Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry Measurement of Caffeine in Caffeine-Laced Pants and in Urine and Skin of a Pants User

Manuela Pellegrini 1, Daniela De Orsi 2, Carmine Guarino 2, Maria Concetta Rotolo 2, Rita di Giovannandrea 1, Roberta Pacifici 1 and Simona Pichini 1,*

1 Department of Therapeutic Research and Medicines Evaluation, National Institute of Health, V.le Regina Elena 299, 00161 Rome, Italy; E-Mails: manuela.pellegrini@iss.it (M.P.); rita.digiovannandrea@iss.it (R.G.); roberta.pacifici@iss.it (R.P.)
2 Centro Nazionale Organismo Notificato Dispositivi e Cosmetici (ONDICO), National Institute of Health, V.le Regina Elena 299, 00161 Rome, Italy; E-Mails: daniela.deorsi@iss.it (D.O.); carmine.guarino@iss.it (C.G.); Mariaconcetta.rotolo@iss.it (M.C.R.)

* Author to whom correspondence should be addressed; E-Mail: simona.pichini@iss.it; Tel.: +39-06-4990-3682; Fax: +39-06-4990-2016.

Received: 31 January 2014; in revised form: 31 March 2014 / Accepted: 6 April 2014 / Published: 15 April 2014

Abstract: A fast and sensitive ultra-performance liquid chromatography tandem mass spectrometry method was developed for the measurement of caffeine in caffeine-laced pants and in urine and skin of a pants user. The substance and its internal standard (N-ethylnorcotinine) were separated by reversed phase chromatography with 5 mM ammonium formate pH 3.0 and 0.3% formic acid in acetonitrile mobile phase (83:17 v/v) by isocratic elution and detected by tandem mass spectrometry operated in multiple reaction monitoring mode via positive electrospray ionization. Linearity was studied from 1.4 to 100 ng/mL range for urine, from 5 to 100 ng/cotton swab for skin caffeine and from 1.3 to 100 µg/samples for 4 cm² textile samples. Good determination coefficients ($r^2 = 0.99$) were found in all cases. At three concentrations spanning the linear dynamic ranges of different samples mean recoveries of caffeine were always higher than 80% and intra-assay and inter-assay imprecision and inaccuracy were always better than 105%. For the first time, caffeine content in this cosmetotextile was determined together with the measurement of caffeine released on the user skin, the absorbed amount with resulting urinary concentrations.
1. Introduction

Gynoid lipodystrophy, also known as cellulite is a localized complex skin disorder caused by changes in the subcutaneous adipose layer and in the extracellular matrix, with the protrusion of subcutaneous fat into the dermis resulting in structural and architectural alterations that are visually characterized by an uneven dimpling appearance of the skin [1]. Those changes include enhanced lipogenesis, decreased lipolysis, and increased lipid storage within the adipocytes as well as changes in the dermal architecture [2,3].

Several approaches have been proposed and applied to treat cellulite appearance, such as massages, controlled weight-loss, laser treatments, liposculpture, and surgery, but the most diffused is the use of reducing cellulite cosmetic products (creams, mud-creams, and gels) [4,5].

Topical treatments for cellulite incorporate many substances, such those that increase the microcirculation flow, agents that reduce lipogenesis and promote lipolysis, ingredients that restore the physiological structure of dermis and subcutaneous tissue, and components that scavenge free radicals or prevent their formation [6].

Several active ingredients are plant-derived and with the mostly used “active” ingredients being methylxanthines known to inhibit phosphodiesterase enzymes and increase lipolysis of triglycerides [3]. The most useful and safest methylxanthine is caffeine (1,3,7-trimethyl xanthine), which is normally used at a concentration from 1% to 2% and acts directly on adipocytes, promoting lipolysis with a stimulating effect on skin microcirculation [3,6]. Studies on cosmetic products containing higher percentages of caffeine, from 4% to 7%, showed a significant reduction of adipocytes and consequent diameter decrease on a treated body zone [7,8]. Ultrasound treatment appears to increase absorption and efficacy of topical caffeine [9].

Recently caffeine has been encapsulated in “cosmetotextile” products, such as leggings, pants, and girdles, meant to be worn for their slimming effects.

Cosmetotextile is a technology merging cosmetics and textiles through the process of micro-encapsulation. Microencapsulation is achieved by the use of cyclodextrins, cyclic oligosaccharides, resulting from the enzymatic hydrolysis of starch. They appear as small cylinders with the outer part hydrophilic and a hydrophobic central cavity, of well-defined size, that encompasses partially or completely the guest molecule to form inclusion complexes with it. Substances microencapsulated inside cyclodextrins are then released over time [10,11].

The European Commission (EC) for Standardization published, in 2009, a technical report on cosmetotextiles [12], but a specific European Regulation on this product is still absent. This is due to the fact that cosmetotextile should conform to both, the EC Regulation on cosmetic products, and that on textile products [13,14].
The great interest and the increasing market of caffeine-based cosmetotextiles raised the need to develop test methods to firstly assess the presence and the content of caffeine and then demonstrate and the effectiveness and duration of the declared properties.

Whereas the results advertised by manufacturers include the slimming effect (reduction in the circumference of the thighs and hips) due to controlled release of caffeine during its wearing, and the “massage” created by the elastic fibers constituting the garment, there are no scientific data about the amount of caffeine present in the textile, nor about the amount of released caffeine and eventual toxic effects due to total absorbed quantify of this substance.

As a first approach to this new cosmetic product, we aimed to measure caffeine content in a type of caffeine laced pants commonly sold in United States, Europe, and Italy, the amount of caffeine released on the skin and into the urine of a pants-user during its wearing using a newly developed and validated ultra-performance liquid chromatography-tandem mass spectrometry analysis.

2. Experimental Section

2.1. Chemicals and Reagents

Caffeine and N-ethylnorcoctinine (NENC) used as internal standard (IS) were supplied by Sigma-Aldrich (Milan, Italy). Ammonium formate, ultrapure water, and all other reagents of analytical grade were obtained from Carlo Erba (Milan, Italy). All solvents and solutions, before use for ultra-performance liquid chromatography tandem mass spectrometry, underwent a 10 min ultrasound treatment.

2.2. Cosmetic Caffeine-Laced Pants

Cosmetic slimming caffeine-based laced pants (also known as slimming leggings, covering both thighs to the knee), distributed by a French manufacturer, were purchased at a local pharmacy. The ingredients listed in the label include pure caffeine and coffea arabica seed oil and many other plant extracts (leaf extracts of Ginkgo biloba, Aesculus hippocastanum, Fagopyrum esculentum, Ruscus aculeatus, Sichuan pepper, Coleus forskohlii).

The pants are recommended for specific reduction and firming treatment for abdomen and hips, intensive contour-treatment, body sculpture, anti-cellulite strategy, fluidifying slimming, thermo-active action specific for abdomen and hips against adipose blotches, body shaping, fat reducing treatment, etc. These specific pants are described to be worn for eight hours/day (preferably during night rest) for 10 days.

2.3. Study Design

A healthy forty-five years old female volunteer (161 cm high, 54 kg weight, non-smoker, not using medication during the study), who did not consume any of caffeine, was asked to wear the caffeine laced pants as indicated on the label and above reported. Urine samples were collected at 8.00 p.m. on day zero, before wearing the pants and then daily at 8.00 a.m. and at 8.00 p.m. for the 10 days of pants wearing, and finally at 8.00 a.m. and at 8.00 p.m. of day 11. Urine samples were collected in plastic tubes and stored at −20 °C until analysis. At the same time points, two cotton swabs soaked with ethyl
alcohol were used to clean each day the same area of 4 cm² of the left and right thigh skin to measure daily caffeine present on the total skin area covered by laced pants. For this purpose, the caffeine measured, as reported in subsequent sections, in the two 4 cm² areas cleaned by the swab was divided by four for both thighs to obtain caffeine in 1 cm², and then multiplied for the area of the two user’ upper legs (thighs to the knee) calculated as two truncated cones (one circle at the top of the thigh, one circle at the knee and the height from top tight to the knee). Finally, to assess total caffeine released from laced pants in the 10 wearing days, three squared areas of 4 cm² each were cut from a new pair of laced pants (up, center, and low part of pants) and from the laced pants worn for 10 days at the end of wearing and caffeine measured as reported in subsequent sections. The mean caffeine value of these three textile areas for each of pants pair was divided by four to obtain caffeine per 1 cm² and then multiplied for the total area of the pants calculated as two cylinders (circle diameter was the same at top tights and at the knee). As a control volunteer, the same experiment was performed in a healthy forty-seven year-old female (158 cm high, 56 kg weight, non-smoker, not using medication during the study), not consuming any source of caffeine, was asked to wear anticellulite pants containing seaweeds and safflower seeds oil, but not caffeine.

2.4. Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry (UPLC-MS/MS) for Caffeine Determination

The ultra-performance liquid chromatography-tandem mass spectrometry analyses were carried out using an ultra-high performance liquid chromatography system (Waters Acquity UPLC, Waters Corporation, Milano, Italy) coupled with a triple quadrupole mass spectrometer (Waters Xevo TQ, Waters Corporation). Data acquisition and analysis were carried on using standard software supplied by the manufacturer. Chromatographic separation achieved by an Acquity UPLC HSS C18 column (150 mm × 2.1 mm, 1.8 μm) using a isocratic elution with a mobile phase formed by: 5 mM ammonium formate pH 3 (solvent A, at 83% v/v) and 0.3% formic acid in acetonitrile (solvent B, at 17% v/v). The flow rate was kept constant at 0.3 mL/min during the analysis.

The separated analytes (caffeine and IS) were detected with a triple quadrupole mass spectrometer operated in multiple reaction-monitoring (MRM) mode via positive electrospray ionization (ESI). The applied ESI conditions were the following: capillary voltage at 3000 V, desolvation temperature at 550 °C, source temperature at 150 °C, cone gas flow rate at 20 L/h, desolvation gas flow rate at 1000 L/h, and collision gas flow rate at 7.8 mL/h. Cone energy voltage and collision energy voltage were set at 30 V and 20 V, respectively. A fragmentation voltages (30 V) was applied in order to obtain a quantifying and two significant qualifying ions for the two analytes under investigation. Dwell time was set at 0.078 s. More specifically, at 30 V, MRM chosen transitions were: m/z 195.1–110.0 and 195.1–138.0 for caffeine and m/z 190.9–120.0 and 190.9–79.9 for NENC (IS). The ions in bold [M + 1]⁺ were used for compounds quantification, the transitions for qualification.

2.5. Calibration Standards and Quality Control Samples

Standard stock solutions of caffeine and IS at 1 mg/mL were prepared in methyl alcohol and stored at −20 °C. Calibration standards containing 10 ng IS and different caffeine amounts were prepared for each analytical batch by adding suitable amounts of standard stock solutions to 1 mL blank urine
(4.5, 10, 50, 75, 100 ng/mL), blank cotton swab (5, 10, 50, 75, 100 ng/swab) and blank textiles (4, 10, 50, 75, 100 μg/textile sample) pre-checked for caffeine absence. Calibration samples, injected in triplicate, were treated and processed as unknown samples, which were also analyzed in triplicate. Several aliquots of caffeine quality control (QC) samples (low, medium, and high, respectively) at 7, 40, and 85 ng/mL urine or 7, 40, and 85 ng/swab, or 5, 40, and 85 μg/textile sample were prepared to be used for calculation of validation parameters.

2.6. Analysis Validation Protocol

Prior to application to real samples, ultra-performance liquid chromatography tandem mass spectrometry was tested in a validation protocol following the accepted criteria for bioanalytical method validation [15,16].

Validation protocol applied in the present study included linearity, limits of detection (LOD) and quantification (LOQ), imprecision, inaccuracy, selectivity, carryover, matrix effect, ion suppression, recovery, and stability, as elsewhere described [17]. Specifically, LOD was defined as the lowest concentration with acceptable chromatography, the presence of all transitions with signal-to-noise ratios of at least 3, and a retention time within ± 0.2 min of the average retention time of the calibrator. LOQ was the lowest concentration that met LOD criteria and a signal-to-noise ratio of at least 10. Validation parameters were calculated using five different daily replicates of quality control samples (low, medium, and high quality control) along five subsequent working days.

2.7. Urine Sample Extraction

A 100 μL urine aliquot added with 10 μL IS (10 μg/mL) was transferred into 15-mL screw-capped glass tube and 890 μL of UPLC mobile phase (5 mM ammonium formate pH 3-formic acid in acetonitrile 83:17 v/v) were added. A 10 μL aliquot of this solution was injected in UPLC-Xevo TQ.

2.8. Cotton Swab Extraction

Cotton swabs were placed in a glass tube with 10 μL IS (10 μg/mL) and 4 ml ethyl alcohol and sonicated for a 15 min period. Subsequently, ethyl alcohol collected in another tube was evaporated to dryness under a nitrogen stream and the dry residue was resuspended with 1 mL UPLC mobile phase. A 10 μL aliquot was injected into UPLC-Xevo TQ.

2.9. Textiles Sample Extraction

Squared samples of 4 cm² from new and used pants were placed in a glass tube with 10 μL IS (10 μg/mL) and 4 mL ethyl alcohol and sonicated for a 15 min period. Subsequently, ethyl alcohol collected in another tube was evaporated to dryness under a nitrogen stream and the dry residue was resuspended with 1 mL UPLC mobile phase. A 10 μL aliquot was injected into UPLC-Xevo TQ.
3. Results and Discussion

3.1. UPLC-MS/MS for Caffeine Determination and Validation Parameters

Representative chromatograms obtained following the extraction of a urine sample containing 11.8 ng/mL caffeine (A), a cotton swab sample containing 27.2 ng/swab (B), and textile sample containing 17.2 µg/sample (C), are shown in Figure 1A–C. Total run time of analysis is 3.1 min.

![Figure 1](image.png)

Figure 1. UPLC-MS/MS chromatograms obtained following the extraction of a urine sample containing 11.8 ng/mL caffeine (A), a cotton swab sample containing 27.2 ng/swab (B) and textile sample containing 17.2 µg/sample (C).

Linear calibration curves for caffeine in three different samples (urine, cotton swabs, and textile samples) showed determination coefficients ($r^2$) equal or higher than 0.991. LOD and LOQ values calculated for all the different samples were adequate for the purpose of the present study and absolute analytical recoveries (mean ± standard deviation, SD) obtained for the three different quality control samples were always around 85% (Table 1). The intra- and inter-assay imprecision (measured as coefficient of variation, CV%) and inaccuracy (measured as % error) values were always lower than 10% (Table 2).
Table 1. Method calibration data and recovery of analyte under investigation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Determination of coefficient ($r^2$)</th>
<th>LOD</th>
<th>LOQ</th>
<th>Mean recovery (%) b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low QC sample</td>
<td>Medium QC sample</td>
<td>High QC sample</td>
<td></td>
</tr>
<tr>
<td>Urine caffeine (ng/mL)</td>
<td>0.999 ± 0.004</td>
<td>1.4</td>
<td>4.5</td>
<td>(7 ng/mL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>85.2 ± 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>85.2 ± 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>87.3 ± 1.8</td>
</tr>
<tr>
<td>Cotton swabs caffeine (ng/swab)</td>
<td>0.995 ± 0.003</td>
<td>1.5</td>
<td>5.0</td>
<td>(7 ng/swab)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>85.8 ± 2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>86.6 ± 1.8</td>
</tr>
<tr>
<td>Textiles sample caffeine (µg/sample)</td>
<td>0.996 ± 0.005</td>
<td>1.3</td>
<td>4.0</td>
<td>(5 µg/sample)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>85.3 ± 3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>86.4 ± 2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>86.1 ± 1.2</td>
</tr>
</tbody>
</table>

a Mean ± SD of three replicates of calibration curves; b Mean ± SD of five replicates; LOD, limits of detection; LOQ, limits of quantification; QC: quality control.

Table 2. Intra- ($n = 5$ for each QC sample) and inter-assay ($n = 15$) precision and accuracy for the analyte under investigation in quality control samples. QC, quality control.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Precision (CV%)</th>
<th>Accuracy (% error)</th>
<th>Precision (CV%)</th>
<th>Accuracy (% error)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low QC sample</td>
<td>Medium QC sample</td>
<td>High QC sample</td>
<td>Low QC sample</td>
</tr>
<tr>
<td>Urine (ng/mL)</td>
<td>7</td>
<td>40</td>
<td>85</td>
<td>7</td>
</tr>
<tr>
<td>CV% or % error (e)</td>
<td>8.0</td>
<td>8.8</td>
<td>5.7</td>
<td>3.2</td>
</tr>
<tr>
<td>Cotton swabs (ng/swab)</td>
<td>7</td>
<td>40</td>
<td>85</td>
<td>7</td>
</tr>
<tr>
<td>CV% or % error (e)</td>
<td>7.8</td>
<td>9.4</td>
<td>7.6</td>
<td>3.9</td>
</tr>
<tr>
<td>Textiles sample (µg/sample)</td>
<td>5</td>
<td>40</td>
<td>85</td>
<td>5</td>
</tr>
<tr>
<td>CV% or % error (e)</td>
<td>5.2</td>
<td>e 3.2</td>
<td>e 3.9</td>
<td>3.8</td>
</tr>
</tbody>
</table>

No additional peaks due to endogenous substances, which could have interfered with the detection of the analytes under investigation, were observed in caffeine-free urine, cotton swab, and textile samples (data not shown). No psychoactive drugs other than the compounds under investigation interfered with the assay. Blank samples injected after the highest points of the calibration curve (100 ng caffeine/ml urine, 100 ng caffeine/swab and 100 µg caffeine/textile) did not present any traces of carryover (data not shown).

For the evaluation of ion suppression/enhancement due to matrix effect, extracts of five different blank urine, cotton swab, and textile samples were injected into the system while a solution of caffeine at concentrations of 1000 ng/mL in formic acid (0.1%) was continuously infused at a flow rate of 50 µL/min through a T-mixer connected to the mobile phase just before the ion source. In addition, the
comparison between peak areas of caffeine spiked in extracted blank urine, cotton swab, and textile samples, versus those for pure diluted standards were compared. Experiments were carried out in triplicate. No significant ion suppression/enhancement (less than 10% analytical signal suppression due to matrix effect), evaluated by both the procedures occurred during chromatographic runs (data not shown).

No relevant degradation was observed after any of the three freeze/thaw cycles at room temperature for one hour applied to the different samples, with differences in the initial concentrations of less than 10%.

3.2. Caffeine Content in Laced Pants, on the Skin and into the Urine of a Pants-User

The total initial amount of caffeine in the two cylinders of laced pants resulted to be 36 mg, equally distributed along the leggings-as resulted by the homogeneous quantity measured in three different 4 cm² pieces of laced pants: 50.2, 53.2, and 55.33 µg caffeine/textile sample. After 10 days of wearing, total caffeine remaining in used pants was 10.7 mg. In addition, in this case, caffeine was equally distributed being 15.7, 16.8, and 16.2 µg caffeine in the three different textile samples. This means that total amount of caffeine released by the pants in 10 days was 25.3 mg, being on average 38.3 µg per 4 cm² textile sample (Table 3).

Table 3. Amount of caffeine present in laced pants before and after using, in urine samples at baseline and during the wearing, in the skin swabs collected during wearing and in the skin area.

<table>
<thead>
<tr>
<th>Matrices where caffeine was measured</th>
<th>Caffeine amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine laced pants before using</td>
<td>Total amount: 36 mg.</td>
</tr>
<tr>
<td>Urine samples</td>
<td>Baseline: 1.2 ± 0.1 ng/mL; First day after night wearing: 6.0 ± 0.9 ng/mL; Maximum value at day 8: 50.5 ± 1.4 ng/mL; First day after the end of treatment: 5.7 ± 0.9 ng/mL.</td>
</tr>
<tr>
<td>Skin swabs</td>
<td>Baseline: Not detected in the right and left thigh; First day after night wearing: 91.0 ng/swab on right thigh; 85.2 ng/swab on the left thigh; Value at day 7: 89.3 ng/swab on right thigh; 84.3 ng/swab on the left thigh; First day after the end of treatment: 27.0 ng/swab on right thigh; 30.2/swab on the left thigh; Total amount of caffeine in the skin area: 708 ng for right thigh and 735 ng for left thigh.</td>
</tr>
<tr>
<td>Caffeine laced pants after 10 days using</td>
<td>Total amount: 10.7 mg.</td>
</tr>
</tbody>
</table>

The amount of caffeine detected by daily analysis of the swabs, used for rubbing the same 4 cm² section of skin, ranged from 91.0 ng/swab in the first treatment day to 27.0 ng/swab at the end of treatment in case of right thigh, and of 85.2 ng/swab in the first treatment day to 30 ng/swab at the end of the treatment for left thigh, with a total amount of caffeine present in the skin area during the whole treatment of 708 ng for right thigh and 735 ng for left thigh (Table 3 and Figure 2). Thus, theoretically, since 38.3 µg caffeine were released by the laced pants in ten days of treatment, in a 4 cm² skin area, and just around 708–735 ng were not absorbed, as they remained on thighs skin surface, more than 98% of released caffeine was absorbed by the skin and entered the dermis. This resulted in urinary
caffeine concentration ranging from $6.0 \pm 0.9$ ng/mL the first morning after the pants-wearing, increasing to $21.7 \pm 1.2$ ng/mL the first evening after the treatment and then fluctuating between $30.2 \pm 1.0$ and $50 \pm 1.2$ ng/mL during the following days, to decrease at baseline concentrations starting from the ninth day of pants-wearing (Table 3 and Figure 3).

**Figure 2.** Time trend concentration of caffeine in the swabs used for rubbing the same 4 cm$^2$ section of skin of caffeine laced pants user during and after wearing (right thigh: solid line, left thigh: dashed line).

**Figure 3.** Time trend concentration of urinary caffeine during and after caffeine laced pants wearing (values are expressed as mean of three different determinations and Standard Deviation).

In case of control volunteer, not consuming any source of caffeine and wearing anticellulite pants that did not contain this substance, caffeine was absent in any of urine or swab samples, collected within the ten experiment days.

The interest in this study generate from the fact that in recent times there has been a significant increase in sales of cosmetotextiles containing caffeine as adjuvant for the treatment of cellulite [7],...
because of the potential lipolytic and thermogenic effect lasting all the day after nightly wearing of laced pants or leggings.

This was just a preliminary study conducted in a single pants user that evidenced an amount of 25.3 mg caffeine released by the micro cyclodextrin spheres of the pants in 10 days of wearing. Recently, we measured caffeine content of most commonly sold anticellulite creams and we found caffeine concentration, per gram of cream, varying from 0.3 to 5.4 mg/g [18]. If we consider that an average amount of cream used for tights and hips is 5 g per day (single application), in 10 day of treatment a quantity of caffeine ranging from 15 to 270 mg is used. Thus, the amount of caffeine released by laced pants and consequently absorbed by the users appears to be by this first experiment in the low range of caffeine-containing cosmetics. As a confirmation, urinary caffeine during the whole treatment was lower than 50 ng/mL (Figure 3). A recent study which determined urinary caffeine to estimate dietary caffeine exposure and metabolic phenotyping in 115 anonymous individuals (unknown caffeine consumption), found a mean urinary value of about 6.5 corresponding to 1300 ng/mL (95% confidence interval from about 4.9 to 8.0 µmol/L corresponding to 950–1750 ng/mL), being the lowest value around 20 ng/mL [19]. In the light of these results we can conclude that, in this first experiment caffeine released by a cosmetic textile is a low amount and that absorbed by the skin resulted in low range of urinary concentrations reported for consumers of caffeinated drinks.

4. Conclusion

We developed and validated a fast and sensitive ultra-performance liquid chromatography tandem mass spectrometry method for the measurement of caffeine in caffeine-laced pants and in urine and skin of a pants user. For the first time, caffeine content in this cosmetotextile was determined together with the measurement of caffeine released on the user skin, the absorbed amount with resulting urinary concentrations. From this first experiment, it seems that not toxic effect are predictable when using this cosmetotextile slimming product.

Acknowledgments

The authors acknowledge the contribution of Antonella Bacosi, Simonetta di Carlo and Silvia Graziano.

Author Contributions

Daniela De Orsi, Carmine Guarino, Roberta Pacifici and Simona Pichini designed the study, verified and analyzed the study results, wrote the first draft of the article and approved the last version of the manuscript. Manuela Pellegrini, Maria Concetta Rotolo and Rita di Giovannandrea set up and developed the analytical method, analyzed samples, prepared the material for the article and approved the last version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.
References


© 2014 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).