Research Progress on Salicylic Acid Content Detection Technology in Aspirin

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Abstract. Aspirin is a common drug. During storage, the influence of acids, bases, or incomplete acetylation can lead to the production of free salicylic acid. Salicylic acid serves as a key basis for measuring the decomposition rate of aspirin tablets. Salicylic acid can cause discomfort to the human body and lead to diseases. Therefore, detecting the salicylic acid content in aspirin is of vital importance. Currently, the primary methods for detecting salicylic acid include colorimetry, chromatography, and spectrophotometry. This study summarizes and generalizes the main detection methods and research progress of salicylic acid content in aspirin.

Keywords: Aspirin, Salicylic acid detection.

1. Introduction

Aspirin (also known as acetylsalicylic acid, Aspirin, 2-(acetyloxy) benzoic acid) is a non-steroidal anti-inflammatory drug (NSAID) with a long history, widely used in antipyretic, analgesic, anti-inflammatory, and antiplatelet treatments. Aspirin is prone to produce free Salicylic acid (2-hydroxybenzoic acid) during storage. Therefore, salicylic acid serves as a key basis for measuring the decomposition rate of aspirin tablets. Aspirin itself is unstable and often decomposes into free salicylic acid under moist, acidic, or alkaline conditions. Studies have shown that inhaling a small amount of salicylic acid into the human body can cause coughing or chest discomfort. Prolonged or repeated contact with the skin can cause skin inflammation and even burns. Excessive intake can cause gastrointestinal irritation and may lead to tinnitus and kidney damage.[1]

Currently, the primary methods for detecting salicylic acid content include colorimetry, chromatography, and spectrophotometry. This study aims to summarize and generalize the current detection methods and research progress on salicylic acid content in aspirin, promote research in the field of salicylic acid detection, and provide new ideas and approaches for future salicylic acid detection.

2. Colorimetric method

Colorimetry is a classic quantitative analysis technique. It determines the content of a specific substance by using the corresponding relationship between the depth of the solution's color (or absorbance) and the concentration of the specific substance in the solution. Zhao Chaoming prepared ethylenediaminized polymethyl methacrylate (EDA-PMMA) by reacting polymethyl methacrylate (PMMA) with ethylenediamine (EDA) [2]. Salicylic acid selective detection test strips were prepared using filter paper as the substrate by taking advantage of the hydrogen bond and other interactions between EDA-PMMA and salicylic acid molecules, as well as the interaction between EDA-PMMA and PMMA molecules. When the concentration of salicylic acid ranges from 50 to 2,200 μmol/L, the detection limit is 48 μmol/L.

3. Liquid chromatography

3.1 High-performance liquid chromatography

High performance liquid chromatography (HPLC) is a modern separation and analysis technique that uses a high-pressure pump to push the mobile phase through a chromatographic column filled with highly efficient particulate stationary phase, enabling the components in the mixture to be separated efficiently, rapidly and with high resolution due to different interaction forces with the stationary phase and the mobile phase. It is also combined with a highly sensitive detector for online detection. Zhao Yong[3] et al. Through high-performance liquid chromatography (HPLC), with a C18 column (4.6 mm×250 mm,5μm), and using phosphorus-acetonitrile-water (2:400:600) as the mobile phase; Flow rate: 1 ml/min, ultraviolet detection wavelength: 237 nm, injection volume: 10μl. Result: Under these chromatographic conditions, aspirin, salicylic acid, and their various impurities in aspirin tablets can be completely separated. The linear relationship of aspirin within the concentration range of 10-500μg/ml was good (r=0.9998); The average recovery rate was 100.04% (RSD=0.54%); The linear relationship of salicylic acid within the concentration range of 2-50μg/ml is good (r=0.9999); The average recovery rate was 98.47% (RSD=0.97%).

Although high-performance liquid chromatography is widely used and highly sensitive for determining salicylic acid content, it also has some limitations. The cost of the instrument is relatively high, and the technical requirements for the operators are also relatively high. Biological samples (such as plasma and urine) or complex matrices (such as cosmetics and plant extracts) need to go through steps such as protein precipitation, liquid-liquid extraction, and solid-phase extraction. These operations are cumbersome and prone to introducing errors. Salicylic acid is prone to oxidation. During the pretreatment process, it is crucial to avoid exposure to light and operate at low temperatures, as this can lead to reduced recovery rates. Impurities in the samples may interfere with the test results. Therefore, more effective pretreatment methods are needed to eliminate matrix interference.

3.2 Gas chromatographic method

Gas chromatography is an analytical technique for the separation and analysis of volatile substances or those that can be converted into volatile compounds.

The basic principle is based on the different distribution coefficients of various substances between the stationary phase (usually a liquid or solid coated on a solid carrier) and the mobile phase (a carrier gas, such as nitrogen or helium). Through repeated adsorption and desorption, as well as dissolution and volatilization processes within the chromatographic column, different substances can be separated. Zhao Huanhuan et al. used a SH-Rxi-5Sil (30 m × 0.25 mm ID, 0.25 μm) gas chromatography column, with methanol serving as both the sample solvent and the methyl ester derivatization reagent[4]. Derivatization and gas phase separation were carried out in the high-temperature liner at the gas injection port. The content of salicylic acid, aspirin derivatives, and clopidogrel in the sample is quantitatively detected by mass spectrometry. The results showed that salicylic acid, aspirin, and clopidogrel had good linearity within the ranges of 0.26-127.5μg·mL-1,1.93-965μg·mL-1, and 5.45-2180ng·mL-1, respectively. The lower limits of quantification for the three analyzed substances were 0.26, 1.93, and 5.45 ng ·mL-1, respectively.

There are certain limitations in the determination of salicylic acid content by gas chromatography. Salicylic acid exhibits poor thermal stability and volatility, making it prone to decomposition at high temperatures. Therefore, the chromatographic conditions need to be optimized to reduce decomposition losses. The polarity of gas chromatography is relatively high, and the separation capacity of conventional gas chromatography columns is limited, resulting in poor performance. This may cause peak deformation to be wide and tailing, affecting the accuracy of quantification. The requirements for derivatization treatment are high. Salicylic acid needs to be converted into volatile derivatives. The reaction conditions for derivatization are difficult to control, and the technical requirements for operators are high. Moreover, the process is cumbersome and time-consuming, and may also introduce additional errors. Additionally, the detection limit of gas chromatography is relatively high, and its sensitivity for detecting trace amounts of salicylic acid is limited. Typically, the sample needs to be concentrated to enhance sensitivity; however, this process may introduce errors and compromise the accuracy of the results.

3.3 Liquid Chromatography-Tandem Mass Spectrometry

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS /MS) is a powerful analytical technique. Combining the separation capability of liquid chromatography (LC) with the identification and quantification capabilities of tandem mass spectrometry (MS/MS), it is widely applied in the analysis of trace components in complex samples. The core principle of the mass spectrometry section is to ionize the sample, generating charged ions. Under the action of electric and magnetic fields, these ions are separated based on their mass-to-charge ratio (m/z), and the signals are detected and recorded by the detector. Tandem mass spectrometry involves setting up two or more mass analyzers in a mass spectrometer. After the sample ions are selected as specific parent ions in the first analyzer (MS_1), they enter the collision chamber, where they are cleaved into daughter ions. Then, the mass-to-charge ratio of the child ions is analyzed by the second analyzer (MS_2) to obtain rich structural information, which is used for qualitative and quantitative analysis.

It was directly extracted with 1% formic acid-acetonitrile solution[5] . Acetonitrile was saturated with n-hexane for the removal and purification of oil. After passing through the membrane, the extract was treated with C18 (1.7 μ m, 2.1 mm × 50 mm). Separation was carried out using a chromatographic column. Gradient elution was performed with acetonitrile -0.10% formic acid aqueous solution as the mobile phase. Electrospray ionization was performed, and detection was conducted using a negative ion multi-reaction monitoring mode. Quantitative analysis was performed using the external standard method. The results show that under the established method conditions, salicylic acid has a good linear relationship within the range of 0.5-50ng/mL, and the correlation coefficient is 0.999. The detection limit is 2.5μg/kg; The average spiked recovery rates of different aquatic products (eel, money fish, grass carp and lobster) at high, medium and low addition levels ranged from 69.10% to 97.30%, and the relative standard deviations ranged from 2.30% to 13.40% (n=6).

Similar to the above two methods, liquid chromatography-tandem mass spectrometry has overly high requirements for laboratory conditions and operators, and the pretreatment of samples is also very cumbersome, which can easily lead to errors

4. Spectrophotometric method

4.1 Ultraviolet spectroscopy

Ultraviolet spectrophotometry (UV-Vis Spectroscopy) is an optical analysis method that analyzes the absorption characteristics of substances to ultraviolet or visible light. When a substance molecule absorbs ultraviolet light or visible light, its electrons will undergo energy level transitions, from the ground state to the excited state. Due to the different molecular structures and electronic energy levels of various substances, they have characteristic absorptions of light of specific wavelengths. Therefore, the type and content of a substance can be determined by measuring the degree to which it absorbs light of different wavelengths (i.e., absorbance).

Li Mengyao[6] adopted visible spectrophotometry. Under the conditions of pH=2.4 and ammonium ferric sulfate as the chromogenic agent, the purple-red complex formed by salicylic acid and Fe3+ had the maximum absorption at λ=526 nm. The results indicated that the optimal conditions for determining the absorbance of this complex were as follows: the absorption wavelength was 526 nm, and the pH value was 2.4. The color development time is 5 minutes, and the complexation ratio is 2. The absorbance of the series of salicylic acid standard solutions was measured under the optimal conditions, and the equation of the standard curve was obtained as A=0.0052c+0.0312, R2=0.9927. In the precision measurement, the RSD was 0.59%. In the determination of recovery rate, the average recovery rate was 92.18% and the RSD was 7.92%. The content of salicylic acid in Isatidis Radix was calculated to be 1.36×10-5g/mL.

Based on the additive principle of ultraviolet absorbance values, a linear regression analysis was conducted on the mass concentrations of aspirin and salicylic acid, two components in the solution, at 273 nm and 297 nm, respectively[7]. With CASP and CSP as unknowns, a system of equations was formed, and the mass concentrations of each component were obtained by solving the system of equations.

The sensitivity of this method is relatively low, and it is not accurate enough for detecting trace or ultra-trace amounts, making it difficult to meet the requirements of high-precision analysis. Secondly, the absorption peak of salicylic acid may be disturbed by other substances with similar ultraviolet absorption characteristics, resulting in insufficient specificity of the detection and a high risk of false-positive or false-negative results in complex samples. Since the absorbance coefficient of salicylic acid is influenced by factors such as solution pH and temperature, the control requirements for experimental conditions are relatively stringent. Even a slight deviation will affect the accuracy and repeatability of the measurement. During the preparation and processing of samples, several factors must be considered, including the selection of solvents and the removal of impurities. Even the slightest mistake can affect measurement results, increase operational difficulty and uncertainty, and to a certain extent, limit their application in the analysis of complex samples.

4.2 Fluorescence spectrophotometry

Fluorescence spectrophotometry is an optical method that analyzes substances based on their absorption and emission of light. When certain substances absorb ultraviolet or visible light, the electrons in their molecules transition to an excited state, and then they return to the ground state. During this process, fluorescence is emitted. Fluorescence spectrophotometry is a method that determines the type and content of a substance by measuring the intensity of the fluorescence it emits. Sun Yantao[8] et al. investigated conditions such as the acidity and alkalinity, stability, and linear range of the solution. It was concluded that the linear relationship of salicylic acid content was good within the range of (0.387-1.935) $\times 10^{-8}$mol·L^{-1}, and the content of salicylic acid in salicylic acid tablets was 1.636×10^{-8}mol·L^{-1}.

However, salicylic acid itself has a weak fluorescence intensity, limited detection sensitivity, and its fluorescence is easily affected by external factors such as temperature and pH, leading to deviations in the results. Other fluorescent substances in the sample can also cause interference, affecting the accuracy of the results. Therefore, this method also has certain limitations.

4.3 Anti-kinetic spectrophotometry

The principle of inhibitory kinetics spectrophotometry is based on the fact that specific chemical reactions accelerate under the action of a catalyst. In contrast, the reaction rate slows down when an inhibitor is present in the system. The amount of the inhibitor is determined by measuring the change in absorbance of the reaction system over time with and without the inhibitor. Specifically, when an inhibitor combines with a catalyst or reactant, it will change the active center or reaction pathway of the reaction, thereby reducing the reaction rate. This change can be observed by measuring the variation in absorbance over time at a specific wavelength using a spectrophotometer.

In the medium of dilute hydrochloric acid, the presence of salicylic acid has a strong inhibitory effect on the decolorization reaction of alizarin red oxidized by potassium bromate. Zuo Miaomiao et al. monitored the changes in alizarin red concentration during the reaction process using the photometric method. They established a method for determining the content of salicylic acid by the photometric inhibition kinetics method of the salicylic acid-potassium bromate-alizarin red system[9] . Under the selected conditions, the linear range of the technique is 0.008 μg/L to 1.2 μg/L, with a detection limit of 0.0017 μg/L.

The reaction system of kinetic inhibition spectrophotometry is complex. It requires the selection of appropriate catalysts, substrates, and reaction conditions. During the detection process, the change in absorbance needs to be monitored to determine the reaction rate. External conditions are also a very important influencing factor. Substances coexisting in the sample may also interfere with the reaction. This method has relatively low sensitivity and accuracy, and it requires high operational standards.

5. Conclusion

Salicylic acid detection has been extensively studied in the fields of drug research and development, cosmetics, video, and agriculture.

Currently, the detection methods for salicylic acid primarily employ chromatography and spectrophotometry, which are novel and diverse. For simple samples, spectrophotometry is easy to operate and has a relatively low cost. However, for complex substances such as food and cosmetics and in industrial production, the most widely used sample detection methods for salicylic acid at present are liquid chromatography and liquid chromatography-tandem mass spectrometry. These two methods have high sensitivity; however, in actual operation, other interfering factors may cause abnormal data. When selecting a method for sample treatment prior to detection, several factors should be considered. When choosing a detection method, a comprehensive consideration should be made based on multiple requirements, and various methods can also be flexibly combined for detection.

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