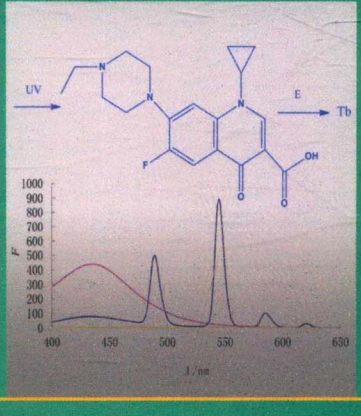
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EXPERIMENT OF PHARMACEUTICAL ANALYSIS



药物分析实验

戚雪勇
（Qi Xueyong） 主编



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Experiment of Pharmaceutical Analysis

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PREFACE

Pharmaceutical analytics is a practical course. To meet the teaching needs for oversea students, we have compiled this experimental guidance. This book is composed of three sections, including pharmaceutical analysis experiment, pharmaceutical analysis experiment *in vivo* and pharmaceutical analysis experiment B in School of Pharmacy at Jiangsu University. Originated from initial scattered experimental notes, this newly revised book has been used in oversea students for three years and shows good effects. Most of the experiments in this book are original, and the others are derived from the ***Chinese Pharmacopoeia***. Through long-term teaching practice and improvement, these experiments has shown some advantages, such as clear principles, advanced and extensive technical methods, easy to handle, and less reagent consumption. In this book, most operation norms and general methods for pharmaceutical analysis are included in different experiments, which enables students grasp a variety of analytical methods, enhances their understanding of concepts and helps to standardize their experimental operations.

This book consists of several chapters, including general instructions, description, identification tests, assays, drug analysis, determination of drug in bio-sample, the validation of analytical methods. It also discusses some conventional techniques, such as volumetric method, ultraviolet spectrophotometry, fluorescence spectrum, infrared spectrum, GC, HPLC, capillary electrophoresis

and so on. Emphasis on the pretreatment process of samples will let students have a train in many ways, and help students learn the basic principles and fundamental skills of pharmaceutical analysis.

The experimental project is arranged based on the orders of chemical raw materials, Chinese herbal medicines, chemical and antibiotic agents, proprietary Chinese medicine, and *in vivo* pharmaceutical analysis. Experiment 9, 21 and "Guidelines for Validation of Analytical Method Adopted in Pharmaceutical Quality Specification", "Determination of Residual Solvents" were written by Xueyong Qi. Experiment 1, 2, 4, 22, 23 were written by Shanshan Tong. Experiment 3, 15 were written by Yuping Shen. Experiment 5, 10, 16 were written by Li Wang. Experiment 6, 7, 12, 14, 18, 20 were written by Qi Zhang. Experiment 11, 13 were written by Yuanrong Xin. Experiment 8, "Rounding of Significant Figures" and "Experiment Record and Report Format" were written by Daoquan Tang. Experiment 17 was written by Xueming Li. Experiment 19 and "Protocol for Using Balance" were written by Guohua Xia.

Thanks for the attention and funding of the School of Pharmacy and Overseas Education College of Jiangsu University. We are grateful to Professor Jiangnan Yu for her help and instructions. We are also grateful to MD Chengyun Zheng, Anran Yuan, Maierhaba Maimaiti and PhD Michael Adu-Frimpong who comes from Ghana for their collecting and collating information, providing and editing illustrations.

As an experiment instruction of pharmaceutical analysis for students of pharmacy, this book is also a good reference for those engaged in research, manufacturing, and quality control of drugs.

Any comments are welcomed in this book. Your suggestions for further improvement are greatly appreciated!

Editors

Mar, 2018

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Lab Safety Rules for Students

1. Read through the protocols before you start the experiment so that you can be familiar with them.

2. Know how to locate and use all safety equipments in the laboratory, including the fume hood, emergency shower, first aid kit, fire blanket, fire extinguisher, and eyewash.

3. Know what you are doing by reading the assigned experiment before you start to work. Pay close attention to any cautions described in the laboratory exercises. If you have any questions, ask your teacher before proceeding.

4. Dress properly in a laboratory. A lab coat or smock should be worn during laboratory experiments. Long hair, dangling jewelry, and loose or baggy clothing are a hazard in the laboratory. Long hair must be tied back, and dangling jewelry and baggy clothing must be secured. Shoes must completely cover the foot. No open toe shoes or sandals are allowed in the laboratory. Wear safety goggles when handling all hazardous chemicals, working with an open flame, or when otherwise instructed.

5. Perform only those experiments authorized by your teacher. Restrictly follow all instructions, both written and oral. Unauthorized experiments are not allowed.

6. Be serious and rational at all times in the laboratory. Do not play around in the lab.

7. Examine glassware before use. Never use chipped or cracked glassware.

8. Do not taste or smell chemicals. Do not lean or sit on the laboratory tables.

Never point a test tube being heated at another student or yourself. Never return unused chemicals to their original container.

9. Work areas should be kept clean and tidy at all times. Keep the work area free of any unnecessary items.

10. Do not eat food, drink beverages, or chew gum in the laboratory. Do not use laboratory glassware as containers for food or beverages.

11. All data should be recorded correctly and honestly in your lab report. Do not leave your assigned laboratory station without permission of the teacher. Experiments must be personally monitored at all times.

12. No chemicals or equipment may be removed from the laboratory.

13. Report all accidents, injuries, and breakage of glass or equipment to instructor immediately. In the event of a skin burn or a chemical splashing, immediately flush the affected area(s) with water and notify the instructor.

14. Dispose of all chemical waste properly. Sinks are to be used only for water. Check with your teacher for disposal of chemicals and solutions.

15. Leave your work station clean and in order before leaving. Make sure that all Bunsen burners, gas outlets, and water faucets are turned off before leaving.

16. The last person to leave the lab at the end of the day should check all the rooms, confirming that all the doors of the lab are locked and all the instruments have been turned off.

17. Students who are found in violation of the safety rules will be warned and dismissed from the lab, or be withdrawn from the class (lab) depending on the seriousness of the case.

(童珊珊)



Experiment 1

Limit Tests for the Generic Impurities in Glucose

Ⅰ. Purpose

1. To learn how to test for the generic impurities in glucose.
2. To learn how to calculate the limit of impurities.

Ⅱ. Principle

A. Limit test for chlorides

Limit test of chloride is based on the reaction of soluble chloride with silver nitrate in the presence of dilute nitric acid to form silver chloride, which appears as solid particles (opalescence) in the solution.

$$NaCl + AgNO_3 \xrightarrow{HNO_3} AgCl\downarrow + NaNO_3$$

B. Limit test for sulfates

Limit test of sulfate is based on the reaction of soluble sulfate with barium chloride in the presence of dilute hydrochloric acid to form barium sulfate, which appears as solid particles (turbidity) in the solution.

$$SO_4^{2-} + BaCl_2 \xrightarrow{HCl} BaSO_4\downarrow + 2Cl^-$$

C. Limit test for iron

Thiocyanate (-amide) test method This analysis can determine the presence of Fe^{3+} ions. To make these ions in solution visible, thiocyanate ions (SCN^-) are added. SCN^- will react with the Fe^{3+} ions to form a pale pink colored complex.

$$Fe^{3+} + 6SCN^- \xrightarrow{H^+} [Fe(SCN)_6]^{3-}$$

D. Limit test for heavy metals

➢ **Scope:** The term "heavy metals" refers to those metals that react with thioacetamide or sodium sulfide under the specified conditions and produce colored compounds, such as Ag, Pb, Hg, Cd, Bi, As, Sb, Sn, Zn, Co and Ni etc.

Limit test of heavy metals is based on the reaction of metallic impurities with hydrogen sulfide in acidic medium to form brownish color solution. The metallic impurities in substances are expressed as parts of lead per million parts of the substance.

➢ **Thioacetamide method:** Limit test of heavy metals is based on the reaction of metallic impurities with hydrogen sulfide in acidic medium to form brownish color solution. The Chinese Pharmacopoeia has adopted three methods for the limit test of heavy metals. The metallic impurities are expressed as parts of lead per million parts of the substance.

$$CH_3CSNH_2 + H_2O \xrightarrow{pH\ 3.5} CH_3CONH_2 + H_2S$$

$$Pb^{2+} + H_2S \xrightarrow{H^+} PbS\downarrow + 2H^+$$

This method is used for the substance which gives clear colorless solution under the specific condition.

Ⅲ. Apparatuses and Reagents

1. Apparatuses: nessler cylinder, pipette, beaker, analytical balance, weighing paper, etc.

2. Reagents: glucose, sodium chloride, nitric acid, silver nitrate, potassium

sulfate, hydrochloric acid, barium chloride, ammonium thiocyanate, ferric ammonium sulfate, lead nitrate, acetate BS(buffer solution), thioacetamide, etc.

Ⅳ. Procedure

A. Limit test for chlorides

➢ **Sample preparation**: Weigh 0.60 g of sample being examined, dissolve in about 25 mL of water (if the solution is alkaline, neutralize with nitric acid dropwise). Add 10 mL of diluted nitric acid and filter if necessary, transfer the solution to a 50 mL Nessler cylinder, add water to produce 40 mL and mix well.

➢ **Reference preparation**: Transfer 6 mL of sodium chloride standard solution (each milliliter is equivalent to 10 μg of Cl^-) to a 50 mL Nessler cylinder, add 10 mL of diluted nitric acid and sufficient water to produce 40 mL and mix well.

➢ **Procedure**: Add 1.0 mL of silver nitrate TS (test solution) to each of the Nessler cylinders, dilute with water to 50 mL and mix well. Allow to stand in the dark for 5 minutes.

➢ **Observation**: Compare the opalescence produced by viewing down the vertical axis of the cylinders against a black background. The opalescence produced in sample solution should not be greater than the standard solution. If the opalescence produced in sample solution is not greater than the standard solution, the sample passes the limit test of chloride. (0.010%)

The filter paper used in this test, if necessary, should be previously washed with water containing nitric acid until it is free from chlorides.

B. Limit test for sulfates

➢ **Test preparation**: Weigh 2.0 g of the sample, dissolve in about 40 mL of water, neutralize the solution with hydrochloric acid and filter if necessary. Transfer the solution to a 50 mL Nessler cylinder, add 2 mL of dilute hydrochloric acid and mix well.

➢ **Reference preparation**: Transfer 2.0 mL of potassium sulfate standard solution

(each milliliter is equivalent to 100 μg of SO_4^{2-}) as prescribed under individual monographs to a 50 mL Nessler cylinder, dilute with water to about 40 mL, add 2 mL of dilute hydrochloric and mix well.

➢ **Procedure**: Add 5 mL of 25% barium chloride solution to each of the Nessler cylinders described above, dilute with water to 50 mL and mix well, allow to stand for 10 minutes.

➢ **Observation**: Compare the opalescence produced by viewing down the vertical axis of the cylinder against a black background. The turbidity produced in sample solution should not be greater than the standard solution. If turbidity produced in sample solution is not greater than the standard solution, the sample will pass the limit test of sulfate. (0.010%)

C. Limit test for iron

➢ **Procedure**: Dissolve 2.0 g of the substance being examined in 20 mL water, add three drops of nitric acid, boil for 5 minutes and then cool. Transfer the solution to a 50 mL Nessler cylinder, dilute with water to about 45 mL, add 3 mL of 30% ammonium thiocyanate solution and mix well. Compare the color produced with that of a reference preparation containing 2.0 mL of standard iron solution (each milliliter is equivalent to 10 μg of Fe) subjected with the same treatment.

➢ **Observation**: The pink color of sample solution should not be stronger than the standard solution. If the color of sample solution is not darker than the standard solution, the sample will pass the limit test of iron. (0.001%)

D. Limit test for heavy metals

➢ **Procedure**: To cylinder A, add the specified volume of lead standard solution (each milliliter is equivalent to 10 μg of Pb) and 2 mL of acetate BS (pH 3.5), dilute with water to 25 mL. To cylinder B, dissolve 4.0 g of the substance being examined in 23 mL of water, and 2 mL of acetate BS (pH 3.5).

To each cylinder add 2 mL of thioacetamide TS and mix well, allow to stand for 2 minutes.

➢ **Observation**: The color produced in cylinder B should not be more intense than that produced in cylinder A. (5 ppm)

Ⅴ. Notes

A. Nessler cylinders

Nessler cylinders are laboratory tubes with a fixed volume, made of glass with optically plane bottom. Nessler cylinders are used for comparison of color and turbidity between solutions. To minimize the differences of the color of the solution from subjective causes, a series (pair) of cylinders should have the same characteristics (matched tubes) - tubes that are matched as closely as possible in internal diameter and other respects, such as height, color and thickness of glass.

B. Interference and their elimination

a. If the test preparation is opalescent, filter using the filter paper given a negative test for impurities (prior to filter).

b. Colored:

[Method 1] If the original test preparation is colorful, it may be matched by adding a few drops of dilute caramel solution or other suitable solution to the test preparation.

[Method 2] If the original test preparation is colorful, place two aliquots of the test preparation in Nessler cylinders separately. To one cylinder, add test solution, filter the content of the cylinder repeatedly until the filtrate is perfectly clear, then add the prescribed volume of standard solution to the filtrate and use it as the reference preparation. To the other cylinder, use it as the test preparation. Treat the test preparation and the reference preparation in the same manner by compareing the opalescence in the two tubes.

Ⅵ. Discussion

1. Please list all the important considerations when using Nessler cylinders.

2. Please calculate how much of the lead standard solution that should be used in the limit test for heavy metals.

（童珊珊）