LC-MS/MS USE FOR TESTING PESTICIDES IN CANNABINOID-CONTAINING PRODUCTS

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Abstract

As the consumption of oils which contain cannabidiol (CBD) is increasing, the exposure to pesticides of the *Cannabis* genus became an important issue due to the potential side effects on the consumers' health. CBD is one of the main alkaloids of the *Cannabis* plants, together with other 120 identified structures. The current study presents the development of a method for the pesticides determination using the high-performance liquid chromatography in tandem with mass spectrometry (LC-MS/MS) for the analysis of some CBD oils. The complete analytical testing includes cannabinoids quantitation, pesticides and mycotoxins analysis, heavy metals analysis, residual solvents analysis, terpene profiling, and microbial screening. The determination of the pesticides is the most complex of these tests, being also the most important analysis in terms of safety for consumption, especially if we have in view the long term use of CBD products. The developed method showed selectivity, sensitivity and good linearity; its main advantage being that it does not require an extensive sample preparation before the analysis.

Rezumat

Deoarece consumul uleiurilor conţinând canabidiol (CBD) este în continuă creştere, expunerea la pesticide a plantelor de *Cannabis* a devenit o problemă importantă din cauza efectelor adverse potenţiale asupra sănătăţii consumatorilor. CBD este unul dintre principalii alcaloizi din compoziţia plantelor de *Cannabis*, împreună cu alte 120 substanţe identificate. Studiul de faţă prezintă dezvoltarea unei metode pentru determinarea pesticidelor folosind cromatografia de lichide de înaltă performanţă în tandem cu spectrometria de masă (LC-MS/MS) pentru analiza compoziţiei unor uleiuri cu CBD. Testarea analitică completă include cuantificarea canabinoizilor, analiza pesticidelor şi micotoxinelor, analiza metalelor grele, analiza solvenţilor reziduali, determinarea profilului terpenic şi *screening*-ul microbian. Dintre aceste teste, determinarea pesticidelor este analiza cea mai complexă, dar şi cea mai importantă în termeni de siguranţă pentru consum, mai ales dacă avem în vedere utilizarea pe termen lung a produselor conţinând CBD. Metoda dezvoltată a dovedit selectivitate, sensibilitate şi o bună liniaritate, iar principalul avantaj constă în faptul că nu necesită o pregătire elaborată a probelor înainte de analiză.

Keywords: cannabinoids, LC-MS/MS, pesticides, environmental analysis

Introduction

The *Cannabis* plant was used from the earliest times to produce hemp fibres (for clothes, ropes and paper) and seeds that may be used as food for animals but also as medicinal plant [19]. The two major neuro-active components from the *Cannabis* plant are: tetrahydrocannabinol (THC), the main psychoactive alkaloid and CBD, the non-psychoactive alkaloid [9]. Under current legislation, there are differences regarding the quantities of THC admitted in the hemp products that vary between 0.05 and 0.6% [20]. Among the illicit products from *Cannabis*, we can mention the following: marijuana (mixture of hemp leaves, flowers and seeds), hashish (obtained from unfertilized buds) and also the oils that may be easily prepared as the alkaloids are lipophilic [5]. The oil of *Cannabis sativa* L. is the most concentrated oil in the polyunsaturated fatty acids, known for the emollient and moisturizing properties, anti-inflammatory, antibacterial properties accelerating the healing of wounds, regeneration of skin and hair [1].

At present, CBD is used as active ingredient in the following EU approved medicinal products: Epidiolex® - oral solution (contains only CBD) for the treatment of any form of epilepsy and Sativex® - oral-mucosal spray (contains both CBD and THC) for the treatment of multiple sclerosis [3, 8, 11, 14].

The large spectrum pesticides are widely sued in the cultures of plants for the control of insects, weeds and other pests. This strategy is used on the plants cultures from the *Cannabaceae* family, cultivated nowadays on significant areas for the production of the food supplements and pharmaceutical products [17]. A pesticide is any substance that can prevent, destroy, or repel insects, rodents, fungi, weeds, or other forms of plant or animal life or viruses, or any substance use to regulate plant growth, such as a leaf-removing or drying agent. As many of these pesticides are lipophilic, they are soluble in the solvents used for the extraction of cannabinoids, including CBD oils and other products using extracted cannabinoids. These pesticides accumulate in the environment and may be found in the final product and therefore in the human body. The maximum level of pesticide residues in food, food supplements and pharmaceutical products from the entire world is not strictly regulated. Washington State Department of Agriculture (WSDA) has recently released a list of 271 pesticide products approved for use on cannabis plantations [17]. The state of Colorado has compiled a list of 357 pesticide products, many of which contain the same active ingredients that are legal to use on cannabis plantations [17]. California monitors 66 pesticides in hemp cultures and cannabis products [2, 6]. The presence of pesticides in the hemp cultures is a challenging problem nowadays, not only for cultivators, but also for authorities, consumers or researches [17]. The organophosphorus compounds, such as chlorpyrifos or malathion, proved to be very toxic, inducing neuro-behavioural and cognitive disorders, teratogenesis, immunotoxicity or endocrine and metabolic disorders [17, 22]. Daminozide and paclobutrazol are two growing regulators for plants that were forbidden in the U.S. and in many European countries, due to the carcinogenic properties [16].

Due to the fact that the use of *Cannabis* cultures as raw material for the industry of food and pharmaceutical products is relatively new, there is still a not well established expertise in the domain of the quality control of raw material [4], including the testing for the determination of pesticide residues [17]. Therefore, the development of some methods for precise, reliable and sensitive detection and quantification of the residues from pesticides from the oils with CBD represent an actual theme in the pharmaceutical research [15].

Materials and Methods

Equipment
An UHPLC Flexar chromatographic system (Perkin Elmer MA, USA) together with a Kinetex Biphenyl 2.6 μm, 4.6 mm x 150 mm column (Phenomenex CA, USA) was used. The detection was performed by an AB SCIEX Triple Quad 4500 mass spectrometer (Sciex MA, USA). All data analysis and processing were performed using the SCIEX Analyst 2.0 software.

Materials
For the identification of the specific retention times and transitions, three certified reference materials (MRC) containing pesticides were used: California Pesticides Class 1 mix 100 μg/mL in acetonitrile - 21 analytes (LGC, USA), California Pesticides Class 2A Mixture 668 100 μg/mL in Acetonitrile - 21 analytes (LGC, USA) and California Pesticides Class 2B Mixture 669 100 μg/mL in Acetonitrile - 24 analytes (LGC, USA).

For the analysis, 4 types of CBD oils purchased from the internet were used. *Sample 1* - with a content of 8% CBD, 4 mg/drop declared by the manufacturer (information from the label). *Sample 2* - with a content of 10% CBD, 1000 mg/10 mL declared by the manufacturer (information from the label). *Sample 3* - with a content of 1350 mg/100 mL total concentration of cannabinoids declared by the manufacturer (information from the label). *Sample 4* - with a content of 2.5% CBD declared by the manufacturer (information from the label).

Analytical and chromatographic grade chemicals and solvents were used: methanol (HiPerSolv, VWR Chemicals PA, USA), acetonitrile (LiChrosolv Merck KGaA, Germany), ammonium formate (LiChropur Supleco PA, USA), formic acid (Optima LC/MS Fisher Chemical NH, USA), 2-propanol (LiChrosolv Merck KGaA, Germany) and acetone (LiChrosolv Merck KGaA, Germany). Ultrapure water was supplied by a Mili-Q water purification system from Millipore (Bedford, MA, USA).

Analytical Method
Preparing the samples and the standard solutions. A stock standard solution 1000 ng/mL in acetonitrile was prepared by mixing and diluting all three certified reference materials. Nine calibration standards, prepared from the stock standard solution by successive dilutions, were prepared in order to assess the linearity of the method in the range of 0.075 ng/mL - 15 ng/mL. Following the declared concentration of the manufacturer, the corresponding covered range was 52.5 - 10500 ng/g. For the verification of the accuracy, two quality control (QC) solutions were prepared. One QC solution had the concentration of the maximum limit admitted for pesticides (100 ng/g, QC1), and the second was prepared at 25% of the superior limit of the calibration curve (QC2). The sample preparation was carried out as follows: 100 mg of vegetal oil was accurately weighted in a 15 mL centrifuge tube. The extraction was conducted by adding 10 mL of methanol over the sample and vortexing at high speed for 3 min, then centrifuged for 10 minutes at 5000 rpm. 1 mL extract was transferred in a new tube and diluted with 2 mL solvent and filtered through a 0.45 μm nylon filter.

Instrumental Parameters. The analysis was performed using as mobile phases: ammonium formate 5 mM + acid formic 0.2% (Mobile phase A, MPA) and ammonium

formate 5 mM:methanol 2:98 (v/v) + acid formic 0.2% (Mobile phase B, MPB) in a gradient elution mode as shown in Table I.

Table I
Gradient elution program

Time (min)	MPA %	MPB %
0.75	95	5
1.00	50	50
1.50	40	60
2.50	22	78
4.00	12	88
10.00	8	92
12.00	0	100
13.80	0	100
13.90	95	5
16.00	95	5

The injection volume was 20 μL and the flow rate was set at 1 mL/min. The temperature was 10°C in the samples' compartment and 40°C in the column oven. The ionization source was maintained at 500°C. The following gas parameters were used: curtain gas, 35 psi; collision gas, 9 psi; ion spray voltage, 3500 V; ion source gas 1, 60 psi; ion source gas 2, 60 psi. The

analysis were carried out by positive electro-spray ionization using retention time-scheduled multiple reaction monitoring (MRM) to acquire one transition for each analyte (Table II).

Results and Discussion

To cover a broadest spectrum possible of different pesticides in CBD oils, a combination of three certified reference materials (MRC) containing all the 66 pesticides regulated in California were used. As a result of the method development and validation study, there were obtained precise mass measurements for 34 pesticides from the 66 tested (Table II). For each of them, the calibration curves were linear within the studied concentration range 0.075 ng/mL - 15 ng/mL (corresponding covered range 52.5 - 10500 ng/g). The least-squares method was used and the correlation coefficient was determined. The calibration curve exhibited a good linear regression with a value of the correlation coefficient higher than 0.99 for 34 pesticides, as shown in Table II [21].

Table II
MRM parameters used for the acquisition of the transitions and regression equation for each of the target analytes

| ID Transition	Q1	Q3	Time (min)	Regression Equation	Correlation coefficient
Acephate	184.1	143.0	2.89	$y = 7.932720 x - 1.81$	r = 0.9968
Acetamiprid	223.2	126.1	4.42	$y = 2.083852 x - 3.68$	r = 0.9968
Aldicarb	116.0	89.1	4.23	$y = 2.52313 x - 0.749$	r = 0.9962
Azoxystrobin	404.1	372.1	7.13	$y = 2.785535 x - 0.67$	r = 0.9948
Bifenazate	301.1	170.1	6.03	$y = 3.725641 x - 0.07$	r = 0.9956
Boscalid	343.3	307.0	5.71	$y = 699 x + 45.8$	r = 0.9938
Carbaryl	202.1	145.0	4.65	$y = 3.996637 x - 1.05$	r = 0.9958
Carbofuran	222.2	123.1	4.66	$y = 7.44522 x + 293$	r = 0.9925
Chlorpyrifos	350.0	198.0	9.00	$y = 1.329148 x - 0.017$	r = 0.9971
Clofentezine	303.0	138.0	8.15	$y = 3.4839778 x - 0.0807$	r = 0.9954
Diazinon	305.0	169.0	6.44	$y = 1.812474 x - 0.0237$	r = 0.9956
Dichlorvos	221.0	109.0	4.26	$y = 2.119429 x - 0.485$	r = 0.9907
Dimethomorph	388.2	301.1	6.93	$y = 1.0673458 x + 750$	r = 0.9931
Etoxazole	360.1	141.0	8.93	$y = 2.008411 x - 0.615$	r = 0.9949
Fenhexamid	302.0	97.0	5.40	$y = 1.00693 x - 0.0231$	r = 0.9927
Fenoxycarb	302.2	88.1	6.39	$y = 3.9773735 x - 0.141$	r = 0.9944
Fenpyroximate	422.0	366.1	10.88	$y = 3.1892363 x - 0.98$	r = 0.9953
Fipronil	437.0	367.9	4.84	$y = 1.1881774 x - 0.0254$	r = 0.9909
Flonicamid	230.1	203.1	3.32	$y = 4.8634719 x - 0.0661$	r = 0.9934
Fludioxonil	266.0	229.0	4.85	$y = 402 x - 0.00613$	r = 0.9933
Hexythiazox	353.0	228.0	10.0	$y = 7.2196881 x - 0.202$	r = 0.9952
Imazalil	297.1	159.2	5.52	$y = 3.0107207 x - 0.0253$	r = 0.9954
Imidacloprid	256.2	209.0	4.20	$y = 6.852821 x + 4.09825$	r = 0.9965
Malathion	331.0	127.0	6.12	$y = 1.520464 x - 0.024$	r = 0.9963
Metalaxyl	280.2	220.2	5.62	$y = 8.4179348 x - 0.162$	r = 0.9943
Methiocarb	226.1	169.2	5.43	$y = 4.5714622 x - 0.0815$	r = 0.9948
Methomyl	163.1	88.1	3.65	$y = 2.664425 x - 0.31$	r = 0.9942
Paclobutrazol	294.0	70.0	4.98	$y = 5.2763132 x - 0.157$	r = 0.9950
Propoxur	210.1	111.0	4.43	$y = 4.4810788 x - 1.14$	r = 0.9947
Spinosyn A	732.6	142.1	8.0	$y = 4.1183437 x - 0.091$	r = 0.9914
Spinosyn D	746.6	142.1	8.65	$y = 1.429841 x - 0.0136$	r = 0.9977
Spirotetramat	374.2	330.2	6.43	$y = 2.1850381 x - 0.0331$	r = 0.9963
Spiroxamine	298.4	144.2	4.98	$y = 4.632466 x - 1.88$	r = 0.9925
Thiamethoxam	292.0	211.0	3.88	$y = 9.920947 x + 716$	r = 0.9929
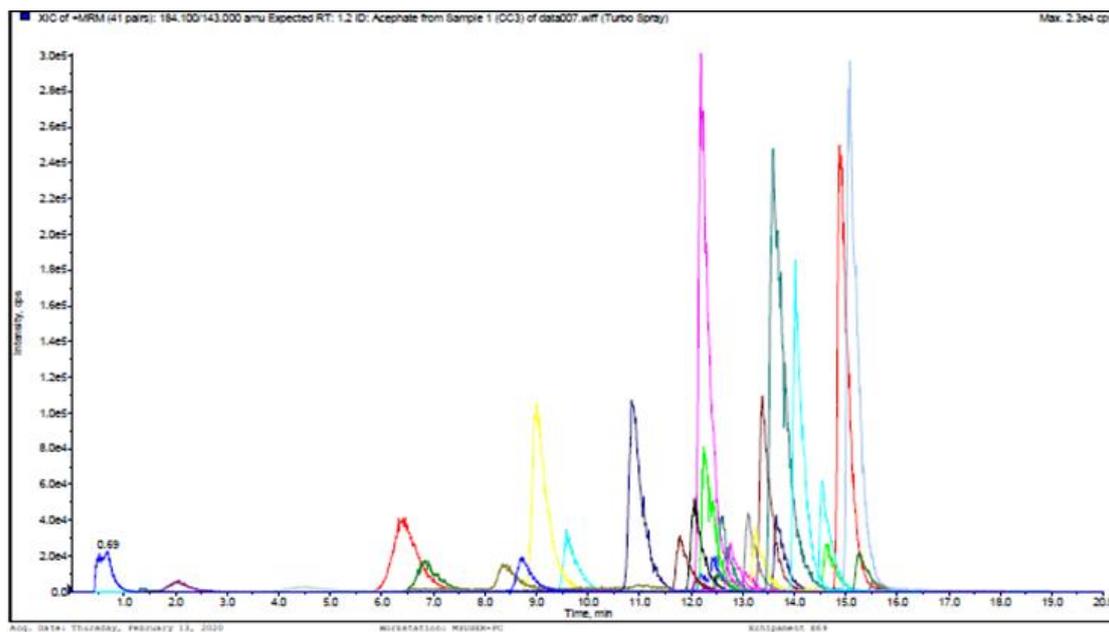
Figure 1.
LC-MS chromatograms of the CBD oils with the MRC pesticides

The specificity of the method is assured by the MS/MS technique, which allows the analysis of complex mixtures, since we used a specific fragment derived from the parent compounds as identification method [18]. A total ion chromatogram of a CBD oils with the selected pesticides is shown in Figure 1.

Limit of Quantification (LOQ = 0.075 ng/mL) was estimated from the data acquired for linearity, as the signal-to-noise ratio for the lowest concentration of the linearity range is more than 10:1 [10].

The accuracy of the method was assessed on the two quality control (QC) solutions as percent recovery. All 34 pesticides analysed (Table II) had a good average recovery, ranging from 80 to 120%.

The method developed in this study was applied for the analysis of the pesticides in four samples of CBD oil. The following sequence was injected into the chromatographic system: 1 x blank; 1 x Standard solution 52.5 µg/mL; 1 x QC1 solution; 1 x QC2 solution; 1 x sample solution; 1 x QC1 every 10 samples; 1 x QC1 after all samples; 1 x Blank; 1 x Wash. There were no pesticides detected in any of the CBD oil samples tested. Testing of medical cannabis products in Canada detected unregistered pesticides in 26 samples from a total of 144 tested [13], while in central California pesticides were identified in 49.3% cannabis samples [12] and in Oregon approximately 6.7% of recreational cannabis samples failed pesticide testing regulations [7]. Those, the results are in line with other reported studies and more CBD oil samples will be purchased and tested using the developed LC-MS/MS method. Sensitive and selective detection such as GC-MS/MS and LC-MS/MS should be used for pesticide residue analysis of cannabis plant material or cannabis products. GC and LC are both needed because some pesticides

are reproducibly detected only at low enough levels by one or the other. However, LC is preferred over GC as some of the compounds require derivatization prior to a GC analysis. LC-MS/MS methods are very sensitive and may detect compounds at concentrations almost two measure orders below the acceptance limits. Most of the compounds from the list of pesticides controlled in CBD oils are easily analysed through LC-MS/MS (Table II), but some of them, such as Avermectin B1b, Chlorantraniliprole, Dimethoate, Spiromesifen require a careful optimization of the method to reduce the interferences of the matrix of target analytes. The developed LC-MS/MS method offered a proper sensitivity for the analysis of 34 pesticides from CBD oils and does not require an extensive sample preparation before the analysis.

For the safety of users, both medicinal and recreational, a set of guidelines and methods for testing of Cannabis products is necessary.

Conclusions

A LC-MS/MS method was developed for the determination of the residues of pesticides in CBD oils. Three certified reference materials with a total content of the mixture of 66 pesticides were used as standard. The developed LC-MS/MS method may be used for the analysis of 34 compounds from the list of pesticides, starting from a concentration of 0.075 ng/mL. Expressed as the label of CBD oil samples, the range covered is 52.5 - 10500 ng/g. The LC-MS/MS method developed showed selectivity, sensitivity and good linearity for the compounds from the list of target analytes; its main advantage is that it does not require an extensive sample preparation before

the analysis. Four CBD oils purchased online were verified using the developed method and no pesticides were detected. The CBD oils, along with other *cannabis* medicinal products, require stricter regulations, such as mandatory labelling requirements, safety assessment, testing and pre-market approval.

Acknowledgement

Conflict of interest

The authors declare no conflict of interest.

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