Analysis Fatty Acids Profile in *Tabanus bivittatus* Mats with Gas Chromatography-Mass Spectrometry

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**Abstract:** *Tabanus bivittatus* Mats., a traditional Chinese medicine, is commonly used for cardiovascular disorders treatment including atherosclerosis. There have been only a few researches on its chemical components, and no detailed report has appeared on its fatty acids. To develop a simple and effective method for the extraction of total fatty acids from *Tabanus bivittatus* Mats., the Soxhlet extraction (SE) condition was optimized with response surface methodology. The fatty acid composition of the extract were determined by GC–MS with previous derivatization to fatty acid methyl esters (FAMEs). The major fatty acids in *Tabanus bivittatus* Mats. were oleic acid, palmitic acid, linoleic acid, palmitoleic acid, and stearic acid, and the unsaturated fatty acids occupy 63.9% of the total fatty acids.

**Keywords:** Fatty acid, gas chromatography, mass spectrometry, response surface methodology, *Tabanus bivittatus* Mats.

1. INTRODUCTION

As a traditional Chinese medicine, *Tabanus bivittatus* Mats. is known to have a regulatory property for blood circulation and inflammatory disease [1, 2]. This natural product is one of the major drugs used in the Chinese traditional medicine Da Huang Zhe Chong pill to treat hepatic cirrhosis [3], atherosclerosis, and menstrual disturbance [4]; these actions are probably produced by pharmacodynamic activity on vascular system. Up to now, there have been only a few researches on the chemical components of *Tabanus bivittatus* Mats. [5], but no detailed report has appeared on its fatty acid components.

A variety of chromatographic techniques have been employed in the analysis of fatty acids, such as high performance liquid chromatography [6, 7], gas chromatography[8, 9]. A way to decrease the limit of quantification and provide a higher level of information is to use a gas chromatograph (GC) equipped with an MS detector, which allows quantification of each individual compound [10].

Soxhlet extraction (SE) is a classical method for decades in extraction of organic compounds from solid sample, and this apparatus has been developed to several types for special used. It is considered to be a “thorough” extraction method because the organic phase cooled from condensation tube continuously passed through the target solid sample for hours. Therefore it is a popular technique to analysis the minor composition file of the solid sample although high organic solvent volume, extensive extraction time, and intensive manpower are required [11].

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In this paper, SE followed by GC–MS was developed for the rapid analysis of fatty acids in Tabanus bivittatus Mats.. The experimental parameters were optimized with RSM in order to obtain the greatest extraction yield of fatty acids, and the fatty acid compositions of extract were determined by GC–MS with previous derivatisation to FAMEs.

2. EXPERIMENT DESIGN OF THE OPTIMIZATION OF EXTRACTION CONDITION WITH RSM

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes in which a response of interest is influenced by several variables [12], and has been widely applied to the optimization of the extraction procedure parameters [13, 14].

A three-level-three-variable Box–Behnken design (BBD) was adopted to optimize the extraction procedure. The independent variables were extractant volume (V, mL), extraction time (T, min), and ratio of acetone and petroleum ether (R). Three levels of each variable were coded as -1, 0, and +1 (Table 1). The extraction yield, represented as the weight of oil extracted form *Tabanus bivittatus* Mats., was taken as response, Y. A regression analysis was carried out in order to fit the experimental data into an empirical second-order polynomial model.

The variables and levels for each variable in BBD were determined according to the results of preliminary experiments. Based on the experimental results of the BBD (Table 1), the extraction yield followed a second-order polynomial model.

Where Y represented the extraction yield, calculated by the weight of oil extracted form *Tabanus bivittatus* Mats.; V, R and T correspond to three independent variables,
extractant volume (mL, for 10.0 g sample), ratio of acetone and petroleum ether, and extraction time (min).

The statistical analysis of our model variance and experimental results was included in Table 2. The F-value of our model is 3.96 which is significant. The chance that a ‘Model F-Value’ this large could occur due to noise was only 4.15%. The linear terms of extractant volume, and quadratic terms of proportion of acetone and petroleum ether had statistically significant effects on extraction yield. This is indicated by the B, A2 were significant model terms (p < 0.05). The coefficient determination in our model was 0.8360.

The best way to visualize the influence of independent variables on the dependent one is to draw a surface response plot of the model. With a fixed third independent variable at the central experimental level of zero, Fig. (1) showed the
effect and interaction of two independents on the responding variable, extraction yield. The extraction yield was increased with the increase of extractant volume up to about 70.0 ml, and then decreased. In contrast, the effects of ratio of acetone and petroleum ether and extraction time were not as important as the extractant volume. These results revealed that the extraction yield of total fatty acids was depended more on extractant volume than on ratio of acetone and petroleum ether and extraction time.

3. IMPACT OF EXTRACTING TIMES

The influence of multiple extracting was explored on the total extraction rate of fatty acids at the optimized condition. Fig. (2) showed that the total extraction rate of fatty acids increased with the extracting times, and the fatty acids were extracted entirely after 5 times. Considering the extraction cost and the total extraction rate increasing slightly over 3 times, extraction 3 times was selected as the optimum condition, at which the rate was 98.9% of that of 5 times.
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4. SYSTEM ANALYSIS OF CERAMIC DESIGN SYSTEM

After soxhlet extraction, the fatty acids in the extract were derivatized to FAMEs with sulphuric acid–methanol complex, and then analyzed with GC-MS. The analytical results were listed in Table 3. The relative contents were calculated by using the area normalization method, without considering response factors. Twenty-one kinds of fatty acids in the SE extract were identified and the sum of identified fatty acids occupied 95.95% of the area normalized. It can be seen from Table 3, that Tabanus bivittatus Mats. is remarkably rich in oleic acid (33.38%) and palmitic acid (20.96%) followed by linoleic acid (19.03%), palmitoleic acid (7.59%), stearic acids (7.53%). The unsaturated fatty acids occupy 63.92% of the total fatty acids. This result is consistent with the regulatory property of Tabanus bivittatus Mats. for blood circulation. Researches have identified that unsaturated fatty acids are effective in preventing cardiovascular events, cardiac death and coronary events, especially in persons with high cardiovascular risk. The high content of unsaturated fatty acids in Tabanus bivittatus Mats maybe the reason for it was used in cardiovascular disease treatment in China.

5. Analysis of Fatty Acids in Tabanus bivittatus Mats.

The current ceramic enterprises in ceramic product design process and production process of detailed study, the design process of ceramic products for further analysis and decomposition. Exploring the project design of ceramic needs human interaction steps in the 3d CAD system, and needs to be done by the system automatically in order to better improve enterprise’s key steps in the efficiency of product design and in the decomposition on the basis of the design process to classify modeling of ceramic products, and finished components decomposition of complex products. Ceramic products decomposition is different from the mechanical parts and components industry and other industry products, ceramic products decomposition lies mainly in the design of the components in the process of decomposition, and the final product in general is not an integral whole, for example, in the design process for more complex products such as “pot” will be broken down parts into the pot body, the pot, a spout, the lid and lid knob, a girder of the pot, an ear piece [10]. And set up all kinds of parts in the three-dimensional model of the material library.

CONCLUSION

To extract fatty acid of Tabanus bivittatus Mats., the best condition including extractant volume of 69.71 mL, ratio of acetone and petroleum ether of 5.3 and extraction time of
98.4 min was optimized using Response surface methodology. The unsaturated fatty acids occupy 63.92% of the total fatty acids. The analytical results of GC-MS showed that abundant unsaturated fatty acids such as oleic acid and linoleic acid accounted for 63.92% of total fatty acids.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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