

## 아연피리치온을 유효성분으로 표기한 화장품류에서 미표기 성분인 베타메타손 유도체의 검출

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### Detection of Undeclared Betamethasone Derivatives in Cosmetic Products Labeled to Contain Zinc Pyrithione as the Active Ingredient

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**요약:** 스테로이드 함유 표시가 없는 화장품에서 항염증 효과가 있는 글루코코티코스테로이드인 베타메타손프로피오네이트 성분이 검출되었다. 이 화장품은 외용스프레이 및 샴푸로서 주성분으로 아연피리치온을 함유하는 것으로 표기되어 있었다. 화장품에서 스테로이드 구조와 활성을 갖는 물질의 존재를 확인하기 위하여 실리카겔 박층판을 이용한 박층크로마토그래프를 사용하였으며 이 성분을 분리하기 위해 high-performance liquid chromatography (HPLC)를 이용하여 확인 및 정량을 수행하였다. 분취용 HPLC를 이용하여 스테로이드를 함유한 것으로 판단되는 분획을 모은 다음 nuclear magnetic resonance (NMR) 및 mass spectrometry (MS)를 이용하여 스테로이드 성분을 확인하였다. 스테로이드 표준물질로 베타메타손 17-프로피오네이트 및 베타메타손 21-프로피오네이트를 합성하여 사용하였고 이 표준물질과 HPLC 크로마토그램을 비교하여 스테로이드 성분의 함량을 분석하였다. 이 방법으로 아연피리치온 제제와 같은 일부 시판 화장품에서 스테로이드 성분을 확인하였고 reversed-phase high-performance liquid chromatography (RP HPLC) 상의 유지시간 비교를 통하여 스테로이드 성분을 정량한 결과 시험한 총 8종의 화장품 시료 중 2개 제품에서 0.005 ~ 0.02 %의 베타메타손프로피오네이트가 검출되었다.

**Abstract:** Betamethasone propionate, an anti-inflammatory glucocorticosteroid, was detected in cosmetics with no indication on the label of this compound as an ingredient. The product was formulated as a topical spray or shampoo and labeled to contain zinc pyrithione as the active ingredient. A thin-layer chromatographic analysis was carried out on silica gel plates to provide a first indication about the presence of a compound with steroid structure and reactivity; then high-performance liquid chromatography (HPLC) separation allowed the identification of the corticosteroid agent and its quantification. To identify the corticosteroid agent from these commercial samples we collected the fractions suspected to have ketol steroids by prep HPLC and identified the compound as betamethasone propionate by NMR and MS spectrometry. Then we synthesized the standard for the betamethasone 17-propionate and 21-propionate and quantitate the corticosteroids from the sample by HPLC with that standards. By this method we identified the corticosteroid compounds from some commercial cosmetics such as zinc pyrithione sprays. The finding of betamethasone propionate in the products was shown by comparison to an authenticated standard of betamethasone propionate by retention time on reverse-phase HPLC. Two of the tested products contained betamethasone propionate at the levels of 0.005 ~ 0.02 % and the others were free of betamethasone propionate.

**Keywords:** glucocorticosteroid, cosmetic, betamethasone propionate, chromatography, zinc pyrithione

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## 1. Introduction

Corticosteroids produced in the adrenal cortex are involved in many physiological systems such as stress and immune reactions, regulation of inflammatory states, metabolism of carbohydrates, catabolism of proteins and levels of blood electrolytes[1]. Synthetic corticosteroids are used in human and veterinary medicine but can be misused in many cosmetics. Our laboratory recently detected new betamethasone derivatives: betamethasone 17-propionate and betamethasone 21-propionate (Figure 1), an anti-inflammatory glucocorticosteroid, in a cosmetic product labeled to contain zinc pyrithione as its active ingredient. This product was formulated as a shampoo or a topical spray. Betamethasone propionate is not mentioned on the label of these products, and yet, it was found in them at therapeutic levels. These products were advertised and sold via the internet; they were indicated for the treatment of red, itchy, flaky skin caused by eczema, seborrhea, and psoriasis. The active ingredient listed for the spray was zinc pyrithione at 0.2 %; the active ingredient listed for the shampoo was zinc pyrithione at 1.0 % and menthol at 0.25 %. Zinc pyrithione is the active ingredient in various shampoos used for the treatment of dandruff and seborrhea of the scalp.

All corticosteroids have potential side effects[2-5]. The risk of this side effects increases with both long term use and increasing corticosteroid potency, especially if used under no medical supervision. Side effects are caused either by local effects on the skin or sys-

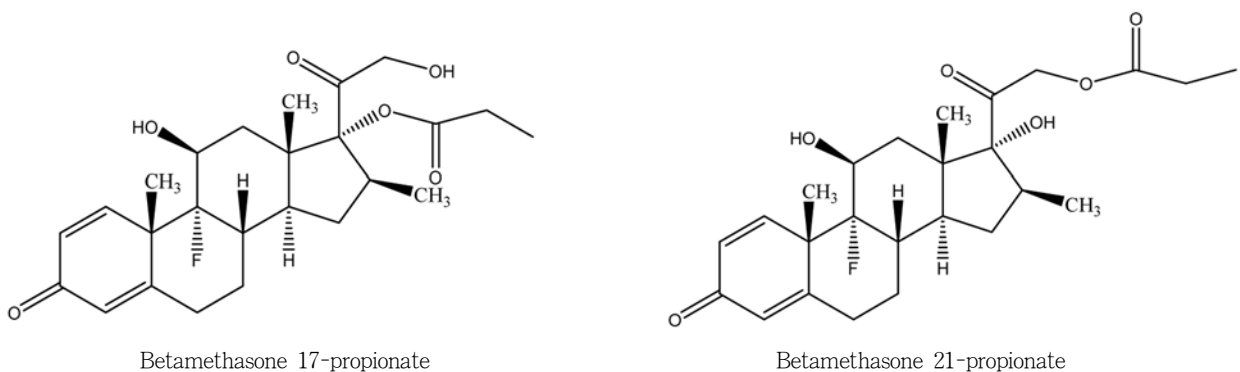
temic effects after the drug is absorbed through the skin. One of the local side effects of the topical corticosteroids is skin atrophy in which the skin becomes thinner and small blood vessels dilate, become visible and appear as a network of red wires[3,4]. These atrophic conditions are often permanent. Topical steroids are recommended for use for only short durations and on small areas of the skin. It is not recommended for children since children are particularly susceptible to side effects of corticosteroids, especially the potent ones. Precautions must be considered before use of topical betamethasone propionate by pregnant women, nursing mothers or older adults.

This paper describes the characterization of betamethasone propionate in these cosmetics by retention times on reversed-phase high-performance liquid chromatography (RP HPLC). It also presents a identification method for the betamethasone propionate in zinc pyrithione formulations and the results of assays on commercial samples.

## 2. Materials and Methods

### 2.1. Materials

All reagents were of analytical grade and were used without further purification. Methanol, diatomaceous earth, toluene, dichloromethane, ethyl ether, water and silica gel were obtained from MERCK (Germany). Tetrazolium blue chloride, sodium hydroxide, dimethyl sulfoxide- $d_6$  (DMSO- $d_6$ ), formic acid, acetonitrile, tetrahydrofuran (THF), triethyl amine, propionic anhydride,



**Figure 1.** Structures of betamethasone 17-propionate and 21-propionate.

calcium chloride anhydride, ethyl acetate, magnesium sulfate, hexane and *p*-toluenesulfonic acid monohydrate were obtained from SIGMA-ALDRICH (USA).

## 2.2. Preliminary TLC

A preliminary screening of commercial cosmetic products was carried by thin layer chromatography (TLC). Standard solutions (0.1 %) of all the corticosteroid standards studied in this work were prepared in methanol. Aliquots (10  $\mu$ L) of the standard solutions were applied to the silica gel plates and chromatographed in two solvent systems successively, first in toluene, second in dichloromethane, ethyl ether, methanol and water (77 : 15 : 8 : 1).

After development the plates were air dried at room temperature and observed under UV light. Finally it was sprayed with detection reagents : the mixture of one volume of 0.5 % tetrazolium blue in methanol and three volumes of 10.7 % NaOH in methanol.

Aliquots (5.0 g) of the sample were exactly weighed, transferred to a 100 mL volumetric flask and taken to volume with methanol. The resulting suspension was stirred at room temperature for about 1 h, mixed with 5 g of diatomaceous earth and placed in -20  $^{\circ}$ C for 2 h. A portion of the clearer supernatant liquid was decanted and then filtered. The final solutions (10  $\mu$ L) were applied to the silica plates, which were processed as for the standards.

## 2.3. Extraction and Purification of Betamethasone Esters from Unknown Samples

About 50.0 g of the sample were exactly weighed, transferred to a 500 mL volumetric flask and taken to volume with methanol. The resulting suspension was stirred at room temperature for about 1 h, mixed with 50 g of cellite and placed in -20  $^{\circ}$ C for 2 h. The clearer supernatant liquid was decanted, filtered. The solvent was evaporated under vacuum and the residue redissolved in 50 mL of methanol. 500  $\mu$ L of methanol solution were injected into the liquid chromatograph.

The analytical column used for reversed-phase chromatography was a J'sphere ODS-H80 column (250  $\times$  10.0 mm, 4  $\mu$ m particle size, YMC Co., Ltd., Japan).

The signal was scanned from 210 to 395 nm in order to record UV spectra and monitored at 240 nm. The mobile solvents were methanol-water (60 : 40) and the flow-rate was 4 mL/min.

The fractions of retention time 26.827 min (fraction A) and 29.360 min (fraction B) was checked by TLC and turned out to be containing ketol steroids, pointed out by the characteristic red-blue spots on the TLC plates after detection with tetrazolium blue solutions. The corticosteroid compounds obtained were concentrated under reduced pressure and used without further purification as sample for the spectroscopic analysis.

## 2.4. Identification of Betamethasone Esters by NMR and LC-MS

The sample obtained from fraction B was concentrated further under reduced pressure. The residue was mixed with 1 mL of DMSO- $d_6$  and analyzed by  $^1$ H and  $^{13}$ C-NMR (300 Hz). The spectra were compared with that of betamethasone.

The sample from fraction A and B was also injected to LC-MS. The chromatographic analysis was carried out at room temperature on a Phenomenex Luna 5  $\mu$  C18 (2) column (150  $\times$  2.0 mm, 5  $\mu$ m, USA). The mobile solvents were formic acid-acetonitrile (8 : 2) and the flow rate was 0.2 mL/min. The mass spectrometer operated with the APCI interface in positive ion mode.

## 2.5. Synthesis of Betamethasone 17-Propionate and 21-Propionate

### 2.5.1. Betamethasone 21-Propionate

About 1.0 g of betamethasone was dissolved in 40 mL of THF and 0.245 g of triethylamine and 3.25 g of propionic anhydride were added. The mixture was stirred for 6 h in a drying tube. The solvent was evaporated under reduced pressure and extracted with ethylacetate (150 mL) and water (50 mL). The organic phase was washed with water 3 times. Magnesium sulfate was added to eliminate water and filtered to eliminate magnesium sulfate. Crystallization from hexane provided expected propionate.

MS ( $m/z$ ): 449 ( $M^+ + 1$ ), 431 ( $M^+ - OH$ ), 429 (449 - HF),

**Table 1.**  $R_f$  Values Obtained for the Analyzed Corticosteroids<sup>a</sup> and Cosmetic Samples

Compound	$R_f$ value	Compound	$R_f$ value
Triamcinolone acetonide	0.51	Dexamethasone	0.39
Triamcinolone	0.26	Cortisone	0.41
Prednisolone	0.33	Betamethasone 17,21-propionate	0.80
Prednisolone 21-acetate	0.63	Dexamethasone decananoate	0.85
Prednisolone acetate valerate	0.79	Betamethasone	0.43
Prednisolone succinate	0.15	Hydrocortisone 21-sodium succinate	0.19
Hydrocortisone aceponate	0.83	Hydrocortisone 17-valerate	0.67
Spray A	0.56	Shampoo A	0.57, 0.68
Spray B	-	Shampoo B	-
Cream A	-	Bath gel A	-
Cream B	-	Bath gel B	-

<sup>a</sup>The values are the mean of three developments.

375 ( $M^+$  - 73, propionate), 355 (375 - HF), 319, 284, 122

### 2.5.2. Betamethasone 17-Propionate

Betamethasone 17,21-propionate (0.2 g) was dissolved in methanol (20 mL) and *p*-toluene sulfonic acid anhydride (about 20 mg) was added. The mixture was stirred for 5 h in a drying tube. After purification by silicagel column the solvent was evaporated under reduced pressure.

MS ( $m/z$ ): 449 ( $M^+$  + 1), 431 ( $M^+$  - OH), 429 (449 - HF), 375 ( $M^+$  - 73, propionate), 355 (375 - HF), 337, 319, 217, 187, 137

### 2.6. Assay of Betamethasone Propionate in Zinc Pyrithione Topical Products

About 10 mg of betamethasone 17-propionate and betamethasone 21-propionate were accurately weighed, placed in a 10 mL volumetric flask, dissolved in methanol and diluted to volume. About 1.0 g of each samples was dissolved in methanol and diluted to 10 mL. The solutions were filtered through a 0.45  $\mu$ m membrane filter (Millipore, USA) and used as sample for the spectroscopic analysis. These standard and sample solutions were applied to the silica gel plates and processed as described above in 2.2.

These standard and sample solutions were diluted further and injected to the LC system for assay. The analytical column was XTerra RP-18 (250  $\times$  4.6 mm,

5  $\mu$ m, Waters, USA), the mobile solvents were acetonitrile-water (2 : 3) and the flow rate was 0.4 mL/min.

## 3. Results and Discussion

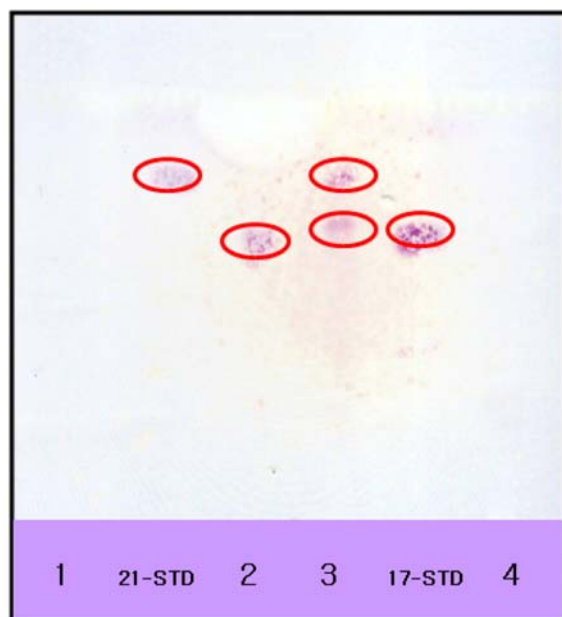
### 3.1. Preliminary TLC

In order to screen a product for the presence of corticosteroid agents, a preliminary TLC separation was carried out. We used tetrazolium blue as a colour detection reagent since steroids with an  $\alpha$ -ketol group (a keton at the carbon 20 adjacent to the primary hydroxyl group at carbon 21) have reducing properties and can be, therefore, identified as red-blue spots after spraying with this reagent. The  $R_f$  values obtained for 17 corticosteroid standards and commercial cosmetic samples under investigation are reported in Table 1.

The red-blue spots unique to corticosteroid were found in the TLC plates for the sample but there was no corticosteroid standard having the same RF value equal to that of the sample.

### 3.2. Identification of Betamethasone Propionate by NMR and LC-MS

To identify the corticosteroid from cosmetic samples we collected the two fractions suspected to have ketol steroids, pointed out by the characteristic red-blue spots on the TLC plates after detection with tetrazolium blue solutions. Both of two fractions were concentrated and



**Figure 2.** TLC chromatogram for the commercial cosmetic samples and betamethasone propionate standards (1 : Cream A, 2 : Spray A, 3 : Shampoo A, 4 : Bath gel A, 17-STD : betamethasone 17-propionate standard, 21-STD : betamethasone 21-propionate standard).

analyzed by  $^1\text{H}$  and  $^{13}\text{C}$  NMR. Comparing with the spectrum of betamethasone the unknown material in the fractions was supposed to have the betamethasone skeleton. That concentrated samples were also analyzed by LC-MS and the two spectra show a signal at  $m/z$  449. Betamethasone propionate has a molecular mass of 448. Because the peak at  $m/z$  449 is due to the molecular ion  $[\text{M} + \text{H}]$  we concluded that purified samples would be betamethasone propionate. So we synthesized betamethasone 17-propionate and betamethasone 21-propionate as the standards to quantitate corticosteroids in the cosmetic products. These two betamethasone propionate standards and commercial cosmetic samples were applied to a silica gel plate (Figure 2). The red-blue spots for the samples have the same RF values equal to that of the standards.

### 3.3. Assay of Betamethasone Propionate in Zinc Pyrithione Topical Products

Samples of zinc pyrithione spray, shampoo, gel and cream were assayed by reversed-phase HPLC. Figure 3

**Table 2.** Analytical Results of Commercial Cosmetic Products<sup>a</sup>

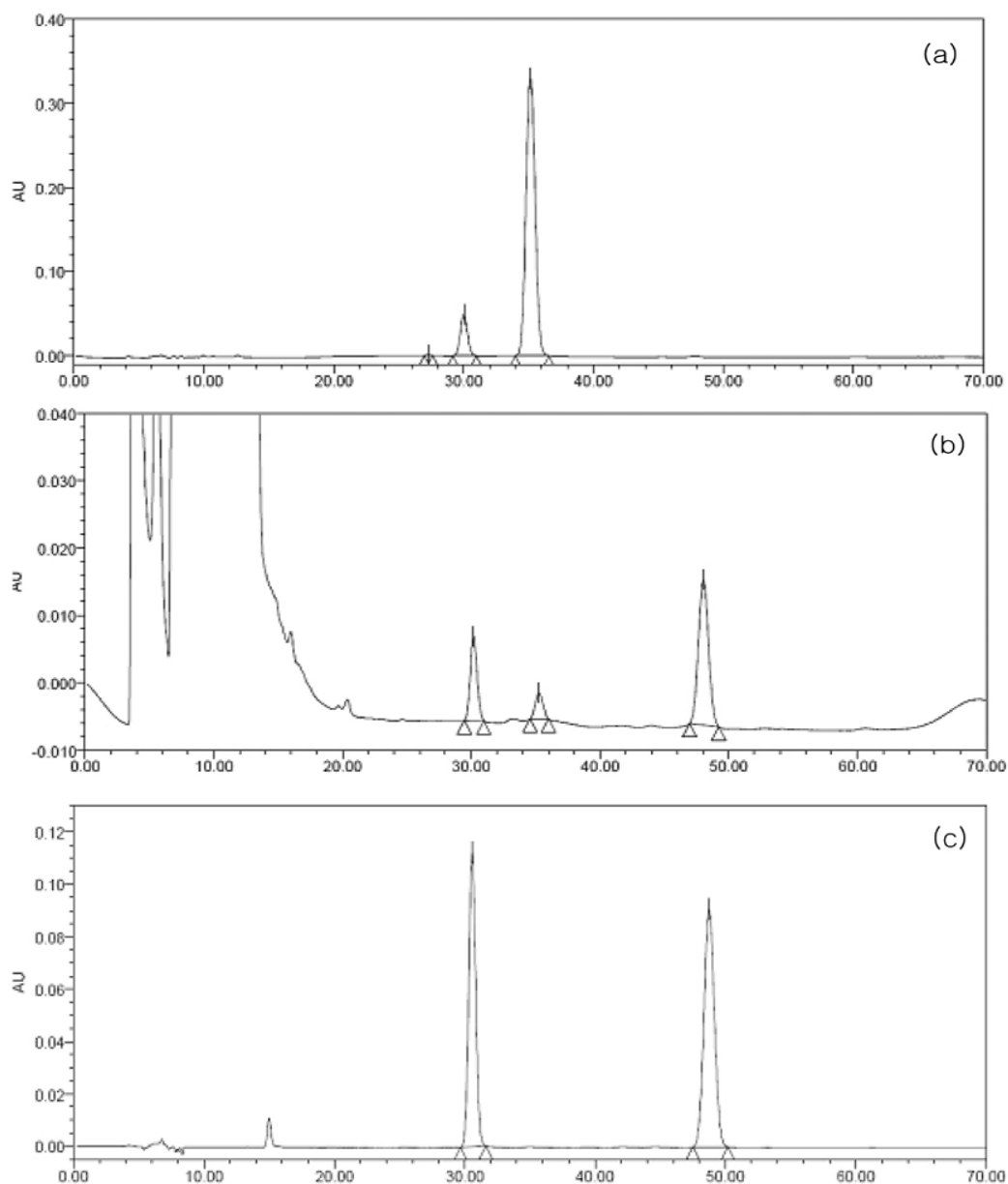
Cosmetics	Identified corticosteroid	Found % (m/m)
Spray A	Betamethasone 17-propionate	0.02
Spray B	ND	-
Shampoo A	Betamethasone 17-propionate	0.005
	Betamethasone 21-propionate	0.01
Shampoo B	ND	-
Cream A	ND	-
Cream B	ND	-
Bath gel A	ND	-
Bath gel B	ND	-

<sup>a</sup>Each value is the mean of three determinations.  
ND : Not detected

shows chromatograms of the spray, shampoo and the betamethasone propionate standards. The retention time for betamethasone 17-propionate and 21-propionate were about 30.5 and 48.7 min respectively. Two of them resulted positive for betamethasone propionate. The results obtained are reported in Table 2. As can be seen some formulation contained the betamethasone 17-propionate and the other contained both of betamethasone 17-propionate and 21-propionate, whose identity was confirmed on the basis of the standards synthesized in our laboratory. Betamethasone 17-propionate and 21-propionate were not found in the zinc pyrithione gel and cream.

## 4. Conclusion

In order to provide a simple and rapid method to verify that the cosmetic contains prohibited corticosteroid agents with anti-inflammatory activity, we decided to utilize chromatographic techniques, in particular TLC and HPLC, since suitable equipments are easily available. When a sample was suspected to contain a corticosteroid, the TLC analysis provided a first indication about the presence of a compound with steroid structure and reactivity; then HPLC separation allowed the identification of the corticosteroid agent and its quantification. Sometimes we are not confident whether the cosmetic sample has the corticosteroid agent and all of the corticosteroid standard may not be available. Then we recommend that the fractions suspected to



**Figure 3.** Chromatograms of the (a) spray A, (b) shampoo A, (c) betamethasone 17-propionate and 21-propionate standards.

have ketol steroids are collected from cosmetic sample by prep HPLC and the compound is identified by NMR and MS spectrometry. Then we can synthesize the standard for that corticosteroid agents and quantitate the corticosteroids from the cosmetic sample by HPLC with the standards. By this method we identified the corticosteroid compounds from some commercial cosmetics such as zinc pyrithione sprays.

## References

1. J. H. Andersen, L. G. Hansen, and M. Pedersen, Optimization of solid phase extraction clean up and validation of quantitative determination of corticosteroids in urine by liquid chromatography-tandem mass spectrometry, *Anal. Chim. Acta*, **617**, 216 (2008).
2. USP DI, Drug information for the health care professional, United States Pharmacopeial Convention,

- 17th ed., 2486 (1997).
3. H. C. Korting and H. I. Maibach (Eds.), Topical glucocorticoids with increased benefit/risk ratio, 6, 122, 192, Karger, Basel (1993).
  4. J. C. Reepmeyer, L. K. Revelle, and I. Vidavsky, Detection of clobetasol propionate as an undeclared steroid in zinc pyrithione formulations by high-performance liquid chromatography with rapid-scanning ultraviolet spectroscopy and mass spectrometry, *J. Chromatogr. A*, **828**, 239 (1998).
  5. L. Gagliardi, D. De Orsi, M. R. Del Giudice, F. Gatta, R. Porra, P. Chimenti, and D. Tonelli, Development of a tandem thin-layer chromatography high-performance liquid chromatography method for the identification and determination of corticosteroids in cosmetic products, *Anal. Chim. Acta.*, **457**, 187 (2002).