SIMULTANEOUS DETERMINATION OF HYDROQUINONE, RETINOIC ACID AND CORTICOSTEROIDS IN WHITENING PRODUCTS BY HPLC-MS

LAWENG NILMANEE

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of Science Degree in Medical Sciences
May 2012

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This thesis entitled "Simultaneous determination of hydroquinone, retinoic acid and corticosteroids in whitening products by HPLC – MS" submitted by Laweng Nilmanee in partial fulfillment of the requirements for the Master of Science Degree in Medical Sciences is hereby approved.

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Title	SIMULTANEOUS DETERMINATION OF HYDROQUINONE, RETINOIC ACID AND CORTICOSTEROIDS IN WHITENING PRODUCTS BY HPLC-MS
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ABSTRACT

Hydroquinone, retinoic acid and corticosteroids are sometimes used in cosmetic products, especially those claimed to have whitening effects. These compounds are prohibited substances and can cause long-term health problems for consumers. Routinely, they can be identified initially by thin layer chromatography, and confirmed by HPLC method. However, these methods of detection are low in efficiency and very much time-consuming. The objective of this study was to establish new strategies to simultaneously detect, confirm and determine hydroquinone (HQ), retinoic acid (RA), betamethasone (BM), betamethasone 17-valerate (BMV), dexamethasone (DM), hydrocortisone acetate (HCA), prednisolone (PRL) and triamcinolone acetonide (TA) in whitening products using HPLC – MS in a single test. A reversed phase high performance liquid chromatography/electrospray ionization mass spectrometry (HPLC/ESI-MS) method has been developed and validated for the simultaneous determination of hydroquinone, retinoic acid, betamethasone, betamethasone 17-valerate, dexamethasone, hydrocortisone acetate, prednisolone and triamcinolone acetonide in cosmetic products. The presence of these substances in cosmetic products is prohibited. Analytes were directly extracted by sonication in a methanol. The separation was achieved on a Hypersil BDS C8 column (150 mm x 3 mm, 3 μm) using a step gradient elution with mobile phase of 0.1% formic acid in water and acetonitrile at a flow rate of 0.5-0.55 mL/min. The column temperature was maintained at 25 °C. The samples are analyzed in selected ion monitoring using the

typical fragmentation in positive mode where the molecular related ions $(M+H)^+$ were generated for each analyte, at *m/z* 111.1, 301.2, 393.1, 477.2, 393.1, 405.2, 361.2 and 435.2 for HQ, RA, BM, BMV, DM, HCA, PRL and TA, respectively. Protonated molecular ions were monitored for all analytes. The retention times of HQ, PRL, BM, DM, TA, HCA, BMV and RA were 2.8, 15.9, 25.1, 26.8, 33.1, 33.8, 47.0 and 60.3 minutes, respectively. The validation data showed the system linearity over the concentration range of 1.5-60, 0.05-2, 0.4-16.3, 0.1-4.2, 0.4-16.7, 0.2-8.3, 0.2-8.1 and 0.2-8.2 µg/mL for HQ, RA, BM, BMV, DM, HCA, PRL and TA, respectively, with correlation coefficients (r) higher than 0.995 and correlation coefficients of method linearity were higher than 0.998. Average recoveries were ranged from 95.5-109.3%. For repeatability, relative standard deviations were ranged of 0.84-1.81%. There is no significant difference between test results of different time ($p>0.05$). The limits of detection were from 0.0001-0.003% w/w and the limits of quantitation were ranged from 0.0004-0.01% w/w. For specificity, there was no interfering peak due to matrix blank. The expanded relative measurement uncertainties were ranged from 3.03-8.86% of reported concentration at 95% confidence level. The validation data was within the specification of the analytical method and showed the validity of the method. The new method could probably be further developed for ASEAN cosmetic standard method (ACM).

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ABBREVIATIONS

ANOVA	=	analysis of variance
AOAC	=	Association of Official Analytical Chemists
APCI	=	atmospheric pressure chemical ionization
APPI	=	atmospheric photo ionization
BM	=	betamethasone
BMV	=	betamethasone17-valerate
BP	=	boiling point
BSA	=	*N*,*O*-bis (trimethylsilyl) acetamide
°C	=	degree celsius
DC	=	direct-current
DM	=	dexamethasone
EIC	=	extracted ion chromatogram
ESI	=	Electrospray ionization
eV	=	Electron volt
FID	=	flame ionization detection
fmol	=	femtomole
FDA	=	Food and Drug Administration
g	=	gram
GC	=	gas chromatography
g/cm^3	=	grams per cubic centimeter
GC-MS	=	gas chromatography mass spectrometry
HCA	=	hydrocortisone acetate
HPLC	=	High performance liquid chromatography
HPLC-MS	=	High performance liquid chromatography-mass spectrometry
HPLC-ESI-MS	=	High performance liquid chromatography-elecrospray ionization-mass spectrometry
HQ	=	hydroquinone
I.D.	=	inside diameter
ISO/IEC	=	International Organization of Standardization/International Electrotechnical Commission

ABBREVIATIONS (CONT.)

IUPAC	=	International Union of Pure and Applied Chemistry
kV	=	kilovolt
LC	=	liquid chromatography
LC-MS	=	liquid chromatography tandem mass spectrometry
LC-MS/MS	=	liquid chromatography mass spectrometry- mass spectrometry
LD_{50}	=	Median lethal dose, the quantity of a chemical that is estimated to be fatal to 50% of the organisms tested
L/min	=	liter per minute
LOD	=	limit of detection
LOQ		limit of quantitation
m	=	meter
MALDI	=	matrix assisted laser desorption ionization
$(M - H)^-$	=	deprotonated molecular ion
$(M + H)^+$	=	protonated molecular ion
mg	=	milligram
mg/kg	=	milligram per kilogram
min	=	minute
mL	=	milliter
mL/min	=	milliliter per minute
mm	=	millimeter
MP		melting point
MS	=	mass spectrometry
MSD	=	mass selective detector
MSDS	=	Material Safety Data Sheet
MS/MS	=	Mass spectrometry/mass spectrometry
MU	=	measurement uncertainty
m/z	=	mass to charge ratio
n	=	number of replicate
N_2	=	nitrogen
NATA	=	National Association of Testing Authorities, Australia

ABBREVIATIONS (CONT.)

No.	=	number
ng/mL	=	nanogram per milliliter
nm	=	nanometer
pg/mL	=	picogram per milliliter
ppb	=	part per billion
ppm	=	part per million
PRL	=	prednisolone
psi	=	pounds per square inch
r	=	correlation coefficient
RA	=	retinoic acid
RF		radiofrequency
RI	=	Refractive index
RP-18		reversed phase C18-bonded silica
RSD	=	relative standard deviation
SD	=	standard deviation
SIM	=	selected ion monitoring
S/N	=	signal to noise ratio
SOP		Standard Operating Procedure
TA	=	triamcinolone acetonide
TIC	=	total ion chromatogram
TLC	=	Thin layer chromatography
™	=	trademark
TMCS	=	trimethylchlorosilane
TMSim	=	trimethylsilyimidazole
TOF	=	Time-off-flight
UPLC-MS/MS	=	Ultra Performance Liquid Chromatography
U.S.A.	=	The United States of America
USP	=	United States Pharmacopeia
UV	=	ultraviolet
UV-VIS	=	Ultraviolet and Visible

ABBREVIATIONS (CONT.)

V	=	volt
v/v	=	volume by volume
w/w	=	weight by weight
µg/L	=	microgram per liter
µg/mL	=	microgram per mililiter
µL	=	microliter
µm	=	micrometer
>	=	more than
≤	=	less than or equal
≥	=	more than or equal
/	=	per
%	=	percentage