IDENTIFICATION OF PROHIBITED SKIN LIGHTENING AGENTS IN COSMETIC PRODUCTS USING UHPLC WITH PDA AND MASS DETECTION

Marian Twohig[1], Paula Hong[1] Helene Boiteux[2] and Chris Stumpf[1]
[1]Waters Corporation, 34 Maple Street, Milford, MA 01757;
[2]Waters S.A.S. BP 608, 78056 Saint-Quentin, En Yvelines Cedex, France

INTRODUCTION

Skin lightening agents including nicotinamide, arbutin and salicylic acid (Figure 1) are often used to produce a more even skin tone. The products are positioned as either a cosmetic or a treatment for improving the appearance of the skin and are usually applied to the face and neck.[1]

The use of pharmaceutical active ingredients (AIs) such as corticosteroids and tretinoin is prohibited in cosmetics due to the potential side effects that can occur.[2,3] Hydroquinone is widely used in dermatology to treat uneven skin tone however, it is prohibited for use in cosmetics in the EU and at over the counter levels exceeding 2% w/w in the US. Long term use of corticosteroids can cause side effects including permanent skin atrophy and other systemic effects. Prolonged use of hydroquinone can cause permanent discolouration of the skin. These AIs can be found in cosmetic products available online.

In this study, cosmetic products (creams and gels) obtained from online vendors were extracted and analysed using UHPLC with PDA and mass detection.
The CORTECS™ stationary phase used in the separation contained a 2.7 µm particle designed to give maximum efficiency and exceptional retention for both polar and non-polar analytes. With an optimized pore size, C_{18} ligand density, and endcap, the CORTECS T3 phase is compatible with 100% aqueous mobile phases and provides the perfect balance of polar and non-polar analyte retention.
Several samples tested positive for prohibited skin lightening agents. The packaging labels were often misleading, in some cases the AI was not listed on the enclosed product information increasing the likelihood of improper long term use and adverse side effects to consumers.

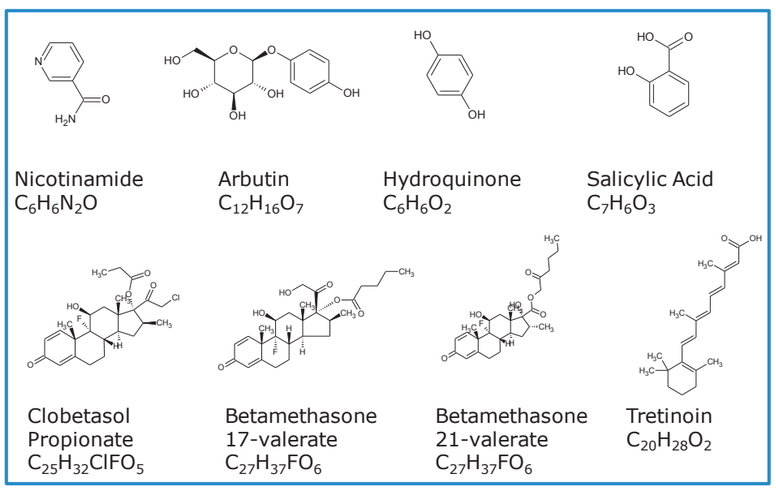

Figure 1. Empirical formulas and structures for the skin lightening agents analysed in the study.

METHODS

Instrumentation and software

All separations were performed on the ACQUITY Arc System equipped with a 2998 Photodiode Array (PDA) Detector and the ACQUITY QDa Detector. Empower 3 Software was used for data acquisition and processing.

Sample preparation

The standard compounds including 8 skin whitening agents, (Figure 1) and four parabens were dissolved in methanol and sequentially diluted to prepare the spiking solutions. Matrix matched calibration curves were prepared using blank cream or gel cosmetic bases obtained from Making Cosmetics Inc. An aliquot (1g) of the matrices were weighed into 15 mL centrifuge tubes and spiked with 100 µL of acetonitrile spiking solutions. The mixture was initially vortex mixed and then shaken for 10 minutes to equilibrate the spiked analytes. A volume of acetonitrile (4.9 mL) was added and the samples were shaken again for 25 minutes. The samples were then centrifuged at 3000 rpm for 10 minutes. An aliquot of the supernatant was syringe filtered using a 0.2 µm PVDF filter and placed in a vial in preparation for sample analysis. The cosmetics samples (1g) were extracted in the same way using 5 mL of acetonitrile in the extraction step.

LC conditions

Column: CORTECS T3, 3.0 x 100 mm, 2.7 µm
Solvent A: 0.1% formic acid in water **Solvent B:** Methanol
Flow rate: 0.80 mL/min: **Column temp.:** 30 °C:
Injection volume: 0.5-1 µL
Gradient conditions: 0 min 0% B, 0.5 min 0% B, 2.2 min 2% B, 6.0 min 95% B, 8.0 min 99% B, 9.0 min 99% B, return to initial conditions.

PDA detection: 210 to 400 nm

MS conditions

MS system : ACQUITY QDa
Ionization mode: ESI + and/or ESI -
MS scan range: 100 to 600 *m/z*
Sampling rate: 5 Hz

RESULTS AND DISCUSSION

- Figure 2 shows the chromatogram resulting from the separation of a standard mix of 12 compounds encountered during the study using the ACQUITY Arc system with PDA detection.
- An Empower processing method was developed to identify the analytes using the retention times (t_R) of the standard compounds which were determined experimentally.
- Four parabens, which are frequently used as preservatives, were detected in many of the cosmetics samples analysed (Figure 2, peaks 4,6,7,8).

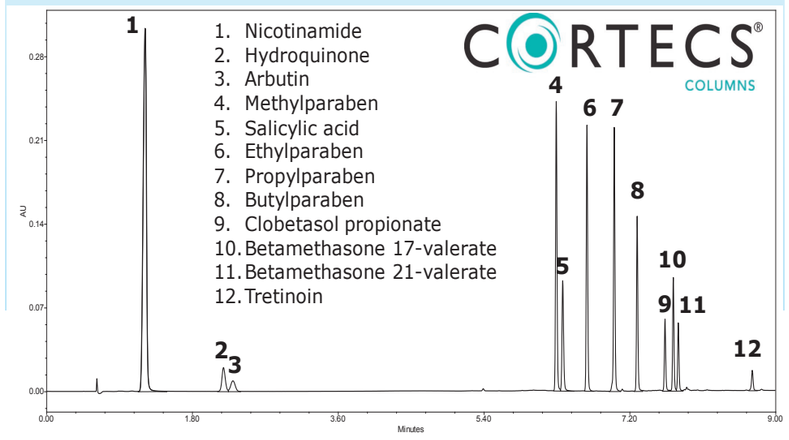

Figure 2. ACQUITY Arc UV chromatogram at 254 nm resulting from the separation of a standard mixture of compounds encountered in the study using a CORTECS T3 3.0 x 100 mm, 2.7-µm column, 1 µL injection.

Clobetasol propionate identified in a cosmetic sample

- Figure 3 (bottom) shows the chromatogram resulting from the separation of the extracted lightening gel sample which was obtained from an internet shop in the US.
- The sample was found to contain arbutin, the corticosteroid clobetasol propionate as well as four parabens which are labeled using the Empower processing method.
- The t_R of the peaks matched with those in the standard (Figure 3 top). The label of the product did not disclose the presence of clobetasol propionate.

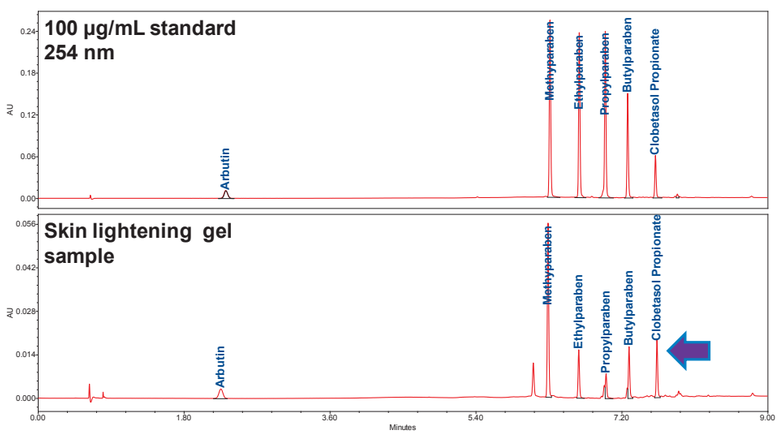

Figure 3. ACQUITY Arc chromatogram resulting from the separation of a skin lightening gel sample (bottom) at 254 nm using a CORTECS T3 3.0 x 100 mm, 2.7-µm column. Standard compounds for t_R matching are also shown (top), 100 µg/mL, 0.5 µL injection. Clobetasol propionate in the sample is indicated by the arrow.

- Figure 4 shows a comparison of both the UV and mass spectra for clobetasol propionate in the gel sample and in the standard.
- The UV spectrum and *m/z* of 467 for clobetasol propionate was the same in both standard and sample.
- The isotopic pattern reflects the chlorine present in the chemical structure providing extra confirmation and increased confidence in the identification.

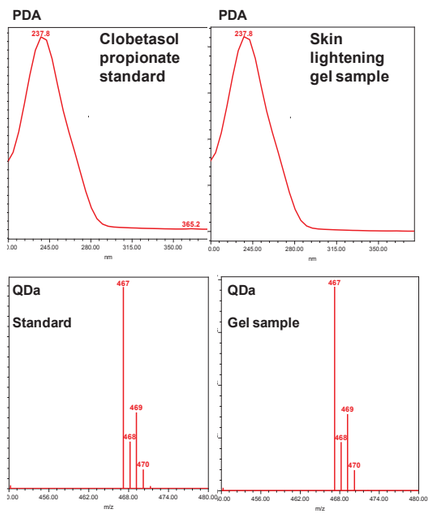

Figure 4. Comparison of the PDA and mass spectra for the clobetasol propionate in the standard and the sample. The same UV spectra, m/z and isotopic patterns were observed.

Betamethasone 17-valerate, hydroquinone and tretinoin identified in a cosmetic sample

- Figure 5 (bottom) shows the chromatogram resulting from the separation of a sample named MJ-1 which was obtained from an internet shop in the US.
- This sample was supplied as a number of skin treatments to be used together. Hydroquinone, betamethasone 17-valerate and tretinoin were detected in the sample. The t_R of the peaks matched with those in the standard (Figure 5 top).
- The second sample MJ-2, (chromatogram not shown) was found to contain betamethasone 17-valerate and its structural isomer betamethasone 21-valerate as well as tretinoin.
- The included product information did not disclose the presence of the AIs. The risk of side effects to a consumer is much higher when the AIs are not disclosed on the label. Mass detection was used to confirm the identifications.

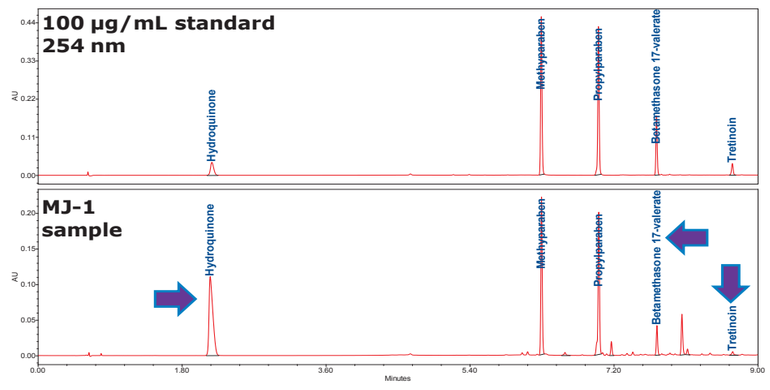

Figure 5. *ACQUITY Arc UV chromatogram at 254 nm resulting from the separation of the MJ-1 sample (bottom). Standard compounds for t_R matching are shown (top), 100 µg/mL, 1 µL injection. Hydroquinone, betamethasone 17-valerate and tretinoin are indicated by the arrows.*

Quantitation

Matrix matched calibration curves (R^2 > 0.999), were used to quantify the samples analysed (Table 1). The results in table 1 show that the levels of most components detected in the samples were in the typical usage range.[2] The linearity was studied for clobetasol propionate, betamethasone 17-valerate, hydroquinone, tretinoin and arbutin using spiked matrix samples ranging in concentration from 0.01-1% w/w or in the case of hydroquinone 0.01-5% w/w. The NS sample was diluted 1:1 to bring the detected hydroquinone into the calibration range.

Compounds	Typical usage level % w/w	Lightening Gel	Lightening Cream	MJ-1	MJ-2	NS	TG
Clobetasol propionate	0.05	**0.038**	**0.060**	-	-	-	-
Betamethasone 17-valerate	0.05	-	-	**0.035**	**0.103**	-	-
Betamethasone 21-valerate	N/A	-	-	-	**0.038**	-	-
Hydroquinone	1-5	-	-	**3.74**	-	**7.20**	**3.00**
Tretinoin	0.01-0.1	-	-	**0.015**	**<0.01**	-	-
Arbutin	4-7	**0.203**	-	-	-	-	-

Table 1. Summary of the quantitation results for selected skin lightening compounds in the samples and the typical usage levels.[2]

CONCLUSION

- **The CORTECS T3 column is designed to give maximum efficiency and balanced retention for both polar, and non-polar analytes. UHPLC separations using this column. facilitated a single analysis for a wide range of the skin lightening agents and parabens.**
- **An Empower processing method was developed with standard compounds and used in the identification and quantitation of the test compounds.**
- **UV and MS spectral matching were used to confirm the identification of samples that tested positive for the skin lightening agents.**
- **Four samples analyzed were found to contain corticosteroids, two of which also contained tretinoin. Quantified amounts were frequently in the typical usage range or above.[2]**
- **Hydroquinone was detected in three samples at >3% w/w which violated both EU and US regulations.**
- **In some of the samples analysed, the presence of the AIs was not declared on the label or the enclosed product information. Inaccurate or insufficient labeling of the cosmetics products increases the likelihood of adverse side effects, as cosmetics are usually used over long time periods with no medical supervision.**

References

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