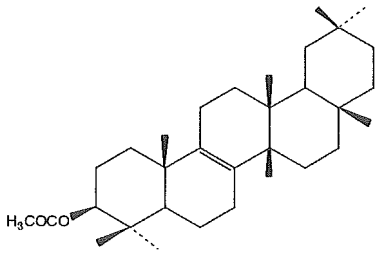
和が必要である。

そこで本研究では各国で用いられている生薬のうち、現在まだ日本薬局方には収載されておらず、将来的に収載される可能性のある生薬を選び、その生薬の成分に着目し、各国で同様に用いられている共通生薬の成分を産地別に検討し、指標成分を探し薬局方におけるもっとも有効な確認試験法案を検討する。それにより各国における薬局方の確認試験法の調和をはかることを目的とする。今年度は検討資料としてトウガシ（冬瓜子）を用いた。トウガシ（冬瓜子）はトウガン（冬瓜）(*Benincasa cerifera* Savi)の成熟種子であり、熱帯アジア原産で、中国中南部、日本などで栽培される。成分としてはサポニン、脂肪、有機酸などが報告されている。日本薬局方外生薬規格には、基原植物として *Benincasa cerifera* Savi が規定されている。他のアジア諸国の薬局方には韓国では*Benincasa hispida* Cogniaux が規定されている。中国の薬局方である中国薬典にはトウガシ（冬瓜子）はまだ収載されていないが、冬瓜の果皮である冬瓜皮については収載されており、その基原植物は *Benincasa hispida* Cogniaux が規定されている。またトウガシについては近い将来収載される可能性が高いと考えられる。学名については、本来 *Benincasa cerifera* Savi は*Benincasa hispida* Cogniaux のシノニムであるとされており、よっていずれの国におけるトウガシも同一の基原植物である。韓国薬局方のトウガシには確認試験の記載はまだない。また、その成分についてはほとんど報告はなく、1991 年に新規骨格を有するトリテルペン isomultiflorenyl acetate (**1**) が報告されているのみである。



Structure of Isomultiflorenyl acetate

B.研究方法

試　料

現在日本に流通しているトウガシはすべて中国産のものである。中国における産地としては安徽省、浙江省、四川省、江蘇省、河南省、河北省、広東省などに多く産するが、日本に流通しているものはそのうち安徽省、河北省、四川省、広東省のものだけである。今回の研究では現在日本で流通しているトウガシを全種類入手し（全部で９種類）、それらの薄層クロマトグラフィーによる確認試験を検討した。入手した検討資料は以下のようである。

No.1　安徽省産、No.2　河北省産、No.3　河北省産、No.4　安徽省産、No.5　四川省産、No.6　中国産（産地不明）、No.7　広東省産、No.8　広東省産、No.9　浙江省産

薄層クロマトグラフィー展開溶媒の検討

各試料をそれぞれサンプルミルにて粉砕した。粉砕した試料をそれぞれ試験管中に500mg 量り取り、溶媒としてメタノールを 5ml 加え、室温下で約 30 分間振とうした。その後濾過し、濾液を薄層クロマトグラフィー用のサンプルとした。

（倫理面への配慮）本研究はいずれも動物等の倫理面を考慮すべき研究材料は使用しない。

C. 研究結果

展開溶媒として、ヘキサン：酢酸エチル=1：1、およびブタノール：水：酢酸=6：3： 1、酢酸エチル：メタノール：水=12：2：1 を検討した。また、検出はほとんどの有機化合物について検出可能であり、化合物の基本骨格情報等を呈色からある程度類推できる希硫酸（10％硫酸噴霧後ホットプレート中で加熱）を採用した。

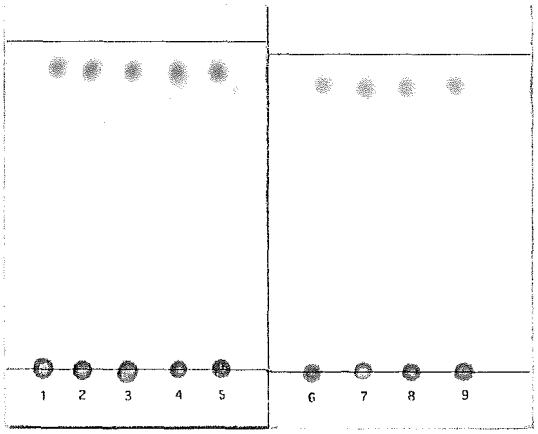


図１ 各試料の TLC クロマトグラム

（展開溶媒 **Hexane:AcOEt=1:1**、検出 **10％硫酸噴霧後加熱**）

ヘキサン：酢酸エチル=1：1 においては、Rf 値 0.5 付近にいずれの試料中にも見られるスポットが検出された（図１）。しかしその TLC 上の挙動等からβ-sitosterol と考えられ、この化合物を指標物質とすることはできないと判断した。

また、日本薬局方で配糖体などの極性物質の検出に用いる系である次にブタノール：水：酢酸混液について検討した。比率は6：3：1 とした。その結果、図２に示すように低極性の脂質類は上部に上がりきり、糖質（加熱すると黒く呈色）が下部に確認された。また、硫酸噴霧後加熱すると赤紫色に呈色するスポットがRf 値約0.3～0.7 付近に現れた。

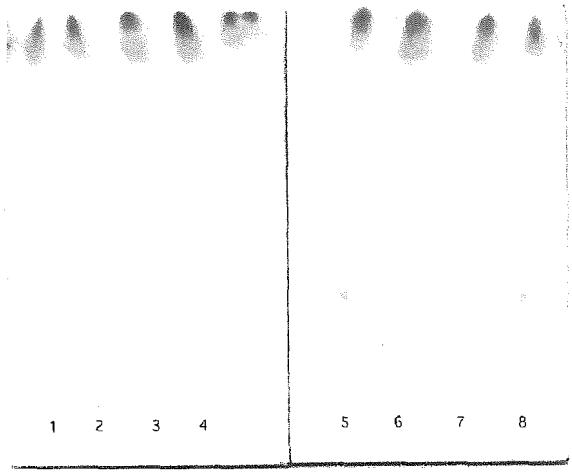


図 **2** 各試料の TLC クロマトグラム

（展開溶媒 **BuOH:H_2O:AcOH=6:3:1**、検出 **10％硫酸噴霧後加熱**）

0.7 付近にはすべての試料に共通のスポットが現れたが、周辺におおくのスポットが現れ分析を困難にすると考えられたので、さらに条件を検討し、次に酢酸エチル：メタノール：水=12：2：1 での検討を行った。その結果、図3 に見られるように、Rf 値0.5 付近にすべての試料に共通のスポットが明確に現れた。このスポットはその Rf 値と 10％硫酸を噴霧後加熱すると、赤紫色に呈色した。しかしながらこの化合物を精製単離し構造を決定した結果、植物界に広く分布しているβ-sitosterolglucoside であることが判明し、この化合物を指標にはできないことがわかった。

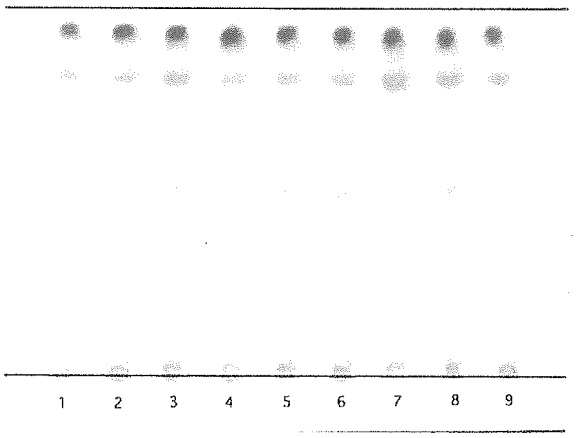


図3　各試料の TLC クロマトグラム

（展開溶媒 AcOEt:MeOH:H_2O=12:2:1、検出

10％硫酸噴霧後加熱）

そこで文献で報告されている isomultiflorenyl acetate を指標とすべく、この化合物を単離することを行った。文献記載の精製方法に従い、No.5 の試料 35 g を粉砕後、クロロフォルムで抽出した。濾過濃縮後シリカゲルカラムクロマトグラフィーにて精製し、主成分である化合物（図4の Rf 値 0.6）を単離したが、NMR 解析により不飽和脂肪酸であると判明した。不飽和脂肪酸は植物種子中には普遍的に分布する成分であるため指標物質にすることはできないと考えられる。

また、図4における Rf 値 0.9、0.5 の化合物も精製単離を行い、NMR にて構造を解析したが、いずれもそのシグナルから脂肪酸と思われ、文献記載の isomultiflorenyl acetate はまだ得られていない。現在さらに精製を進めており isomultiflorenyl acetate を同定することにより、それがトウガシの薄層クロマトグラフィーを用いた確認試験における指標物質となるかどうかを検討する。

図4　メタノール抽出エキスとクロロフォルム抽出エキスとの TLC 比較

（展開溶媒　n-hexane:AcOEt=9:1、10％硫酸噴霧後加熱）

D. 考　察

各種展開溶媒において、薄層クロマトグラフィーを検討したが、すべての試料においてほぼ成分は一致していることがわかった。よって産地によるばらつきはあまりないと考えられた。指標物質の探索においてはまだ単離に至っていないが、各展開溶媒においてもいずれの試料もほぼ同様なパターンを示すことから、指標物質を用いた確認試験法の確立は可能であると考えられる。

E. 結　論

今回、生薬の局方規格の国際調和に関する研究の一環として、日本薬局方外生薬規格収載で韓国薬局方収載のトウガシについて薄層クロマトグラフィーを用いた確認試験法を検討した。トウガシは日本のみならず、韓国や中国でも同じ基原植物が生薬トウガシとして用いられているが、韓国薬局方において

は確認試験の記載はまだされておらず、また中国薬典では未だ収載されていないが今後収載される可能性が高い品目である。このようなことを踏まえ本生薬の品質規格を明確にするべく検討を行ったが、種子であるため脂質類が多く含まれており現在指標となる化合物はまだ得られていないが、この薄層クロマトグラフィー法による確認試験が確立できれば他国に先駆けて本生薬の品質規格を定めることになり、他国の薬局方収載のための参考になりうると考えられ、生薬局方規格の国際調和の一助になると考えられる。また、今後はトウガシにおける指標物質の探索を続けるとともに、他の生薬においても各国薬局方において確認試験における調和のとれていない品目について検討していく。

E. 健康危機情報

特に問題はなし

G. 研究発表

1. 論文発表　特になし

2. 学会発表　特になし

H. 知的財産権の出願・登録状況

1. 特許取得　特になし

2. 実用新案登録　特になし

3. その他　特になし

厚生労働科学研究費補助金（特別研究事業）

分担研究報告書

「生薬規格の国際調和に関する研究事業」

—生薬の品質規格と情報伝達に関する研究—

分担研究者　渕野　裕之　　国立医薬品食品衛生研究所筑波薬用植物栽培試験場

—東アジア地区の薬局方調和における一般試験法—

協力研究者　　関田　節子　　国立医薬品食品衛生研究所筑波薬用植物栽培試験場

医薬品の安全・有効な使用を目的に、その規格書である薬局方の国際的な調和が必要とされている。生薬の分野でも FHH が組織され生薬の品質の向上が図られている。同組織の Working Group の活動のスタートとなる東京会議が開催された。同会議の議題の一つとして一般試験法の比較が提案されたことから第 14 改正日本薬局方の生薬関連一般試験法について東アジア地区への情報の公開を検討した。

A.研究目的

1998 年に開始された日本と中国 2 国間の薬局方—特に生薬の規格—に関する調和会議は、参加を希望する国が増加したことから WHO 西太平洋地域事務所の仲立ちで新たに FHH (Forum for the Harmonaization of Herbal Medicines) として 2001 年より発足した。参加国の増大により各国間の薬局方の方針や表現等を認識し理解することがより重要な課題となり、sub-committee として 2002 年 5 月に東京会議が、7 月にソウル会議が計画された。

東京会議において検討されたテーマのうち、情報部門の課題として、それぞれの薬局方の一般試験法を参加国間に情報公開することになったので、日本薬局方収載の一般試験法の英語版について検討を行った。

B. 研究方法

第 14 改正日本薬局方の一般試験法のうち生薬に関連する一般試験法の項目を抜粋しさらに生薬通則を加えてまとめた。これらに対応する英語版をスキャナーで取り込み OCR ソフトで文字情報に置き換えテキストファイルとした。

C. 研究結果

生薬に関連する一般試験法として液体クロマトグラフ法、核磁気共鳴スペクトル測定、ガスクロマトグラフ法、乾燥減量試験法、吸光度比法、紫外可視吸光度測定法、重金属試験法、生薬の微生物限度試験法、赤外線吸収スペクトル測定法、旋光度測定法、滴定終点検出法、薄層クロマトグラフ法、ヒ素試験

法、崩壊試験法、融点測定法、溶出試験法の 16 項目を抜粋した。また生薬通則も合わせて収録した。

D. 考察

薬局方に生薬を収載している日本、中国、韓国、ベトナムはそれぞれの国で使用している生薬のモノグラフ中で規格を設定している。これらを横断的に比較する場合、正確な数値の読み取りには基本となる試験法の相違を理解することが必須である。今回収録した試験法は日本薬局方の生薬モノグラフを理解する上で必須となる項目だけではなく生薬製剤の評価に必要な項目や他国で検討されている項目についても対象とした。他の部門で行っている規格値の比較は、試験方法によって数値の扱いが異なることから、一般試験法の内容が今後活用されるものと考えられる。今回検討項目とされなかったが、さらに関連するものとして通則がある。例えば、日本薬局方における標準温度、冷水、温湯等温度に関する規定や医薬品の切度及び粉末度の名称等は中国薬典の規定とはかなりの違いが見受けられる。さらに参考情報には「アリストロキア酸」や「非無菌医薬品の微生物学的品質特性」の「生薬及び生薬を配合した製剤の微生物限度基準」の重要項目があるので次回の継続テーマとしたい。また、真に理解するためにはその内容を十分に把握するための解説が行なわれるべきで、今後の FHH の大きなテーマになりうるものと思う。

GENERAL NOTICES

General Notives in Part I are followed in Part II.

GENERAL RULES FOR CRUDE DRUGS

1 . Crude drugs in the monographs include medicinal parts obtained from plants or animals, cell inclusions and secretes separated from the origins, their extracts, and minerals. General Rules for Crude Drugs and the Crude Drugs in General Tests, Processes and Apparatus are applicable to the followings: Acacia, Achyranthes Root. Agar, Agar Powder, Akebia Stem, Alisma Rhizome, Aloe, Amomum Seed, Anemarrhena Rhizome, Angelica Dahurica Root, Apricot Kernel, Areca, Artemisia Capillaris Flower, Asiasarum Root, Astragalus Root, Atractylodes Lancea Rhizome, Atractylodes Rhizome, Bear Bile, Bearberry Leaf, Belladonna Root, Benzoin, Bitter Cardamon, Bitter Orange Peel, Bupleurum Root, Calumba, Capsicum, Cardamon, Cassia Seed, Catalpa Fruit, Chuling, Cimicifuga Rhizome, Cinnamon Bark, Citrus Unshiu Peel, Clove, Cnidium Rhizome, Coix Seed, Condurango, Coptis Rhizome, Corn Starch, Cornus Fruit, Corydalis Tuber, Cyperus Rhizome, Digenea, Digitalis, Dioscorea Rhizome, Ephedra Herb, Evodia Fruit, Fennel, Forsyihia Fruit, Gambir, Gardenia Fruit, Gentian, Geranium Herb, Ginger, Ginseng, Glehnia Root, Glycyrrhiza, Gypsum, Hoelen, Honey, Houttuynia Herb, Immature Orange, Imperata Rhizome, Ipecac, Japanese Angelica Root, Japanese Gentian, Japanese Valerian, Jujube, Lithospermum Root, Longgu, Magnolia Bark, Mallotus Bark, Mentha Herb, Moutan Bark, Mulberry Bark, Nuphar Rhizome, Nux Vomica, Ophiopogon Tuber, Oriental Be2:oar, Oyster Shell, Panax Rhizome, Peach Kernel, Peony Root, Perilla Herb, Pharbitis Seed, Phellodendron Bark, Picrasma Wood, Pinellia Tuber, Plantago Herb, Plantago Seed, Platycodon Root, Polygala Root, Potato Starch. Powdered Acacia, Powdered Alisma Rhizome, Powdered Aloe, Powdered Amomum Seed, Powdered Atractylodes Lancea Rhizome, Powdered Atractylodes Rhizome, Powdered Calumba, Powdered Capsicum, Powdered Cinnamon Bark, Powdered Clove, Powdered Cnidium Rhizome, Powdered Coix Seed, Powdered Coptis Rhizome, Powdered Cyperus Rhizome, Powdered Digitalis, Powdered Dioscorea Rhizome, Powdered Fennel, Powdered Gambir, Powdered Gardenia Fruit, Powdered Gentian, Powdered Geranium Herb, Powdered Ginger, Powdered Ginseng, Powdered Glycyrrhiza, Powdered Hoelen, Powdered Ipecac, Powdered Japanese Angelica Root, Powdered Japanese Gentian, Powdered Japanese Valerian, Powdered Magnolia Bark,

Powdered Moutan Bark, Powdered Oyster Shell, Powdered Panax Rhizome, Powdered Peach Kernel, Powdered Peony Root, Powdered Phellodendron Bark, Powdered Picrasma Wood, Powdered Platycodon Root, Powdered Polygala Root, Powdered Polypourus Sclerotium, Powdered Rhubarb, Powdered Rose Fruit, Powdered Scutellaria Root, Powdered Senega, Powdered Senna Leaf, Powdered Smilax Rhizome, Powdered Sophora Root, Powdered Sweet Hydrangea Leaf, Powdered Swertia Herb, Powdered Tragacanth, Powdered Zanthoxylum Fruit, Prunella Spike, Pueraria Root, Red Ginseng, Rehmannia Root, Rhubarb, Rice Starch, Rose Fruit, Rosin, Safilower, Saffron, Saposhnikovia Root, Saussurea Root, Schisandra Fruit, Schizonepeta Spike, Scopolia Rhizome, Scutellaria Root, Senega, Senna Leaf, Sinomenium Stem, Smilax Rhizome, Sophora Root, Sweet Hydrangea Leaf, Swertia Herb, Toad Venom, Tragacanth, Trichosanthes Root, Wheat Starch, Zanthoxylum Fruit, Zedoary.

2. Crude drugs are usually used in the forms of whole crude drugs, cut crude drugs or powdered crude drugs.

Whole crude drugs are the medicinal parts or their ingredients prepared by drying and/ or simple processes, as specified in the monographs. Cut crude drugs are small pieces or small blocks prepared by cutting or crushing of the whole crude drugs, and also coarse, medium or fine cutting of the crude drugs in whole, and, unless otherwise specified, are required to conform to the specifications of the whole crude drugs used as original materials. Powdered crude drugs are coarse, medium, fine or very fine powder prepared from the whole crude drugs or the cut crude drugs; usually powdered crude drugs as fine powder are specified in the monographs.

3. Unless otherwise specified, crude drugs are used in dried form. The drying is usually carried out at a temperature not exceeding 60'C.

4. Crude drugs are as free as possible from contaminants and other impurities due to molds, insects and other animals and from other foreign matters, and are required to be kept in a clean and hygienic state.

5. Such statements as 'other species of the same genus' and 'allied plants' or 'allied animals' appearing in the origin of crude drugs usually indicate plants or animals which may be used as materials for crude drugs containing the same effective constituents.

6. The description in each monograph of crude drugs usually covers the crude drug derived from its typical original plant or animal and includes statements of

characteristic properties of the crude drug to serve as the criteria. The values given therein are to serve as reference values, except those obtained by microscopic observation.

7. Powdered crude drugs do not contain fragments of tissues, cells, cell inclusions or other foreign matter alien to the original crude drugs or cut crude drugs.

8. Powdered crude drugs, otherwise specified, may be mixed with diluents so as to attain proper content and potency.

9. Crude drugs are preserved under protection from moisture and insect damage, unless otherwise specified. In order to avoid insect damage, suitable fumigants may be used to preserve crude drugs, provided that the fumigants are so readily volatilized as to be harmless at the usual dosage of the crude drugs, and such fumigants that may affect the therapeutic efficacy of the crude drugs or interfere with the testing are precluded.

10. Crude drugs are preserved in well-closed containers unless otherwise specified.

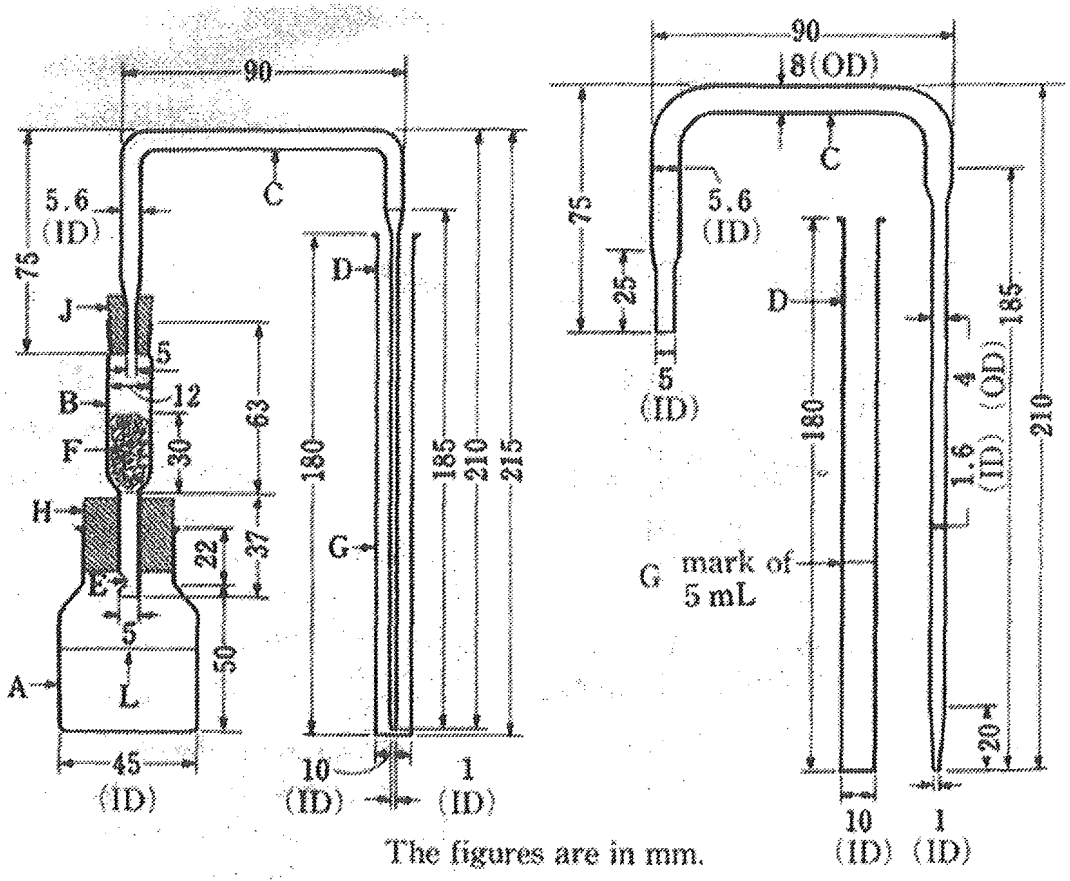
Arsenic Limit test

The Arsenic Limit Test is a limit test for arsenic contained in drugs. The limit is expressed in terms of arsenic(III) trioxide(As_2O_3).

In each monograph, the permissible limit for arsenic (as As_2O_3) is described in terms of ppm in parentheses.

Apparatus B

Use the apparatus illustrated below.



The figures are in mm.

Fig. 2

A: Generator bottle (capacity up to the shoulder : approximately 70mL)

B: Exit tube

C: Glass tube (inside diameter: 5.6mm, the tip of the part to be inserted in the absorber tube D is drawn out to 1mm in diameter)

D: Absorber tube (inside diameter:10 mm)

E: Small perforation

F: Glass wool (about 0.2g).

G: Mark of 5mL

H andJ : Rubber stoppers

L: Mark of 40mL

Place glass wool F in the exit tube B up to about 30 mm in height, moisten the glass wool uniformly with a mixture of an equal volume of lead(II) acetate TS and water, and apply gentle suction to the lower end to remove the excess of the mixture. Insert the tube vertically into the center of the rubber stopper H, and attach the tube to the generator bottle A so that the small perforation E in the lower end of B extends slightly below. At the upper end of B, attach the rubber stopper J to hold the tube C vertically. Make the lower end to the exit tube of C level with that of the rubber stopper J.

Preparation of the test solution

Unless otherwise specified, proceed as directed in the following.

(1) Method 1

Weigh the amount of the sample directed in the monograph, add 5 ml of water, dissolve by heating if necessary, and designate the solution as the test solution.

(2) Method 2

Weigh the amount of the sample directed in the monograph, add 5 mL of water, and add 1 mL of sulfuric acid except in the cases that the samples are inorganic acids. Add 10 mL of sulfurous acid solution, transfer to a small beaker, and evaporate the mixture on a water bath until it is free from sulfurous acid and is reduced to about 2 mL in volume. Dilute with water to make 5 mL, and designate it as the test solution.

(3) Method 3 Weigh the amount of the sample directed in the monograph, and place it in a crucible of platinum, quartz or porcelain. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), ignite the ethanol, and heat gradually to incinerate. If carbonized material still remains by this procedure, moisten with a small quantity of nitric acid, and ignite again to incinerate. After cooling, add 3 mL of hydrochloric acid, heat on a water bath to dissolve the residue, and designate it as the test solution.

(4) Method 4

Weigh the amount of the sample directed in the mono-graph, and place it in a crucible of platinum, quartz or porcelain. Add 10 mL of a solution of magnesium nitrate hexahydrate

in ethanol (95) (1 in 10), burn the ethanol, heat gradually, and ignite to incinerate. If carbonized material still remains by this procedure, moisten with a small quantity of nitric acid, and ignite again to incinerate in the same manner. After cooling, add 3 mL of hydrochloric acid, heat on a water bath to dissolve the residue, and designate it as the test solution.

(5) Method 5

Weigh the amount of the sample directed in the monograph, add 10 mL of N,N-dimethylformamide, dissolve by heating if necessary, and designate the solution as the Test solution.

Test solutions

Absorbing solution for hydrogen arsenide: Dissolve 0.50 g of silver N.N-diethyldithiocarbamate in pyridine to make 100 mL. Preserve this solution in a glass-stoppered bottle protected from light, in a cold place.

Standard Arsenic Stock Solution: Weigh accurately 0.100 g of finely powdered arsenic (III) trioxide standard reagent dried at 105oC for 4 hours, and add 5 mL of sodium hydroxide solution (1 in 5) to dissolve. Add dilute sulfuric acid to neutralize, add further 10 mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 1000 mL.

Standard Arsenic Solution: Pipet 10 mL of Standard Arsenic Stock Solution, add 10 mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 1000 mL. Each mL of the solution contains 1μ g of arsenic (III) trioxide (As_2O_3). Prepare Standard Arsenic Solution just before use and preserve in a glass-stoppered bottle.

Procedure

Unless otherwise specified, proceed using Apparatus B. Carry out the preparation of the standard color at the same time.

Place the test solution in the generator bottle A and, if necessary, wash down the solution in the bottle with a small quantity of water. Add 1 drop of methyl orange TS, and after neutralizing with ammonia TS, ammonia solution (28) or dilute hydrochloric acid, add 5 mL of diluted hydrochloric acid (1 in 2) and 5 mL of potassium iodide TS, and allow to stand for 2 to 3 minutes. Add 5 mL of acidic tin (II) chloride TS, and allow to stand for 10 minutes. Then add water to make 40 mL, add 2 g of zinc for arsenic analysis, and immediately connect the rubber stopper H fitted with B and C with the generator bottle A. Transfer 5 mL of the absorbing solution for hydrogen arsenide to the absorber tube D,

insert the tip of C to the bottom of the absorber tube D, then immerse the generator bottle A up to the shoulder in water maintained at 25 'C, and allow to stand for 1 hour. Disconnect the absorber tube, add pyridine to make 5 mL, if necessary, and observe the color of the absorbing solution: the color produced is not more intense than the standard color.

Preparation of standard color: Measure accurately 2 mL of Srandard Arsenic Solution in the generator bottle A. Add 5 mL of diluted hydrochloric acid (1 in 2) and 5 mL of potassium iodide TS, and allow to stand for 2 to 3 minutes. Add 5 mL of acidic tin (II) chloride TS, allow to stand at room temperature for 10 minutes, and then proceed as directed above. The color produced corresponds to 2 μg of arsenic (III) trioxide (As_2O_3) and is used as the standard.

Note: Apparatus, reagents and test solutions used in the test should contain little or no arsenic. If necessary, perform a blank determination.

Crude Drugs Test

The Crude Drugs Test is applied to the crude drugs mentioned in the General Rules for Crude Drugs.

Sampling

Unless otherwise specified, sample should be taken by the following methods. If necessary, preserve the samples in tight containers.

(1) When crude drugs to be sampled are small-sized, cut or powdered, 50 to 250 g of sample should be taken after mixing thoroughly.

(2) When crude drugs to be sampled are large-sized, 250 to 500 g of sample should be taken after mixing thoroughly.

(3) When the mass of each single piece of the crude drugs is not less than 100 g, not less than 5 pieces should be taken for a sample, or not less than 500 g of the sample should be taken after cutting to a suitable size and mixing thoroughly.

Foreign matter

Unless otherwise specified, weigh 25 to 500 g of the sample, spread out in a thin layer, and separate the foreign matter by inspecting with the naked eye or with the use of a magnifying glass of 10 magnifications. Weigh, and determine the percentage of foreign matter.

Preparation of the test sample for analysis

Preparations are to be made by mixing the sample well. Powdered drugs should be used as they are, and in the case of unpowdered drugs, unless otherwise specified, grind the sample into powder. If the sample cannot be ground into powder, reduce it as finely as possible, spread it out in a thin layer, and withdraw a typical portion for analysis . If necessary, preserve the test sample in a tight container.

Loss on drying

Unless otherwise specified, transfer 2 to 6 g of the test sample for analysis to a tared weighing bottle, and weigh accurately. Dry at 105'C for 5 hours, allow to cool in a desiccator (silica gel), and weigh accurately. Continue the drying at 105'C, and weigh accurately at 1-hour intervals. When the mass of the sample becomes constant, the loss of mass represents the percentage of loss on drying (%). When the period of time for drying is specified, weigh accurately after drying for the period of time specified, and determine the loss on drying (%).

Total ash

Ignite previously a crucible of platinum, quartz or porcelain between 500'C and 550'C for 1 hour. Cool, and weigh accurately the crucible. Unless otherwise specified, weigh accurately 2 to 4 g of the test sample for analysis in this crucible, take off the lid or keep it open a little if necessary, heat the crucible at a low temperature at first, then gradually heat to a temperature between 500"C and 550'C, ignite to incinerate the residue for more than 4 hours until no carbonized substance remains in the ash, cool, and weigh accurately the ash. Incinerate repeatedly to constant mass, cool, weigh accurately, and determine the amount (%) of total ash. If a carbonized substance remains and a constant mass cannot be obtained in the above-mentioned method, extract the charred mass with hot water, collect the insoluble residue on filter paper for assay, and incinerate the residue and filter paper until no carbonized substance remains in the ash. Then add the filtrate, evaporate it to dryness, and incinerate. Cool, weigh accurately, and determine the mass (%) of the total ash. If a carbon-free ash cannot be obtained even in this way, moisten the ash with a small amount of ethanol (95), break up the ash with a glass rod, wash the rod with a small amount of ethanol (95), evaporate carefully, and determine the mass of the total ash as described above. A desiccator (silica gel) is used for cooling.

Acid-insoluble ash

Add carefully 25 mL of dilute hydrochloric acid to the total ash, boil gently for 5 minutes, collect the insoluble matter on filter paper for assay, and wash thoroughly with hot water Dry the residue together with the filter paper, and ignite to incinerate in a tared crucible of platinum, quartz or porcelain for 3 hours. Cool in a desiccator (silica gel), weigh, and determine the amount (%) of acid-insoluble ash. When the amount determined exceeds the limit specified, incinerate repeatedly to a constant mass.

Extract content

The test for the extract content in crude drugs is performed as directed in the following methods:

(1) Dilute ethanol-soluble extract-Unless otherwise specified, weigh accurately about 2.3 g of the sample for analysis, extract with 70 mL of dilute ethanol in a suitable flask with intermittent shaking for 5 hours, and allow to stand for 16 to 20 hours. Filter, and wash the flask and residue with small portions of dilute ethanol until the filtrate measures 100 mL. Evaporate a 50 mL aliquot of the filtrate to dryness, dry at 105'C for

4 hours, and cool in a desiccator (silica gel). Weigh accurately the amount, multiply it by 2, and determine the amount of dilute ethanol-soluble extract Calculate the ex-tract content (%) with respect to the amount of the sample on the dried basis, obtained under the loss on drying.

(2) Water-soluble extract-Proceed as directed in (1).using water instead of dilute ethanol, weigh accurately the amount, multiply by 2, and determine the amount of water-soluble extract. Calculate the extract content (%) with respect to the amount of the sample on the dried basis, obtained under the loss on drying.

(3) Diethyl ether-soluble extract-Unless otherwise specified, dry the test sample for analysis in a desiccator (silica gel) for 48 hours, weigh accurately about 2 g of it, and place in a suitable flask. Add 70 mL of diethyl ether, attach a reflux condenser to the flask, and boil gently on a water bath for 4 hours. Cool, filter, and wash the flask and the residue with small portions of diethyl ether until the filtrate measures 100 mL. Evaporate a 50 mL aliquot of the filtrate to dryness on a water bath, dry in a desiccator (silica gel) for 24 hours, weigh accurately the amount, multiply it by 2, determine the amount of diethyl ether-soluble extract, and calculate the extract content (%).

Essential oil content

The test of essential oil content in crude drugs is performed as directed in the following method:

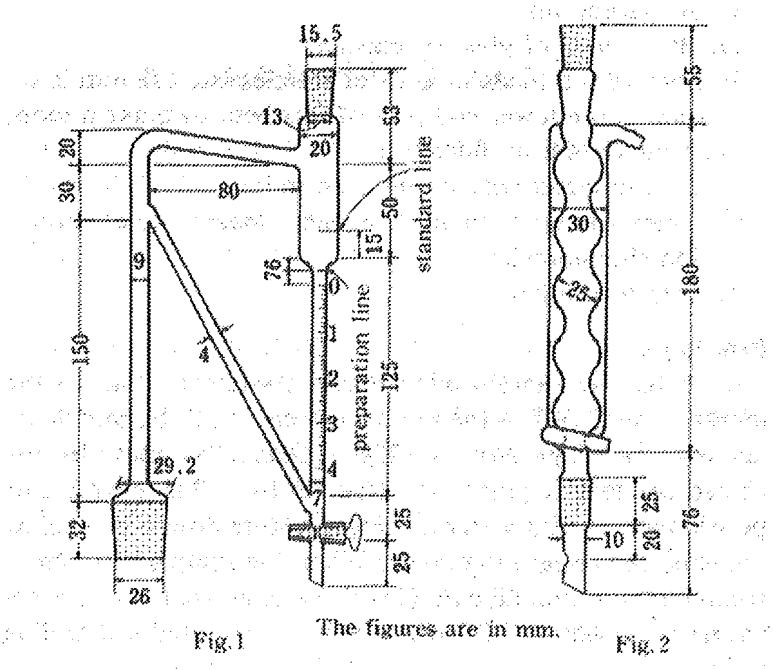


Fig. 1 The figures are in mm. Fig. 2

Essential oil determination: Weigh the quantity of the test sample for analysis directed in the monograph in a 1-L hard glass-stoppered flask, and add from 5 to 10 times as much water as the drug. Set up an apparatus for essential oil determination (Fig. 1), inserting a reflux condenser (Fig. 2) in the upper mouth of it, and heat the content of the flask in an oil bath between 130'C and 150'C to boiling. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2.0 mL of xylene is added to the graduated tube. Unless otherwise specified, continue boiling for 5 hours, allow to stand for some time, and open the stopper of the apparatus. Draw off the water slowly until the surface of the oil layer corresponds to the preparation 1line, and allow it to stand for more than 1 hour at ordinary temperature. Then lower the surface of the oil layer to the zero line, and read the volume (mL) of the oil at ordinary temperature. Subtract the volume (mL) of xylene from the volume of the total oil.

Microscopic examination

(1) Apparatus

Use an optical microscope with objectives of 10 and 40 magnifications, and an ocular of 10 magnifications.

(2) Preparation for microscopic examination

(i) Section: To a section on a slide glass add 1 to 2 drops of a mounting agent, and put a cover glass on it, taking precaution against inclusion of bubbles. Usually use a section 10 to 20 μm in thickness.

(ii) Powder: Place about 0.1 g of powdered sample in a watch glass containing 2 to 3 drops of a swelling agent, stir well with a small rod preventing inclusion of bubbles, and allow to stand for more than 10 minutes to swell the sample. Smear, using a small glass rod, the slide glass with a small amount of the swollen sample, add 1 drop of the mounting agent, and put a cover glass on it so that the tissue sections spread evenly without overlapping each other, taking precaution against inclusion of bubbles.

Unless otherwise specified, use a mixture of glycerin and water (1 : 1) as mounting agent and swelling agent.

(3) Observation of components in the Description

In each monograph, description is usually given of the outer portion and the inner portion of a section in this order, followed by a specification of cell contents. Observation should be made in the same order. In the case of a powdered sample, Description is given of a characteristic component or a matter present in large amount,

rarely existing matter, and cell contents in this order. Observation should be made in the same order.

Heavy Metals Limit Test

The Heavy Metals Limit Test is a limit test of the quantity of heavy metals contained as impurities in drugs. The heavy metals are the metallic inclusions that are darkened with sodium sulfide TS in acidic solution, as their quantity is expressed in terms of the quantity of lead (Pb).

In each monograph, the permissible limit for heavy metals (as Pb) is described in terms of ppm in parentheses.

Preparation of test solutions and control solutions

Unless otherwise specified, test solutions and control solutions are prepared as directed in the following:

(1) Method 1

Place an amount of the sample, directed in the monograph, in a Nessler tube. Dissolve in water to make 40 mL. Add 2 mL of dilute acetic acid and water to make 50 mL, and designate it as the test solution.

The control solution is prepared by placing the volume of Standard Lead Solution directed in the monograph in a Nessler tube, and adding 2 mL of dilute acetic acid and water to make 50 mL.

(2) Method 2

Place an amount of the sample, directed in the monograph, in a quartz or porcelain crucible, cover loosely with a lid, and carbonize by gentle ignition. After cooling, add 2 mL of nitric acid and 5 drops of sulfuric acid, heat cautiously until white fumes are no longer evolved, and incinerate by ignition between 500'C and 600'C. Cool, add 2 mL of hydrochloric acid, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10mL of hot water, and warm for 2 minutes. Then add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution develops a pale red color, add 2 mL of dilute acetic acid, filter if necessary, and wash with 10 mL of water. Transfer the filtrate and washings to a Nessler tube, and add water to make 50 mL. Designate it as the test solution.

The control solution is prepared as follows: Evaporate a mixture of 2 mL of nitric acid, 5 drops of sulfuric acid and 2 mL of hydrochloric acid on a water bath, further evaporate to dryness on a sand bath, and moisten the residue with 3 drops of hydrochloric acid. Hereinafter, proceed as directed in the test solution, then add the

volume of Standard Lead Solution directed in the monograph and water to make 50 mL.

(3) Method 3

Place an amount of the sample, directed in the monograph, in a quartz or porcelain crucible, heat cautiously, gently at first, and then increase the heat until incineration is completed. After cooling, add 1 mL of aqua regia, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and warm for 2 minutes. Add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution develops a pale red color, add 2 mL of dilute acetic acid, filter if necessary, wash with 10 mL of water, transfer the filtrate and washings to a Nessler tube, and add water to make 50 mL. Designate it as the test solution.

The control solution is prepared as follows: Evaporate 1 mL of aqua regia to dryness on a water bath. Hereinafter, proceed as directed for the test solution, and add the volume of Standard Lead Solution directed in the monograph and water to make 50 mL.

(4) Method 4

Place an amount of the sample, directed in the monograph, in a platinum or porcelain crucible, mix with 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol to burn, and carbonize by gradual heating. Cool, add 1 mL of sulfuric acid, heat carefully, and incinerate by ignition between 500'C and 600'C. If a carbonized substance remains, moisten with a small amount of sulfuric acid, and incinerate by ignition. Cool, dissolve the residue in 3 mL of hydrochloric acid, evaporate on a water bath to dryness, wet the residue with 3 drops of hydrochloric acid, add 10 mL of water, and dissolve by warming. Add 1 drop of phenolphthalein TS, add ammonia TS dropwise until a pale red color develops, then add 2 mL of dilute acetic acid, filter if necessary, wash with 10 mL of water, transfer the filtrate and the washing to a Nessler tube, add water to make 50 mL, and use this solution as the test solution.

The control solution is prepared as follows: Take 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and fire the ethanol to burn. Cool, add 1 mL of sulfuric acid, heat carefully, and ignite between 500'C and 600' C. Cool, and add 3 mL of hydrochloric acid. Hereinafter, proceed as directed in the test solution, then add the volume of Standard Lead Solution directed in the monograph and water to make 50 mL.