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CASE REPORT

Toxicology

Barbie drug identification: Not a child's play

Marine Deville PhD | **Corinne Charlier PhD**

Laboratory of Clinical, Forensic and Environmental Toxicology, Center for Interdisciplinary Research on Medicines (CIRM), University Hospital of Liege, Liège, Belgium

Correspondence
Marine Deville, Laboratory of Clinical, Forensic and Environmental Toxicology, Center for Interdisciplinary Research on Medicines (CIRM), University Hospital of Liege, Avenue de l'Hopital,1, Liège 4000, Belgium.
Email: m.deville@chuliege.be

Abstract

Various samples—including two vials with a pharmaceutical appearance—were submitted to the laboratory for identification. The aim of this work was to describe the unique characteristics observed during the analysis of the powder contained in the vial. Samples were submitted to HPLC-DAD, UHPLC-TOF-MS, and/or UPLC-MS–MS analysis. The majority of the samples were easily identified as standard drugs of abuse. The main difficulty lay in identifying the powder in the vials. No match was found in the library through HPLC-DAD analysis. Fortunately, the vials were labeled as "Melanotan II", although the UV spectrum was not available. Mass spectrometric analysis of melanotan II was challenging, as it is a small peptide with a molecular weight of 1024 Da, which is significantly heavier than classical drugs that the laboratory usually handles. As a result, mass spectrometer's parameters can be limited to detect masses up to 1000 Da. Additionally, melanotan II is multi-charged which is also unusual for compounds typically targeted in our daily work. Finally, the reference standard allowed us to confirm the identification with both instruments, and determine the purity of 30%. Melanotan II is not approved on the market due to safety concerns. It is used illegally mainly for tanning, explaining its nickname "Barbie drug". To conclude, analysis of melanotan II was challenging as it is heavy and doubly charged. Moreover, its UV spectrum was initially not available in the literature. The difficulties faced by forensic scientists in detecting this drug may explain its popularity on the illicit market.

KEYWORDS
Barbie drug, drug identification, forensic toxicology, image enhancing drug, mass spectrometry, melanotan II

Highlights

- Melanotan II is an illegal and uncommon product used for tanning, explaining its nickname "Barbie drug"
- Melanotan II is a high molecular weight compound which is multiply charged, making it challenging for analysis.
- Melanotan II spectra were not easily found in the literature and can now be found in this paper.

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1 | INTRODUCTION

Performance and image enhancing drugs (PIEDs) are used to enhance both appearance and skills. PIED primarily encompasses stimulants, such as methylphenidate, steroids (with anabolic androgenic steroids being the most prevalent), and other hormones. Additionally, drugs for erectile dysfunction, specifically phosphodiesterase type 5 inhibitors, are included in the category of PIEDs. These drugs become PIEDs when they are employed outside the context of prescription use for a medical condition; it is more about lifestyle drugs being diverted from their intended use than genuine medicines.

Melanotan II is a cyclic heptapeptide, corresponding to the synthetic analog of α-melanocyte stimulating hormone (α-MSH). It mimics the hormone's effect on melanocortin 1 receptor (MC1R) which is located on melanocytes, leading to an increase in melanin production resulting in skin pigmentation [1]. Compared to α-MSH, the peptide size of melanotan II was reduced to keep only the part that binds the melanocortin receptor. Some amino acids were substituted (Figure 1), and combined with the cyclic structure of melanotan II, its stability and resistance to proteolytic enzymes are increased as well as its duration of action [2, 3].

Melanotan I (also known as afamelanotide) is being studied as a medicine for treating certain skin conditions exacerbated by sunlight exposure. It is already approved in Europe and the USA for erythropoietic protoporphyria in adult patients [4]. In contrast, melanotan II is banned in most countries and is no longer being tested as a medicine due to its adverse effects. Because of a lack of specificity, melanotan II binds to melanocortin 2 receptor in the gut, causing nausea and vomiting, but also to melanocortin 3 receptor in the brain, responsible for spontaneous erection [5, 6]. Other adverse events are anxiety and facial flushing [7], or more serious complications such as chest pain and kidney failure [8]. Obviously, some adverse events concern the skin, with the risk of new naevi or darkening or enlargement of existing naevi [9–11]. The drug can also lead to changes in the appearance and shape of moles [12] and has been associated with melanoma [13].

Despite these potential adverse events and subsequent ban in the drug market, melanotan II remains illegally accessible through the online market and is sold in gyms and beauty salons. Appreciated by users of PIEDs who are driven through appearance ideals, melanotan II is a tanning peptide, explaining its nickname "Barbie drug" [14]. Melanotan II is primarily administered through subcutaneous injection [9] to achieve tanned skin without exposure to UV rays. Some websites even recommend combining its use with tanning bed for a quicker or more even tan, despite consumer arguments citing its affordability compared to tanning beds [7, 15]. To a lesser extent, melanotan II is also used as an appetite suppressant and aphrodisiac [14].

Next to the intrinsic toxicity of the substance itself, the use of melanotan II without medical supervision is compounded by the inherent risks of the black market. Production of the drug in clandestine laboratories that do not respect good manufacturing practice regulations fails to guarantee quality, safety, and efficacy. This can result in mislabelled products, exposure to non-sterile products, receiving a different product than what is purchased, or even receiving a product with no active substance at all. There is also the possibility of receiving a product with an incorrect concentration or an unintended combination with another active compound. Furthermore, the injection route poses the potential risk of transmitting infectious diseases if needles are shared.

Melanotan II is not the most commonly used PIED [16–20]. Among these, human growth hormone (hGH), with a much higher molecular weight, is the most frequently encountered peptide. Others include human chorionic gonadotrophin, insulin-like growth factor-1, and the growth hormone-releasing peptides [21]. Nevertheless, in a study conducted in Belgium, melanotan II was one of the most frequently seized peptides [22], which was also confirmed in Denmark [23]. In this context, laboratories must be prepared to identify it.

There is no reference method for analyzing peptides and proteins. Gas chromatography methods cannot be applied due to the high molecular weight and low volatility of peptides. Peptide analysis can require enzymatic digestion (trypsin, e.g.,) and incubation before LC–MS/MS analysis. With high-resolution instruments, intact proteins can be screened for and structural information can be obtained. In this context, Mestria et al. [24] have previously described the fragmentation pathways and structure of fragment ions found in the mass spectrum of melanotan II.

The objective of this paper is to describe the pitfalls encountered during the analytical confirmation of melanotan II and to provide analytical data that can be useful to other laboratories.

2 | MATERIALS AND METHODS

2.1 | Seized samples

The police conducted a raid at a location suspected of arms trafficking and prostitution. They confiscated numerous pieces of evidence related to drug trafficking, all of which were subsequently submitted to our laboratory for identification.

Approximately 500 pink–orange domino-shaped tablets were received, as well as other individual tablets and various quantities of white-beige powders. Additionally, cannabis residues were discovered in a plastic bag. Most importantly, a couple of glass vials with a pharmaceutical appearance was received. These vials were equipped with a rubber seal plug and a metal over-seal, with one containing a white powder and the other containing a transparent liquid (Figure 2).

α- MSH : Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH2
Melanotan I: Ac-Ser-Tyr-Ser-NLe-Glu-His-DPhe-Arg-Trp-Gly-Lys-Pro-Val-NH2
Melanotan II : Ac-NLe-[Asp-His-DPhe-Arg-Trp-Lys]-NH2

FIGURE 1 Amino acid composition of α-MSH, melanotan I, and melanotan II.

FIGURE 2 Vials of pharmaceutical appearance.

Furthermore, the police seized various items related to consumption and trafficking, including a batch of plastic bags and funnels, a cannabis grinder containing residues, a precision scale, and various cards and straw.

Except for cannabis which was analyzed with a dedicated method, all samples were submitted to an HPLC screening method. Amphetamine derivatives are then quantified with an UPLC-MS/MS method, whereas NPS and melanotan II requested an analysis with UHPLC-TOF-MS.

2.2 | Standards and reagents

Melanotan II acetic acid salt was purchased from TRC.

All of the other analytical standards were purchased from Cerilliant, except for THC-A and MDMA-d5 which were purchased from LoGiCal (LGC Standards). Prazepam, used as internal standard for UHPLC-TOF-MS analysis and HPLC-DAD, was purchased from Certa. All solvents were supplied by J.T. Baker and LC–MS grade (methanol, water) or HPLC grade (acetonitrile, methanol) depending on the instrument for which they were intended.

2.3 | Sample preparation

In the case of tablets, samples were crushed before analysis. Then, 100 mg of powder was diluted in 10-mL methanol before agitation, centrifugation, subsequent dilutions, and analysis using the methods detailed below.

2.4 | HPLC-DAD analysis

Screening for classical drugs of abuse and medicines was performed with a method inspired by Gaillard et al. [25] using high-performance liquid chromatography coupled to a diode array detector (HPLC-DAD).

The HPLC–DAD equipment was an Alliance® 2695 combined with PDA 2996 from Waters. Injections of 40 µL of the samples were done on a Symmetry® C8 column (5 µm, 250 × 4.6 mm) from Waters, in gradient mode, with mobile phase A consisting of 43.5 mM phosphate buffer (pH 3.8) and acetonitrile as mobile phase B. Initial settings were a flow rate of 1 mL/min and 13% of mobile phase B, which was linearly increased to 35% B over 9 min, then increased to 80% B in 19 min. This mobile phase composition was held for 2 min with a flow rate of 1.5 mL/min, before setting back to the initial conditions in 1 min and re-equilibrate for 13 min prior to the next injection. Data were acquired and processed with Empower 3 software, including a comparison to the NewToxicol library (Waters) and an in-house developed library.

For melanotan II quantification in the powder (purity determination), a calibration curve including seven calibration standards diluted in methanol (5%, 12.5%, 25%, 37.5%, 50%, 75%, and 100%) was prepared. Prazepam (100 mg/L) was used as internal standard.

2.5 | UHPLC-TOF-MS analysis

To confirm HPLC-DAD identification, extend the research, and try to identify unknowns, a screening method with a high-resolution instrument was used, and the screening method was advised by Sciex [26]. The UHPLC-TOF-MS apparatus was an Eksigent® LC 100 XL combined with a Triple TOF 4600® (Sciex). Injection of 10 µL of the sample was done on a Kinetex® C18 column (2.6 mm, 100 Å, 50 × 3.00 mm) from Phenomenex. A gradient was applied at 30°C, with mobile phase A made of 10 mM ammonium formate while mobile phase B consisted of a mix of acetonitrile and methanol with 0.1% formic acid (50/50). A constant flow of 0.4 mL/min was applied using the following gradient: the initial condition of 98% of mobile phase A was held for 1 min. Then, the gradient linearly decreased to 0% of A in 10 min, held for 3 min. Finally, the gradient returned to initial conditions and was maintained for 2.5 min prior to the next injection. The TOF was equipped with a DuoSpray Ion source working in positive electrospray ionization mode. Source conditions were the following: ion source gas 1 (GS1) 40 psi, ion source gas 2 (GS2) 60 psi, curtain gas (CUR) 30 psi, source temperature 500°C, and ion spray voltage floating 5500 V. The mass acquisition method was a TOF survey scan from 50 to 1100 Da (cycle time 1 s, accumulation time 0.150 s), combined with a second experiment of product ion scan with an information-dependent acquisition (IDA) method on a maximum of 20 candidates per cycle. Data were acquired with Analyst 1.7.1 software and finally processed with the PeakView software 2.2, including a comparison to a library purchased by Sciex and regularly updated in-house. To be considered as present in the sample, the observed mass error for the compound must be less than 5 ppm, the error percentage on the retention time must be <5.1%, and the percentage difference on the isotope ratio must be <10%. Moreover, the score related to the mass spectrum comparison with the library must be >70. The compound will be identified with less confidence

if these criteria are respectively <10 ppm for the mass error, less than 15% for the retention time, <20% for the isotope ration, and >31 for the library score. If these criteria are not fulfilled, the probability to find the compound is low.

2.6 | UHPLC-MS/MS analysis

Due to insufficient HPLC-DAD specificity, amphetamine derivatives were confirmed and quantified with ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS). More details can be found in the supplemental information.

2.7 | Cannabis analysis

Cannabis residues were analyzed with a dedicated method, published elsewhere [27] and detailed in Data S1.

3 | RESULTS

Most of the samples were readily identified and quantified as amphetamine, ketamine, MDMA and cocaine. One new psychoactive substance was also detected (chloromethcathinone). The results are detailed in Table S2 in the supplemental information.

Cannabis residues discovered in the cannabis grinder, and in a plastic bag, contained 11.7% and 7.94% of total THC, respectively. Additionally, trace amounts of cocaine and cutting agents (caffeine, phenacetin, levamisole) were detected in selected items, including the precision scale, a metallic card, and a blue box.

Among this variety of standard drugs of abuse was a very atypical compound. Identification of the compound within the vials with a pharmaceutical appearance was not so easy. One vial contained 2 mL of liquid, while the other contained 8.7 mg of powder, contrary to the 10 mg indicated on the label. The powder and the liquid were initially analyzed separately. However, a chromatographic peak was only observed in the powder.

After easy dissolution in methanol, chromatographic analysis revealed a compound that strongly absorbed in the UV range, as evidenced by its intense chromatographic peak (retention time of 11.4 min) associated with the UV spectrum depicted in Figure 3. The same chromatographic peak (identical retention time and UV spectrum) was observed after dissolution of the powder with the liquid contained in the other vial.

However, no match was found in our library (New Toxicol® purchased by Waters). Fortunately, the vials were labeled with the name Melanotan II but its UV spectrum could not be found in conventional

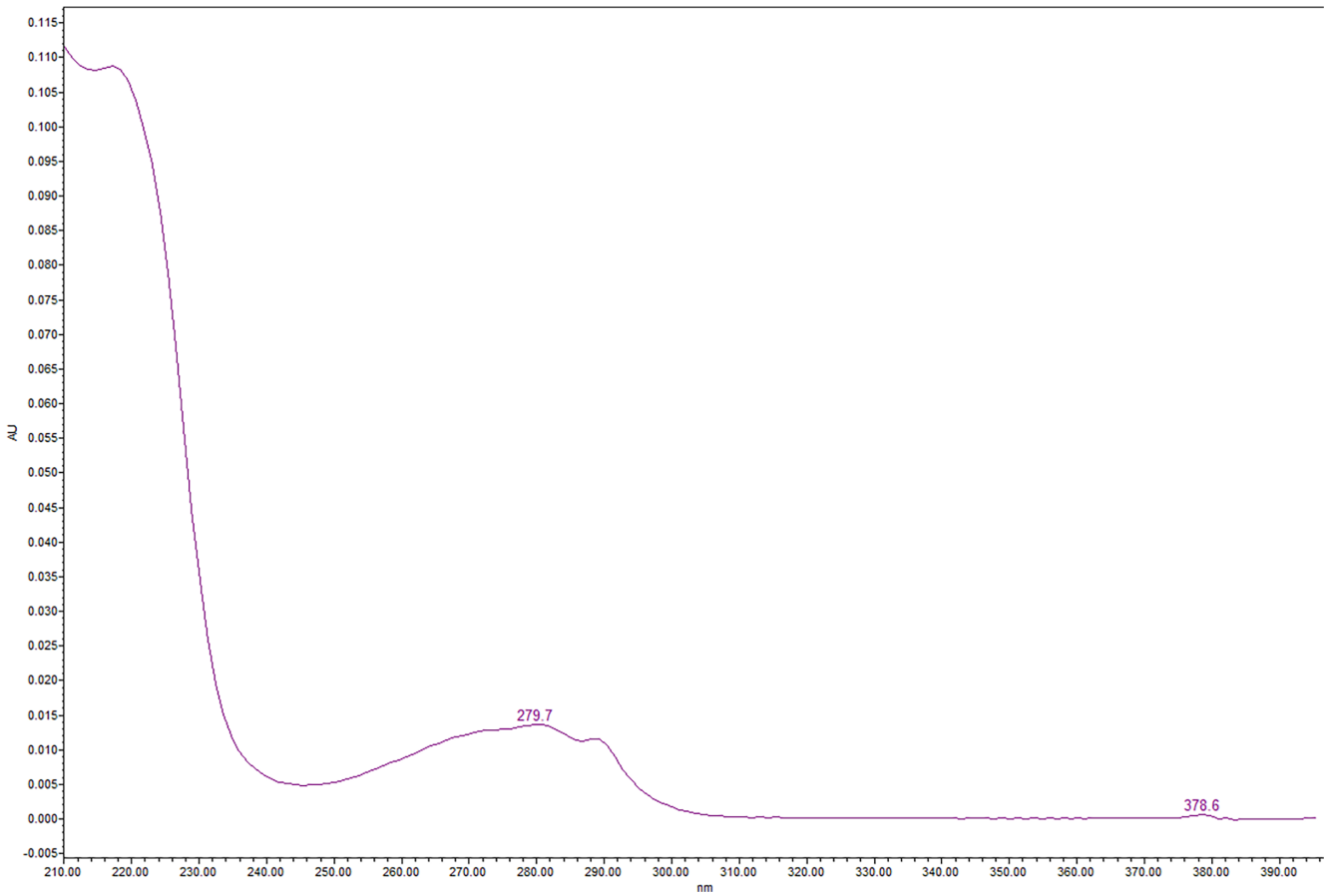

FIGURE 3 UV spectrum of a 50 mg/L methanolic solution of melanotan II showing maxima at 220 and 280 nm.

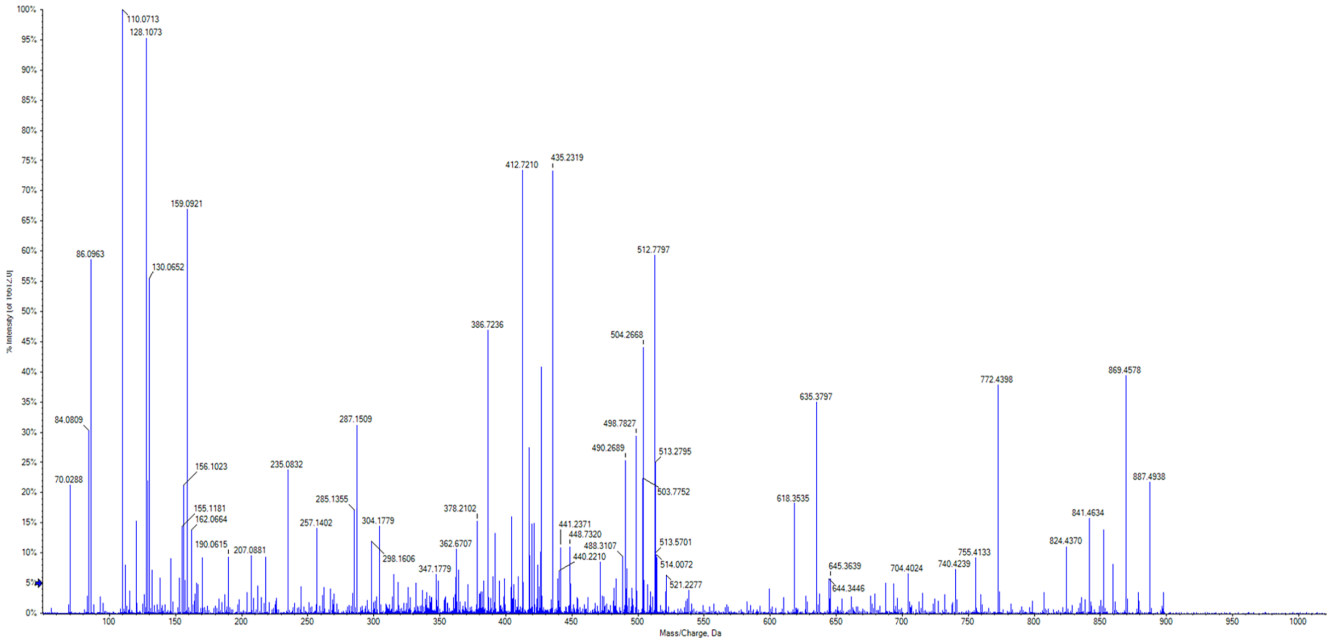

FIGURE 4 Complex MSMS spectrum of a 1 mg/L methanolic solution of melanotan II. Main fragments are 110.0713, 128.1073, 412.7210, 435.2319, 412.7797, 635.3797, 772.4398, and 869.4578.

databases or the existing literature. Consequently, it was imperative to purchase the reference standard to confirm both the retention time and the UV spectrum.

As illustrated in Figure 4, the MS/MS spectrum is complex, it was not present in our library, but it matched with *Chemspider*®, which is integrated with our software.

3.1 | Purity

Using the calibration curve obtained with the reference standard, it was determined that the purity of the powder was only 30% (2.61 mg).

However, when a chromatographic comparison was conducted between the reference standard and the sample (Figure 5), no peaks corresponding to impurities were detected with HPLC-DAD, even if the seized sample confirmed a signal intensity of 30% when compared to the reference standard, as can be observed in the same figure.

The same was observed in mass spectrometry (Figure 6).

4 | DISCUSSION

Identification of the compound within the vials with a pharmaceutical appearance was challenging.

The absence of chromatographic peak in the liquid suggested—as expected—that the liquid (likely water for injection) was used solely to dissolve the powder in the other vial before injection. Indeed, the two vials were contained in a plastic holder with two slots specifically shaped for the vials, strongly suggesting that they were meant to be used together. Furthermore, while the powder was labeled, the liquid was not.

The compound in the powder showed an intense chromatographic peak associated with an unrecognized UV spectrum including 2 maxima at 200 and 280 nm.

As Melanotan II was indicated on the label, a search for its UV spectrum was conducted but it was unsuccessful. Consequently, it was imperative to purchase the reference standard to confirm both the retention time and the UV spectrum.

Mass spectrometric analysis of melanotan II was challenging, as it is a small peptide with a molecular weight of 1024 Da, which is notably heavier than classical drugs of abuse and medicines (although lighter than other peptides used as PIED). Actually, only 5% of the 2000 compounds included in our library possess a molecular weight exceeding 500 Da. Consequently, the parameters of the mass spectrometer can be limited to detect masses up to 1000 Da. In addition, melanotan II is also multi-charged which is unusual for compounds typically targeted in our daily work. As a result, a significant increase in signal intensity (a sevenfold increase) is observed when it is protonated twice. When specifically searching for a compound, such as melanotan II in this case, a significant advantage of TOF mass spectrometry is the ability to input its molecular formula (in this case: C50H69N15O9) and the suspected adduct. The software then scans the chromatographic data for the calculated mass of interest. In our daily practice, the adduct is consistently a single proton. There was a potential risk associated with melanotan II being undetectable if the search had been made only for the mono-protonated compound instead of the doubly charged form, as the latter is highly unusual. As a result, the analysis would have been negative, and the offenders would not have been prosecuted (if this was the only seized product of the raid).

In addition to these analytical specificities, PIEDS are regulated by other legislation [28] than drugs of abuse and medicine,

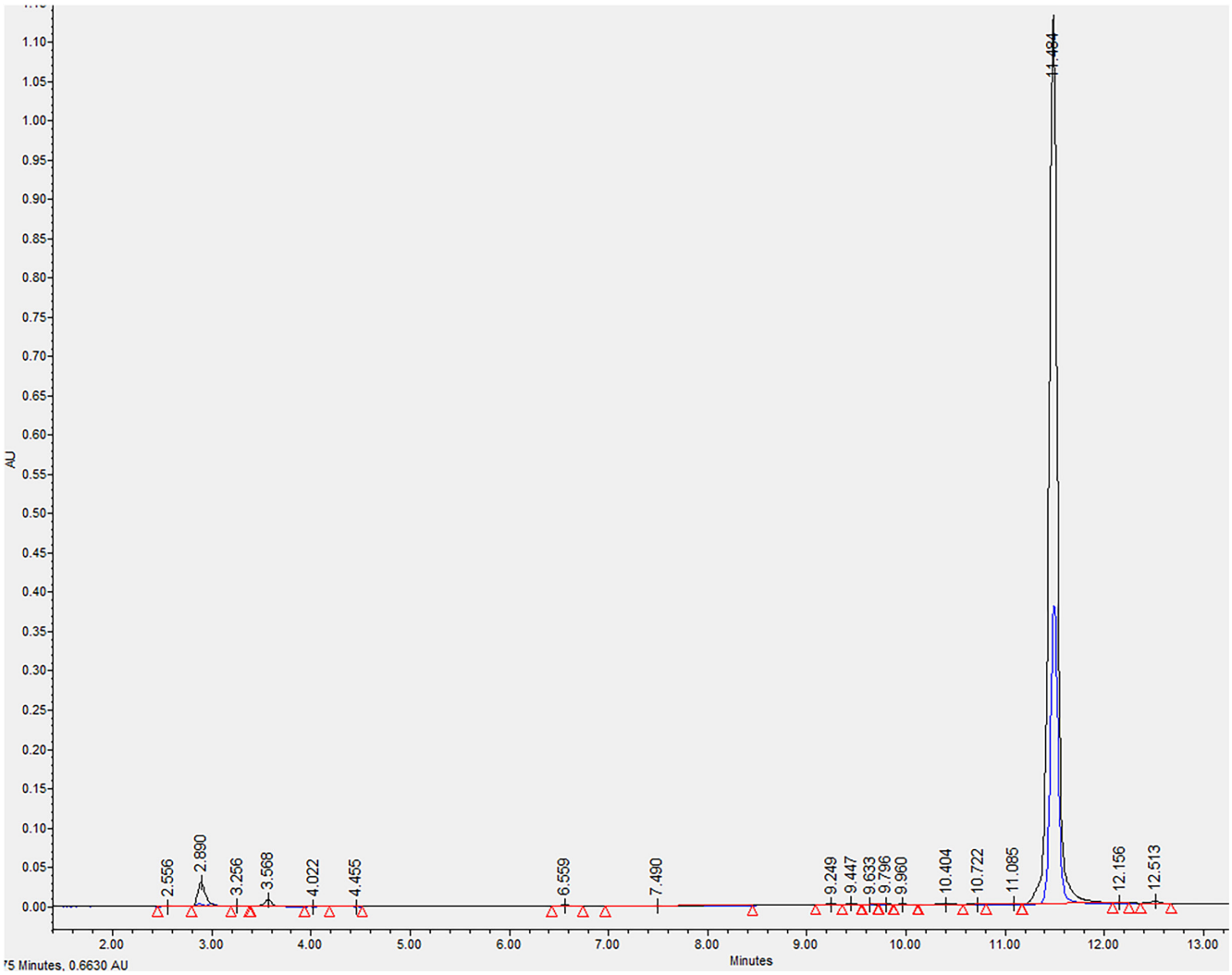

FIGURE 5 Comparison between the reference standard (in black) and the seized sample (in blue) analyzed with HPLC-DAD, working at a wavelength between 210 and 400 nm.

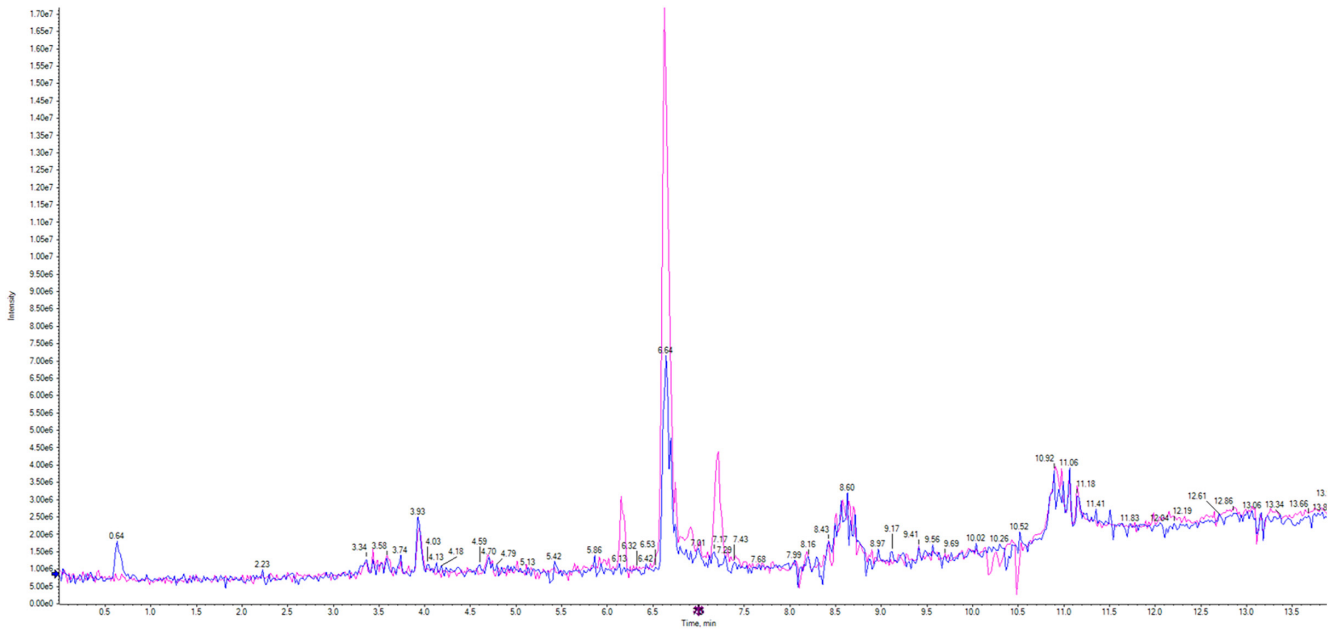

FIGURE 6 Total ion current comparison between the reference standard (in pink) and the seized sample (in blue) analyzed with UHPLC-TOF-MS.

considerably increasing the number of substances that the laboratory must be able to detect.

The purity of the powder was only 30% (2.61 mg). This amount is lower than the quantities observed in the study conducted by Breindahl et al. [29], which ranged from 4.32 to 8.84 mg. The absence of peaks corresponding to impurities with HPLC-DAD and mass spectrometry may suggest inorganic impurities (elemental impurities are not rare), such as buffers or salts, that are frequently found in lyophilized peptides alongside sugars and polyethylene glycol [30, 31].

5 | CONCLUSION

It was a large seizure of drugs of abuse, but one of the products seized was not intended to act on the mind, but rather on the body. Melanotan II is an example of the numerous PIEDs that are sold illegally, and represents a threat to public health.

For toxicologists, melanotan II has a high molecular weight and double charges in mass spectrometric analysis, which is unusual. Consequently, when a compound does not seem easy to detect, analysts need to broaden the mass ranges detected by their spectrometers and not overlook the possibility of a multi-charged compound. On one hand, analytical confirmation is essential to ensure that products implicated in case reports are indeed the substances they are suspected to be. On the other hand, difficult analytical confirmation can also be an argument to explain the popularity of some products on the illicit market.

The methods described in this paper proved successful for the identification and quantification of melanotan II in solid samples. Melanotan II UV spectrum is now available in the literature.

CONFLICT OF INTEREST STATEMENT

The authors have no competing interests to declare that are relevant to the content of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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