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THE PRODUCTION OF STANDARDS AND REAGENTS FOR THE QUALITY CONTROL OF MEDICINES

DP/VIE/84/006

SOCIALIST REPUBLIC OF VIET NAM

Technical report: Production of Standards Antibiotics and other Reference Substance*

Prepared for the Government of the Socialist Republic of Viet Nam
by the United Nations Industrial Development Organization
acting as executing agency for the United Nations Development Programme

Based on the work of Dr. Francis Marffy, expert in the preparation and manufacturing high purity chemicals, reagents and standards

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Vienna

* This document has not been edited.

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ABSTRACT

THE STRENGTHENING OF THE STANDARDS AND REAGENTS SECTION OF THE INSTITUTE OF DRUG QUALITY CONTROL.

DP/VIE/'84/006/11-04

Objective: Train counterpart staff in the production of standard antibiotics...

Duration: 7 Febr - 21 March 1990.

To improve production and quality of reference and standard substances the equipments and raw materials available bas been taken into account in order to purify 8 substances. All substances produced were submitted qualification tests according to the international requirements. Some effort has been made to introduce methods for determining the prefered conditions regarding packing operations. First of all the effect of air humidity has been determined, the effect of oxygen decreased and measures to decrease the influence of light has been proposed. The elements of the packing operation has been studied and proposals were put forward to introduce some simple devices, which assist in increasing the production of reference substances.

Three lectures were delivered on aspects of chemical purification, on diferent chromatographic methods and equipments and some of their applications. According to the interest of the counterpart, questions of industrial production of ionexchangeresins and organic molecular sieves were also treated.

INTRODUCTION

The Institute of Drug Quality Control produces for the pharmaceutical industry, provincial drug quality control laboratories and other health services reference substances, reagents and absorbent in good quality.

The government attaches great importance to local production of drugs for attaining relative self-sufficiency and saving scarce foreign exchange. The pharmaceutical industry consists of one factory manufacturing pharmaceutical chemicals and 10 central formulation plants, approximately 25 % of the production being of traditional system. The central factories, provincial production units, trading companies, depots, provincial and district services form a network of quality control laboratories at the provincial and central levels. The provicial laboratories are small to medium size and carry out some essential testing. The central level is directly under the control of Ministry of Health and deals with samples which require detailed analysis through advanced techniques. The quality control network, the Institute of Drug Quality Control (IDQC) heretoafter referred to as the "Institute" has been established in Hanoi in 1971, with a sub-Institute in Ho Chi Minh city in the South. The main functions of the Institute are:

- To control the quality of medicines (including the traditional ones), their intermediates and raw materials with regard to their production, imports, exports, purchase, storage and untilization;
- To study various methods of testing, controlling and stadardizing the quality of drugs such as: metrology, pharmaceutical regulations, legal chemistry, production and supply of standards and reference substances and analytical and biochemical reagents;
- To disseminate scientific and technical information;
- To provide training in drug quality control.

As a member of international experts assigned to the Institute of Drug Quality Control, under the Chief Technical Adviser and in collaboration with the National Project Co-ordinator and counterpart staff, the expert is expected to

- Train counterpart staff in the production of standard antibiotics, hormones, vitamins and other reference substances.
- Assist in quality improvement of secondary antibiotic standards to be in level with primary ones.

- Advise on new and advanced technologies, processes and methods for the production, standardization, quality control and stability testing of reference substances.

- Recommend methods for the increased production of the present reference substances and the introduction of additional ones and sensitivity discs.

The expert arrived in Hanoi on 7th February 1990 and reported at the Institute on 8th February 1990. The activity was finished on 21 March 1990.

The main original objectives of the activity were not revised. In accordance with the counterpart a working plan was drawn up based on the list of substances already prepared or planned in the future. If the raw materials or reagents needed were not available in due time after mutual agreement a new item on the list were aimed at.

Considering the circumstances the method to be seen realizable was chosen and demonstrated. Although scientifically and technically not always on the most economic level it gives a useful tool to the counterpart in widening the production. In order to assist in preparing more advanced method the chemicals and devices needed were discussed and the copies of publication concerned were handed over.

During the stay in the Institute, purification of eight substances were demonstrated. It seemed to be necessary to discuss with the counterpart the manufacturing technology of Silica gel thin layer chromatographic plates, cellulose ionexchangers and gel besed molecular sieves. It may give new hints in the training programme aimed at studying the production of adsorbents.

I. ASPECTS INFLUENCING THE CHOICE OF TECHNOLOGY

A. Selection of equipment:

The laboratory used for the manufacturing of standard substances contains a larger room for classical preparative works and a small chamber intended for filling the vials with high purity substances. Both rooms are provided with airconditioner (dehumidifyer) but exhaustion (esp.fume hood) has not yet been mounted. The counterpart being fully conscious of the importance widening the electric power supply with grounding did his best to prevent any accident. Considering the increasing use of solventes in purification processes, this precaution is reasonable. During the visit the tap water and destilled water supply were satisfactory. The refrigerators satisfied all the needs. The glassware coming in guarantees an efficient basis of laboratory activity. The counterpart is pinched for devices needed for executing chromatographic separations and laboratory freeze dryer to handle heat sensitive substances (vitamins, hormones and some antibiotics). During our detailed discussions the counterpart set forth that he had already done preparatory steps to acquire the necessary equipment in appropriate time. One can fully agree the selection of the items demand due consideration. At the last stage may emerge the question of getting an appropriate preparative high performance liquid chromatograph. Although from engineering point of view it is a necessity in manufacturing standard substances the investment and operating being rather high, it needs a careful consideration.

B. Selection of raw materials:

The preparation of fine chemicals and standard substances is a laborious process. The end product being a precious substance with substancial stability and passing many complicated chemical and physical tests needs a rather intricate evaluation of raw materials. If some parts of the molecule contain chemically reactive groups, it may restrict the number and sort of purification procedures. All these considerations lead to the conclusion: the raw materials should be chosen first of all according to their chemical and physical charateristics.

The first step in the evaluation is a competent test of substances offered by different manufacturers. In this respects the pharmacopea of industrialised countries (or the World Health Organisations) contains all the

tests needed. In case of different regulations comparative tests with an accepted method give a rather favourable orientation. It is recommendable to choose a substance with a rather high value of assay. The different manufacturers offer sometime phamaceuticals with more than 99 p.c purity. Even in these cases the purification, the qualifying tests and control of stability are complicated laboratory process lasting many months. Use of pharmaceuticals for purification after their expiration dates can occur only in extreme situations.

This conception was agreed upon by the Institute as well. All efforts were undertaken to obtain the samples needed. The coming in of pharmaceuticals is continuous. In order to demonstrate important methods the expert felt his duty to use expired pharmaceuticals (some ampicillin, erytromycin, neomycin). Although the raw materials not always achieving the standards the performance of the methods could be estimated. Receiving the appropriate sample the counterpart can apply the method demonstrated.

C. Selection of purification methods:

The treatment of the pharmaceuticals in order to achieve a rather high purity needs reliable reagentes and careful, complicated activity. As a general rule there are different procedures published during the years. The later it has been published the higher is the need for advenced equipment and reagents. Although certain processes are available, but the human inventiveness is always of unlimited value outlining the different methods available in the literature and practice. An animated discussion developed with the counterpart. During the evaluation the counterpart made every effort to produce the necessary equipment and reagents. Owe to this effort, the assistance of the other institutions and factories, the helping hand of the local UNDP officials always led to a satisfactory solution. The counterpart has been building a rather extensive development project to upgrade the production capacities. As far as the expert informed this include preparations for introducing chromatographic methods (ion-exchange chromatography and physical separation methods especially freeze drying and fast drying procedures). These ambitions are highly estimated by the expert, which will contribute to the counterpart 's capacity and enable him to adopt the purification method needed to remove the last traces of isomers or decomposited substances.

0. Selection of assay methods:

At the beginning of the activity a working plan was drawn up. It consisted demonstration of the purification methods for the following substances: Ampicillin, Chloramphenicol, Sulphadimidin, Sulphadimedoxin . It was extended with erythromycin, streptomycin and neomycin and sulfaguanidin. For the assay of ampicillin the mercurimetric me hod (1) and a HPLC method were used. Recommendations of the International Pharmacopoeia, 3rd Edition 1981 (later: IP) were not applied. By chloramphenicol IP suggests a photometric method. As the counterpart has been applying this assay and all its previous data were available, it was later granted to accept it. (2)- data gained by HPLC were also evaluated. Sulphadimetoxin (3)- was assayed by amperometric titration. Erythromycin was assayed by micro-biological method, (4)- but HPLC was also applied. The streptomycin sulphat was assayed according to British Pharmacopoeia with photometric method. (5)- For neomycin sulphat the regulations of British Pharmacopoeia (6)- were applied. It was also assayed by microbiological method (7)- for testing its potency. Data by HPLC were also evaluated. All assays were carried out by the counterpart's experienced staff. The expert pays tribut for the correct and competent activity.

II. EFFORTS TO INCREASE STABILITY OF REFERENCE SUBSTANCES

Little information has been published on the degradation of longestablished pharmaceutical substances (except for obviously unstable products, and their behaviour, when exposed to extreme climatic condition, is uncertain). By contrast, much information on the stability of newly introduced substances is available, since this information is a mandatory requirement in many countries for registration of a new product and for determining expiry dates.

The work done so far was carried out under the following standardised conditions: 30 day's exposure to air at a temperature of 50°C and a relative humidity of 100 %. The appearance of degradation was detected by thin-layer chromatography, supplemented by spectrophotometry, high-pressure liquid chromatography and chemical determinations. The substance was additionally exposed to a temperature of 70°C under the same humidity condition for a ratherlong period of 3-5 days. When negative, these results provided conclusive

proof of the stability of the substance even under highly adverse conditions. The tests were carried out with light excluded, because it is easy to protect the substance from light during storage.

256 substances were tested by different laboratories of which 96 were degradable under the conditions employed. Test for each of these 96 substances were developed which reliably demonstrated 10 % of degradation. These data will be considered when specifications are prepared for the International Pharmacopoeia.

Since the substances mentioned are mainly of sensitive charater the meauseres employed in handling, distributing chemical reference substances must provide for assurance that their integrity will be safeguarded and maintained through their period of use. The containers for reference substances should afford protection from moisture, light and oxigen.

The selection of suitable analytical methods for monitoring the stability of reference substances depends on the nature of the substances. Simple tests, such as determination of water content or assay are useful for recognizing the onset of degradation When quantitive estimation of the degree of degradation is needed, more complicated techniques such as chromatography with quantitative determination of the separated components must be used.

Change in the moisture content of reference substances is a phenomenon that is difficult to control. To establish suitable conditions for packing operations and storage that might minimize such changes, it is recommended that, for each substance, data be obtained relating to moisture content and relative humidity.

These data have not yet been established for any of the reference substances at the Institute. The most favourable stability can not be expected under tropical conditions. To evaluate stability with various moisture contents samples of ampicillin, steptomycin and penicillin were placed in desiccators with determined and unchanging humidity. The values of the latter were controlled regularly. Through weight change of the samples the moisture content, through regular assays the level of degradation were evaluated. After 4 weeks the ampicillin trihydrate did not changed, the weight of the other two substances showed significant variation by 40-50 % relative humidity. It is recommended to follow the changes at least for six months. On the basis of data gained the packing operations can be accomplished by humidity needed for each substance. In the premises used for packing

operations a rather low humidity is essential. Although the dehumidifyer installed was of low performance, setting its variables favourable it guarantees the wide range of humidities. At least 3 hours before starting the packing operations the dehumidifier must be turned on.

To improve stability the oxigen must be removed from the vials containing the standard substances. From economic reasons it seems to be most reasonable to fill the space over the substances with nitrogen of controlled humidity. During efforts to obtain industrial nitrogen gas a nitrogen generator ordered previously by the counterpart came in. The control of its performance confirmed the expectations. The relative humidity of the nitrogen gas does not exceed 2 %. The oxigen level is extremly low. During 10 hours of operation no breakdown was experienced. The output of nitrogen is sufficient.

Some difficulties appeared in connecting the generator to the vacuum oven containing the filled vials. The low pressure in the oven increases the flow rate of nitrogen causing the increase of humidity and oxigen content in the latter. Obtaining a flow-meter during the short stay seemed to be questionable. A gas bubble-column and glasswool filter gave a useful controlling system. Although it takes some time to control the gas flow its use is inevitable in filling the vials with nitrogen of appropriate humidity and oxigen content. Increasing the flow rate results in decreasing stability of the standard substances.

III. PRODUCTION AND QUALITY IMPROVEMENT OF REFERENCE SUBSTANCES

The total collection of International Reference Substances now contains more than hundred substances. To support all the specifications contained in volumes 2 and 3 of the International Phamracopoeia a further at least 50 new reference substances will be needed. However, because of limited resources of WHO work on the remaining substances is unlikely to be completed until 1990. It is recommended, that establishment of national reference substances concerned must be very carefully calibrated against the International Chemical Reference Substances if reliable, internationally effective standards of quality in pharmaceutical substances are to be maintained.

According to the priorities set by the Institute, the purification methods of some antibiotics has to be demonstrated or improved. Ampicillin standards are in great number needed in the Institute but without satisfactory purification method and by difficulties in packing technology some voices of concerns were expressed. In agreement with the counterpart after taking into account the accessories of the laboratory a dissolution in slightly acidic environment followed by an extraction step and precipitation in alcalic solution was applied. Sample originating from 1983 was purified. An improvement from 86 % to 96 % was achieved (assayed bei high performance liquid chromatography). As comparision a substance of 99,1 % content improved by 0,1 % measured by high performance liquid chromatography and reached 100,4 % measured by the absolute mercuri (II) method. The details of the procedure are reported in the Annex (7). The analytical methods applied are also expounded. In order to give some hints toward impurities contained in the substance, a short summary of manufacturing technology was given.

The chloramphenicol standard substance vials has already been produced by the Institute. It is planned to increase its output significantly. The raw material available is yellow in colour. The assay of 104 % confirms the fact it contains degradation products. It can be purified by recrystallisation in water. By saturating at 90^{o}C the need for a heated funnel was eliminated and the procedure was easily carried out. The substance was dried in room-temperature to avoid any degradataion (8). The assay was carried out with photometric method.

Sulphadimethoxin standard has so far not been produced in the laboratory of the Institute. It is chemically a new group of substances. In order to enable the counterpart in the future to investigate small traces of impurities resulting from the synthesis thin layer chromatography methods were given (9). At the same time an evaluation of the fundamental synthesis gave some direction towards the methods of purification. Because of its solubility in ethanol a recrystallisation was chosen. Although the molecule is rather stable at the temperature of 200^{o}C, a drying at room temperature was recommended. The last step the synthesis was carried out at 100^{o}C, but the evaluation of gas removed the air present in the reaction-vessel. If the production should be increased significantly a fast drying at higher temperature could be introduced.

Sulfadimidin has been also on the schedule of chemical reference substances projected for production. The method of purification was demonstrated. On the proposal of the counterpart streptomycin and neomycin were also chosen for demonstration.

Numerous processes often rather complex, have been used to recover the streptomycin present in solution, and to purify it in the form of various salts (sulfate, chlorhydrate, calcium complex, etc.). They always involve several steps requiring some of the following techniques : adsorption on support, followed by elution: the support can be activated carbon, with elution with a lower alcohol in aqueous acid solution (such as methanol-water-formic acid, for instance) or a non-ionic resin (such as Amberlite XAD1 or SAD2) with elution with methanol or methyl-ethyl-ketone, fixation by ion exchange on a carboxylic type of weak cationic resin (Amberlite IRC, XE 222, Wofatit CP 300) with elution with a diluted mineral acid, HCl or H_2SO_4, chromatography on alumina, followed by elution with a diluted mineral acid, hydrochloric acid or sulphuric acid; chromatography on alumina, followed by elution with methanol; extraction with a water immiscible solvent in the presence of a carrier (for instance a 5 % mono-2-ethylhexyl-phtalate solution in chloroform followed by a reisolation with water having acid pH; selective precipitation as helianthate, picrate, silicotungstate, reinectrate, various sulfonates, etc, which are then converted into chlorohydrate or sulfate; formation of a schiff base reaction with an amine (benzylamine, β -phenetylamine, dibenzylmethylamine, etc); the crystallized product obtained is recovered and later subjected to the action of an acid, sulfuric acid, for instance, which leads to streptomycin sulfate. In additions to those operations, in which streptomycin is involved, there are a certain number of other processing steps which are intended to eliminate impurities without directly interfering with the antibiotic: precipitating calcium ions, to eliminate the heavy metals or with aqueous solutions of carbon dioxide under pressure, to eliminate sodium ions for the reason mentioned earlyer. A process, appliable at present was chosen.

The streptomycin sulfate used as raw material is of yellow colour and assayed for the active ingredient. Being the only raw material (no base obtainable) and considering the local condition of equipment (lack of chromatographic column and resins) a direct precipitation procedure was used

to remove the degradation product (10). Using streptomycin base the process is more simple and with chromatography more effective separation could be achieved. All the methods were discussed in detaile and the literature were handed over. Having the data needed an objective evaluation of situation and direction of development are now possible.

The neomycin sulfat obtained is of yellow shade. The method of choice for neomycin as well as most other aminoglycosides is the ion exchange procedure. Several variations of this procedure have been reported with yields in the 70 % or higher range. Because of the reasons mentioned above by streptomycin a simple method of precipitation was demonstated (11). With the process a significant change of colour and some purification was achieved.

Streptomycin, manufactured in 1978 was also purified. The method of choice is a recrystallisation from alcohol (11). The product obtained was assayed. Purification of sulfadoxin and sulfathiazol and sulfaquanidin were also demonstrated.

IV. IMPROVEMENT OF PRODUCTION TECHNOLOGY

The packing operation at present applies glass tubes sealed at one end as containers and after having filled sealed then at the other end.

The tubes are treated at first with chromosulfonic acid to remove any fat from the surface. It follows rinsing with tap water and scrubbing the internal surface and rinsing again many times with deionized water. Having dryed in an oven the cooled tubes are filled with the substance needed, in flame pulled out into a capillary tube and placed into a vacuum dryer. Following drying each tube are sealed in a flame of a gas burner. Every operation mentioned are carried out manually. To increase the output, using this method is hardly to imagine, considering the reliability and acceptable cost level of production. Without changing or investing significantly the process, the following proposals are to consider:

1. The tubes (20-40 pcs) placed in a polypropylene rack with removable front plate (like polypropylene holder for serological tubes 10 mm in diameter, Fisher 1986, Cat. No.14-809A, used as slant rack) in a slight angle to the horizontal position might be dipped in the chromosulfonic acid. After some minutes the acid might spilled out by lifting the closed end of the tubes

with the rack. By the same way follows the rinsing in tap water but giving up scrubbing with the brush. Reaching a neutral rinsing water follows the washing with deionised water. Lifting the rack the tubes could be transferred into glass beakers as-present and put into an oven for drying.

2. The tubes are filled through a glass funnel. Although there are filling devices available, at present practically no need getting it. Baecause of many substances packed in rather small numbers, the washing up after every change is too laborious.

3. After filling the open end of the tubes has been pulled out. Having checked and putting the nitrogen-generator in operation (see Chapter III) the capillary has lost its previous significance. In order to decide whether with capillary or without it one can achieve more stability, ten tubes were sealed with each method. After having assayed them in six-twelve months interval the fundamental knowledge could be gathered. In both cases a simple sealing device is suggested, because of the rising number of tubes and the strains on the person's eyes and lungs executing the operation. Operation of the device has been discussed.

4. The label ensures identity and the same time safegard the substance from the degrading effect of light. It is proposed by the expert to use some synthetic glue (for example some polivinyacetat suspension with antifungal additive) and place the label such, it will cover the lower part of the vial.

5. Some of the many abled artists of this country certainly could easily assist in drawing up labels reflecting the high professional skill characterizing the Institute.

RECOMMENDATIONS

The reference substances are badly needed in the Health Service of Republic Vietnam.

1. The Institute has displayed considerable professional skill in producing reference substances. It's ambicious project requires raw materials of good quality for reference substances which must be carefully chosen and as soon as possible used for the production. Great effort has already been done and the assistance of the UNDP Field Office is highly approciated.

2. The aspiration of the Institute to introduce new chromatographic devices and method meets the expert's proposals. The ionexchangers, later the gel- and at last the preparative high performance liquid chromatography and it's simplest accesscries play an important role in the purification of antibiotics and vitamines.

3. The methods demonstrated and worked out together for purification of drugs can be applied at the present condition and the products prepared are immidiately used.

4. Stability tests of three substances at five different humidity values need only assays in the next six months before evaluation.

5. The changes introduced in filling the vials under the nitrogen are now an organic component of the production.

6. Some proposal concerning the cleaning and sealing proccess are able to increase the output of the reference substances.

7. Lectures given on economical, technical and managing aspects of the production of high purity solvents, celluloses ionexchangers and different gels are shaping the working conceptions. After reaching decision it is recommended to concentrate all effort for introducing only the production of one substance in a given time.

ANNEXES

1. The International Pharmacopoeia (1981)

Ampicilin

A.Dissolve 50 mg in 10 ml of boric buffer pH 9,0 and 0,2 ml of acetic anhydride and stir for three miutes. Add 10 ml of 1M sodium hydroxide and allow to stand for 15 minutes. Add 10 ml of 1 M nitric acid and 20 ml of acetate buffer PH 4,6 and immidiately titrate with 0,02 M mercury (II) nitrate. Titrate slowly so that the titration takes about fifteen minutes. Determine the end-point potentiometrically using a platinium or mercury indicator electrode and a mercury-mercury(I) sulphate reference electrode. Ignore any preliminary inflection on the titration curve. Each ml of 0,02 M mercury (II) nitrate VS is equal to 0,006988 g of degradation product, calculate the percentage content of total penicillins and the percentage content of degradation products; the difference between the two percentages is the content of ampicillin $C_{16}H_{19}N_3O_4S$.

B.HPLC. The following conditions were used:

Elment: Acetonitrile Phosphate buffer pH=6,8(12:88)

Column:Spherisorb 8588

Detector: Schimadzu SPD-2A operated at 225 mm.

Pump: Waters 600 multisolvent delivery system operated at a flow rate of 1 ml/min

Intergrater: Hewlett Packard 3390A

Sample: 1 mg/ml dissolved in the eluent.

20 ml corresponding to 20 mg were injected.

A comparison was made with the control which contanained about 0,6 % impurities.

2. The International Pharmacopoeia (1981)

Chloramphenicol

Dissolv about 20 mg accurately weighed, in sufficent water to produce 100 ml; dilute 10,0 ml of this solution to 100 ml with the same solvent. Measure the absorbance of a 1 cm layer of the diluted solution at the maximum at about 278 mm. Calculate the amount of $C_{11}H_{12}CL_2N_2O_5$ in the substance being tested by comparison with Chloramphenicol similarly and concurrently examined. In an adequatly calibrated spectrophotometer, the absorbance of the reference solution should be 0,60 $\pm$ 0,03.

3. British Pharmacopoeia 1980.

Sulphadimethoxine

Assay:Dissolve 0,5 g in a mixture of water and 10 ml of hydrocloric acid and carry out the method for amperometric titration, Appendix VIII B.Each ml of 0,1 M sodium nitrite VS is equivalent to 0,03103 g of $C_{12}H_{14}N_4O_4S$.

4. British Pharmacopoeia 1980.

Erythromycin Estolate

Content of $C_{12}H_{26}O_4S$ 22,0 to 25,5 percent, calculated with reference to the anhydrous substance when determined by the following method. Dissolve 0,5 g in 25 ml of dimethylformamide and carry aut Method II for non-aqueous titration, Appendix VI.I A,using 0,1 M sodium methoxide VS as titrant and a 0,2 percent W/V solution of thymol blue in methanol as indicator. Each ml of 0,1 M sodium methoxid VS is equivalent to 0,02664 g of $C_{12}H_{26}O_4S$.

Assay: Dissolve 0,4 g in 400 ml of methanol and add 200 ml of sterile phosphate buffer pH 7,0 and sufficient water for injections to produce 1000 ml. Maintain the solution 60^0 for three hours, cool and carry out the biological assay of antibiotics, erythromycin Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95 percent and not more than 105 percent of the estimated potency. The upper fiducial limit of error is not less than 610 Units per mg, calculated with reference to the anhydrous substance.

5. British Pharmacopoeia 1980.

Streptomycin sulphate

Assay For Potency. Carry out the biological assay of antibiotics, Appendix XIV A.The precision of the assay is such that the fiducial limits of error are not less than 95 percent and not more than 105 percent of the estimated potency. The upper fiducial limit of error is not less than 720 units pge mg, calculated with reference to the dried substance.

For streptomycin sulphate. Dissolve 0,1 g in sufficient water to produce 100 ml to 5 ml add 5 ml of 0,2 M sodium hydroxide and heat for exactly 10 minutes in a water-bath. Cool in ice for exactly five minutes, add 3 ml of 1,5 percent w/v solution ammonium iron (III) sulphate in 0,25 M sulphuric acid and sufficient water to produce 25 ml, and mix. Exactly twenty minutes after the addition of the ammonium iron (III) sulphat, measure the absorbance

of a 2 cm-layer at the maximum at about 525 nm, Appendix II B,using as the blank solution prepared in the same manner, omitting the substance being examined. The absorbance is not less than 90,0 percent of that obtained by carrying out the operations simultaneously using streptomycin sulphate instead of the substance being examined, both absorbances being calculated with reference to the dried material.

6. British Pharmacopoeia 1980.

Neomycin Sulphate

Sulphate 27,0 to 31,0 percent, calculated with reference to the dried substance and determined by the following method. Dissolve 1 g in 200 ml of water, add 3 ml of hydrochloric acid, heat to boiling, and add 15 ml of hot barium chloride solution. Heat on a water-bath for four hours with stirring, collect the precipitate, wash with water, dry, ignite, and weigh. Each g of residue is equivalent to 0,4116 g of sulphate. Assay:Carry out the biological assay of antibiotics, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 90 percent and not more than 110 percent of the estimated potency. The fiducial limit of error is not less than 650 Units per mg, calculated with reference to the dried substance.

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9. Journal of Organic Chemistry 26,2764 (1961)

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