An alternative definition of premature ejaculation (PE) has recently been proposed by The International Society for Sexual Medicine (ISSM). It is the first evidence-based definition of lifelong PE, and it specifies it as a male sexual dysfunction characterized by ejaculation which always or nearly always occurs prior to or within about one minute of vaginal penetration; the inability to delay ejaculation on all or nearly all vaginal penetrations; and negative personal consequences, such as distress, bother, frustration and/or the avoidance of sexual intimacy (1). There are three other definitions that function in the field simultaneously. However, although all of them describe the time to ejaculation, the inability to control or delay ejaculation and negative consequences of PE like bother or distress (2), there is still no consensus about which of them is the most adequate. Despite the lack of knowledge about the etiology and approved treatments, PE is the most prevalent male sexual dysfunction. It is estimated that 23% of men suffer from the dysfunction (3), and it varies only slightly among age groups. According to the Global Study of Sexual Attitudes and Behaviors, depending on the region of the world, 12–30% (4) of men aged 40–80 suffer from PE. It is, however, believed that the majority of cases of PE are not diagnosed, as only 9% of sufferers consult their physicians (3, 5). Men with PE are embarrassed at discussing their condition and doubt in the success of treatment (6). Because of the subtlety of the issue, they prefer helping themselves on their own, than visiting a specialist. Polish sex shops make efforts to meet the needs of these men. They offer creams and aerosols which are supposed to delay ejaculation and elongate intercourse, and, what is more, they can be purchased discreetly without embarrassment. Some of these products have local anesthetics (lidocaine and benzocaine) declared on the label. The composition seems adequate, as one of the possible etiological factors of PE is penile hypersensitivity (7, 8). Although local anesthetic agents reduce the sensitivity of the glans penis and consequently delay ejaculatory latency, there is only one drug designed for PE indications – dapoxetine – which has been approved.

DETERMINATION OF LOCAL ANESTHETICS IN ILLEGAL PRODUCTS USING HPLC METHOD WITH AMPEROMETRIC DETECTION

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Abstract: An HPLC method with amperometric detection was developed for analysis of two local anesthetics (lidocaine and benzocaine) in products for delaying ejaculation illegally marketed in Polish sex shops. Chromatographic elution on an RP column C18 with mobile phase composed of acetate buffer with acetonitrile, provides an optimal separation not only of active substances but also electroactive preservatives which are occasionally added to cosmetic creams (methylparaben and propylparaben). Application of glassy carbon electrode as a working electrode and a procedure with pulsed potential waveforms enables a sensitive, accurate measurement within a relatively short analysis time (250 s). This method has been successfully employed for the determination of local anesthetics in products under investigation. The obtained results show that most samples contained therapeutic concentrations of lidocaine or benzocaine. According to European law, a sale of products containing lidocaine or benzocaine outside the pharmacy sector is forbidden.

Keywords: forensic science, premature ejaculation, illegal products, local anesthetics, high performance liquid chromatography, amperometric detection, glassy carbon electrode, lidocaine, benzocaine
for treatment of PE in ten countries (9), while all other drugs have to be administered off-label, topical anesthetics and selective serotonin reuptake inhibitors SSRI (daily use) are still recommended as the first-line treatment option in lifelong PE (2). Several studies have reported the efficiency and safety of local anesthetics in treatment of PE, such as ointments (10–12) and spray formulations (13, 14). Newly developed PSD-502 (Shionogi Pharma, Inc.) also known as TEMPE (Topical Eutectic Mixture for Premature Ejaculation) is a metered-dose aerosol containing an eutectic-like mixture of base forms of lidocaine and prilocaine with an oily texture. The results of two double-blind placebo-controlled phase III clinical trials are encouraging. Its advantages are dose-controlled delivery system and rapid effect onset (15–17). However, the majority of authors emphasize that education and psychological support must be provided by a physician to both partners, particularly to formulate the rules, which have to be applied to prevent possible adverse reactions to the treatment. The most common side-effects are “burning” sensation on application, penile irritation, loss of sensitivity (penile and/or vaginal), difficulties in reaching orgasm or loss of erection due to numbness of the penis. Such treatment should be avoided when the partner is pregnant. If a cream is applied, the use of a condom is required to prevent transfer of residual cream to the partner. Topical anesthetics are contraindicated for patients and/or their partners with allergies to any component of the product (13, 18, 19). While purchasing such products in sex shops, no information about usage and side-effects is provided. Quality of such preparations is thus questionable.

Categorization of these products is not obvious. Under Polish law (20), they cannot be pharmaceutical products without marketing authorization. On the other hand, they simultaneously cannot be classified as cosmetic products as the presence of lidocaine and benzocaine in such products is strictly prohibited (21).

The most frequent technique of determination of lidocaine and benzocaine is HPLC with spectrophotometric detection (22–25). Mass spectrometry (26–28) and electrochemistry (29–33) are also used. Authors implemented their methods not only for the variety of pharmaceutical formulations but also for biological samples. In ointment formulation parabens as preservatives are often added. Barbato et al. described simultaneous quantitation of some local anesthetics, antihistamines and preservatives in skin cosmetics (34). In the literature, electroactivity of parabens has been confirmed (35).

Our purpose was to develop a method with amperometric detection capable of chromatographic separation of local anesthetics and parabens as well as to determine the presence of lidocaine and benzocaine in samples obtained from Polish sex shops. There is no study that reports the possibility of such quantitation. Only Daniela de Orsi et al. have investigated illegal adulterants (local anesthetics among others) in cosmetic creams sold on the Internet websites using HPLC-MS and HPLC-UV-DAD (36). Amperometric detection, chosen in our study, guarantees selective measurements with high sensitivity, often greater than spectrophotometric procedures. Pulse potential waveforms, applied in the method, are useful for repeatable and reproducible determinations.

EXPERIMENTAL

Chemicals and reagents

Reagents: acetonitrile and methanol (Rathburn Chemicals Ltd.), acetic acid 100% (AppliChem), anhydrous sodium acetate (Fluka) — all of them of HPLC grade and lithium chloride (Merck), redistilled water additionally purified in the Nanopure Diamond UV Deionization System (Barnstead).

Reference standards: lidocaine hydrochloride — 2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide hydrochloride (LID) was purchased from AstraZeneca. Benzocaine — ethyl p-aminobenzoate (BNZ), methyl 4-hydroxybenzoate — methylparaben (MP), propyl 4-hydroxybenzoate — propylparaben (PP) were from Sigma-Aldrich.

Cosmetic products: four different creams (“Orgasmus-Stopper”, “LANGZEIT”, “COR rige” produced by MILAN Arzneimittel GmbH, Germany and “Men stop-stop” manufactured by INVERMA Johannes Lange GmbH + CO KG, Germany) and one spray (STUD 100 — Pound International Ltd., England). All samples were obtained from Polish sex shops. They were offered as effective remedies for delaying ejaculation.

Equipment and chromatography conditions

The analysis were carried out using liquid chromatograph composed of an LC Ultimate 3000 system (Dionex, Germering, Germany) equipped with a pump, a degasser, an autosampler coupled with a pulse damper and a column heater (ESA, Chelmsford, MA, USA). This part was controlled by Chromelone 6.8 software (Dionex). For amperometric detection the following equipment was used: ESA analytical cell 5040 with a glassy carbon electrode GCE (working electrode), a solid-state palla-
Determination of local anesthetics in illegal products using HPLC method...

The analysis was performed in an isocratic mode on a C18 analytical column Hypersil Gold 150 × 4.0 mm; 3 µm with a guard column (Thermo Fisher Scientific). The mobile phase consisting of acetonitrile : 0.01 M sodium acetate adjusted to pH = 4.80 with acetic acid (54:46, v/v) with an addition of lithium chloride (0.05 M in mobile phase) was filtered through 0.22 µm nylon filters. The column was maintained at 25°C in a column block heater. A flow rate was set at 1.0 mL/min, the injection volume was 10 µL, electrode potential +0.85 V.

**Standard solution preparation**

Stock standard solutions were prepared by dissolving separately a weighed amount (about 10 mg) of each tested substance with acetonitrile : water (50:50, v/v) in 10 mL volumetric flask. Obtained solutions were diluted with mobile phase to the appropriate concentrations.

**Sample solutions preparation**

Weighed amounts of cosmetic creams equivalent to 0.2 mg LID or BNZ, transferred to 50 mL volumetric flask and made up to volume with mobile phase were heated to 40°C and shaken to obtain homogenous dispersion. After cooling down, solutions were filtered through a hard filter paper. Filtrates were use to prepare final concentrations (2 µg/mL of active substance). For aerosol sample, the concentration of LID in the liquid from a container and the amount of LID delivered in a single dose (3–8 sprays) were tested. For this purpose, 120 mg of liquid or 5 sprays were dissolved in acetonitrile.

### Table 1. Summarized validation parameters for lidocaine and benzocaine determined by HPLC-ED.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Lidocaine</th>
<th>Benzocaine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity [µg/mL]</td>
<td>0.15–4.99</td>
<td>0.05–2.51</td>
</tr>
<tr>
<td>LOD [µg/mL]</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>LOQ [µg/mL]</td>
<td>0.15</td>
<td>0.05</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = 0.1799 x + 0.0036</td>
<td>y = 0.8436 x + 0.0235</td>
</tr>
<tr>
<td>r²</td>
<td>0.9999</td>
<td>0.9995</td>
</tr>
<tr>
<td>Range [µg/mL]</td>
<td>1.60–2.40</td>
<td>1.67–2.51</td>
</tr>
<tr>
<td>RSD Intra-day [%]</td>
<td>0.09–0.76</td>
<td>0.05–0.30</td>
</tr>
<tr>
<td>RSD Inter-day [%]</td>
<td>0.80–1.31</td>
<td>0.45–0.84</td>
</tr>
<tr>
<td>Recovery [%]</td>
<td>99.81–100.31</td>
<td>100.00–100.01</td>
</tr>
</tbody>
</table>

### Table 2. Determination of active substances in investigated products.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Form</th>
<th>Active substance</th>
<th>Concentration declared</th>
<th>Concentration found</th>
<th>RSD [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Orgasmus-Stopper”</td>
<td>cream</td>
<td>lidocaine HCl</td>
<td>0.18 g/100 g</td>
<td>0.200 ± 0.006 g/100 g</td>
<td>4.24</td>
</tr>
<tr>
<td>“STUD 100”</td>
<td>aerosol</td>
<td>lidocaine</td>
<td>9.6%</td>
<td>8.88 ± 0.04%</td>
<td>0.59</td>
</tr>
<tr>
<td>“LANGZEIT”</td>
<td>cream</td>
<td>benzocaine</td>
<td>8.00 mg/100 g</td>
<td>7.583 ± 0.006 g/100 g</td>
<td>1.15</td>
</tr>
<tr>
<td>“COR rige”</td>
<td>cream</td>
<td>benzocaine</td>
<td>8.00 mg/100 g</td>
<td>7.531 ± 0.009 g/100 g</td>
<td>1.81</td>
</tr>
<tr>
<td>“Men stop-stop”</td>
<td>cream</td>
<td>benzocaine</td>
<td>-</td>
<td>3.93 ± 0.07 mg/100 g</td>
<td>2.60</td>
</tr>
</tbody>
</table>
and diluted in mobile phase, then filtered and treated as cream solutions. Six replicates of each sample were analyzed.

RESULTS AND DISCUSSION

Method optimization

The composition of mobile phase was optimized to obtain good resolution of peaks ($R_s = 2$) (Fig. 1). Suitable separation of all electroactive substances (LID, BNZ, MP, PP) was achieved with the mobile phase consisting of acetate buffer : acetonitrile (46:54, v/v) within 250 s.

To prevent electrode fouling due to reaction product adsorption on the surface of working electrode, pulsed potential waveforms were applied. The optimal detection procedure consisted of four distinct potential steps. At the detection potential ($E_{det} = +0.85 \text{ V}$) applied during a period $t_{det} = 0.4 \text{ s}$ the components to be analyzed are oxidized. The electrode surface was cleaned afterwards by application of negative potential ($E_{red} = -0.55 \text{ V}$) for $t_{red} = 0.2 \text{ s}$ at first and positive potential ($E_{ox} = +1.0 \text{ V}$) over $t_{ox} = 0.07 \text{ s}$ subsequently. Additionally, stabilization step with a potential $E_{stab}$ equal to measuring potential is applied for $t_{stab} = 0.2 \text{ s}$, before the next cycle of waveform. As a result, detection is constantly sensitive and electrode responses are very reproducible.

Method validation

The validity of the method was investigated regarding the ICH guidelines [37]. The data concerning method validation are summarized in Table 1. In order to determine the linearity of LID and BNZ, at least 5 concentrations of standard solutions were tested under the optimum conditions, injected in triplicate. The linear response was obtained for both analytes with the regression equations $y = 0.1806x - 0.0031$, $r^2 = 0.9999$ for LID and $y = 0.8436x - 0.0235$ for BNZ, $r^2 = 0.9995$. The detection limit (LOD) and quantitation limit (LOQ) were calculated as signal-to-noise ratio of 3:1 and 10:1. The repeatability and the accuracy of the recovery were assessed using linear response function at three concentrations injected in triplicate, covering the specified range 80–120% (1.6 – 2.4 µg/mL). The within-day variation was minimal with RSD ranging from 0.05 to 0.76%. Intermediate precision was calculated from 3 days. The mean recoveries were in the range of 99.8–100.3%. To evaluate the efficiency of the extraction procedure, standard addition method was applied. Because the composition of ointment base was unspecified in most samples, standard solution was spiked with the analyzed product. The efficiency was determined as 92%.
Application of the method

One cream and one spray with declared lidocaine in its content, two creams with benzocaine declared and one cream which should not contain any local anestetic (“Men stop-stop”) were investigated (Fig. 2 a,b). Under Polish law (38, 39), all of them were marketed illegally. Although they contained local anesthetics, they were sold outside pharmacies. Moreover, none of them enclosed leaflet in Polish language. Except for “Orgasmus-Stopper”, none of products contained any information about contraindications, side effects and precautions which should be taken while using them. The content of active substance in all tested prod-

Figure 2. Chromatograms obtained from, samples with lidocaine (a), samples with benzocaine (b); analyzed solutions (2 µg/mL of active substance): 1 – “Orgasmus-stopper”, 2 – “Stud 100”, 3 – “Langzeit”, 4 – “Cor rige”, 5 – “Men stop-stop”
The actual and declared concentrations in tested creams were similar. High differences among single results in “Orgasmus-Stopper” cream, in comparison with other cream preparations, suggest lack of homogeneity and poor quality of this product. Considerably better results were obtained for spray formulation as it is produced in the form of oily solution. However, dosing device did not work properly; it provided over 21% more lidocaine per dose than it was stated on the label. It is worth adding that pharmaceutical product in aerosol formulation with similar concentration of lidocaine authorized for marketing in Poland (Lidocain-EGIS) is dispensed only on the basis of medical prescription. In “Men stop-stop” cream, theoretically without any local anesthetics, low content of benzocaine was detected (Table 2). Its presence was confirmed by LC-ESI-TOF-MS/MS experiment (Fig. 3).

CONCLUSION

In this work, HPLC method with amperometric detection for the determination of two local anesthetics, most frequently used in cream preparations, has been developed. Simple preparation of samples and optimized conditions of analysis provide a high sensitivity and suitable separation of active compounds (benzocaine, lidocaine) and electroactive preservatives (methylparaben, propylparaben) within short analysis time. Above advantages suggest that this method may be successfully applied as a screening method to verify the absence of forbidden compounds – benzocaine and lidocaine in cosmetic creams. Apart from creams for premature ejaculation, the developed method can be also used for the control of anesthetics illegally added to after-sun cosmetics to soothe pain of damaged skin after sun exposure.

Although the usage of local anesthetics as treatment method for PE is fully justified, proved and developing (PSD-502 after 2 phase 3 clinical trials, “Stud 100” also known as “Premjact” spray available over-the-counter in some countries (5)) it should be applied under physician control. This kind of products, marketed in sex shops, do not always ensure compliance with declared composition, content and good quality of medicament. They can be misused and become healthy hazardous.

REFERENCES


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