Quantitative Analysis of Sibutramine in Diet drugs by High Performance Liquid Chromatography and Evaluation of the Risk of Sibutramine in Diet drugs by BP Neural Network

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Abstract. Purpose to quantitatively analyse harm of 6 diet drugs containing sibutramine. Method Firstly, we used high performance liquid chromatography to determine the content of sibutramine in six diet drugs and designed experiment to obtain datas after injection of different drug samples in rats. Next, we used these datas for BP neural network training and obtained the harm values of the samples at different doses and times to human. Result The model has been tested with high accuracy. Conclusion People should stay away from illegal drugs to scientifically lose weight.

1. Introduction
In recent years, food safety has become a hot topic of concern. The chemical sibutramine has been banned by the state due to its high risk of heart disease. However, many businessmen and people who love beauty still turn a blind eye to its harm.

In this paper, firstly, we used high performance liquid chromatography to determine the content of sibutramine in six diet drugs and designed experiment to obtain datas after injection of different drug samples in rats. Next, we used these datas for BP neural network training and obtained the harm values of the samples at different doses and times to human.

2. Literature Review
2.1. Progress in the method of HPLC for detecting prohibited components in diet drugs
High-resolution liquid chromatography-mass spectrometry [1] can be used to quantitatively detect complex and trace chemical components in diet drugs, the solvent used was mainly methanol [2] [3] and ethanol [4]. But there exists the situation [5] that acetonitrile-acetic acid is used as a solvent. But the experimental results obtained from acetonitrile as an extract [6] are more accurate.

Qing Gao [7] ensured the reliability of qualitative results by comparing chromatographic retention time, ultraviolet spectrum and mass spectrometry information of samples and control groups. Wending Cao [8] found using the injection volume (x) as the abscissa and the peak area (y) as the ordinate for regression has a good linear relationship in the range of 0.0106-0.2110 mg/ml.

Therefore, the HPLC can be used to quantitatively analyze the sibutramine in the six diet drugs.
2.2. Progress of BP neural network training and data evaluation

Z. Zhang [9] discussed the optimization of BP. J. Y. Zhong [10] elaborated on the deviation of BP neural network. L. W. Fan [11] describe the activation layer selection of BP neural network in detail.

BP neural network has been applied to many analysis, such as the convergence rate of a dilute emulsion with a non-uniform surface tension [12], the effect of different temperatures on the surface tension of branched paraffins [13], simulating and estimating ion flow in liquids [14] the interaction between different alkanes [15]. Ren C [16] and Xiao Z [17], also applied BP neural network in different occasions.

In the multi-factor data processing, BP neural network can obtain satisfactory prediction results.

3. The experimental content

3.1. Determination of sibutramine content of the drug by HPLC

3.1.1. Instruments and drugs

Instruments: beaker, 100ml volumetric flask, 2L volumetric flask, glass rod, rubber head dropper, mortar, pipette gun, centrifuge tube, analytical balance, HPLC, ultrasonic cleaner, centrifuge.

Drugs: sibutramine, potassium dihydrogen phosphate, acetonitrile (chromatographically pure), methanol (chromatographically pure), secondary treatment of distilled water, samples(DC diet drug(id:1), yanhee diet drug(id:2),thin fast tea powder(id:3),tomato fat burning capsules(id:4),159 Meal replacement powder(id:5)&Taobao secret recipe black pill(id:6).

3.1.2. Experimental steps

(1) Prepare potassium dihydrogen phosphate solution.(2) Prepare standard solutions of the five concentration gradients.(3) Process the sample to be tested.(4) Select chromatographic conditions.(5) Draw standard curve of standard solutions.(6) Record the chromatogram of the sample to be measured.

3.1.3. Standard curve fitting results

![Chromatogram](image)

**Figure 1. Chromatogram**

Through the results, we obtained the relative retention time of sibutramine: 6.460min.
By linear fitting (Figure 2), we get the function between peak area and sibutramine concentration: $y = 684486x - 2 \times 10^6$, and the correlation coefficient is: $R^2 = 0.9961$, indicating that the fitting effect is good. Then we substituted the peak area of experimental sample measured at a mass of 0.100 g to obtain the following concentrations (Table 1).

### Table 1. Relationships between peak area and concentration

| id of drugs | Peak area | Concentration (μg/ml) |
|-------------|-----------|-----------------------|
| 1           | 4972311   | 10.1862               |
| 2           | 5927397   | 11.5815               |
| 3           | 927382    | 4.27676               |
| 4           | 3791730   | 58.3172               |
| 5           | 1389744   | 4.95225               |

3.2. Analysis of the effect of sibutramine on rats

In this experiment, firstly, we set three kinds of rats as experimental groups for each of five different gradients: normal-weight group (200-250 g), lean group (less than 150 g), and obese group (more than 300 g), all Eight-week-old female rats, 6 rats each group. Besides, we select 3 rats in 3 different groups as control groups.

Secondly, we injected different concentrations of drugs along the tail vein of the rats and injected them in multiple injections with a total injection volume of 1 ml. We continued to observe the condition of the rats every 6 hours for three days, and plotted the drug concentration and heart rate changes.

Finally, experimental observations showed that the three groups of rats had a certain degree of excitement and abnormal behaviour after injection of drugs, including: no eating, abnormal aggression, chaos and so on. Abnormal behaviour will be alleviated over time, but the higher the drug concentration, the slower the response. Compared with the three groups of rats, the recovery rate of the normal group was faster than that of the lean group and the obese group. Sibutramine had the most obvious effect on the obese group. The obesity group index had the largest difference before and after injection, and the recovery rate was the slowest. In addition to the previously described behaviour, rats injected with 80 μg/ml of drug also developed convulsions and vomiting. The mice in the obese group died suddenly after 72 hours of measurement.

3.3. BP neural network to estimate the harm of drugs to organisms

3.3.1. Experimental data preprocessing. To increase the calculation accuracy and the running speed of the training model, we use the function premnmx in MATLAB data processing software to normalize the input and output values of the original test samples and convert the data to the range between [-1,1]. We use the following equation to get the input and output sample values $P_n$ and $T_n$:

$$P_n = \frac{2(i - \min i)}{\max i - \min i} - 1$$  (1)

![Figure 2. Relationship between concentration and peak area](image)
\[ T_n = \frac{2(o - \min o)}{\max o - \min o} - 1 \]  \hspace{1cm} (2)

I is the total amount of original test samples to be processed, o is the sample value to be output, and the maximum value maxi and maxo of the input and output samples is calculated by corresponding premnmx function, so as the corresponding minimum values mini and mino.

3.3.2. **Corresponding training model establishment.** In this paper, we set the momentum factor mc 0.9, the maximum number of iteration steps 9999 and calculated error value 0.0001. Formula (3) shows a principle of establishing the training model.

\[ H_n = \sqrt{In + Ot + \alpha} \]  \hspace{1cm} (3)

\(H_n\) represents the number of hidden layer nodes, \(In\) is the corresponding number of input layer nodes, \(Ot\) is the corresponding number of output layer nodes, and \(\alpha\) is an integer between 1 and 10.

In the process, the number of neurons in the input layer is 5, and the number of neurons in the output layer is 1, we set the final selected number of hidden layers 3 \([10-14]\) and use Sigmoid function as the activation function of each hidden layer.

3.3.3. **Evaluation criteria for harm values.** In this training, healthy weight rats in the control group were taken as the full score of 1, obesity and emaciated rats were taken as the benchmark index, set as 0.9, heart rate and blood pressure of the three groups were taken as the benchmark, and the index of rats with the highest drug concentration and the longest time was taken as the minimum score of the training: 0.

After substituting the processed data as samples into the training, we obtain the BP neural network scoring system's benchmark score, and then we substitute the non-benchmark index data into the training, we obtain the score value of rats in other cases, and then the harm value (1 minus the score) was obtained in Table 2.

| Drugs | Thin harm | Normal harm | Obesity harm |
|-------|-----------|-------------|--------------|
| 1     | 0.212     | 0.198       | 0.224        |
| 2     | 0.355     | 0.327       | 0.393        |
| 3     | 0.098     | 0.093       | 0.103        |
| 4     | 0.527     | 0.523       | 0.538        |
| 5     | 0.101     | 0.100       | 0.113        |
| 6     | 0.832     | 0.803       | 0.856        |

3.3.4. **Assessment of the effects of drugs on humans.** Firstly, we chose tomato fat burning capsules and four volunteers, then we made the weight, drug dosage, heart rate and blood pressure of the volunteers equivalent to the data of the rats and substituted them into BP neural network to predict the blood pressure, heart rate and harm of the four volunteers. The comparison result of predicted and measured values is as following image: except for one data error of 16.7%, other data deviations are within 10%, indicating that the model has a relatively high level of accuracy.
4. Conclusion
In this paper, we quantitatively analyze the harm of diet drugs containing sibutramine through BP Neural Network Training, and we find that the drugs contained sibutramine has a huge influence on the rise of heart rate and blood pressure, and the recovery is long. High concentrations of sibutramine can cause heart rate to be too high and even cause sudden death. Therefore, people should scientifically lose weight, stay away from illegal drugs, and care more about health.

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