Published in : Journal of Chromatography B (2019), vol. 1092, pp. 332-342
doi.org/10.1016/j.jchromb.2018.05.049
Status : Postprint (Author's version)

IMPLEMENTATION OF A GENERIC SFC-MS METHOD FOR THE QUALITY CONTROL OF POTENTIALLY COUNTERFEITED MEDICINAL CANNABIS WITH SYNTHETIC CANNABINOIDS

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Abstract

In this study, we describe the development of a SFC-MS method for the quality control of cannabis plants that could be potentially adulterated with synthetic cannabinoids. Considering the high number of already available synthetic cannabinoids and the high rate of development of novel structures, we aimed to develop a generic method suitable for the analysis of a large panel of substances using seventeen synthetic cannabinoids from multiple classes as model compounds. Firstly, a suitable column was chosen after a screening phase. Secondly, optimal operating conditions were obtained following a robust optimization strategy based on a design of experiments and design space methodology (DoE-DS). Finally, the quantitative performances of the method were assessed with a validation according to the total error approach. The developed method has a run time of 9.4 min. It uses a simple modifier composition of methanol with 2% H_2O and requires minimal sample preparation. It can chromatographically separate natural cannabinoids (except THC-A and CBD-A) from the synthetics assessed. Also, the use of mass spectrometry provides sensitivity and specificity. Moreover, this quality by design (QbD) approach permits the tuning of the method (within the DS) during routine analysis to achieve a desirable separation since the future compounds that should be analyzed could be unknown. The method was validated for the quantitation of a selected synthetic cannabinoid in fiber-type cannabis matrix over the range of 2.5% – 7.5% (w/w) with LOD value as low as 14.4 ng/mL. This generic method should be easy to implement in customs or QC laboratories in the context of counterfeit drugs tracking.

Keywords

SFC-MS, Supercritical fluid chromatography (SFC), Synthetic cannabinoids, Cannabis, Method validation, Quality by Design

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1. Introduction

The cannabis plant (Cannabis sativa) is undoubtedly one of the most controversial plant in modern society [1, 2]. On one hand, it has a long history of use as a recreational drug which brought concern for public health and prompted various regulations and policies all around the world [3]. On the other hand, it was used for centuries for its supposed medicinal properties. Modern medicine shows that there is substantial evidence for its therapeutic effects in the treatment of various conditions [4, 5] and is currently gathering a lot of attention from the pharmaceutical field. The latter context is the focus of this study.

The cannabis plant is a rather complex matrix containing various compounds such as terpenoids, flavonoids, amino-acids, alkaloids and more [6]. The main active components are called cannabinoids, of which tetrahydrocannabinol (Δ^9-THC) is usually the most known, it is also the most responsible for the psychoactive effects of the plant. This molecule is nonetheless therapeutically relevant, as well as cannabidiol (CBD), another major cannabinoid. They are synthetized in the plant in acidic form as tetrahydrocannabinolic acid (THC-A) or cannabidiol acid (CBD-A) from the precursor cannabigerolic acid (CBG-A). The neutral forms are obtained by non-enzymatic decarboxylation by sunlight, heat or combustion during consumption [7]. There are however other less desirable compounds that can be detected in the cannabis plant that result from contaminations or adulterations. Expected natural contaminants can be degradation products, microbes or heavy metals and are usually introduced during cultivation or storage [8]. Cases of adulterations however are rather more obscure. Busse et al. [9] reported a case of lead poisoning that resulted from a malicious adulteration of cannabis with lead. They hypothesized that the perpetrators performed it to increase the weight of the product and therefore maximize profit margin. McPartland [10] and McPartland et al. [11] reported the adulteration of cannabis with psychotropic substances such as tobacco, calamus and other cholinergic compounds with the intent to enhance the cannabimimetic effects or reduce the adverse effects associated with the use of cannabis. Such aspects are of interest in this study, considering that ensuring the quality of the cannabis plant is essential to produce safe cannabis medicines.

Few cannabis-based medicines have been approved for use around the world [4, 12]. Most formulations make use of natural cannabinoids from the cannabis plant material (e.g. Bedrocan®), extracts from the plant or purified extracts containing only THC, CBD or a combination of both. There are other formulations that make use of synthetic cannabinoids such as dronabinol (Marinol®), a structural synthetic analogue of THC. However, with few exceptions such as the case of dronabinol, the most predominant use of synthetic cannabinoids currently is for recreational purposes. Historically, those substances were initially developed and studied for therapeutic use but were later misappropriated and misused [13, 14]. One key issue surrounding those substances is that their legal status is not always clear, bringing their manufacturers to produce novel substances at a high rate in an effort to circumvent the law whenever a substance becomes controlled. There are however model of legislation that could be appropriate for their regulation [15]. They are ultimately formulated to resemble natural cannabis by spraying the synthetic cannabinoids on material plant, marketed as "legal highs" and sold on the internet and other various shops [16]. Since their number is so high, there has been no safety and toxicological evaluation for most of them and therefore pose a serious threat to their users [17]. These issues highlight the need to develop methods for the screening of synthetic cannabinoids [18, 19].

Gas chromatography with mass spectrometry detection (GC–MS) is one of the most widely used technique for the determination of synthetic cannabinoids [18, 20] as well as natural cannabinoids [2, 21]. Second to

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GC are LC-based techniques, especially for the analysis of metabolites in biological samples [2, 18]. For this study we assessed the use of supercritical fluid chromatography (SFC). This green technique has gained a lot of interest during the last few years following recent technological advancements and could be presented as a bridge between gas chromatography and liquid chromatography [22, 23]. Its applications for the determination of synthetic cannabinoids has shown promising results for seized illegal drugs [24, 25] and bioanalysis [26]. It has also been evaluated for the determination of major cannabinoids in cannabis plant materials and products [27].

The objective of this study was to develop a SFC-MS method that could permit the detection of adulterations of the cannabis plant with synthetic cannabinoids. Hyphenation of SFC to MS was selected to improve method sensitivity and also to reach a more specific detection technique, especially in the context of counterfeiting drugs. Considering the great number of synthetic cannabinoids, many of which there is a lack of analytical standards, our focus was therefore on assessing few representative compounds from different classes in order to develop a fast-generic method that could be applicable on a large range of compounds. Indeed, the analytical method should be able to analyze each potentially adulterated sample, whatever is the nature of added synthetic cannabinoid(s). For this purpose, seventeen representative compounds from seven main classes were selected, they are presented in Section 2.4.1 and structures are available in supplementary data. Six natural cannabinoids were also included to access method selectivity/ specificity since they are endogenous compounds in the plant, but their quantitative determination was not the focus of this study. They were selected based on their prevalence in the plant and their therapeutic interest.

To optimize a high throughput SFC-MS method able to separate synthetic cannabinoids, a robust optimization strategy based on design of experiments (DoE) and Bayesian design space (DS) methodology was used. The usefulness of this strategy for the computation of the ICHQ8 (R2) design space (also named Method Operable Design Region) has already been demonstrated and is increasingly recommended for the robust optimization of analytical methods [28–31]. Several studies highlight its reliable performance in the optimization of chromatographic methods [32–34]. A detailed description of the DoE-DS method is provided in reference literature [28,29,31]. Ultimately, the quantitative performances of the SFC-MS method were assessed thanks to the total error approach method validation [35–38].

2. Materials and methods

2.1 SFC-MS instrumentation

The experiments were carried out on a Waters Acquity UPC^2 system equipped with a binary solvent delivery system, an autosampler with a 10 µL loop, a two stage back-pressure regulator and a PDA detector. A Waters Acquity QDa mass detector (ESI source) was used for mass spectrometry detection. Mass spectrometer was hyphenated to chromatographic system using a Waters Isocratic Manager (ISM) as a makeup solvent delivery system with a dedicated SFC-MS splitter interface.

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2.2 Chemicals and reagents

Synthetic cannabinoids JWH-018, JWH-073, JWH-122, JWH-019, JWH-081, JWH-200, AM-2201, MAM-2201, JWH-250, XLR-11, UR144, APINACA (also known as AKB-48), AB-FUBINACA, AB-PINACA, RCS-4, PB-22, HU-210 as well as natural cannabinoids CBG, (-)-Delta-9THC, CBD and internal standard deuterated cannabidiol (CBD-D3) were obtained from Lipomed AG (Arlesheim, Switzerland). Acidic natural cannabinoids CBG-A, THC-A and CBD-A were purchased from SigmaAldrich (St. Louis, MO, USA).

Methanol (MeOH, HPLC gradient grade), 2-Propanol (LC-MS grade) were purchased from J.T. Baker (Deventer, Netherlands). Water (ULC/ MS SFC/CC grade) and acetonitrile (ACN -LC-MS grade) were obtained from Biosolve BV (Valkenswaard, Netherlands). Ammonium formate (AF, analytical grade, > 98.7%) and ammonium acetate (AA, analytical grade, > 98%) were purchased from VWR chemicals (Leuven, Belgium). Carbon dioxide (99.995%) was sourced from Westfalen BVBA (Aalst, Belgium).

2.3 Plant material samples

Five different fiber-type cannabis samples were kindly provided by the Laboratory of Pharmacognosy of the University of Liege.

2.4 Sample preparation for method development

2.4.1 Stock standard solutions

A stock solution of each cannabinoid at a concentration of 1 mg/mL in methanol was prepared by weighing 5 mg of standard in a volumetric flask of 5 mL. (-)-Delta-9-THC, MAM-2201, HU-210, THC-A, CBD-A, and CBG-A standards were already provided as a solution at 1 mg/mL or 10 mg/mL in methanol or acetonitrile and were used as is or diluted to attain a concentration of 1 mg/mL in methanol. All stock solutions were stored at −20 °C.

2.4.2 Column screening samples

For the column screening experiments, only ultraviolet (UV) detection was available. Therefore, the seventeen cannabinoids were separated into five groups to simplify data analysis and method development. Those groups were devised to include synthetic cannabinoids of different classes in the following way:

Group 1: JWH-018 (naphtoyindole), JWH-200 (naphtoyindole), AB-FUBINACA (indazole).

Group 2: JWH-019 (naphtoyindole), UR-144 (ketone indoles), ABPINACA (indazole).

Group 3: JWH-073 (naphtoyindole), JWH-250 (phenylacetylindole), AM-2201(naphtoyindole), APINACA (indazole).

Group 4: JWH-081 (naphtoyindole), XLR-11 (ketone indoles), MAM-2201 (naphtoyindole).

Group 5: JWH-122 (naphtoyindole), RCS-4 (benzoylindole), PB-22 (quinolinyl ester), HU-210 (dibenzopyrane).

For each group, the stock standard solutions were diluted to prepare a solution containing the cannabinoids at a concentration of 50 µg/mL in methanol.

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2.4.3 Method optimization samples

After choosing a suitable column from the screening, a design of experiments was carried out. The stock standard solutions were diluted to prepare a solution containing all the seventeen synthetic cannabinoids as well as the six natural cannabinoids (23 compounds) at a concentration of 40 µg/mL in methanol.

2.5 Sample preparation for method validation

2.5.1 Validation protocol

The first step in preparing the validation protocol was to choose a matrix and concentration range, in such a way to represent as closely as possible real samples. For the matrix, fiber-type cannabis was found to be suitable. It is a variety of cannabis that has a low concentration of THC and generally contains a higher concentration of the other nonpsychoactive cannabinoids CBD or CBG [1]. These plants are the most representative of real samples that could be used legally in the lab.

Choosing an appropriate range however can be quite challenging in the case of synthetic cannabinoids since the concentration at which they are added to the plant material can vary tremendously, some authors report a range from 0.1% to 10% w/w [39, 40]. Such variations can be expected considering the illicit nature of the products and clandestine working conditions during their manufacture. It was therefore chosen to validate the method for a target concentration of 5% w/w and a range covering 50% – 150% of the target concentration (i.e. 2.5% – 7.5%) for the quantitative determination of the synthetic cannabinoid XLR-11.

The protocol was built to use efficiently the available quantity of analytical standard. Therefore, the first series was performed using the standard stock solution prepared for method development. For the three following series, a fresh stock solution dedicated to the validation (SVS) was prepared by weighing accurately 2.5 mg of XLR-11 analytical standard and dissolving it in a volumetric flask of 10 mL (250 µg/mL) with methanol.

Moreover, an internal standard (CBD-D3) was added in all the final calibration and validation solutions at a concentration of 250 ng/mL to control ionization variability and potential matrix effect. This internal standard was selected as a potential generic one that could be used for the quantification of a large panel of synthetic cannabinoids. However, it must be evaluated compound per compound basis since matrix effect (s) can depend on retention times and compound chemical properties.

2.5.2 Calibration standards

For calibration standards (three concentration levels), a calibration stock solution (CSS) was prepared by diluting ten times the validation stock solution (SVS) to obtain a concentration of 25 µg/mL. For each series, independent dilutions of this stock solution (CSS) were performed to obtain final concentrations of 125, 250 and 375 ng/mL.

2.5.3 Validation standards

For validation standards (five concentration levels), three independent repetitions were prepared for each series by spiking 5 mg of three different plant material with 1 mL of the validation stock solution (SVS - 250 µg/mL) which corresponds to the target concentration of 5% w/w. Samples were sonicated for 15 min with vortex at T0, T5, T10 and T15 min, centrifugated at 14000 rpm for 1 min and filtrated on chromafil Xtra PTFE

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20/13 0.20 um (Macherey & Nagel, Berlin, Germany). Finally, the resulting solution was diluted to obtain final concentrations of 125, 188, 250, 313 and 375 ng/mL.

2.5.4 Matrix sample solutions

Matrix sample solutions were also prepared for each available plant matrix by adding 1 mL of methanol to 5 mg of plant material and applying the same preparation protocol used for validation standards.

2.6 Analytical method

2.6.1 SFC method

The following parameters for the chromatographic system were kept constant throughout the whole study: a flow rate of 1.5 mL/min, an injection volume of 2 µL and the autosampler temperature at 6 °C. After the column screening experiments, the modifier composition employed for the rest of the study (optimization and validation) was MeOH/H_2O 98/2 v/v (without additive).

2.6.2 Column screening

Seven different SFC-dedicated chromatographic columns were considered namely Acquity UPC^2 Torus Diol, Torus 2-PIC, Torus DEA, Torus 1-AA, Viridis CSH-FP, Viridis BEH and Viridis BEH-2EP. All columns had a dimension of 100 × 3.0 mm and a particle size of 1.7 µm.

A generic gradient was used with MeOH as a modifier from 2% to 25% in 5 min, followed by a 2 min isocratic step, a return to initial conditions in 0.5 min and finally a 2 min reequilibration step before the next injection with a flow rate of 1.5 mL/min. An additive was added in the modifier, either 2% H_2O + 10 mM ammonium formate or 2% H_2O + 10 mM ammonium acetate except for 1-AA column testing. Indeed, no additive was used on the 1-AA column to minimize the risk of excessive UV baseline noise from column bleeding due to the degradation of the amino-anthracene stationary phase [41].

2.6.3 MS parameters

Initial MS conditions were based upon generic conditions provided by the equipment software. The cone voltage was set at 15 V and the capillary voltage at 0.8 kV in positive and negative mode. Data acquisition was performed in centroid mode at a sampling rate of 10 points/s and the probe temperature was set at 600 °C. The make-up solvent was delivered at a flow rate of 0.3 mL/min. Its composition was based upon the work of Andri et al. [42], consisting of a mixture of methanol and water (80:20 v/v) with 10 mM of ammonium formate. Several tests were performed to reduce MS ionization variability before method validation. Indeed, in-source fragmentation was highlighted mainly caused by the cone voltage. This parameter was found to have the most influence and it was set at 10 V to reach the lowest signal variability.

ESI source in positive and negative mode were used in scan mode from 280 to 400 *m/z* for method optimization. MS detection parameters for each targeted analyte are presented in Table 1. Finally, for method validation, SIR mode was selected to target only the analyte and internal standard.

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2.7 Design of experiments

Following the initial tests and column screening experiments, a method optimization was carried out using the design of experiments (DoE) and Bayesian design space (DS) methodology. A brief description of the steps of its implementation in the current study is provided as follows. Four Critical Method Parameters (CMP) were selected after the preliminary study: the pressure (*P*), the temperature (*T*), the isocratic time (T_I) and the gradient time (T_G). The parameters and their respective levels are summarized in Table 2. The DoE selected to perform the optimization of the method is a four-parameter rotatable inscribed central composite design with five levels for each parameter. The central point of this design was independently repeated 12 times enabling a model-independent estimation of the pure experimental error and allowing a lack-of-fit test to validate the model. The resulting design is composed by 36 experiments (*N* = 36). The experiments were randomized and performed over the course of three days (i.e. 12 experiments per day). For each day, a fresh modifier mobile phase and make-up solvent were prepared.

Table 1

Targeted *m/z* ratio. Acid cannabinoids THC-A, CBD-A and CBG-A were monitored in negative ionization mode.

Synthetic cannabinoids							
Compound	*m/z*	Compound	m/z	Compound	*m/z*	COMPOUND	*m/z*
AB-FUBINACA	369.40	JWH-019	356.15	MAM-2201	374.16	THC-A (ESI -)	357.12
AB-PINACA	331.05	JWH-073	328.09	PB-22	359.12	CBD-A (ESI-)	357.12
AM-2201	360.14	JWH-081	372.16	RCS-4	322.12	CBG-A (ESI-)	359.16
APINACA	366.10	JWH-122	356.15	UR-144	312.06	THC	315.12
HU-210	387.23	JWH-200	385.5	XLR-11	330.06	CBD	315.12
JWH-018	342.50	JWH-250	336.13			CBG	317.07

Table 2

DoE factors and their respective levels.

Factors	Levels				
T° (°C)	35	40	45	50	55
P (psi)	1595	1668	1740	1813	1885
t_{iso} (min)	0.50	0.88	1.25	1.62	2
t_{grad} (min)	6	7.5	9	10.5	12

2.7.1 Choice of the modelled responses and the critical method attributes

Three responses were measured to describe the chromatographic behavior of each compound. These are the retention times measured in minutes at the beginning ($t_{B,m}$), the apex ($t_{R,m}$), and the end ($t_{E,m}$) of each chromatographic peak (where *m* = 1, …, *M* and *M* = 23 is the total number of compounds). The measured retention times were normalized and log-transformed, and thus modelled as (log-transformed) retention factors as follows,

$$y_{B,m} = \log\left(\frac{t_{B,m} - t_0}{t_0}\right), y_{R,m} = \log\left(\frac{t_{R,m} - t_0}{t_0}\right), and y_{E,m} = log\,\frac{t_{E,m} - t_0}{t_0} \quad (1)$$

where $y_{B,m}$, $y_{R,m}$ and $y_{E,m}$ are the (log-transformed) retention factors at the beginning, apex and end of the peak corresponding to compound m; t_0 is the measured dead time of the system in minutes. The separation criterion (*S*) was selected as Critical Method Attributes (CMA) to determine the best achievable separation conditions. It is defined as the difference between the beginning of the second peak and the end of the first peak of the critical pair, which is defined as the two closest peaks.

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2.7.2 Model

The postulated statistical model to describe each of the three retention times for each compound ($3M = 69$ responses) as function of the four parameters was a second-order response surface model, written as follows:

$$y = b_0 + b_1P + b_2T + b_3T_G + b_4T_I + b_{12}P.\,T. + b_{13}P.\,T_G + b_{14}P.\,T_I + b_{23}T.\,T_G + b24T.\,T_I + b_{34}T_GT_I + b_{11}P^2 + b22T^2 + b_{33}T_G^2 + b_{44}T_I^2 + \varepsilon \quad (2)$$

where y is any of the modelled responses; P, T, T_I and T_G are the CMP; b_i, b_{ij}, and b_{ii} are the model coefficients ($i = 1, 2, 3, 4$ and $i < j$); and ε is the random error. This model is quite flexible and allows providing a good approximation to the true relationships between the responses and the CMP with moderate size experimental domain [42]. Moreover, this model has already yielded satisfactory description of the specific chromatographic behaviors of SFC [42, 43].

2.7.3 Model fitting

Model (2) was fitted jointly to the data including $N = 36$ observations of the $3M = 69$ responses, using the Bayesian standard multivariate regression as follows,

$$\boldsymbol{Y} = \boldsymbol{XB} + \mathbf{E}, \quad (3)$$

where $\boldsymbol{Y}$ is the matrix of observed retention factors values, $\boldsymbol{X}$ is the model matrix, $\boldsymbol{B}$ and $\boldsymbol{E}$ are the coefficients and errors matrices respectively; non-informative priors were considered for the coefficients in $\boldsymbol{B}$, while errors of the three retention factors of each peak were a priori assumed correlated [29].

A key output of the Bayesian multivariate linear regression is the joint posterior predictive distribution of the retention times, which accounts for both model errors and uncertainties in the estimation of model parameters. This approach properly fulfills ICH Q8 (R2) requirements. The closed-form of this distribution is a multivariate Student-t [29]. It is useful to obtain predictions of the retention times at any point within the experimental domain.

2.7.4 Definition of the Bayesian design space

The design space was determined using the random search method proposed by Lebrun et al. [44]. This method proceeds as follows. First, a sample of size $N_s = 10000$ values was randomly drawn from a uniform probability distribution, independently for each of the four parameters. This resulted in $N_s = 10000$ random points, densely filling the experimental domain, each point corresponding to a combination of four random factors' settings. Second, a Monte Carlo sample of the joint predictive distribution of the retention times was generated for each point by drawing randomly from the multivariate Student-t distribution. Third, the predictive distribution of the criterion S at each point filling the experimental domain was computed by propagating error from the Monte-Carlo sample of the retention times to S. Given the predictive distribution of S, regions of experimental domain where most or almost all points fulfilled the criterion S with a sufficient probability level were identified as regions of interests. Fourth, the above described search strategy was iterated on these smaller and interesting subspaces, until an area of robustness was found. This resulted in a sequential and multi-resolution search strategy. Fifth, within the design space, a working point corresponding to experimental domain that maximize the probability of meeting the criterion S was identified, corresponding to the optimal working condition. This random search approach has several substantial advantages compared to the classical grid search and probability surface approach. For a fixed number of points to be evaluated in the experimental domain, it enables to evaluate more levels per

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parameters than the grid search approach. Furthermore, it generally localizes more efficiently small and local patterns of the probability of meeting a specified CMA, compared with the classical grid search method [44].

2.8 Software

Empower 3 (Waters Corp., Milford, USA) was used to control the UPC^2 and QDa as well as data handling. JMP 13.0 was used to generate the design of experiments.

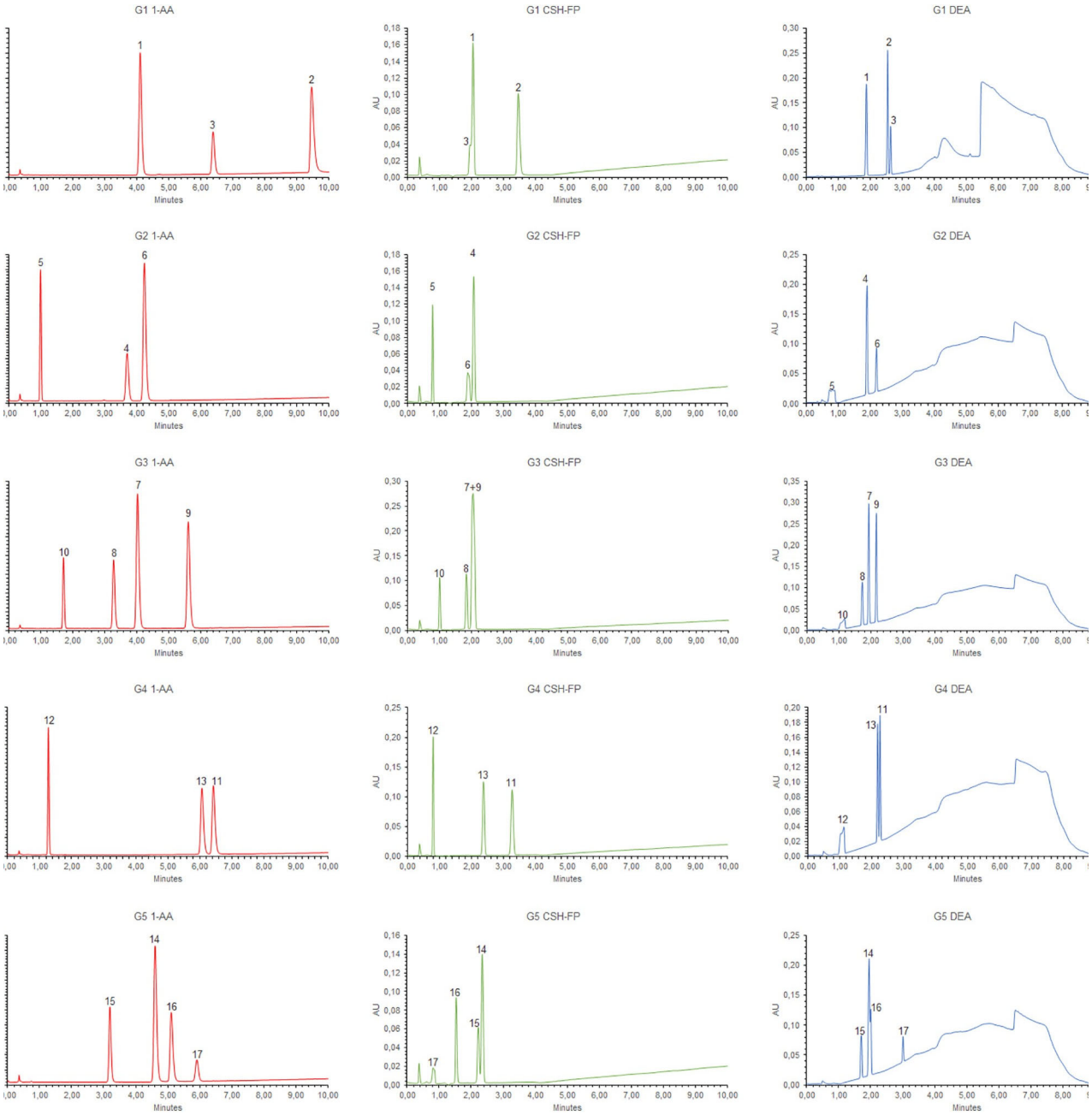

Fig. 1. UV chromatograms of the five synthetic cannabinoids groups on three columns: 1-AA, CSH-FP and DEA. Peaks label: 1. JWH-018; 2. JWH-200; 3. AB-FUBINACA; 4. JWH-019; 5. UR-144; 6. AB-PINACA; 7. JWH-073; 8. JWH-250; 9. AM-2201; 10. APINACA; 11. JWH-081; 12. XLR-11; 13. MAM-2201; 14. JWH-122; 15. RCS-4; 16. PB-22; 17. HU-210. Chromatograms obtained with during a generic test with a gradient that started from 6% modifier to 15% in 6 min at a flow rate of 1.5 mL/min. ABPR was set to 1740 psi and the column temperature at 40 °C.

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An algorithm was developed in R 3.4.0 to fit a Bayesian model and to compute the DS using Monte-Carlo simulations. E-Noval 4.0 (Arlenda S.A., Liege, Belgium) was used to assess the validation data and compute the accuracy profile.

3. Results and discussion

3.1 Method development

3.1.1 Column screening

The choice of the stationary phase is a critical step that will determine the success of the separation. Therefore, method development started with a stationary phase screening process using the protocol described in Section 2.6.2. The selectivity, global resolution and peak shape were selected as criteria to evaluate the stationary phases. Three columns (DEA, 1-AA and CSH-FP) were selected for further evaluation as they provided the best results to the criteria assessed. For subsequent tests, different modifier composition and gradient were evaluated using methanol, ethanol or acetonitrile with ammonium formate or ammonium acetate as additives, except on the 1-AA column (additive free conditions as explained in Section 2.6.2). Fig. 1 shows an example of UV chromatograms obtained for each of the groups using those three columns.

The DEA and CSH-FP performed rather similarly in the sense that they showed the lowest retention times and resolution for all the compounds in comparison to the 1-AA column. The DEA column did however show overall higher efficiency. Poor peak shapes could also be observed for the least retained compounds AKB-48, XLR-11, UR-144 on the DEA column and HU-210 on the CSH-FP. Indeed, using additive is generally mandatory when using this stationary phase [43]. The higher retention times observed on the 1-AA column can be attributed to π- π interactions from the indole, naphthalene or indazole moieties with the amino anthracene stationary phase. Such behavior was also reported by Breitenbach et al. [24]. The 1-AA column was therefore chosen for its overall better performances and could be presented as a really interesting stationary phase for cannabinoids. Furthermore, working with additive-free conditions present several advantages such as (i) shorter equilibration times, (ii) higher column lifetime, (iii) avoid stationary phase degradation/modification, (iv) provide easier mobile phase preparation and (v) reduce method qualitative variability. It is also important to notice that simple chromatographic conditions will be more easily implemented in different laboratories such as customs or QC control labs in the context of medicines counterfeiting.

3.1.2 Method optimization

3.1.2.1. Adequacy of the fitted model.

The adequacy of the model was proven by the good agreement between the observed and the predicted retention times. The adjusted coefficients of determination were above 0.98 for 65 out-of-the 69 modelled retention factors. The coefficients of determination of the remaining four retention factors were between 0.92 and 0.95. Moreover, the relationships between the observed values and the corresponding residuals showed no issue of lack-of-fit and heteroscedasticity. The raw residuals present a narrow distribution between −0.2 and 0.2 min (see Appendix 1).

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3.1.2.2. Design space and working point.

In previous publications, separation criterion S is often set at S > 0 in order to optimize a method with complete separation of all compounds. In the present study, the objective is a bit different. Indeed, it is important to remind that involved compounds are representative of the different synthetic cannabinoids classes. Therefore the future compounds that will be analyzed during routine analysis of real samples could be more diverse and some of them could be unknown (as counterfeiters are often ahead in comparison with regulatory agencies and QC labs). In this context, method optimization was focused on the development of a high throughput generic method able to analyze with adequate retention a large panel of synthetic cannabinoids. Moreover, high concentrations (40 µg/mL) were used as a worst case regarding separation criterion (leading to quite 'large' peaks) because the concentration of future samples is unknown. To obtain the method with the higher separation capacity (maximizing the number of peaks that could be separated), several levels of S criterion were investigated. Finally, two main points should be reminded at this stage: (i) regarding the method final objective (routine analyses of QC or seized samples), the nature of potentially added compound(s) is not known, thus there is no need to accurately separate critical pairs of peaks for model compounds. (ii) MS detection provides specificity (except for isobaric compounds) especially in the case of (partially) co-eluted analytes. Then, negative values of separation criterion were selected in order to fulfill method objectives and to avoid unrealistic DS computation due to one pair of peaks coelution (as natural cannabinoids were the more difficult to separate in the chromatographic conditions tested).

The steps and results of the DS computation strategy are shown on Fig. 2. On this Figure, the four-dimensional factors' space filled with the N_s = 10000 random points is unfolded and represented as a scatter matrix, and the patterns of probability of meeting various values of the criterion S were examined for every pair of factors. As shown on Fig. 2, A-1 and A-2, with a minimum S = −0.3, no subspace of the factors' space provided a probability of meeting S equal to or above 0.40. With S = −0.5, the subspace corresponding approximately to a pressure of 1675–1750 psi, a temperature of 35–42 °C, a gradient time of 7.5–10 min and an isocratic time of 0.5–1.0 min appeared as a stable region where the probability of meeting S was above 0.40 for most points (Fig. 2, B-1 and B-2). An in-depth investigation of this subspace using the search strategy described in the method showed that it is a region of robustness where almost all sampled points met the criterion S ≥ −0.5 with a minimum probability of 0.40 (Fig. 2, C-1 and C-2).

Hence, in this study, the design space is this subspace in which a separation between peaks (S) is superior to −0.5 min. A working point has been identified as the point corresponding to a pressure of 1738 psi, a temperature of 39 °C, a gradient time of 8.5 min and isocratic time of 0.90 min. At these conditions, the expected probability of meeting the criterion S = −0.5 was 0.81.

In the present study, optimal working condition should be considered as generic chromatographic conditions for samples screening. Subsequently, if critical pair(s) is (are) observed in the routine sample, working within the DS area should be investigated in order to maximize peak separation while keeping in mind that the detection technique is selective.

With the exception of THC-A and CBD-A, it is possible to chromatographically separate the natural cannabinoids from the synthetic cannabinoids under these optimal conditions. This is an important aspect for future analysis because it should be possible to directly highlight the presence of an unexpected compound in cannabis plant. For synthetic cannabinoids, it was also possible to achieve near baseline resolution for the separation of isobaric compounds JWH-019 and JWH-122 as depicted in Fig. 3. This fast

Published in : Journal of Chromatography B (2019), vol. 1092, pp. 332-342
doi.org/10.1016/j.jchromb.2018.05.049
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generic method enables the screening of samples in a run time of 9.4 min (reequilibration time not included).

3.2 Method validation

To demonstrate the quantitative performances, the optimized SFCMS method was validated for the quantification of a selected synthetic cannabinoid (XLR1-11). Method validation was performed using a total-error validation approach by considering the accuracy profile based on β-expectations tolerance intervals. Data were computed using ratio between XLR1-11 peak area and internal standard peak area. The accuracy profile obtained is presented in Fig. 4. The dashed blue lines are the β-expectation tolerance limits that model an interval in which future analytical results are expected to fall with a pre-defined risk. The method can be considered valid if the β-expectation tolerance limits are included in the acceptance limits represented by the dashed black lines. For this study, the acceptance limits were set at ± 15% and the β-expectation tolerance limits at 90% probability level. One outlier value (inconsistent result) from series 4 (level 5) was discarded. The profile obtained shows that developed method is valid for the quantification of XLR-11 in cannabis over the whole considered range. The validation data are presented in Table 3.

3.2.1 Selectivity

The selectivity of the method was demonstrated by analyzing the matrix sample solutions, no interfering compounds could be detected at the same retention time with the analyte of interest. Moreover, selectivity/specificity of the SFC-MS method regarding the other cannabinoids was highlighted during method optimization step. In the same context, method selectivity towards cannabinol (CBN - the degradation product of THC) was demonstrated.

3.2.2 Trueness and precision

Trueness, expressed in terms of mean relative bias (%), provides information on systematic errors. It is a measure of the agreement between the main results with the true value of the spiked samples and was assessed for each concentration level by comparing the mean introduced concentrations and the mean back calculated concentrations from the validation standards. Results show a slight negative bias across the whole range (from −0.57% to – 2.81%) that is totally acceptable and highlights the recovery efficiency of the method. For trueness criterion, validation data were also computed using absolute value (XLR111 peak area instead of ratio). The bias values reported are lower than |3%| meaning that the use of internal standard normalization did not impact method trueness. No ionization enhancement or suppression was highlighted for this compound. The large dilution of the sample and the retention properties of SFC method could partially explain these results.

Precision, on the other hand, provides information on random errors. It is expressed in terms of relative standard deviation (RSD (%)) and was evaluated at two different levels: repeatability (intra-series) and intermediate precision (intra and inter-series). The RSD values obtained showed the good precision of the method as RSD lower than 5 and 6% were obtained for repeatability and intermediate precision, respectively. The within-series variability (independent replicates) has a major impact on the overall method variability than the between-series (days). It could be easily explained by two main reasons: (i) different cannabis plant matrix were used to prepared independent validation standard, (ii) extraction step is also a source of method variability. For diagnosis purpose, validation data were also computed using absolute value (XLR1–11 peak area instead of ratio) and larger RSD values were obtained. The MS ionization

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variability could explain this observation and therefore the internal standard was useful in minimizing (at least partially) its effect.

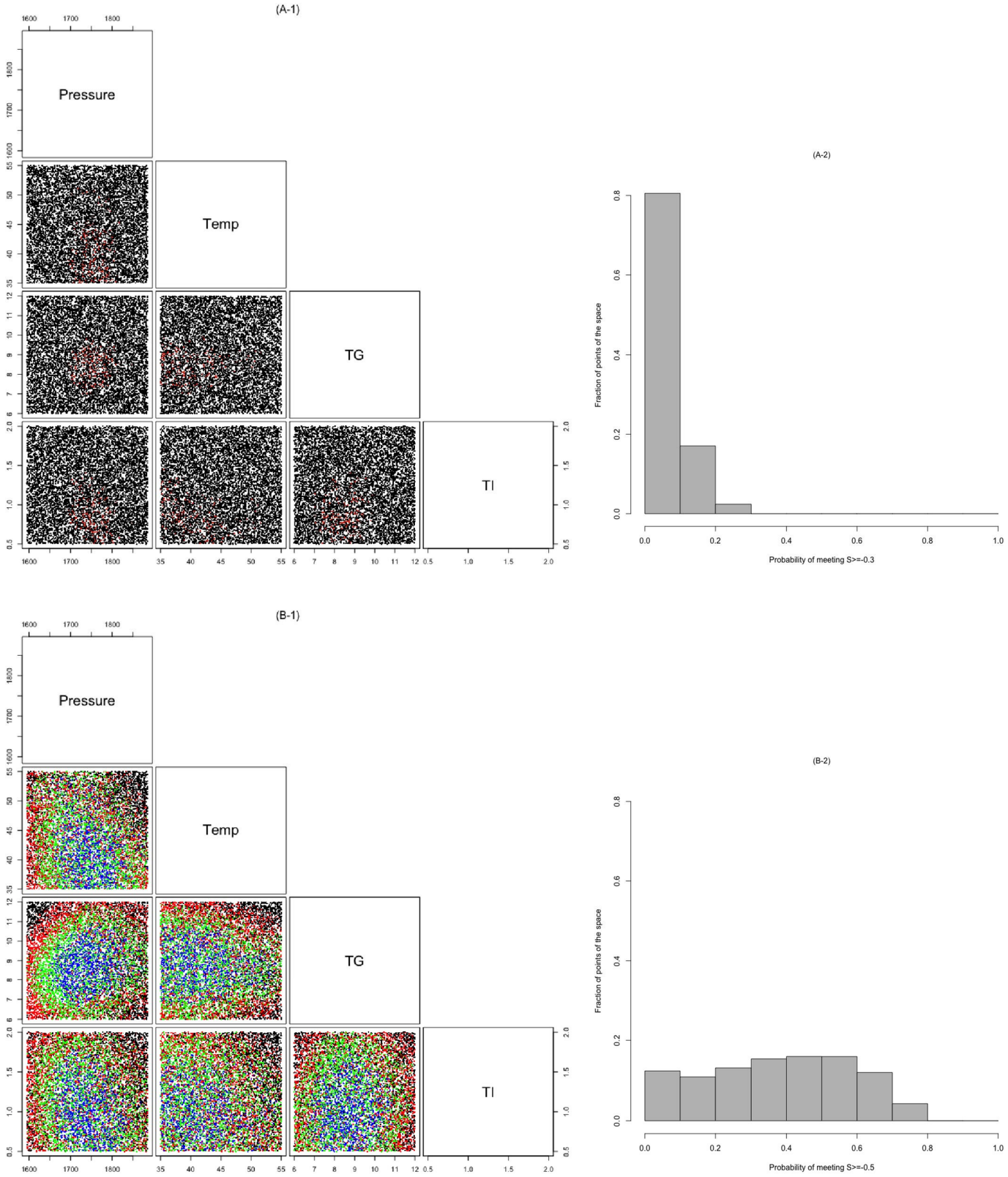

Fig. 2. Patterns and distributions of the probability of meeting separation criteria in the factors' space and identification of the design space. N_s = 10,000 new points were randomly sampled in each space or subspace and evaluated the probability of meeting a specified acceptance criteria. (A-1): Patterns of the probability of meeting S ≥ −0.3 in the factors' space (Pressure = 1595–1885

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psi, Temperature = 35–55 °C, TG = 6.0–12.0 min, TI = 0.50–2.00 min,) with p_max = 0.297. (A-2): Frequency distribution of the probability of meeting S ≥ −0.3 in the factors' space. (B-1): Patterns of the probability of meeting S ≥ −0.5 in the factors' space (Pressure = 1595–1885 psi, Temperature = 35–55 °C, TG = 6.0–12.0 min, TI = 0.50–2.00 min), p_max = 0.809. (B-2): Frequency distribution of the probability of meeting S ≥−0.5 in the factors' space. (C-1): Design space (Pressure = 1675–1750 psi, Temperature = 35–42 °C, TG = 7.50–10.00 min, TI = 0.50–1.00 min), p_max = 0.821. (C-2): Frequency distribution of the probability of meeting S ≥ −0.5 in the design space.

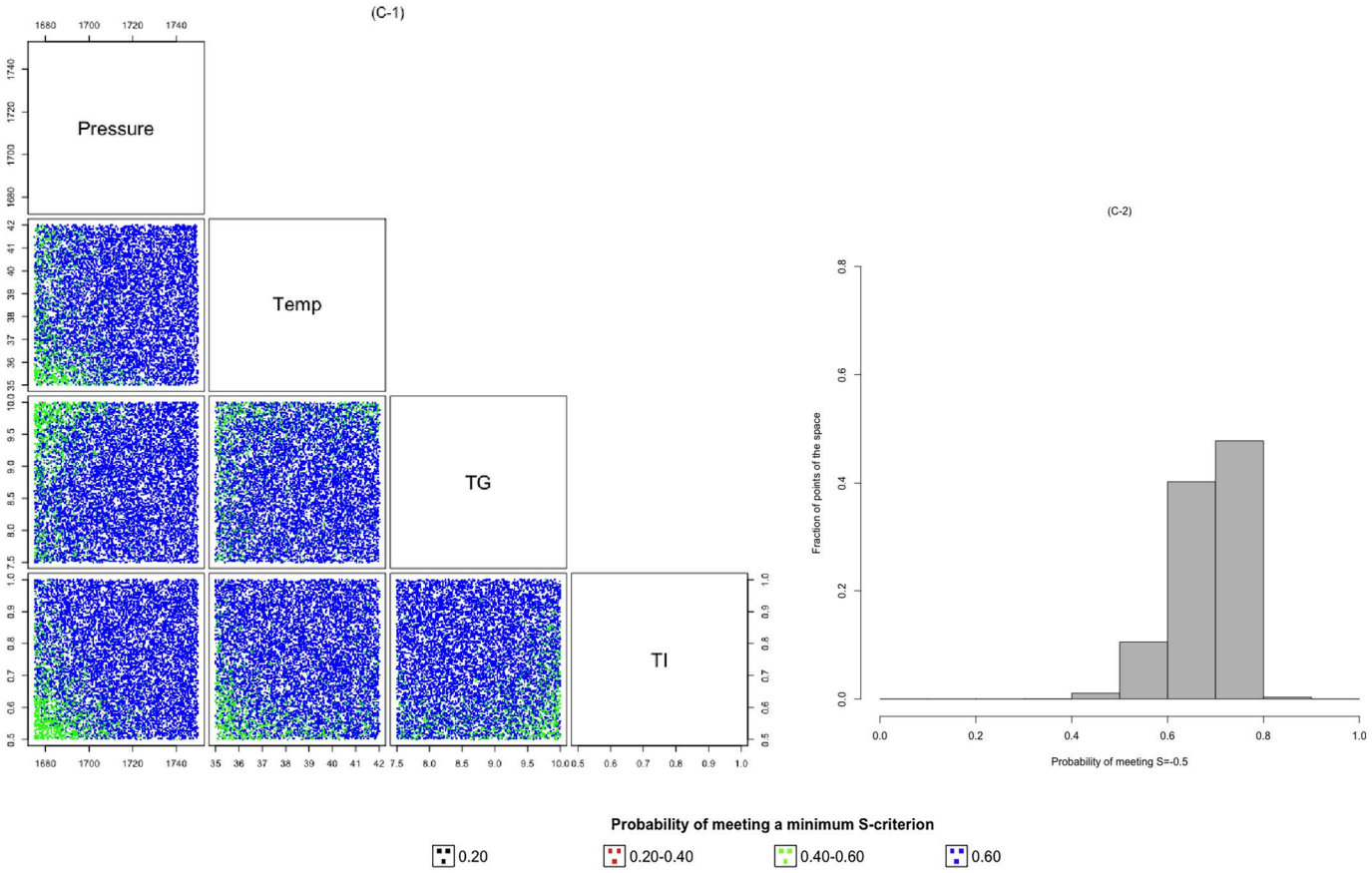

Fig. 2. (continued)

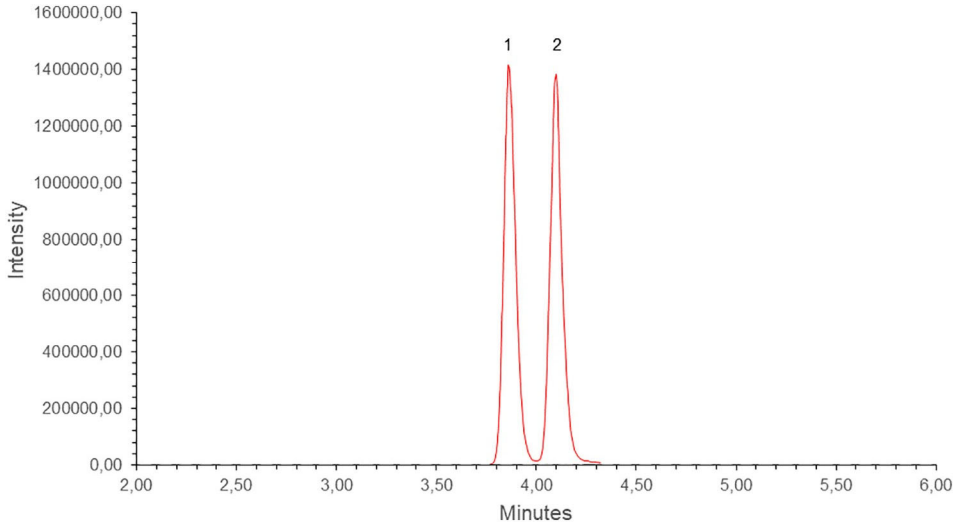

Fig. 3. Separation of isobaric compounds JWH-019 (1) and JWH-122 (2) under optimal conditions.

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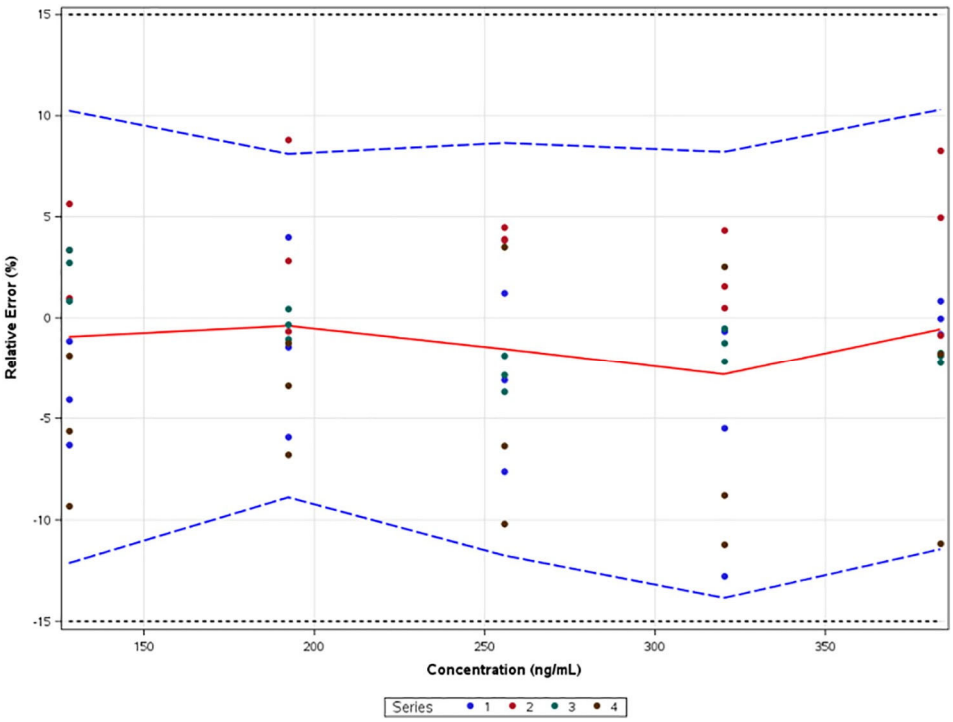

Fig. 4. Accuracy profiles obtained for the validation of the quantification of XLR-11 in fiber-type cannabis plant by considering weighted (1/ X2) linear regression as model for the calibration curve. The acceptance limits have been fixed at ± 15%. The plain red line represents the relative bias, the dashed blue lines are the βexpectation tolerance limits, the black dotted curves are the acceptance limits and the colored circles represent the relative back-calculated concentrations and are plotted in respect to their targeted concentration. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3. Method validation results.

Trueness		
Concentration (ng/mL)	Relative bias (%)	
125	−0.94	
188	−0.39	
250	−1.55	
313	−2.82	
375	−0.57	
Precision		
Concentration (ng/mL)	Repeatability (%RSD)	Intermediate precision (%RSD)
125	2.6	4.9
188	3.7	4.4
250	4.2	5.1
313	4.9	5.7
375	3.6	5.1
Accuracy	Relative β-expectation tolerance limits (%)	
Concentration (ng/mL)		
125	[−12.13, 10.25]	
188	[−8.80, 8.11]	
250	[−11.76, 8.66]	
313	[−13.84, 8.21]	
375	[−11.45, 10.30]	
Linearity		
Range (ng/mL)	[125–375]	
Slope	0.9830	
Intercept	0.8622	
R2	0.9784	
LOD (ng/mL)	LLOQ (ng/mL)	ULOQ (ng/mL)
14.40	127.9	383.7

3.2.3 Accuracy

Accuracy asserts the closeness of agreement between the test results and an accepted reference value. It is the resultant of the systematic and random errors. The accuracy of the method is demonstrated over the whole dosing range since the beta expectation tolerance intervals are included in the acceptance limits set at 15% according to the method objectives.

3.2.4 LOD and LOQ

The limit of detection (LOD) represents the smallest quantity that can be detected by the method but not accurately quantified. Its estimation was performed according to Miller & Miller methodology and is equal to 14.40 ng/mL. Considering the injected volume (2 µL) and an approximated flow rate of 30 µL/min into the mass spectrometer, this LOD value corresponds to a detectable quantity of about 0.5 pg showing the high sensitivity of this method.

The limits of quantification are classified as lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ). They represent respectively the smallest and highest quantity of the targeted substance that can be quantified with accuracy. Since the whole β-expectation tolerance interval is included in the acceptance limits, the LOQs are therefore equal to the calculated range limits assessed with LLOQ equal to 127.9 ng/mL and ULOQ equal to 383.7 ng/mL (i.e. 4 and 14 pg into MS).

The validated dosing range corresponds to 50–150% of a target concentration of 5% w/w (i.e. 2.5% - 7.5%). It means that cannabis samples adulterated with 0.30% of XLR1-11 could be detected using this SFC-MS method.

3.2.5 Linearity

The linearity is the ability of an analytical technique to obtain results proportional to the concentration of the analyte in the sample. It is assessed by fitting the back calculated concentrations using the calibration model selected on a linear regression model. A good linearity was observed since the slope was close to 1 with a r^2 value close to 0.98. 4.

4. Conclusion

The present work demonstrates the applicability of SFC-MS as a tool for the detection of adulteration of the cannabis plant with synthetic cannabinoids. Because of the high number of structurally diverse synthetic cannabinoids, it is impossible to predict which compounds will be found. Therefore our aim was to develop a fast generic method using selected representatives from multiple classes.

A robust optimization strategy (DoE-DS) was used to develop an optimal method that can be used for the screening of samples. This strategy also permits the testing of various conditions within the DS to tune the separation. Additionally, the use of mass spectrometry brings high sensitivity and specificity. This SFC-MS method permits high throughput analysis, makes use of a simple modifier (MeOH/H_2O 98/ 2 v/v) and requires minimal sample preparation. Therefore, its implementation should be straightforward.

Its quantitative performances have been demonstrated with a validation using a total error approach for a dosing range of 2.5% – 7.5% (w/w) with LOD equal to 14.40 ng/mL. However, sample preparation and matrix effects should be evaluated if less concentrated samples must be quantified.

Published in : Journal of Chromatography B (2019), vol. 1092, pp. 332-342
doi.org/10.1016/j.jchromb.2018.05.049
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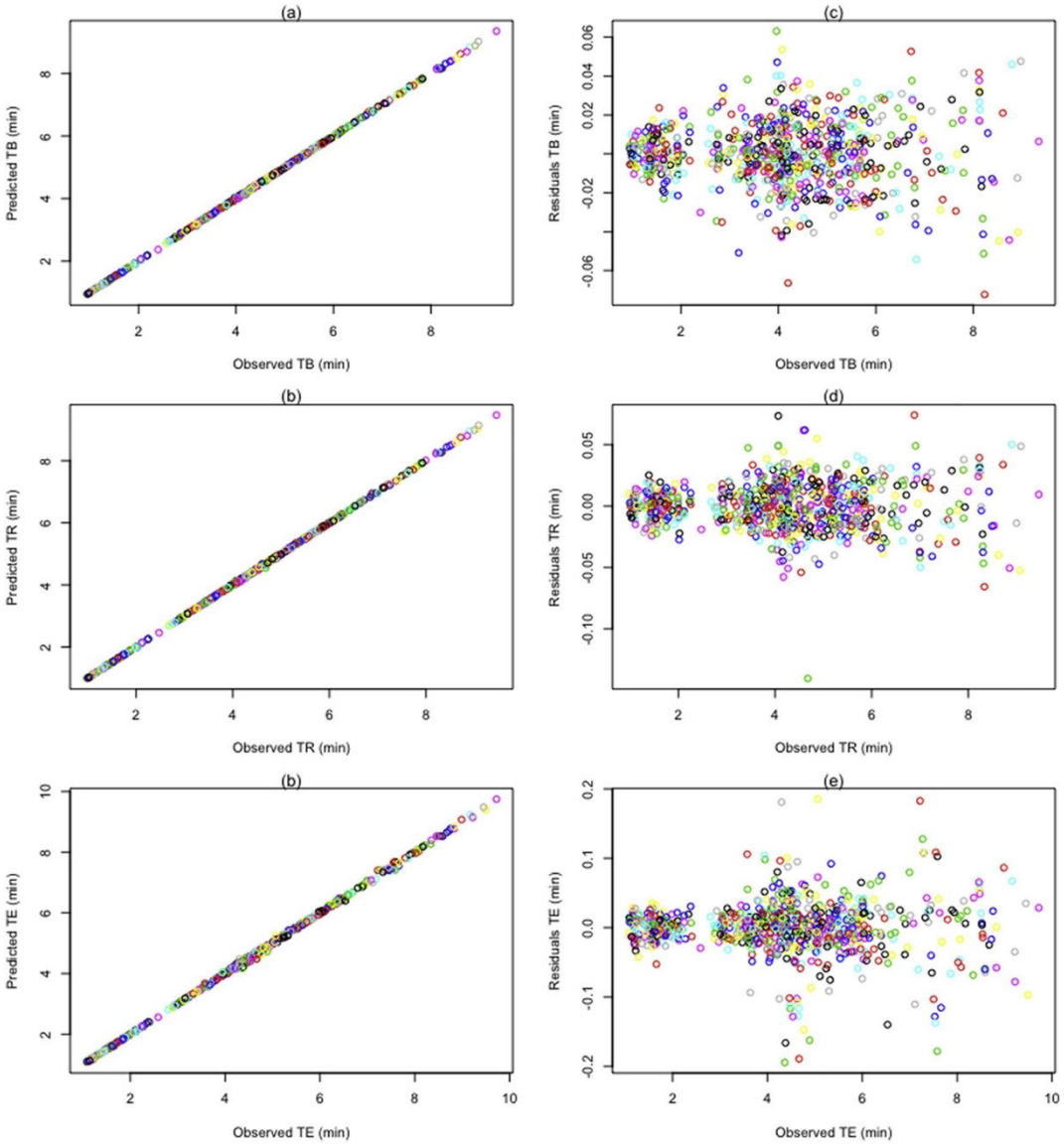
For unknown synthetic cannabinoids, this SFC-MS could be able to retain or separate the compounds but the hyphenation of SFC to a highresolution mass spectrometer (e.g. Q-TOF) will be necessary for structure elucidation.

Appendix 1. Model quality



Acknowledgements

Research grants from Walloon Region of Belgium and EU Commission (project FEDER-PHARE) to Amandine DISPAS are gratefully acknowledged.

Research grants from the Walloon Region of Belgium (project Vibra4Fake) to Hermane AVOHOU are likewise gratefully acknowledged.

Financial support from Fond Leon Fredericq (ULiège) is gratefully acknowledged for the student research grant of Sebastien ANDRÉ.

The authors thank the ULiège laboratory of pharmacognosy (Prof. M. Frédérich) for providing the fiber-type cannabis samples.

Appendix 2. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2018.05.049

References

[1] B. De Backer, B. Debrus, P. Lebrun, L. Theunis, N. Dubois, L. Decock, A. Verstraete, P. Hubert, C. Charlier, Innovative development and validation of an HPLC/DAD method for the qualitative and quantitative determination of major cannabinoids in cannabis plant material, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 877 (2009) 4115–4124, http://dx.doi.org/10.1016/j.jchromb.2009.11.004.

[2] C. Citti, D. Braghiroli, M.A. Vandelli, G. Cannazza, Pharmaceutical and biomedical analysis of cannabinoids: a critical review, J. Pharm. Biomed. Anal. 147 (2018) 565–579, http://dx.doi.org/10.1016/j.jpba.2017.06.003.

[3] EMCDDA, EMCCDA Monographs - A Cannabis Reader: Global Issues and Local Experiences, (2008).

[4] The National Academies of Science Engineering Medicine, The Health Effects of Marijuana: The Current State of Evidence and Recommendations for Research, (2017), http://dx.doi.org/10.17226/24625.

[5] P.F. Whiting, R.F. Wolff, S. Deshpande, M. Di Nisio, S. Duffy, A.V. Hernandez, J.C. Keurentjes, S. Lang, K. Misso, S. Ryder, S. Schmidlkofer, M. Westwood, J. Kleijnen, Cannabinoids for medical use: a systematic review and meta-analysis, JAMA 313 (2015) 2456–2473, http://dx.doi.org/10.1001/jama.2015.6358.

[6] S. Farag, O. Kayser, The Cannabis Plant: Botanical Aspects, Elsevier Inc., 2017, http://dx.doi.org/10.1016/B978-0-12-800756-3.00001-6.

[7] F. Degenhardt, F. Stehle, O. Kayser, Chapter 2 - The Biosynthesis of Cannabinoids, (2017), http://dx.doi.org/10.1016/B978-0-12-800756-3/00002-8.

[8] J. McLaren, W. Swift, P. Dillon, S. Allsop, Cannabis potency and contamination: a review of the literature, Addiction 103 (2008) 1100–1109, http://dx.doi.org/10.1111/j.1360-0443.2008.02230.x.

[9] F.P. Busse, G.M. Fiedler, A. Leichtle, H. Hentschel, M. Stumvoll, Lead poisoning due to adulterated marijuana in Leipzig, Dtsch Arztebl Int 105 (2008) 757–762, http:// dx.doi.org/10.3238/arztebl.2008.0757.

[10] J.M. McPartland, Adulteration of cannabis with tobacco, calamus, and other cholinergic compounds, Can. Underwrit. 3 (2008) 16–20 www.cannabis-med.org , Accessed date: 29 September 2017.

[11] J.M. McPartland, D.J. Blanchon, R.E. Musty, Cannabimimetic effects modulated by cholinergic compounds, Addict. Biol. 13 (2008) 411–415, http://dx.doi.org/10.1111/j.1369-1600.2008.00126.x.

[12] EMCDDA, Cannabis Legislation in Europe, http://www.emcdda.europa.eu/system/files/publications/4135/TD0217210ENN.pdf_en , Accessed date: 9 October 2017.

[13] M.S. Castaneto, D.A. Gorelick, N.A. Desrosiers, R.L. Hartman, S. Pirard, M.A. Huestis, Synthetic cannabinoids: epidemiology, pharmacodynamics, and clinical implications, Drug Alcohol Depend. 144 (2014) 12–41, http://dx.doi.org/ 10.1016/j.drugalcdep.2014.08.005.

[14] L.K. Brents, P.L. Prather, The K2/spice phenomenon: emergence, identification, legislation and metabolic characterization of synthetic cannabinoids in herbal incense products, Drug Metab. Rev. 46 (2014) 72–85, http://dx.doi.org/10.3109/ 03602532.2013.839700.

[15] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Legal approaches to controlling new psychoactive substances - updated 2016, Perspect. Drugs 4 (2016), http://www.emcdda.europa.eu/topics/pods/controlling-newpsychoactive-substances.

[16] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Synthetic cannabinoids in Europe - update 2017, Perspect. Drugs 9 (2017), http://dx.doi.org/ 10.2810/32306.

[17] C.R. Harris, A. Brown, Synthetic cannabinoid intoxication: a case series and review, J. Emerg. Med. 44 (2013) 360–366, http://dx.doi.org/10.1016/j.jemermed.2012.07.061.

[18] E.L. Øiestad, R. Karinen, K. Haugland, Å.M.L. Øiestad, Chapter 101 – screening of synthetic cannabinoids, Handb. Cannabis Relat. Pathol, 2017, pp. 981–997, , http://dx.doi.org/10.1016/B978-0-12-800756-3.00118-6.

[19] C. Mazoyer, J. Carlier, C. Sastre, V. Di Fazio, Synthetic cannabinoids: analytical methods|Cannabinoïdes de synthèse: méthodes analytiques, Toxicol. Anal. Clin. 27 (2015) 184–194, http://dx.doi.org/10.1016/j.toxac.2015.03.112.

[20] UNODC, Recommended Methods for the Identification and Analysis of Synthetic Cannabinoid Receptor Agonists in Seized Materials, UNODC, 2013.

[21] B. De Backer, K. Maebe, A.G. Verstraete, C. Charlier, Evolution of the content of THC and other major cannabinoids in drug-type cannabis cuttings and seedlings during growth of plants, J. Forensic Sci. 57 (2012) 918–922, http://dx.doi.org/10.1111/j.1556-4029.2012.02068.x.

[22] V. Desfontaine, D. Guillarme, E. Francotte, L. Nováková, Supercritical fluid chromatography in pharmaceutical analysis, J. Pharm. Biomed. Anal. 113 (2015) 56–71, http://dx.doi.org/10.1016/j.jpba.2015.03.007.

[23] A. Dispas, H. Jambo, S. André, E. Tyteca, P. Hubert, Supercritical fluid chromatography: a promising alternative to current bioanalytical techniques, Bioanalysis 10 (2018) 107–124, http://dx.doi.org/10.4155/bio-2017-0211.

[24] S. Breitenbach, W.F. Rowe, B. McCord, I.S. Lurie, Assessment of ultrahigh performance supercritical fluid chromatography as a separation technique for the analysis of seized drugs: applicability to synthetic cannabinoids, J. Chromatogr. A 1440 (2016) 201–211, http://dx.doi.org/10.1016/j.chroma.2016.02.047.

[25] T. Toyo'oka, R. Kikura-Hanajiri, A reliable method for the separation and detection of synthetic cannabinoids by supercritical fluid chromatography with mass spectrometry, and its application to plant products, Chem. Pharm. Bull.(Tokyo) 63 (2015) 762–769, http://dx.doi.org/10.1248/cpb.c15-00170.

[26] T. Berg, L. Kaur, A. Risnes, S.M. Havig, R. Karinen, Determination of a selection of synthetic cannabinoids and metabolites in urine by UHPSFC-MS/MS and by UHPLC-MS/MS, Drug Test. Anal. 8 (2016) 708–722, http://dx.doi.org/10.1002/ dta.1844.

[27] M. Wang, Y.H. Wang, B. Avula, M.M. Radwan, A.S. Wanas, Z. Mehmedic, J. van Antwerp, M.A. Elsohly, I.A. Khan, Quantitative determination of cannabinoids in cannabis and cannabis products using ultra-high-performance supercritical fluid chromatography and diode array/mass spectrometric detection, J. Forensic Sci. 62 (2017) 602–611, http://dx.doi.org/10.1111/1556-4029.13341.

[28] J.J. Peterson, M. Yahyah, A Bayesian design space approach to robustness and system suitability for pharmaceutical assays and other processes, Stat. Biopharm.Res. 1 (2009) 441–449, http://dx.doi.org/10.1198/sbr.2009.0037.

[29] P. Lebrun, B. Boulanger, B. Debrus, P. Lambert, P. Hubert, A Bayesian design space for analytical methods based on multivariate models and predictions, J. Biopharm. Stat. 23 (2013) 1330–1351, http://dx.doi.org/10.1080/10543406.2013.834922.

[30] A. Dispas, H.T. Avohou, P. Lebrun, P. Hubert, C. Hubert, 'Quality by Design' approach for the analysis of impurities in pharmaceutical drug products and drug substances, TrAC Trends Anal. Chem. (2017), http://dx.doi.org/10.1016/J.TRAC. 2017.10.028.

[31] J.J. Peterson, M. Yahyah, K. Lief, Predictive distributions for constructing the ICH Q8 design space, Comprehensive Quality by Design for Pharmaceutical Product Development and Manufacture, 2017, pp. 55–70.

[32] B. Debrus, P. Lebrun, J.M. Kindenge, F. Lecomte, A. Ceccato, G. Caliaro, J.M.T. Mbay, B. Boulanger, R.D. Marini, E. Rozet, P. Hubert, Innovative high-performance liquid chromatography method development for the screening of 19 antimalarial drugs based on a generic approach, using design of experiments, independent component analysis and design space, J. Chromatogr. A 1218 (2011) 5205–5215, http://dx.doi.org/10.1016/J.CHROMA.2011.05.102.

[33] A. Dispas, P. Lebrun, E. Ziemons, R. Marini, E. Rozet, P. Hubert, Evaluation of the quantitative performances of supercritical fluid chromatography: from method development to validation, J. Chromatogr. A 1353 (2014) 78–88, http://dx.doi. org/10.1016/J.CHROMA.2014.01.046.

[34] C. Hubert, P. Lebrun, S. Houari, E. Ziemons, E. Rozet, P. Hubert, Improvement of a stability-indicating method by Quality-by-Design versus Quality-by-Testing: a case of a learning process, J. Pharm. Biomed. Anal. 88 (2014) 401–409, http://dx.doi. org/10.1016/j.jpba.2013.09.026.

[35] P. Hubert, J.-J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P.-A. Compagnon, W. Dewé, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, Harmonization of strategies for the validation of quantitative analytical procedures: a SFSTP proposal—part I, J. Pharm. Biomed. Anal. 36 (2004) 579–586, http://dx.doi.org/10.1016/J.JPBA.2004.07.027.

[36] P. Hubert, J.-J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P.A. Compagnon, W. Dewé, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, E. Rozet, Harmonization of strategies for the validation of quantitative analytical procedures: a SFSTP proposal – part II, J. Pharm.Biomed. Anal. 45 (2007) 70–81, http://dx.doi.org/10.1016/J.JPBA.2007.06.013.

[37] P. Hubert, J.-J. Nguyen-Huu, B. Boulanger, E. Chapuzet, N. Cohen, P.A. Compagnon, W. Dewé, M. Feinberg, M. Laurentie, N. Mercier, G. Muzard, L. Valat, E. Rozet, Harmonization of strategies for the validation of quantitative analytical procedures: a SFSTP proposal–part III, J. Pharm. Biomed. Anal. 45 (2007) 82–96, http://dx.doi.org/10.1016/J.JPBA.2007.06.032.

[38] P. Hubert, J.-J. Nguyen-Huu, B. Boulanger, E. Chapuzet, N. Cohen, P.A. Compagnon, W. Dewé, M. Feinberg, M. Laurentie, N. Mercier, G. Muzard, L. Valat, E. Rozet, Harmonization of strategies for the validation of quantitative analytical procedures: a SFSTP proposal: part IV. Examples of application, J. Pharm. Biomed. Anal. 48 (2008) 760–771, http://dx.doi.org/10.1016/J.JPBA.2008.07. 018.

[39] N. Langer, R. Lindigkeit, H.M. Schiebel, U. Papke, L. Ernst, T. Beuerle, Identification and quantification of synthetic cannabinoids in spice-like herbal mixtures: update of the German situation for the spring of 2016, Forensic Sci. Int. 269 (2016) 31–41, http://dx.doi.org/10.1016/j.forsciint.2016.10.023.

[40] L. Ernst, K. Brandhorst, U. Papke, A. Altrogge, S. Zodel, N. Langer, T. Beuerle, Identification and quantification of synthetic cannabinoids in 'spice-like' herbal mixtures: update of the German situation in early 2017, Forensic Sci. Int. 277 (2017) 51–58, http://dx.doi.org/10.1016/j.forsciint.2017.05.019.

[41] V. Desfontaine, J.L. Veuthey, D. Guillarme, Evaluation of innovative stationary phase ligand chemistries and analytical conditions for the analysis of basic drugs by supercritical fluid chromatography, J. Chromatogr. A 1438 (2016) 244–253, http:// dx.doi.org/10.1016/j.chroma.2016.02.029.

[42] B. Andri, A. Dispas, R. Klinkenberg, B. Streel, R.D. Marini, E. Ziémons, P. Hubert, Is supercritical fluid chromatography hyphenated to mass spectrometry suitable for the quality control of vitamin D3 oily formulations? J. Chromatogr. A 1515 (2017) 209–217, http://dx.doi.org/10.1016/J.CHROMA.2017.07.057.

[43] A. Dispas, V. Desfontaine, B. Andri, P. Lebrun, D. Kotoni, A. Clarke, D. Guillarme, P. Hubert, Quantitative determination of salbutamol sulfate impurities using achiral supercritical fluid chromatography, J. Pharm. Biomed. Anal. 134 (2017) 170–180, http://dx.doi.org/10.1016/j.jpba.2016.11.039.

[44] B.B. Lebrun, P. Sondag, X. Lories, J.-F. Michels, E. Rozet, Quality by design applied in formulation development and robustness, in: T. Coffey, H. Yang (Eds.), Stat. Biotechnol. Process Dev, 2018.