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Fluorescence Automatic Sampling Instrument for Drug Toxicity Detection Based on the Information Angle of Binding with the Carrier Protein

Xiangshuai Li, Xiangfen Li, Zishi Wang*, Hongliang Xu*

Engineering Research Center of Pesticide of Heilongjiang Province, College of Advanced Agriculture and Ecological Environment, Heilongjiang University, Harbin 150080, China
E-mail address: xuhongliang@hlju.edu.cn (H. Xu), 2016048@hlju.edu.cn (Z. Wang)
**Corresponding Author*

Abstract: *Considering the requirements of high precision, automation, fast speed and batch production for sample injection detection by fluorescence spectrophotometer, a fluorescence automatic sampling instrument for drug toxicity detection based on the information angle of binding with the carrier protein are developed for automatic sample injection. Firstly, through the "carrier protein binding information-toxicity relationship", the fluorescence automatic sampling instrument is controlled online to realize automatic sampling and online collection of spectral signals. Then, based on the changes in the manual sampling of the traditional fluorescence spectrophotometer, a hands-free and airtight sampling method was proposed to avoid measurement errors caused by manual sampling. Next, Sterilization thermostats and temperature regulators are added to ensure that the samples are not cross-contaminated and reduce the rate of protein deformation. Finally, using the "carrier protein binding information-toxicity relationship" to control sample injection in real-time, predict the acute toxicity LD50 and toxicity level of the sample, and provide suggestions for modification of the test substance structure. The fluorescence automatic sampling instrument for drug toxicity detection based on the information angle of binding to the carrier protein is suitable for online collection and analysis of large quantities of drug toxicity.*

Keywords: *Fluorescence spectroscopy. Automatic sampling instrument. Automation.*

1. Introduction

Fluorescence spectroscopy is a commonly used instrument in modern laboratories that provides advantages of simple operations, many kinds of measurable substances, fast detection speed, highly sensitive, non-invasive and low analysis cost. It also provides information on the existence of fluorescent molecules in biological samples. At present, fluorescence spectrophotometers have been widely used in the fields of chemical industry, agriculture, medicine and biology, and are the main analytical equipment for studying molecular structure and function. Compared with other absorption spectroscopy techniques, fluorescence spectroscopy has high sensitivity and low detection limit in identifying fluorophores[1-3]. Toxicity is the key to the success of drug development. Fluorescence spectrophotometer is often used to explore the toxic effects of drugs on carrier proteins, and the binding characteristics such as the fluorescence quenching mechanism, the binding constants (Ka), the number of binding sites (n) and the thermodynamic parameters can be obtained, and then used the "carrier protein binding information-toxicity relationship(CPBITR)[4]" to predict the acute toxicity(LD_{50}) and toxicity level of the substance, and provide suggestions for the modification of the structure of the substance.

Fluorescence automatic sampling instrument for drug toxicity detection based on the information angle of binding with the carrier protein (The device has applied for a utility model patent, Patent No. 202110178985.7.) is a fluorescence spectrophotometer sample injector controlled by "carrier protein binding information-toxicity relationship." The instrument mainly includes position sensors, Sterilization 18℃ thermostat, temperature regulator, Constant temperature water bath tube, solution storage box, USB jack and other components. Temperature regulator, constant temperature water bath tube and Sterilization 4℃ thermostat, which greatly reduces the protein denaturation rate, improves the detection accuracy and shortens the fluorescence spectrum detection time. The "carrier protein binding information-toxicity relationship" manipulates the sampling process in real-time and transmits the

ISSN 2616-5767 Vol.4, Issue 4: 41-48, DOI: 10.25236/AJETS.2021.040408

fluorescence spectrum information obtained by the fluorescence spectrophotometer to the platform. The platform automatically processes the data to predict acute toxicity LD_{50} of the substance, toxicity level and provide suggestions for modification of the structure of the test substance. Compared to the drug toxicity fluorescences automatic sample instrument based on the information of the carrier protein, the traditional fluorescence spectrophotometer relies on manual sampling, which has the disadvantages of easy cross-contamination of the solution, complicated operation, long detection time and the accuracy is lower. Therefore, the fluorescence automatic sampling instrument for drug toxicity detection based on the information angle of binding with the carrier protein has the characteristics of automation, high accuracy and fast detection speed,and can be used for a large number of drug toxicity predictions.

The purpose of this paper is to collect fluorescence spectrum information by the "carrier protein binding information-toxicity relationship" and fluorescence automatic sampling instrument for drug toxicity detection based on the information angle of binding with the carrier protein. And then the combination of "carrier protein binding information-toxicity relationship" to solve the error caused by the fluorescence spectrophotometer, manual sample injection, indoor temperature changes, the interference of the cleanliness of the quartz cell, and finally use the "carrier protein binding information-toxicity relationship" to predict and analyze the fluorescence spectrum information to achieve rapid detection of a large number of drugs, and lay the foundation for early prediction of drug toxicity in drug development.

2. Injector Structure

As shown in figure 1, the sterilization 18℃ thermostat (9) and the sterilization 4℃ thermostat (13) are used to keep the inner box of the box in a constant temperature state. As shown in figure 2, the sterilization 18℃ thermostat (9) is fixedly connected with the drug solution to be tested storage box (8) and the mixing gun (12) to be tested on both sides of the inside. The upper end of the mixing gun (12) is processed with the mixing gun cleaning port (11).

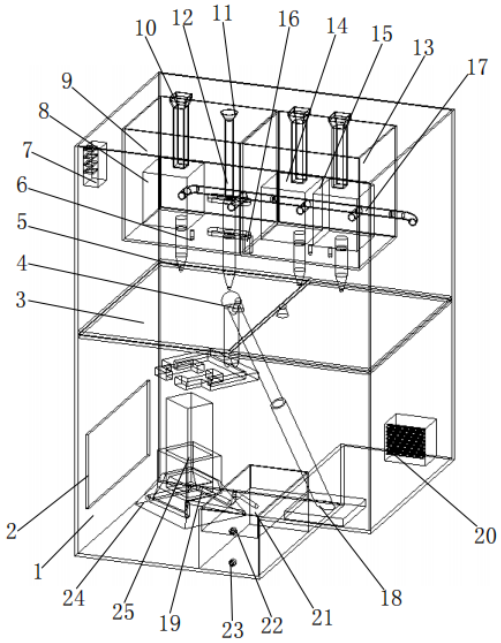

Figure 1: Assembly drawing of fluorescence auto-sampler for drug toxicity detection based on the information angle of binding to carrier protein. (1.Autosampler housing,2.Light passing hole, 3.Insulation baffle,4.Obstacle sensing switch,5.Injection gun head,6.Position Sensor,7.USB jack,8.Drug solution to be tested storage box,9.Sterilization 18℃ thermostat,10.Filling cleaning port,11.Mixing gun cleaning port,12.Mixing gun,13.Sterilization 4℃ thermostat,14.Carrier protein storage box,15.Buffer storage box,16.Temperature Sensor,17.Clean drain,18.Quartz cell gripper,19.Constant temperature water bath tube,20.temperature regulator,21.Ultrasonic water bath,22.Ultrasonic water bath filling port,23.Ultrasonic water bath drainage port,24.Quartz cell base,25.Quartz cell)

ISSN 2616-5767 Vol.4, Issue 4: 41-48, DOI: 10.25236/AJETS.2021.040408

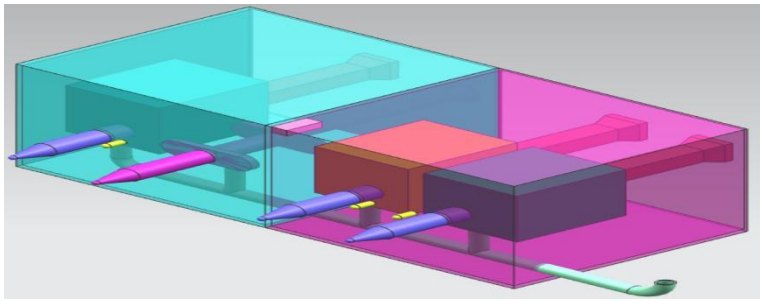

Figure 2: Sterilization 18℃ thermostat and sterilization 4℃ thermostat parts diagram.

The prepared human serum albumin carrier protein or other carrier protein solutions can be filled in the carrier protein storage box (14). The upper ends of the drug solution to be tested storage box (8) to be tested, the carrier protein storage box (14) and the buffer storage box (15) are processed with the Filling cleaning port (10). As shown in figure 3, the lower ends of the drug solution to be tested storage box (8), the mixing gun (12), the carrier protein storage box (14), and the buffer storage box (15) are machined with the injection gun head (5). As shown in figure 4, the clean drain (17) is installed inside the sterilization 18℃ thermostat (9) and the sterilization 4℃ thermostat (13). As shown in figure 5, the left end of the autosampler housing (1) is equipped with the USB jack (7).

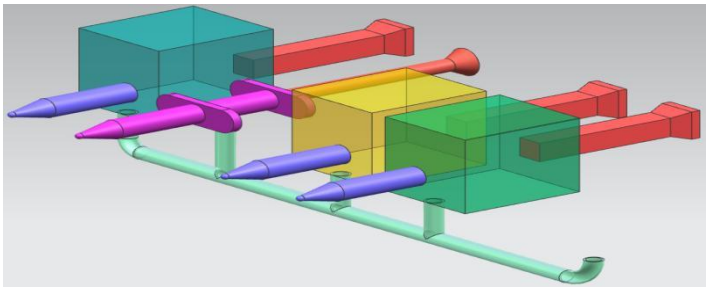

Figure 3: Storage box and drain connection diagram.

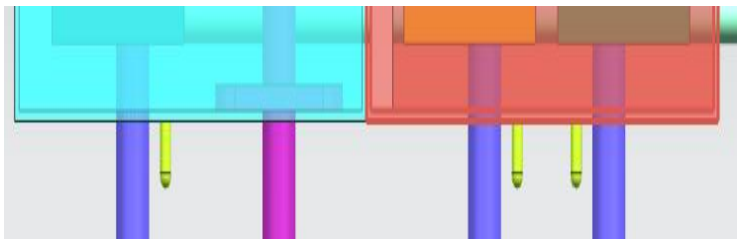

Figure 4: Installation position diagram of the position sensor.

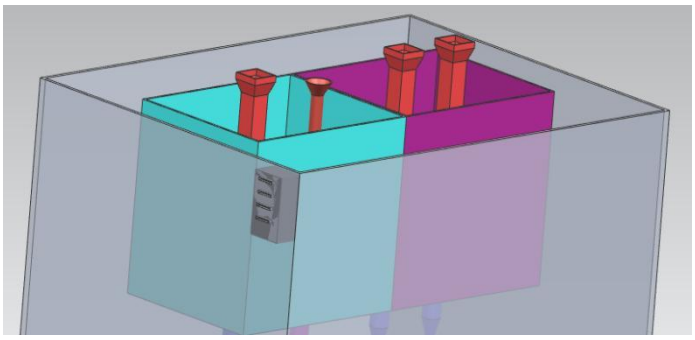

Figure 5: Installation location of the USB jack.

As shown in figure 6, the ultrasonic water bath (21), the quartz cell gripper (18) and the quartz cell base (24) are installed on the inner wall of the autosampler housing. The outer wall of the ultrasonic water bath (21) is connected with the constant temperature water bath tube (19). The constant temperature water bath tube (19) passes through the bottom of the quartz cell base (24) to provide a test condition of the corresponding temperature for the liquid to be tested in the quartz cell (25). As shown in figure 7, the light passing hole (2) is machined on the inner wall of the autosampler housing (1) and

ISSN 2616-5767 Vol.4, Issue 4: 41-48, DOI: 10.25236/AJETS.2021.040408

the light passing hole (2) can correspond to the spectrum scanning hole of the LS-55 fluorescence spectrophotometer.

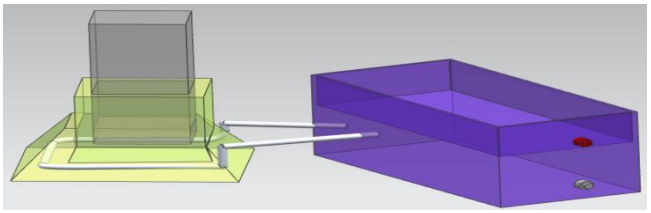

Figure 6: Connection diagram of constant temperature water bath tube.

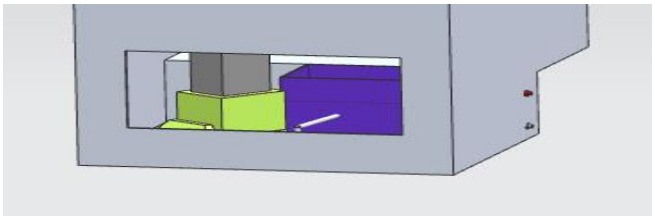

Figure 7: Light passing hole partial view.

As shown in figure 8, the sterilization 4℃ thermostat (13) and the sterilization 18℃ thermostat (9) are equipped with the temperature sensors (16) that transmit their internal temperatures to the "platform for predicting drug toxicity based on the information angle of binding with carrier protein." (The platform has registered the computer software copyright in the China Copyright Protection Center, the registration number is 2021SR0226101) It can not only monitor the storage temperature of its internal substances in real-time, but also accurately determine whether the temperature of the water bath required by the experiment is reached. The temperature regulator (20) is installed on the other side of the inner wall of the autosampler housing (1) and the temperature regulator (20) has two modes of space temperature regulation and partial drying.

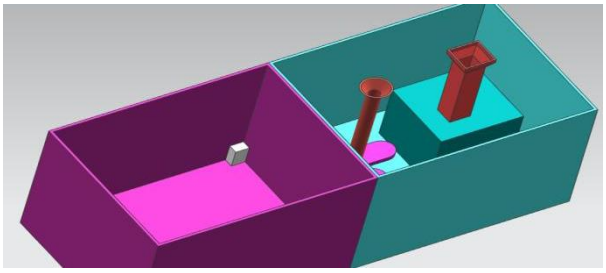

Figure 8: Temperature sensor installation location diagram.

As shown in figure 9, the insulation baffle (3) is installed at the center of the inner wall of the autosampler housing (1) and the insulation baffle (3) can be automatically folded and retracted. Whenever the quartz cell gripper (18) grips the quartz cell (25) for sample addition, the obstacle sensing switch (4) will automatically switch on or off.

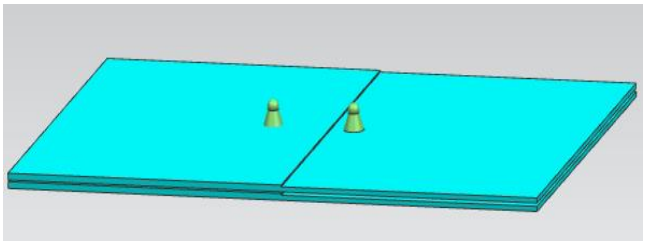

Figure 9: Insulation baffle parts diagram.

ISSN 2616-5767 Vol.4, Issue 4: 41-48, DOI: 10.25236/AJETS.2021.040408

3. Sample Injector Experiment Method

3.1 Installation of the Sampler

Open the sample chamber cover of the "LS-55 Fluorescence Spectrophotometer". Place the "Fluorescence automatic sampling instrument for drug toxicity detection based on the information angle of binding with carrier protein" on the external table of the sample chamber of the "LS-55 Fluorescence Spectrophotometer".

Combine the "LS-55 Fluorescence Spectrophotometer" and the "Fluorescence automatic sampling instrument for drug toxicity detection based on the information angle of binding with carrier protein," and then connect them to the same computer through a USB jack.

3.2 Software Platform Installation

Open the instrument status menu of "LS-55 Fluorescence Spectrophotometer". Import the "Platform for predicting drug toxicity based on the information angle of binding with carrier protein" software installation package into the computer, install and run.

3.3 Preparation before Testing

3.3.1 Cleaning of the Device

The connection port of the storage box and the injection gun head (5) will be automatically closed. Pour appropriate distilled water from the filling cleaning port (10) and the mixing gun cleaning port (11) to the carrier protein storage box (14), the buffer storage box (15), the drug solution to be tested storage box (8) and the mixing gun (12) is cleaned, and the waste liquid is discharged from the drain (17) to the outside of the device. Repeat three times. Start the drying function of the sterilization 4℃ thermostat (13) and the sterilization 18℃ thermostat (9), and set the carrier protein storage box (14), buffer storage box (15) and the drug solution to be tested storage box (8), the mixing gun (12) and the clean drain pipe (17) are completely dried.

The quartz cell gripper (18) clamps the quartz cell (25) and puts it in the ultrasonic water bath (21). The ultrasonic water bath (21) starts the ultrasonic function for 5 minutes to clean the quartz cell (25). The cleaning waste liquid is discharged from the ultrasonic water bath drainage port (23). Inject new distilled water back and forth into the ultrasonic water bath (21), and repeat the cleaning of the quartz cell (25) three times. After three repeated cleanings, the quartz cell gripper (18) clamps the quartz cell (25) out of the ultrasonic water bath filling port (22), and clamps it to the drying port of the temperature regulator (20) for drying. The quartz cell gripper (18) puts the cleaned and dried the quartz cell (25) back into the quartz cell base (24).

3.3.2 Filling of Test Reagents

Put the configured human serum albumin or other carrier protein solution into the carrier protein storage box (14) in the Sterilization 4℃ thermostat (13) through the filling cleaning port (10). Put the configured PBS solution or HEPES buffer into the buffer storage box (15) in the sterilization 4℃ thermostat (13) through the filling cleaning port (10). The compound to be tested is dissolved in ethanol, and after being completely dissolved at a certain concentration, it is loaded into the drug solution to be tested storage box (8) through its corresponding the filling cleaning port (10). Then pour distilled water into the ultrasonic water bath (21) from the ultrasonic water bath filling port (22) of the ultrasonic water bath.

3.3.3 Device Placement and Replacement

Put the washed and dried the 1cm four-way quartz cell (25) into the sample room of the "LS-55 Fluorescence Spectrophotometer". Replace the 4 injection pipette tips (5) under the storage box of the "Fluorescence automatic sampling instrument for drug toxicity detection based on the information angle of binding with carrier protein."

3.4 PBS Buffer-fluorescence Detection of Drug Solution to be Tested

After cleaning by automatic cleaning function 1 and 2,the platform controls the quartz cell holder (18) to clamp the quartz cell (25) directly below the injection pipette tip (5) of the buffer storage tank

ISSN 2616-5767 Vol.4, Issue 4: 41-48, DOI: 10.25236/AJETS.2021.040408

(15). The buffer storage box (15) sequentially injects 2000 μL of PBS buffer, 1 μL of the drug solution to be tested and 2 μL of the drug solution to be tested into the quartz cell (25) through the injection gun head (5). Then the quartz cell gripper (18) puts the quartz cell (25) containing the PBS buffer solution back into the quartz cell base (24), and waits for the temperature to stabilize for 2 minutes. The spectrophotometer is driven by the platform to perform the first three fluorescence spectrum scans.

The platform controls the quartz cell holder (18) to move the quartz cell (25) containing the PBS buffer and 3 μL of the drug solution to be tested to Just below the injection gun head (5) of the drug solution to be tested storage box (8), and then inject 3 μL the drug solution to be tested into it. Clamp the quartz cell (25) containing the PBS buffer and 6 μL of the mixed solution of the drug to be tested directly under the mixing gun (12), and make the injection gun head (5) of the mixing gun (12) deep into the bottom of the quartz cell (25). The mixing gun (12) draws the mixed solution of PBS buffer and drug to be tested in the quartz cell (25) back and forth 10 times to ensure that the PBS buffer and drug to be tested solution are well mixed. The quartz cell gripper (18) puts the quartz cell (25) containing the mixed solution of PBS buffer and 6 μL of the drug to be tested back into the quartz cell base (24), and waits for the temperature to stabilize for 2 minutes. The spectrophotometer was driven by the platform to perform the fourth fluorescence spectrum scan.

The control step 4 of the platform control step 4 was repeated 5 times to complete the fluorescence detection experiment of the PBS buffer and the drug to be tested at a temperature of 296k.

3.5 Carrier Protein—Fluorescence Detection of Drug Solution to be Tested

Click the fluorescence detection button of the "Platform for predicting drug toxicity based on carrier protein binding information angle". First start the ultrasonic water bath (21) and set the water temperature to 296k. After that, start the temperature regulator (20) to adjust the ambient temperature in the autosampler housing to 296k. Finally, the temperature stabilized for 5 minutes.

The platform controls the quartz cell gripper (18) to clamp the quartz cell (25) from the quartz cell base (24). When passing the insulation baffle (3), the obstacle sensing switch (4) on the baffle will automatically open the insulation baffle (3) through induction to ensure that the quartz cell gripper (18) clamps the quartz cell (25) to pass smoothly take a sample. When the quartz cell gripper (18) clamps the quartz cell (25) to sample and returns, the obstacle sensing switch (4) on the baffle will automatically close the insulation baffle (3) through induction. The insulation baffle (3) can ensure that the ambient temperature of the lower chamber of the entire device is maintained at 296k without affecting the experimental results.

The quartz cell gripper (18) precisely clamps the quartz cell (25) directly below the injection gun head (5) of the buffer storage box (15) by means of the position sensor (6) below the buffer storage box (15). The platform controls the injection gun head (5) below the buffer storage box (15), and sequentially injects 1990 μL PBS buffer, 10 μL of carrier protein solution, 1 μL of the drug solution to be tested and 2 μL of the drug solution to be tested into the quartz cell (25). The quartz cell gripper (18) then placed the quartz cell (25) with PBS buffer back into the quartz cell base (24) and allowed the temperature to stabilize for 2 min. The "LS-55 Fluorescence Spectrophotometer" was driven by the platform to perform the first four fluorescence spectrum scans.

The platform controls the quartz cell holder (18) to move the quartz cell (25) containing the mixed solution of PBS buffer, carrier protein and 3 μL of the drug solution to be tested to Just below the injection gun head (5) of the drug solution to be tested storage box (8), and then inject 3 μL of the drug solution to be tested into it.Clamp the quartz cell (25) containing the PBS buffer, carrier protein, and 6 μL of the mixed solution of the drug to be tested directly under the mixing gun (12), and make the injection gun head (5) of the mixing gun (12) deep into the bottom of the quartz cell (25). The mixing gun (12) draws the mixed solution of PBS buffer, carrier protein and drug to be tested in the quartz cell (25) back and forth 10 times to ensure that the PBS buffer, carrier protein and drug to be tested solution are well mixed. The quartz cell gripper (18) puts the quartz cell (25) containing the mixed solution of PBS buffer, carrier protein and 6 μL of the drug to be tested back into the quartz cell base (24), and waits for the temperature to stabilize for 2 minutes. The spectrophotometer was driven by the platform to perform the fifth fluorescence spectrum scan.

The control step 7 of the Platform is repeated 5 times to complete the fluorescence detection experiment of the carrier protein and the drug to be tested at a temperature of 296k. The platform and then separately control the water temperature of the ultrasonic water bath (21) and the ambient temperature of the temperature regulator (20) to 303k. After the temperature is stabilized for 5 minutes,

ISSN 2616-5767 Vol.4, Issue 4: 41-48, DOI: 10.25236/AJETS.2021.040408

repeat the above steps, namely It can complete the fluorescence toxicity detection experiment of the drug under test at a temperature of 303k. The platform controls the water temperature of the ultrasonic water bath (22) and the ambient temperature of the temperature regulator (20) to 310k. After the temperature is stabilized for 5 minutes. The platform will automatically control all the above processes, and repeat the 3.3 and 3.4 operations three times to complete all fluorescence spectrum drug toxicity testing experiments for the drug to be tested.

3.6 Data Processing Results in Toxicity

The "Platform for predicting drug toxicity based on carrier protein binding information" will automatically process the data through the Stern-Volmer equation, Van't Hoff and other equations to obtain the Ksv (quenching constant), Ka (binding constant), n (binding constant) and ΔG (free energy) of the drug to be tested 4 spectroscopic parameters. Then substitute the obtained 4 spectroscopy parameters into the toxicity detection model of the platform to obtain the acute toxicity LD50 value, toxicity and changes of the surrounding environment of the amino acid residues of the test compound. Finally, give the modification opinions on the compound structure of the drug to be tested.

4. Result and Discussion

Based on the "carrier protein binding information-toxicity relationship," the fluorescence automatic sampling instrument for drug toxicity detection based on the information angle of binding with the carrier protein was prepared for automatic injection. the fluorescence automatic sampling instrument for drug toxicity detection based on the information angle of binding with the carrier protein adopts an automatic sampling design, which can automatically complete a series of operations such as adding solution, cleaning the instrument, controlling the temperature and other experimental background conditions. the "carrier protein binding information-toxicity relationship" controls the sample injection status in real-time through intelligent manipulation of fluorescence automatic sampling instrument for drug toxicity detection based on the information angle of binding with the carrier protein, collects fluorescence spectrum information online, analyzes and processes the fluorescence spectrum data and realizes online collection and processing of spectrum information. The fluorescence automatic sampling instrument for drug toxicity detection based on the information angle of binding with the carrier protein has the characteristics of automation, low cost, high accuracy and fast detection speed, and can be used for a large number of drug toxicity predictions. Solve most of the common problems of fluorescence spectroscopy injection.

Traditional fluorophotometers mainly rely on manual sample injection. Different experimental operators have different levels of skill and a little irregular operation will lead to large errors in experimental results and longer detection time, such as manual addition or mixing of the drug solution to be tested. Each operation produces a slight difference, which will have a greater impact on the experimental results. The operation of this sampling device is specific, which effectively improves the accuracy of experimental results and reduces errors.

The fluorescence spectrum information collected by most fluorescence spectrophotometers needs to be analyzed by other data analysis software. The sampling device is operated by the "carrier protein binding information-toxicity relationship," and the background of this platform automatically converts the collected fluorescence spectrum data is analyzed and processed. Finally, the relevant index of toxicity prediction is obtained. This device reduces the complexity of manual data processing and improves the convenience of experimental data processing.

The quartz cell is an important part of the fluorescence spectrophotometer, and the cleanliness of the quartz cell directly determines the accuracy of the experimental results. The quartz cell of the traditional fluorescence spectrophotometer will have the following problems: the cleaning method of the quartz cell is complicated; after the quartz cell is cleaned, you need to wait for a period of time to allow the inside of the quartz cell to dry naturally, which greatly wastes experimental time; During the experiment, the rough surface of the quartz cell is held to avoid contamination or abrasion of the translucent surface due to improper operation, which greatly affects the accuracy of the toxicity detection of the sample to be tested. The device automatically cleans, dries the quartz cell, and clamps the quartz cell for position movement, which solves the risk caused by holding the cuvettes and greatly improves the accuracy of the experiment. Fluorescence automatic sampling instrument for drug toxicity detection based on the information angle of binding with the carrier protein is a closed device, which avoids the contamination of buffer, protein solution and samples, and it is easy to control experimental

ISSN 2616-5767 Vol.4, Issue 4: 41-48, DOI: 10.25236/AJETS.2021.040408

background conditions such as temperature.

In this paper, based on the new perspective of artificial intelligence, Fluorescence automatic sampling instrument for drug toxicity detection based on the information angle of binding with the carrier protein is designed. The fluorescence automatic sampling instrument for drug toxicity detection based on the information angle of binding with the carrier protein has the functions of automatic cleaning of instruments, automatic addition and discharge of solutions, temperature control and so on, which solves the test error caused by the complicated operation, solution storage environment, temperature changes and background interference, satisfies the need for the detection of the toxicity of a variety of drugs and carrier proteins. Fluorescence automatic sampling instrument for drug toxicity detection based on the information angle of binding with the carrier protein has the characteristics of automation, low cost, high accuracy and fast detection speed, which can realize rapid detection of a large number of drugs and lay the foundation for early toxicity prediction of drug development.

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