



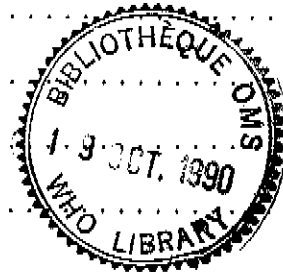
WHO COLLABORATING CENTRE FOR CHEMICAL REFERENCE SUBSTANCES

Report on the work in 1989

by M. Westermark

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Distribution of reference substances in 1989

During 1989 the total number of International Chemical Reference Substances distributed from the Centre were 1157 and 46 sets of Melting Point Reference Substances. The substances were distributed to drug control laboratories in 35 different countries. Compared to the figures for 1988 this corresponds to an increase of about 7 per cent. The five most frequently requested substances during 1989 were in order of demand Dicloxacillin sodium, Ampicillin trihydrate, Melting point reference substances, Oxacillin sodium and Benzylpenicillin sodium. Detailed figures for the distribution of the individual substances are given in Appendix 1.

Establishment of reference substances in 1989

In accordance with the procedure recommended by the WHO Expert Committee on Specifications for Pharmaceutical Preparations in its Twenty-fifth report (Technical Report Series No 567), 6 International Chemical Reference Substances were established in 1989. The substances are listed in Appendix 2 to this report. Ampicillin sodium, Dexamethasone, Dexamethasone acetate, 4-Epi-anhydrotetracycline hydrochloride and Folic acid are replacement batches as the former stocks were depleted during 1989.

A complete list of all International Chemical Reference Substances available from the Centre in January 1990, with information about package sizes and control numbers for the current batches, is given in Appendix 3 to this report. The list also includes 7 substances mentioned below, which are expected to be formally adopted during the first half of 1990.

Work on new reference substances completed in 1989.

Work is being continued on new reference substances required to support specifications in the third edition of the International Pharmacopoeia. During 1989 four new reference substances for volume 3 were examined, they were Carbamazepine, Levothyroxine sodium, Oxytetracycline dihydrate and Oxytetracycline hydrochloride. The analytical reports for these materials are given in Appendices 7 - 10. These substances were considered suitable for their intended use and were proposed for adoption as International Chemical Reference Substances.

The stocks of the following three International Chemical Reference Substances were depleted and have been replaced by new batches during 1989. Bupivacaine hydrochloride No 172054 was replaced by No 289054, Prednisolone No 283029 was replaced by No 389029 and Prednisolone acetate No 167030 was replaced by No 289030.

Stability testing

Each year a number of the International Chemical Reference Substances held in stock at the Centre are being reexamined to control their storage stability. During 1989 the reexamination was performed on thirteen substances.

The selection of analytical methods to be used for the stability monitoring requires careful reflection. The choice of method is, of course, much depending on the nature of the substance concerned. However, a generally applicable guiding principle is to use methods of high reproducibility and to adhere as closely as possible to the same methods and the same experimental conditions for the reexamination of a reference material as were used in the initial analysis. This will reduce the influence from analytical errors and facilitate early detection of degradation of the material. It is, however, also prudent to consider from time to time the progress of analytical chemistry and to introduce new methods if they are considered to be more informative and/or more convenient.

The results obtained in the reexamination together with the results from earlier studies are summarized in Appendix 4 to this report. Details about the methods used can be obtained from the Centre.

Work in progress and future work

Work on the establishment of new chemical reference substances is being continued. There is still one substance remaining to support a monograph in volume 2 of the International Pharmacopoeia. To support the monographs in volume 3 there is today a need for 36 new reference substances. Sixteen of these are already under development at the Centre. Older batches also have to be replaced because the stocks are depleted. At present 4 substances have to be replaced during 1990-1991 but this figure may increase depending on the distribution. A great deal of the work load originates from the growing demand for regular reexamination of already existing reference substances. Some substances are very old and the extended total amount of reference substances results in still more work. The projected reference substances to be established by the Centre are listed in Appendix 5 to this report. The substances in progress are indicated with an asterisk.

During 1989 computerization of the activities concerning the work on reference substances has continued. The system consists of an IBM XT Personal Computer. Today information about bulk ordering, analytical schemes, dispensing worksheets and a plan for regular reexamination are available in the computer. Plans for computerized orders and an inventory of the stock of existing reference substances are in progress. Collaboration with other laboratories to decrease the workload on the Centre in Stockholm has continued.

Administrative and financial matters

The total cost for running the Centre in 1989 was estimated at 418.500 US\$. The income from sales of reference substances was about 42.300 US\$ and the contribution received from the WHO Headquarters was 21.000 US\$, which leaves a deficit of 355.200 US\$. The management board of the National Corporation of Swedish Pharmacies has agreed to support the continued operation of the Centre at an unchanged level, provided all possibilities to reduce the deficit would be investigated.

The fee is US\$ 40 per package and a freight and handling charge of US\$ 10 is added to each order.

In order to alleviate the financial deficit of the Centre, National Research Centres have been requested to offer analytical support and WHO Regional Offices have been approached for financial assistance.

Acknowledgements

The Centre wants to thank the laboratories that have contributed to the work during 1989, namely the United States Pharmacopoeial Convention Inc. in Rockville, USA and the European Pharmacopoeial Laboratory in Strasbourg, France. The Centre would also like to express its sincere gratitude to all pharmaceutical industries who have assisted the Centre by provision of candidate reference materials as well as by participation in the analytical testing. This year we particularly want to thank Astra Läkemedel in Södertälje, Sweden; Ciba-Geigy AG in Basel, Switzerland; Fermtech Prochim in Milano, Italy and Pfizer in Groton, Connecticut, USA.

APPENDIX 1

DISTRIBUTION OF CHEMICAL REFERENCE SUBSTANCES IN 1989

| | | | | | |
|--|----|-------|--|----|-------|
| Aceclidine salicylate | 1 | items | Diazepam | 14 | items |
| p-Acetamidobenzalazine | -- | " | Diazoxide | 1 | " |
| Acetazolamide | 2 | " | Dicloxacillin sodium | 64 | " |
| Allopurinol | 8 | " | Dicolinium iodide | 1 | " |
| 2-Amino-5-nitrothiazole | -- | " | Dicoumarol | 1 | " |
| 3-Aminopyrazole-4-carbox- amide hemisulfate | 1 | " | Diethylcarbamazine dihydrogen citrate | 9 | " |
| Amitriptyline hydrochloride | 5 | " | Digitoxin | 7 | " |
| Ampicillin | 4 | " | Digoxin | 18 | " |
| Ampicillin sodium | 13 | " | NN' -Di-(2,3-xylyl)anthra- nilamide | 1 | " |
| Ampicillin trihydrate | 59 | " | Emetine hydrochloride | 1 | " |
| Anhydrotetracycline hydro- chloride | 12 | " | 4-Epianhydrotetracycline hydrochloride | 9 | " |
| Atropine sulfate | 15 | " | 4-Epitetracycline ammonium salt | 8 | " |
| Azathioprine | 1 | " | Ergometrine hydrogen maleate | 18 | " |
| Bendazol hydrochloride | 1 | " | Ergotamine tartrate | 32 | " |
| Benzobarbital | 1 | " | Estradiol benzoate | 8 | " |
| Benzylamine sulfate | 2 | " | Estrone | 2 | " |
| Benzylpenicillin potassium | 8 | " | Etacrynic acid | -- | " |
| Benzylpenicillin sodium | 40 | " | Ethambutol hydrochloride | 8 | " |
| Bephenium hydroxynaphthoate | -- | " | Ethinylestradiol | 8 | " |
| Betamethasone | 8 | " | Ethisterone | 1 | " |
| Betanidine sulfate | 1 | " | Ethosuximide | 2 | " |
| Bupivacaine hydrochloride | 7 | " | Etocarlide | -- | " |
| Caffeine | 6 | " | Flucytosine | -- | " |
| Carbenicillin monosodium | 3 | " | Fluorouracil | 4 | " |
| Chloramphenicol | 18 | " | Fluphenazine decanoate dihydrochloride | 3 | " |
| Chloramphenicol palmitate | 4 | " | Fluphenazine enantate dihydrochloride | 2 | " |
| Chloramphenicol palmitate (Polymorph A) | 8 | " | Fluphenazine hydrochloride | 2 | " |
| 5-Chloro-2-methylamino- benzophenone | 2 | " | Folic acid | 21 | " |
| 2-(4-Chloro-3-sulfamoyl- benzoyl)benzoic acid | 2 | " | Furosemide | 16 | " |
| Chlorphenamine hydrogen maleate | 5 | " | Griseofulvin | 13 | " |
| Chlorpromazine hydro- chloride | 10 | " | Haloperidol | 7 | " |
| Chlortalidone | -- | " | Hydrochlorothiazide | 8 | " |
| Chlortetracycline hydrochloride | 7 | " | Hydrocortisone | 11 | " |
| Clomifene citrate | 2 | " | Hydrocortisone acetate | 11 | " |
| Clomifene citrate Z-isomer (Zuclomifene) | 1 | " | (-)-3-(4-Hydroxy-3-methoxy- phenyl)-2-methylalanine | 1 | " |
| Cloxacillin sodium | 19 | " | Ibuprofen | 9 | " |
| Cortisone acetate | 10 | " | Imipramine hydrochloride | 8 | " |
| Dapsone | 15 | " | Indometacin | 17 | " |
| Desoxycortone acetate | 9 | " | o-Iodohippuric acid | -- | " |
| Dexamethasone | 29 | " | Isoniazid | 6 | " |
| Dexamethasone acetate | 4 | " | Lanatoside C | -- | " |
| | | | Levodopa | 5 | " |

| | | | |
|---------------------------------------|---------|--|----------|
| Lidocaine | 9 items | Prednisone | 28 items |
| Lidocaine hydrochloride | 13 " | Prednisone acetate | 2 " |
| Mefenamic acid | 8 " | Procaine hydrochloride | 5 " |
| Metazide | 1 " | Procarbazine hydrochloride | -- " |
| Methaqualone | 4 " | Progesterone | 11 " |
| Methyldopa | 12 " | Propicillin potassium | 27 " |
| Methyltestosterone | 6 " | Propranolol hydrochloride | 6 " |
| Meticillin sodium | 7 " | Propylthiouracil | -- " |
| Metronidazole | 22 " | Pyridostigmine bromide | 3 " |
| Nafcillin sodium | 1 " | Reserpine | 4 " |
| Neostigmine metilsulfate | 3 " | Riboflavin | 12 " |
| Nicotinamide | 8 " | Sodium cromoglicate | -- " |
| Nicotinic acid | 4 " | Sulfamethoxazole | 22 " |
| Niridazole | -- " | Sulfamethoxypyridazine | 4 " |
| Niridazole-chlorethyl- carboxamide | -- " | Sulfanilamide | 5 " |
| Norethisterone | -- " | Testosterone propionate | 1 " |
| Norethisterone acetate | -- " | Tetracycline hydrochloride | 27 " |
| Ouabain | -- " | Thioacetazone | 10 " |
| Oxacillin sodium | 43 " | 4,4'-Thiodianiline | -- " |
| Papaverine hydrochloride | 5 " | Tolbutamide | 2 " |
| Phenethicillin potassium | 1 " | Tolnaftate | -- " |
| Phenoxymethylpenicillin | 6 " | Trimethadione | -- " |
| Phenoxymethylpenicillin calcium | 1 " | Trimethoprim | 23 " |
| Phenoxymethylpencillin potassium | 25 " | Trimethylguanidine sulfate | 1 " |
| Phenytoin | 12 " | Tubocurarine chloride | 2 " |
| Prednisolone | 35 " | Vitamin A acetate (solution) (à 25000 IU) (Retinol) | 23 " |
| Prednisolone acetate | 8 " | Warfarin | 5 " |

Melting Point Reference Substances 46 x 13 substances.

APPENDIX 2

INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES ESTABLISHED IN 1989

| Reference Substance | Control Number | Analytical Report | Remarks |
|---|----------------|---------------------------------|-----------------------|
| Ampicillin sodium | 388002 | WHO/PHARM/89.544 Appendix 6 | Replaces No 274002 |
| Dexamethasone | 388008 | WHO/PHARM/89.544 Appendix 7 | Replaces No 279008 |
| Dexamethasone acetate | 288009 | WHO/PHARM/89.544 Appendix 8 | Replaces No 168009 |
| 4-Epianhydrotetracycline hydrochloride | 288097 | WHO/PHARM/89.544 Appendix 9 | Replaces No 180097 |
| Folic acid | 388019 | WHO/PHARM/89.544 Appendix 10 | Replaces No 277019 |
| Sodium cromoglicate | 188140 | WHO/PHARM/89.544 Appendix 11 | |

APPENDIX 3

LIST OF AVAILABLE INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES

1990

General information

International Chemical Reference Substances are established upon the advice of the WHO Expert Committee on Specifications for Pharmaceutical Preparations. They are supplied primarily for use in physical and chemical tests and assays described in the specifications for quality control of drugs published in *The International Pharmacopoeia* or proposed in draft monographs.

International Chemical Reference Substances may also be used in tests and assays not described in *The International Pharmacopoeia*. However, the responsibility for assessing the suitability of the substances then rests with the user or with the pharmacopoeia commission or other authority that has prescribed these substances to be used.

Directions for use and analytical data as required for the use intended in the relevant specifications of *The International Pharmacopoeia* are given in the certificates enclosed with the substances when distributed. More detailed analytical reports on the substances may be obtained on request from the WHO Collaborating Centre for Chemical Reference Substances.

It is generally recommended that the substances should be stored protected from light and moisture and preferably at a temperature of about +5 °C. When special storage conditions are required, this is stated on the label or in the accompanying leaflet.

The stability of the International Chemical Reference Substances kept at the Collaborating Centre is monitored by regular reexamination and deteriorated materials are replaced by new batches when necessary. Lists giving control numbers for the current batches are issued in the annual reports from the Centre and may be obtained on request.

Ordering Information

Orders for the International Chemical Reference Substances should be sent to:

WHO Collaborating Centre for Chemical Reference Substances
APOTEKSBOLAGET AB
Centrallaboratoriet
S-105 14 STOCKHOLM
SWEDEN

(Telex: 115 53 APOBOL S)
(Telefax: + 46 8 740 60 40)

The International Chemical Reference Substances are only supplied in standard packages as indicated in the following list.

| <u>Reference substance</u> | <u>Package size</u> | <u>Control Number</u> |
|---|---------------------|-----------------------|
| Aceclidine salicylate | 100 mg | 172048 |
| p-Acetamidobenzalazine | 100 mg | 171042 |
| Acetazolamide | 100 mg | 186128 |
| Allopurinol | 100 mg | 287049 |
| 2-Amino-5-nitrothiazole | 25 mg | 186131 |
| 3-Aminopyrazole-4-carboxamide hemisulfate | 100 mg | 172050 |
| Amitriptyline hydrochloride | 100 mg | 181101 |
| Ampicillin | 200 mg | 274001 |
| Ampicillin sodium | 200 mg | 388002 |
| Ampicillin trihydrate | 200 mg | 274003 |
| Anhydrotetracycline hydrochloride | 25 mg | 180096 |
| Atropine sulfate | 100 mg | 183111 |
| Azathioprine | 100 mg | 172060 |
| Bendazol hydrochloride | 100 mg | 173066 |
| Benzobarbital | 100 mg | 172051 |
| Benzylamine sulfate | 100 mg | 172052 |
| Benzylpenicillin potassium | 200 mg | 180099 |
| Benzylpenicillin sodium | 200 mg | 280047 |
| Bephenium hydroxynaphthoate | 100 mg | 183112 |
| Betamethasone | 100 mg | 183113 |
| Betanidine sulfate | 100 mg | 172053 |
| Bupivacaine hydrochloride | 100 mg | 289054 |
| Caffeine | 100 mg | 181102 |
| Carbamazepine | 100 mg | 189143 |
| Carbenicillin monosodium | 200 mg | 383043 |
| Chloramphenicol | 200 mg | 486004 |
| Chloramphenicol palmitate | 1 g | 286072 |
| Chloramphenicol palmitate (Polymorph A) | 200 mg | 175073 |
| 5-Chloro-2-methylaminobenzophenone | 100 mg | 172061 |
| 2-(4-Chloro-3-sulfamoylbenzoyl)benzoic acid | 50 mg | 181106 |
| Chlorphenamine hydrogen maleate | 100 mg | 182109 |
| Chlorpromazine hydrochloride | 100 mg | 178080 |
| Chlortalidone | 100 mg | 183114 |
| Chlortetracycline hydrochloride | 200 mg | 187138 |
| Clomifene citrate | 100 mg | 187136 |
| Clomifene citrate Z-isomer (Zuclomifene) | 50 mg | 187137 |
| Cloxacillin sodium | 200 mg | 274005 |
| Cortisone acetate | 100 mg | 167006 |
| Dapsone | 100 mg | 183115 |
| Desoxycortone acetate | 100 mg | 167007 |
| Dexamethasone | 100 mg | 388008 |
| Dexamethasone acetate | 100 mg | 288009 |
| Diazepam | 100 mg | 172062 |
| Diazoxide | 100 mg | 181103 |
| Dicloxacillin sodium | 200 mg | 174071 |
| Dicolinium iodide | 100 mg | 172055 |
| Dicoumarol | 100 mg | 178077 |
| Diethylcarbamazine dihydrogen citrate | 100 mg | 181100 |
| Digitoxin | 100 mg | 277010 |

| | <u>Package size</u> | <u>Control Number</u> |
|--|-------------------------|---------------------------|
| Digoxin | 100 mg | 587011 |
| NN'-Di-(2,3-xylyl)anthranilamide | 50 mg | 173067 |
| Emetine hydrochloride | 100 mg | 187134 |
| 4-Epianhydrotetracycline hydrochloride | 25 mg | 288097 |
| 4-Epitetracycline ammonium salt | 25 mg | 180098 |
| Ergometrine hydrogen maleate | 50 mg | 277012 |
| Ergotamine tartrate | 50 mg | 385013 |
| Estradiol benzoate | 100 mg | 167014 |
| Estrone | 100 mg | 279015 |
| Etacrynic acid | 100 mg | 281056 |
| Ethambutol hydrochloride | 100 mg | 179081 |
| Ethinylestradiol | 100 mg | 167016 |
| Ethisterone | 100 mg | 167017 |
| Ethosuximide | 100 mg | 179088 |
| Etocarlide | 100 mg | 172057 |
| Flucytosine | 100 mg | 184121 |
| Fluorouracil | 100 mg | 184122 |
| Fluphenazine decanoate dihydrochloride | 100 mg | 182107 |
| Fluphenazine enantate dihydrochloride | 100 mg | 182108 |
| Fluphenazine hydrochloride | 100 mg | 176076 |
| Folic acid | 100 mg | 388019 |
| Furosemide | 100 mg | 171044 |
| Griseofulvin | 200 mg | 280040 |
| Haloperidol | 100 mg | 172063 |
| Hydrochlorothiazide | 100 mg | 179087 |
| Hydrocortisone | 100 mg | 283020 |
| Hydrocortisone acetate | 100 mg | 280021 |
| (-)-3-(4-Hydroxy-3-methoxyphenyl)- 2-methylalanine | 25 mg | 179085 |
| Ibuprofen | 100 mg | 183117 |
| Imipramine hydrochloride | 100 mg | 172064 |
| Indometacin | 100 mg | 178078 |
| o-Iodohippuric acid | 100 mg | 171045 |
| Isoniazid | 100 mg | 185124 |
| Lanatoside C | 100 mg | 281022 |
| Levodopa | 100 mg | 172065 |
| Levothyroxine sodium (Thyroxine sodium) | 100 mg | 189144 |
| Lidocaine | 100 mg | 181104 |
| Lidocaine hydrochloride | 100 mg | 181105 |
| Mefenamic acid | 100 mg | 173068 |
| Melting Point Reference Substances (set of 13 substances with melting tempera- tures ranging from +69 °C to +263 °C) | 13 x 4 g | |
| Metazide | 100 mg | 172058 |
| Methaqualone | 100 mg | 173069 |
| Methyldopa | 100 mg | 179084 |
| Methyltestosterone | 100 mg | 167023 |
| Meticillin sodium | 200 mg | 274024 |
| Metronidazole | 100 mg | 183118 |
| Nafcillin sodium | 200 mg | 272025 |

| | <u>Package size</u> | <u>Control Number</u> |
|--|-------------------------|---------------------------|
| Neostigmine metilsulfate | 100 mg | 187135 |
| Nicotinamide | 100 mg | 179090 |
| Nicotinic acid | 100 mg | 179091 |
| Niridazole | 200 mg | 186129 |
| Niridazole-chlorethylcarboxamide | 25 mg | 186130 |
| Norethisterone | 100 mg | 186132 |
| Norethisterone acetate | 100 mg | 185123 |
| Ouabain | 100 mg | 283026 |
| Oxacillin sodium | 200 mg | 382027 |
| Oxytetracycline dihydrate | 200 mg | 189142 |
| Oxytetracycline hydrochloride | 200 mg | 189141 |
| Papaverine hydrochloride | 100 mg | 185127 |
| Phenethicillin potassium | 200 mg | 167028 |
| Phenoxymethylpenicillin | 200 mg | 179082 |
| Phenoxymethylpenicillin calcium | 200 mg | 179083 |
| Phenoxymethylpenicillin potassium | 200 mg | 176075 |
| Phenytoin | 100 mg | 179089 |
| Prednisolone | 100 mg | 389029 |
| Prednisolone acetate | 100 mg | 289030 |
| Prednisone | 100 mg | 167031 |
| Prednisone acetate | 100 mg | 169032 |
| Procaine hydrochloride | 100 mg | 183119 |
| Procarbazine hydrochloride | 100 mg | 184120 |
| Progesterone | 100 mg | 167033 |
| Propicillin potassium | 200 mg | 274034 |
| Propranolol hydrochloride | 100 mg | 187139 |
| Propylthiouracil | 100 mg | 185126 |
| Pyridostigmine bromide | 100 mg | 182110 |
| Reserpine | 100 mg | 186133 |
| Riboflavin | 250 mg | 382035 |
| Sodium cromoglicate | 100 mg | 188140 |
| Sulfamethoxazole | 100 mg | 179092 |
| Sulfamethoxypyridazine | 100 mg | 178079 |
| Sulfanilamide | 100 mg | 179094 |
| Testosterone propionate | 100 mg | 167036 |
| Tetracycline hydrochloride | 200 mg | 180095 |
| Thioacetazone | 100 mg | 171046 |
| 4,4'-Thiodianiline | 50 mg | 183116 |
| Tolbutamide | 100 mg | 179086 |
| Tolnaftate | 100 mg | 176074 |
| Trimethadione | 200 mg | 185125 |
| Trimethoprim | 100 mg | 179093 |
| Trimethylguanidine sulfate | 100 mg | 172059 |
| Tubocurarine chloride | 100 mg | 170037 |
| Vitamin A acetate (solution) (Retinol) | 5 caps. (*) | 686038 |
| <u>Warfarin</u> | 100 mg | 168041 |

(*) About 9 mg in 250 mg oil per capsule

APPENDIX 4

STABILITY TESTING

The storage stability of the International Chemical Reference Substances is monitored by regular reexamination of the substances held in stock at the Centre. The results obtained for the substances reexamined in 1989 are summarized below. For comparison results obtained at earlier occasions are included in the summaries. The substances have been stored at +5 °C. The following abbreviations are used in the tables:

| | |
|------|--|
| DSC | Differential Scanning Calorimetry |
| DTA | Differential Thermal Analysis |
| HPLC | High Performance Liquid Chromatography |
| IR | Infrared Spectrophotometry |
| KF | Karl Fischer titration |
| LOD | Loss on drying |
| TLC | Thin-layer Chromatography |
| PSA | Phase Solubility Analysis |
| TGA | Thermogravimetric analysis |

The estimates of total solid impurities by HPLC and by TLC are expressed as area per cent (area %), if not otherwise stated, by DSC and by DTA as mole per cent (mole %), and by PSA as weight per cent (w/w %). LOD and TGA (loss in weight) are expressed as weight per cent (w/w %). Assay values are calculated with reference to the dried or the anhydrous substance.

More details about the analytical methods used can be obtained from the Centre.

Bupivacaine hydrochloride, Control No 172054

Initial analytical report: WHO/PHARM/72.471, Appendix 16

| Examination year: | 1972 | 1977 | 1979 | 1981 | 1989 |
|----------------------------------|--------------|--------------|--------------|--------------|---------------------|
| IR | conforms | - | - | - | conforms |
| TLC, % | no sec spots | no sec spots | no sec spots | no sec spots | no sec spots < 0.25 |
| HPLC, % | - | - | - | - | no impurities < 0.1 |
| TGA, % | - | - | - | - | 5.17 |
| LOD, % | 5.1 | 4.7 | 5.1 | - | 5.2 |
| Assay, % (potentiometric) | 100.5 | - | 100.6 | - | 99.5 |
| UV, 263 nm | 0.55 | 0.54 | 0.54 | - | 0.57 |
| 271 nm | 0.45 | 0.44 | 0.45 | - | 0.46 |
| Assay, % (spectrophotometric) | | | | | 99.8 % |

NN'-Di-(2,3-xylyl)anthranilamide. Control No. 173067

Initial analytical report: WHO/PHARM/74.478, Appendix 6

| Examination year: | 1973 | 1976 | 1982 | 1989 |
|-------------------|--------------|----------|------|--------------|
| IR | conforms | conforms | - | - |
| TLC, % | no sec spots | - | - | no sec spots |
| TGA, % | - | - | - | < 0.1 |
| LOD, % | 0.2 | - | - | - |
| DSC, % | 1.4 | - | - | 0.7 |
| UV, 281 nm | 0.68 | 0.68 | - | - |
| 349 nm | 0.41 | 0.41 | - | - |

Ergometrine hydrogen maleate. Control No 277012

Initial analytical report: WHO/PHARM/78.494, Appendix 5

| Examination year: | 1977 | 1982 | 1989 |
|------------------------------|--------------------|-----------------|----------------------|
| IR | conforms | - | - |
| TLC, % | 1 (3 sec spots) | (2-3 sec spots) | 0.8 (4 sec spots) |
| HPLC, % | 0.8 | 0.9 | - |
| TGA, % | - | - | 0.3 |
| LOD, % | 0.8 | - | 0.3 |
| Assay, % (potentiometric) | 100.0 | 99.9 | - |

Hydrocortisone, Control No 283020

Initial analytical report: WHO/PHARM/84.513, Appendix 11

| Examination year: | 1983 | 1989 |
|----------------------------------|----------------------|----------------|
| TLC, % | 0.3 (4 sec spots) | - |
| HPLC, % | 0.3 4 peaks | 0.6 6 peaks |
| TGA, % | - | <0.1 |
| LOD, % | 0.0 | - |
| Assay, % (spectrophotometric) | 99.9 | 99.7 |
| PSA, % | 0.5 | - |
| UV, 242 nm | 0.45 | 0.45 |

Hydrocortisone acetate, Control No 280021

Initial analytical report: WHO/PHARM/81.508, Appendix 11

| Examination year: | 1980 | 1989 |
|----------------------------------|----------------------|----------------------|
| TLC, % | 0.4 (4 sec spots) | 0.4 (3 sec spots) |
| TGA, % | - | 0.1 |
| LOD, % | - | 0.1 |
| Assay, % (spectrophotometric) | 99.6 | 99.9 |
| PSA, % | <0.5 | - |
| UV, 242 nm | | |
| E (1%, 1 cm) | 401 | 402 |

o-Iodohippuric acid. Control No 171045

Initial analytical report: WHO/PHARM/71.464, Appendix 6

| Examination year: | 1971 | 1989 |
|-------------------|----------|------------------------|
| IR | conforms | conforms |
| TLC, % | <0.5 | no impurities observed |
| TGA, % | - | 0.2 |
| LOD, % | 0.5 | - |
| DSC, % | 0.5 | 0.4 |

Levodopa. Control No 172065

Initial analytical report: WHO/PHARM/73.475, Appendix 8

| Examination year: | 1972 | 1976 | 1979 | 1989 |
|----------------------|------------------------|------------------------|------------------------|------------------------|
| IR | conforms | conforms | - | conforms |
| TLC, % | no impurities observed | no impurities observed | no impurities observed | no impurities observed |
| TGA, % | - | - | - | <0.1 |
| LOD, % | 0 | 0 | 0 | - |
| Assay, % (titration) | 100.1 | 99.7 | 99.9 | - |
| UV, 280 nm | 0.70 | 0.72 | 0.70 | - |

Mefenamic acid, Control No 173068

Initial analytical report:WHO/PHARM/74.478, Appendix 7

| Examination year: | 1973 | 1979 | 1989 |
|----------------------|--------------|------------|-------------------|
| IR | conforms | - | - |
| TLC, % | no sec spots | 1 sec spot | 0.5 (2 sec spots) |
| TGA, % | - | - | 0.16 |
| LOD, % | 0.2 | 0.14 | - |
| DSC, % | 0.4 | - | - |
| Assay, % (titration) | 100.0 | 100.1 | - |

Prednisolone, Control No 283029

Initial analytical report:WHO/PHARM/84.513, Appendix 15

| Examination year: | 1983 | 1987 | 1988-89 |
|----------------------------------|--------------------|--------------------------|--------------------|
| Light absorption, 263 nm | 0.417 | 0.416 | - |
| TLC, % | 1.6 2 sec spots | about 1.5 2 sec spots | 1.5 2 sec spots |
| LOD, % | 0.08 | - | - |
| KF (water), % | - | 0.2 | - |
| IR | conforms | conforms | |
| HPLC, % | 1.4 | 2.1 | 1.3 |
| Assay, % (spectrophotometric) | 100.0 | 99.9 | - |

Prednisolone Acetate, Control No 167030

Initial analytical report: WHO/PHARM/66.431, Appendix 7

| Examination year: | 1966 | 1975 | 1984 | 1987 | 1988-89 |
|---------------------------------------|-------------|------------|-------------|-------------|----------------------|
| UV-absorption 242 nm, E (1%, 1 cm) | 382 | 377 | 377 | 386 | - |
| Loss on drying, % | 0.0 | 0.2 | - | 0.0 | - |
| TLC, % | 2 sec spots | 1 sec spot | 3 sec spots | 3 sec spots | 2.4 (3 sec spots) |
| IR | conforms | | | conforms | |
| HPLC, % | - | - | 1.8 | 2.2 | 1.2 |
| PSA, % | 0.5 | - | - | - | - |

Tetracycline hydrochloride, Control No 180095

Initial analytical report: WNO/PHARM/81.508, Appendix 12

| Examination year: | 1980 | 1985 | 1989 |
|--|--------------|------|-------|
| HPLC, weight % of 4-epiantetracycline | 1.3 | 0.7 | 0.7 |
| anhydrotetracycline | 0.1 | 0.1 | <0.05 |
| 4-epianhydrotetracycline | <0.05 | 0.05 | <0.05 |
| unidentified, area % | ~0.05 | 0.1 | - |
| Loss on drying, % | 0.25 | - | - |
| Water (KF), % | 0.4 | 0.3 | - |
| TLC | one sec spot | - | - |
| Assay (potentiometric) | 99.6 | - | - |
| TGA, % | - | - | 0.3 |

Tubocurarine chloride, Control No 170037

Initial analytical report: WHO/PHARM/72.471, Appendix 3

| Examination year: | 1971 | 1977 | 1989 |
|----------------------|------|-------------|-------------|
| IR | - | conforms | conforms |
| TLC | - | 3 sec spots | 4 sec spots |
| HPLC, % | - | - | 0.9 |
| KF | - | 11.5 | 11.6 |
| LOD | - | 11.4 | - |
| Assay, % (titration) | 99.2 | 100.2 | 99.1 |

Warfarin, Control No 168041

Initial analytical report: WHO/PHARM/69.452, Appendix 3

| Examination year: | 1967 | 1980 | 1984 | 1989 |
|----------------------------------|--------------|--------------|--------------|----------------------|
| IR | conforms | - | - | conforms |
| TLC | no sec spots | no sec spots | no sec spots | <0.5 no sec spots |
| HPLC, % | - | - | <0.1 | <0.1 |
| TGA, % | - | - | - | <0.1 |
| LOD, % | <0.1 | <0.1 | - | - |
| Assay, % (titration) | 100.0 | 99.4 | - | - |
| Assay, % (spectrophotometric) | - | - | - | 99.6 |

INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES - PROJECT LIST 1990

The following additional International Chemical Reference Substances are required to support specifications in the third edition of the International Pharmacopoeia:

Volume 2

Colecalciferol (*)

Volume 3

Amodiaquine hydrochloride (*)

Amphotericin B (*)

Bacitracin zinc (*)

Beclomethasone dipropionate

Betamethasone valerate (*)

Calcium folinate

Cimetidine (*)

Dexamethasone sodium phosphate (*)

Dopamine hydrochloride

Doxorubicin hydrochloride

Ergocalciferol (*)

Fludrocortisone acetate

3- Formylrifamycin SV (*)

(impurity in Rifampicin)

Gentamicin sulfate

Hydrocortisone sodium succinate

(-)-3-(4-Hydroxy-3-methoxyphenyl)-2-hydrazino-2-methylalanine (impurity in Carbidopa)

Levonorgestrel

Liothyronine

(impurity in Levothyroxine sodium)

Loperamide hydrochloride

Methotrexate

Neamine (*)

(impurity in Neomycin sulfate)

Neomycin B sulfate

(impurity in Neomycin sulfate)

Nifurtimox

Noroxymorphone hydrochloride

(impurity in Naloxone hydrochloride)

Nystatin (*)

Paromomycin sulfate

Praziquantel

Prednisolone sodium phosphate

Probenecid (*)

Pyrantel embonate (*)

Rifampicin quinone (*)

(impurity in Rifampicin)

Spectinomycin hydrochloride

Sulfacetamide

Sulfasalazine (*)

Testosterone enantate

Vincristine sulfate (*)

Replacements

The following existing International Chemical Reference Substances should be replaced by new batches in 1990-1991.

p-Acetamidobenzalazine (*)

Ampicillin (anhydrous) (*)

Anhydrotetracycline hydrochloride (*)

Ethinylestradiol (*)

(*) Denotes that work on the substance is in progress at the Centre.

APPENDIX 6

BUPIVACAINE HYDROCHLORIDE

Control No 289054

Analytical Report

INTENDED USE

The stock of the current batch of the International Chemical Reference Substance for bupivacaine hydrochloride, Control No 172054, is depleted and has to be replaced.

The monograph for bupivacaine hydrochloride in the International Pharmacopeia 3rd Ed. Vol 2 requires a reference substance to be used in the infrared spectrophotometric test for identity.

MATERIAL

About 100 g of the sample (manufacturers batch No 48598-01) were received at the WHO Centre in October 1987. The material is being stored protected from light in tightly closed containers at +5 °C.

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTUREInfrared spectrum

An infrared spectrum is given in Figure 1 (Control No 289054). The spectrum is concordant with the spectrum obtained from ICRS Control No 172054. No difference was observed between the spectra run in KBr and KCl respectively.

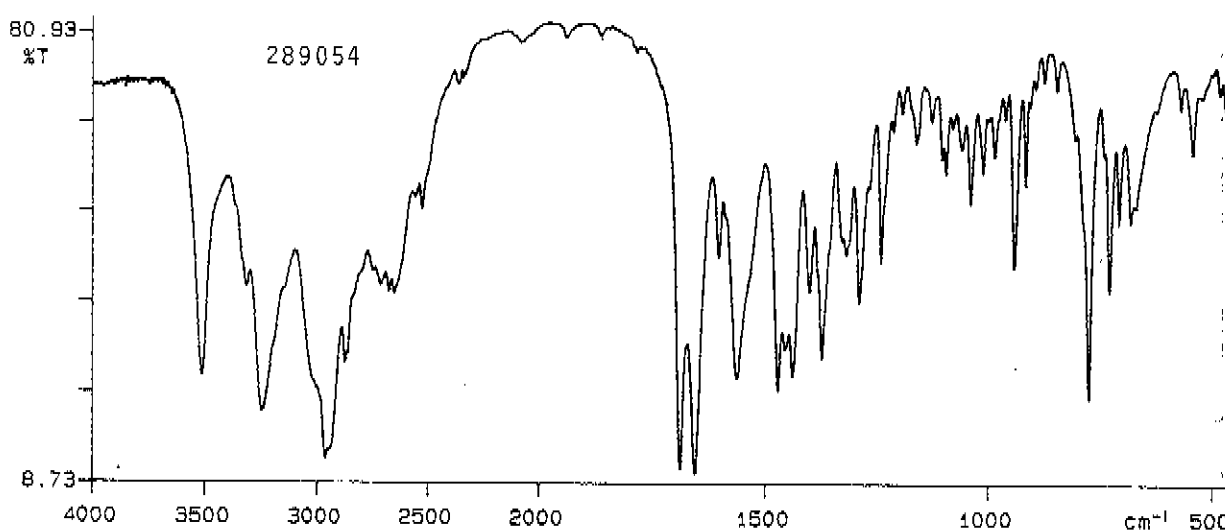


Figure 1. IR-spectrum of 1.5 mg of bupivacaine hydrochloride Control No 289054 in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 1600 FTIR.

UV-spectrum

A UV-spectrum in hydrochloric acid (0.01 M) is given in Figure 2.

λ max in hydrochloric acid (0.01 M) = 262 and 271 nm.

E (1%, 1 cm) = 14 (262 nm), E (1%, 1 cm) = 11 (271 nm) (n = 4).

The result is calculated with reference to the dried substance.

The absorbance of a 0.40 mg/ml solution was 0.56 (262 nm) and 0.45 (271 nm).

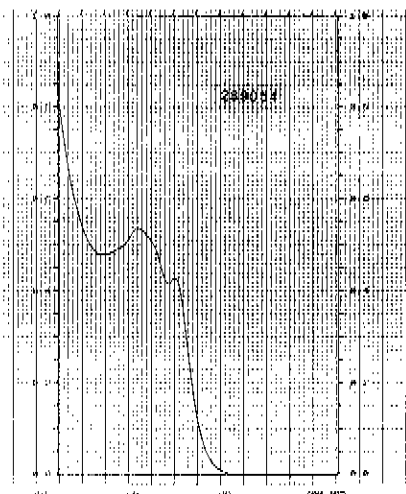


Figure 2. UV-spectrum of bupivacaine hydrochloride, Control No 289054, 0.40 mg/ml in hydrochloric acid (0.01 M).

ASSAY

Spectrophotometric assay: 99.8% (n = 2), ICRS Control No 172054 was used as reference and regarded as 100%. The result is calculated with reference to the dried substance.

Titrimetric assay: 99.8% (n = 3), s rel % = 0.20.

Determined by potentiometric titration with 0.1 M perchloric acid according to Ph Int 3rd Ed, Vol 2. The result is calculated with reference to the dried substance.

Thermogravimetric analysis: When the substance was heated to 150 °C a loss of weight of 5.26% was observed.

Instrument: Perkin-Elmer TGA 7 Thermogravimetric analyzer.

Sample weight: About 5 mg.

Heating rate: 10 °C/minute

Water: 5.24%, determined by Karl Fischer titration.

PURITY

Thin-layer chromatography

No impurities were detected.

The following thin-layer chromatographic system was used:

According to the International Pharmacopoeia 3rd Ed, Vol 2. but examined in UV (254 nm) before spraying.

Thin-layer: Silica gel 60, F-254 (Merck) and Silica gel 60 (Merck).

Eluent: Ethanol (95%)

Sample: 100 - 200 µg of bupivacaine hydrochloride were applied.

Visualization: UV-light of 254 nm, evaluation by densitometry at 254 nm with a Desaga Densitometer CD 60, followed by spraying with potassium iodobismuthate TS and examination in day-light and also evaluation by densitometry at 480 nm with a Desaga Densitometer CD 60.

Result: No extra spots were detected by visual inspection. When evaluating by densitometry at 254 nm as well as at 480 nm no impurities were observed. The detection limit of this system was about 0.5 µg (0.25%) when scanned at 254 nm. R_f (bupivacaine hydrochloride) = about 0.5.

A comparison was made with ICRS Control No 172054 in which no impurities were detected.

High performance liquid chromatography

No impurities were detected. A chromatogram is shown in Figure 3. The peak eluting after 6.3 minutes corresponds to bupivacaine hydrochloride.

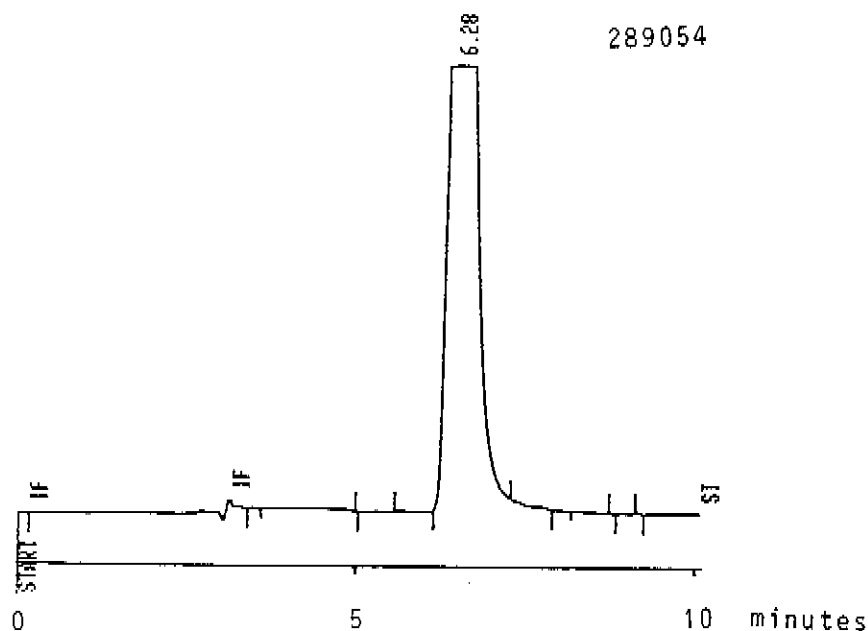


Figure 3. Chromatogram of bupivacaine hydrochloride, Control No 289054.

The following conditions were used.

Eluent: Methanol/Phosphate buffer (0.05 M) mixed with pentanesulfonic acid (0.5 mmol) at pH = 3.3 (50:50)

Column: Spherisorb S5 CN
Detector: Shimadzu SPD-2A operated at 263 nm.
Pump: Waters 600 Multisolvent Delivery System operated at a flow rate of 1 ml/min.
Integrator: Hewlett Packard 3390 A. Attenuation: 4.
Sample: 0.5 mg/ml dissolved in the eluent. 20 µl corresponding to 10 µg were injected.

A comparison was made with ICRS Control No 172054 in which no impurities were detected.

Diode-array detection

The chromatogram was also evaluated with a LKB 2140 Rapid Diode Array Detector. The same chromatographic system as described above was used. An isogram is given in Figure 4.

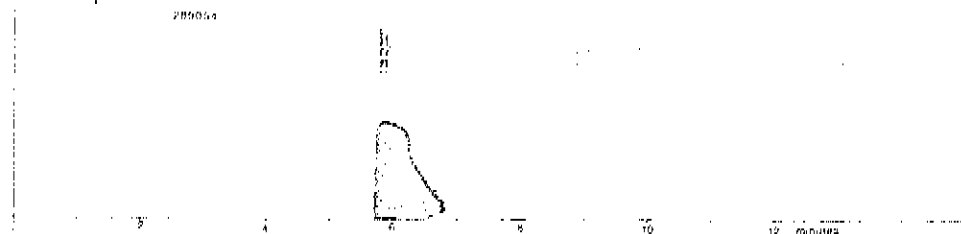


Figure 4. *Isogram of bupivacaine hydrochloride, Control No 289054. Sensitivity: 0.002.*

As seen from the figure no impurities are observed at any wavelength. Bupivacaine hydrochloride eluting after about 5.5 minutes has its absorbance maxima at 210 nm and 264 nm.

DATA GIVEN BY THE MANUFACTURER

IR: Conforms with reference spectra.
TLC: No single impurity exceeds 1%.
HPLC: 0.1% impurities by peak area measurements.
LOD: 5.2%
Assay: 99.8% calculated as Bupivacaine hydrochloride monohydrate.

STABILITY

No special stability studies were performed as we have good experience of the stability of this substance from earlier batches. Bupivacaine hydrochloride Control No 172054 showed no tendency of degradation when stored for 17 years at + 5 °C at our Centre.

CONCLUSION

Bupivacaine hydrochloride, Control No 289054, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 7

CARBAMAZEPINE

Control No 189143

Analytical Report

INTENDED USE

The monograph for carbamazepine in the International Pharmacopoeia 3rd Ed. Vol 3 requires a reference substance to be used in the infrared spectrophotometric test for identity and thin-layer chromatographic test for related substances and in the spectrophotometric assay.

MATERIAL

About 200 g of the sample (manufacturers batch no 002 183.4, Prod. stand. 83) were received at the WHO Centre in April 1985. The material is being stored protected from light in tightly closed containers at + 5 °C.

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (Control No 189143). The spectrum is concordant with the spectrum obtained from the USP reference substance Lot G-1.

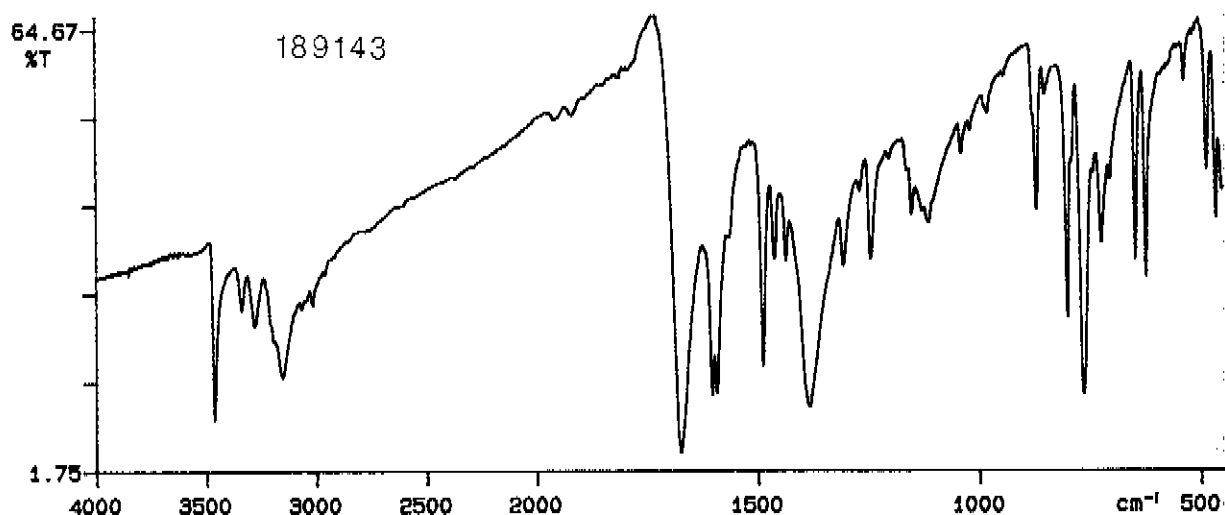


Figure 1. *IR-spectrum of 1.5 mg of carbamazepine Control No 189143 in 300 mg KBr recorded against a KBr disc.*

Instrument: Perkin-Elmer 1600 FTIR.

UV-spectrum

A UV-spectrum in ethanol (750 g/l) is given in Figure 2.

λ max in ethanol = 237 and 285 nm

E (1%, 1 cm) = 491 (n = 5) at 285 nm

The result is calculated with reference to the dried substance.

The absorbance of a 13.6 μ g/ml solution was 0.67.

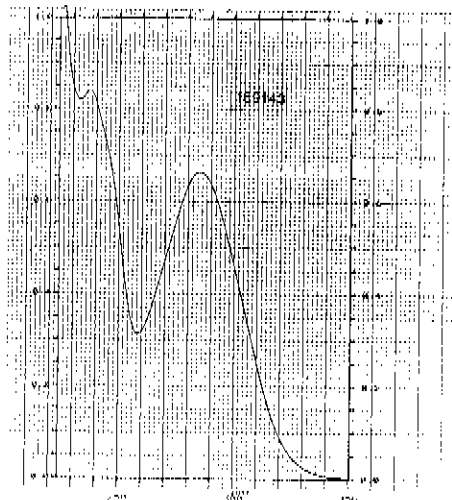


Figure 2. UV-spectrum of carbamazepine
Control No 189143 13.6 μ g/ml in ethanol.

ASSAY

Spectrophotometric assay: 100.2% (n=5), s rel % = 0.4% determined according to Ph. Int. 3rd Ed. Vol 3. USP reference substance Lot G-1 was used as reference and regarded as 100%. The result is calculated with reference to the dried substance. The substance seems difficult to dissolve in ethanol. It is important to use an ultrasonic bath and to check that the sample is totally dissolved.

Thermogravimetric analysis: When the substance was heated to 150 °C no loss of weight was observed. A loss of 0.2% occurred on further heating to 200 °C.

Instrument: Perkin-Elmer TGA 7 Thermogravimetric analyzer.

Sample weight: 7 mg

Heating rate: 10 °C/minute

Decomposition temperature: 189 - 193 °C

PURITY

Total solid impurities

1) Differential Scanning Calorimetry (DSC): About 0.1 mol % (n=6). The determination was carried out on 2.3 mg using a heating rate of 5.5 °C per minute.

Melting temperature: 191.5 °C (onset)

Instrument: Perkin-Elmer DSC 7 Differential Scanning Calorimeter.

Polymorphism

Seven polymorphs of carbamazepine have been identified according to the manufacturer. This reference substance consists mainly of the β -form (recrystallised from methanol) which is the anhydrous form.

The thermograms did show a small exotherm at about 180 °C which indicates the presence of another polymorphic form. However the reference substance mainly consists of the β -form. The melting point (onset) is 191.5 °C and the peak maxima is about 193 °C. See figure 3.

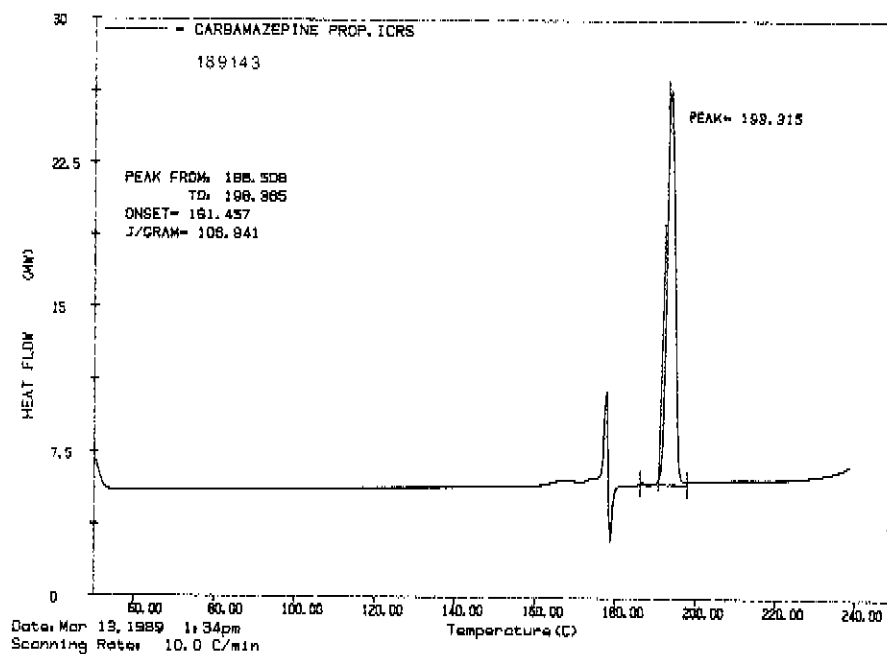


Figure 3. Thermogram of carbamazepine Control No 189143.

Thin layer chromatography

The total amount of impurities was estimated to less than 0.01%. No extra spots were detected.

The following thin-layer chromatographic system was used:

System: According to the International Pharmacopeia 3rd Ed, Vol 3.

Thin-layer: Silica gel 60, F-254 (Merck)

Eluent: Toluene: methanol (86:14)

Sample: 100 μ g of carbamazepine were applied.

Visualization: UV-light of 254 nm. Evaluation by densitometry at 254 nm with a Desaga Densitometer CD 60 and spraying with potassium dichromate and examined in daylight followed by heating to 140 °C for 15 minutes and examination in ultraviolet light (254 nm).

Result: No extra spots were detected either by visual inspection or when evaluating by densitometry at 254 nm. The detection limit of this system was about 0.01 µg (0.01%) when scanned at 254 nm.
Rf (carbamazepine) = 0.3. The same Rf was observed for the ICRS and the USP Reference Standard Lot G - 1.

Iminodibenzyl was also applied as being a potential impurity.
Rf (iminodibenzyl) = 0.6

A comparison was made with USP reference substance Lot G-1 which did not show any extra spots.

High performance liquid chromatography

No impurities were detected, which means less than 0.005%. A chromatogram is shown in Figure 4. Two possible impurities iminodibenzyl and iminostilben were also injected but not observed in the sample. If present iminostilben elutes at about 18 minutes and iminodibenzyl at about 31 minutes.

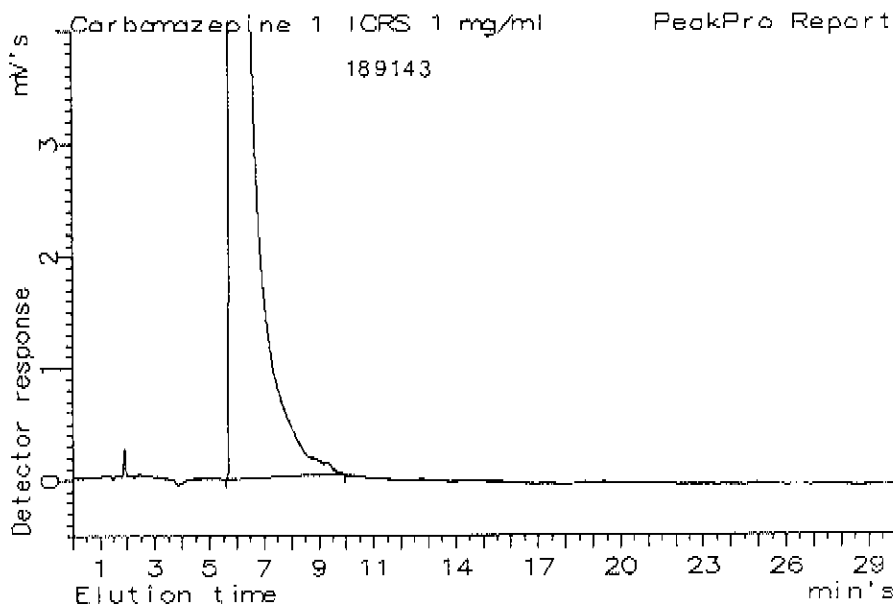


Figure 4. *Chromatogram of carbamazepine Control No 189143.*

The following conditions were used: (Modified from USP XXI)

- Eluent: Methanol/Water/Acetonitrile (40:40:20)
- Column: RP-18, Spheri-5 (Brownlee)
- Detector: Varian UV 200 operated at 230 nm
- Pump: Varian 5560 operated at a flow rate of 1.3 ml/min

Integrator: Varian 4270. Attenuation: 1

Sample: 1 mg/ml dissolved in the eluent.
10 μ l corresponding to 10 μ g were injected.

A comparison was made with USP reference substance Lot G-1 which contained about 0.01% impurities.

Diode-array detection

The chromatogram was also evaluated with a LKB 2140 Rapid Diode Array Detector. The same chromatographic system as described above was used, except for the injection volume that was increased to 100 μ l. An isogram is given in Figure 5.

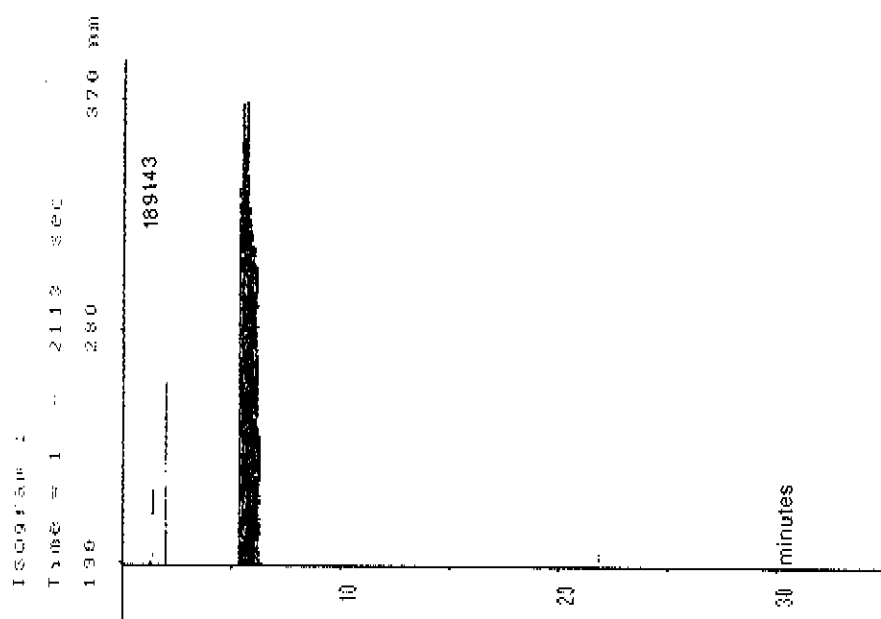


Figure 5. *Isogram of carbamazepine Control No 189143.*
Sensitivity: 0.001

As seen from the figure no impurities are observed at any wavelength. Carbamazepine has two absorbance maxima at 211 and 285 nm.

The two potential impurities were also studied with the Diode Array Detector. In figure 6 UV- spectra for carbamazepine, iminostilben and iminodibenzyl in the eluent used are given. All substances have maxima between 202 - 210 nm. Iminodibenzyl and carbamazepine have one further maximum at 284 - 285 nm but iminostilben has a maximum at 252 nm. The best wavelength to detect the impurities is probably 210 nm but due to the low content of impurities in this particular reference substance no extra peaks were observed at 210 nm.

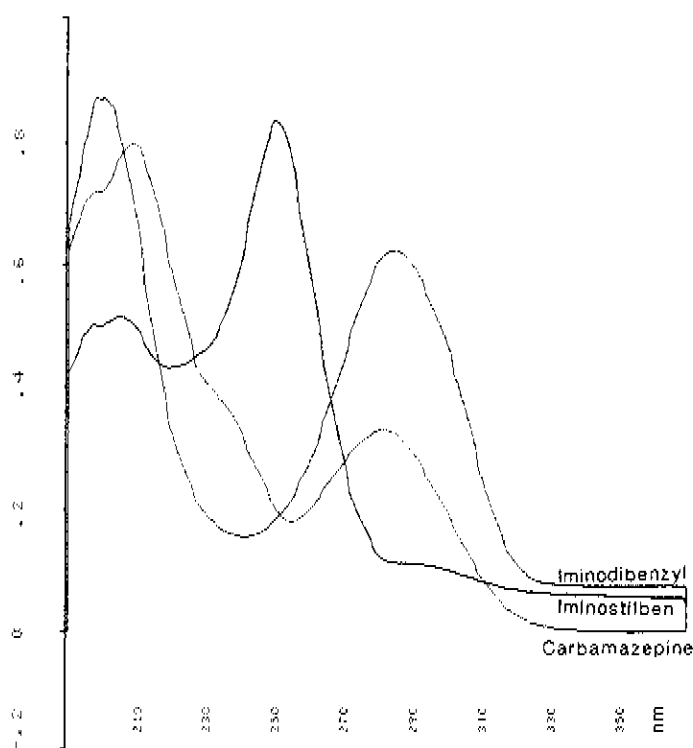


Figure 6. *UV-spectra of carbamazepine and potential impurities.*
Solvent: Methanol/Water/ Acetonitrile (40:40:20)

DATA GIVEN BY THE MANUFACTURER

| | |
|---------------------------|--------------------------|
| Appearance: | Practically white powder |
| Crystal modification: | B |
| Absorbance in chloroform: | 0.006 |
| LOD: | 0.00% |
| Sulphated ash: | 0.00% |
| Lead: | < 2 ppm |
| Copper: | < 2.5 ppm |
| TLC Iminodibenzyl: | not visible |
| TLC Iminostilben: | 0.0004% |
| Total byproducts: | < 0.001% |

STABILITY

No special stability studies were performed as no signs of degradation was observed when comparing the analytical results from this sample of the proposed ICRS with analytical results from 1983 obtained from the manufacturer. It has been stored for six years at + 5 °C.

CONCLUSION

Carbamazepine, Control No 189143, can be considered suitable as International Chemical Reference Substance for the intended purpose. The content of carbamazepine when used in the spectrophotometric assay is taken to be 100.0% calculated with reference to the dried substance.

APPENDIX 8LEVOTHYROXINE SODIUM
(THYROXINE SODIUM)

Control No 189144

Analytical Report

INTENDED USE

The monograph for Levothyroxine sodium in the International Pharmacopeia 3rd Ed. Vol 3 requires a reference substance to be used in the thin-layer chromatographic test for identity.

MATERIAL

About 10 g of the sample (EPCRS 1) were received at the WHO Centre in October 1987. The material is being stored protected from light in tightly closed containers at + 5 °C.

ANALYTICAL DATA

Description: An almost white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTUREInfrared spectrum

An infrared spectrum is given in Figure 1 (Control No 189144). The spectrum is concordant with the spectrum obtained from another sample of levothyroxine sodium (batch 1381/96).

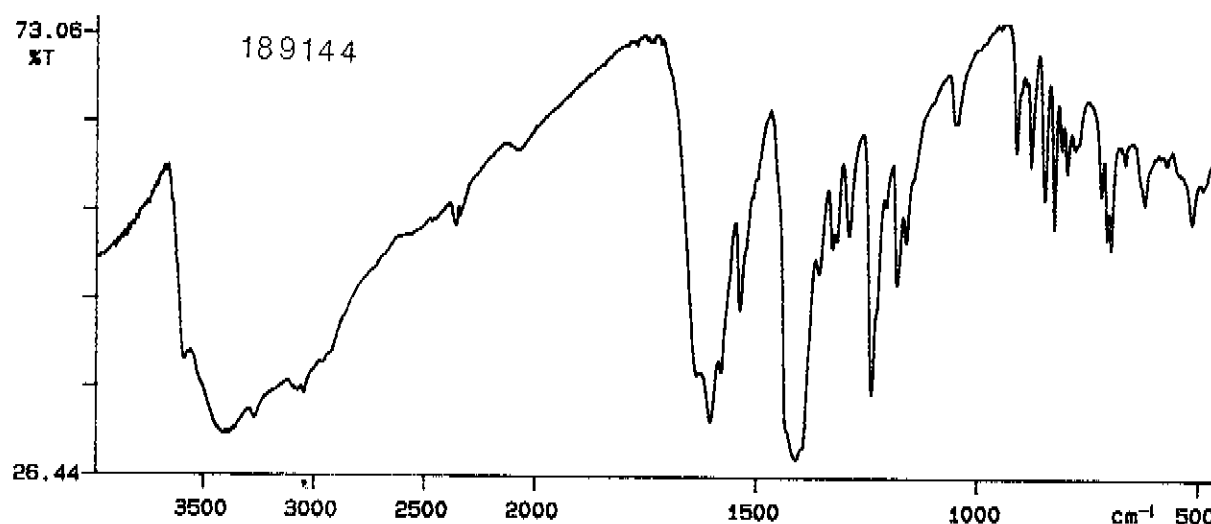


Figure 1. IR-spectrum of 1.2 mg of levothyroxine sodium Control No 189144 in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 1600 FTIR.

The spectrum from USP Reference standard levothyroxine Lot H is not concordant with the above mentioned spectra for levothyroxine sodium.

Thin-layer identity test

When examined according to the identity test (c) in the Ph. Int. 3rd Ed, Vol 3 levothyroxine sodium showed one major spot with $R_f = 0.2$. Liothyronine (EPCRS) had $R_f = 0.3$. This result was obtained with a home-made plate. For Merck ready-made plate see purity.

ASSAY

Thermogravimetric analysis: When the substance was heated to 200 °C a loss of 9.1% of weight occurred.

Instrument: Perkin-Elmer TGA 7 Thermogravimetric analyzer.
Sample weight: About 8 mg
Heating rate: 10 °C/minute
Decomposition temperature: 225 - 281 °C (Merck Index)

Sodium: 2.87% (n=3) determined by atomic absorption.

PURITY

Thin-layer chromatography

The following thin-layer chromatographic system was used:

System: According to the International Pharmacopeia 3rd Ed, Vol 3.
Thin-layer: Silica gel 60, F-254 (Merck)
Eluent: Ammonia (260 g/l): 2-propanol: ethylacetate (20:35:55)
Sample: 100 µg of levothyroxine sodium were applied.
Visualization: UV-light of 254 nm. Spraying with ferric chloride/ferricyanide/arsenite and examination in daylight.
Result: In UV-light only one extra spot corresponding to liothyronine was observed. It was estimated to less than 0.1%.
 R_f (levothyroxine) = 0.11

After spraying 4 secondary spots were detected with $R_f = 0.19$ (liothyronine) 0.25, 0.37 and 0.41. The detection limit was less than 0.05 µg (0.05%) for liothyronine.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.2%. A chromatogram is shown in Figure 2. Four faint impurities are observed. The peak eluting after 8.7 minutes corresponds to liothyronine. It was estimated to 0.02% against USP reference substance Lot I (0.03% by peak area measurement).

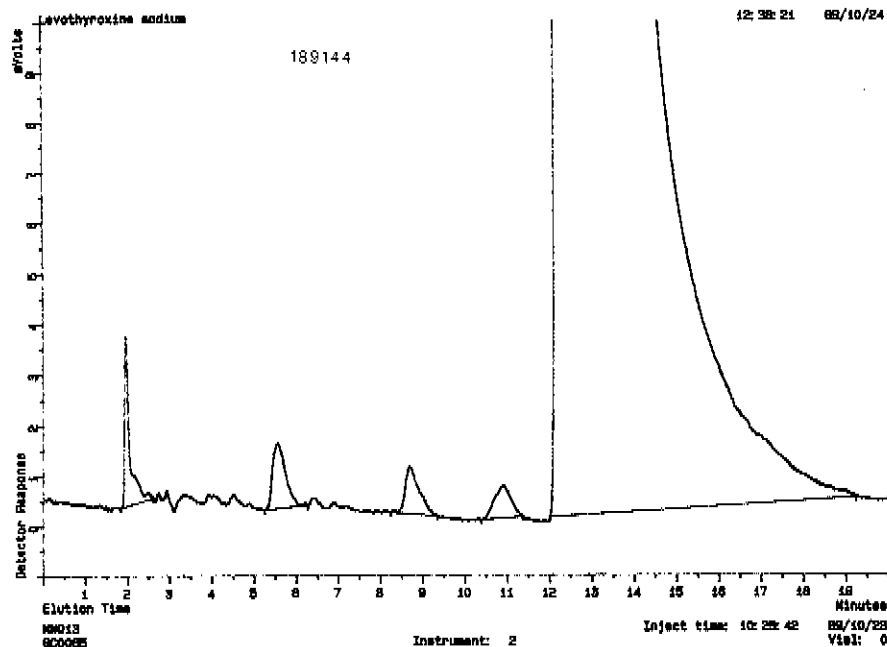


Figure 2. Chromatogram of levothyroxine sodium Control No 189144.

The following conditions were used:

Eluent: Acetonitrile/Water containing 0.02 M SDS (60:40)

Column: RP-18, Spheri-5 (Brownlee)

Detector: Shimadzu SPD-2A operated at 225 nm

Pump: Waters 600 Multisolvant Delivery System operated at a flow rate of 1. ml/min.

Integrator: Hewlett Packard 3390 A. Attenuation: 4

Sample: 0.5 mg/ml dissolved in the eluent.
20 μ l corresponding to 20 μ g were injected.

A comparison was made with USP Reference standard for levothyroxine Lot H which contained about 0.05% impurities. The observed retention time was the same for the USP reference standard and the ICRS.

Diode-array detection

The chromatogram was also evaluated with a LKB 2140 Rapid Diode Array Detector. The same chromatographic system as described above was used, except for the injection volume that was increased to 100 μ l to get maximum sensitivity. An isogram is given in Figure 3.

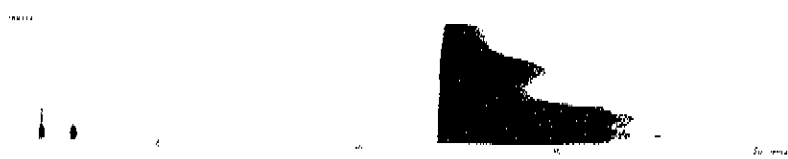


Figure 3. *Isogram of levothyroxine Control No 189144.*
Sensitivity: 0.001

As seen from the figure no further information about impurities are observed. The sensitivity of this diode array detector is lower than the UV-detector used in the determination of impurities described on page 31, where 0.2% were found. They are not detected here. Levothyroxine sodium eluting after about 12.4 minutes has a maximum absorbance at 214 nm and a weaker at 301 nm. The peak purity of levothyroxine sodium was tested by recording spectra at the up- and downslope of the peak. They were all identical with maxima at 214 nm and 301 nm. The peaks eluting before 3 minutes originates from the blank.

DATA GIVEN BY COLLABORATING LABORATORIES

Being an EPCRS the following data was found in reports from EP.

| | |
|-------------------|--|
| Water content: | 9.67% |
| TLC: | No secondary spot due to liothyronine (100 µg applied) |
| HPLC: | 98.6% Liothyronine 0.6% other impurities 0.7% (1986) |
| Optical rotation: | + 17.6° (1976) |
| Loss on drying: | 9.9%, 9.12%, 9.3% (3 Labs) |

STABILITY

No special stability studies were performed as this substance is an established EPCRS and no significant degradation has been observed compared to results from 1986 obtained from EP.

CONCLUSION

Levothyroxine sodium No 189144, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 9

OXYTETRACYCLINE HYDROCHLORIDE

Control No 189141

Analytical Report

INTENDED USE

The monograph for oxytetracycline hydrochloride in the International Pharmacopoeia 3rd Ed. Vol 3 requires a reference substance to be used in the thin-layer chromatographic test for identity.

MATERIAL

About 100 g of the sample (manufacturers batch no 435/2) were received at the WHO Centre in June 1989. The material is being stored protected from light in tightly closed containers at + 5 °C.

ANALYTICAL DATA

Description: A yellow, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTUREInfrared spectrum

An infrared spectrum is given in Figure 1 (Control No 189141). The spectrum is concordant with the spectrum obtained from the EPCRS oxytetracycline hydrochloride Lot 1.

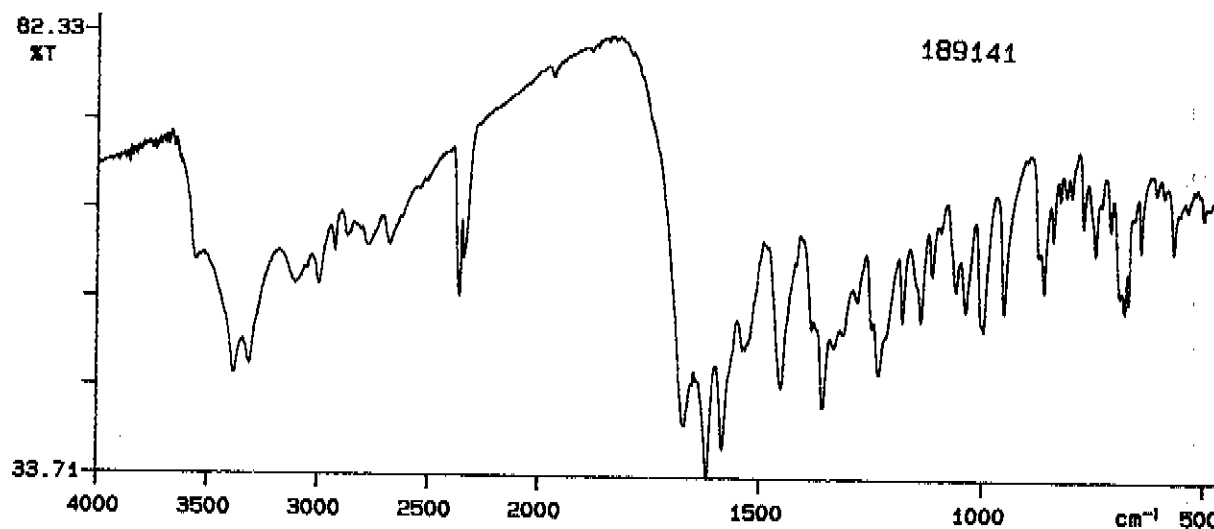


Figure 1. IR-spectrum of 0.9 mg of oxytetracycline hydrochloride Control No 189141 in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 1600 FTIR.

Spectra were also recorded in nujol but no significant difference was observed.

To show the difference between spectra from oxytetracycline dihydrate and oxytetracycline hydrochloride a part of the IR-spectrum in KBr is enlarged and compared in Figure 2.

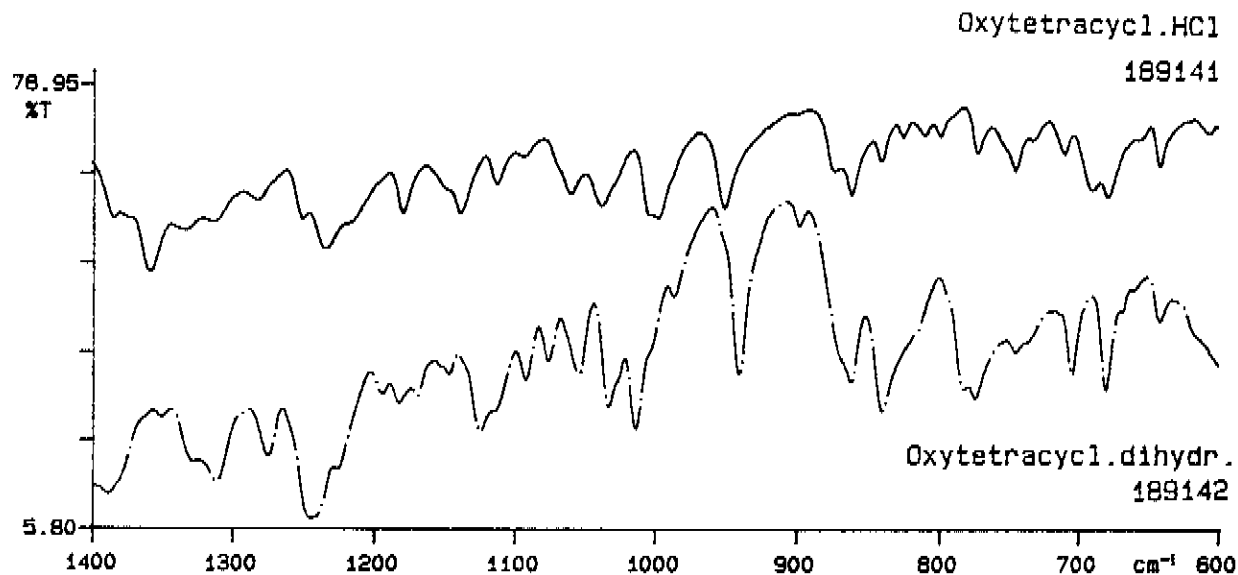


Figure 2. Comparison of IR-spectra of oxytetracycline hydrochloride and oxytetracycline dihydrate in KBr.

Differences are observed for example in the regions 950, 800-850 and 700 cm⁻¹.

UV-spectrum

A UV-spectrum in 0.1 M hydrochloric acid is given in Figure 3.

λ max in 0.1 M HCl = 216, 268 and 353 nm

E (1%, 1 cm) = 290, 384 and 279 (n=6)

The result is calculated with reference to the anhydrous substance.

The absorbance of a 20 μ g/ml solution was 0.56 at 353 nm performed on the substance as is (according to Ph. Int).

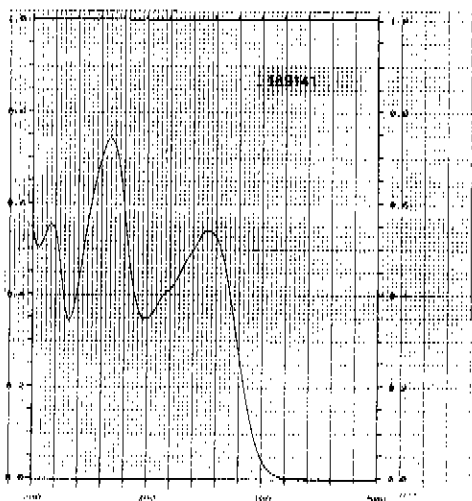


Figure 3. UV-spectrum of oxytetracycline hydrochloride Control No 189141 19.5 μ g/ml in 0.1 M HCl.

Thin-layer chromatography (- identity test)

The following thin-layer chromatographic system was used:

- System: According to the International Pharmacopoeia 3rd Ed. Vol 2 for tetracycline.
- Thin-layer: Cellulose Avicel (Merck). Impregnated with phosphate/citrate buffer pH 4.5 TS.
- Eluent: Ethylacetate:acetone:water (60:30:6)
- Sample: 3 μg of oxytetracycline dihydrate or hydrochloride and a mixture of oxytetracycline dihydrate, tetracycline hydrochloride and chlortetracycline hydrochloride 0.05 μg of each were applied. Before elution the plate was sprayed with trimethylpyridine (cold) 50 mg/ml water and saturated for one hour in the vapours from the eluent.
- Visualization: Exposure to vapour of ammonia and detection at 365 nm UV-light.

A chromatogram is shown in Figure 4.

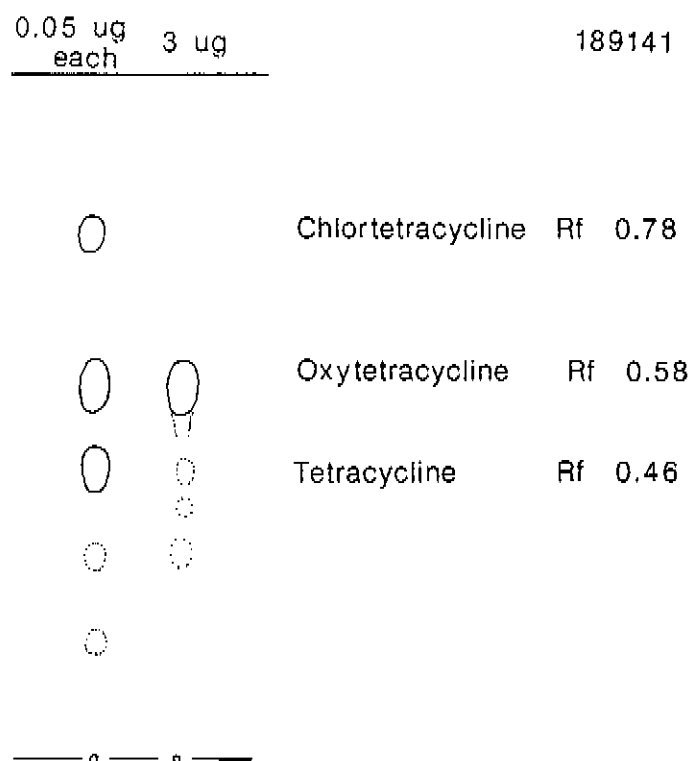


Figure 4. *Thin-layer chromatogram from identity test for oxytetracycline (dihydrate and hydrochloride).*

Result: As can be seen from the figure the three substances are clearly separated, with the following retention factors. Chlortetracycline Rf = 0.78, oxytetracycline dihydrate (or hydrochloride) Rf = 0.58 and tetracycline Rf = 0.46. The extra spots observed for oxytetracycline dihydrate shows that it contains small amounts of tetracycline which is more thoroughly investigated by liquid chromatography. Oxytetracycline dihydrate and hydrochloride have the same retention factor.

Chloride identity: The identity of chloride was confirmed by ion-chromatography. The chloride standard was prepared according to Ph. Eur.

ASSAY

Spectrophotometric assay: 100.8% (n=5, rsd 0.5%) determined at 268 nm. The EPCRS Lot 1 was regarded as 100% and used as standard. Calculations were performed with reference to the anhydrous substances.

Liquid chromatographic assay: 101.7% oxytetracycline when assayed against the EPCRS Lot 1 which was regarded as 100% (n=6, rsd 2%). However when these two substances were investigated for purity they were found to be 97.8% (ICRS) and 98.8% (EPCRS) by liquid chromatography see below.

Thermogravimetric analysis: It was not possible to use thermogravimetric analysis as the substance decomposes.

Water: 0.3% determined by Karl Fischer titration.

PURITY

Light-absorbing impurities: According to Ph. Int.
430 nm: 0.20 (max 0.5) and 490 nm: 0.10 (max 0.2)

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 2%. A chromatogram is shown in Figure 5.

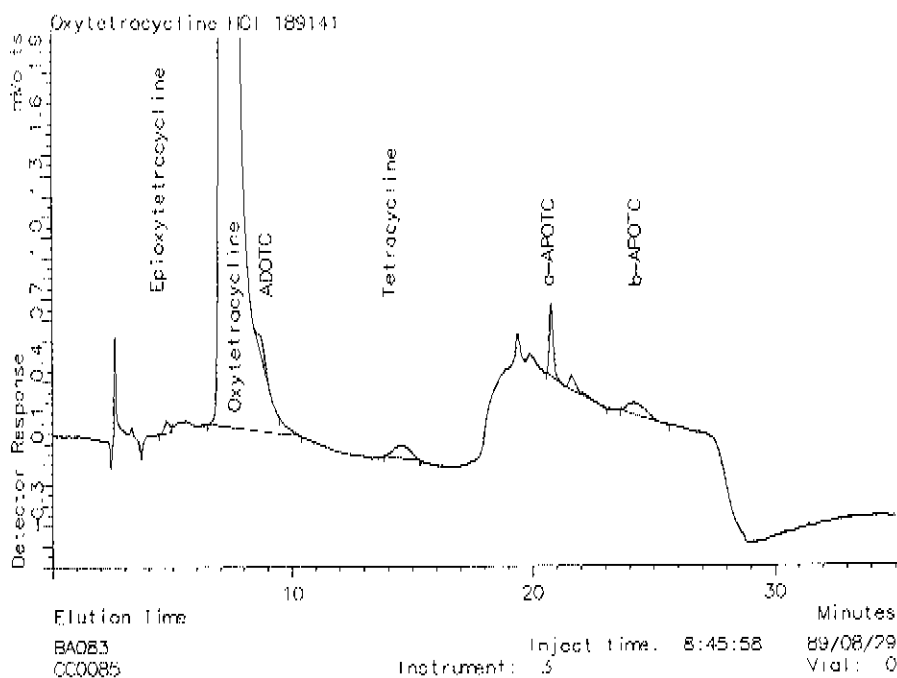


Figure 5. Chromatogram of oxytetracycline hydrochloride Control No 189141.

As can be seen from the figure six impurities are observed. They are 4-epioxytetracycline 0.1%, 2-acetyl-2-decarboxamido-oxytetracycline (ADOTC) 0.3%, tetracycline 0.5%, α -apooxytetracycline (α -APOTC) 0.6%, unknown 0.3% and β -apooxytetracycline (β -APOTC) 0.4%. The baseline drift at 19 minutes originates from the gradient profile.

A comparison with EPCRS showed that it contained about 1.2% impurities identified as 0.5% ADOTC and 0.7% tetracycline. BPCRS contained 3.2% impurities identified as 0.2% ADOTC, 0.5% tetracycline, 1.4% α -APOTC, 0.2% unknown and 0.9% β -APOTC.

The following conditions were used:

Eluent: Solution A (25 g tert. butanol/1 000 ml, 0.02 M Phosphate buffer pH 7.5, 0.001 M TBAH-sulfate pH 7.5, 0.00001 M Na-EDTA pH 7.5).

Solution B (100 g tert. butanol/1 000 ml, 0.02 M phosphate buffer pH 7.5, 0.001 M TBAH-sulfate pH 7.5, 0.00001 M Na-EDTA pH 7.5).

| Time, minutes | Solution A % | Solution B % |
|---------------|--------------|--------------|
| 0 - 12 | 65 | 35 |
| 12 - 22 | 10 | 90 |
| 22 - 35 | 65 | 35 |

Column: Brownlee polymer PRP-10 A

Detector: Varian UV-100 operated at 254 nm

Pump: Varian 5560 operated at a flow rate of 1 ml/min

Integrator: Varian 4270

Attenuation: 2 and Peak Pro (Beckman)

Sample: 0.2 mg/ml dissolved in the 0.01 M HCl.
10 μ l corresponding to 2 μ g were injected.

Diode-array detection

The chromatogram was also evaluated with a LKB 2140 Rapid Diode Array Detector. The same chromatographic system as described above was used. The sample concentration was 2 mg/ml.

Due to disturbances from the gradient profile after 18 minutes it was not possible to obtain any extra information about the late eluting impurities. From earlier studies of oxytetracycline dihydrate (see report for ICRS 189142) we know that the impurities ADOTC and tetracycline have the same UV-maxima as oxytetracycline i. e. 205, 270 and 360 nm.

DATA GIVEN BY THE MANUFACTURER

A yellow crystalline powder

Identification: Satisfactory

Solubility: Complete

Absorbance: (0.2% MeOH) 430 nm: 0.146

Absorbance: (1% MeOH) 490 nm: 0.026

Water = 0.2%

pH 2.5

Assay 981 I.U./mg

STABILITY

Oxytetracycline hydrochloride was exposed to air of different relative humidity at room temperature (about 20 °C) for a period of 10 weeks as described in WHO/PHARM/82.509. The samples stored at 98% RH were discoloured (brown) after two weeks. Only small increases of weight were observed. Samples stored at 55% RH - 100% RH gained in weight between 0.4% - 1.4% already after one day. After 8 weeks the sample stored at 98% RH was strongly discoloured and had gained 3% in weight. The results are shown in Figure 6.

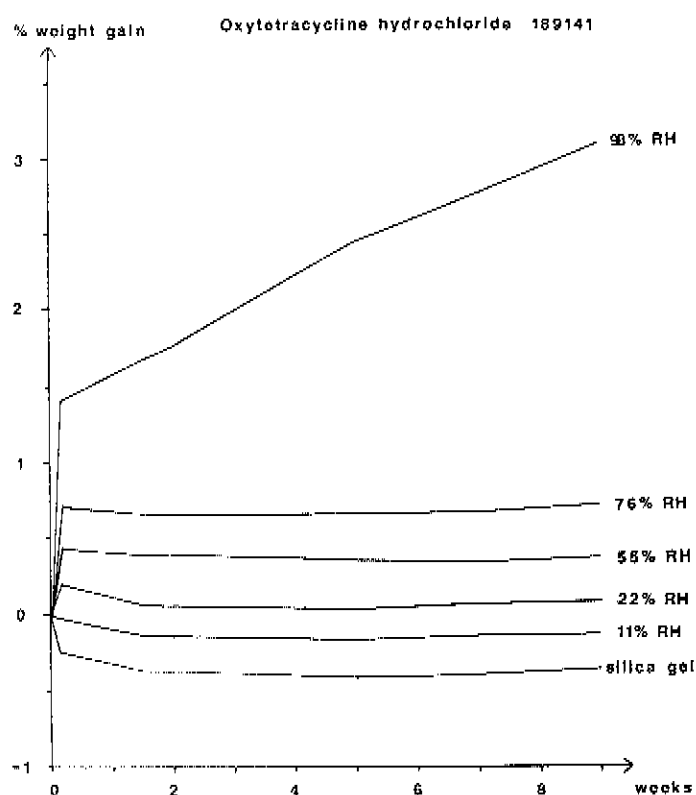


Figure 6. Stability of oxytetracycline hydrochloride, Control No 189141 stored at different relative humidity at 20 °C.

No signs of degradation were observed when the samples were analyzed by the liquid chromatographic method described above. However in the discoloured samples stored at 98% humidity the content of α -APOTC and β -APOTC decreased. When the brown samples were tested for

light-absorbing impurities the absorbance increased from 0.1 to 0.26 at 490 nm. Liquid chromatographic investigations at 490 nm showed no peaks at all. Diode-array detection gave no further information about the degradation products.

Thin-layer chromatography also showed less impurities for the samples stored at 98% RH and no new spots were observed. It is difficult to explain the discolouration of the samples.

Different crystal forms of oxytetracycline hydrochloride show different hygroscopicity as reported by Burger et al. in *Acta Pharmaceutica Technologica* 31 (4) 1985 page 230 - 235. The present ICRS 189141 is probably form A which was found to be more stable concerning the uptake of water.

CONCLUSION

Oxytetracycline hydrochloride Control No 189141, can be considered suitable as International Chemical Reference Substance for the intended purpose.

OXYTETRACYCLINE DIHYDRATE

Control No 189142

Analytical Report

INTENDED USE

The monograph for oxytetracycline dihydrate in the International Pharmacopoeia 3rd Ed. Vol 3 requires a reference substance to be used in the thin-layer chromatographic test for identity.

MATERIAL

About 200 g of the sample (manufacturers batch no B68111-53011) were received at the WHO Centre in June 1987. The material is being stored protected from light in tightly closed containers at + 5 °C.

ANALYTICAL DATA

Description: A pale yellow, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (Control No 189142). The spectrum is concordant with the spectrum obtained from the International Biological Standard (2nd WHO).

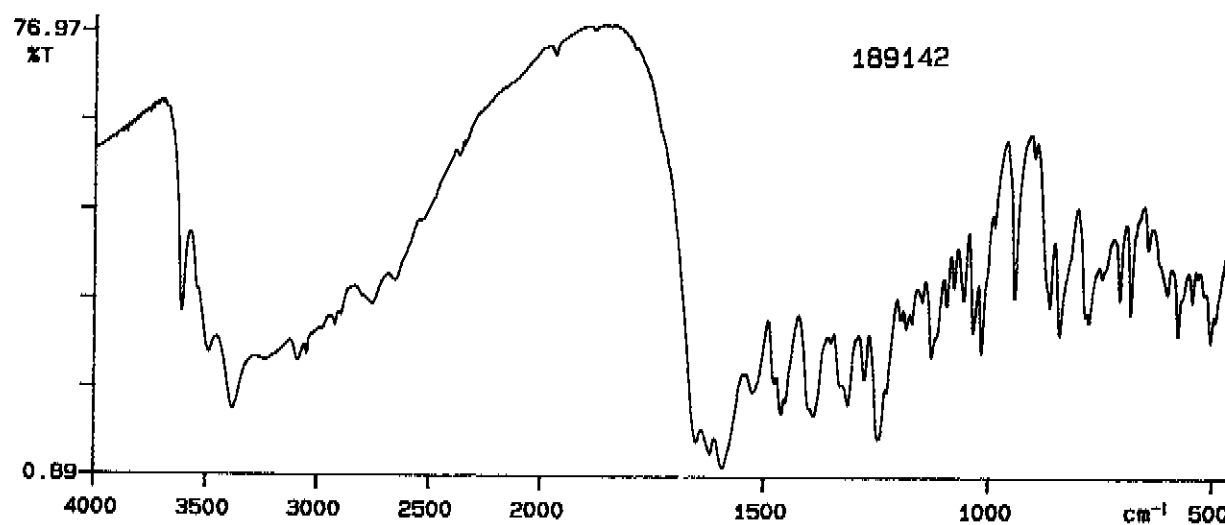


Figure 1. IR-spectrum of 1.3 mg of oxytetracycline dihydrate Control No 189142 in 300 mg KBr recorded against a KBr disc.
Instrument: Perkin-Elmer 1600 FTIR.

Spectra were also recorded in nujol but no significant difference was observed.

To show the difference between spectra from oxytetracycline dihydrate and oxytetracycline hydrochloride a part of the IR-spectrum in KBr is enlarged and compared in Figure 2.

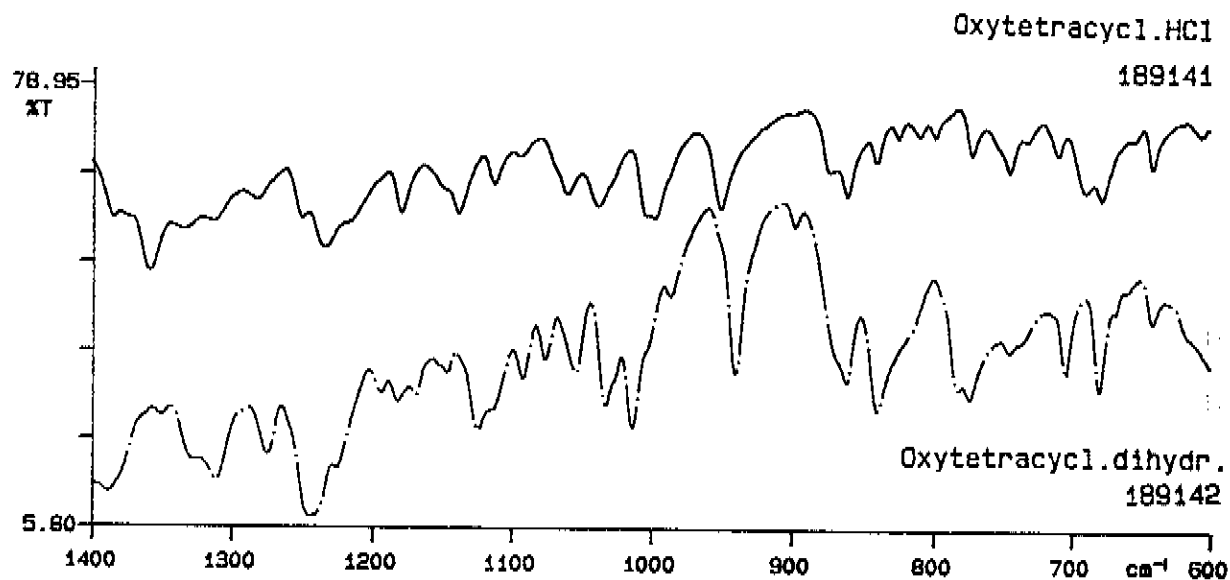


Figure 2. Comparison of IR-spectra of oxytetracycline hydrochloride and oxytetracycline dihydrate in KBr.

Differences are observed for example in the regions 950, 800-850 and 700 cm^{-1} .

UV-spectrum

A UV-spectrum in 0.1 M hydrochloric acid is given in Figure 3.

λ max in 0.1 M HCl = 216, 268 and 353 nm

E (1%, 1 cm) = 309, 413 and 303 (n=6)

The result is calculated with reference to the anhydrous substance.

The absorbance of a 20 $\mu\text{g/ml}$ solution was 0.56 at 353 nm performed on the substance as is (according to Ph. Int).

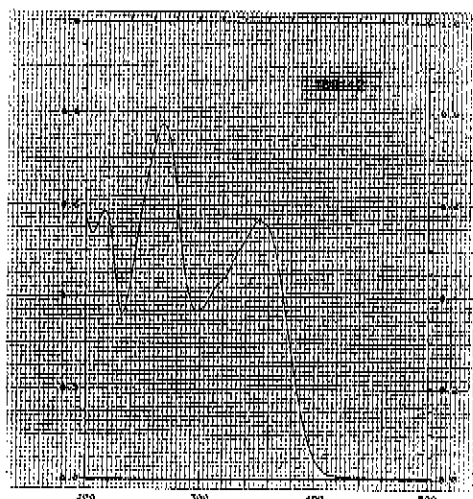


Figure 3. UV-spectrum of oxytetracycline dihydrate Control No 189142
20 $\mu\text{g/ml}$ in 0.1 M HCl.

Thin-layer chromatography (- identity test)

The following thin-layer chromatographic system was used:

- System: According to the International Pharmacopoeia 3rd Ed. Vol 2 for tetracycline.
- Thin-layer: Cellulose Avicel (Merck). Impregnated with phosphate/citrate buffer pH 4.5 TS.
- Eluent: Ethyl acetate:acetone:water (60:30:6)
- Sample: 3 µg of oxytetracycline dihydrate or hydrochloride and a mixture of oxytetracycline dihydrate, tetracycline hydrochloride and chlortetracycline hydrochloride 0.05 µg of each were applied. Before elution the plate was sprayed with trimethylpyridine (cold) 50 mg/ml water and saturated for one hour in the vapours from the eluent.
- Visualization: Exposure to vapour of ammonia and detection at 365 nm UV-light.

A chromatogram is shown in Figure 4.

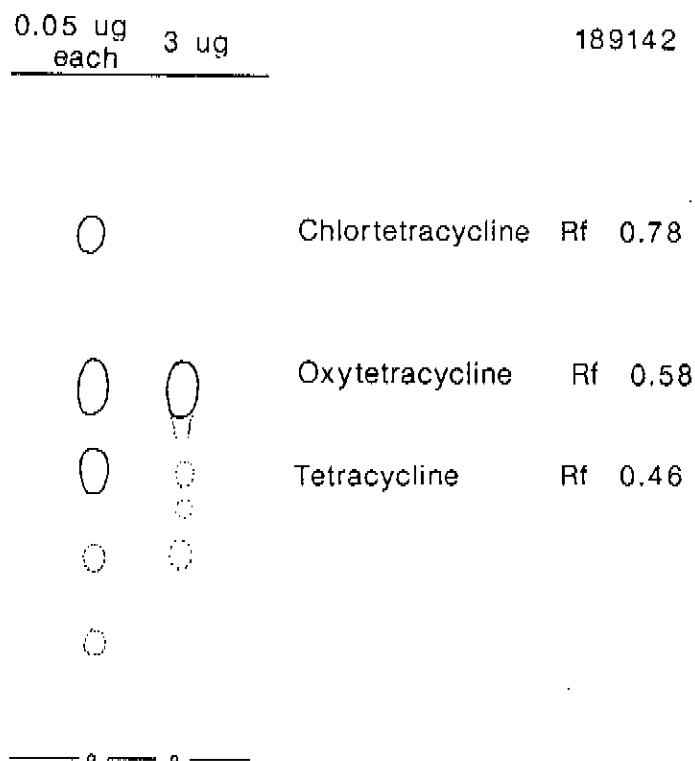


Figure 4. *Thin-layer chromatogram from identity test for oxytetracycline (dihydrate and hydrochloride).*

Result: As can be seen from the figure the three substances are clearly separated with the following retention factors. Chlortetracycline Rf = 0.78, oxytetracycline dihydrate (or hydrochloride) Rf = 0.58 and tetracycline Rf = 0.46. The extra spots observed for oxytetracycline dihydrate shows that it contains small amounts of tetracycline which is more thoroughly investigated by liquid chromatography. Oxytetracycline dihydrate and hydrochloride has the same retention factor.

ASSAY

Spectrophotometric assay: 104% (n=6, rsd 0.2%) determined at 353, 268 and 254 nm. The Biol. 2nd WHO standard which was regarded as 100% was used as standard. Calculations were performed with reference to the anhydrous substances.

Liquid chromatographic assay: 104.6% oxytetracycline when assayed against the Biol. 2nd WHO which was regarded as 100% (n=6, rsd 2%). However when these two substances were investigated for purity they were equal 98.5% by liquid chromatography see below.

Thermogravimetric analysis: It was not possible to use thermogravimetric analysis as the substance decomposes and the water is strongly bound.

Water: 7.4% determined by Karl Fischer titration.

PURITY

Light-absorbing impurities: According to Ph. Int.
430 nm: 0.10 (max 0.5) and 490 nm: 0.04 (max 0.2)

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 1.5%. A chromatogram is shown in Figure 5, which also shows the 2nd Int. Biol. Stand.

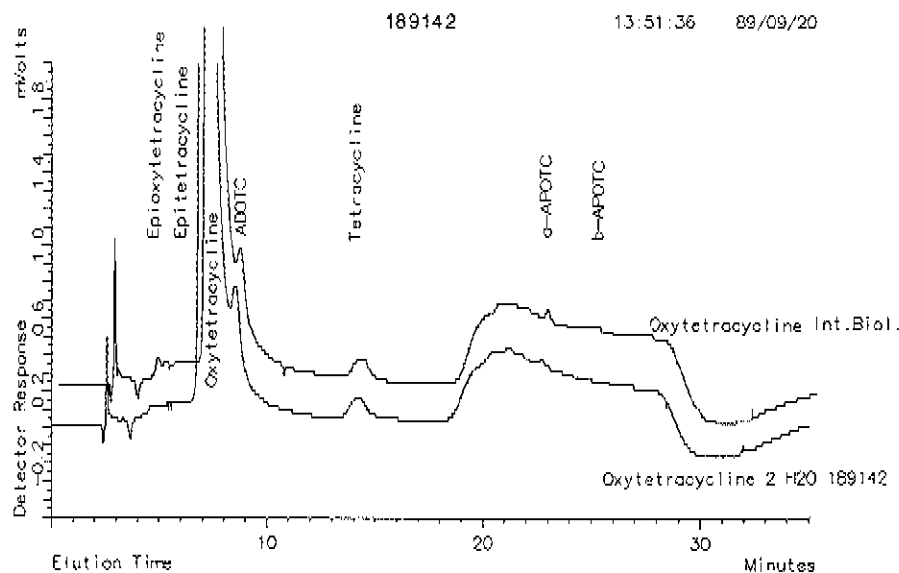


Figure 5. Chromatogram of oxytetracycline dihydrate Control No 189142 and the 2nd Int. Biol. Stand.

As can be seen from the figure two main impurities are observed. In the proposed ICRS they are 2-acetyl-2-decarboxamido-oxytetracycline (ADOTC) 0.7%, tetracycline 0.7%. Only traces of α -apo-oxytetracycline (α -APOTC) were found. 4-Epioxytetracycline (EOTC), epitetracycline and β -apo-oxytetracycline (β -APOTC) were not found in the proposed ICRS. The baseline drift at 19 minutes originates from the gradient profile.

The comparison with the 2nd Int. Biol. Stand showed that it contained about 1.5% impurities identified as 0.5% ADOTC, 0.1% EOTC, 0.7% tetracycline and 0.1% a-APOTC. EPCRS, oxytetracycline dihydrate was also included in the comparison it contained 2.6% impurities.

The following conditions were used:

Eluent: Solution A (25 g tert. butanol/1 000 ml, 0.02 M Phosphate buffer pH 7.5, 0.001 M TBAH-sulfate pH 7.5, 0.00001 M Na-EDTA pH 7.5)

Solution B (100 g tert. butanol/1 000 ml, 0.02 M phosphate buffer pH 7.5, 0.001 M TBAH-sulfate pH 7.5, 0.00001 M Na-EDTA pH 7.5).

| Time, minutes | Solution A % | Solution B % |
|---------------|--------------|--------------|
| 0 - 12 | 65 | 35 |
| 12 - 22 | 10 | 90 |
| 22 - 35 | 65 | 35 |

Column: Brownlee polymer PRP-10 A

Detector: Varian UV-100 operated at 254 nm

Pump: Varian 5560 operated at a flow rate of 1 ml/min

Integrator: Varian 4270

Attenuation: 2 and Peak Pro (Beckman)

Sample: 0.2 mg/ml dissolved in the 0.01 M HCl.
10 μ l corresponding to 2 μ g were injected.

Diode-array detection

The chromatogram was also evaluated with a LKB 2140 Rapid Diode Array Detector. The same chromatographic system as described above was used. An isogram is given in Figure 6. The sample concentration was 2 mg/ml.

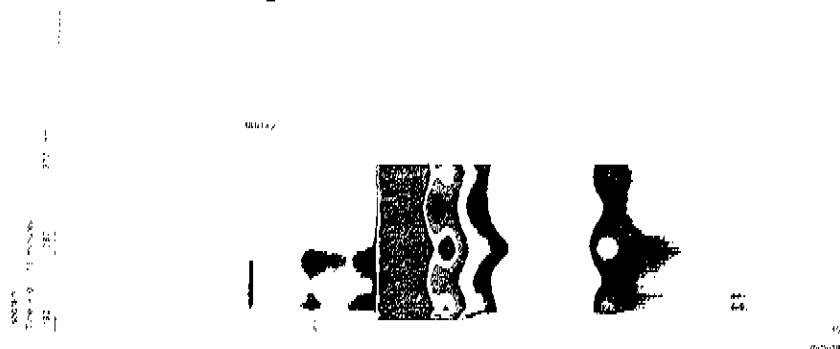


Figure 6. *Isogram of oxytetracycline dihydrate Control No 189142.*

Sensitivity: 0.025

Sample: 2 mg/ml

In this figure the dominating impurity is ADOTC eluting after about 7.2 minutes and tetracycline eluting after 10.3 minutes. No further impurities were observed.

In figure 7 UV-spectra for oxytetracycline, ADOTC and tetracycline in the eluent used are given. All substances have similar UV-curves with maxima at 205, 270 and 360 nm.

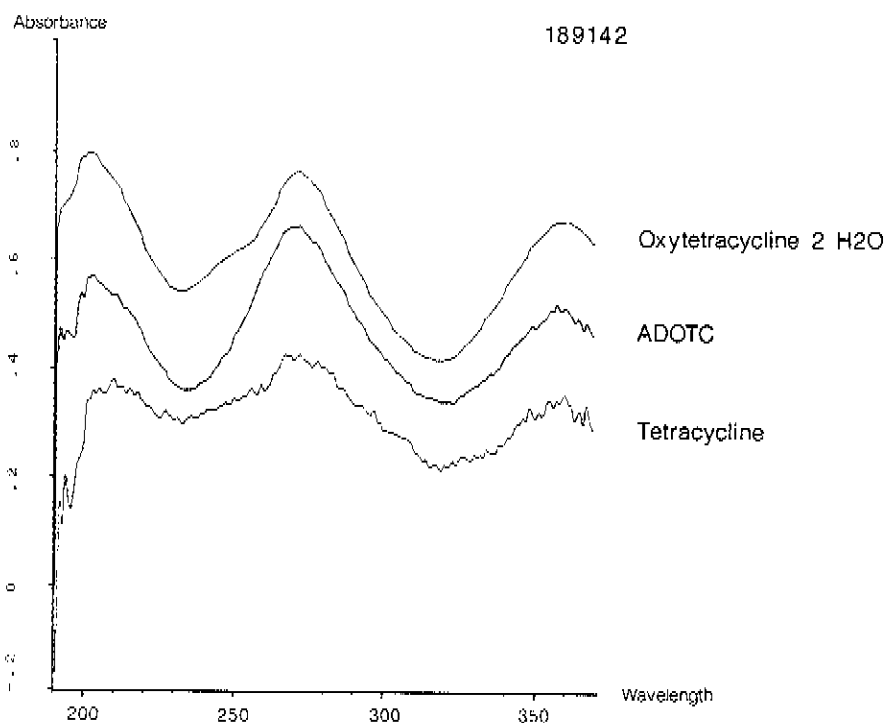


Figure 7. UV-spectra of oxytetracycline and the impurities ADOTC and tetracycline.

DATA GIVEN BY THE MANUFACTURER

Oxytetracycline (turbidimetric assay, *Klebsiella pneumoniae*) 918 µg/mg (as is basis)

Water 7.3%

pH (1%) 5.1

Crystallinity meets test

Identification UV 353 nm: 99.4% oxytetracycline dry basis against a Pfizer standard.

Identification, sulfuric acid chromagen: meets test.

STABILITY

Oxytetracycline dihydrate was exposed to air of different relative humidity at room temperature (about 20 °C) for a period of 10 weeks as described in WHO/PHARM/82.509.

All samples were unchanged at visual inspection and a small increase of weight was observed already after 1 week. Samples stored at 22% RH - 100% RH gained between 0.5% to 2% in weight over a period of 10 weeks. No signs of degradation were observed when the samples were analyzed by the liquid chromatographic method described above.

CONCLUSION

Oxytetracycline dihydrate Control No 189142, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 11

PREDNISOLONE

Control No 389029

Analytical Report

INTENDED USE

The stock of the current batch of the International Chemical Reference Substance for prednisolone, Control No 283029, is depleted and has to be replaced.

The monograph for prednisolone in the International Pharmacopoeia 3rd Ed. Vol 2 requires a reference substance to be used in the infrared spectrophotometric and thin-layer chromatographic tests for identity and in the spectrophotometric assay.

MATERIAL

About 50 g of the sample (manufacturers lot no K-1) were received at the WHO Centre in April 1988. The material is being stored protected from light in tightly closed containers at + 5 °C.

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (Control No 389029). The spectrum is concordant with the spectrum obtained from ICRS Control No 283029.

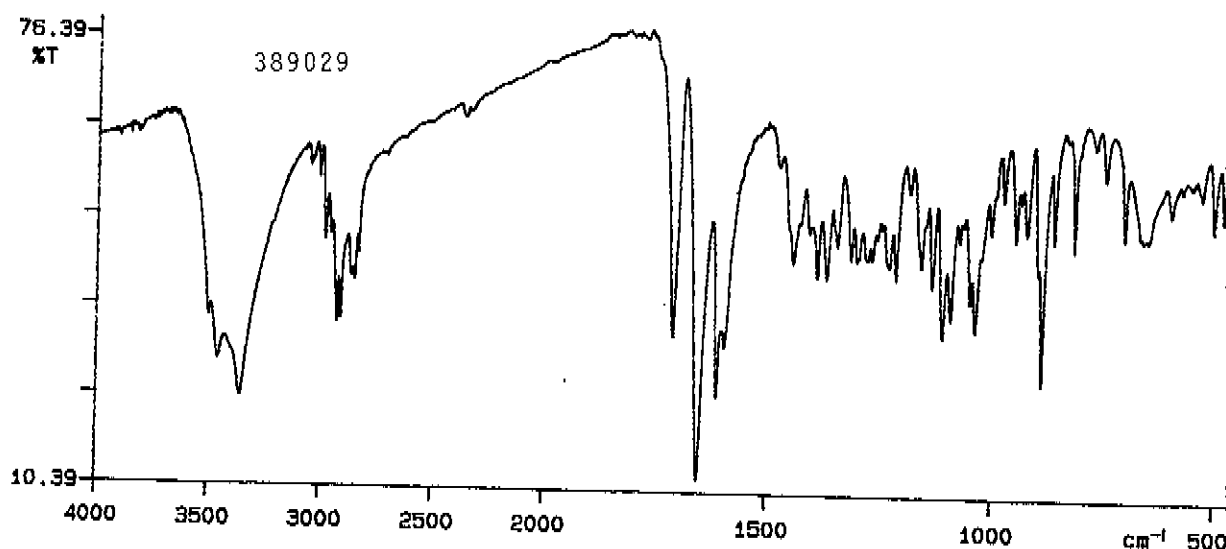


Figure 1. IR-spectrum of 1.5 mg of prednisolone Control No 389029 in 300 mg KBr recorded against a KBr disc. Instrument: Perkin-Elmer 1600 FTIR.

UV-spectrum

A UV-spectrum in ethanol is given in Figure 2.

λ max in ethanol = 243 nm

E (1%, 1 cm) = 417 (n=7), s rel % = 2.0

The result is calculated with reference to the dried substance.

The absorbance of a 14 μ g/ml solution was 0.61.

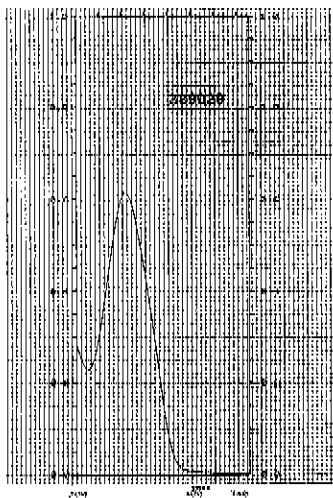


Figure 2. *UV-spectrum of prednisolone Control No 389029 14 μ g/ml in ethanol.*

ASSAY

Spectrophotometric assay: 100.1% (n=7), s rel % = 2.0 determined according to Ph. Int. 3rd Ed. Vol 2. The ICRS Control No 283029 was used as reference and regarded as 100%. The result is calculated with reference to the dried substance.

Thermogravimetric analysis: When the substance was heated to 160 °C a loss of weight of 0.24% was observed.

| | |
|----------------------------|--|
| Instrument: | Perkin-Elmer TGA 7 Thermogravimetric analyzer. |
| Sample weight: | 9 mg |
| Heating rate: | 10 °C/minute |
| Decomposition temperature: | 241 °C |

Water: 0.28% determined by Karl Fischer titration.

PURITY

Total solid impurities

1) Differential Scanning Calorimetry (DSC): It was not possible to estimate the purity by this method as the substance melts with decomposition.

Thin-layer chromatography

The total amount of impurities was estimated to about 0.2%.

The following thin-layer chromatographic system was used:

System 1: According to the International Pharmacopeia 3rd Ed, Vol 2.

Thin-layer: Silica gel 60, F-254 (Merck)

Eluent: Dichloromethane:Ether:Methanol:Water (77:15:8:1.2)

Sample: 15 µg and 105 µg of prednisolone were applied.

The plate was developed twice.

Visualization: UV-light of 254 nm, evaluation by densitometry at 240 nm and spraying with blue tetrazolium/ethanol TS followed by heating to 105 °C and examination in daylight, and evaluation by densitometry at 525 nm.

One secondary extra spot with $R_f=0.68$ was detected visually at 254 nm and after spraying with blue tetrazolium when applying 105 µg. When applying 15 µg (the amount prescribed in the monograph) also one small secondary spot was detected. The detection limit of this system was about 0.2 µg (0.2%). When scanned at 240 nm by densitometry, the amount of impurities was estimated to about 0.2%.

R_f (prednisolone) = 0.62

A comparison was made with ICRS Control No 283029. It showed two secondary spots, earlier estimated to about 1.5%.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.30%. A chromatogram is shown in Figure 3.

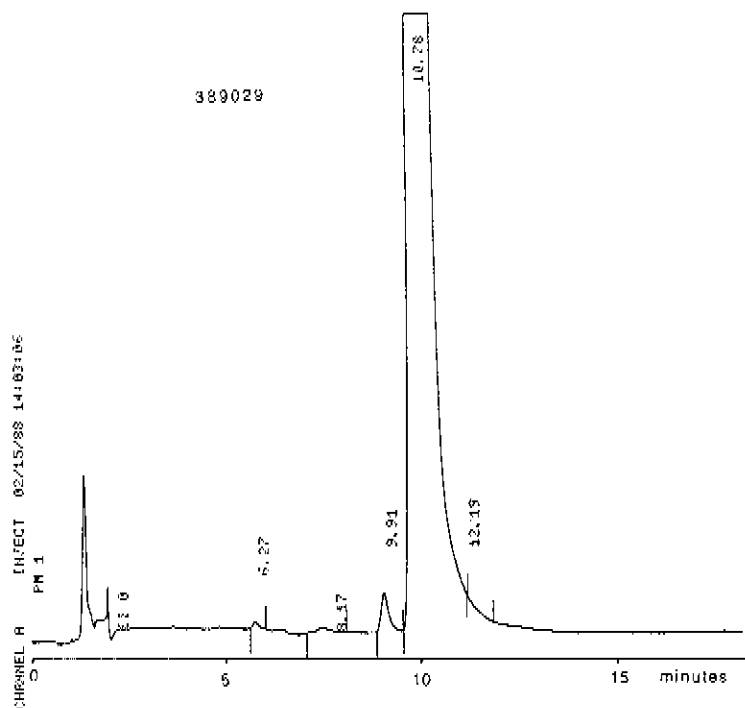


Figure 3. Chromatogram of prednisolone Control No 389029.

The following conditions were used:

Eluent: Hexane/Dichloromethane/Methanol/Water (70.3+23.3+6.3+0.1)

Column: Spherisorb S5W, silica

Detector: Varian UV 200 operated at 240 nm

Pump: Varian 5560 operated at a flow rate of 1.80 ml/min

Integrator: Varian 4270

Attenuation: 4

Sample: 0.5 mg/ml dissolved in 2 ml of methanol and diluted to 10 ml with the eluent.
10 μ l corresponding to 5 μ g were injected.

A comparison was made with ICRS Control No 283029 which contained about 1.3% impurities.

DATA GIVEN BY THE MANUFACTURER

Identity IR: conforms

Ultraviolet absorption in methanol 242 nm

Assay HPLC 100.1%

Specific optical rotation = + 99° (in dioxan)

LOD 0.05%

Water = 0.2% (KF)

HPLC purity 254 nm 0.27% (cortisone 0.03%, prednisone 0.02%, hydrocortisone 0.16%, unknown 0.06%)
TLC purity < 0.1%

STABILITY

No special stability studies were performed as we considered this substance not to be of first priority regarding stability based on experience from earlier batches. Prednisolone, Control No 283029, showed no tendency of degradation when stored for 6 years at + 5 °C at the Centre.

CONCLUSION

Prednisolone, Control No 389029, can be considered suitable as International Chemical Reference Substance for the intended purpose. The content of prednisolone when used in the spectrophotometric assay is taken to be 100.0% calculated with reference to the dried substance which corresponds to 99.7% calculated on the "as is" basis.

PREDNISOLONE ACETATE

Control No 289030

Analytical Report

INTENDED USE

The stock of the current batch of the International Chemical Reference Substance for prednisolone acetate, Control No 167030, is depleted and has to be replaced.

The monograph for prednisolone acetate in the International Pharmacopoeia 3rd Ed. Vol 3 requires a reference substance to be used in the infrared spectrophotometric and thin-layer chromatographic tests for identity and in the spectrophotometric assay.

MATERIAL

About 50 g of the sample (manufacturers lot no H-2) were received at the WHO Centre in April 1988. The material is being stored protected from light in tightly closed containers at + 5 °C.

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (Control No 289030). The spectrum is concordant with the spectrum obtained from ICRS Control No 167030.

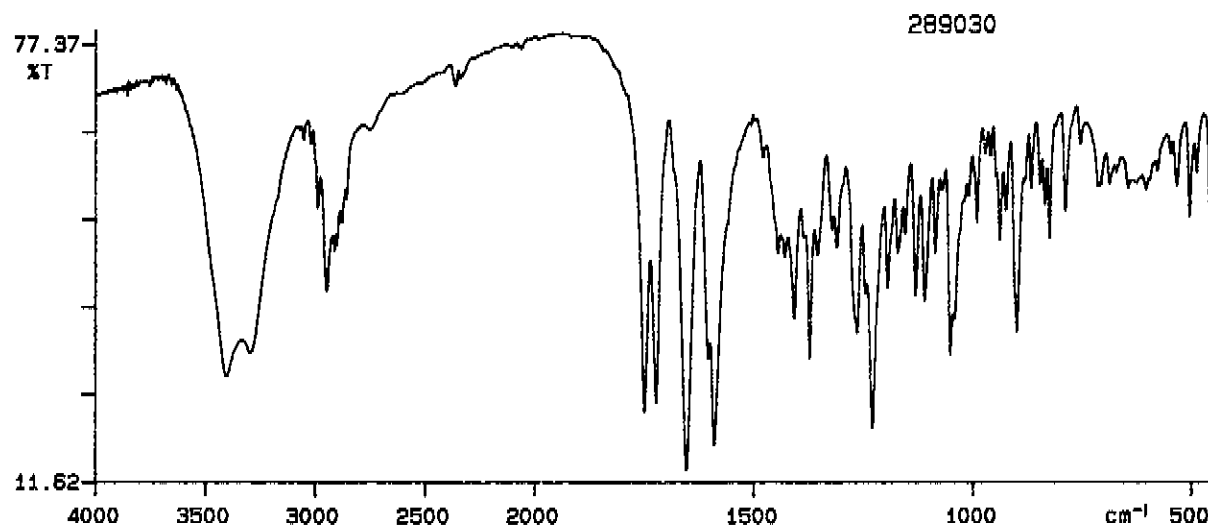


Figure 1. IR-spectrum of 1.5 mg of prednisolone acetate Control No 289030 in 300 mg KBr recorded against a KBr disc. Instrument: Perkin-Elmer 1600 FTIR.

UV-spectrum

A UV-spectrum in ethanol is given in Figure 2.

λ max in ethanol = 243 nm

E (1%, 1 cm) = 375 (n=9), s rel % = 1.6

The result is calculated with reference to the dried substance.

The absorbance of a 15 μ g/ml solution was 0.59.

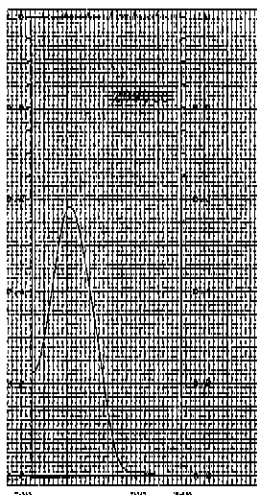


Figure 2. *UV-spectrum of prednisolone acetate Control No 289030 15 μ g/ml in ethanol.*

Thin-layer chromatography: See purity.

ASSAY

Spectrophotometric assay: 99.9% (n=9), s rel % = 1.5 determined according to Ph. Int. 3rd Ed. Vol 3, but using ethanol 95% instead of methanol. The ICRS Control No 167030 was used as reference and regarded as 100%. The result is calculated with reference to the dried substance.

Thermogravimetric analysis: When the substance was heated to 180 °C no loss of weight was observed (<0.1%).

| | |
|----------------------------|--|
| Instrument: | Perkin-Elmer TGA 7 Thermogravimetric analyzer. |
| Sample weight: | 7 mg |
| Heating rate: | 10 °C/minute |
| Decomposition temperature: | 230 °C |

Water: 0.08% determined by Karl Fischer titration.

PURITY

Total solid impurities

1) Differential Scanning Calorimetry (DSC): It was not possible to estimate the purity by this method as the substance melts with decomposition.

Thin-layer chromatography

The total amount of impurities was estimated to about 0.3%.

The following thin-layer chromatographic system was used:

System 1: According to the International Pharmacopeia 3rd Ed, Vol 2 (prednisolone).

Thin-layer: Silica gel 60, F-254 (Merck)

Eluent: Dichloromethane:Ether:Methanol:Water (77:15:8:1.2)

Sample: 5 µg, 15 µg and 105 µg of prednisolone acetate were applied.

The chamber was unsaturated.

Visualization: UV-light of 254 nm after heating to 105 °C for 10 minutes followed by evaluation by densitometry at 240 nm. Also spraying with blue tetrazolium/sodium hydroxide and evaluating visually in daylight.

One secondary extra spot with $R_f=0.7$ was detected visually at 254 nm and after spraying with blue tetrazolium when applying 105 µg. When applying 15 µg (the amount prescribed in the monograph) no secondary spot was detected. The detection limit of this system was about 0.1 µg (0.1%). When scanned at 240 nm by densitometry, the amount of impurities was estimated to about 0.3%.

R_f (prednisolone acetate) = 0.62

A comparison was made with ICRS Control No 167030. It showed two secondary spots estimated to about 2.4%.

It is also possible to use this system for identification in applying 5 µg prednisolone acetate ICRS.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.3%. A chromatogram is shown in Figure 3.

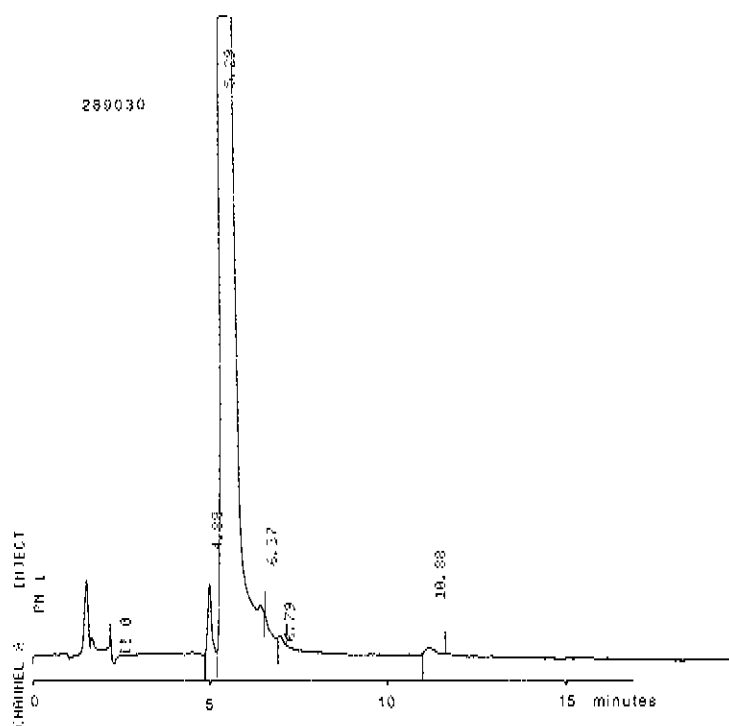


Figure 3. Chromatogram of prednisolone acetate Control No 289030.

The following conditions were used:

Eluent: Hexane/Dichloromethane/Methanol/Water (70.3+23.3+6.3+0.1)

Column: Spherisorb S5W, silica

Detector: Varian UV 200 operated at 240 nm

Pump: Varian 5560 operated at a flow rate of 1.8 ml/min

Integrator: Varian 4270

Attenuation: 4

Sample: 0.5 mg/ml dissolved in 2 ml of methanol and diluted to 10 ml with the eluent.
10 μ l corresponding to 5 μ g were injected.

DATA GIVEN BY THE MANUFACTURER

Identity IR: conforms (collaborator A)

Ultraviolet absorption in methanol 243 nm, E (1%, 1 cm) 375

Assay HPLC 99.9%

Specific optical rotation = + 114° (in dioxan)

LOD 0.1%

Water = 0.2% (KF)

HPLC purity 254 nm 0.1% (no prednisone was found)

TLC purity one spot (about 0.25%)

STABILITY

No special stability studies were performed as we considered this substance not to be of first priority regarding stability based on experience from earlier batches. Prednisolone acetate, Control No 167030, showed a very slight tendency of degradation when stored for 22 years at + 5 °C at the Centre.

CONCLUSION

Prednisolone acetate, Control No 289030, can be considered suitable as International Chemical Reference Substance for the intended purpose. The content of prednisolone acetate when used in the spectrophotometric assay is taken to be 99.9% calculated with reference to the dried substance which corresponds to 99.8% calculated on the "as is" basis.

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