European Journal of Pharmaceutics and Biopharmaceutics 72 (2009) 275–281

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Analytical, biopharmaceutical and regulatory evaluation of topical testosterone preparations

B. Baert [a], S. Annavarapu [a], C. Burvenich [b], B. De Spiegeleer [a,*]

[a] Drug Quality and Registration (DruQuaR) group, Ghent University, Ghent, Belgium
[b] Department of Physiology and Biometrics, Ghent University, Merelbeke, Belgium

ARTICLE INFO

Article history:
Received 19 June 2008
Accepted in revised form 27 October 2008
Available online 6 November 2008

Keywords:
Comparative quality
Topical pharmaceuticals
Testosterone
LC–UV/ESI-MS
Specifications
In vitro drug release
Dissolution
Assay
Impurities
Regulatory affairs

ABSTRACT

Testosterone-containing pharmaceutical products for topical use were obtained from the pharmacist or through the internet. The legal status of the different products obtained is discussed: some products through the internet were clearly a medicinal product according to the current definitions, while they are not registered as such. Assay and impurity profiles of each of the marketed samples were obtained using HPLC–UV and ESI-iontrap MS. The analytical results were evaluated relative to the reporting, identification and qualification thresholds as defined by the the International Conference on Harmonisation (ICH) and the European Pharmacopoeia (Ph. Eur.). Preparations with impurities above the qualification threshold were observed. Moreover, *in vitro* release profiles over an artificial membrane were obtained using a standardised cell in a paddle dissolution bath as well as in a static Franz diffusion cell, using phosphate buffered saline (PBS; pH 7.0) containing 5% bovine serum albumin (BSA) as dissolution or receptor fluid. This biopharmaceutical quality attribute differs significantly between the preparations tested. In conclusion, the equivalency of topical testosterone preparations is not assured, nor on their legal status, nor on their impurity profiling nor on their biopharmaceutical behaviour. This calls for an urgent transnational product-class harmonisation approach.

1. Introduction

The analytical characterisation and quality verification of semi-solid, topical products involve many elements specific to this dosage form. The Ph. Eur. and the United States Pharmacopoeia (USP), supported by international regulatory guidelines, provide guidance and procedures for these dosage forms. General quality considerations are forming the basis, including assay and impurity profiling of related substances. Dosage form-specific quality attributes are added which include *in vitro* release profiling. The "SUPAC-SS Guidance" recommends *in vitro* diffusion cell testing for semi-solid dosage forms when manufacturing or material changes occur to compare the pre- and post-change release rates of the product [1]. Nevertheless, the value and application of such *in vitro* release systems are not as widely accepted as for oral dosage forms, i.a. due to the use of several cell types, designs and operational protocols (including solvents used) although the "Franz cell" has emerged as the most popular design for testing the *in vitro* release of topical semi-solid dosage forms [2–4].

From a regulatory quality perspective, several texts have to be considered in setting proper specifications against which the quality of pharmaceutical products is evaluated with the final purpose to make a release decision. Releasing a pharmaceutical product implies that the quality is sufficiently high and consistent, as justified in the approved marketing authorisation (MA) dossier, so that the probability of unwanted and unexpected harm to the patient is minimised. At a global level, the ICH quality guidelines, adopted by the European Committee for Proprietary Medicinal Products (CPMP), describe how to deal with impurities and specification settings in an international regulatory environment, i.e. sensu stricto only at the phase of preparing the submission of a MA application [5–7]. Recently, the Ph. Eur. has extended its mandatory general monograph "Substances for pharmaceutical use" [8] with an explanatory general chapter 5.10 "Control of impurities in substances for pharmaceutical use" [9]. While these are sensu stricto only applicable on substances specifically monographed in the Ph. Eur., the analytical procedures and/or acceptance criteria (APAC) described therein are clearly a rational basis for other drug substances and drug products as well [10].

Testosterone topical formulations are suspected for their intensive off-label use [11,12] and their distribution occurs not only in a controlled way through pharmacists, but also in rather uncontrolled ways via the internet; hence the importance of the pharmaceutical quality characterisation and comparison of a series of

---

* Corresponding author. Drug Quality and Registration (DruQuaR) group, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium. Tel.: +32 9 264 81 00; fax: +32 9 264 81 93.
E-mail address: Bart.DeSpiegeleer@UGent.be (B. De Spiegeleer).

0939-6411/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2008.10.014

marketed topical testosterone preparations from different sources. For the current study, six commercially available testosterone-containing formulations were investigated using LC–UV(DAD)/ESI-MS for assay and impurity profiling, as well as dissolution equipment and Franz cells for the *in vitro* release characterisation. The analytical findings are compared and evaluated versus regulatory specifications as currently defined.

2. Materials and methods

2.1. Products examined

Four testosterone-containing topical products were purchased from the Ghent university hospital pharmacy: Testim® 50 mg gel (Ipsen, Merelbeke, Belgium), Androgel® 50 mg gel (Laboratoires Besins International, Paris, France), Tostrex® 2% gel (Strakan, Tredegar, UK) and Intrinsa® 300 µg/24 h transdermal patch (Proctor & Gamble Pharmaceuticals, Egham, UK). Two products were obtained from the internet: Androtiv® 2% gel (Anastore, Geneva, Switzerland) and Libido Edge™ “testosterone” cream (Libido Edge Labs, Newark, USA). Their qualitative composition from the packaging labelling is stated in Table 1.

2.2. Reagents

Phosphate buffered saline (PBS, 0.01 M) was purchased from Sigma (St. Louis, MO, USA). Bovine serum albumin (BSA) was obtained from Merck (Darmstadt, Germany). HPLC gradient grade acetonitrile and methanol were purchased from Fisher Scientific (Leicestershire, UK). LC-MS grade formic acid was purchased from Fluka (Buchs, Switzerland). Water was purified using an Arium 611 purification system (Sartorius, Göttingen, Germany) resulting in ultrapure water of 18.2 MΩ cm quality.

2.3. Liquid chromatography

The testosterone content was assayed using a validated HPLC–UV method. The HPLC apparatus consisted of a Waters Alliance 2695 separation module and a Waters 2996 photodiode array detector (DAD) controlled by Empower 2 software (all Waters, Millford, USA). LC separations were performed using a Lichrospher 100 RP C_{18} (125 × 4 mm, 5 µm particle size) column (Merck, Darmstadt, Germany) maintained at 30 °C, with a mobile phase consisting of a mixture of 45% (A) 0.1% m/V formic acid in water, and 55% (B) 0.1% m/V formic acid in acetonitrile. The flow rate was set at 1.0 ml/min. UV detection was done at 254 nm.

Table 1
Qualitative composition of the formulations analysed (as given by the supplier).

Formulation	Composition
Testim®	Testosterone, ethanol, carbomer 980, carbomer 1342, propylene glycol, glycerol, macrogol 1000, trometamol, pentadecalactone, purified water
Androgel®	Testosterone, ethanol, carbomer 980, isopropyl myristate, sodium hydroxide, purified water
Tostrex®	Testosterone, ethanol, carbomer 1382, isopropyl alcohol, propylene glycol, oleic acid, trolamine, butylhydroxytoluene, purified water
Intrinsa®	Testosterone, sorbitan oleate, 2-ethylhexylacrylate-1-vinyl-2-pyrrolidone co-polymer. Backing layer: translucent polyethylene backing film printed with proprietary ink containing sunset yellow FCF, latolrubine BK and copper phtalocyanine blue pigment. Protective release liner: siliconised polyester film
Androtiv®	Testosterone, alcohol, water, carbomer, tea, phenoxyethanol, methylparaben, ethylparaben, propylparaben, butylparaben
Libido Edge™	Purified water, lanol, homeopathic testosterone in 10×, 30× and 100× potencies, somatropin (hHG) in 10×, 30× and 100× potencies, montanov, liposome delivery system, triple strength herbal complex of horny goat weed, *Tribulus terrestris*, *Mucuna pruriens*, saw palmetto, natural omega oils, 1% micronised dihydroepiandrosterone, diindolylmethane, sepigel, Germall

Related impurities were determined by liquid chromatography electrospray ionization mass spectroscopy (LC-ESI-MS). The LC–UV/MS apparatus consisted of a Spectra System SN4000 interface, a Spectra System SCM1000 degasser, a Spectra System P1000XR pump, a Spectra System AS3000 autosampler and a Finnigan LCQ classic ion trap mass spectrometer (all from Thermo, San Jose, CA, USA) equipped with a SPD-10A UV-VIS detector set at 254 nm (Shimadzu, Kyoto, Japan) and Xcalibur 1.2 software (Thermo, San Jose, CA, USA) for data acquisition. LC separations were performed using a Lichrospher 100 RP C_{18} (250 × 4 mm, 5 µm particle size) column (Merck, Darmstadt, Germany) maintained at 40 °C. The mobile phase consisted of a gradient mixture of (A) H_2O/methanol (45:55, V/V), and (B) pure methanol. The flow rate was set at 1.0 ml/min. Mass spectroscopy was performed in positive ion mode, and spectra were acquired in the mass/charge range of 100.0–2000.0.

2.4. Determination of testosterone solubility in the dissolution medium

Before injection of the samples, BSA was precipitated by adding an equal volume of acetonitrile. After 10 min of shaking at room temperature, the samples were centrifuged for 10 min at 20,000*g* and the clear supernatant was transferred into glass HPLC vials. Additionally, the solubility of testosterone in the dissolution medium at 32 °C was determined using this HPLC method on suitable dilutions of the saturated solution obtained after 48 h incubation.

2.5. Microscopy

The different preparations were examined for their morphology by light microscopy (Zeiss Axiostar Plus, Göttingen, Germany). A small amount of the gels was placed in a Fuchs-Rosenthal counting chamber (Superio Marienfield, Lauda-Königshofen, Germany) and examined at 50× magnification.

2.6. Dissolution experiments

Dissolution of testosterone into a PBS aqueous solution containing 5% m/V of BSA was measured using a standard Ph. Eur./USP dissolution apparatus with paddles (VK 8000 dissolution bath, VK 750 D pump and VK 7010 autosampler; all Varian, USA). Because of the size of the extraction cell, the volume of dissolution medium used was reduced from the standard 900 to 750 ml. Bath temperature was maintained at 32 ± 1 °C and the paddles rotated at 75 rpm. The products examined were placed into an in-house developed extraction cell similar to the one described in the Ph. Eur. “paddle over extraction cell” method (see Fig. 1) [13]. Hydrophilic mixed cellulose esters filter sheets with 0.05 µm pore size (VMWP; Millipore, Schwalbach, Germany) were used as diffusion membrane. Two ml samples were automatically taken at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10 and 12 h for testosterone content determination by HPLC–UV (see Section 2.4). The testosterone values were correspondingly corrected for the sample amount removed without replenishing.

2.7. Franz cell experiments using artificial membranes

Permeation experiments were carried out in 5 ml static Franz diffusion cells (Logan Instruments Corp., New Jersey, USA). The glass cells consisted of a donor compartment, containing the drug preparation and a receptor compartment filled with the dissolution medium as described above. The hydrophilic mixed cellulose esters

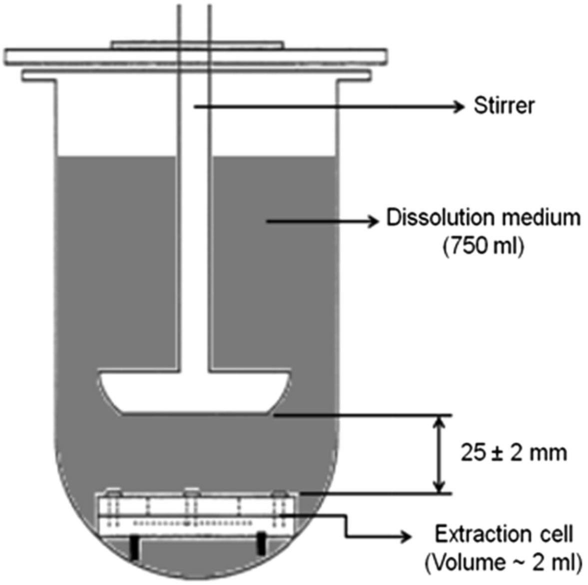

Fig. 1. Paddel over extraction cell dissolution test.

filter sheets, identical to those used in the dissolution experiments, were positioned between both cell compartments and held together with a clamp. A quantity of the formulation corresponding to 3 mg of testosterone was topically applied to the surface of the membrane and carefully spread to achieve complete coverage. The donor compartment was covered with parafilm (American National Can™, Chicago, USA) and the temperature of the acceptor compartment was kept at 32 ± 2 °C by a water jacket. The acceptor fluid (5 ml) was mixed with a magnetic stirring bar (400 rpm). The available diffusion area was 0.64 cm^2. Samples (200 μl) were drawn at regular time intervals from the sample port (0, 0.5, 1.0, 2.0, 4.0, 8.0 and 12.0 h) and were immediately replaced with 200 μL fresh solution. The testosterone values were correspondingly corrected for the replenishments.

2.8. Kinetic analysis of dissolution and Franz cell data

Mean dissolution time (MDT) was considered as a basis for comparison of the dissolution rate and was estimated by the following equation [14]:

$$\mathrm{MDT} = \frac{\int_0^\infty t \cdot M_\mathrm{d}(t) \cdot \mathrm{d}t}{\int_0^\infty M_\mathrm{d}(t) \cdot \mathrm{d}t} = \frac{ABC}{M_0} \tag{1}$$

where *ABC* is the area between the release curve and its asymptote, calculated by the trapezoidal rule from time zero to the time-point where the amount of dissolved drug is equal to a reference value M_0. In this study, an arithmetic estimation of *ABC* was made using trapezoidal rule.

3. Results

3.1. Liquid chromatography

The assay method was validated for specificity, linearity, accuracy and precision. The chromatograms obtained for testosterone, progesterone and triamcinolone showed that the method was specific for testosterone, as related steroids were clearly separated. Moreover, no interference was found between testosterone and different excipients present in the formulations under investigation (ethanol, propylene glycol, isopropyl myristate, glycerol, methylparaben, ethylparaben, propylparaben and butylparaben). Specificity was again confirmed by DAD peak purity testing. Linearity was obtained in the range of 0.05–100 μg/mL. Recovery experiments showed no statistical significant loss of testosterone due to sample treatment, confirming the accuracy. Precision was determined by performing three replicates of three different concentrations covering the specified range, and a mean RSD of 0.51% was found. The limit of quantitation was found to be 0.17 μg/ml.

The method used for detecting impurities was based upon the related substances test described in the European Pharmacopeia 5th edition monograph on testosterone [15]. The system suitability was verified using the appropriate chemical reference substances obtained from the European Directorate for the Quality of Medicines (EDQM): a baseline separation between the peaks due to testosterone and 17β-hydroxyandostra-4,6-dien-3-one (impurity I) was achieved. After calibration of the mass spectrometer according to internal procedures, the measured mass of the reference impurities corresponded to their calculated mono-isotopic mass. In conclusion, our methods were found suitable for their use of quantification and identification.

3.2. Assay values and impurity profiles

The testosterone assay results obtained on the products examined are given in Table 2. All formulations were found to comply with standard acceptance criteria (i.e. 95–105% of the label claim), except for Libido Edge™ "testosterone" cream, where the testosterone content was below the limit of quantification of 0.17 μg/ml. Although the name of this latter product suggests the presence of testosterone in higher concentrations, this finding complies with the ingredients list stating "homeopathic testosterone in 10×, 30× and 100× potencies".

Results on related substances are given in Table 3. The reporting threshold for impurities was set at 0.05%. The active pharmaceutical product (API) Ph.Eur. specification criterion for the epimeric testosterone impurity was maximally 0.50%, while for other individual impurities a specification limit of 0.20% was applied. Based

Table 2
Assay values.

Formulation	Origin	Label claim	Assay (% label claim);[a]
Testim®	Pharmacy	1% m/m	102.82 (±0.98)
Androgel®	Pharmacy	1% m/m	101.38 (±0.95)
Tostrex®	Pharmacy	2% m/m	102.61 (±2.20)
Intrinsa®	Pharmacy	8.4 mg/patch	102.66 (±3.37)
Androtiv®	Internet	2% m/m	100.18 (±0.18)
Libido Edge™	Internet	Homeopathic	<LOQ[b]

[a] % Label claim ± standard deviation ($n = 3$).
[b] Limit of quantification = 0.17 μg/ml (assuming a density of 1).

Table 3
Related impurities.

Formulations	n[a]	Impurities (%)			
		Oxidised testosterone	Epitestosterone	Highest unidentified impurity	Sum
Androgel®	0	ND	ND	ND	0.00
Testim®	3	0.07	0.28	0.06	0.41
Tostrex®	7	0.05	0.41	0.19	1.01
Intrinsa®	8	0.12	0.28	0.18	0.98
Androtiv®	4	0.30[b]	0.55[b]	0.09	0.99

ND: Not detected.
[a] *n*, number of impurities above reporting threshold of 0.05%.
[b] Above specification limit.

upon the relative retention times, the UV-spectrum and the mass spectrum, two of the impurities were identified in the products examined: epitestosterone and oxidised testosterone. For the Intrinsa® transdermal patch, an initial result of 1.45% was obtained for the sum of impurities. However, upon careful examination of the LC–UV/MS data, some of the peaks observed could be attributed to non-testosterone-related compounds, probably arising from the patch itself, leading to a sum of testosterone-related impurities of 0.98%.

3.3. Microscopy

Microscopical investigation of the four testosterone-containing gel formulations (see Fig. 2) revealed a significant difference between Androtiv® and the other products: presence of highly abundant, large needle-like crystals *vs.* a minor amount of spherical particles, respectively.

3.4. Dissolution experiments

Due to the very low solubility of testosterone in water, a solubilizer had to be added to the dissolution medium. From a regulatory quality point of view, solvent-based dissolution/receptor media are a last choice. Although ethanol-based solvents are quite often used, this solvent was hence not retained for this investigation. As the use of a biorelevant dissolution medium may be helpful in product development and in establishing *in vitro*–*in vivo* correlations [16], and BSA-containing receptor media are recommended for *in vitro* transdermal investigations [17], we chose to add BSA to the physiological PBS buffer used as dissolution medium. In order to determine a suitable BSA concentration in the medium, the solubility of testosterone at 32 °C (i.e. the physiological skin temperature [18]) was determined at different levels. All experiments were performed in duplicate and a mean RSD value of 3.88% was obtained, which was considered acceptable for our purpose. The results showing a linear relation between testosterone solubility and the BSA concentration are given in Fig. 3. Based upon extraction cell volume, testosterone concentration in the formulation and dissolution medium volume, the theoretical maximum testosterone concentration in the dissolution medium was calculated to be 0.23 mM (which is equivalent to 50 mg = 0.17 mmol in 750 ml). Although theoretical sink conditions (i.e. solubility exceeding 2.3 mM) would require higher BSA concentrations if all testosterone was to be released from the drug product, 5.0% m/V of BSA was added to the dissolution medium. This BSA concentration corresponds to a testosterone solubility of 1.6 mM, and is generally considered to be the mean albumin concentration in human serum [19], resulting in a physiologically relevant dissolution medium.

Dissolution experiments on Libido Edge™ and Intrinsa® were not performed as the former does not contain detectable amounts of testosterone, and the latter is a transdermal patch which cannot be compared with the gels under our experimental conditions. The remaining four testosterone gel products were tested three times each in a randomised way. Results of the dissolution tests are shown in Fig. 4, where the mean cumulative amount of testosterone in the dissolution medium is plotted against time. At the end of the experiment (i.e. after 12 h) the rank order of the dissolution kinetics was as follows (from high to low): Tostrex®, Androgel®, Testim® and Androtiv®. It should be noted that for Testim® a very high variability in the dissolution results was observed in comparison to the other preparations. For a quantitative comparison of the release data from the different formulations, MDT was calculated. In principle, estimation of MDT requires the time at which the dissolution process is complete [20]. However, our formulations are topical gels and therefore, the completeness of the dissolution/Franz cell process cannot be truly considered. The amount of testosterone released from Androgel® at the end of the experiment (i.e. after 12 h) was chosen as a reference value (M_0). This can be done because at this time-point, a steady-state condition was reached for all formulations, resulting in a linear

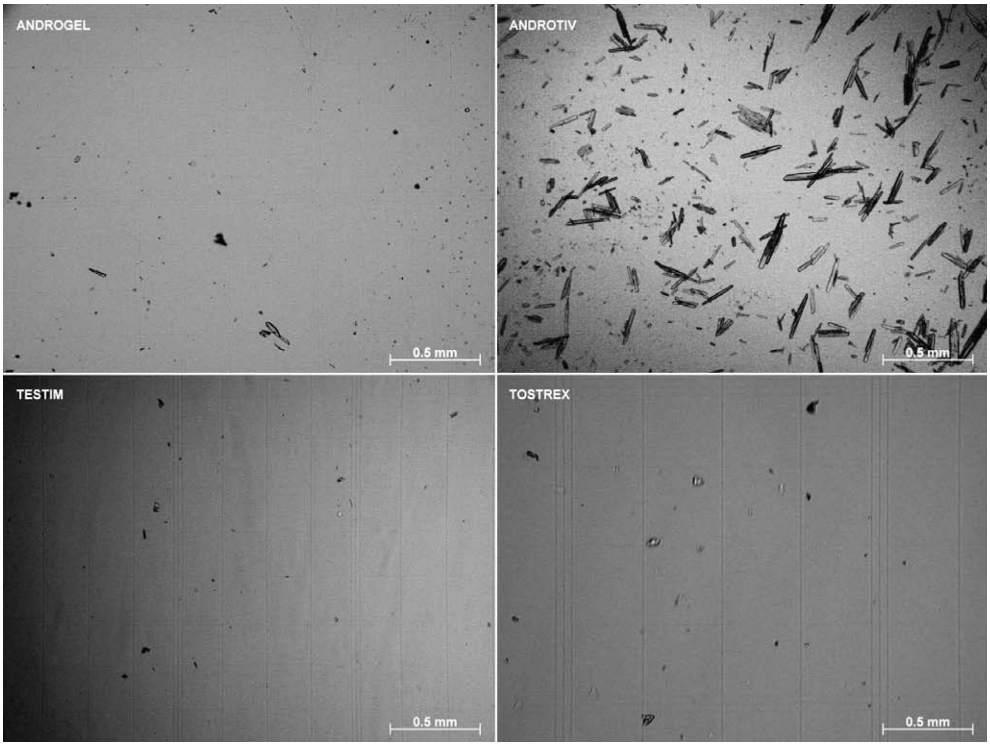

Fig. 2. Microscopic examination of testosterone gels.

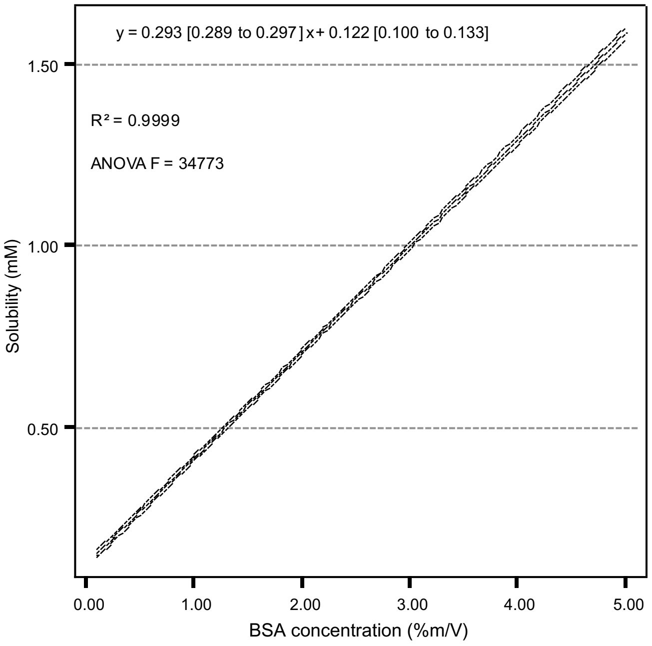

Fig. 3. Testosterone solubility (mM) in function of BSA concentration (95% confidence intervals on the coefficients obtained from linear regression are given between brackets).

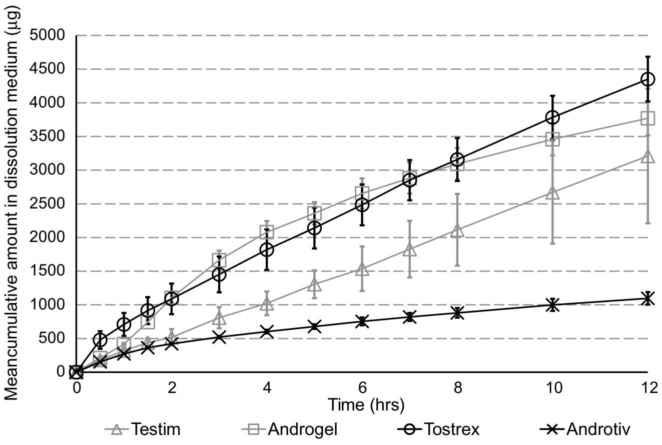

Fig. 4. Drug release profiles of the different testosterone preparations investigated using a standard dissolution apparatus (mean cumulative amount of testosterone ± SEM, *n* = 3).

relationship between amount of drug released versus time. The *ABC* was calculated for each dissolution profile. If the reference value was not yet reached, the corresponding time-point was estimated by performing linear regression on the last four time-points (i.e. 7, 8, 10 and 12 h). The results obtained are given in Table 4 and correspond well with the rank order obtained at the end of the experiment. When comparing the two 2% gels, testosterone release from Androtiv® was five times lower compared to Tostrex®. Although the two 1% gels showed a much smaller difference, their kinetic behaviour is completely different. Testosterone release from Testim® is linear over the whole time-curve, while the dissolution profile for Androgel® shows a bending point [21] at approximately 4 h after which the drug release rate is lowered. While Testim® did not show a bending point at all and Androgel® had a very late bending point of approximately 4 h, both Tostrex® and Androtiv® showed a short bending point of less than 1 h.

Table 4
Drug release kinetics of the formulations investigated.

Formulations	MDT in hours (± STDEV)	
	Dissolution	FDC
Androgel®	4.45 (±0.67)	7.21 (±2.48)
Tostrex®	4.39 (±0.65)	1.88 (±0.68)
Testim®	7.09 (±1.27)	17.41 (±7.17)
Androtiv®	26.82 (±5.91)	9.71 (±3.23)

3.5. Franz diffusion cell experiments

In addition to the "paddle over extraction cell" dissolution approach, the same four gels were tested in triplicate on Franz diffusion cells in a randomised fashion. Results are shown in Fig. 5, where the mean cumulative amount of testosterone in the receptor fluid is plotted against time. The ranking order at 12 h was (from high to low): Tostrex®, Androgel®, Androtiv® and Testim®. The high variability noticed for Testim® in the paddle over extraction cell dissolution tests could not be observed in the Franz diffusion cell experiments. MDT values for each curve were calculated using the amount of testosterone released from Androgel® at the end of the experiment as a reference value and are given in Table 4. If the reference value was not yet reached, the corresponding time-point was estimated by performing linear regression on the last three time-points (i.e. 4, 8 and 12 h). Both 2% gels behaved differently: testosterone diffusion from Tostrex® is much faster compared to Androtiv®. Testim® and Androgel® showed less pronounced differences resulting in similar profiles, although the diffusion rate of testosterone in the receptor medium was significantly higher for Androgel®.

4. Discussion

HPLC–UV/DAD analysis, i.e. the traditional method for quality control of drug substances and products, was used to determine the testosterone content of six different commercially available topical testosterone products obtained from a pharmacy and via the Internet. Even though all testosterone-containing products met conventional acceptance criteria on active pharmaceutical ingredient content, some regulatory-legal issues still remain for the internet-obtained products. Article 1 of Directive 2001/83/EC [22] gives the formal definition of a medicinal product as currently applied in Europe, consisting of two parts: any substance (or combination) presented as having disease treating-preventing properties or having a physiological, pharmaceutical, immunological

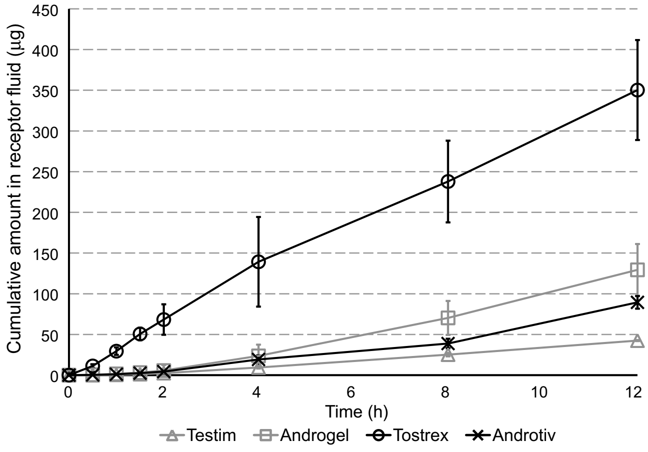

Fig. 5. Drug release profiles of the different testosterone preparations investigated using a Franz cell approach (mean cumulative amount of testosterone ± SEM, *n* = 3).

metabolic or diagnostic action. Libido Edge™ did not contain a detectable amount of testosterone, but nevertheless it is presented as a testosterone cream claiming therapeutic effects such as blood pressure regulation and prevention of depression (although there is an explicit statement that FDA has not evaluated these statements). On the other hand, Androtiv® was easily obtained via internet and did contain testosterone at a 2% concentration as stated on the product label. However, this product was clearly not a formal pharmaceutical product as it did not contain a license number, nor was the labelling and packaging conform to general pharmaceutical requirements. The product was sent to us from Spain, although the declared marketing responsibility was from Switzerland. Last, in most EU countries, including Belgium (where the product was ordered), testosterone is a prescription only medicine (POM), while nothing was requested or informed on the Internet on ordering. This demonstrates that steroid hormone products can be easily obtained without proper control by competent authorities or by medical supervision, while the internet user cannot be expected to be aware of those health risks and legal violations.

Impurity profiling is an important aspect of chemical quality control. Therefore we investigated the five testosterone-containing products for related substances by HPLC. UV detection alone may be misleading, as unrelated substances may be extracted and detected as well. Therefore, MS is an additional requirement in this type of testing as it can differentiate the API-related impurities from the matrix or excipient compounds. All Ph.Eur. monographs are legally binding as stated in Directive 2001/83/EC [22]. The Ph.Eur. gives limits of impurities in its API-specific monographs, based upon the impurity profiles found in APIs of products in the market. Therefore, the API-specific monograph reflects the current status of API quality. Besides the API-specific monographs, there are the mandatory general monographs which need to be combined with the API-specific monograph. If this specific or general monograph is explicitly referring to a general chapter, then this general chapter becomes mandatory as well. Hence, the specific monograph of testosterone API [15] is to be combined with the general monograph of "Substances for pharmaceutical use", which is also mandatory and which refers to the general chapter "Impurities". This general chapter on impurities reflects the principles of the ICH guideline Q3A and gives a decisional flowchart for interpretation. Last ICH-Q3B deals with API impurities, arising from degradation or interaction, in the finished drug products. While the settings of specifications was, and still can be, a challenge and often a matter of debate, there has been an evolution towards a consensus around the above ICH guidelines. In principle, the API-synthesis impurities which cannot be degradants in the forced degradation products (FDPs) are sensu stricto not required to be incorporated in the FDP specifications as they are verified at the API level. However, for transparent consistency and analytical interpretation reasons, the FDP may encompass all related impurities, i.e. not only the degradants and interaction products, but also the API-synthesis impurities. Moreover, independent, external inspections will consider all related impurities as well. The Ph.Eur. monograph on testosterone defines 10 specified impurities, which are thus controlled by the monograph and limited as well. No other detectable impurities (ODIs) are given. The general acceptance criterion defined by the specification limit of 'any other impurities' is 0.1%, while the disregard limit is 0.05% (identical to the reporting threshold of ICH-Q3A). As this is identical to the applicable identification threshold (maximum daily dose (MDD) of testosterone equals 100 mg), unspecified impurities in API testosterone is limited to 0.1%. Limits for each specified impurity is explicitly given in the monograph: 0.1% (impurities A, B, E, F, G, H and J), 0.2% (impurities D and I) and 0.5% (impurity C). As the general qualification threshold for impurities in API for this MDD is 0.15% (ICH-Q3A), impurities C, D and I are considered safe at higher levels. Next, evaluation of API impurities in drug products is done according to ICH-Q3B, with a reporting threshold of 0.10% and an identification and qualification threshold of 0.20%. Impurities above the qualification threshold may possibly present a risk, which should in principle be assessed by geno- and sub-chronic toxicity tests. Of course, when the Ph.Eur. specifies a limit above the qualification threshold, then this higher level is considered safe. As such, we can propose 0.20% for our investigation for individual specified and unspecified impurities, except for impurity C (epitestosterone) which has a limit of 0.50% (identical to Ph.Eur. limit in the API). Acceptable levels of impurities were found for the drug products with proper manufacturing authorisation, i.e. Androgel®, Testim® and Intrinsa®. However, for Androtiv®, epitestosterone and a testosterone-related impurity identified as an oxidised compound were observed above the acceptance thresholds of 0.50 and 0.20%, respectively.

Dissolution testing is a very important tool to characterise active ingredient release from drug products, and is well established for testing solid dosage forms [23]. Nevertheless, its usage is currently gaining more acceptances for other dosage forms, such as semi-solid topical preparations [24]. In the latter case, a Franz diffusion cell approach as recommended by the FDA is most often used [25]. The composition of the dissolution and receptor medium used for investigation of products containing water-insoluble products requires special attention as the maintenance of sink conditions is a challenge. Use of surfactants can be problematic, especially in the Franz diffusion cells, as they result in foaming and formation of air bubbles during mixing [3]. On the other side, the use of organic solvents as aqueous mixtures diminishes the physiological relevance. The chemical characteristics of the membrane do not generally influence the release rate of drugs from topical dosage forms [2], with polysulfone, cellulose acetate, nylon, Teflon and polycarbonate membranes being frequently used [25]. We investigated the dissolution behaviour of four testosterone topical preparations, and each clearly showed a significantly different behaviour independent of the concentration, both in a standard dissolution apparatus as in Franz cell experiments. The observed differences between the formulations are due to the qualitative as well as quantitative excipient differences. However, as the exact quantitative compositions of these commercially obtained products are not known, the observed differences cannot be further assigned to formulation differences. Interestingly, both the biopharmaceutical approaches used did not result in the same ranking order or dissolution profile, although Tostrex® and Androgel® showed the highest dissolution/diffusion rates in both the techniques. This confirms that, given the variety of formulations, sites of application and different release rates, no single test procedure would be suitable for complete biopharmaceutical characterisation and quality control of semi-solid topical dosage forms [24]. As no *in vivo* pharmacokinetic data is available, the risk of overdiscriminating is acknowledged. However, for quality control purposes and from a regulatory point of view, an overdiscriminating test is preferred as it guarantees more consistency in quality, irrespective of its *in vivo* relevance.

5. Conclusions

Due to the continually increasing marketing techniques in a globalized environment, it becomes very easy for patients/customers to obtain pharmaceutical products, defined by content or presentation. Especially, the Internet provides opportunities to circumvent established safeguards. Findings of this study suggest that topical preparations obtained via the Internet cannot be considered interchangeable with the prescribed drugs in terms of legal status or impurity profiling. Moreover, none of the testosterone topical products investigated showed an equivalent biopharmaceutical behaviour.

Acknowledgements

The authors thank Roosmarijn De Cock, Samuel Bodé, Valentijn Vergote, Vera Huys and Ann De Meulemeester for their technical assistance.

References

[1] FDA, SUPAC-SS Guidance for Industry Nonsterile Semisolid Dosage Forms Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In vitro Release Testing and In vivo Bioequivalence Documentation. in: U.S.D.o.H.a.H. Services (Ed.), Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 1997.

[2] G.L. Flynn, V.P. Shah, S.N. Tenjarla, M. Corbo, D. DeMagistris, T.G. Feldman, T.J. Franz, D.R. Miran, D.M. Pearce, J.A. Sequeira, J. Swarbrick, J.C. Wang, A. Yacobi, J.L. Zatz, Assessment of value and applications of in vitro testing of topical dermatological drug products, Pharm. Res. 16 (9) (1999) 1325–1330.

[3] V.P. Shah, J.S. Elkins, R.L. Williams, Evaluation of the test system used for in vitro release of drugs for topical dermatological drug products, Pharm. Dev. Technol. 4 (3) (1999) 377–385.

[4] W.W. Hauck, V.P. Shah, S.W. Shaw, C.T. Ueda, Reliability and reproducibility of vertical diffusion cells for determining release rates from semisolid dosage forms. Pharm Res 24 (11) (2007) 2018–2024.

[5] ICH, Q6A: Specifications: Test Procedures and Acceptance Criteria for New Drug Products: Chemical Substances, 1999.

[6] ICH, Q3A (R2): Impurities in New Drug Substances, 2006.

[7] ICH, Q3B (R2): Impurities in New Drug Products, 2006.

[8] Monograph 01/2008:2034 Substances for pharmaceutical use, in: European Pharmacopoeia sixth ed., vol. 1, 2007, pp. 703–705.

[9] Monograph 01/2008:51000 Control of impurities in substances for pharmaceutical use, in: European Pharmacopoeia 6th Edition, vol. 1, 2007, pp. 653-656.

[10] B. De Spiegeleer, V. Vergote, A. Pezeshki, K. Peremans, C. Burvenich, Impurity profiling quality control testing of synthetic peptides using liquid chromatography-photodiode array-fluorescence and liquid chromatography-electrospray ionization-mass spectrometry: the obestatin case, Anal. Biochem. 376 (2) (2008) 229–234.

[11] D.J. Handelsman, Testosterone: use, misuse and abuse, Med. J. Aust. 185 (8) (2006) 436–439.

[12] B. Dickinson, L. Goldberg, D. Elliot, D. Spratt, A.D. Rogol, L.H. Fish, Hormone abuse in adolescents and adults – a review of current knowledge, Endocrinologist 15 (2) (2005) 115–125.

[13] Monograph 01/2008:20904 Dissolution test for transdermal pathes, in: European Pharmacopoeia 6th Edition, vol. 1, 2007, pp. 275–277.

[14] J. Emami, N. Tavakoli, Formulation of sustained-release lithium carbonate matrix tablets: influence of hydrophilic materials on the release rate and in vitro–in vivo evaluation, J. Pharm. Pharmaceut. Sci. 7 (3) (2004) 338–344.

[15] 01/2008:1373 corrected 6.0 Testosterone, in: European Pharmacopoeia 6th Edition, vol. 2, 2007, pp. 3030–3031.

[16] V.P. Shah, The role of dissolution testing in the regulation of pharmaceuticals: the FDA perspective, in: J. Dressman, J. Kramer (Eds.), Pharmaceutical Dissolution Testing, Taylor & Francis Group, New York, 2005, pp. 81–96.

[17] S.E. Cross, Y.G. Annisimov, B.M. Magnusson, M.S. Roberts, Bovine-serum-albumin-containing receptor phase better predicts transdermal absorption parameters for lipophilic compounds, J. Invest. Derm. 120 (4) (2003) 589–591.

[18] OECD, Guidance document for the conduct of skin absorption studies, in: OECD Series on Testing and Assessment No. 28, Paris, 2004, p. 21.

[19] B.T. Veering, A.G. Burm, J.H. Souverijn, J.M. Serree, J. Spierdijk, The effect of age on serum concentrations of albumin and alpha 1-acid glycoprotein, Br. J. Clin. Pharmacol. 29 (2) (1990) 201–206.

[20] P. Macheras, C. Reppas, J.B. Dressman, Biopharmaceutics of Orally Administered Drugs, Ellis Horwood, New York, 1995.

[21] L. Van Vooren, G. Krikilion, J. Rosier, B. De Spiegeleer, A novel bending point criterion for dissolution profile interpretation, Drug Dev. Ind. Pharm. 27 (8) (2001) 885–892.

[22] Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the community code relating to medicinal products for human use, 2001.

[23] S. Azarmi, W. Roa, R. Lobenberg, Current perspectives in dissolution testing of conventional and novel dosage forms, Int. J. Pharm. 328 (1) (2007) 12–21.

[24] M. Siewert, J. Dressman, C.K. Brown, V.P. Shah, FIP/AAPS guidelines to dissolution/in vitro release testing of novel/special dosage forms, AAPS Pharm. Sci. Tech. 4 (1) (2003) E7.

[25] K.D. Thakker, W.H. Chern, Development and validation of in vitro release tests for semi-solid dosage form-case study, Dissolution Technologies 10 (2) (2003) 10–15.