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Monitoring of clobetasol propionate and betamethasone dipropionate as undeclared steroids in cosmetic products manufactured in Korea

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ABSTRACT

Some cosmetic products manufactured in Korea have been suspected to contain anti-inflammatory corticosteroids, such as clobetasol propionate and betamethasone dipropionate, for the treatment of eczema, seborrhea and psoriasis, without any indication on the label of the cosmetic products. Due to their severe side effects, such as permanent skin atopy, these two corticosteroids in cosmetic products need to be monitored from a forensic point of view. Cosmetic product samples (number of samples = 47) of manufacturers charged by consumers have been collected in local and online markets of Korea, and they were validated and analyzed by a simple high performance liquid chromatography (HPLC) method with ultraviolet diode array (UV–DAD). LC–MS/MS and LC–MS were used to confirm these steroids in cosmetic samples with diagnostic ions (m/z) and isotope ratio.

Linearity was studied with 0.5–10 $\mu\text{g/mL}$ range in both steroids. Good correlation coefficients ($r^2 \geq 0.999$) were found, and their limits of quantifications were 0.59 $\mu\text{g/mL}$ and 0.66 $\mu\text{g/mL}$ in clobetasol propionate and betamethasone dipropionate, respectively. At three different concentrations spanning the linear dynamic ranges, mean recoveries were always higher than 93%, and precisions for intra-day and inter-day analyses were both less than 3.5%.

The results show 32–96.4 $\mu\text{g/g}$ levels of clobetasol propionate in five different cosmetic products. Also, betamethasone dipropionate in a sample was monitored at the level of 195.1 $\mu\text{g/g}$. This fact reveals that some manufacturers have added these steroids in their cosmetic products to advertise the treatment effect for skin atopy. Thus, these cosmetic products need to be monitored carefully, and ultimately removed from the market.

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1. Introduction

Corticosteroids have been garnering attention as highly effective drugs widely used in dermatology for the treatment of inflammatory diseases, in the form of creams, gel, solution and ointments [1–3]. Clinical studies have shown that the use of corticosteroids, such as clobetasol propionate (CP) and betamethasone dipropionate (BP), has significantly enhanced the treatment of patients with dermatoses, such as psoriasis and eczema [4–6]. CP and BP (Fig. 1), analogs of prednisolone, are kinds of synthetic anti-inflammatory glucocorticosteroids [7] with a high degree of activity and a slight degree of mineralocorticoid activity [8]. CP and BP reduce inflammation and can temporarily relieve the symptoms of inflammatory skin problems of severe plaque psoriasis [4,8–11].

These corticosteroids have severe potential side effects, such as permanent skin atopy and pustular psoriasis. Also, some systematic side effects of corticosteroids are hypertension, diabetes

mellitus, osteoporosis, allergic contact dermatitis, Cushing's syndrome, etc. [12]. The risk of these side effects increases with long-term use and high dosage, especially if used without medical supervision. These steroids should be kept away from the face, eyes, nose and mouth [13]. Also, chronic use of potent topical corticosteroid can be of high risk, especially for babies and children.

Thus, cosmetic products absolutely should not contain any corticosteroids. However, some manufacturers of cosmetic products have advertised their products with the indication for the improvement of red, itchy, flaky skin caused by eczema, seborrhea and psoriasis. Consumers were not aware of the presence of these steroids in cosmetic products, which led to improper long-term use and over-dosage, as they believed that their troubled skin would get better [1,2].

Although earlier papers reported a number of different LC methods for these steroids in pharmaceutical formulations or biological matrices, simple validated LC methods were not usually reported in these kinds of cosmetic products [6,14–17].

Therefore, this study aimed to develop and validate analytical methods allowing for the separation and rapid determination of CP

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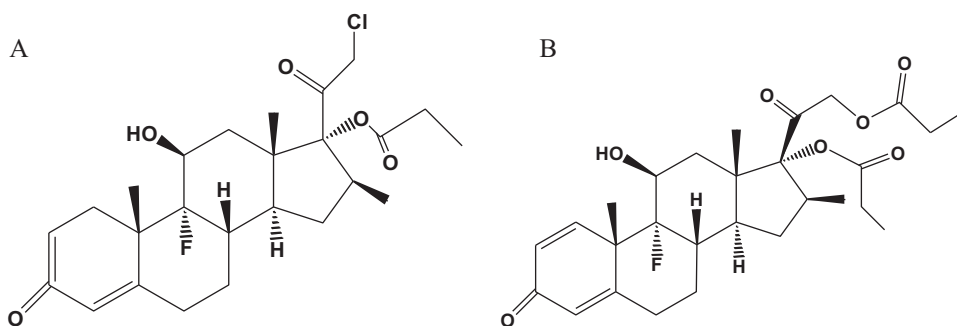


Fig. 1. Structures of [A] clobetasol propionate and [B] betamethasone dipropionate.

and BP, which are added illegally in a large number of cosmetic products. For this research, simple HPLC-UV-DAD method for the rapid screening of CP and BP in cosmetic products (cream, skin, emulsion and essence lotions) was developed, validated and monitored. Also, cosmetic samples which were monitored to include these steroids by LC, were further confirmed using LC-MS/MS and LC-MS [11,14–22].

2. Materials and methods

2.1. Chemicals, solvents and samples

Distilled water was provided by Milli-Q water purification system from Millipore (Bedford, MA, USA), and was used throughout the study. HPLC grade acetonitrile was obtained from J.T. Baker (Philipsburg, NJ, USA). Clobetasol propionate (purity > 98%) was purchased from Fluka (St. Louis, MO, USA). Betamethasone dipropionate (purity > 97%) was obtained from Sigma (St. Louis, MO, USA). 47 different kinds of cosmetic products with indication of containing active ingredients of traditional herbal clinic plants were purchased from local and online markets; these cosmetic samples were made from 22 different manufacturers. A 100 µg/mL stock solution was made by diluting the standards in acetonitrile.

2.2. Validation procedure

Calibration standards of quality control (QC) samples (0.5, 2.5 and 10 µg/mL) containing different amounts of CP and BP were prepared for calculation of validation parameters. The prepared solutions were then stored at 4 °C. Calibration curves were prepared for each analytical substance by adding suitable amount of standard stock solution to blank samples in triplicate. Recovery, matrix effect, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy were measured.

Analytical recoveries were calculated by comparing the peak areas obtained when calibration samples were analyzed by adding the QC samples. The recoveries were obtained by QC samples using seven replicates at each level.

For the evaluation of matrix effects, the peak areas of extracted blank creams spiked with QC samples, after the extraction procedure was compared to the peak areas of pure diluted substances.

Specificity was checked on both standards and samples, by the function “peak purity” of the UV-peak match software (ChemStation), which was based on the analysis of the UV-spectra in various regions of the peak. Linearity was studied in the range of 5-point calibration curve for both steroids. Standard deviation (SD) of the mean noise level over the retention time window of each analyte was used to determine the detection limit (LOD = 3SD) and the quantification limit (LOQ = 10SD). Precision is expressed as the relative standard deviation (RSD) of concentrations calculated for QC samples, and accuracy as the relative error of the calculated concentrations. Both parameters were measured in intra- and inter-day.

2.3. Sample preparation

About 2 g sample was mixed to attain a homogeneous mixture, and 1 g was accurately weighed into a 10 mL volumetric flask. It was then diluted with 10 mL acetonitrile, shaken vigorously and precipitated by centrifuge, filtered through a 0.45 µm filter paper, and transferred into a 1 mL vial.

2.4. Instrumental conditions

The HPLC system was an Agilent 1200 series (quaternary pump, UV-DAD detector, autosampler and column oven). The Agilent ChemStation was utilized to control the system and to acquire and analyze the data. All separations were carried out on ZORBAX® Eclipse XDB-C₁₈, a 5 µm C₁₈ column (150 mm × 4.6 mm, Agilent

Technologies, USA), with a flow rate of 1 mL/min, column temperature of 27 °C, injection volume of 10 µL, and a total running time of 25 min. HPLC method for separation of standard and samples were modified as following. The elution was carried out using mobile phase A (0.1% phosphoric acid in water) and B (acetonitrile) at 240 nm. UV scan mode, which ranges from 210 to 400, attempted to take spectra, useful for comparison of standards as well as samples.

Standards and samples were separated by an initial 20% with solution B, and a subsequent gradient grew up to 50% of solution B in 10 min and increased to 100% until 20 min, and then solution B was changed back to 20% in 25 min.

LC-MS/MS and LC-MS analyses were carried out on a Varian 1200L quadrupole MS/MS equipped with ProStar 430 autosampler and ProStar 335 PDA. Varian workstation version 6.5 software was used for data acquisition and analysis. Chromatographic separation was done using a ZORBAX® Eclipse XDB-C₁₈ column (4.6 mm × 50 mm, 1.8 µm, Agilent Technologies, USA). The flow rate was 0.3 mL/min for 25 min. 0.1% formic acid (solution A) and acetonitrile (solution B) were used as the mobile phases. Eluent composition used for separation under the above HPLC conditions.

Electrospray ionization (ESI) with positive ion mode was selected for mass spectral analysis, and the detector mode as extended dynamic range (EDR). LC-MS/MS or LC-MS was operated with a needle potential of 5 kV, a source temperature of 400 °C, a desolvation temperature of 200 °C, a nebulizing gas pressure of 60 psi, and drying gas pressure of 20 psi.

The API housing and drying gas temperatures were kept at 40 °C and 200 °C. The argon pressure in the collision cell was kept at 1.5 mTorr for MS/MS measurements. The collision energy was 16 V for CP, and 15 V for BP. The scan time was 0.5 s, and the detector multiplier voltage was set to 1500 V. Scan width in SIM and MRM mode was 0.70 amu.

The parameter optimization of MS/MS is as follows. The scan range was performed by 467 (Q1FM), 300 (Q3FM)–500 (Q3LM) for CP; and 505 (Q1FM), 300 (Q3FM)–600 (Q3LM) for BP. Mass spectra were monitored using product scan mode and the following diagnostic ions (*m/z*: 355, 373, 447, and 467 for CP; and 393, 411, 485, and 505 for BP) [20,21].

LC-MS was used to detect the ratio of relative abundance of *m/z* 467 to *m/z* 469 in CP, and was scanned from 300 (Q1FM) to 500 (Q1LM).

3. Results and discussion

3.1. Chromatography and validation results

The presence of CP and BP in cosmetic samples was validated by comparing it to a high purity standard, using the retention time of HPLC-UV-DAD. The presence of a 3-keto-4-ene in A-ring of the steroids causes the strong absorption at 240 nm, and was monitored at 240 nm [22]. Fig. 2 shows the HPLC chromatograms of a standard and two real cosmetic samples. These chromatograms indicate that cosmetic samples have many peaks, including CP, BP and other compounds. Peaks with the retention times of 15.5 min and 16.2 min in samples were coincided with signals of standard CP and BP. Peaks with similar retention time of HPLC in solvent gradient for cosmetic samples and steroid standards were confirmed, with the degree of similarity based on the match of normalized UV spectra to one another, using the ChemStation. UV spectra for peaks of standard CP and cosmetic cream sample with similar retention times in HPLC show similarity factors of 999.9 and 165.5, which indicates that a signal with the similarity factor of 999.9 coincides exactly with a standard signal, as shown in Fig. 3.

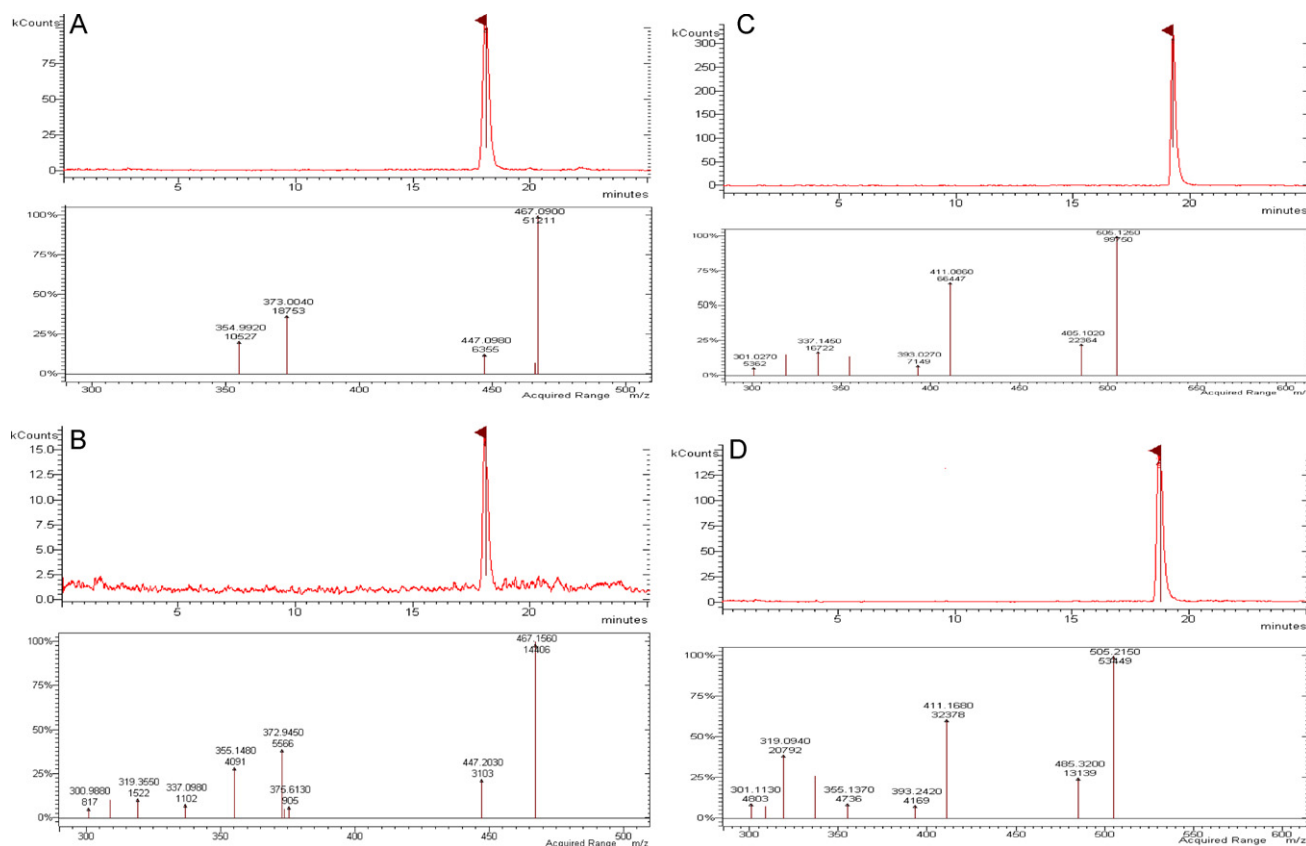


Fig. 4. Typical LC–MS/MS chromatogram of [A] a standard CP with 467 m/z and its mass spectrum, [B] a real cream sample containing CP and its mass spectrum, [C] a standard BP with 505 m/z and its mass spectrum [D] a real cream sample containing BP and its mass spectrum.

3.2. Confirmation of analytes by LC–MS/MS and LC–MS

The presence of CP or BP in real cosmetic samples was further confirmed through LC–MS and LC–MS/MS. Two peaks were observed at 18.1 and 19.2 min, of relative retention times in the LC–MS/MS analysis of standards and cosmetic cream samples added with CP and BP (Fig. 4). CP has a molecular formula of $C_{25}H_{32}ClFO_5$ and a molecular mass of 466. In the MS/MS spectrum of CP (Fig. 4A and B), protonated molecular ion (m/z 467) was observed along with several fragment ions produced in collision cell, which is protonated molecular ion, m/z 447 (loss of HF), m/z 373 (loss of HF and $CH_3CH_2CO_2H$) and m/z 355 (loss of HF, $CH_3CH_2CO_2H$ and H_2O). BP has a molecular formula of $C_{28}H_{38}FO_7$ and a molecular mass of 504. In the MS/MS spectrum of BP (Fig. 4C and D), protonated molecular ion (m/z 505) was observed along with several fragment ions produced in collision cell, which is protonated molecular ion, m/z 485 (loss of HF), m/z 411 (loss of HF and $CH_3CH_2CO_2H$) and m/z 393 (loss of HF, $CH_3CH_2CO_2H$ and H_2O). Table 2 summarizes the diagnostic ions from the chemical formula for the identification of CP and BP. Mass fragmentation patterns in real cosmetic cream samples matched well with a standard mass spectrum of CP and BP. Furthermore, signal of CP with a chlorine

atom was also confirmed, with relative abundance of chlorine, using LC–MS. The ratio of the relative abundance of m/z 467 to m/z 469 in LC–MS of CP was approximately 3:1, which corresponds to the isotopic ratio of ^{35}Cl – ^{37}Cl , confirming the presence of one chlorine atom in the molecule [20] (Supplementary Fig. 1).

3.3. Analysis of cosmetic products

Although a number of different LC methods for these steroids in pharmaceutical formulations or biological matrices were published, simple validated LC methods were not usually reported in cosmetic lotion samples. These methods have been applied to the analysis of four different cosmetic products (cream, skin, emulsion and essence lotions) sold in local and Internet markets of Korea. A total of 47 cosmetic samples suspected to contain anti-inflammatory corticosteroids by consumers were monitored for CP and BP, using the method described above. It was found that CP was detected in five cream lotion samples with the concentration levels of 32.9, 42.3, 56.4, 70.6 and 96.4 $\mu g/g$, including BP monitored in a cream lotion sample with a level of 195.1 $\mu g/g$. These steroids were not detected in skin, emulsion and essence lotions.

Although the levels of two steroids added in cream lotions are not as high as those added in medical drugs, such as in forms of creams, gel, solution and ointments, for the treatment of patients with dermatoses, such as psoriasis and eczema, cream lotions are usually used long-term and are not supervised by physicians. Thus, it should not be allowed to contain these steroids in cosmetic products.

4. Conclusion

Some cosmetic products purchased in local and online markets of Korea have been accused of severe side effects by consumers.

Table 2
MS measurement of molecular ions and fragmented ions for CP and BP in LC–MS/MS.

Fragmentation	CP (m/z)	BP (m/z)	Product ions
1	467	505	Protonated molecular ion
2	447	485	Loss of HF
3	373	411	Loss of HF and $CH_3CH_2CO_2H$
4	355	393	Loss of HF, $CH_3CH_2CO_2H$ and H_2O

These cosmetic products have been advertised for manufacturing with extracts of medicinal natural herbs, and for the treatment of psoriasis and eczema. Although these products reduced inflammation and temporarily relieved the symptoms of inflammatory skin problems of severe plaque psoriasis, it later brought severe side effects, such as permanent skin atopy and pustular psoriasis for long-term use consumers.

The assay of CP and BP forbidden in these cosmetic products was performed by simple HPLC–UV–DAD, and the presence of these steroids was confirmed by product ions and isotope ratio, using LC–MS/MS and LC–MS. The present method allowed for analysis of a large number of samples in a short period of time for the presence of illegal steroids in four different lotions.

Although CP and BP were generally found at therapeutic levels of 0.02–0.06% in ointment, emollient cream and shampoo with indication on the label of these products, these steroids should not be contained in cosmetic creams for the treatment of skin problems. As a result of our laboratory findings, cosmetic creams containing these steroids sold in local markets of Korea have been withdrawn. However, regular and consistent monitoring of the use of steroids in cosmetic products is still needed.

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Appendix A. Supplementary data

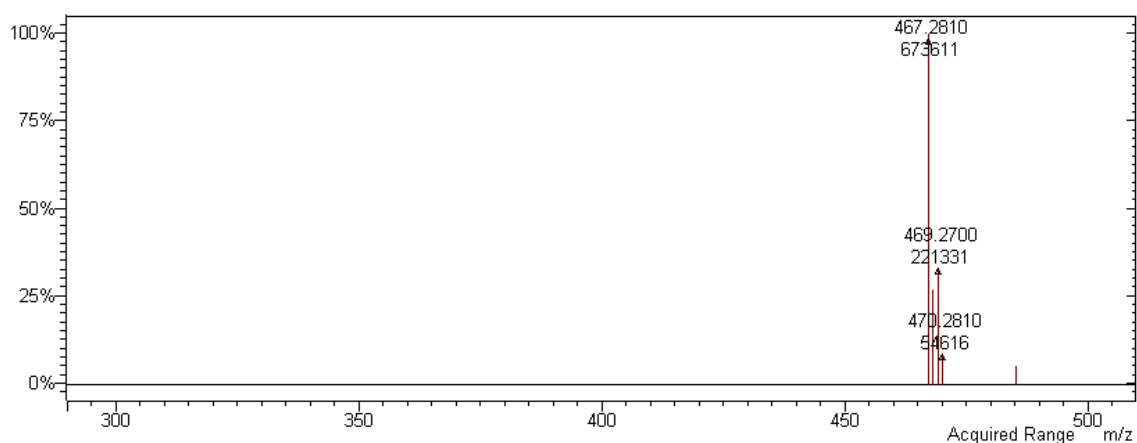
Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.forsciint.2011.02.027.

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Supplementary Figure Legend

Supplementary Figure 1. The ratio of the relative abundance of m/z 467 to m/z 469 is approximately 3:1, [A] a standard of CP and [B] sample with CP through LC/MS.

Supplementary Fig. 1.**[A]****[B]**