Welcome back To Pharmaceutical Analysis !

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Task: What is the drug and how much is the drug ?

Qualitative analysis & Quantitative analysis

The three important procedures in Pharmaceutical analysis

True or fake drug ?

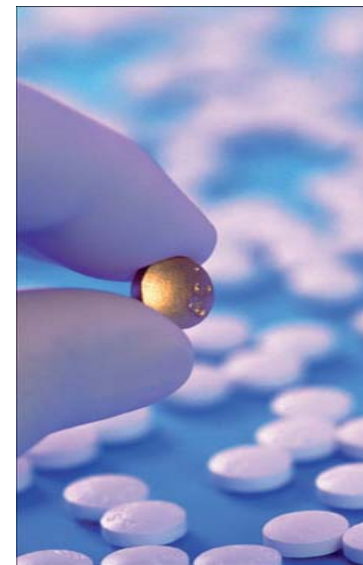**Identification Test**
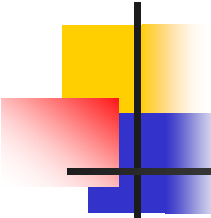
Is the drug safe?

Impurity test

Is the drug efficient ?

Assay of content

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Chapter 2: *Identification*

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Teaching objectives & requirement of this chapter:

- *To master:* identification and its object, classification of identification, characteristics of identification methods commonly used in CH.P

- *To be familiar with:* items of identification, sensitivity for identification test

- *To comprehend:* the impact factors of identification reaction, the principle of selecting identification method.

Content of this section

Including:

- ➢ **Identification and its object**
- ➢ **Items of identification**
- ➢ **Classification of Identification**
- ➢ **The impact factors of identification reaction**
- ➢ **Sensitivity for identification test**
- ➢ **Characteristics of identification methods commonly used in CH.P ***
- ➢ **The principle of selecting identification method**

Identification

> To estimate whether a drug is <u>true or fake</u> by the methods of chemistry, physical chemistry or biology according to its molecular structure or physical & chemical properties

Notice ***

✓ Object of identification <u>is not unknown drug but definite drug</u> stored in a container <u>with label</u>

✓ The test for impurity and assay of content can be done <u>only after the test for identification has be checked out</u>

Items of identification

> ## <u>Description:</u>

Appearance, solubility, physical constants (including melting point, specific rotation, absorption coefficient)

> ## <u>General identification test</u>

> To use <u>the common chemical structures and common physical & chemical properties of a certain sort of drug</u> as characteristics by physical & chemical reactions

> ## <u>Specific identification test</u>

> To use <u>the differences in chemical structure between various kinds of drug</u> as characteristics on the basis of general identification test

Classification of Identification

➢ **Chemical identification**

➢ **Spectrometric identification**

➢ **Chromatographic identification**

➢ **Biological identification**

250   300

wavelength (nm)
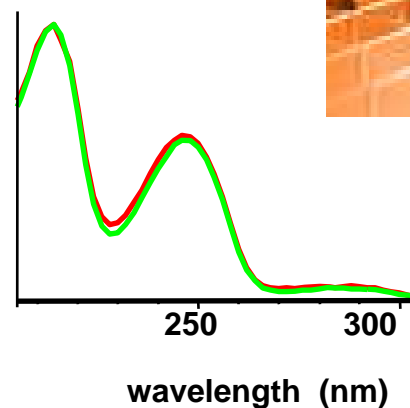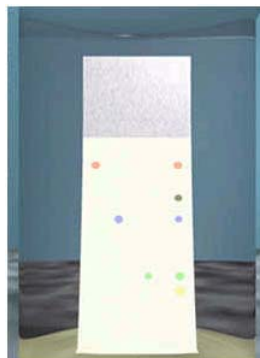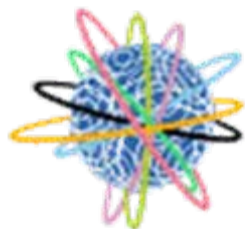
1. Chemical identification

（1）Identification by color reaction
呈色反应鉴别法

- **Trichloride ferric color reaction(三氯化铁呈色反应):** characteristic reaction of phenolic hydroxyl group(酚羟基) and the structure which can produce phenolic hydroxyl group by hydrolysis (水解);

- **Hydroxamic acid reaction(异羟肟酸反应):** characteristic reaction of aromatic acid and their esters(芳酸及其酯类) and acid amides (酰胺类);

- **Diazotizing-coupling reaction(重氮化-偶合反应):** characteristic reaction of aromatic primary amino-group or the structure with potential aromatic primary amino-group;

- **Oxidation-reduction reaction or other color reaction**

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1．Chemical identification

(2) Identification by sedimentation reaction
沉淀生成反应鉴别法

✓ Sedimentation reaction with heavy metals

与重金属离子的沉淀反应

✓ Other sedimentation reactions

1. Chemical identification

(3) Identification by fluorescence reaction
荧光反应鉴别法

- ✓ Fluorescence emission of drug being exposed to ultraviolet or visible radiation;

- ✓ Fluorescence emission of drug being exposed to ultraviolet or visible radiation by the contribution of sulfuric acid;

- ✓ Fluorescence emission of drug being exposed to ultraviolet or visible radiation by the contribution of other reactions.

1. Chemical identification

(4)Identification by gas production reaction
气体生成反应鉴别法

- ✓ The production reaction of alkaline air by strong alkali treatment is used for identification of most amines or <u>acid amines(酰胺) drug.</u>

- ✓ The production reaction of hydrogen sulphide gas by strong alkali treatment is used for identification of sulfurate chemical structure of drug.

- ✓ The fragrance production reaction of ethyl acetate by acid hydrolysis(酸水解) with sulfuric acid at first and then addition of ethanol is used for identification of drug containing <u>acetic ester(醋酸酯)</u>or <u>acetic amide(乙酰胺)</u>

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2. Spectrometric Identification

(1) UV spectrometry
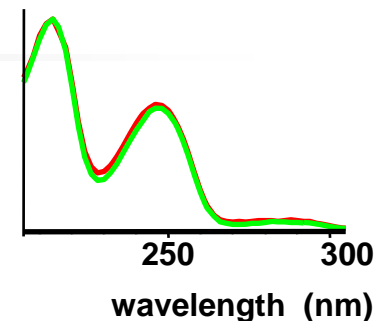
wavelength (nm)

➢ **In Ch.P: standard comparison method**

✓ Method of prescriptive absorption wavelengths (simultaneously measuring λ_{max} and λ_{min})
✓ Method of prescriptive maximum wavelength and corresponding absorbance
✓ Method of prescriptive wavelength and absorption coefficient
✓ Method of prescriptive wavelength and absorbance ratio
✓ Method of absorptive spectrum characteristics measurement after chemical treatment

➢ **In USP：reference substance method**

✓ A_{max} and A_{min} at the same wavelength (λ) with the same specific absorptivity(E), or $A_{\lambda max}/A_{\lambda min}$ in prescribed limit is all stipulated.

2. Spectrometric Identification

For example:

In Ch.P: Identification of VB_{12} is defined as light absorption of VB_{12} exhibits A_{max} at λ_{278} , λ_{361} , λ_{550},

$$\frac{A_{361}}{A_{278}} = 1.70\sim1.88 \ , \ \ \frac{A_{361}}{A_{550}} = 3.15\sim3.45$$

In USP: Identification of furosemide is defined as absorption coefficient of furosemide at 271 nm at the concentration of 8μg/mL in the solution of sodium hydroxide(0.02mol/L) within the difference of 3.0% being compared with the reference subs tan *ce by calculation of dried materials*

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2. Spectrometric Identification

(2) Infrared spectrometry

- In Ch.P or BP: Standard spectrum contrast method

- In USP: Reference substance contrast method

- In JP: Characteristic absorptive peak method

✓ the most commonly used method for identification by spectrometry.
✓ with better specificity.

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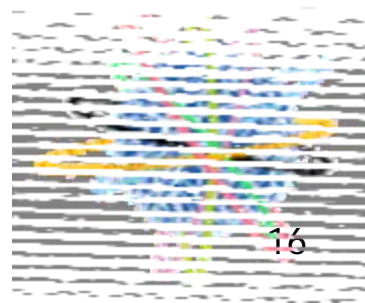
3.Chromatographic identification

(1) HPLC: high performance liquid chromatography

(2) GC: gas chromatography

➢ Both methods can be used for identification of drug by <u>comparing the retention time</u> of substance being examined to that of reference substance in coincident chromatographic condition. but not as popular as TLC.

(3) TLC: thin-layer chromatography

· <u>Reference substance contrast method</u>

· apply and develop the substance being examined and the reference substance in the meantime on the same plate, then <u>compare their R_f (rate of flow) and color</u> of the spots.

· The most commonly used method for identification by chromatography
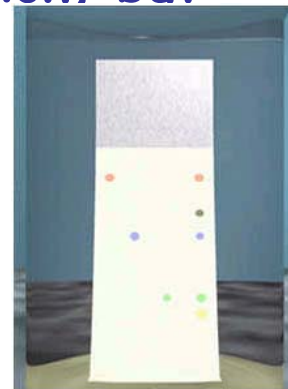
Notes:

➤ **some identification test, such as functional group reaction or color reaction, is only a certain characteristic of a drug and shouldn't be used as the only judgment standard;**

➤ **A set of experiments is needed for identification of drug to overall evaluate a drug and obtain a precise conclusion.**
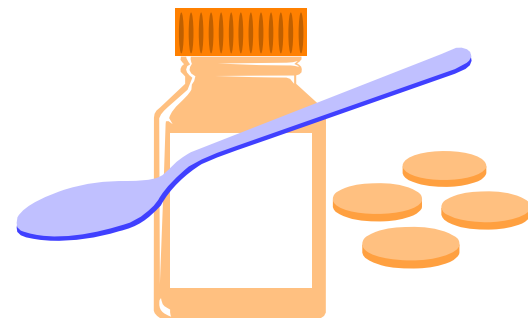
The impact factors of identification reaction

✓ **Concentrations of substance being examined: precipitation, color, parameters *A***

✓ **Dosage of reagent**

✓ **Temperature**

✓ **Reaction time**

✓ **Acidity: pH value**

✓ **Coexistent interferent**

Software for the Pharmaceutical Industry that will Boost Your Immunity
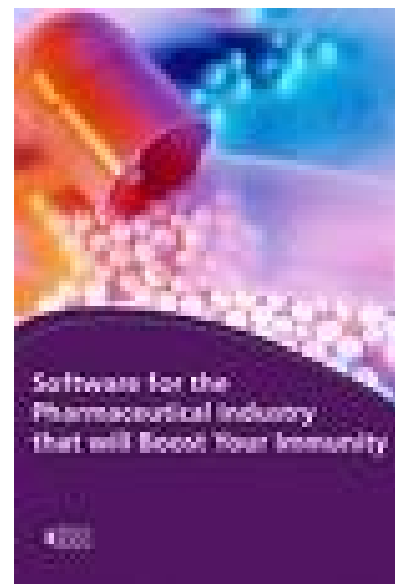
Sensitivity for identification test *

✓ *Reaction sensitivity* :

refers to the degree being fit for the reaction requirement which is likely to determine the substance being examined as little as possible in a solution as diluted as possible in a definite condition .

✓ *Blank test* ***

refers to a test carried out in the similar condition without the substance being examined but all the other reagents at the same amount being added (in order to eliminate the effect of instrument and utensil).

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Characteristics of identification methods commonly used in CH.P ***

✓ **Chemical method:**

• convenient, cheap & extensive application, <u>bad specificity.</u>

✓ **UV spectrometry:**

• <u>qualitative analysis by characteristic value</u>, such as $E_{1cm}^{1\%}$, λ_{max}, λ_{min}, $A_{\lambda max}/A_{\lambda min}$ from spectrum of conjugated structure in organic drug molecule.

✓ **IR spectrometry:**

✓ provides vibration-rotation spectrum, each group in the molecule can produce corresponding infrared absorption peak with <u>remarkable characteristics</u>, and has <u>better</u> <u>specificity and reliability than UV spectrometry</u>

Characteristics of identification method commonly used in CH.P

✓ **TLC:**

• a method with convenient operation, practical use and good specificity; a kind of identification method with the most extensive application in chromatography

✓ **GC and HPLC:**

• compared with TLC, relatively seldom adopted as identification methods.

✓ **Application order in Ch.P:**

✓ chemical method (with the most extensive application); UV spectrometry; IR spectrometry; TLC; HPLC and GC (with the least application)

The principle of selecting identification method:

① To select a method with definite specificity, sensitivity, and being easily popular

② Generally, to make up for each other's deficiencies, 2~4 methods are selected for identification test of a kind of drug, while chemical methods are combined with instrumental methods

③ To select the methods which are recorded in Ch.P

250   **300**

wavelength (nm)

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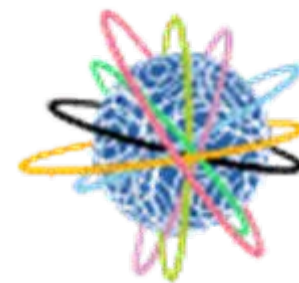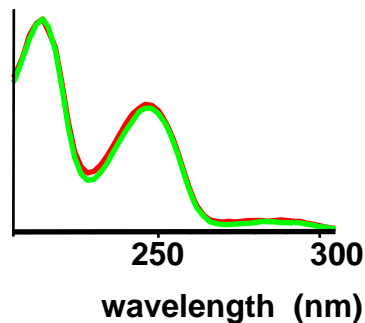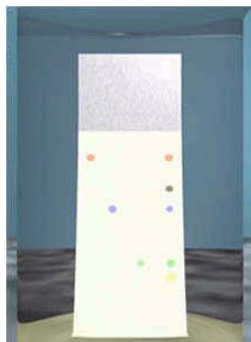
Practices
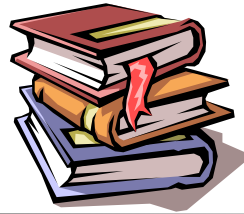
Multiple Choice：

* Identification of drug is to judge (   )

A. The purity of drug

B. Whether the drug is true or false

C. Whether the drug is good or bad

D. Therapeutic effect of drug

E. Toxicity and side effect of drug

➢ The End

Any Questions?

Please review!!!

And For The Next section

➢ *Impurity test*