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Identification and characterization by LC-UV-MS/MS of melanotan II skin-tanning products sold illegally on the Internet

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Q1 New methods were developed and validated to determine the identity, contents, and purity of samples of melanotan II, a synthetic melanocortin receptor agonist, sold in vials as injectable skin-tanning products that were purchased from three online shops. Methods were based on liquid chromatography with ultra-violet detection (LC-UV) at wavelength 218 nm, and tandem mass spectrometric detection (MS/MS) after collision-induced fragmentation of the double charged $[M+2H]^{2+}$ precursor ion (m/z 513). Identification of melanotan II was verified by correct chromatographic retention time, and relative abundance ratios of five qualifying fragment ions. LC-UV was used to quantify melanotan II as well as impurities. Method validation was performed with reference to guidelines for assessing active substances in authorized medicinal products to reach acceptable accuracy and precision. Vials from two shops contained unknown impurities ranging from 4.1 to 5.9%; impurities from one shop were below the quantification limit. The total amount of melanotan II in vials ranged between 4.32 and 8.84 mg, although each shop claimed that vials contained 10 mg melanotan II. A broad range of drugs used for enhancement purposes can be obtained from the illicit market. However, users of these drugs may be exposed to a range of potential harms, as shown in this study, given that these products are manufactured, distributed and supplied from an illicit market. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: melanotan II; liquid chromatography; tandem mass spectrometry; identification; assay; impurities

Introduction

Data from non-clinical and clinical studies have demonstrated that administration of the synthetic peptide melanocortin receptor agonists melanotan I or melanotan II can increase skin pigmentation.^[1–4] The mechanism of action of melanotan I is thought to be mediated by mimicking the effects of endogenous α -melanocyte stimulating hormone (α -MSH) on the melanocortin 1 receptor that is expressed on melanocytes. This results in the up-regulation of brown-black eumelanin synthesis, leading to increased skin pigmentation.^[5,6] Melanotan II, a cyclic, lactam-bridged heptapeptide with the sequence Ac-Nle-cyclo[Asp-His-D-Phe-Arg-Trp-Lys]-NH₂, is thought to exert a comparable action; however, data are limited as clinical studies of this peptide in human skin pigmentation were not pursued after it was found to induce penile erection.^[1,7–11] Nevertheless, skin-tanning products that claim to contain melanotan II are being advertised and sold on the illicit drug market. This includes through Internet shops as well as being sold and administered in gyms, hairdressers, tanning salons, and beauty salons.^[12,13]

Most of the information about the sale and use of these products comes from regulatory action against retailers, seizures made by police and custom authorities, Internet monitoring of shops and user discussion forums, case reports/series of suspected adverse reactions, and media reports. Triangulation of this information suggests that over the past few years the use of melanotan II has been growing amongst people who want to increase their skin pigmentation usually for cosmetic reasons; to a lesser degree the drug is also used for weight loss, as an

aphrodisiac, and as a self-directed treatment for diseases such as rosacea.^[12,13]

Medicine regulatory authorities in a number of countries in Europe as well as Australia and the United States have classed such 'melanotan products' as unauthorized medicinal products. As a result, marketing, distribution, and advertisement are unlawful without authorization in these jurisdictions. Warnings have been issued related to the sale and use of these products,^[14–17] with the Medicines and Healthcare Products Regulatory Agency in the United Kingdom closing down 72 online shops in mid-2013.^[16] Even so, a search for 'buy melanotan' on Google, that we conducted at the time of writing this paper, found a number of online shops selling products claiming to contain melanotan II, and, to a lesser degree, melanotan I.

The acute adverse reactions associated with administration of melanotan II reported from phase I and II clinical studies include spontaneous erections, facial flushing, nausea, and vomiting.^[8,9,18]

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Little is known about the potential for chronic adverse reactions, although there are obvious concerns given that melanotan II is a potent non-selective melanocortin receptor agonist with high affinity for MC1, MC3, MC4, and MC5 receptor subtypes – which are involved in the regulation of a number of physiological systems such as the pigmentary system, energy homeostasis, sexual functioning, the immune system, inflammation, and the cardiovascular system.^[19] Suspected adverse reactions associated with the use of melanotan or melanotan II bought from the illicit market have been reported through national pharmacovigilance systems and clinical case reports.^[20–29] These include changes in pre-existing moles (such as dysplastic growth) and one case of systemic toxicity.^[29] However, causal assessment of most of these cases is, in part, confounded by a lack of analytical identification of the actual substance used (either from toxicological screening of a biological sample from the patient or forensic analysis of the product used). In this respect, as far as we are aware, analytical confirmation of the substance used – melanotan II – was only reported in the case report of systemic toxicity.^[29]

Additional hazards posed by the use of these products arise from the fact that they are administered by injection. As a result users may be exposed to non-sterile products and/or other forms of product contamination; while from a broader public health perspective the sharing of injecting equipment and/or drug vials can transmit bacterial as well as viral infections such as HIV, hepatitis B, and hepatitis C.

In previous research, analytical methods have been used to characterize melanotan I and melanotan II in biological samples from animals and humans. One study used liquid chromatography with ultra-violet detection (LC-UV) to quantify melanotan II in rat plasma.^[30] In other studies, liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to quantify melanotan II in plasma and brain tissue in mice^[31] and in rat plasma.^[32] Another study used LC-UV to quantify melanotan I in human plasma, although the recovery and sensitivity was low.^[33] These methods are not directly applicable for analysing lyophilized solids in drug vials. One study used MS to identify melanotan II in samples seized by law enforcement agencies, although details of the applied methods were not provided.^[34]

No published validated analytical methods are currently available to identify and quantify active substance and impurities of products claiming to contain melanotan II. As melanotan II are produced outside formal regulatory systems and thus without the necessary quality assurance systems, methods to determine their composition are needed to assess their potential health hazards. This need was further highlighted to us when the substance from an unlabelled vial, seized from a drug treatment programme in Denmark, was analyzed by our group and found to contain melanotan II.^[35]

The present study aimed at developing, validating, and applying analytical methods for melanotan II products, based on liquid chromatography with ultraviolet detection and tandem mass spectrometry (LC-UV-MS/MS). Data are presented from analysis of products claiming to contain melanotan II that were purchased from three online shops. Results include identification of the active substance, total content of active substance in vials (mg) and unknown impurities (area-%). While a small number of studies have identified melanotan II in vials seized by law enforcement agencies,^[34] and substances used by patients,^[29,35] as far as we know, these are the first published quantitative data based on a validated analysis of melanotan II.

Methods and materials

Sample acquisition

In mid-2011, the term ‘buy melanotan’ was used to search google.co.uk for online shops that were selling ‘melanotan’ skin-tanning products. The search gave a ranked list of websites based on importance/relevance determined by Google which is likely to provide measure of the popularity of these sites amongst consumers looking to buy these products.^[36,37] Each hyperlink to a website was followed to select shops that: (1) claimed to offer vials with 10 mg of melanotan II; (2) accepted credit card payment through a secure website; and (3) offered delivery to the United Kingdom. Three shops that met the inclusion criteria were then selected from the first 20 results. A total of 73 vials were purchased from the shops, hereafter referred to as Shop A, Shop B, and Shop C (Table 1). While it is difficult to determine whether these shops were different storefronts for the same retailer, an examination of the websites (e.g. comparing shop contact information and packaging/labelling of the products), suggests that this did not appear to be the case.

On receipt of the products, the contents of each package were inspected by us to document the contents including the number of vials that were provided. The vials were then shipped to the Department of Clinical Biochemistry (Vendsyssel Hospital,

Table 1. Melanotan II product information, contents of melanotan II in vials (mg) and sum of impurities (area-%)

Internet shop and product number ^a	Total content of melanotan II in vial (mg)	Mean (mg); % RSD	Sum of impurities (area-%)	Mean (area-%); % RSD
A1	4.70	4.91; 2.2	4.7	4.5; 4.9
A2	4.83		4.8	
A3	5.03		4.3	
A4	4.90		4.6	
A5	4.89		4.7	
A6	4.96		4.1	
A7	5.12		4.3	
A8	4.82		4.6	
A9	4.78		4.2	
A10	4.81		4.7	
A11	4.99		4.7	
A12	4.92		4.3	
A13	4.97		4.6	
A14	4.95		4.6	
A15	4.97		4.4	
B1	6.06	7.08; 14.5	4.9	5.03; 9.2
B2	7.73		5.1	
B3	6.67		4.6	
B4	6.50		4.7	
B5	8.84		5.0	
B6	6.67		5.9	
C1	4.32	5.20; 10.1	< 0.05	N/A
C2	5.13		< 0.05	
C3	5.58		< 0.05	
C4	5.58		< 0.05	
C5	5.40		< 0.05	

^aTotal number of vials purchased from each shop; Shop A, n=32; Shop B, n=21; Shop C, n=20

Denmark). Upon arrival, each vial was labelled with a unique identifier, and vials from each retailer were assigned to three different studies. This paper reports on the analysis of 26 vials. All vials were stored at room temperature in the dark. Analysis commenced within one week of arrival in Denmark. The study had approval from the Danish Health and Medicines Authority.

Reference standards and chemicals

Melanotan II reference standards (CAS number: 121062-08-6) were purchased from Sigma-Aldrich (Munich, Germany), Tocris Bioscience (Bristol, UK), and Bachem (Bubendorf, Switzerland). All melanotan II standards used in this study were prepared in aqueous solutions. For all quantitative data shown below, the 'industrial grade' standard from Sigma-Aldrich (Cat. No. M8693) was assigned as primary calibration standard. Trifluoroacetic acid (TFA), used for preparation of mobile phase A, was LC grade from Thermo Scientific (Loughborough, UK). Acetonitrile with 0.1% TFA (mobile phase B) was LC-MS grade from Fluka (Buchs, Switzerland). Ultrapure water (18.2 M Ω) used for preparation of standards, samples, blanks and mobile phases was prepared on an Elga Centra RDS system (Buckinghamshire, United Kingdom). A Mettler Toledo analytical scale (Greifensee, Switzerland) was used to prepare standard solutions for reference.

Sample preparation

An aqueous stock of each sample was prepared by adding ultrapure water to the vial through a 5 mL syringe. The syringe was filled with 1.00 mL water, using a calibrated pipette, and its content transferred to the vial using a surplus of air. From this solution, an aliquot of 100 μ L was transferred to a micro vial for determination of impurities by LC-UV. Another 50 μ L was diluted with 450 μ L water and 100 μ L transferred to a micro vial for assay by LC-UV to determine the vial's content (mg). Finally, 50 μ L of the remaining assay solution was diluted with 950 μ L water, and used for unambiguous identification of melanotan II by LC-UV-MS/MS.

Instrumentation and conditions

Chromatography

LC was performed using Agilent Technologies 1200 series LC modules (Waldbronn, Germany). Auto sampler injection volume was set to 10 μ L for detection of impurities, otherwise to 1 μ L. The analytical column was an Agilent Zorbax 300SB-C8, 100 mm x 2.1 mm i.d., packed with 3.5 μ m particles. Flow rate was 450 μ L/min. Column temperature was 50°C. Mobile phase A was 0.1 % TFA in aqueous solution. Mobile phase B was 0.1% TFA in acetonitrile. The binary pump gradient went from 5% B for 0.1 min to 95% B in 7 min, where it was maintained for 2 min, and was brought back to equilibration at initial conditions for 3 min. Total run time: 15 min. UV detection was performed using an Agilent 1100 diode array detector. Wavelength was 218 nm; bandwidth 30 nm; slit width 16 mm; and sampling rate 0.62 Hz with no reference wavelength.

Mass spectrometry

The MS/MS after electrospray ionization was performed on a Sciex QTRAP 3200 mass spectrometer (Applied Biosystems, USA) equipped with a Turbo Ion Source (electrospray) operated in positive mode. Parameters for MS/MS were optimized during infusion of a melanotan II reference standard solution using the

Analyst 1.5 software. Ion spray voltage was set to 2500 V; source temperature: 550°C; ion gas 1: 55 p.s.i.; ion gas 2: 50 p.s.i.; curtain gas: 22 p.s.i.; and declustering potential was 81 V. Multiple reaction monitoring (MRM) parameters and collision energies (CE) for the six collision induced dissociations were m/z 513 \rightarrow m/z 86.0 (CE: 59), 110.1 (CE: 59), 83.8 (CE: 59), 70.4 (CE: 61), 120.1 (CE: 61) and 130.1 (CE: 61), listed in order of decreasing relative intensity. Dwell time was 150 ms at unit resolution.

Method validation

The validation was based in part on the international conference of harmonization of technical requirements for registration of pharmaceuticals for human use (ICH) guideline TOPIC Q2 (R1).^[38] Acceptance criteria are listed in Table 2. Intermediate precision was not tested.

Assay method

Linearity for the assay method was assessed by analysis of five melanotan II standard solutions prepared by diluting an aqueous stock solution of melanotan II (Sigma-Aldrich, Cat. No. M8693) to 0.205, 0.41, 0.82, 1.23 and 1.64 mg/mL. Linear regression analysis was performed on the basis of peak areas with no weighting factor and curves not forced through origin. Accuracy and precision was assessed by determination of method recovery (%) and relative standard deviation (% RSD) at 80, 100 and 120% from the target value for assay (0.82 mg/mL). System precision was expressed as % RSD from five injections of a 0.82 mg/mL standard.

Method for impurities

Linearity was assessed by analysis of six melanotan II standard solutions in the range 3.28 μ g/mL to 205 μ g/mL prepared by diluting an aqueous stock solution of melanotan II (Sigma-Aldrich, Cat. No. M8693). Linear regression analysis was performed from peak areas with no weighting factor and not forced through origin. To determine accuracy and precision at the required limit of quantification, five different standard solutions at 4.1 μ g/mL (equal to 0.05% of the target concentration: 8.2 mg/mL) were analyzed. Limit of detection was defined as the concentration at which the peak signal-to-noise ratio was above five. Impurities were reported in area-% of the base peak (melanotan II) in the chromatogram. This practise is generally applied in pharmaceutical analysis of unknown impurities in medical products.

Identification by MS/MS

Melanotan II was identified in samples by LC retention time (\pm 0.5 min relative to standard) and six fragment ions after collision induced dissociation of the double charged $[M+H]^{2+}$ molecular ion (m/z 513). These ions are either specific immonium fragments from amino acid side chains of norleucine, histidine, phenylalanine and tryptophane, or ions related to the amino acids in melanotane II^[39] as seen in the product ion spectrum of m/z 513 (Figure 1).

For unambiguous mass spectral identification of melanotan II, the relative ion intensities of five qualifying ions, expressed as the percentage of the intensity of the most intense MRM transition (m/z 513 \rightarrow m/z 86), must be within \pm 20% compared to averaged values for calibrators ($n=3$) in the same analytical sample run.^[40] The acceptance criteria were tested for multiple injections of samples ($n=5$).

Stability study of standards and samples

Short-term stability was assessed by placing a standard and sample solutions in vials (1 mL, 0.82 mg/mL) at 4°C in dark, -22°C in dark

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Table 2. Validation data and ICH guidelines acceptance criteria for methods of identification, assay and impurities

Method and parameter	Acceptance criteria	Results in this study		
IDENTIFICATION				
Retention time	Retention time should match calibration standards within ± 0.5 min	All samples within ± 0.03 min		
Mass spectral data	The relative ion intensities of 5 qualifying MRM transitions should match the average of reference standards ($n=3$) in the analytical sample run within $\pm 20\%$	All relative ion intensities for qualifying ions within $\pm 10\%$		
ASSAY				
Linearity	Correlation coefficient, $r \geq 0.995$	$r = 0.9993$		
Range	No criteria. For information only	0.205–1.64 mg/mL		
Accuracy and precision	Mean recovery: $100 \pm 2\%$ at 80–100% of target sample concentration (0.82 mg/mL), $n=5$.	Level	Recovery (%)	RSD
(%)		80%	101	1.65
		100%	100	0.75
		120%	99	1.62
System precision	$\leq 2\%$ RSD for $n=5$ injections	RSD = 0.79%		
Stability (standard /sample) 48 hours	Change in response of 0.82 mg/mL solution $\leq 5\%$	$< 2.1\%$		
IMPURITIES				
Linearity	Correlation coefficient, $r \geq 0.995$	$r = 0.9997$		
Range	No criteria. For information only	4.1–205 $\mu\text{g/mL}$		
Accuracy	Mean recovery: $100 \pm 20\%$ at 0.05%	100%		
Precision	RSD-% $\leq 10\%$ at 0.05% of base peak ($n=5$)	0.39%		
Limit of quantification	0.05%	4.1 $\mu\text{g/mL}$ (or 0.05%)		
Limit of detection	No criterion. For information only	3 $\mu\text{g/mL}$ (or 0.03%)		

and in light by room temperature. After 48 h, the peak response at 218 nm was compared to a new and recently prepared Sigma standard (0.82 mg/mL). The acceptance criterion for response (peak area) change was $\pm 5\%$. No forced stability studies were conducted.

Analysis of other reference standards

The Sigma-Aldrich standard stock solution (0.82 mg/mL) was used to quantify the two other commercially available reference standards (Tocris and Bachem) using the assay method with triplicate injections of each standard in 1 mg/mL solutions of the white, lyophilized solids. The stated peptide content on the respective certificates of analysis was used to compare the quantitative results.

Results and discussion

Method development

During method development in the present study, a number of anionic ion pairing reagents were tested unsuccessfully as mobile phase modifiers, including formic acid, acetic acid, and heptafluorobutyric acid. The latter has been found useful in reversed-phase separations of proteins and peptides.^[41] However, only TFA as a mobile phase modifier could produce chromatograms with symmetric, non-tailing peaks by LC-UV (Figure 2). Gradient elution was chosen to achieve optimal conditions for separation of impurities. The spectrum of melanotan II in the mobile phase showed a local absorbance optimum at 218 nm,

which was used as detection wavelength to ensure that the sensitivity criterion was met.

Method validation results

The method validation data for precision, accuracy, sensitivity, and stability were acceptable for the purpose of the study (Table 2). Retention time varied more than ± 0.5 min between different preparations of mobile phases, but retention time was always within ± 0.03 min for standards and confirmed melanotan II samples in analytical series. Blanks were free from interfering signals (Figure 2). It was concluded that standards and samples were fully stable in solution within the time frame of analysis (48 h).

Limitations of the methods

The ICH guideline Topic Q2 (R1) lists standards for identification as well as quantitative testing of active substances, and impurities for registration applications for new drugs submitted within the European Union, Japan, and the United States.^[38] Synthetic peptides for medical treatment, however, fall between high molecular weight biopharmaceuticals and classical organic molecules for which ICH regulatory guidelines do not apply directly,^[42] and there is no compendial monograph available for melanotan II. Certain criteria of the ICH guideline could not be met in this study (Table 2). For example, synthetic by-products and degradation products for melanotan II are not available as reference compounds and their response factors are unknown. The impurities

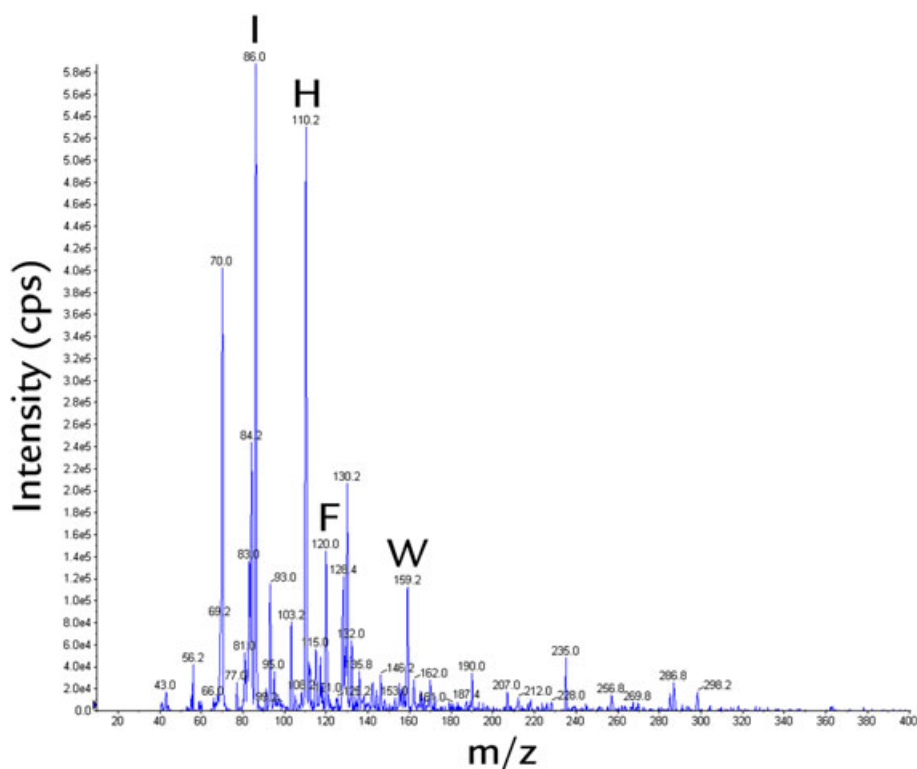


Figure 1. Product ion spectrum of the double charged $[M+2H]^{2+}$ precursor ion (m/z 513) of melanotan II standard using direct sample infusion at 10 μ l/min. The spectrum is dominated by immonium ions of norleucine (m/z 86, label: I), histidine (m/z 110, label: H), phenylalanine (m/z 120, label: F), tryptophane (m/z 159, label: W) and related ions characteristic of arginine (m/z 70) lysine (m/z 84) and tryptophane (m/z 130). Above m/z 300 only ions with very low abundances were observed due to the high level of collision energy (CE: 55). The immonium ions of lysine (m/z 101) and aspartic acid (m/z 88) are not observed as these are lactam-bridged in melanotan II.

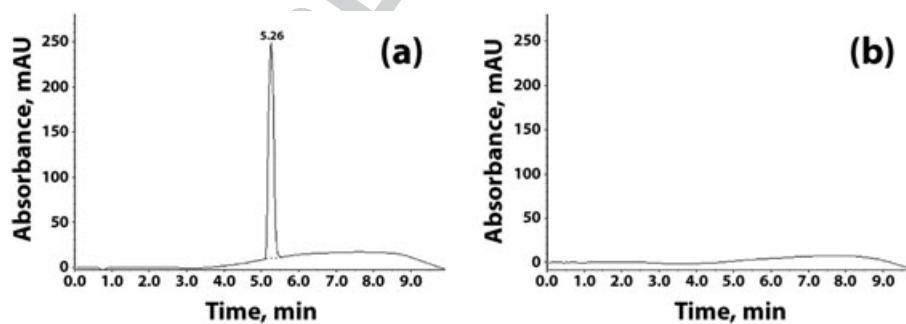


Figure 2. LC-UV assay method showing (a) chromatogram (218 nm) of a Sigma-Aldrich Melanotan II standard; (b) blank sample.

detected in this study were not fully separated from the base peak. It is questionable whether a full separation can be achieved by reversed-phase chromatography. Furthermore, impurities that co-elute with the base peak will not be detected with the methods presented here. Quantification of impurities was based on careful, manual integration of peak areas using the tangent skimming method. No attempts were made to determine the presence of salts, residual solvents, and water in samples. Hence, this study only reports organic compounds detectable with LC-UV at 218 nm as impurities.

Moreover, the ICH guidelines apply to substances that are produced under strict supervision to meet specifications for active substances and consistency in each batch. In contrast, melanotan II products obtained from online shops are manufactured outside of formal regulatory systems. Consequently, vials purchased from

the same shop could have been manufactured differently, for example originating from different synthetic routes or produced in different manufacturing plants/laboratories resulting in variation in content.

Finally, it should be noted that methods developed in this study do not apply to biological samples.

Reference standards

All three analytical grade standards purchased for the study stated the peptide purity on their respective certificates of analysis. No impurities were detected in the Sigma (peptide content: 82%), Tocris (peptide content: 69.4%), or Bachem standard (peptide content: 88.4%). Quantification of the Tocris and Bachem standard, relative to the Sigma standard, showed

deviations from the theoretical value of +1.0% and +1.1%, respectively, which is within the method imprecision. Thus, it was concluded that all three chemical standards were quantitatively compatible.

Application to melanotan II products

Assay and impurities

For all standards and samples the relative ion intensity ratios measured by MS/MS were within the acceptance criteria of $\pm 20\%$. Accordingly, it was concluded that all sample vials contained melanotan II. The total amount of melanotan II in the vials ranged from 4.32 to 8.84 mg.

The UV chromatographic profiles of melanotan II products from Shop A and Shop B (Figure 3) showed impurities between 4.1 to 5.9% (Table 1), whereas impurities in samples from Shop C were below the quantification limit. Methods applied in this study neither identify nor discriminate between impurities from synthesis, degradation products, or contaminants.

Vials and supplied accessories

Shop A only supplied vials of melanotan II. None of these had any form of marking to identify the active substance (Figure 4 shows pictures of vials from all three shops). Of note is that we discovered that a vial from Shop A had a damaged metal overseal (a small but visible dent) suggesting that future studies should undertake sterility testing of products from the illicit market.

Shop B supplied vials, alcohol skin wipes, injecting equipment, as well as an unlabelled excipient. Vials from Shop B were unmarked. Shop C supplied vials, alcohol skin wipes, injecting equipment, as well as a vial of excipient labelled 'bacteriostatic water'. Here it appeared that the shop had produced labels for their vials of melanotan II (labelled 'MT2 Melanotan Powder') using the same branding as the website from which they were sold. Products from Shop C were delivered with instructions that provide dosage regimens as well as instructions on how to inject the product. This, along with testimonies from 'satisfied customers' posted on the websites of retailers, may go some way towards legitimizing these products and their use. It is notable that samples bought from Shop C had a sticker on the packages with the words: 'Item checked'. It is possible that such marketing techniques are used to give the products an air of authenticity and quality.

Implications for user safety and public health

Although each of the tested vials contained a lower amount (4.32–8.84 mg) of melanotan II than the 10 mg claimed on the shops' websites, it is important to highlight that the results from this study should not be generalized beyond this sample; users cannot assume that other melanotan II products are under strength and should not subsequently increase the dose used as a result of these findings.

High levels of impurities were detected in samples from Shop A and Shop B; while from Shop C they were below the limit of quantification. Since most users inject melanotan II, the presence

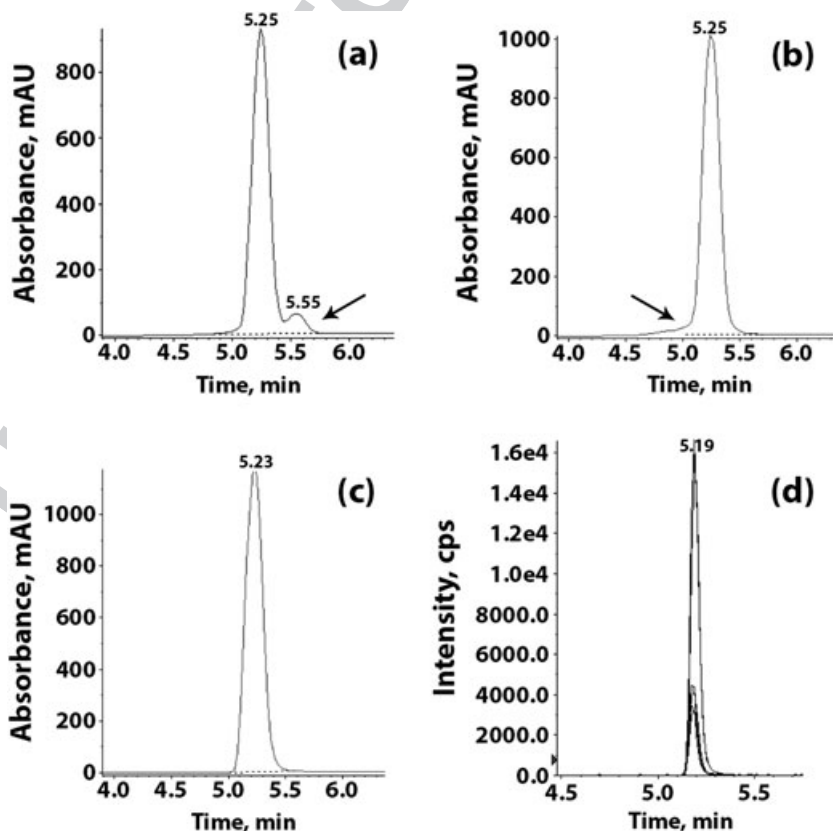


Figure 3. Examples of LC-UV impurity profiles of (a) products from Shop A; (b) products from Shop B; (c) products from Shop C; (d) data for Sigma melanotan II standard showing the six overlaid MRM transitions (m/z 513 \rightarrow m/z 86, 110, 84, 70, 120 and 130) used for mass spectrometric identification. Arrows indicate those impurities which are quantified in Table 1.

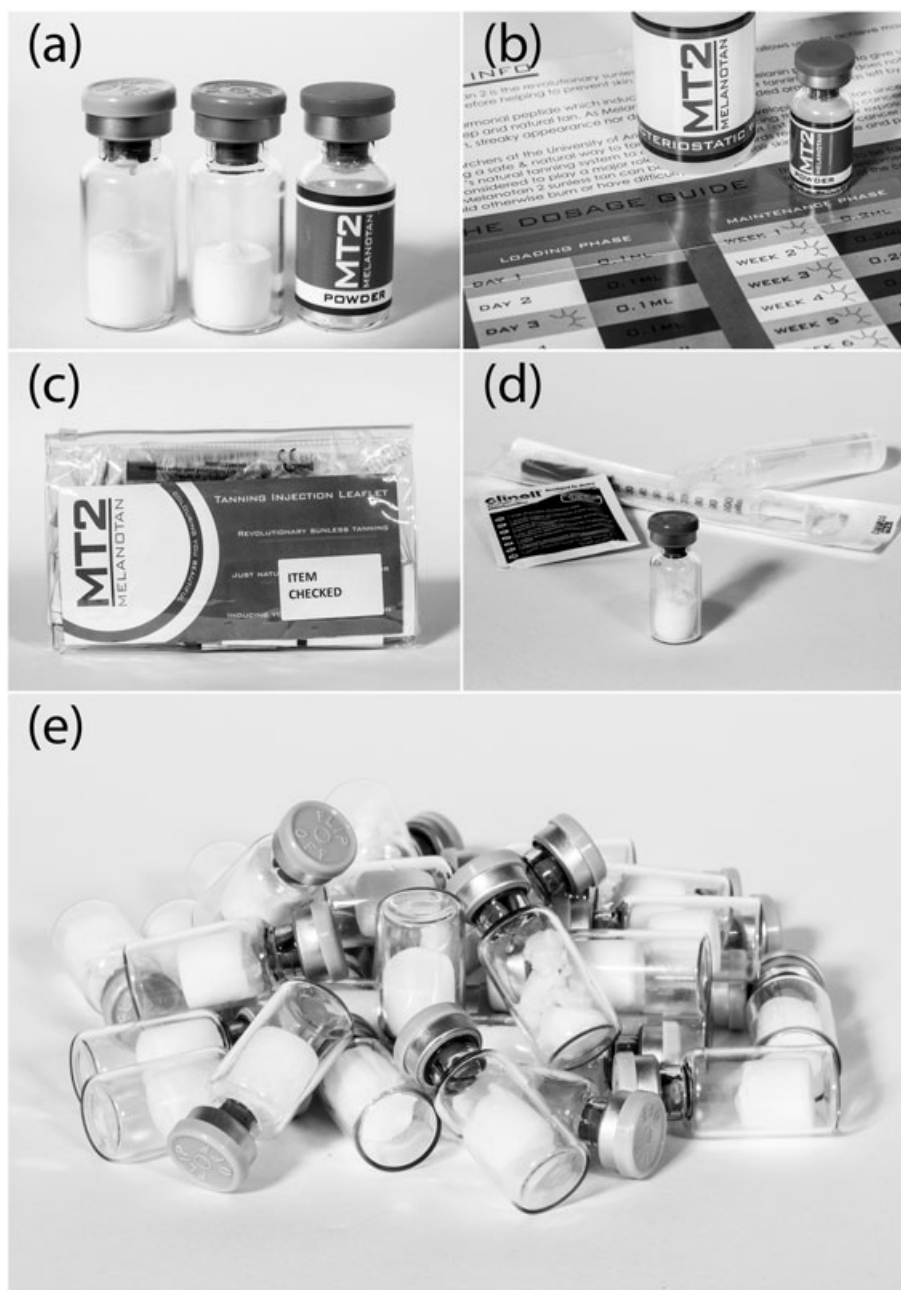


Figure 4. Melanotan II products. (a) vials from Shop A, Shop B and Shop C (from left to right); (b) products from Shop C and 'bacteriostatic water'; (c) 'Tanning injection leaflet' with information on dosage supplied with vials from Shop C; (d) an example of a vial delivered from Shop B with alcohol wipes, excipient (unlabelled), and injecting equipment; (e) unlabelled vials from Shop A shown after being unwrapped from a cushioned envelope.

of impurities may pose a hazard. Further work is required in order to characterize these impurities as well as the hazards that they pose.

A broad range of substances and medicinal products used for enhancement purposes can be obtained without prescription from online shops, bricks-and-mortar shops, and dealers. These include products such as melanotan II which are primarily used to increase skin pigmentation, as well as those used to enhance muscle, cognitive function, modify mood and social behaviour, lose weight, and stimulate sexual function.^[13] However, users may be exposed to a range of potential harms from such products, as highlighted in this study, in part because the manufacturers of these substances are usually beyond of regulatory

oversight. The transnational nature of the global market of enhancement drugs makes it particularly difficult to control and reduce supply. In part this appears to be due to the growing role of the sale of such drugs through the Internet. In the latter case this is as the legal regulation of the Internet is largely based on national law whereas manufacturers, suppliers, retailers, website hosting and payment processing services may all be based in different countries. In addition, there are often few restrictions on consumers buying these drugs for their own use.^[13]

Melanotan II is just one of a growing range of human enhancement drugs being sold illegally both online as well as through bricks-and-mortar shops and street-level dealers. While it is not yet possible to estimate the potential for acute and chronic

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health harms of many of these enhancement drugs, including melanotan II, analytical methods such as those provided in this study play an essential role in the public health response to these drugs by providing tools to identify and quantify the active substance and impurities. In turn this helps develop both our understanding of composition of such drugs and their quality, and, from this, some of the hazards that they may pose.

Conclusions

Analytical methods to unambiguously identify melanotan II, quantify vial content and determine the level of impurities were developed and validated. Furthermore, this study demonstrated for the first time that melanotan II is readily available on the illicit market, being identified in all vials that were tested.

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