

# Development and validation of a solid-phase extraction method using anion exchange sorbent for the analysis of cannabinoids in plasma and serum by gas chromatography-mass spectrometry

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**Abstract** The aim of this work was to develop and validate a solid-phase extraction (SPE) method for the analysis of cannabinoids with emphasis on a very extensive and effective matrix reduction in order to ensure constant good results in selectivity and sensitivity regardless of the applied measuring technology. This was obtained by the use of an anion exchange sorbent (AXS) and the purposive ionic interaction between matrix components and this sorbent material. In a first step, the neutral cannabinoids  $\Delta^9$ -tetrahydrocannabinol (THC) and 11-hydroxy- $\Delta^9$ -tetrahydrocannabinol (11-OH-THC) were eluted, leaving 11-nor-9-carboxy- $\Delta^9$ -tetrahydrocannabinol (THC-COOH) and the main interfering matrix components bound to the AXS. In a second step, exploiting differences in pH and polarity, it was possible to separate matrix components and THC-COOH, thereby yielding a clean elution of THC-COOH into the same collecting tube as THC and 11-OH-THC. Even when using a simple measuring technology like gas chromatography with single quadrupole mass spectrometry, this two-step elution allows for an obvious decrease in number and intensity of matrix interference in the chromatogram. Hence, in both plasma and serum, the AXS extracts resulted in very good selectivity. Limits of detection and limits of quantification were below 0.25 and 0.35 ng/mL for the neutral cannabinoids in both matrices, 2.0 and 3.0 ng/mL in plasma and 1.6 and 3.3 ng/mL in serum for THC-COOH. The recoveries were  $\geq 79.8$  % for all analytes. Interday and intraday imprecisions ranged from 0.8 to 6.1 %

relative standard deviation, and accuracy bias ranged from -12.6 to 3.6 %.

**Keywords** Cannabinoids · SPE · Anion exchange sorbent · GC-EI-MS

## Introduction

With cannabis being the most commonly used illicit drug worldwide [1], the cannabinoids  $\Delta^9$ -tetrahydrocannabinol (THC), 11-hydroxy- $\Delta^9$ -tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy- $\Delta^9$ -tetrahydrocannabinol (THC-COOH) represent very frequently detected substances in cases of driving under the influence of drugs (DUIDs) and other medico-legal subjects [2, 3]. Therefore, the analyses of these cannabinoids require a sensitive and robust high-throughput method. One important requirement for optimal analyses is a low matrix load in the sample extracts as it often interferes with analytical results. Especially the heterogeneity in blood collecting systems used by the police [4] and their differences in storage length and conditions increase the complexity of the matrices. Depending on the time of storage before centrifugation, the physiological properties of matrices can change drastically, e.g. due to haemolysis [5].

When applying the still regular in use single quadrupole mass spectrometry (MS), the matrix load can cause immense interfering peaks both in liquid chromatography (LC) and gas chromatography (GC). By the use of a tandem quadrupole mass spectrometry (MS/MS) or a two-dimensional GC (2D-GC), the obvious matrix interference in the chromatogram can be reduced. However, a high level of matrix pollution in the chromatographic system still should be prevented. For the LC-MS system, this is important in order to avoid matrix effects like ion suppression or ion enhancement. For the GC-MS

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system, it is necessary in order to avoid matrix contamination which can cause column performance degradation and thereby drastically shorten the column lifetime. Depending on the instrument sensitivity and the scientific or forensic issue, a reduction of the amount of sample in order to reduce the matrix load is not always possible. Therefore, constant further development of efficient extraction methods is necessary.

To date, many different extraction procedures have been published in combination with both GC-MS and LC-MS, all pursuing the objective of improving the chromatographic results. Besides liquid-liquid extraction (LLE) [6–8] and solid-phase extraction (SPE) with nonpolar (e.g. C18) sorbent material [9–12], SPE with anion exchange sorbent (AXS) received increasing attention in the field of cannabinoid extraction out of urine, plasma and serum. Most published extraction methods for cannabinoids in serum and plasma using SPE with AXS deploy a single-elution step [13–19]. However, to utilize the potential of an anion exchange sorbent, a two-step elution might be promising.

According to literature, less clean up is required if the two-step elution is applied without combining the eluates [20]. However, this implies duplication in measurement time. Therefore, extraction methods for whole blood using AXS in combination with a two-step elution with combined eluates have been published [21, 22]. However, without using an additional washing step between the elution steps, still major matrix problems occur. In the LC-MS analyses, this becomes apparent by matrix effects up to –88.5 % [21]. In the GC-MS analyses, it can be seen in the need for further chromatographic separation e.g. with 2D-GC [22]. In another published method for plasma using AXS in combination with a two-step elution and an additional washing step in between, the recovery of THC-COOH appeared to vary with the lot of SPE columns and showed a recovery of only 17 % in the worst case [23].

In this work, we examine and utilize the extraction potential of a strong AXS with a C-8/quaternary amine/silica-material in a two-step elution, in combination with gas chromatography-electron impact-mass spectrometry (GC-EIMS) for the analysis of cannabinoids. Particular emphasis is placed on clean chromatograms with very little interfering matrix peaks. The advantage of the developed and validated analytical method is demonstrated by comparing it with a validated C18-SPE method used in our laboratory and with a representative published AXS-SPE with only a single-elution step validated for a 2D-GC-MS system [17].

## Material and methods

### Materials and reagents

THC (1 mg/mL), 11-OH-THC and THC-COOH (100 µg/mL) were purchased from Lipomed AG (Switzerland). Deuterated

internal standards THC-d<sub>3</sub>, 11-OH-THC-d<sub>3</sub> and THC-COOH-d<sub>9</sub> (100 µg/mL) were obtained from Sigma-Aldrich (Germany). Acetonitrile (ACN), acetone, ethyl acetate, ethanol and methanol were purchased from Sigma-Aldrich (Germany). Sodium acetate, potassium dihydrogen phosphate, sodium hydrogen phosphate, acetic acid, hydrochloric acid and hexane were from Merck (Germany). Double distilled water was purchased from AppliChem (Germany). All solvents were analytical or HPLC grade. The derivatization reagent *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) and the SPE columns Chromabond® Drug II and Chromabond® C18 ec (200 mg, 3 mL) were from Macherey-Nagel (Germany). The SPE columns Clean Screen® ZSTHC020 (200 mg, 10 mL) were from United Chemicals Technologies (USA).

Phosphate buffer (0.15 M, pH 6.0) was prepared with 0.15 M potassium dihydrogen phosphate and 0.15 M sodium hydrogen phosphate. Sodium acetate buffer (2.0 M, pH 4.0) was prepared with 2.0 M sodium acetate and 2.0 M acetic acid. For method development and validation, drug-free fluoride/oxalate plasma collected from healthy volunteers and bovine serum purchased from Sigma-Aldrich (Germany) were used. In addition, selectivity was determined in drug-free serum collected from healthy volunteers. All SPE methods were manually performed on BAKER SPE-12G column processors from J.T. Baker.

### Standard solutions

An internal standard (I.S.) working solution containing 0.1 µg/mL THC-d<sub>3</sub>, 11-OH-THC-d<sub>3</sub> and 1 µg/mL THC-COOH-d<sub>9</sub> in methanol was made. For the validation parameters, a working solution with 0.1 µg/mL THC, 11-OH-THC and 1 µg/mL THC-COOH in methanol, as well as a tenfold and a 100-fold dilution of this solution was prepared.

### Sample preparation and SPE (AXS, two-step elution)

One millilitre plasma or serum was diluted with 2 mL phosphate buffer (0.15 M, pH 6.0). After the addition of 50 µL I.S. working solution, 250 µL ACN was added under vortex-mix. Samples were centrifuged at 2576×*g* for 10 min. Chromabond® Drug II columns were conditioned with 3 mL acetone, 3 mL methanol and 3 mL phosphate buffer (0.15 M, pH 6.0). After sample application, the columns were washed with 2 × 3 mL water, followed by two drying steps of 5 min in a stream of nitrogen with a custom-designed nitrogen manifold and a pre-wash of 100 µL acetone in between. After the elution of THC and 11-OH-THC with 1.6 mL acetone, each column was washed with 2 mL ACN/0.1 M acetic acid (30:70, *v/v*) followed by a drying step of 10 min in a stream of nitrogen. After the addition of 1 mL hexane and 1 mL hexane/ethyl acetate (70:30, *v/v*), the columns underwent a

final drying step of 5 min in a stream of nitrogen before the final washing step of 2 mL acetone. THC-COOH was finally eluted with acidic acetone (0.05 M acetic acid in 1.6 mL acetone) into the same collecting tube as THC and 11-OH-THC. After evaporation to dryness at 40 °C in a stream of nitrogen, the extracts were reconstituted with 30 µL MSTFA for derivatization at 70 °C for 20 min.

#### Investigation of extraction procedure (AXS, two-step elution)

Recoveries in plasma with different levels of blood haemolysis and the influence of ACN addition before extraction were investigated. For this, drug-free plasma that had been centrifuged immediately after blood withdrawal, after 3 and after 10 days of storage at 8 °C was prepared. Extraction was carried out both with and without the addition of 250 µL ACN to each sample after the dilution with 2 mL phosphate buffer (0.15 M, pH 6.0). Extraction was carried out in triplicates. The recovery was assessed by comparing the peak area ratios (analytes/I.S.) of plasma samples spiked with analyte (5 ng/mL THC, 5 ng/mL 11-OH-THC and 50 ng/mL THC-COOH) before extraction and I.S. after extraction with the ones of the respective matrix spiked with analytes and I.S. after extraction.

#### Sample preparation and SPE (C18)

One millilitre plasma or serum was diluted with 2 mL phosphate buffer (0.15 M, pH 6.0). After the addition of 50 µL I.S. working solution, samples were centrifuged at 2576×g for 10 min. Chromabond® C18 ec columns were conditioned with 2×3 mL acetone, 3 mL methanol and 3 mL water. After sample application, the columns were washed by subsequent addition of 3 mL water, 1 mL 0.25 M acetic acid and 3 mL methanol/water (50:50, v/v). After 5 min of maximum vacuum, the columns were pre-washed with 100 µL acetone followed by a further drying step under maximum vacuum for 20 min. Finally, THC, 11-OH-THC and THC-COOH were eluted with 1.5 mL acetone. After evaporation to dryness at 40° in a stream of nitrogen, the extracts were reconstituted with 30 µL MSTFA for derivatization at 70 °C for 20 min.

#### Sample preparation and SPE (AXS, single-step elution)

Extraction was carried out on the basis of a published method [17]. To 1 mL plasma, 50 µL I.S. working solution was added. After protein precipitation with 2 mL cold ACN, samples were centrifuged at 2576×g for 10 min. The supernatants were diluted with 4 mL sodium acetate buffer (2 M, pH 4.0). Clean Screen® ZSTHC020 SPE columns were conditioned with 1 mL elution solvent (hexane/ethyl acetate, 80:20, v/v), 3 mL methanol, 3 mL water and 1 mL 0.1 M HCl. After

sample application, the columns were washed by subsequent addition of 3 mL water and 2 mL 0.1 M HCl/ACN (70:30, v/v). After 10 min of maximum vacuum, the columns were primed with 200 µL hexane. Finally, THC, 11-OH-THC and THC-COOH were eluted with 5 mL elution solvent into 10-mL tubes containing 500 µL ethanol. After evaporation to dryness at 40 °C in a stream of nitrogen, the extracts were reconstituted with 30 µL MSTFA for derivatization at 70 °C for 20 min.

#### GC-MS analysis

For the analysis of the cannabinoids, an Agilent Technologies 6890 N GC system equipped with a mass selective detector (5973) and a Combi PAL autosampler from CTC Analytics was used. Detection was accomplished with the mass selective detector operating in electron impact (EI) selective ion monitoring (SIM) and scan mode.

The GC separation was carried out with a capillary column OPTIMA® 5 MS Accent (95 % dimethylpolysiloxane, 5 % diphenylpolysiloxane, 30 m×0.25 mm i.d.×0.25 µm from Macherey-Nagel, Germany) with the following temperature program: 1.5 min at 150 °C, 9°/min up to 260 °C, hold for 6 min; 30°/min up to 300 °C, hold 8 min. The helium gas flow rate was 1.0 mL/min. The temperatures of the injector, the transfer line, the ion source and the quadrupole were 250, 280, 230 and 150 °C, respectively. The injection mode was splitless, and the injection volume was 2 µL. The *m/z* values used for identification and quantification of the trimethylsilyl derivatives in the SIM mode were as follows (target ion underlined): THC-d<sub>3</sub> 374, 389; THC 371, 386, 303; 11-OH-THC-d<sub>3</sub> 374, 462; 11-OH-THC 371, 459, 474; THC-COOH-d<sub>9</sub> 380, 306; THC-COOH 371, 297, 473.

#### Validation

The validation was carried out according to the criteria of the “Society of Toxicological and Forensic Chemistry” (GTFCh) guidelines [24] by using the software Valistat 2.0 (Arvecon GmbH, Germany). Selectivity was analyzed by extracting six blank serum and six blank plasma samples of different human individuals, two blank samples of each matrix with I.S. and two blank serum samples spiked with drugs expected in forensic samples at their highest calibration level (amphetamines, cocaine, morphine, codeine and their metabolites). The linearity was determined by preparing six calibration curves. For this, blank serum samples were spiked with analyte at concentrations of 0.5, 1, 3, 5, 7 and 10 ng/mL for THC and 11-OH-THC and 4, 5, 10, 30, 50, 70 and 100 ng/mL for THC-COOH. To assess precision (expressed as relative standard deviation, RSD) and accuracy (calculated as bias in percent), quality control (QC) pools at a low, medium and high concentration in serum, and low and high concentration in

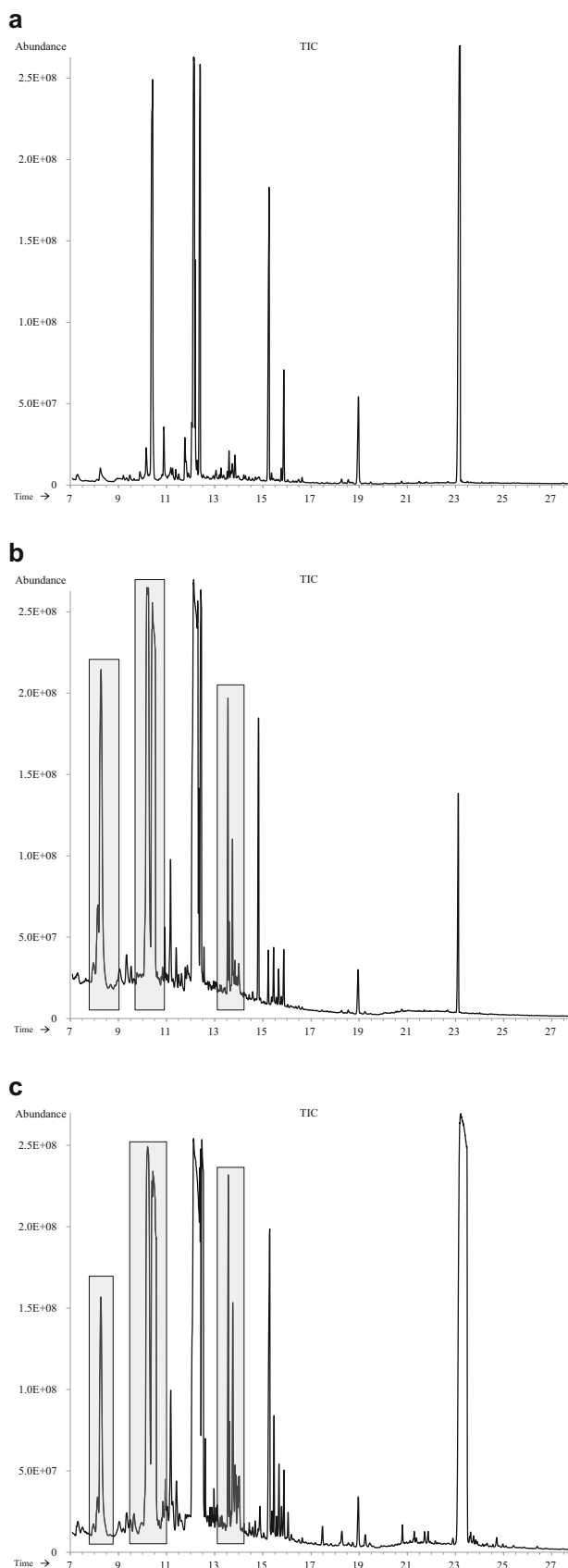
plasma were prepared. QCs of each concentration were extracted in duplicate on eight different days. The limits of detection (LOD) and limits of quantification (LOQ) in serum and plasma were determined with specific calibration curves using five calibrators in the range of the expected LOD. Recovery was evaluated by comparing the peak area ratios (analytes/I.S.) at low and high concentrations of six different serum and plasma samples spiked with analytes (1 and 8 ng/mL THC, 11-OH-THC, 10 and 80 ng/mL THC-COOH) before extraction and I.S. after extraction, with the ones of six different samples of the respective matrix spiked with analytes and I.S. after extraction. In order to evaluate the stability of processed serum samples in the autosampler, repeated injections of six QCs both at the low and high concentration over a period of 2 days were carried out. The stability was assessed by the decrease of the absolute peak areas.

In addition to the criteria of the GTFCh, the stability of the main THC metabolite THC-COOH-glucuronide during sample preparation and chromatographic analysis was evaluated. For this, six blank serum samples were spiked with 300 ng/mL THC-COOH-glucuronide, extracted and analyzed. The hydrolysis rate was calculated as the amount of THC-COOH-glucuronide which was converted to THC-COOH.

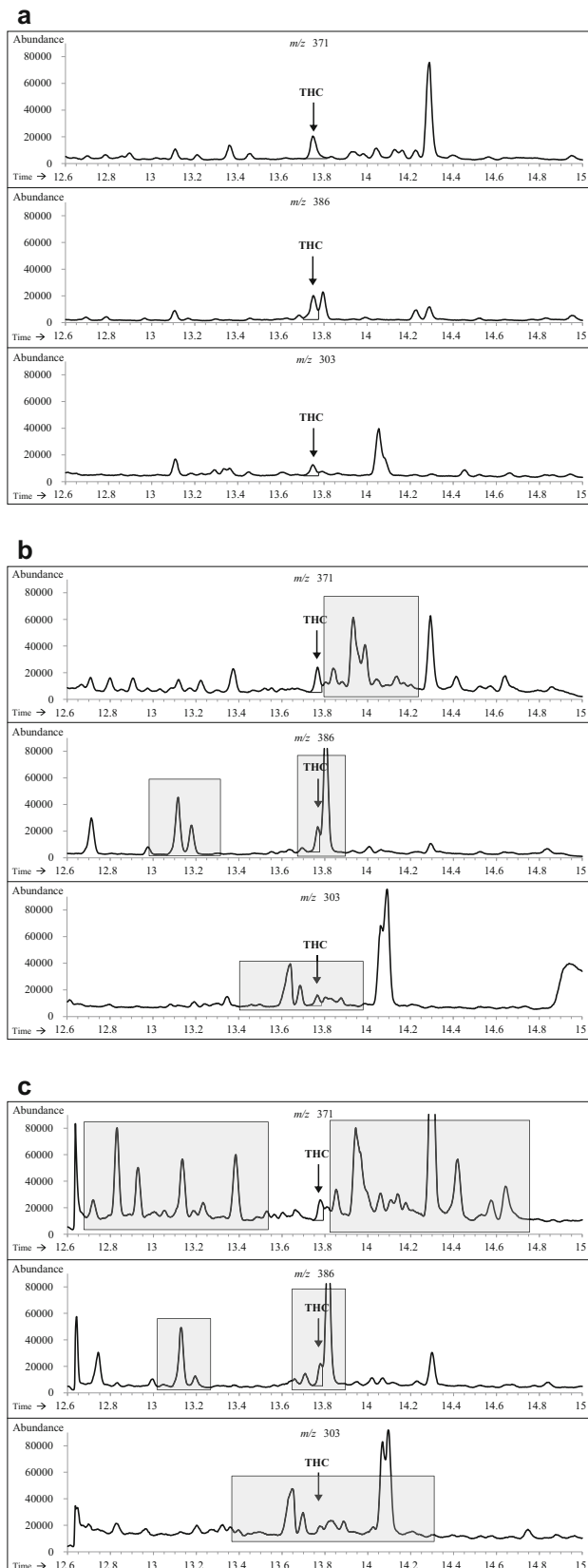
## Results and discussion

### Method development

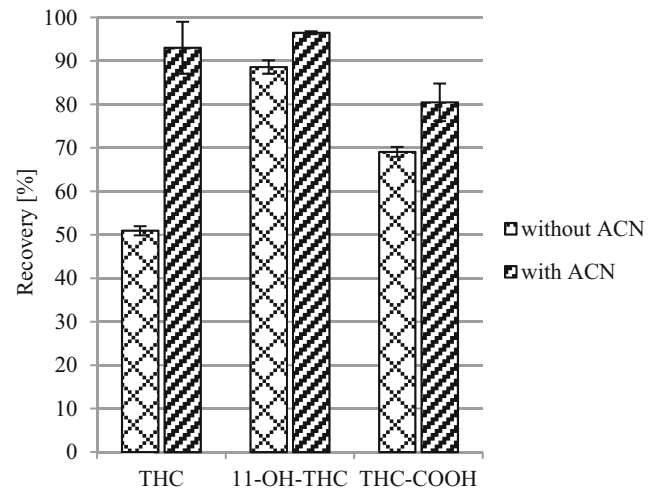
Initially, we compared different sorbent materials (hydrophobic silica-based sorbent, hydrophilic-lipophilic balanced polymer, mixed-mode cation exchange sorbent and mixed-mode AXS) in order to enable the most efficient separation between matrix components and cannabinoids. When using the hydrophobic silica-based sorbent, the hydrophilic-lipophilic balanced polymer and the mixed-mode cation exchange sorbent with a hydrophobic elution step (e.g. acetone), most of the interfering matrix components eluted together with the cannabinoids from the sorbents. A prior separation of the cannabinoids and the matrix components due to different washing steps was not effective because of similar interactions between matrix components or cannabinoids and the sorbent materials. However, when using a strong AXS with a hydrophobic elution step, an extensive retention of major matrix components could be seen demonstrating their anionic character. This provided the opportunity to elute the neutral cannabinoids THC and 11-OH-THC in a first step using acetone at pH 6, leaving anionic THC-COOH and main interfering matrix bound to the AXS. The most challenging part was the separation between THC-COOH and remaining matrix compounds without losses in recovery. Therefore, several washing steps with differences in pH and polarity combined with nitrogen drying steps were investigated. The best results in matters of separating



**Fig. 1** GC-EI-MS total ion chromatograms (TICs) of blank serum samples extracted with Drug II (a), C18 (b) and ZSTHC (c)



**Fig. 2** GC-EI-MS single ion modus (SIM) chromatograms of blank serum spiked with 0.4 ng/mL THC and extracted with Drug II (a), C18 (b) and ZSTHC (c)



**Fig. 3** Mean recovery of THC, 11-OH-THC and THC-COOH in plasma samples with and without the addition of ACN before extraction with standard deviations ( $n=3$ )

remaining matrix compounds from THC-COOH were achieved by using hexane and hexane/ethyl acetate solutions combined with nitrogen drying steps. This result is explainable due to changes in the aqueous film on the sorbent material. The acquired polarity finally enables the removal of the matrix components with pure acetone while at the same time maintaining the interaction between sorbent material and THC-COOH. After this final washing step, a clean elution of THC-COOH with acidic acetone in the same collecting vial as THC and 11-OH-THC was possible.

In order to outline the reduction of matrix interference as the main objective of this work, the chromatographic results were first compared with the C18 extraction previously used in our laboratory. During the application of this C18-SPE, the chromatographic results varied widely with the lot of the GC column. Minimal variations in the film thickness caused critical changes in the column selectivity due to the large numbers of interfering matrix peaks. Therefore, the application of this extraction method implied a high dependency on the column lot. Secondly, we compared the developed AXS-SPE with one representative published AXS-SPE with only a single-elution step validated for a 2D-GC-MS system [17]. The chromatographic results show a major reduction of the matrix load for the developed AXS-SPE (Drug II extracts) when compared to C18-SPE (C18 extracts) and AXS-SPE with only a single-elution step (ZSTHC extracts). The analysis of blank serum measured in scan mode (Fig. 1) obviously demonstrates less matrix peaks and a considerably lower baseline for the Drug II extracts in the total ion chromatogram. The additional matrix load shown in the C18 extract and the ZSTHC extract (highlighted in grey boxes) results in approximately twice the size in number and intensity of peaks compared to the Drug II extract. This leads to a decreased matrix exposure for the injection system, the GC-column and the mass spectrometer when using the Drug II extracts. If we look at the

**Table 1** Mean recovery of THC, 11-OH-THC and THC-COOH in plasma samples after different times of storage before centrifugation with relative standard deviations ( $n = 3$ )

Analyte	Recovery [%]		
	Day 1	Day 3	Day 10
	Mean (RSD)	Mean (RSD)	Mean (RSD)
THC	93.0 (6.4)	92.3 (1.1)	88.4 (3.6)
11-OH-THC	96.5 (0.3)	92.9 (1.2)	87.7 (2.9)
THC-COOH	80.5 (5.4)	78.7 (0.7)	76.9 (1.8)

measurement in SIM mode, most interfering matrix peaks occur in the retention area of THC. In this respect, the analysis of blank serum spiked with 0.4 ng/mL THC measured in SIM mode (Fig. 2) demonstrates considerably less interfering matrix peaks for the Drug II extract, ensuring reliable analyses regardless of the column lot. With the lot used in this experiment, co-eluting matrix interference in the C18 extract and the ZSTHC extract (highlighted in grey boxes) do not allow for quantification in this very low calibration area when using a GC-MS system without a 2D-GC.

During method development with the Drug II columns, the recovery showed variations for THC and THC-COOH in plasma compared to serum. Further investigation suggested varying interaction between these analytes and the sorbent material due to plasma components. In order to avoid this problem, different protein precipitation procedures and solvent additions were tested (e.g. acetone, ACN, methanol and methylene chloride). Very good results were achieved by the addition of ACN. However, in order to avoid the solvation of further matrix components in larger amounts of organic solvents, the addition of ACN was not carried out in form of the already described complete protein precipitation [25]. In this method, only 250  $\mu$ L ACN was added to each sample after the dilution with 2 mL phosphate buffer (0.1 M, pH 6). Due to this small amount of organic solvent, the interaction between analytes and sorbent material is less affected by plasma components without dissolving major matrix components, resulting in constantly good recovery. Figure 3 shows the compensation of the

**Table 2** Recovery ( $n = 6$ ) and LOD/LOQ for Drug II extracts in plasma and serum

Analyte	Conc. [ng/mL]	Recovery [%]		Limit of detection and quantification [ng/mL]			
		Plasma	Serum	Plasma		Serum	
		Mean (RSD)	Mean (RSD)	LOD	LOQ	LOD	LOQ
THC	1	92.8 (2.4)	90.1 (4.2)	0.15	0.30	0.15	0.30
	8	91.4 (2.4)	87.4 (1.9)				
11-OH-THC	1	92.9 (2.4)	91.5 (2.5)	0.25	0.35	0.25	0.35
	8	91.9 (2.3)	89.5 (1.0)				
THC-COOH	10	80.0 (6.4)	83.7 (9.9)	2.00	3.00	1.60	3.30
	80	79.8 (7.8)	86.0 (1.5)				

**Table 3** Accuracy bias, intraday and interday precision data for THC, 11-OH-THC and THC-COOH in serum and plasma

Analyte	Matrix	Conc. [ng/mL]	Accuracy bias [%]	Intraday precision RSD [%]	Interday precision RSD [%]
THC	Serum	1	-8.7	1.8	4.9
		5	-12.6	1.4	2.5
		8	-7.9	2.0	2.9
	Plasma	1	-11.3	1.9	3.5
		8	-11.3	0.8	2.6
		8	-11.3	0.8	2.6
11-OH-THC	Serum	1	-0.9	1.9	3.2
		5	1.3	1.5	2.3
		8	3.6	1.8	2.3
	Plasma	1	-2.4	1.5	3.1
		8	3.0	1.2	2.9
		8	3.0	1.2	2.9
THC-COOH	Serum	10	-5.1	2.2	3.9
		50	-6.3	1.7	2.2
		80	-2.6	1.4	1.9
	Plasma	10	-4.9	4.8	6.1
		80	-2.1	0.9	2.7
		80	-2.1	0.9	2.7

losses in recovery due to the addition of ACN. THC in particular shows a distinct improvement in the mean recovery from 51 % (RSD 2.1 %) to 93 % (RSD 6.4 %) by adding ACN. But also, THC-COOH gains from 69 % (RSD 1.6 %) to 80 % (RSD: 5.4) and 11-OH-THC from 89 % (RSD 1.7 %) to 96 % (RSD 0.3 %) in their mean recovery due to the addition of ACN. Table 1 shows the recoveries in plasma samples after different times of storage at 8 °C before centrifugation, in order to verify the good results due to the addition of ACN even in plasma samples with different levels of haemolysis. The experiments demonstrate that there is only a little decrease in the recoveries despite increasing levels of haemolysis. The mean recoveries still remain  $\geq 77$  % for all analytes. This ensures good and robust results even in samples that could not be centrifuged immediately after blood withdrawal, which is for example the case for blood samples gathered by the police.

## Validation

In both plasma and serum, the Drug II extracts resulted in very good selectivity. Neither matrix nor spiked xenobiotics showed interfering peaks in the retention area of analytes or internal standards.

Calibration range and calibration model were determined with the Grubbs test, Cochran test and Mandel test. The validation showed the applicability of a linear calibration model using a weighting factor of  $1/x$ . Both THC, 11-OH-THC and THC-COOH revealed good linearity with correlation coefficients ( $R^2$ ) not lower than 0.9982. Linear ranges were 0.5–10 ng/mL for THC and 11-OH-THC and 4–100 ng/mL for THC-COOH.

The decrease in interfering matrix peaks in the chromatogram allowed for very low LOD and LOQ in both matrices (Table 2). A good recovery determined at two concentration levels was achieved with  $\geq 79.8\%$  for THC-COOH and  $\geq 87.4\%$  for the neutral cannabinoids in plasma and serum (Table 2).

Accuracy bias and intra- and interday precisions were all below the acceptable value of 15 %, with a maximum bias of  $-12.6\%$  and a maximum RSD of 6.1 % (Table 3). Processed serum samples in the autosampler were stable over at least 2 days with a decrease of the absolute peak areas lying under the maximum acceptable threshold of 25 % for all analytes.

A very important issue concerning the analysis of cannabinoids is the influence of the cleavage from THC-COOH glucuronide to THC-COOH during sample preparation and chromatographic analysis [26]. Our investigation showed that after SPE with Drug II and analysis with GC-MS, only 1.35 % (RSD 1.80 %) of the spiked THC-COOH glucuronide was transformed to THC-COOH. Therefore, when applying the presented method, the cleavage of THC-COOH glucuronide to THC-COOH is negligible.

## Conclusion

In this work, we present a detailed examination of the interaction between matrix compounds, cannabinoids and the AXS. The method development was carried out with serum and plasma as well as plasma samples with different degrees of haemolysis to ensure reproducible results regardless of different storage conditions. This comprehensive investigation led to an effective two-step elution method which exploits the purposive ionic interaction between matrix components and the AXS. The achieved reduction of the matrix load lowers the requirements on the measurement instrument. Even when using gas chromatography-single quadrupole-mass spectrometry instead of two-dimensional gas chromatography or tandem-mass spectrometry, the developed and fully validated analytical method allows for an obvious decrease in number and intensity of matrix interference in the chromatogram.

Besides decreasing interfering matrix peaks, the reduction of the matrix load enables a decreased matrix exposure for the injection system, the GC-column and the mass spectrometer. Our results demonstrate new aspects in the field of effectively matrix-reducing SPE methods. This does not only offer a reliable and constant good analysis of cannabinoids in serum and plasma. It can also be the basis for further method development e.g. with different matrices or different analytes.

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