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Extraction of dl-anabasine from Alangium platanifolium root using emulsion liquid membrane

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Abstract: An experimental study on the extraction of dl-anabasine in Alangium platanifolium root (APR) using an emulsion liquid membranes system (ELMs) has been reported. The ELMs was assembled using a butadiene styrene rubber emulsifier to prepare the emulsion, instead of surfactant Span 80, acetic acid and kerosene as the carrier and diluent solution, respectively. The extraction time, pH, and volume ratio of styrene rubber emulsifier in the membrane phase were examined and the optimum condition was found. Under such condition, an appropriate pH gradient in the feed and strip aqueous phase was set up. Selective separation of dl-anabasine from the matrix of raw APR or processed APR demonstrated that facilitated transport was achieved in the ELMs. A dynamic extraction of dl-anabasine provides a reasonable fit of the amount extracted versus time in the ELMs, which is based on mass transfer across aqueous boundary layer, interfacial chemical reaction, and diffusion in the emulsion globule. A comparison of the amount of dl-anabasine extracted from the raw APR and processed APR between the liquid-liquid extraction (LLE) and ELMs extraction were performed for both qualitative and semi-quantitative analysis using GC/MS. The results showed the relationship between the extraction amount of
dl-anabasine and the extraction time is a bell shape curve, as the extraction amount of dl-anabasine reached its maximum at 2 min, and the amount decreased with either increasing or decreasing extraction time. The amount of dl-anabasine extracted using ELMs is three times as much as that extracted using LLE.

Key words: Alangium platanifolium root (APR); dl-anabasine; emulsion liquid membrane extraction; rubber emulsifier; GC/MS

1. Introduction

APR belongs to the Alangiaceae plant. Its root is a commercial herbal medicine in China, which is used to treat the rheumatism pain, paralysis, heart failure, lumbago, and traumatic injury [1, 2].

The pharmaceutical analysis shows that the dl-anabasine (Fig.1), whose structure is similar to the nicotine, is the major bioactive substance in the APR [3]. The principal method for the recovery of dl-anabasine from herbs is LLE, in which the extracting solution is generally degreased by acidolysis, followed by extraction using organic solvents in alkaline condition [4,5]. Hence, the LLE method is a time and organic solvent consuming process. ELMs extraction attracts attentions once again in recent years due to its high extraction rate. This technology has been applied in the recovery of metal ion from the wastewater [6-14], the removal of organic dye from the water [15], and the extraction of the weak alkaline and acidic substances [16-21].

Fig. 1

Kerosene and Span 80 are normally used as the organic membrane phase for the
preparation of the emulsion [22, 23]. But the emulsions are not suitable for complicated bio-sample due to the donor phase being complex. If they used to pre-treat complicated samples like the herb extractive solution, a long-term stability of the emulsion would not be obtained, leading to unsatisfying the results.

One of the remedies for improvement of the emulsion stability is increasing the emulsion viscosity through adding surfactant. Unfortunately, the membrane mass transfer rates decrease substantially as the emulsion viscosity is increased [24]. However, Hopper found the transfer rates dose not decay as the emulsion contained polymer, despite an increase in fluid consistency [25].

The work reported here was undertaken to isolate dl-anabasine from the APR using ELMs extraction. The emulsion was prepared using the rubber emulsifier without adding any other surfactant. We found the emulsion prepared with rubber emulsifier had good stability and could be used for emulsion membrane extraction of alkaloids from raw herbal medicine. A bell shaped curve was found when plotting the curve of extraction amount with time. This phenomenon is inconsistent with the emulsion liquid membrane extraction principle. The possible reasons were discussed in this paper in detail. GC/MS method was used in the qualitative analysis of the dl-anabasine in the APR. The nicotine was used as the standard in the quantitative analysis with the double internal standard method.

2. Experimental

2.1. Instrumentations and Reagents

GC/MS was performed on an Agilent 7890A GC Plus equipped with a 5975C
mass/selective detector (Agilent Technologies). A fused silica capillary column, HP-5-MS, with 5%-phenyl methylpolysiloxane as a non-polar stationary phase (30 m × 0.32 mm i.d. × 1.05 mm film thickness, Agilent Technologies) was utilized for analysis of dl-anabasine obtained from the processed APR and the raw APR. A high shear emulsifying machine (WX500CY, Shanghai Weiyu) was used to prepare the emulsion.

APR was procured from AnGuo medicinal materials company (authenticated by pharmacists). Acetone (AR), dichloromethane (AR), 1,2-dichloroethane (AR), ethanol (95%), hydrochloric acid (36%), concentrated sulfuric acid (98%), acetic acid (36%), sodium hydroxide were purchased from HuaXin reagent shop. Kerosene (boiling point range of 200–240 °C) and butadiene styrene rubber (BR9000), were obtained from manufacturers (Yanshan Beijing). Butadiene styrene rubber emulsifier was prepared in our lab. The water used in the experiments was double distilled water prepared in our lab.

2.2. Extraction of APR

The raw APR or processed APR (soaked with HCl solution (2 mol/L) for 1 h and dried in the air) was weighed (5 g) and soaked in ethanol aqueous solution (60%) in Soxhlet extractor overnight and then the sample was extracted to the solution until colorless. After ethanol was recovered, the residues (40 mL) of the APR extracting (APRE) solution were obtained.

2.3. Preparation of W/O emulsion

The W/O emulsion was prepared using kerosene and rubber emulsifier (prepared
by butadiene styrene rubber (5 g) dissolved in kerosene (150 mL) as membrane phase and the sulfuric acid aqueous solution (0.1 M) as the strip phase. The volume ratio of the mixture for emulsion was 0.8:12.2:7 (emulsifier: kerosene: sulfuric acid solution v:v:v). The mixture added with 0.4 mL acetic acid (36%) as carrier was agitated at 10000 r/min for a few minutes. The fresh water-in-oil (W/O) emulsion was obtained.

2.4 Emulsion extraction

The donor phase solution was prepared by adjusting pH of APRE solution (20 mL) to 12 with NaOH solution (9.8%) and diluting with water to 40 mL. A water-in-oil-in-water (W/O/W) emulsion liquid membrane extraction cell was formed as freshly prepared emulsion (10 mL) was uniformly dispersed to the donor phase solution (40 mL) under magnetic stirring (150/min). After stopping the magnetic stirrer, the emulsion of W/O/W was spontaneously separated by gravity. The lower water phase was collected and its volume and pH were measured.

The demulsification of W/O emulsion was performed by heating the emulsion with magnetic stirring (150/min) [26]. After demulsification process, the pH of the strip phase was measured; and the residual of dl-anabasine in the kerosene was extracted with 6.5 mL sulfuric acid aqueous solution (0.3 mol/L). After extraction, the mixture of kerosene and aqueous solution were centrifuged (2000 r/min) for 10 min to make the oil phase and water phase separated. The lower layer water solution was separated and adjusted the pH to 12 with NaOH (9.8%). The dl-anabasine in the water solution was extracted by 1,2-dichloroethane (3 mL×3). The organic phase was combined in a 10 mL centrifuge tube as S1 for further GC/MS analysis.
2.5. Liquid-liquid extraction

APRE solution (20 mL) was undertaken acidolysis by heating reflux with adding 20 mL of HCl solution (2 mol/L) for 1 h; then it was extracted with 1,2-dichloroethane (14 mL×3) for degreasing at room temperature. The upper layer water solution (40 mL) was collected and adjusted to pH to around 12 using NaOH solution (9.8%). The dl-anabasine in the water solution was extracted by 1,2-dichloroethane (14 mL×3). The organic solution was combined as S2 for further GC/MS analysis.

2.6. Preparation of the nicotine standard solution

The nicotine stock solution (2000 µL/L) was prepared by diluting the nicotine ethanol solution (nicotine: ethanol; V:V=1:1) with water. Briefly, 0.8 mL of the nicotine ethanol solution was pipette into a brown volumetric flask (200 mL), and then water was added to the marked line of the volumetric flask.

The nicotine standard solution was prepared by diluting the nicotine stock solution with ethanol (95%) in a brown volumetric flask. Briefly, 1.5 mL of the nicotine stock solution was removed into a brown volumetric flask (10 mL), and then ethanol was added to the marked line of the brown volumetric flask. The concentration of 300 µL/L nicotine standard solution was obtained.

2.7. Conditions of gas-chromatography and mass spectrometry

The compositions of alkaloids of APR were analyzed by GC/MS (6890N, Agilent; VG70E-HF, Micromass), equipped with a HP-5 column (32 m × 0.32 mm i.d., 1.05 µm film thickness). The temperature program was as follows: 60°C for 1 min,
then increased by 25 °C/min to 160 °C and further increased by 10 °C/min to 240 °C held for 10 min. The other parameters were as follows: injection temperature, 240 °C; ion source temperature, 220 °C; EI, 70 eV; carrier gas, He at 1.6 mL/min; injection volume, 1 µL; splitless mode; purge time, 0.5 min; and mass range, m/z 35 – 500; accelerated voltage, 6 kV; source temperature, 220 °C; collected current, 200 µA; source vacuum, 10^-4 Pa; analyzer vacuum, 10^-6 Pa. Quantification was obtained from percentage peak areas from the gas chromatogram. National Institute of Standards and Technology Mass Spectral Database Library (NIST-MS, 1998) search were used for dl-anabasine identification.

2.8. Quantification of dl-anabasine

The S1 and S2 were evaporated to dryness under a gentle stream of nitrogen gas. The dry residual was dissolved by 0.2 mL acetone and 0.1 mL of nicotine standard solution (300 µL/L) for injection of GC/MS analysis. All the calibration standards and pharmaceutical samples were run in triplicates. The average peak areas ratios of dl-anabasine and nicotine GC signals were calculated for quantification of dl-anabasine. The dl-anabasine content extracted in per gram raw APR or processed APR sample \( Q \) as shown in the following equations:

\[
Q = \frac{VC}{m} \quad (I)
\]

where \( V \) is the volume of sample solution (mL); \( C \) is the concentration (µg/mL) of the sample solution for dl-anabasine measured by GC/MS and the \( m \) is the grams of the APR.

3. Results and discussions
3.1. Alkaloids emulsion liquid membrane extraction principle

Alkaloids emulsion liquid membrane extraction is similar to the cation exchange (Fig.2). The cation exchange reaction happens in the second interface of the strip phase and the organic membrane phase.

\[ \text{A}^+ + \text{QH} \leftrightarrow \text{QA} + \text{H}^+ \]

Equilibrium coefficient: \( K = \frac{[\text{QA}][\text{H}^+]}{[\text{QH}][\text{A}^+]}) \)

where QH is the carrier and A\(^+\) is protonated alkaloids. Protonated alkaloids (A\(^+\)) are either present as A\(^+\) or A\(^+\)HSO\(_4^-\) in the strip phase depending on solubility of the acidic salt. The protonated alkaloids (A\(^+\)) or the insoluble acidic salt could not return to the organic membrane phase, which helps accomplish the unidirectional transmission from the donor phase to the strip phase.

In our previous work, we found the acetic acid carrier is necessary, in terms of nicotine extraction [27,28]. Since anabasine has similar molecular structure as nicotine (Fig.1), we just apply our previous condition here. In our preliminary data, we also found the extraction efficiency of anabasine with acetic acid carrier is better than without it.

Fig. 2

3.2. Choice of the emulsion

If the emulsion was prepared by the kerosene and Span 80, and the nicotine solution was used as the donor phase, a stable W/O/W emulsion liquid membrane extraction system would be formed when the emulsion was dispersed into the donor phase. However, the W/O emulsion prepared by the kerosene and Span 80 was
instantly coalescing without any stirring when dispersed into the APRE solution, which made the extraction of complicated substances difficult. Hence, in this paper, the emulsion used for the extraction was prepared using the rubber emulsifier. As this emulsion was dispersed into APRE solution, the emulsion was not demulsified obviously after the magnetic stirring for 8 minutes. The volume and pH of the donor and strip phase before and after the emulsion extraction was listed in Table 1. It showed that the change of the volume and pH was not significant for the donor phase before and after the extraction; however, the change of the volume and pH was significant for the strip phase. Even so, the strip phase was still the acidic and the donor phase was the basic, which ensure the dl-anabasine unidirectional transfer. The table 1 also showed a decrease of the recovery volume of the strip phase. The volumetric decrease came from the emulsion swelling and coalescence and the experimental operation loss, which could be observed from the recovered kerosene after the demulsification. There are 6.5 mL of kerosene in the 10 mL of emulsion initial. After the demulsified, the recovered kerosene is 6 mL (recovery rate was 92.3%, recyclable). The experimental operation loss was 0.5 mL.

The increase of the pH was because of the transfer of the H⁺ from the strip phase to the donor phase. In other words, in the dl-anabasine extraction process, the liquid membrane not only transferred the dl-anabasine from the donor phase to the strip phase, but also transferred H⁺ from the strip phase to the donor phase.
Table 1  Change of W/O/W extraction parameters

<table>
<thead>
<tr>
<th></th>
<th>The donor phase</th>
<th>The strip phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>V (mL)</td>
</tr>
<tr>
<td>Before the extraction</td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>After the extraction</td>
<td>10</td>
<td>41</td>
</tr>
<tr>
<td>After the demulsification</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The volume of the emulsion was 10 mL (kerosene, 6.5 mL; sulfuric acid solution, 3.5 mL);
The volume of the donor phase was 40 mL (APRE solution, 20 mL; water, 20 mL); extraction time: 8 min.

3.3. Dynamic extraction of dl-anabasine

The extraction dynamic curve of dl-anabasine was shown in Figure 3, which was the dl-anabasine content extracted in per gram APR plotted against the extraction time. The curve showed that the extraction amount increased with time initial, reached a maximum (19.7 µg) at 3 minutes, and with the extraction time further increasing, decreased to constant up (2 µg). According to the single direction mass transfer extraction principle, the dl-anabasine amount should increase in the strip phase with the increasing of the extraction time. As the extraction reached saturation, the dl-anabasine amount would not decrease with the extraction time. The conflict between the experimental fact showed in Fig. 3 and the single direction mass transfer extraction principle may be caused by three reasons: 1) With the unstable emulsion, the long-time stirring made the emulsion swelling and coalescence, leading to the breakage of the emulsion; 2) With poor selectivity of the emulsion extraction, substances other than dl-anabasine were transferred also, among which might combine with the dl-anabasine and generate the undissolved substance under the acidic environment; 3) There were some organic substances with high molecular weight in the APRE solution, which could penetrate the organic liquid membrane into the strip phase to generate the undissolved substance to adsorb the dl-anabasine in the
acidic aqueous solution.

The first reason could be excluded because Tab. 1 showed that the emulsion was stable. Hence, the results of the conflict were most probably caused by the second or third reason.

Fig. 3 the extraction of compared with the processed APR

3.4. Processing APR

To explore the reason of the dynamic extraction above, the raw APR were undergone a processing handcraft prior to extractions. The processing handcraft of herbal medicine has appreciable enhancement in both the medicine effect increase and toxic decrease. The processing handcraft for APR was soaked in the raw APR in acidic solution. After being soaked for 12 hours under ambient temperature, the soaked APR was removed and dried under room temperature, and then was extracted with ethanol aqueous solution (60%) using Soxhlet extractor. The alcohol extractants of the processed APR was extracted by the ELMs extraction or the LLE.

The time of the ELMs extraction was 3 minute and the extraction results of the processed APR were compared with the raw APR. The results showed that the amount of the dl-anabasine per 1g of the raw APR sample extracted by the ELMs extraction was 19.7 µg/g, and by the LLE was (9.8 µg/g); the amount of the dl-anabasine extracted by ELMs was twice of that by the LLE. While the APR was processed, this amount extracted in the two extraction methods was the same 2.2 µg/g, which was much less than that of raw APR and coincidentally agreed with that of the ELMs extraction of the raw APR after 6 minutes (Figure 3).
These experiments indicated that the amount of the extracted dl-anabasine was decreased when it was exposed in acidic environment for a long time. The reason of these may be the formation of the undissolved substance between dl-anabasine (had been extracted in the strip phase) and other substances co-transport with dl-anabasine or some organic substances with high molecular weight in the APRE solution as seen during the reflux acidolysis of the sample as 2.5 described. These substances make the amount of the extracted dl-anabasine decreased in both of the LLE and ELMs extraction.

Keeping the strip phase in acidic was necessary for the ELM extraction of alkaloids. The long time ELMs extraction would lead to a decrease of the amount of the extracted dl-anabasine. Therefore, the relationship between the amount of extracted dl-anabasine and the extraction time is a bell shape curve with the maximum appearing at 3 min (Fig.3).

3.5. The effect of acidolysis

To study the effect of acidolysis APR extractin solution on the dl-anabasine transfer rate, the contrast test was carried out. The extraction solution of the raw APR sample was divided into two equal parts. One part was undertaken by the reflux acidolysis according to Part 2.5 and the other one did not. By acidifying, the sample solution became darker and cloudy. Some black precipitates were generated in the solution. Table 2 showed the results of the ELMs extraction of dl-anabasine in the sample solution. The dl-anabasine was not found in the acidified sample solution until the solution was filtrated and black precipitates were removed from the solution. The
The extraction amount of dl-anabasine was more than that of the LLE, but less than that of the unacidolysis sample extracted by ELMs extraction. The black precipitates might be the undissolved substances, which could adsorb the dl-anabasine in the extraction solution and prevent dl-anabasine penetrating through the oil membrane into the strip phase. The contrast test above illustrated that: 1) Reactants existed in the APR extraction, which can generate the undissolved substance under the acidic condition; 2) The undissolved substance can adsorb dl-anabasine in the aqueous solution; 3) The concentration of the undissolved substance in the sample solution is not high enough to adsorb all the dl-anabasine.

Table 2 The results of ELMs extraction APRE solution under acidolysis and unacidolysis conditions

<table>
<thead>
<tr>
<th>Samples</th>
<th>The content of dl-anabasine per gram APR (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-filtration</td>
</tr>
<tr>
<td>Raw APR (acidolysis)</td>
<td>0</td>
</tr>
<tr>
<td>Raw APR (unacidolysis)</td>
<td>19.7</td>
</tr>
</tbody>
</table>

Extraction time: 3 min.

3.6. High resolution mass of the black precipitates

To investigate the black precipitate component, a high resolution MS was performed with FTMS using the black precipitates of methanol solution as sample. Fig. 3 was the spectral mass data of the black precipitates. It showed many peaks indicating the black precipitates are a complex mixture of several compounds. Table 3 listed the probable molecular formula for 4 major peaks of the black precipitates. They are all N-contained organic compounds with low vapor pressures and high molecular weight except C_{49}H_{65}O_{3} at m/z of 701. These N-contained compounds, whose molecular structure are not clear, can partially be penetrate through the
organic liquid membrane into the strip phase during ELMs extraction, where it reacted with $\text{H}^+$ under the acid environment and generating the undissolved substances leading to a decrease of the amount of extracted dl-anabasine.

Fig.4

<table>
<thead>
<tr>
<th>m/z</th>
<th>588</th>
<th>701</th>
<th>814</th>
<th>927</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>$\text{C}<em>{31}\text{H}</em>{58}\text{N}_1\text{O}_9$</td>
<td>$\text{C}<em>{37}\text{H}</em>{60}\text{N}<em>2\text{O}</em>{10}$</td>
<td>$\text{C}<em>{43}\text{H}</em>{60}\text{N}<em>3\text{O}</em>{11}$</td>
<td>$\text{C}<em>{62}\text{H}</em>{83}\text{N}_6\text{O}$</td>
</tr>
<tr>
<td>$\text{C}<em>{32}\text{H}</em>{54}\text{N}_5\text{O}_5$</td>
<td>$\text{C}<em>{38}\text{H}</em>{67}\text{N}_{16}$</td>
<td>$\text{C}<em>{44}\text{H}</em>{68}\text{N}_7\text{O}$</td>
<td>$\text{C}<em>{61}\text{H}</em>{87}\text{N}_2\text{O}_5$</td>
<td></td>
</tr>
<tr>
<td>$\text{C}<em>{28}\text{H}</em>{50}\text{N}_{11}\text{O}_3$</td>
<td>$\text{C}<em>{34}\text{H}</em>{61}\text{N}<em>2\text{O}</em>{4}$</td>
<td>$\text{C}<em>{40}\text{H}</em>{76}\text{N}_4\text{O}_7$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{C}<em>{38}\text{H}</em>{63}\text{N}_6\text{O}_6$</td>
<td>$\text{C}<em>{56}\text{H}</em>{82}\text{N}_5$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{C}<em>{39}\text{H}</em>{65}\text{O}_3$</td>
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</table>

3.7. Influence of emulsifier concentration and emulsifying time on the rate of mass transfer

The experiment above showed that the emulsion prepared by the rubber emulsifier could be used in the extraction of dl-anabasine from the APR. To investigate the influence of the rubber emulsifier concentration in the W/O emulsion and emulsification time on the rate of dl-anabasine transfer, the ELMs extraction of dl-anabasine was tested using emulsion prepared in increase emulsifier concentration and in different emulsification time. The emulsifier concentration was increased from 4% to 6% and the emulsification time was 2, 3, and 4 min, respectively. The amount of dl-anabasine extracted using these emulsions were listed in Table 4.
Table 4 The content of dl-anabasine per gram APR (µg/g) extracted

<table>
<thead>
<tr>
<th>Number of test groups ()*</th>
<th>Extraction time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1 (2)</td>
<td>5.0</td>
</tr>
<tr>
<td>2 (3)</td>
<td>0</td>
</tr>
<tr>
<td>3 (4)</td>
<td>0</td>
</tr>
</tbody>
</table>

()*: emulsification time (min.); emulsifier: 5 g of butadiene styrene rubber dissolved in 150 mL of kerosene.

The results of extraction showed that the amounts of dl-anabasine extracted were all zero except for group 1. The highest mass transfer rate was obtained when the emulsifier concentration is 6% (V/V) coupled with a shorter emulsification time (< 3 min). This is because the emulsion droplets could achieve the optimal size, resulting in a good stability. Postponing the emulsification time would cause the emulsion droplets becoming smaller and smaller under the high internal shearing force, which is not favored for dispersion. Therefore, the mass transfer of dl-anabasine was inhibited.

The dynamic curve of extraction was shown in Figure 5. The peak was also appeared with increased extraction time. Compared with Figure 3, the dl-anabasine peak appeared in advance of half minute with increased value as well, which was expected according to the assumption above. The peak shift was due to the short extraction time, which reduced the consumption of dl-anabasine in strip phase, leading to an increase of the extraction amount.

Our study raises the issue of the loss of the alkaloid components of herbs during the extraction. The amount of some alkaloid components extracted from herbal medicine is often less than that of its content as long as the sample was pretreated with acid. Therefore, the alkaloids content in the herbs might be overestimated if
acid treatment is involved in the analysis.

Fig. 5

4. Conclusions

The emulsion prepared from the rubber emulsifier was more stable than that from Span 80, which could be used for extraction of the dl-anabasine from APR. There are the optimal extraction times in the ELMs extraction of the dl-anabasine from APR depending on the parameters of emulsion. Either long or short extraction time could not achieve the optimum extraction amount of dl-anabasine. The effects of the strip phase acidity and acidolysis play an important role in the extraction of dl-anabasine from raw APR. If this phenomenon was not a special case, it could be concluded that the alkaloids content in herbs would be smaller than that of the actual content, which would lead to a deviation on the efficiency and toxic side effect of the herbs. In this study, the amount of dl-anabasine extracted from 1 g raw APR by ELMs was 33.4 µg and by LLE was 9.8 µg; for the processed sample, the amounts of dl-anabasine extracted from 1 g processed APR by both ELMs and LLE were all 2 µg.

References:


2011, 192, 986–994.


Fig. 1 The structure chart of dl-anabasine

60x48mm (96 x 96 DPI)
Schematic diagram of the transport mechanism in ELM process for alkaloids separation

130x85mm (96 x 96 DPI)
The curve of extraction amount of the dl-anabasine per gram with time

Fig. 3

The curve of extraction amount of the dl-anabasine per gram with time
436x337mm (96 x 96 DPI)
Fig. 4 The mass spectrum of the black precipitates (The solvent is methanol.)

202x99mm (96 x 96 DPI)
Dynamic extraction figure (the emulsifier amount was 6% and emulsification time was 2 min)

436x337mm (96 x 96 DPI)