The Applications of Florescence Analysis: Determination of Ephedrine

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Abstract. Fluorescence analysis is a widely used detection method which can be used in many disciplines, including biotechnology and clinical practice. Fluorescence analysis is a strong instrument that can quickly and accurately detect a variety of chemicals. It is a useful approach due to its rapid and low-cost characteristics. Especially in the analysis of pharmaceutical ingredients, the fluorescence analysis method has been widely used by researchers. Ephedrine, a kind of natural medicine, is an indispensable component in the preparation of anaesthetics. Achieving efficient and rapid detection of ephedrine is an important research topic. Fluorescence analysis can precisely meet such needs due to its rapid and accurate detection. Therefore, the use of fluorescence analysis in ephedrine has become a research hotspot in recent years. In this article, various aspects of fluorescence analysis would be introduced from its working principle, characteristics, advantages and disadvantages. This work will contribute to the further development of this field.

Keywords: Ephedrine (EPH), Fluorescence Analysis, Pseudoephedrine.

1. Introduction

Drugs are indispensable in our lives. It usually takes decades from the concept of preparing drugs to commercial products which for people to use. The development of drugs requires financial support for long-term and extensive experiments. Once targeted drugs have been chosen, drug analysis is of great significance for the successful synthesis of drugs. First of all, for drug production, these analysis methods can know the structure of drug molecules and some data on composition and stability. Then to production, pharmaceuticals processed from various raw materials require a series of analyses to control the usability of the drug. After that, in the clinical stage, the patient's urine or tissue can be analyzed by chemical instruments and methods. Drugs are indispensable in our lives, and it usually takes decades from the concept of preparing drugs into commercial products for people to use, it also requires energy and property support. Once targeted drugs have been chosen, in this period drug analysis is an essential part. Firstly, for drug production, these analysis methods can know the structure of drug molecules and some data on composition and stability. Then to production, pharmaceuticals processed from various raw materials require a series of analyses to control the usability of the drug. After that, in the clinical stage, the patient's urine or tissue can be analyzed by chemical instruments and methods.

Fluorescence analysis is an analysis method which has been widely used in many areas. Fluorescence is a type of photoluminescence. During this process, the emission of light occurs within few nanoseconds after the absorption of light. The emitted light in this process has longer wavelengths [1]. The distinction between the excitation state and emission state wavelengths is called Stokes shift. Qualitative and quantitative analyses of substances can be realized based on fluorescence analysis. Many organic drug molecules have their unique fluorescence spectra, and this may be the key reason why fluorescence analysis so useful.

Ephedrine (EPH) is an alkaloid extracted from the ephedraceae plant. Ephedrine has many important applications in medicine. For instance, it can be used to produce narcotics but is mainly used to prevent bronchial asthma attacks and relieve mild asthma attacks [2]. The raw material to obtain ephedrine is from the extraction of ephedra. The molecular formula of ephedrine is $C_{10}H_{15}NO$, which contains benzene, amine and alcohol. This drug seems to have a lot of benefits but one thing that cannot be overlooked is addiction since it has some addictions when people who require the drug

periodically. Pseudoephedrine is a stereoisomer of ephedrine. The structural differences cause pseudoephedrine to lose its ability to metabolise into deoxyephedrine in the body and thus not cause addiction. Fluorescence analysis was reported to identify a variety of substances, including ephedrine.

Using fluorescence analysis to discover the structure of pseudoephedrine is a vital tool for researchers. Fluorescence analysis contains various methods. So, choosing the most efficient way to analyse the drug structure is essential. Fluorescence spectroscopy is a well-known analytical tool used to enhance the simplicity, selectivity, and sensitivity of this method [2]. Overall, fluorescence analysis is a valuable tool for researchers.

2. Fluorescence Detection for Determination of Ephedrine

2.1. Mechanisms with Merits and Demerits

Ephedrine is the name of ((1R,2S)-2-(methylamino)-1-phenylpropan-1-ol), which is a sympathomimetic amine. It is frequently used as a bronchodilator and decongestant. It reduces swelling, narrows blood vessels in the nose, and expands pulmonary airways, all of which ease breathing [2]. By using fluorescence analysis can be separated into various categories, since it contains different effective or easier ways. It is often inevitable to produce pseudoephedrine during the preparation process of ephedrine, so it is important to distinguish the components of ephedrine. Four methods for analyzing ephedrine will be introduced in the following description.

Micellar electrokinetic chromatography (MEKC) and laser-induced fluorescence (LIF) detection can be employed to analyze the results. More specifically is to separate the derivatives, the procedure uses a running buffer containing 10 mM sodium borate and 16 mM SDS. The measurement of ephedrine (E) and pseudoephedrine (PE) derivatized with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole can be done using the straightforward, quick, selective, and sensitive MEKC with LIF detection approach. It has also been reported to derivatize and analyze ephedrine and pseudoephedrine in-capillaries using MEKC and LIF detection. For the separation of red and yellow historical dyes, the MEKC-LIF separation parameters can be tuned. But it's crucial to remember that a recent study determined the presence of pholcodine alone or in conjunction with ephedrine in human plasma using a unique spectrofluorimetric technique [3]. This method is highly beneficial since it has a high recovery rate of about 89.6% to 107%. But what cannot be overlooked is that the additional step can be time-consuming. Moreover, it may introduce errors in the analysis. It is crucial to keep in mind that this procedure is unique to the usage of MEKC with LIF detection and could not work with other fluorescence analysis techniques. Depending on the precise application and sample matrix, alternative derivatization techniques may also be utilized for ephedrine analysis.

Fluorescence spectroscopy can also help to analyze the ephedrine. This method was reported to measure the natural fluorescence of foci-dine at 337nm after excitation at 284nm to determine the presence of ephedrine in human plasma. The introduction of human plasma could make the EPH sample be detected more easily [2]. Simply, synchronous spectrofluorimetry is highly useful in a sample that contains an overlapped spectra mixture. It has a high sensitivity and selectivity. It even increases the spectral resolution. As a result, it can detect many drugs in one sample [4].

Fluorometric analytical data evaluation is another method. This method comprises evaluating multicomponent fluorometric analytical data for the detection of codeine and ephedrine in urine using partial least-squares calibration. For the simultaneous determination of acetylsalicylic acid, codeine, and ephedrine by Spectro fluorescence, a partial least squares calibration (PLS) approach was developed. Furthermore, in chemometrics and related fields, partial least squares calibration (PLS) is a statistical technique for creating calibration models. Projecting the predicted variables and the observable variables to a new space is a step in the approach that leads to the discovery of a linear regression model. By examining the synthetic mixture, the use of PLS in multicomponent fluorescence spectrometry was demonstrated. Even in the presence of heavily overlapping fluorescence signals, analyte mixtures can be separated [5]. It has several benefits, for instance, it allows for the analysis of multicomponent fluorimetric analytical data and also can be used to

determine the concentration of multiple analytes in a single sample. Relatively higher accuracy and precision during analysis. But it has some drawbacks, one of them is the large amount of data to be collected and analyzed. In the study of ephedrine, fluorimetric analytical data assessment is a useful instrument that enables the precise determination of ephedrine in intricate matrices. Two techniques have been tested for the sensitive detection and measurement of ephedrine in biological fluids: PLS calibration and Field-Amplified Sample Injection Capillary Electrophoresis (FASI-CE). The repeatability gained with GC-MS, however, is not always enough.

Fluorescence resonance energy transfer (FRET) is the most effective way to detect ephedrine. In order to identify the presence of ephedrine in the solution, a time-resolved FRET-based method can be employed. It was reported that how ephedrine interacts with telomeric G-quadruplex DNA could be explored by the use of circular dichroism (CD), FRET melting test, and FRET [6,7]. More details are based on competitive binding between ephedrine and a fluorescent probe for a receptor. When the fluorescent probe is bound to the receptor, it emits a fluorescence signal. However, when ephedrine is present in the solution, it competes with the fluorescent probe for binding to the receptor, resulting in a decrease in the fluorescence signal. The amount of ephedrine in the solution has a direct relationship to how much the fluorescence signal decreases. The process is therefore quick and easy. In comparison to other methods, it takes longer time [6]. In conclusion, FRET is a useful method for analyzing ephedrine. It has become a prominent method to assess the dynamic activities of biological molecules at the nanoscale and is a very distance-dependent phenomena.

Overall, fluorescence analysis offers several routes for analyzing ephedrine. The specific method used may depend on the sample matrix, the required sensitivity and selectivity. Ephedrine analysis using fluorescence analysis has a number of benefits, including excellent specificity, sensitivity, and the capacity to identify ephedrine in complicated matrices. However, there are still some drawbacks for fluorescence analysis as well. For instance, derivatization is required to increase the fluorescence of ephedrine for analysis. This may take time and add further sources of error to the study.

2.2. Some Benefits of Fluorescence Analysis in EPH

This method has a high sensitivity because fluorescence analysis can detect very low concentrations of ephedrine in solution, making it a highly sensitive method of analysis [7]. Unlike absorption spectroscopy, which compares two very big signals, the emission signal is measured over a low background level, making it intrinsically more sensitive. Secondly, because of the linear relationship between fluorescence intensity and fluorophore, it can be employed for quantitative analysis to increase sensitivity. Additionally, against a weak background, emission photons can be found thanks to the Stokes shift.

The method can save a lot of time rather than other time-consuming analysis. Since it would only require to accept the fluorescence signals from the fluorophore, FRET only happens in a nanosecond scale. So, these processes are really time-saving methods to detect ephedrine compared to some of the chromatography.

What cannot be overlooked is these processes are non-destructive. Fluorescence analysis is a non-destructive method of analysis, meaning that the sample can be recovered [7]. It only requires minimal sample preparation can start to do the analysis.

It can also do multicomponent analysis, which means it can detect the mixture within several different samples. Since the process has a high selectivity, some overlapped spectra can be separated by the method. In addition, when compared to other analytical methods, fluorescent assays are frequently more affordable. As a result, it is a useful option for the routine analysis of ephedrine in a number of circumstances.

Overall, fluorescence analysis offers several advantages in analyzing ephedrine, including sensitivity, selectivity, rapidity, and cost-effectiveness. These benefits make it a valuable tool for researchers and analysts working with ephedrine-containing samples.

2.3. Drawbacks of Fluorescence Analysis in EPH

Fluorescence analysis in EPH relies on the competitive binding of the fluorescent probe to the receptor. It lacks some specificity since fluorescence analysis may not provide high specificity for ephedrine detection. This means that other compounds with similar binding affinities could interfere with the analysis, leading to potential false positive or false negative results [6]. Due to some cross-reactivity with ephedrine, it may exhibit cross-reactivity with other compounds or analytes. Thus, the fluorescence signal may be impacted by the interfering substances.

The derivatization requirement is also a limitation for fluorescence analysis in EPH. Because ephedrine need derivatization in order to modify the chemical structure of the compound to enhance its fluorescence properties. This step takes some time, and it is hard to ensure some introduction of errors during the derivatization [3]. Derivatization is a time-consuming technique that may add further sources of error to the study. In addition, fluorescence analysis in ephedrine may not be appropriate for the detection of ephedrine in some matrices.

In addition, a large number of precision instruments are required in the operation, which also brings difficulties to the operation process. For FRET, two flophouses are on a nanometer scale so it is relatively hard to control [8].

Moreover, this sort of analysis method is not always suitable for all types of samples [9]. For example, some sample with high turbidity or strong fluorescence background would affect the accuracy of the analysis [10]. In such cases, alternative approaches may need to be considered [3].

It is significant to note that these shortcomings may apply to other analytical techniques as well and are not specific to fluorescence analysis. When choosing a method for ephedrine analysis, researchers and analysts should carefully assess the fluorescence analysis limits and take measures to reduce potential sources of mistakes [10].

3. Conclusion

Fluorescence analysis is a powerful tool which could be used in detecting ephedrine. Till now the utilise of fluorescence analysis has become a research hotspot in drug composition analysis. In this work, four methods for analyzing ephedrine based on fluorescence analysis were introduced. Through comparison and analysis, FRET is considered the most promising method for development. This work also discussed in detail the advantages and disadvantages of fluorescence analysis for detecting ephedrine. Fluorescence analysis has several advantages in analyzing ephedrine, including sensitivity, selectivity, speed, and cost-effectiveness. Meanwhile it also has some limitations. This is also a topic that future researchers should pay attention to and solve. How to optimize steps and improve detection efficiency is a noteworthy issue. In order to further improve this method in the future, reducing the difficulty of instrument operation and improving the universality of detection is worth studying.

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