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Falsified and problematic methandienone products available online: active pharmaceutical ingredient identification by portable Raman spectrometers and quantification by ultra-high-performance liquid chromatography–Fourier transform mass spectrometry

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Abstract

This study aimed on the one hand to clarify the quality, authenticity, safety, and other issues related to products of the anabolic-androgenic steroid methandienone advertised on the Internet and personally imported to Japan and on the other hand to evaluate the use of two portable Raman spectrometers in identifying the active pharmaceutical ingredient (API). The study found that all $n = 15$ samples purchased from 14 websites were problematic regarding their package, labeling, and/or content. Specifically, one sample (6.7%) was confirmed falsified, twelve samples (80%) were found either to be falsified or unlicensed as pharmaceutical product, and two samples (13.3%) were received without information on the manufacturers' physical address or country of origin, with one sample (6.7%) having no labeling or other accompanying information at all. Both Raman spectrometers were able to identify the API in all samples as confirmed and quantified by ultra-high-performance liquid chromatography–Fourier transform mass spectrometry. Twelve samples contained on average less than 90% of the declared API content. By contacting national regulatory authorities in 44 countries, methandienone products were found to be approved in 1 country and not approved in 21 countries. To prevent health hazards and abuse, measures against the acquisition of anabolic-androgenic steroids from unknown sources are required. Portable Raman spectrometers may be suitable for the non-destructive and quick identification of methandienone in tablets.

Keywords Falsified, Anabolic-androgenic steroids (AASs), Methandienone, Personal import, Raman spectroscopy, Portable, Handheld, Raman spectrometer, Ultra-high-performance liquid chromatography–Fourier transform mass spectrometry

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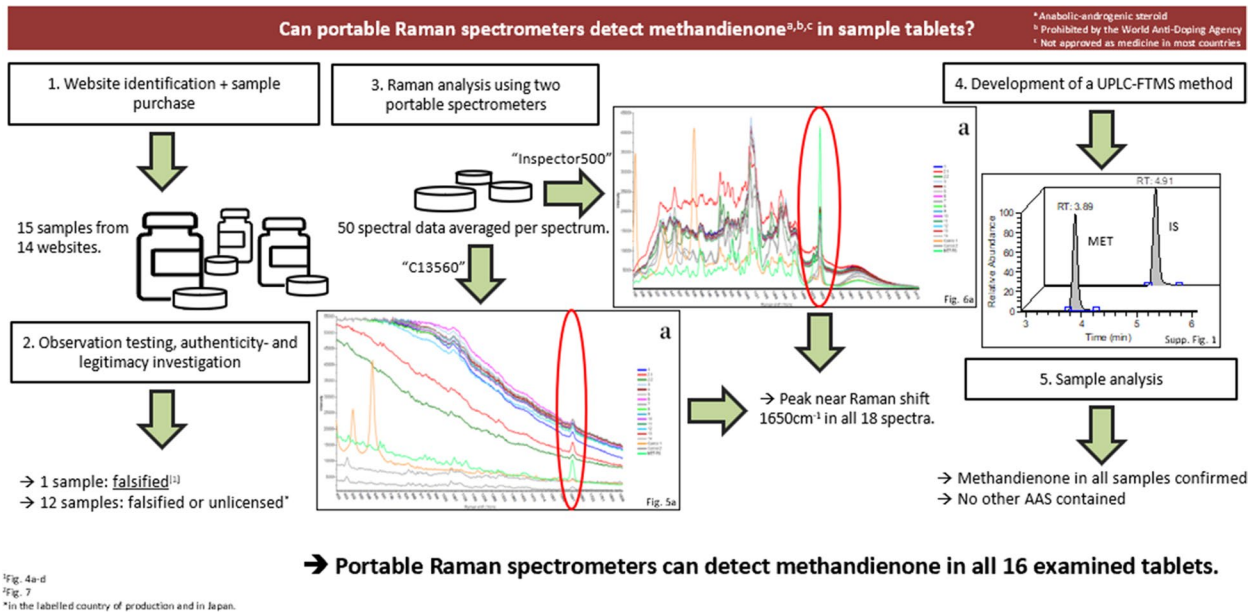
Graphical Abstract



Introduction

Anabolic-androgenic steroids (AASs) are synthetic steroids structurally related to testosterone, the male sex hormone, and well known for their androgenic effects (Ganesan et al. 2023; Bhasin et al. 1996; National Institute on Drug Abuse 2018; Barceloux and Palmer 2013). As prescription medicines, they are used in treating hypogonadism and bone marrow stimulation in leukemia, kidney failure, and aplastic anemia (Ganesan et al. 2023). However, the non-medical use of AASs is a serious global public health concern (Sagoe et al. 2014; Frude et al. 2020) in that AASs are widely abused to increase muscle mass, not only among bodybuilders and athletes for doping purposes but also increasingly among individuals to achieve masculine cosmetic benefits (Magnolini et al. 2022; Rahnema et al. 2014; McBride et al. 2018; Storer et al. 2003).

Non-medical dosages are up to 100 times the therapeutic dose (National Institute on Drug Abuse 2018; Barceloux and Palmer 2013; Solimini et al. 2017) and known to cause psychological changes, addiction, and severe physiological changes (Bhasin et al. 1996; Magnolini et al. 2022; Rahnema et al. 2014; Solimini et al. 2017; van Amsterdam et al. 2010; Liu and Wu 2019; Takayanagi et al. 2008; Nieschlag and Vorona 2015) including hepatotoxicity (Solimini et al. 2017) and AAS-induced hypogonadotropic hypogonadism, which does not improve after the discontinuation of AASs (Rahnema et al. 2014).

Substandard and falsified (SF) medicines have been defined by the World Health Organization since 2017 (World Health Organization 2018). Substandard medicines fail to meet either their quality standards, specifications, or both. Falsified medicines deliberately/fraudulently misrepresent their identity, composition, or source. Unregistered/unlicensed medicines have not been approved for sale under relevant regulations and legislation (World Health Organization 2018). Falsified medicines might contain an altered amount of the active pharmaceutical ingredient (API), no API, or the wrong API and can therefore have serious health effects up to death of the consumer (Rahman et al. 2018).

The online availability of SF medicines is a global challenge and there is a high demand for suitable analytical detection methods and tools for identifying SF medicines (Ahmed et al. 2022), with Japan being no exception to this development (Zhu et al. 2020; Sanada et al. 2020; Rahman et al. 2022; Sanada et al. 2021; Yoshida et al. 2015; Khan et al. 2012). The widespread international online distribution of SF AASs is well documented (Magnolini et al. 2022; McBride et al. 2018; Fabresse et al. 2021; Coopman and Cordonnier 2012), and a recent systematic review estimated the overall mean prevalence of falsified AASs on the black market to be more than one in three (Magnolini et al. 2022). SF medicines are entering Japan through personal importation, often without the

requirement for a prescription (Zhu et al. 2020; Sanada et al. 2020; Rahman et al. 2018).

Although reports of health hazards of AAS products posted by consumers on online forums reduce the consumers' risk (Frude et al. 2020), analytical methods for identification, quantification, and determining quality-related parameters are urgently required to protect consumer health (Piatkowski et al. 2023).

Raman scattering analysis identifies the constituents of a mixture (Rebiere et al. 2016) and is thus a technology for the quick and non-destructive qualitative analysis of APIs (Rebiere et al. 2016; Opuni et al. 2019; Sanada et al. 2020). Portable and handheld Raman spectrometers are comparably inexpensive devices that can be used on site to detect SF medicines, reducing the number of samples requiring expensive laboratory analysis (Sanada et al. 2020; Hajjou et al. 2013; Dégardin et al. 2017).

The present study aimed on the one hand to investigate the quality, harmfulness, and other problems relating to methandienone (also known as methandrostenolone; MET) products advertised and sold over the Internet and personally imported to Japan and to assess whether samples are falsified. MET is an orally available AAS that is explicitly prohibited for use in sports by the World Anti-Doping Agency (WADA) (World anti-doping agency 2023). The availability of officially licensed MET products was investigated in a worldwide search covering 44 countries on five continents. This study aimed on the other hand to explore the use of two portable Raman spectrometers for non-destructive MET analysis that could be useful for the quick on-site detection of MET containing products, e.g. at airports or at customs. To verify the Raman scattering analysis results, a liquid chromatography–mass spectrometry (LC-MS) method was developed for MET identification, quantification, and impurity testing.

Materials

Chemicals

MET (#H1193, Lot H4FHF, purity 97.5%) and methyltestosterone (#M0435, Lot YNV3C) were purchased from Tokyo Chemical Industry (Tokyo, Japan) and used as reference standards. DANABOL 10 mg, an MET product approved in the Republic of Moldova, was imported under an agreement between SC Balkan Pharmaceuticals SRL and Kanazawa University and used as the standard formulation and control.

LC-MS grade ultrapure water was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Methanol (MeOH), acetonitrile (MeCN), isopropanol (IPA), and 0.1% acetic acid were used in LC-MS grade only and purchased from Wako Pure Chemical Industries, Ltd. (Kyoto, Japan).

Devices

Images of the samples were taken with an IXY 650 compact digital camera manufactured by Canon (Tokyo, Japan).

Raman scattering analysis was conducted using a C13560 ultra-compact portable Raman spectrometer (96 mm × 14.5 mm × 60 mm; 90 g) manufactured by Hamamatsu Photonics K.K. (Shizuoka, Japan) with a silicon substrate provided by the manufacturer (Sanada et al. 2020) and an Inspector500 portable Raman spectrometer manufactured by SciAps Inc. (Laramie, WY, USA) with a polystyrene standard provided by the manufacturer. The supplied software and drivers were installed on a personal computer prior to the analysis.

LC-MS experiments were performed adopting ultra-high-performance liquid chromatography–Fourier transform mass spectrometry (UPLC-FTMS; Q Exactive, Thermo Fisher Scientific, Waltham, MA) interfaced with an UltiMate 3000 RS UPLC system.

Methods

Source selection and sample collection

Websites of personal import agents offering MET products to consumers in Japan were searched via the Google Japan search engine (Mountain View, CA, USA) using the term "methandienone AND personal import". The minimum unit number per sample was 60 tablets. All identified samples were purchased and personally imported using a private address as the recipient address. After receipt, samples were stored under suitable conditions and sample identification numbers (IDs) were assigned according to the website of purchase.

Visual observation, authenticity investigation, and legitimacy status request

Visual observation test

For each sample, the packaging, labeling, and dosage units were visually examined and evaluated using the International Pharmaceutical Federation's Tool for Visual Inspection of Medicines (International Pharmaceutical Federation n.d.), and detailed information was collected and documented. This information is presented anonymized in Supplemental Table1. Images of problematic samples were taken.

Authenticity investigation

Each labeled manufacturer was requested to authenticate their samples. A questionnaire with photographs of the

samples and relevant information, including the labeled trade name, batch number, manufacturing and expiry dates, manufacturer name and address, API name, and dosage strength, was sent to the labeled email addresses. If no email address was available, the request was sent via the inquiry form of the labeled website. The authenticity investigation was not possible for unlabeled samples.

Legitimacy status request

The national regulatory authorities (NRAs) of the countries indicated on the sample and of the countries of origin of the shipments were queried about the legitimacy of the samples and whether the labeled manufacturer holds a manufacturing license for the sample product or was generally allowed to handle MET-containing products.

Raman scattering analysis

Measurement conditions

Both the C13560 and Inspector500 Raman spectrometers were set up according to the manufacturers' instructions.

In analysis using the C13560 Raman spectrometer, the output was set at 15 mW ("High"), the excitation laser wavelength was fixed at 785 nm, the scanning time was 1,000 ms/scan, and the spectral X-axis wavenumber interval was defined as 403–1852 cm^{-1}. Before taking measurements, the dark signal was measured by inserting a silicon substrate provided by Hamamatsu Photonics K.K. Subsequent calibration was performed using the Raman shift peak of the substrate near 521 cm^{-1}. Following instructions, the emitted laser wavelength was corrected manually to 785 nm.

In analysis using the Inspector500 Raman spectrometer, the output was set at 300 mW ("High"), the excitation laser wavelength was fixed at 1,030 nm, the scanning time was set to the default "automatic" setup (maximum of 8 s), and the spectral X-axis wavenumber interval was defined as 150–2,450 cm^{-1}. Before taking measurements, a self-test was run to check the calibration status. The device was continually calibrated until it passed this test.

Measurement procedure

The measurement procedure was the same for the two Raman spectrometers. Each spectrometer's attachment for analysis was placed in direct contact with the AAS tablet such that the tablet surface was fully covered. One measurement was the average of five spectral data in the Raman scattering analysis. A total of 10 measurements per tablet were performed on randomly chosen spots of the sample surface. Five measurements were taken from the upside and five measurements from the downside of the tablet. Therefore, a total of 50 spectral data were averaged to give one spectrum for each sample. During analysis, black opaque fabric covered the setup to prevent light contamination.

A methandienone reference standard (MET-RS), only available as powder, was placed in a small plastic film bag and analyzed in the same manner as the sample after smoothing the powder to create a smooth, consistent powder surface. DANABOL 10 mg, an MET product approved in the Republic of Moldova and identified in this study, was twice analyzed by scanning the surface of the coating (Control 1) and scanning the product surface after sufficiently removing the coating (Control 2) to eliminate the effect of the coating.

Determination of AASs by LC-MS

Preparation of standard solutions

MET and methyltestosterone standards were separately dissolved in 100% methanol at 10 mM, and the standard solution of MET was stepwise diluted to 10, 5, 2, 1, 0.5, and 0.2 mM. To quantify the MET contents of the sample tablets, calibration standards were prepared in the same manner as the samples as outlined below. A 2-mL quantity of each diluted MET standard solution was added to a mixture of 1 mL distilled water with 3 mL MeOH, the mixture was shaken for 10 min and centrifuged at 3,000 rpm, and 5 mL of the supernatant liquid was separated. This procedure was conducted thrice and the 3×5 mL was combined for each supernatant solution. A 1-mL quantity of 4 mM methyltestosterone was added to this solution as an internal standard, and the total volume was made up to 20 mL using MeOH. Exactly 1 mL of this solution was taken and diluted with MeOH to 20 mL. Again, exactly 1 mL was taken and MeOH and distilled water at a ratio of 65:35 were added to obtain a total volume of 10 mL. Finally, this solution was filtered through a 0.22-μm filter and used as the standard solution. The final standard solutions for the MET contained 5.0, 2.5, 1.0, 0.5, 0.25, and 0.1 μM MET-RS and 1.0 μM methyltestosterone as an internal standard. Quality control solutions (4, 2, and 0.8 μM MET-RS) were prepared in the same manner as the calibration standards.

Preparation of sample solutions

The MET extraction for each tablet including DANABOL, the standard product obtained from the Republic of Moldova, was performed through the assay pretreatment of estriol tablets and prednisolone tablets listed in the Japanese Pharmacopoeia (The Japanese Pharmacopeia 18th edition - Official Monographs (A to L) 2021; The Japanese Pharmacopeia 18th edition - Official Monographs (M to Z) 2021).

The mass of one tablet of each product was weighed before the tablet was homogenized using a mortar. A quantity corresponding to approximately 2 mg of the

labeled MET content was precisely weighed, exactly 1 mL of distilled water was added, and the solution was sonicated. A 5-mL quantity of 100% MeOH was added, and the solution was then shaken for 10 min and subsequently centrifuged. After centrifugation, 5 mL of the supernatant liquid was separated and another 5 mL of MeOH was added to the remaining solution. This extraction procedure was conducted thrice. The obtained 3 × 5 mL of each supernatant was combined, and 1 mL of a MeOH solution of 4 mM methyltestosterone was added as an internal standard. MeOH was added to obtain a total volume of 20 mL. Exactly 1 mL of this solution was taken and diluted with MeOH to 20 mL. Again, exactly 1 mL was taken, and MeOH and distilled water at a ratio of 65:35 were added to obtain a total volume of 10 mL. Finally, this solution was filtered through a 0.22-µm filter (Millipore, Bedford, MA, USA) and used as the sample solution.

To evaluate the recovery of the extraction, a positive control sample was prepared as follows. A 2-mg quantity of the MET-RS was weighed precisely and extracted three times in the same manner as the sample, and 3 × 5 mL of the resulting supernatant solution was combined. The internal standard solution was added and MeOH was used to make up to a total volume of 20 mL. This solution was diluted in the same manner as the sample solution to obtain the positive control solution.

Screening of testosterone analogous substances

First, all samples were screened for 25 AASs including MET (Table 1) through LC-FTMS following Tircova et al. (2019).

The injection volume of sample solution was fixed at 2 µL, and a Shim-pack FC-ODS column (2.0 mm i.d. × 75 mm, 3-µm particles) was used for separation. The column temperature was maintained at 40 °C. The flow rate of the mobile phase was 300 µL/min. Mobile phase A comprised MeCN/water at a ratio of 3:7 (including 0.1% formic acid) whereas mobile phase B comprised MeCN/isopropanol at a ratio of 9:1. During separation in liquid chromatography (LC), the mobile phases A and B were used in gradient elution. Mobile phase A was initially set at 100% for 0.5 min. The proportion of mobile phase B was then gradually increased from 0 to 95% over 6.5 min. For the next 3 min, the 95% proportion of mobile phase B was maintained. Blank runs were carried out randomly between samples to check that there was no significant chromatographic carryover. Mass spectrometry was performed in synchronous full-scan positive mode with a resolution of 70,000 and data dependent MS/MS with a resolution of 17,500 (full-scan MS^1/dd-MS^2). The scan range of the instrument was set at a mass-to-charge ratio m/z of 100–1,000, and the top three ions were fragmented at a collision energy of 30 eV with a dynamic exclusion time of 10.0 s. Heated electrospray ionization was applied at a spray voltage in positive ion mode of 3.5 kV. The sheath gas (nitrogen) was set at 50 arbitrary units; the auxiliary gas (nitrogen) was set at 10 arbitrary units; and the vaporizer and capillary temperatures were set at 300 and 250 °C, respectively. Data were collected in profile mode using Xcalibur software (Thermo Fisher Scientific).

Table 1 25 AASs used in a study conducted in the Czech Republic and Slovakia (Tircova et al. 2019)

AAS	Molecular formula	m/z (positive ion mode)
Boldenone cypionate	$C_{27}H_{38}O_3$	411.2894
Boldenone undecylenate	$C_{30}H_{44}O_3$	453.3363
Clenbuterol	$C_{12}H_{18}Cl_2N_2O$	277.0869
Drastonolone propionate	$C_{23}H_{36}O_3$	361.2737
Fluoxymesterone	$C_{20}H_{29}FO_3$	337.2173
Chlorodehydromethyltestosterone	$C_{20}H_{27}ClO_2$	335.1772
Mesterolone	$C_{20}H_{32}O_2$	305.2475
Methandienone	$C_{20}H_{28}O_2$	301.2162
Methenolone enanthate	$C_{27}H_{42}O_3$	415.3207
Nandrolone decanoate	$C_{28}H_{44}O_3$	429.3363
Nandrolone phenylpropionate	$C_{27}H_{34}O_3$	407.2581
Nandrolone undebanoate	$C_{29}H_{46}O_3$	443.352
Oxandrolone	$C_{19}H_3O_3$	307.2268
Oxymetholone	$C_{21}H_{32}O_3$	333.2424
Stanozolol	$C_{21}H_{32}N_2O$	329.2587
Testosterone cypionate	$C_{27}H_{40}O_3$	413.305
Testosterone decanoate	$C_{29}H_{46}O_3$	443.352
Testosterone enanthate	$C_{26}H_{40}O_3$	401.305
Testosterone isocaproate	$C_{25}H_{38}O_3$	387.2894
Testosterone phenylpropionate	$C_{28}H_{36}O_3$	421.2737
Testosterone propionate	$C_{22}H_{32}O_3$	345.2424
Testosterone undecanoate	$C_{30}H_{48}O_3$	457.3676
Trenbolone acetate	$C_{20}H_{24}O_3$	313.1798
Trenbolone enanthate	$C_{25}H_{34}O_3$	383.2581
Trenbolone hexahydrobenzylcarbonate	$C_{26}H_{34}O_4$	411.253

m/z mass to charge ratio

The UPLC-FTMS data were processed using Compound Discoverer 3.1 software (Thermo Fisher Scientific), and m/z values of the extracted ion peaks were compared with the known m/z values of AASs (Table 1) to determine whether the products contained these AASs.

Quantification of MET by LC-MS

MET contents in sample solutions were quantified also using calibration standards by LC-FTMS but applying a

gradient program and data acquisition mode as follows. During separation in LC, the mobile phases A and B were used in gradient elution. The proportion of mobile phase B was gradually increased from 5 to 50% for the first 7 min and then rapidly raised to 95% and maintained for the next 3 min. Mass spectrometry was performed in target-single ion monitoring mode at a resolution of 70,000. MET ($C_{20}H_{28}O_2$) and methyltestosterone ($C_{20}H_{30}O_2$) were monitored at an [M+H] ion of m/z 303.2319 and m/z 301.2162, respectively. The UPLC-FTMS data were processed using the Quan Browser (Thermo Fisher Scientific). Areas of the extracted ion peak chromatograms were obtained with a mass tolerance of 5 ppm.

Availability of approved MET products

A total of $n=44$ NRAs on five continents (Africa ($n=1$), Asia ($n=9$), Australia/Oceania ($n=1$), Europe ($n=31$), and North America ($n=2$)) and $n=33$ institutions (including international chemical and pharmaceutical industry associations, the world and Japanese anti-doping agencies WADA and JADA, and the Japanese Ministry of Economy, Trade, and Industry) were emailed about the availability of officially approved, registered, or authorized MET products. In addition, MET products and manufacturers producing MET products were searched for using the Google search engine (Mountain View, CA, USA) for text and images by combining search terms including "Methandienone", "Methandienon", "Methandrostenolone", "medicine", "product", "leaflet", "buying", "manufacturing", "approval/approved", "registration/registered", "supplement", and related terms. The search results were documented, and the companies were queried by email for specific information on the product identified. Concurrently, the NRAs of the manufacturers' countries of location were asked for information on product approval and the results were recorded. All responses are reported in Supplemental Table 2.

Data analysis and visualization

Data were analyzed using Microsoft Excel for Microsoft 365 MSO (Microsoft Corporation; WA, USA). Raman spectra were line plotted and colorized using The Unscrambler X 10.5 (CAMO Software AS; Oslo, Norway). Images were formatted by applying Microsoft Word "Format" equally across each entire image.

Results

Sample collection

$n=15$ samples of four MET products were purchased between December 25, 2019 and January 6, 2020 from 14 identified websites of personal import agents and received by January 31, 2020. Sample 2.2 was provided by a personal import agent as a replacement product for sample 2.1 with a statement that the product of sample 2.1 had sold out: however, the two samples arrived together, with payment claimed for sample 2.1 but not for sample 2.2. $n=12$ (80%) samples were labeled with trade name A, one (6.7%) with trade name B, and one (6.7%) with trade name C, and one sample (6.7%) was received unlabeled. Upon arrival, no sample had exceeded the expiry date indicated on the label; however, no expiry date was given for the unlabeled sample (Supplemental Table 1).

The postal labels on the packages indicated that 12 of the 15 samples (80%) were shipped from Taiwan and the other three (20%) from Thailand (Supplemental Table 1).

Twelve of the 14 websites (85.7%) described and advertised their product as a pharmaceutical product, with eight indicating their product was as an "anabolic steroid" and four that their product was a "pharmaceutical product". Two websites did not describe the purpose, use, or intention of their product (Supplemental Table 1).

Notably, none of the 14 orders required a prescription or similar documentation.

Sample evaluation

Sample observation

All 15 samples (100%) failed the visual observation test. The packaging of two samples (samples 1 and 3) was opened or damaged (Figs. 1 and 2a, b). One sample

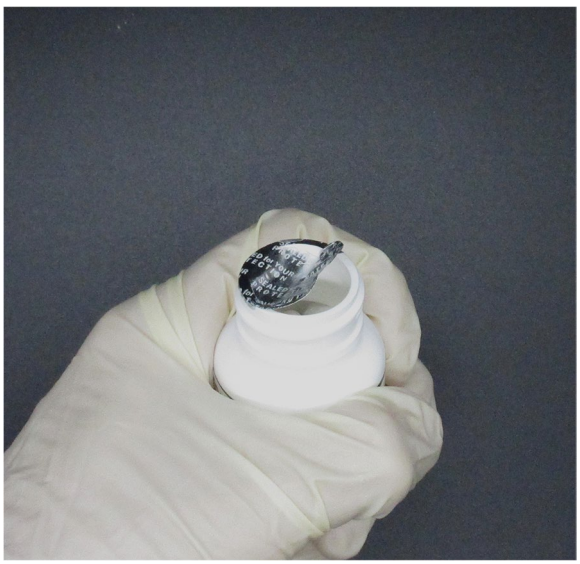


Fig. 1 Opened seal of sample 1. Fig. 1 shows an image of the primary packaging and seal of sample 1, which was found opened when the sample was collected. In Fig. 1, the brightness of the original was reduced by −30%

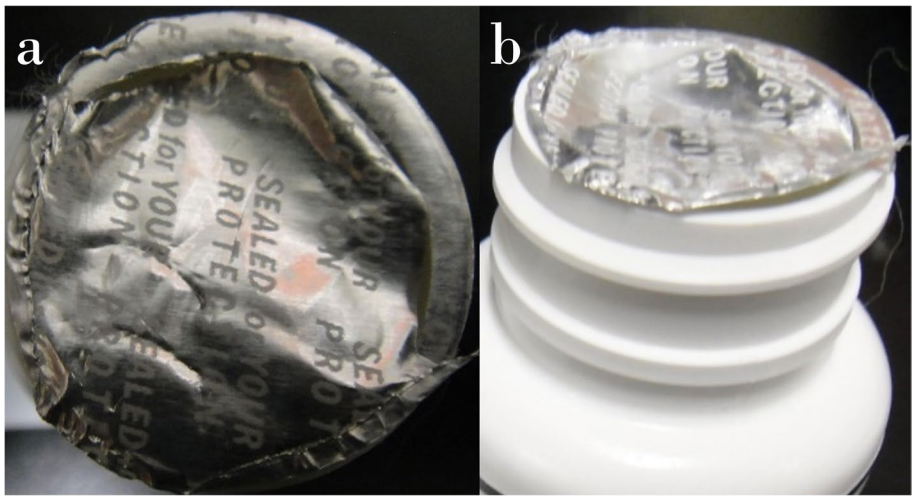


Fig. 2 Damaged seal of sample 3 viewed from two angles. Fig. 2 shows an image of the primary packaging and seal of sample 3, which was found damaged when the sample was collected. **a** top view. **b** side view

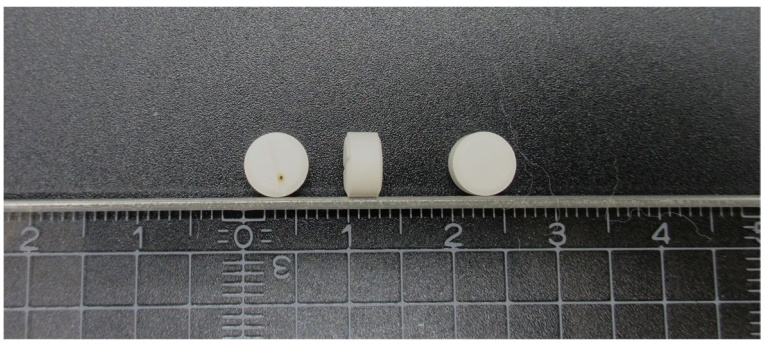


Fig. 3 Tablets of sample 9. Fig. 3 shows an image of tablets of sample 9. Notably, one tablet has a dark spot on the surface. In Fig. 3, the brightness of the original was reduced by −30%

gave a fictitious manufacturer's address, had grammatical and spelling errors, and did not provide a batch number or dosage strength. Twelve samples only had the text "Manufactured for: [Manufacturer A]", leaving the actual manufacturer unclear. Furthermore, one sample was received unlabeled and another did not give the manufacturer's address or country of origin.

In addition, two samples contained more tablets than indicated on the label, and one had a dark spot on the surface of one tablet (Fig. 3).

Two samples did not contain any leaflet. Twelve of the 15 samples had physical leaflets attached, and one sample presented a quick response (QR) code for downloading the leaflet. Notably, in August 2023, it was discovered that this QR code no longer linked to an accessible website, and therefore, the leaflet could not be accessed or downloaded.

All 13 collected leaflets were in English, and none had a Japanese translation.

Authenticity investigation

Repeatedly contacting the manufacturers to inquire about the authenticity did not lead to any response from the manufacturers. Two samples were labeled with a QR code for online authenticity.

These QR codes linked to two websites stating the authenticity of the product. Notably, one of these two samples was confirmed falsified (see Identification of falsified MET section). Remarkably, the website claiming authenticity for the falsified product included the spelling mistake "authenicity" (*sic!*).

Owing to the absence of a label or accompanying information, there was no contact information for one sample.

Legitimacy and registration status

There were no MET products approved in Japan and therefore all 15 samples were prohibited for distribution. The 12 samples labeled "Manufactured for: [Manufacturer A]" were confirmed to be unlicensed in the country

labeled and the country of distribution. Notably, one sample was confirmed falsified (see Identification of falsified MET section). For two samples, it was impossible to inquire about legitimacy owing to the absence of information on the manufacturer's address or country.

Identification of falsified MET

Sample 2.2 did not have a batch number, dosage strength, or registration number. In addition, it gave a fictitious German address, which was confirmed not to exist by the German municipality (83674 Gaißach) responsible for the labeled address (Fig. 4b).

Notably, the label indicated the name of the German municipality using what is believed to be the Latin letter uppercase "B" or the Greek letter uppercase beta "B", whereas the municipality would be correctly written using the German letter "Eszett; sharp S" expressed as "ß", which is different from the Greek letter lowercase beta "β" and therefore a spelling mistake. Furthermore, the storage instruction on the label stated "Donot […]" (*sic!*), which is grammatically incorrect (Fig. 4b).

The sample was confirmed to be a falsified product by the responsible German regulation authority; i.e., no manufacturing license, marketing authorization, or export license was issued in the European Union, including Germany, for any MET-containing product. In addition, the labeled manufacturer was found to be fictitious. Images of falsified sample 2.2 are presented in Fig. 4a, b, c and d. A summary of the information labeled on falsified methandienone product EP.Dbol-10 is presented in Table 2.

Raman scattering analysis

Spectra of all 15 samples, the standard formulation (Control 1 and Control 2), and the MET-RS obtained by Raman scattering analysis using the two spectrometers

Fig. 4 Primary packaging of falsified sample 2.2 viewed from four angles. Fig. 4 shows images taken of the primary packaging and labeling of the falsified sample 2.2 viewed from four angles **a** Front, **b** Product information, **c** Authentication Codes, **d** Hologram and website information. In Fig. 4d, the brightness of the original was increased by + 20%

Table 2 Information on falsified methandienone product EP.Dbol-10 (sample 2.2)

Sample code	Labeled trade name	Labeled strength	Labeled batch number	Labeled Mfg.date,	Labeled Exp.date	Labeled manufacturer	Labeled manufacturer address
Sample 2.2	EP.Dbol-10	N/A	N/A	01. June 2018	01. June 2022	Confirmed fictitious	Confirmed fictitious

N/A Not available / Not labeled

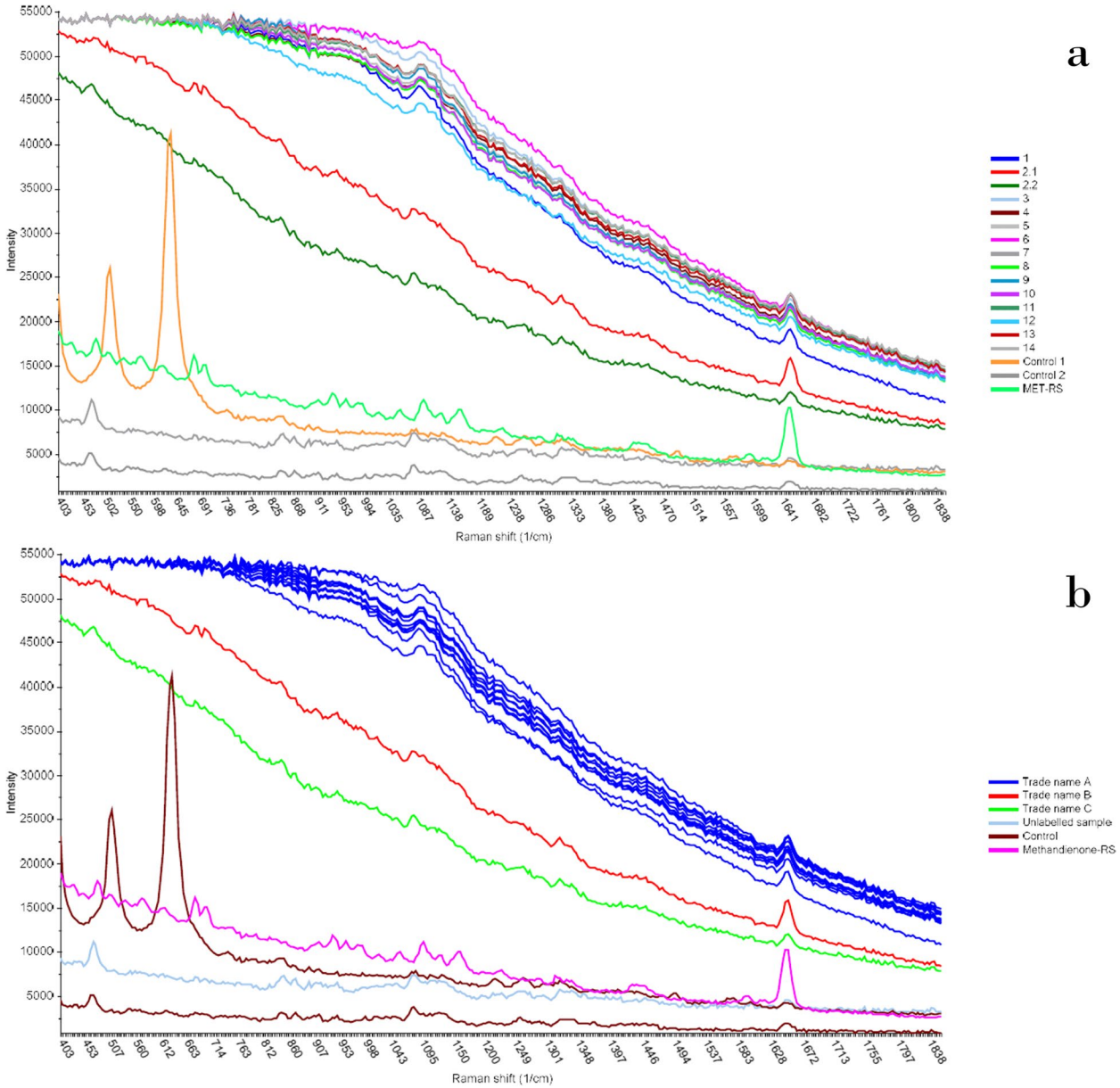


Fig. 5 Raman spectra obtained using a C13560 ultra-compact Raman spectrometer. Fig. 5 shows Raman spectra of the 15 sample products, the standard formulation without and with the sufficient removal of the coating (Control 1, Control 2), and the methandienone-reference standard (MET-RS). For each spectrum, 50 spectral data taken from five randomly selected positions each on the tablet upside and tablet downside were averaged. The MET-RS, available as powder, was placed in a plastic bag and piled up and 50 spectral data were taken with five randomly selected positions from each of the two sides. A peak near a Raman shift of 1650 cm −1 is visible in all spectra. The spectra are presented with (**a**) coloring according to the sample, control, and MET-RS and (**b**) coloring according to the labeled trade name

show a clear peak near the Raman shift wavenumber of 1,650 cm^{-1} as presented in Figs. 5a, b and 6a, b.

LC-MS analysis

Detection of analogues

Twenty-five AASs (Table 1) considered in the investigation of AAS counterfeits in the Czech Republic and Slovakia (Tircova et al. 2019) were screened for through LC-MS with full-scan MS^1/dd-MS^2 mode. Among these 25 AASs, none other than MET was detected.

Quantitative analysis of MET

Under the high-performance liquid chromatography condition, MET eluted at 3.9 min and methyltestosterone

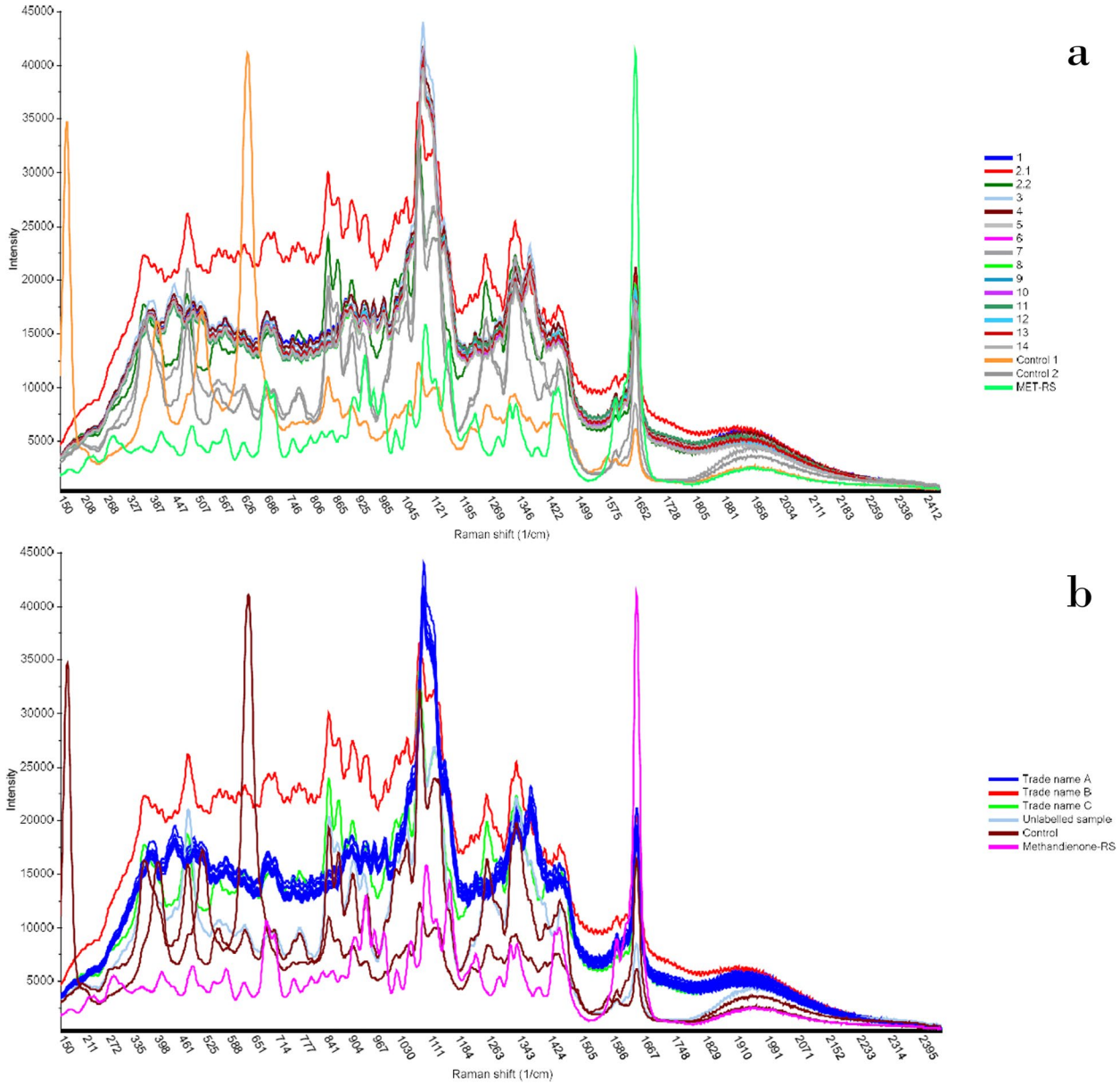


Fig. 6 Raman spectra obtained using an Inspector500 ultra-compact Raman spectrometer. Fig. 6 shows Raman spectra of the 15 sample products, the standard formulation without and with the sufficient removal of the coating (Control 1, Control 2), and the methandienone-reference standard (MET-RS). For each spectrum, 50 spectral data taken from five randomly selected positions each on the tablet upside and tablet downside were averaged. The MET-RS, available as powder, was placed in a plastic bag and piled up, and 50 spectral data were taken at five randomly selected positions from each of the two sides. A peak near a Raman shift of 1650 cm^{-1} is visible in all spectra. The spectra are presented with (**a**) coloring according to the sample, control, and MET-RS and (**b**) coloring according to the labeled trade name

at 4.9 min (Supplemental Fig. 1). Validation data are summarized in Supplemental Table 3. In view of the lack of labeling of sample 7, the MET content (5 mg) indicated on website 7 was chosen as the reference value. The recovery for the MET-RS of the positive control solution was calculated to be 100.7% ± 5.4% ($n=9$). The MET content in the standard prescribed product obtained from the Republic of Moldova was 101.6% ± 2.5% ($n=3$) of the labeled amount. The average API content of 12 samples was less than 90%, and all 15 samples contained at least one tablet with a content of less than 90%, with 11 samples having at least one tablet with a content below 80%. Among the 15 samples, the product with the lowest average content per tablet was sample 7 with 80.6% ± 3.3%

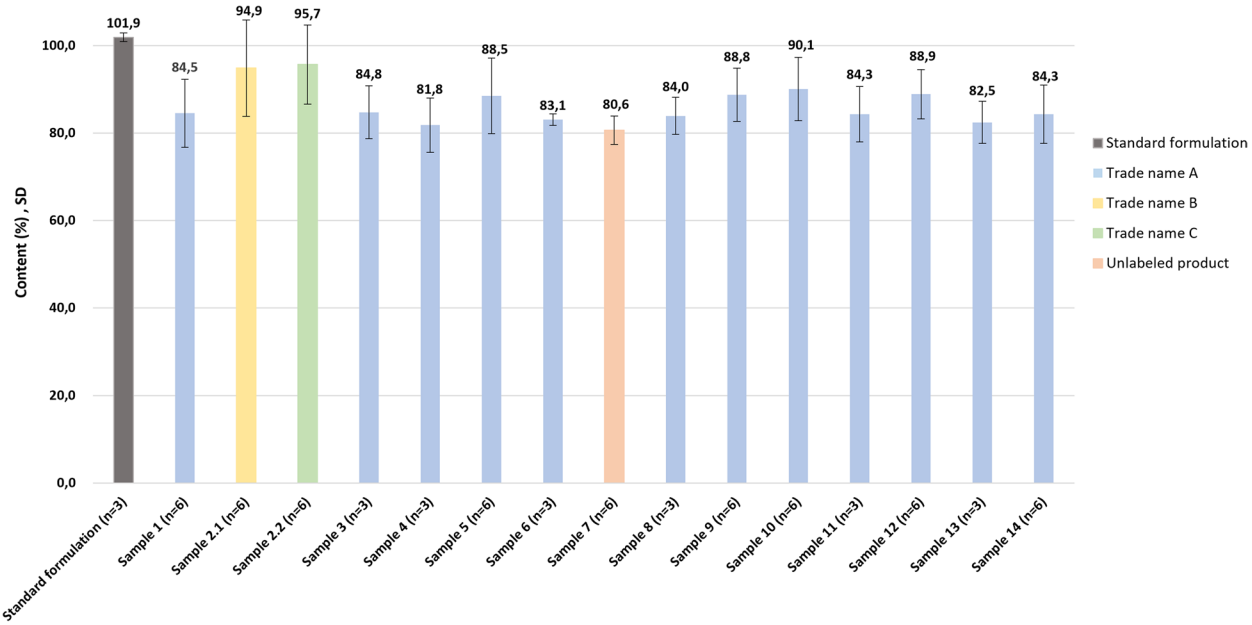


Fig. 7 Content (%) and standard deviation (SD) of methandienone samples and the standard formulation determined by UPLC-FTMS. Fig. 7 shows the average content and the standard deviation (both in percent) of n tablets of each sample and of the standard formulation. The results were determined using the UPLC-FTMS method developed in this study. Bars are colored according to the product trade names

($n=6$). The product with the highest average content was confirmed falsified sample 2.2 with 95.7% ± 9.1% ($n=6$). The results of the quantification analysis are presented in Fig. 7.

Availability of approved MET products

Twenty-two of 44 (50%) NRAs responded to the information request, among which one, the Medicines and Medical Devices Agency of the Republic of Moldova, confirmed that it had approved an MET-containing product. The other 21 NRAs stated that no registered MET-containing pharmaceutical product was authorized or produced in their country. Furthermore, eight of the 33 institutions other than the NRAs replied to the information inquiry, with five institutions responding that their accessible databases did not show any manufacturing or exporting of MET products and the other three institutions stating non-responsibility in this matter (Supplemental Table 2).

Discussion

This study revealed that unapproved MET products could enter Japan through personal importation via the Internet. All $n=15$ collected samples that were personally imported to Japan in this study were found to have defects, and one sample (6.7%) was confirmed as falsified (Fig. 4a–d). All fifteen samples (100%) failed the visual observation test, and two samples (13.3%) did not give information on the origin of manufacture. Two different portable Raman spectrometers were found to identify MET in all $n=16$ tested products, as verified using a developed UPLC-FTMS (Lim et al. 2016) method, which has sufficient mass accuracy for the identification and quantification of MET. This study thus adds an LC-FTMS method to a low number of available quantitative LC-MS AAS analysis methods; i.e., LC-MS/MS with triple–quadrupole mass spectrometers and time-of-flight mass spectrometry (Tircova et al. 2019; Cho et al. 2015; Van Poucke et al. 2007; Prokudina et al. 2015; Mesmer and Satzger 1997).

Raman scattering analysis was performed prior to UPLC-FTMS analysis to explore its possibilities as a rapid screening technology. UPLC-FTMS analysis was required to identify AASs, impurities other than MET, or related compounds to demonstrate that the Raman scattering methods correctly detected MET and have not falsely judged the samples. Furthermore, it is well known that SF medicines may contain no API, the wrong API, or an altered API content among individual units or impurities owing to incorrect manufacturing or storage (Rahman et al. 2018), and the API content or ingredients may differ even between units from the same blister pack or units packaged together. Therefore, the API contents of individual units were accurately determined using LC-MS rather than by performing an assay to report average contents, but the sample material was insufficient to perform further analyses.

Despite not being approved as pharmaceutical products or being licensed for distribution in Japan, all 15

collected samples were ordered without a prescription, as previously observed for other medicines (Zhu et al. 2020; Sanada et al. 2020; Rahman et al. 2018), indicating that personal importation readily enables the bypassing of medical prescriptions and the acquisition of prohibited and unauthorized products. All fifteen samples had problematic packaging, labeling, and/or content, putting consumers at high risk of accidental or unknown misuse and health hazards. Furthermore, opened, damaged or inappropriate primary packaging may lead to insufficient protection of the product from environmental effects and therefore reduced quality, efficacy, and safety, but no impurities that would provide evidence of such degradation processes were detected in this study. Inadequate or missing labeling and leaflets lead to a lack of product-related and health-specific information and can result in medication errors including incorrect application, incorrect dosing, and consumption despite contraindications. Furthermore, it was found that samples having a leaflet that is only downloadable via a labeled QR code are prone to the risk that information may be removed from websites or become inaccessible, as happened for one sample.

Although the presence of API was confirmed, the low and varying API content on average and across individual tablets of the samples (Fig. 7) suggests violations of good manufacturing practice; however, the effect of inadequate primary packaging and the storage conditions in the international distribution chain was not assessable, and therefore, it cannot be completely ruled out that product degradation reduced the content.

Notably, the online authentication message received after scanning the QR code on the label of the falsified product (Fig. 4c) demonstrates the efforts criminals are prepared to take to deceive customers and highlights that QR code authentication is not a trustable method per se. Verifying the authenticity of a sample thus requires direct information from the manufacturer or the responsible pharmaceutical entrepreneur. Remarkably, the website of the falsified product changed its layout between July and October 2023, confirming continuous activities in online product presentation and advertisement and therefore suggesting the continuous trade of falsified products.

As MET is not listed in the main pharmacopoeias (i.e., USP, BP, Int. Ph., EP, and JP), authentic products are crucial for comparison in quality analysis and differentiation from falsified products in Raman scattering analysis and especially in chemical analysis. However, owing to the low response rate of the labeled manufacturers, it was not possible to clarify the authenticity of all samples or acquire authentic products matching the samples. Regardless, problematic samples including a falsified product (Fig. 4a–d) were identified, reaffirming the importance of visual observation testing and legitimacy investigation.

In Raman scattering analysis, there was a peak near a Raman shift wavenumber of 1,650 cm^{-1} for the samples, reference standard, and control (Figs. 5a, b and 6a, b), similar to previous results (Rebiere et al. 2016). Non-destructive Raman scattering analysis can thus detect the presence of MET as confirmed by LC-FTMS (Fig.5). Notably, the spectrum of the coated standard formulation (Control 1) had a peak near 1,650 cm^{-1} for both spectrometers (Figs. 5a, b and 6a, b), suggesting that MET identification is possible despite the presence of a coating, which is known to interfere often with Raman scattering analysis. Raman scattering analysis using C13560 and Inspector500 spectrometers may thus be suitable for the quick and non-destructive API detection of MET and used to screen for SF medicines having a wrong or no API or considerably low API contents, but the detection limit of the API content has not been assessed in this study. As AASs by definition have similar structures (Ganesan et al. 2023; Bhasin et al. 1996; National Institute on Drug Abuse 2018; Barceloux and Palmer 2013), identification of other AASs through portable Raman scattering analysis seems plausible. The differential identification of AASs needs to be evaluated in future work.

In comparison with LC-MS analysis, the Raman scattering methods used in this study are non-destructive, do not require organic solvents during the analytical process, can be used on-site owing to low weight and small size of the apparatuses, and can be learned time effectively by analysts in training as the analytical process is simple and fast to carry out. The methods may thus cost-effectively contribute to supply chain integrity and protection by supporting costly and time-consuming LC-MS analysis through pre-screening products of interest, or even replacing LC-MS analysis in certain scenarios such as the qualitative analysis of known MET products.

Considering that only half of the NRAs responded regarding the availability of approved products (Supplemental Table 2), better cooperation between NRAs, governmental institutions and researchers regarding requests for information on the availability and legitimacy of products should be encouraged. MET was not approved by NRAs in most countries investigated.

Limitations of the present study include that only one AAS was analyzed and evaluated with a limited number of samples collected from websites identified only per generic name and only within a short period of time. The low response rate of manufacturers prevented clarification of the authenticity of all samples, and authentic products could not be obtained for comparison in visual observation testing and analysis.

This study highlights that potent products not registered or approved as medicines in most countries are being distributed globally, in that MET products are

readily available online without a prescription for personal import to Japan, as has been reported for other medicines (McBride et al. 2018; Zhu et al. 2020; Coopman and Cordonnier 2012; Rahman et al. 2018). Substances with strong effects on human physiology, such as AASs (Bhasin et al. 1996; Rahnema et al. 2014; Solimini et al. 2017; van Amsterdam et al. 2010; Liu and Wu 2019; Takayanagi et al. 2008), must be controlled to reduce inadvertent misuse and abuse.

Conclusion

Fifteen samples (100%) of four MET (ASS) products were obtained via personal importation over the Internet without prescription and found to have problematic packaging, labeling, and/or contents, with one sample (6.7%) confirmed as being falsified. Two portable Raman spectrometers identified the presence of MET in all samples, as subsequently confirmed adopting an LC-MS method, suggesting potential on-site use. The API was detected with a content below 90% on average for each of 12 samples. Further research is needed to differentiate AASs using portable Raman spectrometers. The results underline the importance of visual observation testing and legitimacy investigation in addition to chemical analysis. To avoid health hazards, measures of preventing the personal import and abuse of MET and other AASs are suggested for implementation in Japan and internationally.

Abbreviations

AAS	Anabolic–androgenic steroid
API	Active pharmaceutical ingredient
FTMS	Fourier transform mass spectrometry
IPA	Isopropyl alcohol
LC	Liquid chromatography
MeCN	Acetonitrile
MeOH	Methanol
MET	Methandienone
MET-RS	Methandienone reference standard
MS	Mass spectrometry
MS/MS	Liquid chromatography–tandem mass spectrometry
m/z	Mass–to–charge ratio
NRA	National regulatory authority
N/A	Not available
QR code	Quick response code
SD	Standard deviation
SF medicines	Substandard and falsified medicines
UPLC	Ultra–high–performance liquid chromatography

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41120-024-00093-0.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

Authors' contributions

RS carried out the sample investigation, observation testing, authenticity and legitimacy investigations, Raman scattering analysis, data analysis and data interpretation, and visualization of results, wrote the original draft and reviewed and edited the manuscript. MH was majorly involved in the LC-MS method development, validation, sample analysis and visualization of results. CT, MSR, and AN were majorly involved in the LC-MS method development and sample analysis. SZ and FZ collected the samples and carried out observation testing and authenticity investigations. NY supervised and conceptualized the study and its methodology, was involved in the investigation, data curation, and resource provision, and reviewed and edited the manuscript. KM supervised the LC-MS investigation, provided resources, and reviewed and edited the manuscript. KK supervised and conceptualized the study and its methodology, was involved in the investigation and data curation, reviewed and edited the manuscript, and was responsible for funding acquisition and project administration. All authors read and approved the final manuscript.

Declarations

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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References

Ahmed J, Modica de Mohac L, Mackey TK, Raimi-Abraham BT (2022) A critical review on the availability of substandard and falsified medicines online: incidence, challenges and perspectives. J Med Access 6:23992026221074548. https://doi.org/10.1177/23992026221074548

Barceloux DG, Palmer RB (2013) Anabolic-androgenic steroids. Dis Mon 59(6):226–248. https://doi.org/10.1016/j.disamonth.2013.03.010
Bhasin S, Storer TW, Berman N, Callegari C, Clevenger B, Phillips J et al (1996) The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. N Engl J Med 335(1):1–7. https://doi.org/10.1056/NEJM199607043350101
Cho SH, Park HJ, Lee JH, Do JA, Heo S, Jo JH et al (2015) Determination of anabolic-androgenic steroid adulterants in counterfeit drugs by UHPLC-MS/MS. J Pharm Biomed Anal 111:138–146. https://doi.org/10.1016/j.jpba.2015.03.018
Coopman V, Cordonnier J (2012) Counterfeit drugs and pharmaceutical preparations seized from the black market among bodybuilders. Ann Toxicol Anal 24(2):73–80. https://doi.org/10.1051/ata/2012012
Dégardin K, Guillemain A, Roggo Y (2017) Comprehensive Study of a Handheld Raman Spectrometer for the analysis of counterfeits of solid-dosage form Medicines. J Spectrosc (Hindawi) 1:1–13 Article ID 3154035. https://doi.org/10.1155/2017/3154035
Fabresse N, Gheddar L, Kintz P, Knapp A, Larabi IA, Alvarez JC (2021) Analysis of pharmaceutical products and dietary supplements seized from the black market among bodybuilders. Forensic Sci Int 322:110771. https://doi.org/10.1016/j.forsciint.2021.110771
Frude E, McKay FH, Dunn M (2020) A focused netnographic study exploring experiences associated with counterfeit and contaminated anabolic-androgenic steroids. Harm Reduct J 17(1):42. https://doi.org/10.1186/s12954-020-00387-y
Ganesan K, Rahman S, Zito PM (2023) Anabolic steroids. StatPearls. StatPearls, Treasure Island (FL). Available from: https://www.ncbi.nlm.nih.gov/books/NBK482418. Accessed 16 Aug 2023
Hajjou M, Qin Y, Bradby S, Bempong D, Lukulay P (2013) Assessment of the performance of a handheld Raman device for potential use as a screening tool in evaluating medicines quality. J Pharm Biomed Anal 74:47–55. https://doi.org/10.1016/j.jpba.2012.09.016
Khan MH, Tanimoto T, Nakanishi Y, Yoshida N, Tsuboi H, Kimura K (2012) Public health concerns for anti-obesity medicines imported for personal use through the internet: a cross-sectional study. BMJ Open 2:e000854. https://doi.org/10.1136/bmjopen-2012-000854
Lim L, Yan F, Bach S, Pihakari K, Klein D (2016) Fourier Transform Mass Spectrometry: the Transformation of Modern Environmental analyses. Int J Mol Sci 17(1):104. https://doi.org/10.3390/ijms17010104
Liu JD, Wu YQ (2019) Anabolic-androgenic steroids and cardiovascular risk. Chin Med J (Engl) 132(18):2229–2236. https://doi.org/10.1097/CM9.0000000000000407
Magnolini R, Falcato L, Cremonesi A, Schori D, Bruggmann P (2022) Fake anabolic androgenic steroids on the black market - a systematic review and meta-analysis on qualitative and quantitative analytical results found within the literature. BMC Public Health 22(1):1371. https://doi.org/10.1186/s12889-022-13734-4
McBride JA, Carson CC 3rd, Coward RM (2018) The availability and Acquisition of Illicit anabolic androgenic steroids and Testosterone preparations on the internet. Am J Mens Health 12(5):1352–1357. https://doi.org/10.1177/1557988316648704
Mesmer MZ, Satzger RD (1997) Determination of anabolic steroids by HPLC with UV-vis-particle beam mass spectrometry. J Chromatogr Sci 35(1):38–42. https://doi.org/10.1093/chromsci/35.1.38
National Institute on Drug Abuse, National Institutes of Health (2018) Anabolic Steroids. In: U.S. Department of Health and Human Services. https://nida.nih.gov/sites/default/files/drugfacts-steroids.pdf. Accessed 25 Aug 2023
Nieschlag E, Vorona E (2015) Doping with anabolic androgenic steroids (AAS): adverse effects on non-reproductive organs and functions. Rev Endocr Metab Disord 16(3):199–211. https://doi.org/10.1007/s11154-015-9320-5
Opuni KF, Nettey H, Larbi MA, Amartey SNA, Nti G, Dzidonu A et al (2019) Usefulness of combined screening methods for rapid detection of falsified and/or substandard medicines in the absence of a confirmatory method. Malar J 18(1):403. https://doi.org/10.1186/s12936-019-3045-y
Piatkowski T, Puljevic C, Francis C, Ferris J, Dunn M (2023) They sent it away for testing and it was all bunk: exploring perspectives on drug checking among steroid consumers in Queensland, Australia. Int J Drug Policy 119:104139. https://doi.org/10.1016/j.drugpo.2023.104139
Prokudina EA, Prchalová J, Vyšatová E, Kuchař M, Rajchl A, Lapčík O (2015) Analysis of anabolic androgenic steroids by direct analysis in real time ionization with time-of-flight mass spectrometry. Int J Mass Spectrom 392:28–33. https://doi.org/10.1016/j.ijms.2015.08.022
Rahnema CD, Lipshultz LI, Crosnoe LE, Kovac JR, Kim ED (2014) Anabolic steroid-induced hypogonadism: diagnosis and treatment. Fertil Steril 101(5):1271–1279. https://doi.org/10.1016/j.fertnstert.2014.02.002
Rahman MS, Yoshida N, Tsuboi H, Tomizu N, Endo J, Miyu O et al (2018) The health consequences of falsified medicines- A study of the published literature. Trop Med Int Health 23(12):1294–1303. https://doi.org/10.1111/tmi.13161
Rahman MS, Yoshida N, Hanafusa M, Matsuo A, Zhu S, Stub Y et al (2022) Screening and quantification of undeclared PGF2α analogs in eyelash-enhancing cosmetic serums using LC-MS/MS. J Pharm Biomed Anal 219:114940. https://doi.org/10.1016/j.jpba.2022.114940
Rahman MS, Yoshida N, Sugiura S, Tsuboi H, Keila T, Kiet HB et al (2018) Quality of omeprazole purchased via the internet and personally imported into Japan: comparison with products sampled in other Asian countries. Trop Med Int Health 23(3):263–269. https://doi.org/10.1111/tmi.13028
Rebiere H, Ghyselinck C, Lempereur L, Brenier C (2016) Investigation of the composition of anabolic tablets using near infrared spectroscopy and Raman chemical imaging. Drug Test Anal 8(3–4):370–377. https://doi.org/10.1002/dta.1843
Sagoe D, Molde H, Andreassen CS, Torsheim T, Pallesen S (2014) The global epidemiology of anabolic-androgenic steroid use: a meta-analysis and meta-regression analysis. Ann Epidemiol 24(5):383–398. https://doi.org/10.1016/j.annepidem.2014.01.009
Sanada T, Ohnishi M, Yoshida N, Kimura K, Tsuboi H (2021) Quality assessment of Diflucan® tablets distributed online: Diflucan® distributed online. Med Access Point Care 5:23992026211002089. https://doi.org/10.1177/23992026211002089
Sanada T, Yoshida N, Kimura K, Tsuboi H (2020) Discrimination of falsified erectile dysfunction medicines by use of an ultra-compact Raman scattering spectrometer. Pharm (Basel) 9(1):3. https://doi.org/10.3390/pharmacy9010003
Sanada T, Yoshida N, Matsushita R, Kimura K, Tsuboi H (2020) Falsified tadalafil tablets distributed in Japan via the internet. Forensic Sci Int 307:110143. https://doi.org/10.1016/j.forsciint.2020.110143
Storer TW, Magliano L, Woodhouse L, Lee ML, Dzekov C, Dzekov J et al (2003) Testosterone dose-dependently increases maximal voluntary strength and leg power, but does not affect fatigability or specific tension. J Clin Endocrinol Metab 88(4):1478–1485. https://doi.org/10.1210/jc.2002-021231
Solimini R, Rotolo MC, Mastrobattista L, Mortali C, Minutillo A, Pichini S et al (2017) Hepatotoxicity associated with illicit use of anabolic androgenic steroids in doping. Eur Rev Med Pharmacol Sci 21(1 Suppl):7–16
Takayanagi A, Kobayashi K, Hashimoto K, Kato R, Masumori N, Itoh N et al (2008) Case of androgenic anabolic steroid abuse caused hypogonadotropic hypogonadism. Nihon Hinyokika Gakkai Zasshi 99(7):729–32. https://doi.org/10.5980/jpnjurol1989.99.729. Japanese
The Ministry of Health, Labour and Welfare (2021) Official Monographs (A to L), The Japanese Pharmacopeia 18th edition (JP XVIII). 7:960–961. https://www.mhlw.go.jp/content/11120000/000912386.pdf. Accessed 05 Sept 2023
The Ministry of Health, Labour and Welfare (2021) Official Monographs (M to Z), The Japanese Pharmacopeia 18th edition (JP XVIII). 7:1572–1573. https://www.mhlw.go.jp/content/11120000/000912386.pdf. Accessed 05 Sept 2023
Tool for Visual Inspection of Medicines (n.d.) International Pharmaceutical Federation https://www.fip.org/files/fip/counterfeit/VisualInspection/A%20tool%20for%20visual%20inspection%20of%20medicines%20EN.pdf. Accessed 25 Aug 2023
Tircova B, Bosakova Z, Kozlik P (2019) Development of an ultra-high performance liquid chromatography-tandem mass spectrometry method for the determination of anabolic steroids currently available on the black market in the Czech Republic and Slovakia. Drug Test Anal 11(2):355–360. https://doi.org/10.1002/dta.2541
van Amsterdam J, Opperhuizen A, Hartgens F (2010) Adverse health effects of anabolic-androgenic steroids. Regul Toxicol Pharmacol 57(1):117–123. https://doi.org/10.1016/j.yrtph.2010.02.001
Van Poucke C, Detavernier C, Van Cauwenberghe R, Van Peteghem C (2007) Determination of anabolic steroids in dietary supplements by liquid chromatography-tandem mass spectrometry. Anal Chim Acta 586(1–2):35–42. https://doi.org/10.1016/j.aca.2006.09.050

World Health Organization (2018) Substandard and falsified medical products. 2018 Jan 31. https://www.who.int/news-room/fact-sheets/detail/substandard-and-falsified-medical-products. Accessed 25 Aug 2023

World anti-doping agency (2023) Prohibited List, international standard. In: World anti-doping code. https://www.wada-ama.org/sites/default/files/2022-09/2023list_en_final_9_september_2022.pdf. Accessed 25 Aug 2023

Yoshida N, Numano M, Nagasaka Y, Ueda K, Tsuboi H, Tanimoto T et al (2015) Study on health hazards through medicines purchased on the internet: a cross-sectional investigation of the quality of anti-obesity medicines containing crude drugs as active ingredients. BMC Complement Altern Med 15(1):430. https://doi.org/10.1186/s12906-015-0955-2

Zhu S, Yoshida N, Kimura K, Matsushita R, Tsuboi H (2020) Falsified vardenafil tablets available online. J Pharm Biomed Anal 177:112872. https://doi.org/10.1016/j.jpba.2019.112872

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