hungerbühler, K. *The British journal of dermatology* **2014**, *171* (6): 1368–1374.

(45) Schlumpf, M.; Kypke, K.; Vökt, C. C.; Birchler, M.; Durrer, S.; Faass, O.; Ehnes, C.; Fuetsch, M.; Gaille, C.; Henseler, M.; Hofkamp, L.; Maerkel, K.; Reolon, S.; Zenker, A.; Timms, B.; Tresguerres, J. A. F.; Lichtensteigera, W. *CHIMIA* **2008**, *62* (5): 345–351.

(46) Schlumpf, M.; Kypke, K.; Wittassek, M.; Angerer, J.; Mascher, H.; Mascher, D.; Vökt, C.; Birchler, M.; Lichtensteiger, W. *Chemosphere* **2010**, *81* (10): 1171–1183.

(47) Kolossa-Gehring, M.; Fiddicke, U.; Leng, G.; Angerer, J.; Wolz, B. *International journal of hygiene and environmental health* **2017**, *220* (2 Pt A): 103–112.

(48) Modick, H.; Schütze, A.; Pälmke, C.; Weiss, T.; Brüning, T.; Koch, H. M. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences* **2013**, *925*: 33–39.

(49) Li, J.; Chen, H.; Zhang-Negrerie, D.; Du, Y.; Zhao, K. *RSC Adv.* **2013**, *3* (13): 4311.

(50) Holderbaum, M.; Beck, K.; Aumüller, A.; Witzel, T.; Voit, G. *DE19519894 Verfahren zur Herstellung von 2-Cyan-3,3-diarylacrylsäureestern*, 31.05.1995.

(51) Brenner, D. G.; Cavolowsky, K. M.; Shepard, K. L. *Journal of Heterocyclic Chemistry* **1985**, *22* (3): 805–808.

(52) Gilsing, H.-D.; Angerer, J.; Prescher, D. *Monatshefte für Chemie / Chemical Monthly* **2002**, *133* (8): 1147–1155.

(53) Kenda, B. M.; Matagne, A. C.; Talaga, P. E.; Pasau, P. M.; Differding, E.; Lallemand, B. I.; Frycia, A. M.; Moureau, F. G.; Klitgaard, H. V.; Gillard, M. R.; Fuks, B.; Michel, P. *Journal of medicinal chemistry* **2004**, *47* (3): 530–549.

(54) Röder, E.; Krauß, H. *Liebigs Ann. Chem.* **1992**, *1992* (3): 177–181.

(55) Koch, H. M.; Gonzalez-Reche, L. M.; Angerer, J. *Journal of Chromatography B* **2003**, *784* (1): 169–182.

(56) Lessmann, F.; Schütze, A.; Weiss, T.; Brüning, T.; Koch, H. M. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences* **2016**, *1011*: 196–203.

(57) Schutze, A.; Palmke, C.; Angerer, J.; Weiss, T.; Bruning, T.; Koch, H. M. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences* **2012**, *895-896*: 123–130.

(58) Silva, M. J.; Samandar, E.; Preau, J. L.; Reidy, J. A.; Needham, L. L.; Calafat, A. M. *Journal of Analytical Toxicology* **2005**, *29* (8): 819–824.

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