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Original Communication

Multiple-instrument determination of compositions in cosmetics

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ABSTRACT

An analysis method to determine compositions of unknown cosmetic was established. The method is based on multi-instruments analysis technique coupled solvent extraction. The multi-instruments involve high-performance liquid chromatography (HPLC), X-ray diffraction (XRD) and Fourier transform infrared (FTIR). The cosmetic sample was separated and enriched into 4 fractions by solvent extraction in which water, heptanes, chloroform, tetrahydrofuran (THF) were used. The main compounds of the cosmetic were qualitative and quantitative analyzed. The compounds were defined as mercuric ammonium chloride, liquid paraffin, polyethylene glycol 200, 1,2-propanediol, glycerin, stearic acid, linoleic acid, linoleic acid methylester and α-linoleic acid ethyl ester.

KEYWORDS: cosmetic, mercuric ammonium chloride, analysis, HPLC, FTIR, XRD

1. INTRODUCTION

In modern life, there is more and more emphasis on protecting and beautifying facial skin, especially for young women, and more and more types of cosmetic products could be found in cosmetics market. However, it is necessary to point out that some cosmetics used for a long-time may lead to damage of human health due to the presence of certain harmful substances. For example, there are a lot of reports about harmful substances being prohibited or restricted in cosmetics. They could be detected through instruments analysis, including carcinogens-N-nitroso-diethanolamine [1, 2], formaldehyde [3], preservatives [4] hormone [5], antibiotics [6], phenolies [7] and so on.

Many methods to detect specific components [1, 3-7] and heavy metals of cosmetics were reported in lots of literature. For example electrochemical detector detection (EC) [8], cold vapor atomic absorption spectrometry (CVAAS) [9-10], atomic emission spectroscopy (AES) [11], and inductively coupled plasma mass spectrometry (ICP-MS) [12-13], and so on. There are also some reports using capillary electro-phoresis (CE) combining with inductively coupled plasma mass spectrometry (CE-ICPMS) [14-15] and capillary electrophoresis combining with UV-Vis detection for heavy metals speciation [16-17]. These methods have high sensitivity and low selectivity for detection of heavy metals; at the same time, most of these methods were used to determine the total amount of metal rather than the metal form. Majidi [14] and Gudzenko [16] reported the determination of methyl mercury and ethyl mercury, and Evans reported the determination of three forms of mercury including CH_3-, C_2H_5- and C_6H_{13}- [8]. In addition, there is no report about determination of the heavy metal form and simultaneous detection of multi-components in cosmetics.

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In this paper, to know an unknown cosmetic, we developed a method to analyze the form and content of heavy metal, and other major components in cosmetic.

2. EXPERIMENTAL

2.1. Instruments, reagents and samples

HPLC analysis was performed on a Shimadzu LC-3A high performance liquid chromatograph (Shimadzu Co., Japan), with a UVD ultraviolet detector, and a RID 3A differential refractometer. The chromatographic columns used were Zorbax-ODS (25 cm length, 0.46 cm i.d.), Zorbax-NH_2 (25 cm length, 0.46 cm i.d.) and. SHIMPACK GPC-801 (30 cm length, 0.8 cm i.d., poly-styrene 6μm), all provided by Shimadzu Company.

XRD analysis was performed on a Riguku D/Max 2500 system.

FTIR determination was performed on a Bio-Rad Excalibur Series FTS 3000 spectrometer in the range of 4000-400 cm^{-1} using KBr pellets.

Analytical-grade methanol, hexane, tetrahydro-furan, chloroform, benzene and ethylene glycol obtained from Tianjin Chemical Reagent Factory (Tianjin, China). Pure mercuric ammonium chloride, stearic acid, linoleic acid , α-linoleic acid ethyl ester, 1,2-propanediol, 1,3-propanediol, liquid paraffin, polyethylene glycol and glycerol, etc were from Shanghai Chemical and Medical Reagent Company (Shanghai, China).

The cosmetic sample was unknown, which was provided by the relating departments of Cosmetics Company of Taiyuan city in Shanxi Province (China).

2.2. Experimental methods

2.2.1. Preparations of fractions by extraction

The first step is obtaining optimum conditions for enrichment and separation of fractions. A series of experimental factors were investigated involving different reagents and extraction order. The most optimum condition was defined, which were described as follows.

i. Separation of fraction 1

The operation procedure of extraction: 1.5-2 g of cosmetic sample was weighed with accurate of .00001 grams, and then placed into a 250 ml of Separatory Funnels; 100 ml of distilled water was added into the funnels; they were mixed fully by shaking, and then centrifuged. Then they were rested until the both layers appeared. These water-soluble liquids were denoted as fraction 1, which was subjected to fixed volume and analyzed by HPLC. Preparation of sample for FTIR analysis was that 10 ml of fraction 1 was placed into a Petri dish, under nitrogen at 90^0C for remove water and the dried fraction 1 was got.

ii. Separation of fraction 2

The above water-insoluble substances were placed into a Separatory Funnel and mixed with 100 ml hexane. They were fully mixed by shaking, and were centrifuged. Then they were rested until the both layers appeared. Then the hexane-soluble liquid was got and denoted as fraction 2, which was subjected to fixed volume and analyzed by HPLC. After fraction to remove hexane, the dry residuals were analyzed by FTIR.

iii. Separation of fraction 3

The above hexane-insoluble substances were dissolved and extracted with 100 ml trichloro-methane. The other extraction operations were similar to the above-mentioned process. The chloroform-soluble substance was denoted as fraction 3 and analyzed by HPLC.

iv. Preparations of fraction 4

The above chloroform-insoluble substances were extracted with 50 ml THF, and then were filtrated. The insoluble residue with 50 ml THF was extracted again. The THF soluble substances were denoted as fraction 4 and analyzed by HPLC. The dried samples were analyzed by XRD and FTIR.

2.2.2. The conditions of multi- instrument analyses

i. HPLC analysis

For analysis of fraction 1, R.I.D-3A differential refractometer and a Zorbax-ODS column were used. Pure-water was used as mobile phase, with a rate of 0.8 ml/min and column temperature at 20^oC.

For analysis of fraction 2, differential refracto-meter and a Zorbax-NH_2 column were used hexane was used as mobile phase, with a rate of 1.0 ml/min and column temperature at 20^oC.

For analysis of fraction 3, UV detector at 254 nm and a Zorbax-NH_2 column were used. The heptanes/chloroform = 1/1 (V/V) was used as mobile phase, with a rate of 0.9 ml/min and column temperature at 23°C.

For analysis of fraction 4, the flow rate of THF was 1.0ml/min and the temperature of the column was kept at 25°C. The wavelength (λ) of UV detector was at 270 nm.

ii. FTIR analysis

For analysis of fraction 1, fraction 2 and fraction 4, FTIR was used. The spectra of Fourier transform infrared (FTIR) were acquired in the transmission mode as 64 scan in the IR range from 4000 to 500 cm^{-1} at a resolution of 4 cm^{-1}. KBr standard pellets were used, and the samples were dried and then mixed with KBr, ground, and palletized.

iii. XRD analysis

For analysis of dried fraction 4 XRD was used. Diffraction patterns were recorded with Cu Kα (λ = 0.1542 nm) radiation and the X-ray tube was operated at 40 KV and 100 mA. Step scans were taken over the range of 2θ from 10° to 70° at a speed of 2 °/min

3. RESULTS AND DISCUSSION

3.1. Analysis results of fraction 1

The typical HPLC chromatograms of fraction 1 are shown in Fig. 1. The identities of components were checked by retention time of pure reagents. The qualitative results show that the main components of fraction 1 were polyethylene glycol 200, ethylene glycol, 1, 2-propanediol and glycerin.

The IR spectra of fraction 1 and pure reagents including ethanol, glycerol and 1, 3-propanediol was shown in Fig. 2. It can be seen evidently, that IR characteristic of fraction 1 is basically same as that of reagents. The characteristic peaks around 3316.cm^{-1}, 2920.cm^{-1}, 1400.cm^{-1}, and 1044.cm^{-1} respectively are attributed to OH-, CH_2, CH_3 and C-O group, respectively.

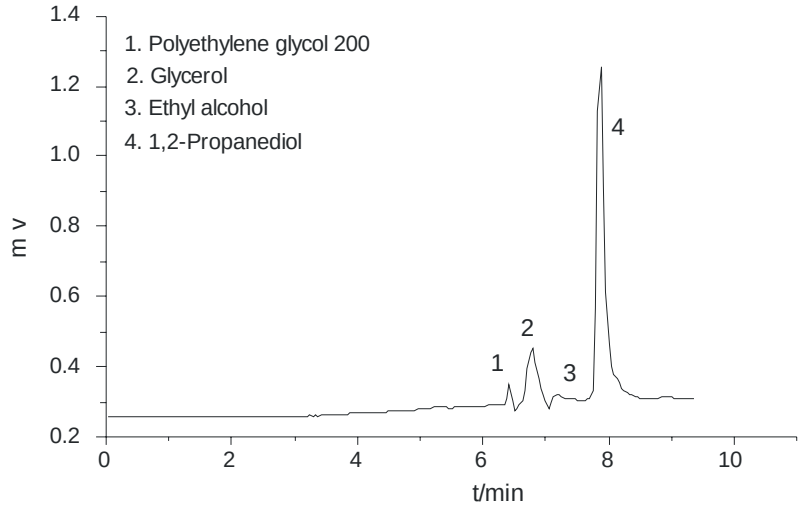

Fig. 1. The HPLC chromatogram of fraction 1.
Chromatographic conditions: Shimadzu LC-3A HPLC chromatograph
Detectors: R.I.D-3A differential refractometer detector
Column: 0.46 × 25 cm, packed with Zorbax-ODS, 5μm
Column temperature: 20°C.
Mobile phase: pure water
Flow rate: 0.8 ml/min.
Peaks order:
[1] Polyethylene glycol 200 [2] Glycerol [3] Ethyl alcohol [4] 1,2-propanediol.

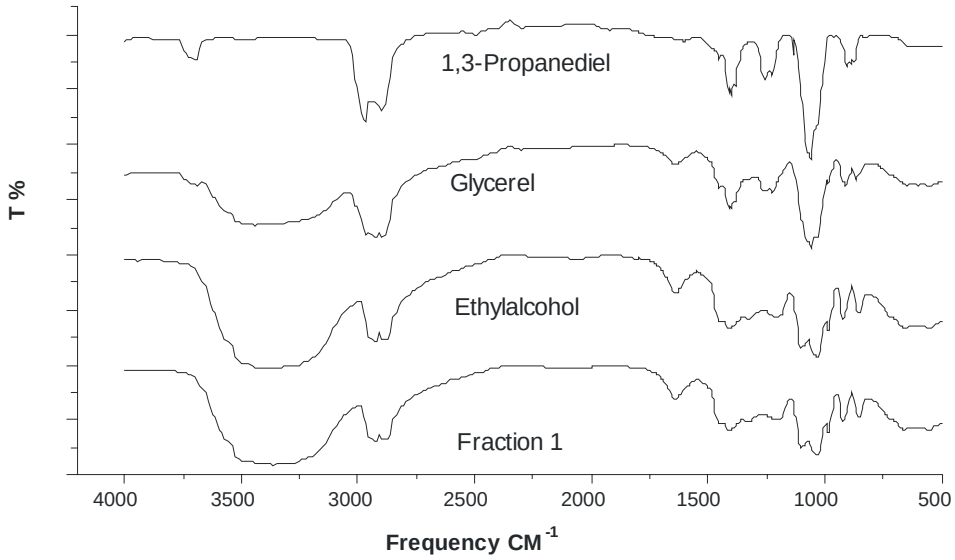

Fig. 2. The IR spectra of fraction 1 and pure reagents.

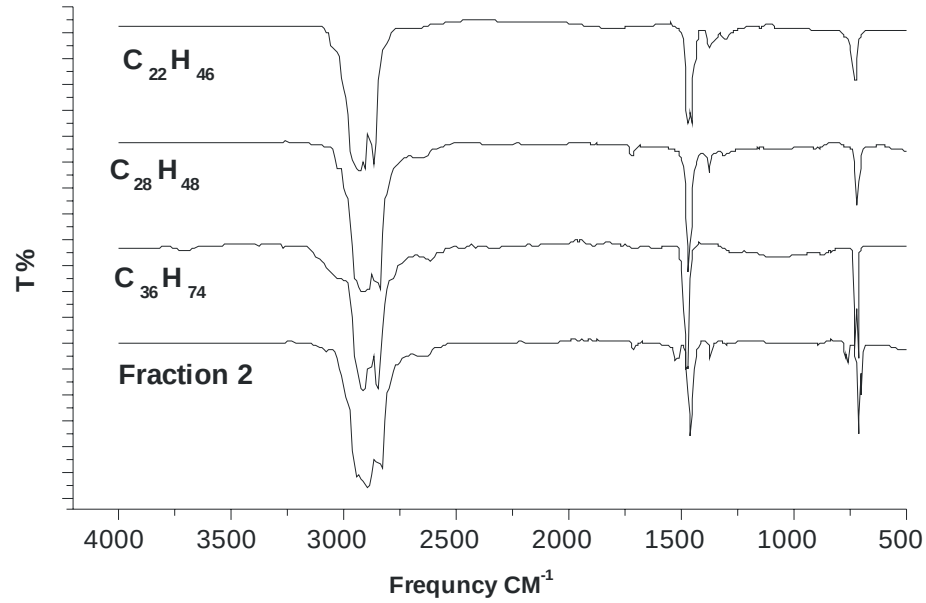

Fig. 3. The IR spectra of fraction 2 and pure reagents.

The enrichment principle of components by extraction method is mainly based on the different solubility of components in solvents. If this component of sample has same group of solvent, then it may be easier to dissolve and concentrate than other components. When water was used as extraction reagent, the solubility alcohols were much higher than other no-soluble compounds, and thus, fraction 1 were mainly substances of alcohol.

3.2. Analysis results of fraction 2

The fraction 2 was analyzed by FTIR. The IR spectra were shown in Fig. 3. It can be seen that the characteristics of fraction 2 and relational pure reagents were basically same. The IR

characteristics peaks of 719.45 cm^{-1}, 1377.17 cm^{-1}, 2850.78 cm^{-1}, 2918.29 cm^{-1} and 2959.79 cm^{-1} were attributed to peaks of alkanes.

In HPLC analysis (Fig. 4), the liquid paraffin in fraction 2 was eluted as one peak firstly and its retention time was less than benzene (added in). While normal phase chromatography systems (Zorbax-NH_2 -hexane system) were used, the non-polar liquid paraffin must be eluted first [18].

Through analysis of FTIR and HPLC, finally, the main composition of fraction 2 was defined as liquid paraffin. These results imply that liquid paraffin has same CH_2 and CH_3 groups of hexane, so it might exhibit better solubility than other components.

3.3. Analysis results of fraction 3

The fraction 3 was analyzed by HPLC and chromatogram was shown in Fig. 5. Through retention time's comparisons of sample with pure reagents, finally, linoleic acid, stearic acid, linoleic acid methyl ester and α-linoleic acid ethyl ester were defined as main components in fraction 3.

In order to further verify the qualitative analysis, the "blank experimental" about solubility was conducted. The pure reagents which involved linoleic acid, stearic acid, linoleic acid methyl ester and α-linoleic acid ethyl ester were selected, and then they were dissolved by water, hexane, and trichloromethane, respectively, to see their dissolution natures. The results indicated they are better soluble in chloroform.

3.4. Analysis results of fraction 4

The fraction 4 was analyzed by XRD and FTIR. Their spectra were shown in Fig. 6 and Fig. 7, respectively. It obviously can be seen that the XRD characteristics of fraction 4 were same as that of corresponding pure mercuric ammonium chloride ($HgNH_2Cl$). In IR spectrums, the characteristics of fraction 4 were also basically same as that of pure reagent $HgNH_2Cl$. The fraction 4 was analyzed by HPLC and

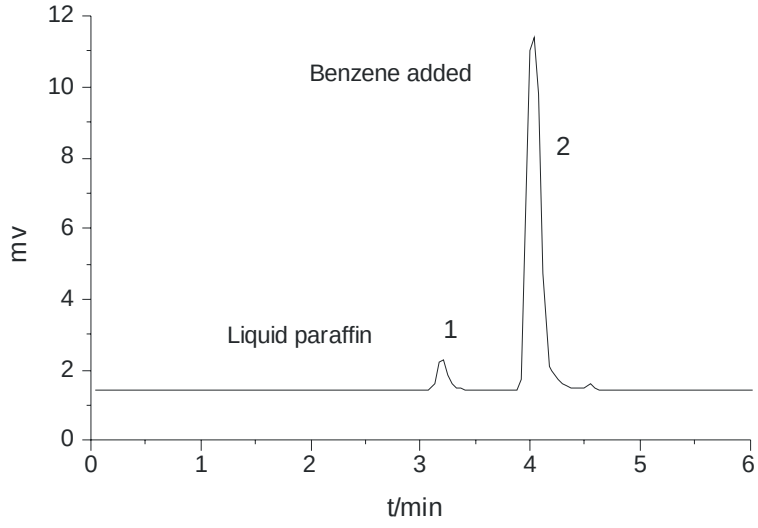

Fig. 4. The HPLC chromatogram of fraction 2.
Chromatographic conditions: Shimadzu LC-3A HPLC chromatograph
Detectors: R.I.D-3A differential refractometer detector
Column: 0.46 × 25 cm, packed Zorbax-NH_2, 5μm
Column temperature: 20°C.
Mobile phase: n-hexane
Flow rate: 1.0 ml/min.
Peak:order
[1] Liquid paraffin [2] Benzene added.

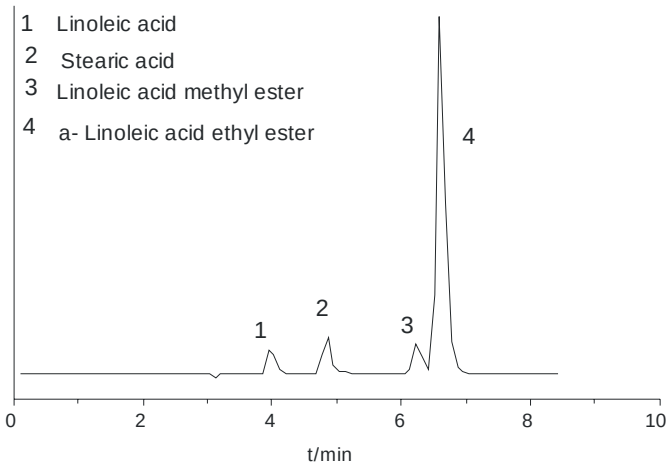

Fig. 5. The HPLC chromatogram of fraction 3.
Chromatographic conditions: Shimadzu LC-3A HPLC chromatograph
Detectors: UV detector, 254nm
Column: 0.46 × 25 cm, packed with Zorbax-NH_2, 5μm
Column temperature: 20°C.
Mobile phase: heptanes/trichloromethane =1/1 (V/V)
Flow rate: 0.9 ml/min.
Column temperature: 23°C
Peak order:
[1] linoleic acid [2] stearic acid [3] linoleic acid methyl ester [4] α-linoleic acid ethyl ester.

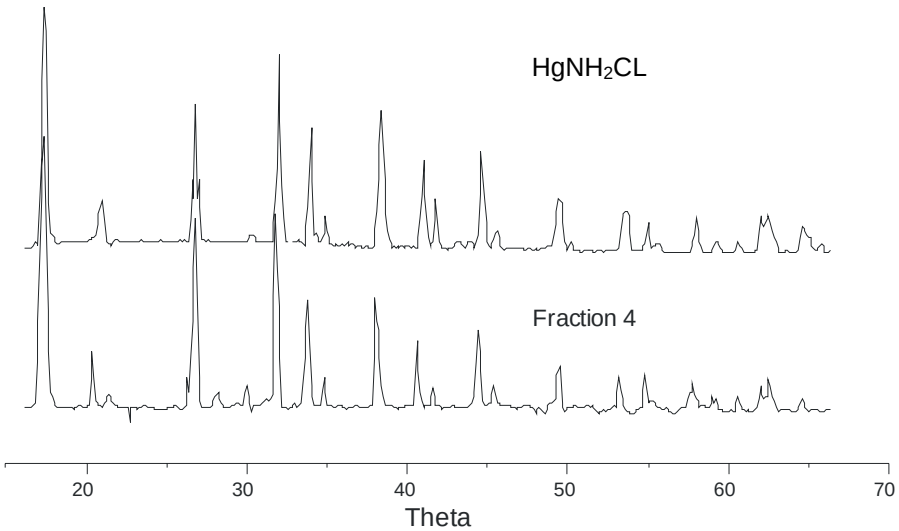

Fig. 6. The XRD spectra of fraction 4 and pure $HgNH_2CL$.

chromatogram was shown in Fig. 8. The "blank" experimental was done and the results indicate the pure mercuric ammonium chloride only can be dissolved in THF.

It is necessary to emphasize that the mercuric ammonium chloride exhibits highly toxic even at much lower concentrations. It can be easily deposited on skin surface and transmitted by

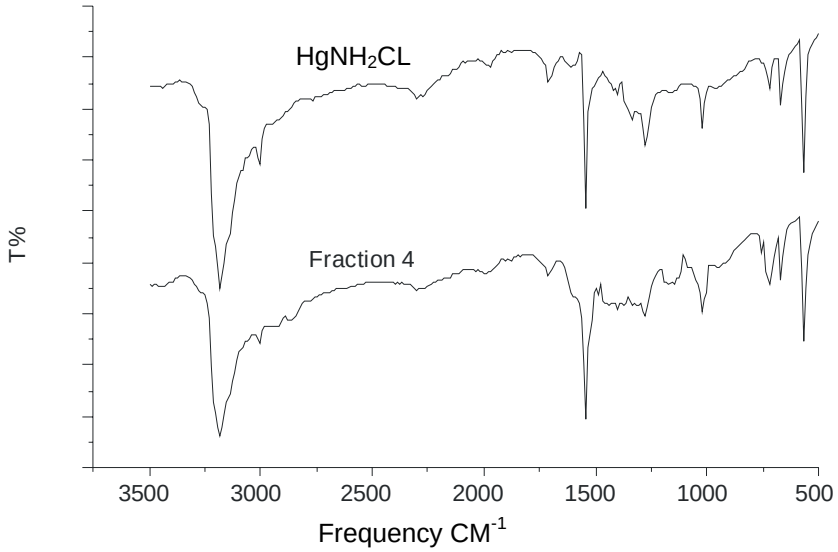

Fig. 7. The IR spectra of fraction 4 and pure $HgNH_2CL$.

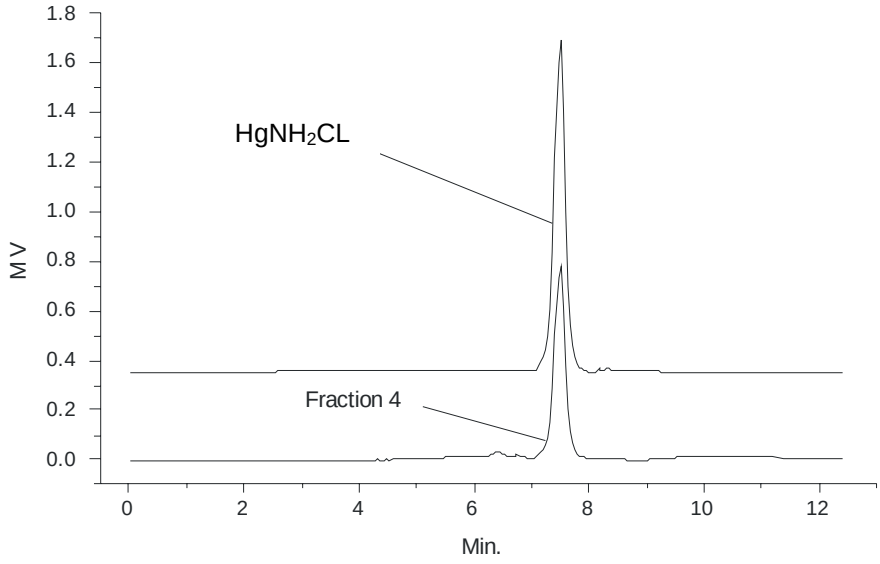

Fig. 8. The HPLC chromatogram of fraction 4 and pure $HgNH_2CL$.
Chromatographic conditions: Shimadzu LC-3A HPLC chromatograph
Detectors: UV detector, 270nm
Column: SHIMPACK GPC-801 (30 cm length, 0.8 cm i.d., polystyrene 6μm)
Mobile phase: THF
Flow rate: 1.0 ml/min.
Column temperature: 25°C.

mobile blood. If they were used for long-time, eventually, it will cause kidney failure and nervous system damage, including loss of motor skills and personality changes, etc. It is a serious problem, so consumers and managers of cosmetics must pay great attention to it.

Table 1. Quantitative result of components.

Components	(W%) (1)	(W%) (2)	(W%)Average
Mercuric ammonium chloride	0.26×10^{-1}	0.24×10^{-1}	0.25×10^{-1}
Polyethylene glycol 200	1.52	1.22	1.27
Glycerin	6.95	7.01	6.98
Ethylene glycol	0.83	0.97	0.90
1,2-Propanediol	13.57	13.62	13.59
Linoleic acid	10.12	10.03	10.07
Stearic acid	11.41	11.63	11.52
Linoleic acid methyl ester	0.43	0.46	0.45
α-linoleic acid ethyl ester	6.54	6.58	6.56
Liquid paraffin	4.52	4.42	4.47
Water	31.83	31.75	31.79

3.5. Quantitative results

The compositions of sample were quantified by the chromatographic external standard method and calculation formula as follows.

$$C_x\% = C_{ex}\% \times S_x / S_{ex} \times V_x / W_x \times I_{ex} / I_x \quad (1)$$

In which C_x% is the content of component x and the unit of C_x% is the weight percentage. C_{ex}% is content of corresponding standard and the unit of C_{ex}% is the weight in 100 ml. S_x and S_{ex} are the chromatographic responses of component x and external standard, respectively. V_x is the volume of fraction containing component x, W_x is the total weight amount of cosmetic. I_{ex} and I_{x-} are the chromatographic injection amounts of component x and external standard, respectively.

The quantitative results of components were listed in Table 1. Through qualitative and quantitative analysis, a more complete profile of a cosmetic was known. It should be noted, these main key compositions of cosmetic were tested only and the quantitative results were only preliminary, and the issues of these deficiencies need further study.

CONCLUSION

The joint application of multiple determination including HPLC, XRD and FTIR determination and solvent extraction was an effective analysis method to study cosmetic.

The main compositions of cosmetic were defined as mercuric ammonium chloride, liquid paraffin, 1,2-propanediol, glycerin, polyethylene glycol 200, stearic acid, linoleic acid, linoleic acid methyl ester and α-linoleic acid ethyl ester.

An analysis method to effectively determine compositions of unknown cosmetics was established. The method has a certain universality in analyzing other cosmetics.

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REFERENCES

1. Matyska, M. T., Pesek, J. J., and Yang, L., 2000, J. Chromatogr. A, 887, 497.
2. Meyer, T. A. and Powell, J. B. 1991, Anal. Chem., 74, 766.
3. René, T. R. and Vinod, T. 2004, J. Chromatogr. A, 1029, 217.
4. Maw-Rong, L., Chueh-Yu, L., Zu-Guang, L., and Tzu-Feng, T. 2006, J. Chromatogr. A, 1120, 244.
5 Xiaofang, W., Wenfang, Z., Jing, W., and Pen, R. 2009, Chin. J. Chromatogr., 27, 328.
6. Hualiang, L., Fang, L., Run, Y., Lianhong, W., and Yongjian, M. 2009, Chin. J. Chromatogr., 27, 237.

7. Padilla, M., Palma, M., and Barroso, C. G. 2005, J. Chromatogr. A, 1091, 83.
8. Evans, O. and McKee, G. D. 1988, Analyst, 113, 243.
9. Fujita, M. and Takabatake, E. 1983, Anal. Chem., 55, 454.
10. Birnie, S. E. 1988, J. Automat. Chem., 10, 140.
11. Costa-Fernandez, J. M. and Pereiro-Garcia, L. R., 1995, J. Anal. At. Spectrom., 10,1019.
12. Lewen, N., Mathew, S., Schenkenberger, M., and Raglione, T. 2004, J. Pharm. Biomed. Anal., 35, 739.
13. Passariello, B., Barbaro, M., Quaresima, S., Casciello, A., and Marabini, A. 1996, Microchem. J., 54, 348.
14. Majidi, V. and Miller-Ihlib, N. J. 1998, Analyst, 123, 803.
15. Tsung-Hung, L. and Shiuh-Jen, J. 2000, Anal. Chim. Acta, 413, 197.
16. Gudzenko, L.V., Pantaler, R. P., and Blank, A. B. 2001, J. Anal. Chem., 56, 721.
17. Edward, P. C., Weigang, Z., Xenia, T., Anett, G. S., Scott, K. K., Tini, M., and Ewa, D. Z. 1998, Anal. Chim. Acta, 364, 63.
18. Changming, Z. Aiying, L. Ying, L., and Linmei, Z. 1999, Chin. J. Chromatogr., 17, 372.