DETERMINATION OF ANTIBIOTICS AND OTHER COMPONENTS IN MEDICINAL-LOTION AND EVALUATION

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ABSTRACT
The aim of this paper is to know the compositions in unknown medicinal-lotion and to evolution its applications. Through a variety of studies, an analysis method was established. This method mainly is that samples were first extracted and pre-treated by concentration of compositions, and then they were separated and qualitatively analyzed by multiple-instruments. These instruments involve Mass Spectrum (MS), Fourier transform infrared (FT-IR) and High Performance Liquid Chromatography (HPLC). The qualitative analysis were done by using four different diagnostic indices using the single stage MS analysis identification; the characteristics of FT-IR; the measurements of the retention time in the HPLC and the choice characteristics of compositions in sample to HPLC detectors. The chloramphenicol was found to be the main antibiotic in the medicinal-lotion. The other compositions were roxithromycin, dexamethasone, ethylene glycol, 1, 2-propanediol and ethanol. The quantitative determination of these compounds was measurement by HPLC external standards method, and this medicinal-lotion was also evaluated.

Keywords: Medicinal-lotion; MS; FT-IR; HPLC; chloramphenicol.

1. INTRODUCTION
In Chinese cosmetics market, there is a kind of medicinal lotion, which can cure acne, kill mites and as functioning of cosmetic. But its composition, especially its effective medicinal component is not disclosed. The question of possible harmful effects in long term use must be answered for people’s health. As it can cure acne, it may contain some antibiotics. But, the variety of antibiotics is numerous, and cosmetics usually have a complex composition. This is a difficult problem for analytical workers. After consulting literatures [1-8] and a series of experimental, we establish a scheme, combining solvent extraction, enrichment and instrumental analysis. This work showed that the effective antibiotic in the medicinal lotion is chloramphenicol. The other primary components were also found.

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-2 ultraviolet detector, and a RID 3A differential refractometer. The chromatographic columns used were Zorbax-ODS and Zorbax-CN, the all of them provided by Shimadzu Company. FTIR determination was performed on An Excalibur Series spectroscope made in USA by Digilb Company. MS analysis was performed on a Quattro combined chromatograph-mass spectrocope manufactured by the British VG Co. Analytical-grade pure reagents such as methanol, 1, 2-propanediol, 1, 3-propanediol, ether and ethanol obtained from Tianjin Chemical Reagent Factory (Tianjin, China). Pure chloramphenicol, roxithromycin and dexamethasone, etc were from Shanghai Chemical and Medical Reagent Company (Shanghai, China). The medicinal-lotion was unknown, which was provided by the relating departments of Cosmetics Company of Taiyuan city in Shanxi Province (China).

2.2. Preparations of fractions of sample
The preparations of fractions was done 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. 1.0-1.5 g of cosmetic sample was weighed with accurate of 0.0001 grams, and then placed into a 250 ml of Separatory Funnels; 100 ml of ether was added into; they were mixed fully by shaking, and centrifuged. Then they were rested until the both layers appeared. This upper ether-soluble liquids was evaporated under a gentle stream of nitrogen at 40 °C to dryness and the dry solid residue was labeled as fraction 1, to be analyzed for antibiotics by FT-IR, MS and HPLC. The fraction 1 contains mainly the antibiotic component.
After the above extraction of samples, the lower fluid (no ether solution) of Separatory Funnels was added with magnesium sulfate to remove water, then the solution was evaporated under a stream of nitrogen at 60 °C until the remaining liquid is constant weight and this liquid was labeled as fraction 2, to be analyzed by FT-IR, MS and HPLC. The fraction 2 contains mainly the alcohols compounds.

2.3. The conditions of multi-instrument analyses

2.3.1. FT-IR analysis
For analysis of fraction 1 and fraction 2, 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, with a weight ratio of sample: KBr = 1:200.

2.3.2. MS analysis
In MS analysis, the ionization potential of the electron-bombardment source (EI) was 70 eV, the emission current was 200 μA, and the temperature of the electron source was 180 °C. Unknown components in the fractions were identified by their mass to charge ratios.

2.3.3. HPLC analysis

Through a series of experimental, the optimum separation conditions of antibiotic compounds are: the Zorbax-ODS (25 cm length, 0.46 cm i.d.) column; mobile phase of methanol/water (85:5, V %); the flow rate of 1.0 ml / min; temperature of column of 25°C; UV detector at 254 nm. It should be noted: the solvent of preparation medicinal-lotion was also same as mobile phase and this concentrations were prepared with 0.0100 -0.0500 % (g/ml) . The optimum conditions of analysis of alcohols compounds are: the Zorbax-CN (25 cm length, 0.46 cm i.d.) column; mobile phase of water, the flow rate of 0.8 ml / min; the column temperature of 25°C; differential refractometer.

The HPLC quantitative determination of the medicinal-lotion was done by external standards. When the injection volume of sample solution and external standard solution were the same, the content (W %) of a component in the sample was calculated by the following equation:

\[
C_{s\%} = \frac{A_{s\%}}{A_{st\%}} \times \left( \frac{C_{st-pre\%}}{C_{sa-pre\%}} \right) \times C_{s\%}
\]

Where, \(C_{s\%}\) and \(C_{s\%}\) represent the contents (w %) of the component in sample and external standard solutions respectively. \(A_{s\%}\) and \(A_{st\%}\) represent the chromatographic responses (peak areas) of the component in the sample solution and standard solution, respectively. \(C_{st-pre\%}\) and \(C_{sa-pre\%}\) are the prepared concentrations of the standard and sample solutions.

When analysis was done by HPLC, some key technologies must be indicated. The analysis of antibiotic compounds was illustrated. Key technologies of medicinal-lotion preparation are that the best solvent for sample is the mobile phase (methanol/water; 85:5, V%), the optimum concentrations of the medicinal-lotion preparation solution are 0.0100 -0.0300 % (g/ml) , preparing the solution of medicinal-lotion need be treatment by ultrasonic technology and sample solutions need filtration prior to the HPLC analysis.

3. RESULTS AND DISCUSSION

3.1. Concentration effect of composition
With fraction 1 as example, its HPLC chromatogram is shown in Fig. 1. It indicated that the concentration of chloramphenicol in this 1 fraction was 99 %. Hence the process of pretreatment of unknown sample is successful. The success of pretreatment ensure that the following accurately qualitative by IR, MS and HPLC.
Chromatographic conditions: Shimadzu LC-3A HPLC chromatograph: Detector: UVD-1 ultraviolet detector; Column: 0.46 cm × 25 cm with Zorbax-ODS. Column temperature: 25°C. Mobile phase: methanol / water = 85/15 (v/v). Flow rate: 1.0 ml / min

Above success of pretreatment ensure the no- know compositions were accurately qualitative determined through IR, MS and HPLC.

3.2. Overview of qualitative methods
The main qualitative methods used of each composition and corresponding figure are summarized in Table 1. The qualitative analysis were done by using four different diagnostic indices using the single stage MS analysis identification; the characteristics of FT-IR; the retention time (RT) quality of HPLC and the choice characteristics of compositions to HPLC detectors.

The so-called RT qualitative method of HPLC is identification of components of the sample, it is done by comparing their retention times (RT) with that of standard reagents. In addition, it should be noted, the type of detector selected can also be a qualitative means. These determination of Chloramphenicol, Roxithromycin and Dexamethasone could be used to illustrate these. The structures of the three compounds are shown in Fig 2. It is could be seen from the figure, these structures have Benzene-rings, in which the performance of UV absorption must be shown. This was confirmed by experimental.

On the contrary, the structures of 1,2-propanediol, ethylene glycol and ethanol do not have Benzene-ring and then without UV absorption performance. To analyze them only the differential detector would be used.

Therefore, the selective-application of the type of detector also is a qualitative means.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Method (1)</th>
<th>Method (2)</th>
<th>Method (3)</th>
<th>Method (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol</td>
<td>FT-IR</td>
<td>RT of HPLC</td>
<td>UV detector</td>
<td>m/z of MS</td>
</tr>
<tr>
<td>Roxithromycin</td>
<td>RT of HPLC</td>
<td>UV detector</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>RT of HPLC</td>
<td>UV detector</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2-Propanediol</td>
<td>FT-IR</td>
<td>RT of HPLC</td>
<td>Differential refract meter</td>
<td>m/z of MS</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>RT of HPLC</td>
<td>Differential refract meter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>RT of HPLC</td>
<td>Differential refract meter</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig.2-a
Chloramphenicol

Fig.2-b
Roxithromycin

Fig.2-c
Dexamethasone

Fig.2. The structures of compounds

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3.3. Results of FT-IR analysis
The 1 fraction is successfully characterized by using FT-IR. The spectra of 1 fraction and pure standard chloramphenicol are shown in Fig. 3 and it can be seen that the characteristic peak in 1 fraction is coincident with the standard pure chloramphenicol reagent.

![Fig. 3 Infrared spectrums of prepared fraction 1 and pure chloramphenicol reagent](image1)

The FT-IR spectra of fraction 2 and pure 1, 2-propanediol reagent are shown in Fig. 4. It can be seen that the characteristic peaks in fraction 2 are coincident with the standard peaks of pure 1, 2-propanediol.

![Fig. 4. Infrared spectrums of fraction 2 and pure 1, 2-propanediol](image2)

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. In Fig.4 the IR characteristics of fraction 2 are also basically same as that of pure 1, 2-propanediol.

Then these results indicated that the medicinal-lotion contained chloramphenicol and 1, 2-propanediol.

3.4. The results of MS analysis
Further confirmation of the presence of chloramphenicol and 1, 2-propanediol was done by MS analysis, as shown in Fig.5 and Fig.6, respectively.
The MS qualitative method depends on the comparison of the mass spectrum obtained with that of a pure reagents standard. As shown in Fig.5, the mainly m/z values of spectrum obtained for the fraction 1 are indicated at 15, 30, 36, 42, 51, 60, 70, 77, 83, 90, 106, 118, 136, 153, 170, 191 and 209, which they are same as the pure chloramphenicol. The mainly m/z values of spectrum obtained for the fraction 2 are indicated at 26, 27, 29, 30, 31, 33, 37, 39, 41, 43, 44, 45, 46, 57 and 61, which they are same as the pure 1, 2- propanediol (Fig. 6). Therefore, through MS analysis, this medicinal-lotion further is defined with containing chloramphenicol and 1, 2- propanediol.

**3.5. Results of HPLC analysis**

Under different chromatographic columns and detectors, the chromatograms of medicinal-lotion are shown in Fig. 7 and Fig. 8, respectively.
The components of sample were identified by comparing their RT with corresponding standard reagents. Such as, in Chromatogram 7, the RT of No. 1 peak of fraction 1, No. 2 peak and No. 3 peak are 4.80 min, 9.88 min and 31.27 min respectively; their corresponding pure reagents (chloramphenicol; roxithromycin; dexamethasone) are 4.80 min, 9.88 min and 31.27 min respectively. In Chromatogram 8, the RT of No. 1 peak of fraction 2, No. 2 peak and No. 3 peak are 6.75 min, 8.70 min and 11.25 min, respectively; their corresponding pure reagents (ethylene glycol; 1,2-propanediol; ethanol) are 6.75 min, 8.70 min and 11.25 min, respectively. By HPLC identification, the components defined in medicinal-lotion as: chloramphenicol, roxithromycin, dexamethasone, ethylene glycol, 1,2-propanediol and ethanol.

Results of quantitative determination by external standards were listed in Table 2.
The determination of chloramphenicol in foodstuff has been reported [5-8]. Long term contact with chloramphenicol may cause aplastic anemia. So the maximum allowed content of chloramphenicol in foodstuffs is 1 ppm in many countries. Then this medicinal-lotion can only be used for short-term to cure acne and skin mites, but if long term use, this should be avoided and forbidden.

4. CONCLUSION

A method was established to analyze the no-know medicinal-lotion in which including solvent extraction, concentration of compositions and multi-instruments identify to components.

In analysis of multi-instruments identify, the qualitative methods of compositions depends on the comparison of MS mass to charge ratios, FT-IR characteristics peak, HPLC retention time and choice characteristics of HPLC detectors of compositions with that of corresponding pure standards.

Through a series of studies, the accurately defined compositions are chloramphenicol; roxithromycin; dexamethasone; ethylene glycol; 1,2-propanediol and ethanol. And these compositions were quantitative analysis by HPLC method.

Our evaluation for medicinal-lotion is that although it can treatment of acne and kill mites, but because it contains up to 16% of chloramphenicol, long-term, the usage should be avoided and prohibited. The identification of unknown compounds is very cumbersome. The present work will be useful to research the composition of un-known sample.

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REFERENCES