Determination of parabens in cosmetics by subcritical water chromatography with salt-assisted homogenous extraction

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ABSTRACT

A green analytical method for the determination of parabens in cosmetic products by subcritical water chromatography (SbWC) with salt-assisted homogeneous liquid liquid extraction (SHLLE) was investigated. After a dilution of cosmetic sample with water, parabens in 5 mL aqueous sample solution (adjusted pH to 6) were extracted into 200 μL isopropanol after adding 4 g of ammonium sulfate in a self-designed glass extraction device. The extractant was collected, dried by nitrogen purging, and re-dissolved in 10 μL of water (pH 10.5), prior to subcritical water chromatographic separation and followed by UV detection. After examining the parameters affecting the performance of SbWC, the best results for the SbWC were obtained by using PLRP-S (100Å 5μm, 15 cm × 4.6 mm I.D.) column with pH 10.5 citric acid buffer as mobile phase with the temperature of preheater and column oven as 80°C and 100°C respectively. Under these conditions, the linear dynamic range for the detection was 0.75-75 μg/L for methyl paraben and ethyl paraben, and 1.5-150 μg/L for propyl paraben, with relative standard derivation (RSD) below 9.2%. Detection limits were achieved at the level of 0.07~0.1 μg/L. Recoveries were ranged in between 92.3% to 109.6% and its RSD less than 9.2%. The proposed method only used 200 μL isopropanol as extraction solvent demonstrated that the proposed method was a simple, inexpensive and eco-friendly approach for the determination of paraben preservatives in cosmetic products.

KEY WORDS:
Salt-Assisted Homogeneous Liquid Extraction; Subcritical Water Chromatography; Parabens; Cosmetics
1. Introduction

As in recent years to enhance the sustainable development and environmental awareness is gradually making the attention of green chemistry. In analytical chemistry, the green analytical techniques must contain green sample pretreatment technology and green chromatographic process. But in conventional sample pretreatment methods and chromatography usually use non eco-friendly process. In this present study developed a green analysis, salt-assisted homogenous extraction couple subcritical water chromatography, and use tree paraben preservatives as model compounds.

Cosmetics are the essential daily supplies of modern humans, but there are a lot of chemical additives in cosmetics. So, monitoring of harmful ingredients which are very important, it also the analyst's responsibility. The preservatives are the most common cosmetic additives, in any kind of cosmetics in order to prevent corruption are preservatives must be added. Most of the preservative is not only harmful to microorganisms but also toxic for the human body. Parabens are most widely used preservatives in cosmetics, especially methyl and propyl paraben. But parabens was found the relationship with breast cancer, so it still have controversy in the safty. In order to protect people's safety, the European Union (EEC) and the safe use of our country are regulated concentration range. EU regulations limit of a single type Paraben 0.4% (w / w), the total content of a maximum of 0.8% (w / w), and our department of health provides for a maximum total content of 1.0% (w / w). However, cosmetic contain lot of matrix such as aromatizer, surfactant or emulsifier etc. The matrix will affect analytical precision and accuracy. Therefore, development a method to determination parabens in cosmetics is very important.

In the previous study, had many analytical methods have been used in the detection of parabens such as liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase microextraction (SPME) and supercritical fluid extraction (SFE). Those method both has some serious drawback such as time-consuming and complicated procedure, LLE and SPE must use large amounts of organic solvent extraction and the sensitivity is poor. Although SPME and SFE without the use of organic solvents, but the their analysis is expensive, especially the SPME fiber are easily broken and carry over problem. In the present study, homogeneous extraction method will be introduced, and use salting out separate organic solvent layer and aqueous layer, it called salt-assisted homogenous liquid-liquid extraction (SHLLE) or salting out extraction.

Homogenous liquid-liquid extraction was first introduced by Matkovich and Christian, they use salting out method to separation acetone and aqueous sample. The method use the water phase and the organic phase homogenous mixing with an infinite interfacbe area to reduce the time required for equilibrium. In previous study,
the phase separate is basic on salting-out phenomenon, temperature change\textsuperscript{13}, pH control\textsuperscript{14} and non-solvent method\textsuperscript{15}. In this study use salting-out phenomenon with the self-designed glass extraction device to reduce the organic solvent volume and time-consuming also increase the sensitivity.

Salt-assisted homogenous liquid-liquid extraction is such an environmentally friendly method, use few organic solvent and . So chromatography method should not be burden on the environment. Prior literature most use high performance liquid chromatography (HPLC) with UV-Vis\textsuperscript{16} or DAD\textsuperscript{17} detector , gas chromatography (GC) with MS\textsuperscript{17}, capillary zone electrophoresis (CZE) with UV-Vis\textsuperscript{11} detector. These are the classic and widely used instrument, but CZE has poor sensitivity, GC-MS analysis is high cost, although HPLC is low cost and high sensitivity but in the analysis process need use a lot of organic solvent witch is not environmentally friendly. Subcritical water chromatography, a fully green chromatography is similar with HPLC will used in present study.

Subcritical water at the first is use on extraction, first introduced by Hawthorne and Yang\textsuperscript{18}. When water temperature increased the dielectric constant of water will be decrease\textsuperscript{18-22}, at this point the properties of subcritical water and water–methanol or water–acetonitrile mixtures are similar.

In this study, methyl, ethyl and propyl parabens ware detected by subcritical water chromatography with salt-assisted homogenous liquid-liquid extraction. Use self-designed glass extraction device combine salt-assisted homogenous liquid-liquid extraction can reduce time-consuming, cost and organic solvent. In the analysis process include chromatography, only use few solvent, is a fully green method.

2. Experimental

2.1. Chemicals

Methyl, Ethyl and Propyl 4-hydroxybenzoate were obtain from sigma-Aldrich. HPLC grade acetone, methanol, acetonitrile and 2-propanol were purchased from Merck. Phosphoric acid, sodium hydroxide, Sodium dihydrogen phosphate, disodium phosphate, phosphoric acid are Analytical grade obtain from Merck. Ultrapure water were produce from Barnstead.

2.2. Subcritical water chromatographic equipment and condition

HPLC system was Shimadzu LC-9A solvent delivery system with Soma S-3702 UV-Vis detector and Rheodyne injection valve (model 7125) fitted with 10 μl sample loop. Column heater and pre-heater were purchased from Jianxin electrothermal. The device show at fig.1. Data collection by SISC 32. Mobile phase use 25mM pH 10.5 phosphate buffer solution and the flow rate keep on 1mL/min with temperature
100°C.

2.3. **Salt-assisted homogenous extraction procedure**

The self-designed glass extraction device fig.2, scale end plugged tightly, contain 5mL aqueous sample and stir bar. Then add extraction solvent and uniform mixing. Following add ammonium sulfate and plugged the end. After centrifugation, the upper organic solvents evaporated by nitrogen, and re-dissolved with mobile phase. Finally inject of 10uL re-dissolved solution into the HPLC for analysis.
Figure 1. Subcritical water system
Figure 2 Self-design extraction device and extraction procedure
3. Results and discussion

3.1. Optimize of subcritical water chromatography

3.1.1. Effect of Mobile phase pH and column temperature

pH value and column temperature in subcritical water chromatography are most important parameter for elution ability. pH value will affect the state of the analyte in molecular state or ionic state. In the reverse phase HPLC the analyte with ionic state is no retention or weak retention, but the analyte with molecular state in the reverse phase column. Temperature changes associated with changes in dielectric constant. With the dielectric constant change the elution ability also changed.

In the experiment investigate subcritical water at different pH and temperature affect of the elution ability. The result are show at fig. 3. At the mobile phase pH 10, the propyl paraben had a strong retention resulting peak broaden and had shoulder. When pH value was greater than 10.5 will get symmetrical peaks and more sharp. When the column more high the retention time will more short result the peak more sharp, but if temperature higher than 100°C methyl paraben was easy overlap with peak from pressure pulse. Considered the best instrument and column operating environment, so choose mobile phase pH 10.5 at column temperature 100°C to complete the following experiment.

3.2. Optimize of salt-assisted homogenous extraction

3.2.1. Extraction solvent selection

Extraction solvent selection in solvent extraction method is very important. The solvent in the salt-assisted homogenous extraction require can be separated from the aqueous solution by salting-out effect, and extraction maximum amount analyte.

In the experiment using 300μL acetone, acetonitrile and isopropyl alcohol as the extraction solvent. The result is show at fig. 4. Isopropyl alcohol could extract the maximum amount of analyte and had the most solvent recovery. In the following experiment will use the isopropyl alcohol as an extraction solvent.

3.2.2. Effect of Extraction solvent inject to the subcritical water chromatography system

Solvent extraction method was used in current study, but in HPLC system inject strong elution solvent will affect peak shape even retention time. In this study use different ratio of extraction solvent dilution with mobile phase to investigate solvent effect in the subcritical water. Results(fig. 5) indicate that injected into the higher ratio of organic solvent effects on the chromatogram higher. So In the following experiment which will evaporate and re-dissolve with the way.
Figure 3 (a) mobile phase pH 10 (b) mobile phase pH 10.5 (c) mobile phase pH 11
Figure 4  Extraction solvent selection
Figure 5 inject Isopropenol ratio: a: 0 %, b: 5 %, c: 10 %, d: 20 %, e: 30 %, f: 40 %
3.2.3. Effect of Solvent volume

Solvent volume in solvent extraction will affect the sensitivity. If solvent volume is too much it will not dilute the analyte concentration, but need more time to evaporate solvent it will increase the deviation, otherwise if solvent volume is too less the solvent is easy to loss result the deviation increased. The result of solvent volume is show at fig. 6, solvent volume in 150uL is too less to easy loss so the standard deviation is high. After 200 uL solvent volume the extraction efficacy are similar, consider about evaporate time so 200uL selected as the best solvent volume.

3.2.4. Effect of salt type and amount

Salt type and amount in salt-assisted homogenous extraction is play an important role, will affect water phase and organic phase separation conditions. In the experiment investigate ammonium sulfate, magnesium sulfate, sodium chloride and potassium chloride in salt-assisted homogenous extraction. The result indicate only ammonium sulfate can let Isopropyl alcohol and sample solution separate. Amount of ammonium sulfate will affect recover organic solvent indirect effect sensitivity. In the experiment investigate ammonium sulfate weight in range 3-4.5 g. The result show at fig. 7, after 4.0g ammonium sulfate was saturation, so in following study 4.0g ammonium sulfate was used.

3.2.5. Effect of sample pH value

pH value of sample solution will affect the analyte in molecular state or ionic state. In solvent extraction, organic solvent only can dissolve analyte in molecular state. In this study investigate pH value in range 2-10, the result show at fig. 8. All the analyte pKa is around 8.4, so below pH 6 all the analyte were in molecular state, but lower than pH 2 the analyte were easier decomposed. According to the experimental results, the pH6 will be selected as the best pH value.

3.3. Analytical performance

3.3.1. Calibration curve parameter

To prove the performance of this method, calibration curve obtained by linear range 0.75-75 mg/L for methyl and ethyl paraben, 1.5-150mg/L for propyl paraben. the result show at Table 1,detection limits ranged from 0.07 ~ 0.1 μg / L, R2 ranges from 0.9986 - 0.9993, pre-concentration factor of 238 ~ 309 and precision below 9.2% .

3.3.2. Determination of real samples

In this study, samples taken from the real market selling cleanser "3 M Nexcare Acne Cleanser". Weighing 0.1 g of cleanser pH 6 by adding 100 ml of phosphate buffer solution, then adjust this solution to pH 6 phosphate buffer solution diluted 200 times. The diluted real sample extracted by salt-assisted liquid-liquid extraction with optimize. For obtain the recovery to definite performance of this method, three level standard spiked into real sample. The result show at Table 2
Figure 6 Effect of Solvent volume

Figure 7 Effect of salt amount
Figure 8 Effect of sample pH value
<table>
<thead>
<tr>
<th>analyte</th>
<th>Linear range(μg/L)</th>
<th>Calibration curve</th>
<th>$R^2$</th>
<th>LOD(μg/L)</th>
<th>RSD</th>
<th>Enrichment factor</th>
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<tbody>
<tr>
<td>Methyl paraben</td>
<td>0.75-75</td>
<td>$y = 116833x + 110199$</td>
<td>0.9986</td>
<td>0.07</td>
<td>2.4-9.2%</td>
<td>238</td>
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<tr>
<td>Ethyl paraben</td>
<td>0.75-75</td>
<td>$y = 147193x + 137219$</td>
<td>0.9992</td>
<td>0.09</td>
<td>3.0-9.1%</td>
<td>264</td>
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<tr>
<td>Propyl paraben</td>
<td>1.5-150</td>
<td>$y = 161361x + 146937$</td>
<td>0.9993</td>
<td>0.1</td>
<td>1.8-7.5%</td>
<td>309</td>
</tr>
<tr>
<td>Analyte</td>
<td>Real sample</td>
<td>Spike level 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Spike level 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Spike level 3&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Dilution (μg/L)</td>
<td>Real&lt;sup&gt;d&lt;/sup&gt; (w/w)</td>
<td>R (%)</td>
<td>RSD (%)</td>
<td>R (%)</td>
<td>RSD (%)</td>
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<tr>
<td>MP</td>
<td>8.26</td>
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<td>92.3</td>
<td>1.7</td>
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<tr>
<td>EP</td>
<td>ND</td>
<td>ND</td>
<td>93.1</td>
<td>5.2</td>
<td>109.4</td>
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<tr>
<td>PP</td>
<td>3.50</td>
<td>0.07%</td>
<td>94.7</td>
<td>1.1</td>
<td>102.7</td>
<td>6.6</td>
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</tbody>
</table>

Note: ND indicates analyte not detected; R is recovery; RSD is relative standard deviation

<sup>a</sup>: Level 1: MP, EP 5μg/L, PP 10μg/L

<sup>b</sup>: Level 2: MP, EP 20μg/L, PP 40μg/L

<sup>c</sup>: Level 3: MP, EP 40μg/L, PP 80μg/L

<sup>d</sup>: After calculating the true sample concentration
4. Conclusion
In this study, successful use of salt-assisted homogeneous liquid-liquid extraction with
subcritical water chromatography system and UV detector to detect the Methyl paraben,
Ethyl paraben, Propyl paraben in the cosmetics. In this experiment, use self-designed
glass device extract 5 ml pH 6 sample add 200μl of Isopropanol after adding 4 grams of
ammomium sulfate can get the best extraction efficiency. Under these conditions, the
linear dynamic range for the detection was 0.75-75 μg/L for methyl paraben and ethyl
paraben, and 1.5-150 μg/L for propyl paraben, with relative standard derivation (RSD)
below 9.2%. Detection limits were achieved at the level of 0.07~0.1 μg/L. Recoveries
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