separations

*Article*

# Determination of Ecdysterone in Dietary Supplements and Spinach by Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry

**Krzysztof Grucza *, Mariola Wicka, Aleksandra Drapała and Dorota Kwiatkowska**

Polish Anti-Doping Laboratory, Księcia Ziemowita 53 bud.4, 03-885 Warsaw, Poland; mariola.wicka@antydopinglab.pl (M.W.); aleksandra.drapala@antydopinglab.pl (A.D.); dorota.kwiatkowska@antydopinglab.pl (D.K.)

* Correspondence: krzysztof.grucza@antydopinglab.pl

**Abstract:** Ecdysterone is a naturally occurring steroid hormone of the ecdysteroid class. This group is widely marketed to athletes in dietary supplements as a "natural anabolic agent", advertised to increase strength and muscle mass during resistance training, reduce fatigue and ease recovery. The aim of the study was to develop and validate a straightforward approach for identifying ecdysterone in dietary supplements by means of ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS). Furthermore, due to the fact that ecdysterone is one of the compounds naturally occurring in spinach, the fit-for-purpose method for extraction and identification of ecdysterone in spinach is proposed. The validity of the developed method was confirmed with the use of a reference standard and the limit of detection (LOD) for ecdysterone was established at 1 mg/g supplement. The presence of ecdysterone was confirmed in all tested supplements at estimated concentrations ranging between 5 mg/g and 383 mg/g.

**Keywords:** ecdysterone; dietary supplements; LC-MS/MS



**Citation:** Grucza, K.; Wicka, M.; Drapała, A.; Kwiatkowska, D. Determination of Ecdysterone in Dietary Supplements and Spinach by Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry. *Separations* **2022**, *9*, 8. https://doi.org/10.3390/separations9010008

Academic Editor: Achille Cappiello

Received: 3 November 2021
Accepted: 21 December 2021
Published: 29 December 2021



## 1. Introduction

Ecdysterone (crustecdysone;beta-ecdysone; 20-hydroxyecdysone; Figure 1), also known as the "Russian secret", is a naturally occurring steroid hormone of the ecdysteroid class [1].

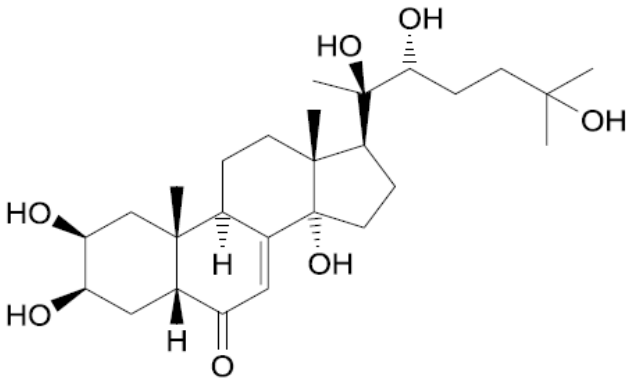



**Figure 1.** The chemical structure of ecdysterone (20-hydroxyecdysone; $C_{27}H_{44}O_7$).

This group is widely marketed to athletes in dietary supplements as a "natural anabolic agent", advertised to increase strength and muscle mass during resistance training, reduce fatigue, and ease recovery [2]. Our study of supplements sold on the internet showed that ecdysterone is listed in special nutrition products as "*Ecdysterone*", "*20beta-hydroxyecdysone*", "*Cyanotisvagaextract*", "*Leuzeacarthamoidesextract*" or as "*spinach extract*".Spinach, a vegetable

known and consumed worldwide, has traditionally been considered to have strength-boosting properties. However, empirical studies have consistently found ecdysteroid content in spinach to be low [3]. Moreover, *Ajugaturkestanica* was one of the early plant species investigated for the presence of ecdysteroids and it was from that plant that ecdysterone and cyasterone were first isolated. *A. turkestanica* is a perennial plant from the family Lamiaceae. It grows naturally in Uzbekistan and Tadzhikistan and has traditionally been valued by the local population for its beneficial effects onmuscle strength, muscle and stomach pain, and its protective activity against cardiovascular diseases [4,5].

In recent years, ecdysterone has become a focus of interest in science, one that is widely commented on in the anti-doping community [6]. Several studies have found a performance-enhancing effect in animals and humans [2]. Ecdysterone appears to promote an anabolic effect that was reported to be even stronger than that of some anabolic androgenic steroids (AAS), e.g., metandienone [1,2]. Moreover, scientific papers concerning using ecdysterone don't indicate any adverse effects for humans.Taking into account these properties, the inclusion of ecdysterone by the World Anti-Doping Agency (WADA) in its 2020 Monitoring Program seems justified.

The aim of the study was to develop and validate a straightforward approach for identifying ecdysteronein dietary supplements by means of ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS). Furthermore, due to the fact that ecdysterone is one of the compounds naturally occurring in spinach, the fit-for-purpose method for extraction and identification of ecdysterone in spinach is proposed.

## 2. Materials and Methods

### *2.1. Spinach, Supplements, Chemicals, and Reagents*

The standards of ecdysterone and mefruside were purchased from ChromaDex (Los Angeles, CA, USA) and from Bayer (Elberfeld, Germany), respectively. LC/MS-grade of methanol and acetonitrile were purchased from Merck Millipore (Darmstadt, Germany) and Fisher Chemical (Hampton, NH, USA), respectively. The Millipore DirectQ UV3 system (Darmstadt, Germany) was used as the source of water (R > 18 MΩcm). Stock solutions of standard substances were prepared at the concentration of 1 mg/mL and its working solutions (100 µg/mL, 10 µg/mL, 1 µg/mL, and 0.1 µg/mL) in methanol and stored at −20 °C.

The fresh spinach package was purchased at a food supermarket. One supplement (No.1 in Table 1) was purchased in a stationary store in Warsaw which specializes in the sales of dietary supplements, another three products (No. 3, 4, and 5 in Table 1) were purchased from the website store, and while the two remaining supplements (No. 2 and 6 in Table 1) were delivered to the lab in previous years as part of an explanation of anti-doping rule violation cases, respectively.

**Table 1.** Estimated concentrations of ecdysterone in the investigated dietary supplements.

| No. | Manufacturer/ Product Name | Product Form/ EcdysteroneAmount | Estimated Concentration of Ecdysterone(mg/g) |
|---|---|---|---|
| 1 | Magnum Nutraceuticals Thrust® Male Amplifier | Capsules 250 mg/portion | 5 |
| 2 | Universal Animal M. Stak™ | Capsules 100 mg/portion | 69 |
| 3 | Activ LAB MACA | Capsules 2.5 mg/portion | 5 |
| 4 | VEM HERB Supplement DietyEcdysterone 95% Beta Ecdysterone | Capsules 490 mg/portion | 64 |
| 5 | PEAK Ecdysterone extract andL-leucine | Capsules 100 mg/portion | 25 |
| 6 | SyntraxSyntra™ Anabolic Agent | Capsules 200 mg/portion | 383 |

In all supplements, ecdysterone was listed as an official ingredient or as a plant extract (*Leuzeacarthamoides* root), and spinach extract. All supplements were in capsule form and the declared contents of ecdysterone or its extract in these products were 250 mg/portion (3 capsules), 100 mg/portion (8 capsules), 2.5 mg/portion (1 capsule), 490 mg/portion (2 capsule), 100 mg/portion (4 capsule), and 200 mg/portion (1 capsule), respectively (Table 1).

### *2.2. Sample Preparation*

#### 2.2.1. Supplement Extraction

Samples were prepared according to the procedure described previously, with modifications [7]. Ten mg of a product was dissolved in 10 mL of water (1 mg/mL). Next, 10 µg/mLof the aqueous solutions of the supplements were prepared. Then, 100 µL of each solution was transferred to 1.5 mL Eppendorf tubes, spiked with Mefruside (internal standard at a final concentration of 300 ng/mL), and diluted with 900 µL of water. Samples were then strongly vortexed for 2 min and 200 µL of each solution was transferred to a 96-well plate.

#### 2.2.2. Spinach Extraction

100 g of fresh spinach was transferred to a slow juicer machine and squeezed. 3 mL out of 60 mL of spinach juice produced were transferred to a tube and 1.1 mL of 0.5 M acetate buffer (Honeywell Fluka, Seelze, Germany) and 5 µL of Mefruside(internal standard at a final concentration of 200 ng/mL, (Bayer Elberfeld, Baycaron) were added. Next, extraction with 4 mL of ethyl acetate was performed for 20min. The sample was then centrifuged (5 min/3000 rpm, Universal 320R Hettich, Germany), and the ether phase was transferred to a new tube. The pH of the aqueous phase was adjusted to 9–10 with approx. 1 g of potassium carbonate (POCH, Gliwice, Poland) and this was followed by the addition of approx. 6 g of anhydrous sodium sulfate (Honeywell Fluka, Seelze, Germany) and the second extraction with 4 mL of ethyl acetate was performed for 20 min. The samples were then shaken and centrifuged, and the organic layer was transferred to the tube and mixed with a previous ether phase. Two extraction phases were evaporated under a nitrogen flow at 55 °C. The dry residue was reconstituted in 150 µL of mobile phase (ACN:$H_2O$: 1:9 *v/v*, ACN Fischer Scientific, Geel, Belgium).

### *2.3. Instrumental Analysis*

#### 2.3.1. Liquid Chromatography Separation

The analysis was performed in a UPLC Acquity chromatograph (Waters, Milford, MA, USA) equipped with an HSST3 column (1.8 µm, 2.1 × 100 mm). The mobile phase consisted of 0.1% formic acid in acetonitrile (A), and 0.1% formic acid in water (B), and the LC gradient was used at a constant flow rate of 0.3 mL/min at 45 °C. The concentration of acetonitrile was gradually increased in a linear fashion: from 0% to 60% within the first 5 min, and then from 60% to 100% in 1 min. Finally, the column was re-equilibrated for 1.5 min with the mobile phase of initial composition.

Samples were stored at 5 °C in the autosampler prior to analysis and the injection volume was fixed at 10 µL and 2 µL for supplements and spinach, respectively.

#### 2.3.2. Mass Spectrometry

Ecdysterone was traced in a multiple reaction monitoring (MRM) mode with Xevo TQ-S (Waters, Milford, MA, USA) mass spectrometer equipped with a new atmospheric pressure ionization source, marketed as UniSpray™. Analytes were investigated in the "positive" mode. The desolvation gas flow was set at 800 L/h at 500 °C and the source temperature was 150 °C. The applied capillary voltage was 3.0 kV. The cone and collision gas flows were set at 150 L/h and 0.20 mL/min, respectively.

Traced MRMs and their corresponding MS settings are listed in Table 2. All data were acquired and processed using MassLynx™ software version 4.1 SCN905 (Waters, Milford, MA, USA).

**Table 2.** MRMs of ecdysterone and internal standard.

| Compound | Precursor Ion | Product Ions | Cone Voltage (V) | Collision Energy (eV) |
|---|---|---|---|---|
| Ecdysterone | 481.32 | 445.08 | 10 | 10 |
| | | 427.07 | | 15 |
| | | 371.06 | | 10 |
| | | 165.07 | | 10 |
| Mefruside | 382.97 | 129.05 | 25 | 20 |

## 3. Results

### *3.1. Method Validation*

The validation process was performed in accordance with, among other things, the WADA technical document TD2021IDCR [8]. The following analytical parameters were evaluated: selectivity, matrix effect (ME), limit of detection (LOD), limit of quantification (LOQ), as well as carry-over effects.

#### 3.1.1. Selectivity

Selectivity of the method was assessed by the analysis of ten dietary supplements bothin powder and capsule form. For each, the results obtained for a blank sample and the same sample spiked with ecdysterone at concentrations of 0.1 mg/g, 1 mg/g, 5 mg/g, and 10 mg/g of the supplements were compared. The extracted ion chromatograms at the retention timesof the studied compound were examined for interfering peaks. Evaluation of chromatograms recorded for four selected precursor ion-product transitions at the retention time of ecdysterone showed the absence of strong interfering components in its identification for concentrations 1 mg/g, 5 mg/g, and 10 mg/g of the supplements. For these concentrations, the ratio of signal to noise (S/N) above 3:1 was observed.

The mean relative standard deviation, an index of the precision of the measurements, was evaluated for samples at concentrations 1 mg/g, 5 mg/g, and 10 mg/g of the supplements.The results are shown in Table 3.

**Table 3.** Results for validation parameters for concentrations 1 mg/g, 5 mg/g, and 10 mg/g of ecdysterone.

| Ecdysterone Concentration (mg/g) | 1 | 5 | 10 |
|---|---|---|---|
| Coefficient of variation (CV) (%) | 8.3 | 6.6 | 6.6 |
| Intra-day precision (N = 3) (CV) (%) | 2–12.8 | 3.9–4.7 | 0.6–6.1 |
| Inter-day precision (N = 9) (CV) (%) | 8.1 | 4.4 | 3.9 |
| Matrix effects (%) | 101 | 83 | 93 |

#### 3.1.2. Matrix Effects, Precision, LOD, and LOQ

Matrix effects (MEs) were evaluated at three concentrations levels (mg/g): 1, 5, and 10 in ten different nutritional supplements by dividing peak areas recorded for spiked samples by peak areas of the corresponding aqueous solution of the standard, which was considered a recovery factor. The values of MEs are presented in Table 3. For these same concentrations of ecdysterone, the intra- and inter-day (three days) precision were established. LOD for ecdysterone was established at 1 mg/g. In turn, LOQ was established at 3 mg/g.

### 3.1.3. Carry-Over

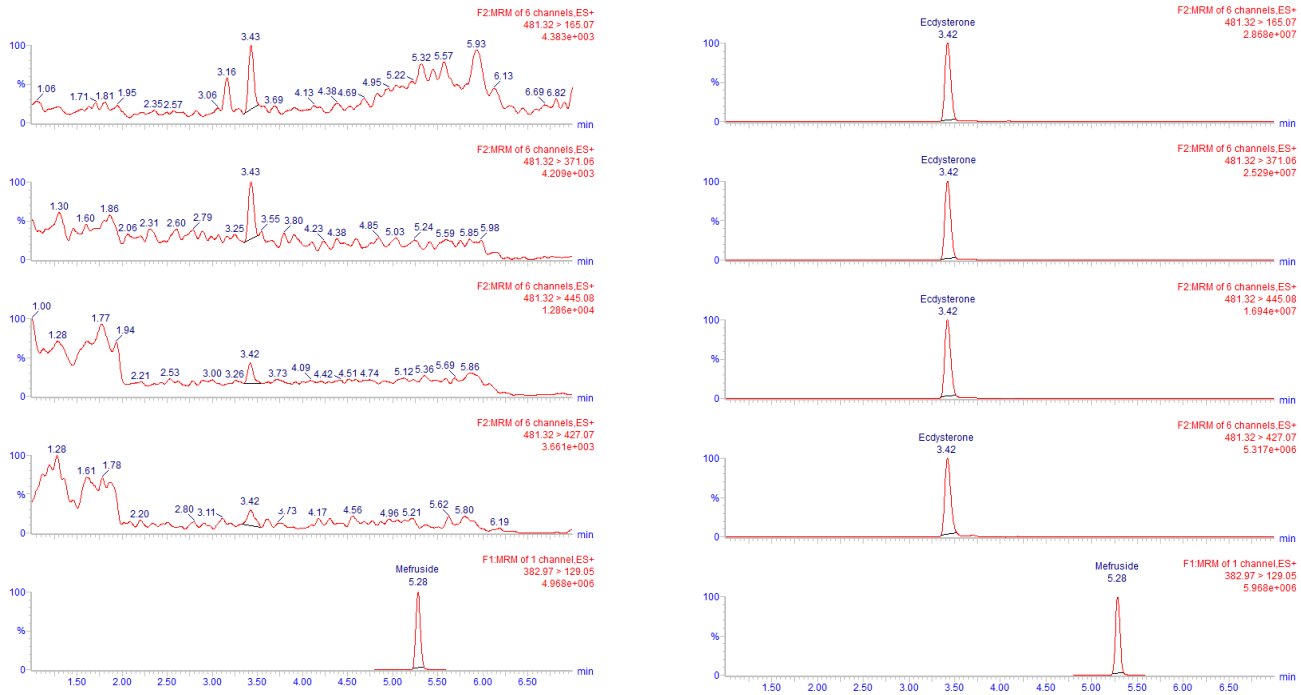
Carry-over was evaluated by injection of three blank supplement samples directly following samples spiked with ecdysterone at 10 mg/g. The analysis was performed for ten different supplements samples. The presence of carry-over was evaluated by visual inspection of the chromatograms obtained for the blank samples and revealed no noticeable carry-over.

## *3.2. Method Application to Dietary Supplement Samples*

The validated method was used to identify ecdysterone in six special products for athletes, which had it listed as an official ingredient (or plant extract) on product labels.

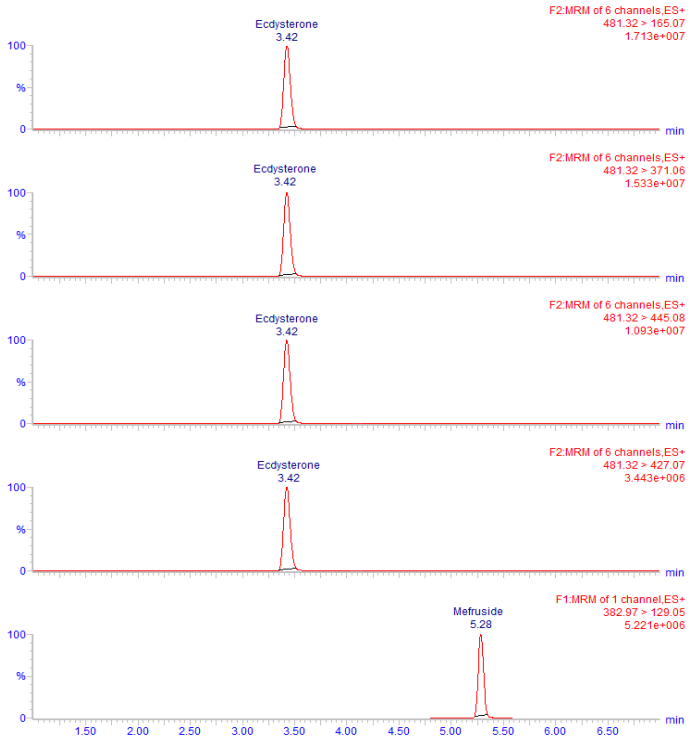
For each of the analyses, a blank sample (not spiked with ecdysterone) and a set of quality control samples spiked with ecdysterone at concentrations of 1 mg/g, 5 mg/g, 10 mg/g, 50 mg/g, 100 mg/g, 250 mg/g, and 500 mg/g were prepared. The samples were prepared using dietary supplements which do not list ecdysterone on their labels and which had previously been tested.

For the confirmation of the identity of ecdysterone in the investigated dietary supplements, the WADA technical document TD2021IDCR [8] was applied. Sample results obtained during the analysis of the dietary supplements prepared by the developed method are shown in Figure 2.




(**a**) (**b**)

**Figure 2.** *Cont.*




(**c**)

**Figure 2.** Chromatograms from the analysis of a sample dietary supplement (**a**) blank sample; (**b**) investigated sample (estimated concentration 383 mg/g); (**c**) QC sample (at 250 mg/g).

The retention time (RT) for ecdysterone and mefruside in the QC sample (250 mg/g) was 3.42 min and 5.28 min, respectively. The same results were obtained in the analysis of left-over investigated dietary supplements. By contrast, in ablank sample, only interference was observed and the ratio of signal to noise compared to all QC samples exceeded 3. The estimated concentrations of ecdysterone in dietary supplements are shown in Table 1.

### *3.3. Spinach Sample*

The spinach sample was prepared according to the protocol described above. Again, the WADA technical document TD2021IDCR [8] was followed to confirm the identity of ecdysterone in this sample. Some results obtained in the analysis of the spinach sample are shown in Figure 3. The retention time (RT) for ecdysterone and mefruside was 3.40 min and 5.24 min, respectively, and the estimated concentration of ecdysterone was 10 µg/100g of spinach.

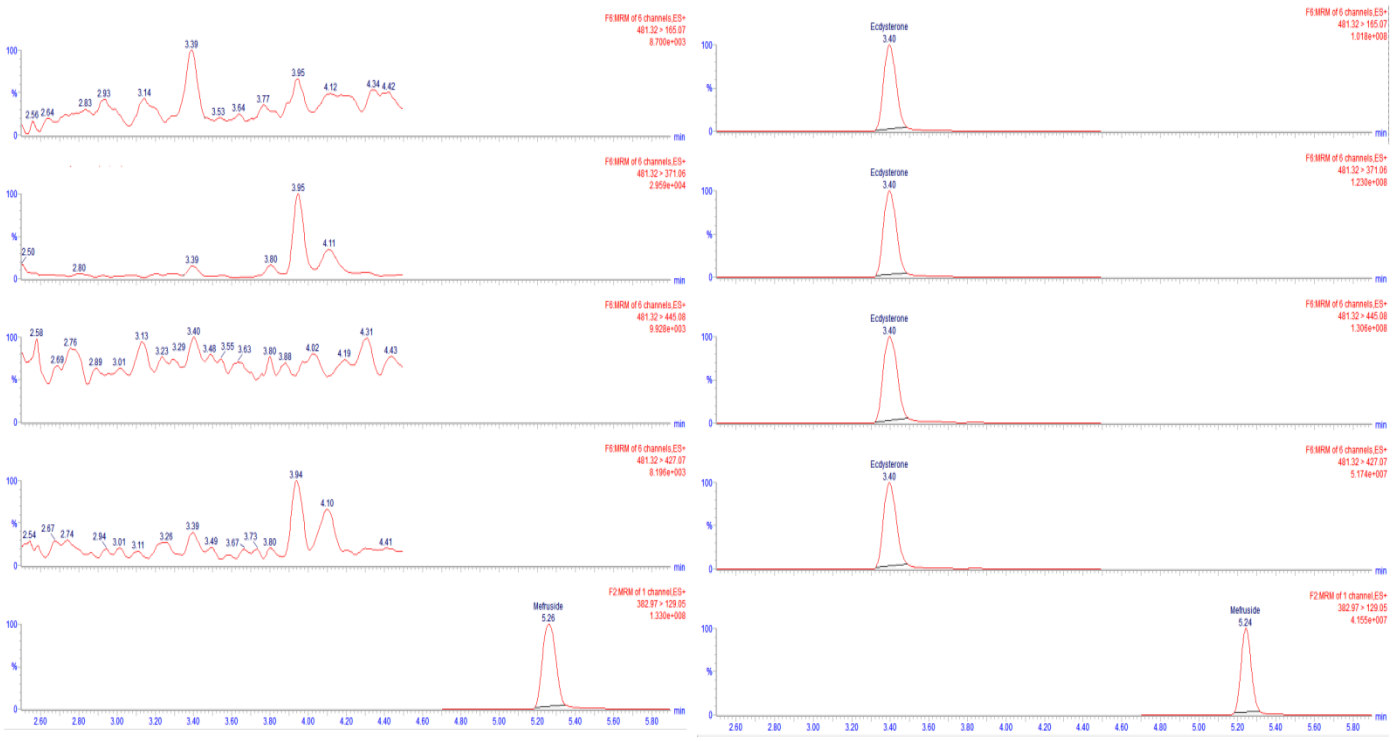



**Figure 3.** Chromatograms from the analysis of a sample dietary supplement (**a**) blank sample; (**b**) fresh spinach sample.

## 4. Discussion

Due to the fact that dietary supplements are complex matrices, UHPLC-MS/MS was selected as a method for ecdysterone determination. Many ingredients from these products may interfere with the tested compound(s), so the choice of a suitable technique of separationis necessary. Results obtained during this study meet all criteria dedicated to the analytical approach. First of all, used chromatographic separation of ecdysterone and used the composition ofphases allowedto obtain a single chromatographic peak with the typical Gauss shape. This is very crucial for the estimate of the concentration of the tested analyte. During the experiment, the stability of RT for ecdysterone was tested and any variation in this aspect was not observed as well. Further, the RT for Ecdysteroneis optimal because is neither in the "dead time" nor at the end of chromatographic separation. Moreover, any interferences in RT for ecdysterone were not observed and the S/N ratio exceeded 3 for all MRMs transitions.

Undoubtedly, the market for special nutrition products for athletes is a global one, and thus, such products are easily available. Although dietary supplements mainly contain minerals, herbs and botanicals, amino acids, and other ingredients, including prohibited substances in sport.This situation is a result of the legal regulations of dietary supplements. Athletes have to remember that these products are not classified as pharmaceuticals and need to be aware of the problems that can follow supplement use. Moreover, sport authorities need to ensure that nutritional education and guidance for athletes is of the highest standard.For example, higenamine, a substance included in class S3 (β2-agonist) of the WADA 2021 Prohibited List [9], is an alkaloid found in many plants including *Nelumbo nucifera*, *Nandinadomestica*, *Aconitum carmichaelii* (*Aconitum napellus*), *Asarumheterotropides*, and *Galiumdivaricatu*, and is present in many dietary supplements under these synonyms [7,10]. Another such case, 1,3-Dimethylamylamine (DMAA), a substance listed in class S6 (Stimulants) of the WADA 2020 Prohibited List [9]. In this case, the labels listed either its synonyms (37 deposited in the PubChem database or named "*Geranamine*", "*geraniumoil*", or "extract" as its source [11,12]. Although a notion that this substance was added to nutritional supple-

ments in a form of synthetic material seems to predominate, there is still an intense debate whether or not these extracts indeed contain 1,3-dimethylamylamine [12–15].In turn, Fleming et al. [16] reported that concentrations of 1,3-DMAA and 1,4-DMAA in a plant depend on the time and region of harvest (*Pelargonium graveolens*) that is often present in dietary supplements. In another case, stimulants such as, N,N-dimethyl-2-phenylpropan-1-amine and β-methylphenethylamine were found in dietary supplement, labelled as containing caffeine and adrenergic amines such as acacia rigidula [17]. It is also worth mentioning that more and more often there are attempts to hide the presence of a banned substance by the use of unusual chemical naming on the label and to make the substance difficult to recognize by the user [18–21].

The problem with the addition of plants or their extract to dietary supplements is widely described in scientific literature and considered an important problem for athletes.The above casesshow that the consumption of dietary supplements on which labels extracts from plants were listed may result in anti-doping rule violations. Seems to be undeniable, that the worst situation for athletes is when the prohibited plant or its extract was added to the dietary supplements without its mention. Moreover, athletes should not suggest a dietary supplement label because the declared ingredients may not have been added to the product, or the concentration of these compounds is varied or they were listed "only" on the label.

At present, ecdysteroneis included in WADA Monitoring Program. Given that ecdysterone is present in vegetables and in special products for athletes and mentioned on their labels under different names, the real scale consumption of this compound by athletes for improving sports performance may be false. Therefore, setting a reporting limit for this substance seems to be necessary (manuscript in preparation).This minimum reporting level would avoid an "unintentional" Adverse Analytical Findings (AAFs) that may occur in the future, in the case when ecdysterone will include in the WADA Prohibited List.Another question that seems to be important is what kind of ingredients may be included on the label of the dietary supplements in this situation, the full name of ecdysterone or only its extracts?

The WADA-accredited laboratory in Warsaw began monitoring of ecdysteronein routine doping control analysis in January 2020. Ecdysterone was confirmed in 81 samples at different concentrations (total number of samples 3270). In turn, since the beginning of 2021, ecdysterone was confirmed in athletes' urine samples multiple times and at different concentrations, as well (*manuscript in preparation*). According to the International Standard for Laboratories, all WADA-accredited laboratories shall analyze each dietary supplement specifically requested by an anti-doping organizations as part of a doping case investigation [22]. Thus, the development and validation of a method for the identification of ecdysterone in special products for athletes werenecessary for future possible doping case investigations.

## 5. Conclusions

This paper presents a simple and rapid method for identifying ecdysterone in dietary supplements, and its presence in the investigated products was clearly confirmed. Thus, this approach may be applied by anti-doping and/or toxicological laboratories. Additionally, the described method may be implemented in explanations of cases regarding violations of anti-doping rules in the future. Furthermore, our current research on ecdysterone focuses on establishing estimated concentrations of ecdysterone following ingestion of a single spinach smoothie (*manuscript in preparation*). Based on the results, a reporting limit for ecdysterone will be proposed.

**Author Contributions:** Conceptualization, K.G. and D.K.; methodology, K.G.; software, K.G., M.W. and A.D.; validation, K.G. formal analysis, K.G. and D.K.; resources, K.G., D.K., A.D. and M.W.; writing—original draft preparation, K.G. and D.K.; writing—review and editing, K.G. and D.K.; Visualization, K.G. and D.K.; Supervision, D.K.; Funding acquisition, D.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** Financial support from the Ministry of Culture, National Heritage and Sport of the Republic of Poland under project number 2021.0417/1575/UDOT/DS/14/AMW aregratefully acknowledged.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References


1. Isenmann, E.; Ambrosio, G.; Joseph, J.F.; Mazzarino, M.; de la Torre, X.; Zimmer, P.; Kazlauskas, R.; Goebel, C.; Botrè, F.; Diel, P.; et al. Ecdysteroids as non-conventional anabolic agent: Performance enhancement by ecdysterone supplementation in humans. *Arch. Toxicol.* **2019**, *93*, 1807–1816. [CrossRef] [PubMed]
2. Parr, M.K.; Ambrosio, G.; Wuest, B.; Mazzarino, M.; de la Torre, X.; Sibilia, F.; Joseph, J.; Diel, P.; Botrè, F. Targeting the administration of ecdysterone in doping control samples. *Forensic Toxicol.* **2020**, *38*, 172–184. [CrossRef]
3. Hunyadi, A.; Herke, I.; Lengyel, K.; Báthori, M.; Kele, Z.; Simon, A.; Tóth, G.; Szendrei, K. Ecdysteroid containing food supplements from Cyanotisarachnoidea on the European market: Evidence for spinach product counterfeiting. *Sci. Rep.* **2016**, *6*, 373322. [CrossRef] [PubMed]
4. Cheng, D.M.; Yousef, G.G.; Grace, M.H.; Rogers, R.B.; Gorelick-Feldman, J.; Raskin, I.; Lila, M.A. In vitro production of metabolism-enhancing phytoecdysteroids from Ajuga turkestanica. *Plant Cell Tiss. Organ Cult.* **2008**, *93*, 73–83. [CrossRef]
5. Guibout, L.; Mamadalieva, N.; Balducci, C.; Girault, J.P.; Lafont, R. The minor ecdysteroids from Ajuga turkestanica. *Phytochem. Anal.* **2015**, *26*, 293–300. [CrossRef] [PubMed]
6. Parr, M.K.; Botre, F.; Nas, A.; Hengevoss, J.; Diel, P.; Wolber, G. Ecdysteroids: A novel class of anabolic agents? *Biol. Sport* **2015**, *32*, 2. [CrossRef] [PubMed]
7. Grucza, K.; Kowalczyk, K.; Wicka, M.; Szutowski, M.; Bulska, E.; Kwiatkowska, D. The use of a valid and straightforward method for the identification of higenamine in dietary supplements in view of anti-doping rule violation cases. *Drug Test Anal.* **2019**, *11*, 912–917. [CrossRef] [PubMed]
8. World Anti-Doping Agency (WADA). Minimum Criteria for Chromatographic-Mass Spectrometric Confirmation of The Identity of Analytes for Doping Control Purposes. Available online: https://www.wada-ama.org/sites/default/files/resources/files/td2021idcrfinaleng0 (accessed on 25 October 2021).
9. World Anti-Doping Agency (WADA). WADA2021 Prohibited List. Available online: https://www.wada-ama.org/sites/default/files/resources/ (accessed on 25 October 2021).
10. Feng, S.; Jiang, J.; Hu, P.; Zhang, J.; Liu, T.; Zhao, Q.; Li, B. A phase I study on pharmacokinetics and pharmacodynamics of higenamine in healthy Chinese subjects. *Acta Pharmacol. Sin.* **2012**, *33*, 1353–1358. [CrossRef] [PubMed]
11. National Center for Biotechnology Information. PubChem Substance Database; CID=7753. Available online: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=7753 (accessed on 13 December 2021).
12. Lisi, A.; Hasick, N.; Kazlauskas, R.; Goebel, C. Studies of methylhexaneamine in supplements and geranium oil. *Drug Test Anal.* **2011**, *3*, 873–876. [CrossRef] [PubMed]
13. Gauthier, T.D. Evidence for the presence of 1,3-dimethylamylamine (1,3-DMAA) in geranium plant materials. *Anal. Chem. Insights* **2013**, *8*, 29–40. [CrossRef] [PubMed]
14. Di Lorenzo, C.; Moro, E.; Dos Santos, A.; Uberti, F.; Restani, P. Could 1,3 dimethylamylamine (DMAA) in food supplements have a natural origin? *Drug Test Anal.* **2013**, *5*, 116–121. [CrossRef] [PubMed]
15. Austin, K.G.; Travis, J.; Pace, G.; Lieberman, H.R. Analysis of 1,3 dimethylamylamine concentrations in Geraniaceae, geranium oil and dietary supplements. *Drug Test Anal.* **2014**, *6*, 797–804. [CrossRef] [PubMed]
16. Fleming, H.L.; Ranaivo, P.L.; Simone, P.S. Analysis and Confirmation of 1,3-DMAA and 1,4-DMAAin Geranium Plants Using High Performance LiquidChromatography with Tandem Mass Spectrometryat ng/g Concentrations. *Anal. Chem. Insights* **2012**, *7*, 59–78. [CrossRef] [PubMed]
17. Kwiatkowska, D.; Wójtowicz, M.; Jarek, A.; Goebel, C.; Chajewska, K.; Turek-Lepa, E.; Pokrywka, A.; Kazlauskas, R. N,N-dimethyl-2-phenylpropan-1-amine—New designer agent found in athlete urine and nutritional supplement. *Drug Test. Anal.* **2015**, *7*, 331–335. [CrossRef] [PubMed]
18. Kazlauskas, R.; Hasick, N. ASDTL supplements project. In *Recent Advances in Doping Analysis(19)*; Schänzer, W., Geyer, H., Gotzmann, A., Mareck, U., Eds.; Sport und Buch Strauß: Köln, Gremany, 2011; pp. 10–14.
19. Van Thuyne, W.; van Eenoo, P.; Delbeke, F.T. Nutritional supplements: Preva-lence of use and contamination with doping agents. *Nutr. Res. Rev.* **2006**, *19*, 147–158.
20. Geyer, H.; Braun, H.; Burke, L.M.; Stear, S.J.; Castel, L.M. A–Z of nutritional supple-ments. *Br. J. Sports Med.* **2011**, *45*, 752–754. [CrossRef] [PubMed]
21. Wójtowicz, M.; Jarek, A.; Chajewska, K.; Turek-Lepa, E.; Kwiatkowska, D. Determination of designer doping agent2-ethylamino-1-phenylbutane—In dietary supplements andexcretion study following single oral supplement dose. *J. Pharm. Biomed.* **2015**, *115*, 523–533. [CrossRef] [PubMed]
22. World Anti-Doping Agency (WADA). International Standard for Laboratories 2021. Available online: https://www.wadaama.org/sites/default/files/resources/files/isl2021.pdf (accessed on 25 October 2021).