



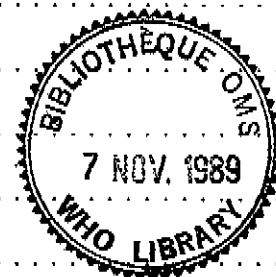
WHO COLLABORATING CENTRE FOR CHEMICAL REFERENCE SUBSTANCES

Report on the work in 1988

by M. Westermark

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Distribution of reference substances in 1988

During 1988 the total number of International Chemical Reference Substances distributed from the Centre were 1096 and 25 sets of Melting Point Reference Substances. The substances were distributed to drug control laboratories in 40 different countries. Compared to the figures for 1987 this corresponds to a decrease of about 46 per cent. The five most frequently requested substances during 1988 were in order of demand Ampicillin, Ampicillin trihydrate, Ergotamine tartrate, Digoxin, Benzylpenicillin sodium and Prednisone. Detailed figures for the distribution of the individual substances are given in Appendix 1.

Establishment of reference substances in 1988

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), 8 International Chemical Reference Substances were established in 1988. The substances are listed in Appendix 2 to this report. Allopurinol and Digoxin are replacement batches as the former stocks were depleted during 1988.

A complete list of all International Chemical Reference Substances available from the Centre in January 1989, with information about package sizes and control numbers for the current batches, is given in Appendix 3 to this report. The list also includes 6 substances mentioned below, which are expected to be formally adopted during the first half of 1989.

Work on new reference substances completed in 1988.

Work is being continued on new reference substances required to support specifications in the third edition of the International Pharmacopoeia. During 1988 one new reference substance for volume 3 was examined, it was Sodium cromoglicate. The analytical report for this material is given in Appendix 11. This substance was considered suitable for its intended use and was proposed for adoption as International Chemical Reference Substance.

The following five stocks of International Chemical Reference Substances were depleted and have been replaced by new batches during 1988. Ampicillin sodium No 274002 was replaced by No 388002, Dexamethasone No 279008 was replaced by No 388008, Dexamethasone acetate No 168009 was replaced by No 288009, 4-Epi-anhydrotetracycline hydrochloride No 180097 was replaced by No 288097 and Folic Acid No 277019 was replaced by No 388019.

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 1988-1989 the re-examination was performed on three penicillins.

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 40 new reference substances. Fifteen of these are already under development at the Centre. Older batches also have to be replaced because the stocks are depleted. At present 6 substances have to be replaced during 1989-1990 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 preparation are indicated with an asterisk.

During 1988 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 financial situation of the Centre remains unsatisfactory. The total cost for running the Centre in 1988 was estimated at 264.300 US\$. The income from sales of reference substances to industrial laboratories was about 28.000 US\$ and the contribution received from the WHO Headquarters was 16.000 US\$, which leaves a deficit of 220.300 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 Regional Offices have been approached for financial assistance.

Acknowledgements

The Centre want to express thanks to the laboratories that have contributed to our work during 1988, these are the National Biological Standards Laboratory in Canberra, Australia 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 American Cyanamid Company in Pearl River, USA; Beecham Pharmaceuticals in Worthing, England; Fisons in Leicestershire, England; Merck Sharp & Dohme in Rahway, USA and Takeda Chemical Industries Ltd in Osaka, Japan.

APPENDIX 1

DISTRIBUTION OF CHEMICAL REFERENCE SUBSTANCES IN 1988

Aceclidine salicylate	1	items	Dapsone	4	items
p-Acetamidobenzalazine	2	"	Desoxycortone acetate	2	"
Acetazolamide	1	"	Dexamethasone	2	"
Allopurinol	13	"	Dexamethasone acetate	4	"
2-Amino-5-nitrothiazole	--	"	Diazepam	7	"
3-Aminopyrazole-4-carbox- amide hemisulfate	1	"	Diazoxide	3	"
Amitriptyline hydrochloride	5	"	Dicloxacillin sodium	29	"
Ampicillin	72	"	Dicolinium iodide	--	"
Ampicillin sodium	21	"	Dicoumarol	--	"
Ampicillin trihydrate	62	"	Diethylcarbamazine dihydrogen citrate	2	"
Anhydrotetracycline hydro- chloride	27	"	Digitoxin	21	"
Atropine sulfate	12	"	Digoxin	38	"
Azathioprine	3	"	NN'-Di-(2,3-xylyl)anthra- niamide	--	"
Bendazol hydrochloride	--	"	Emetine hydrochloride	1	"
Benzobarbital	--	"	4-Epianhydrotetracycline hydrochloride	20	"
Benzylamine sulfate	1	"	4-Epitetracycline ammonium salt	24	"
Benzylpenicillin potassium	8	"	Ergometrine hydrogen maleate	14	"
Benzylpenicillin sodium	38	"	Ergotamine tartrate	43	"
Bephenium hydroxynaphthoate	--	"	Estradiol benzoate	15	"
Betamethasone	4	"	Estrone	8	"
Betanidine sulfate	--	"	Etacrynic acid	--	"
Bupivacaine hydrochloride	7	"	Ethambutol hydrochloride	4	"
Caffeine	4	"	Ethinylestradiol	5	"
Carbenicillin monosodium	15	"	Ethisterone	--	"
Chloramphenicol	23	"	Ethosuximide	1	"
Chloramphenicol palmitate	7	"	Etocarlide	--	"
Chloramphenicol palmitate (Polymorph A)	6	"	Flucytosine	1	"
5-Chloro-2-methylamino- benzophenone	2	"	Fluorouracil	6	"
2-(4-Chloro-3-sulfamoyl- benzoyl)benzoic acid	7	"	Fluphenazine decanoate dihydrochloride	--	"
Chlorphenamine hydrogen maleate	4	"	Fluphenazine enantate dihydrochloride	--	"
Chlorpromazine hydro- chloride	10	"	Fluphenazine hydrochloride	1	"
Chlortalidone	1	"	Folic acid	3	"
Chlortetracycline hydrochloride	--	"	Furosemide	8	"
Clomifene citrate	--	"	Griseofulvin	18	"
Clomifene citrate Z-isomer (Zuclomifene)	--	"	Haloperidol	3	"
Cloxacillin sodium	10	"	Hydrochlorothiazide	6	"
Cortisone acetate	3	"	Hydrocortisone	32	"
			Hydrocortisone acetate	7	"

(-)-3-(4-Hydroxy-3-methoxy-phenyl)-2-methylalanine	4 items	Phenytoin	4 items
Ibuprofen	6 "	Prednisolone	36 "
Imipramine hydrochloride	4 "	Prednisolone acetate	4 "
Indometacin	4 "	Prednisone	38 "
o-Iodohippuric acid	1 "	Prednisone acetat	-- "
Isoniazid	5 "	Procaine hydrochloride	17 "
Lanatoside C	-- "	Procarbazine hydrochloride	2 "
Levodopa	8 "	Progesterone	3 "
Lidocaine	4 "	Propicillin potassium	13 "
Lidocaine hydrochloride	12 "	Propranolol hydrochloride	-- "
Mefenamic acid	6 "	Propylthiouracil	2 "
Metazide	1 "	Pyridostigmine bromide	1 "
Methaqualone	2 "	Reserpine	-- "
Methyldopa	6 "	Riboflavin	15 "
Methyltestosterone	-- "	Sulfamethoxazole	6 "
Meticillin sodium	5 "	Sulfamethoxypyridazine	1 "
Metronidazole	9 "	Sulfanilamide	4 "
Nafcillin sodium	-- "	Testosterone propionate	2 "
Neostigmine metilsulfate	-- "	Tetracycline hydrochloride	17 "
Nicotinamide	11 "	Thioacetazone	2 "
Nicotinic acid	11 "	4,4'-Thiodianiline	2 "
Niridazole	-- "	Tolbutamide	5 "
Niridazole-chlorethyl-carboxamide	-- "	Tolnaftate	6 "
Norethisterone	-- "	Trimethadione	-- "
Norethisterone acetate	2 "	Trimethoprim	7 "
Ouabain	-- "	Trimethylguanidine sulfate	-- "
Oxacillin sodium	19 "	Tubocurarine chloride	1 "
Papaverine hydrochloride	3 "	Vitamin A acetate (solution) (à 25000 IU) (Retinol)	34 "
Phenethicillin potassium	1 "	Warfarin	5 "
Phenoxymethylpenicillin	9 "		
Phenoxymethylpenicillin calcium	3 "		
Phenoxymethylpencillin potassium	8 "		

Melting Point Reference Substances 25 x 13 substances.

APPENDIX 2

INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES ESTABLISHED IN 1988

Reference Substance	Control Number	Analytical Report	Remarks
Allopurinol	287049	WHO/PHARM/88.537 Appendix 6	Replaces No 172049
Chlortetracycline hydrochloride	187138	WHO/PHARM/88.537 Appendix 7	
Clomifene citrate	187136	WHO/PHARM/88.537 Appendix 8	
Clomifene citrate Z-isomer (Zuclomifene)	187137	WHO/PHARM/88.537 Appendix 9	
Digoxin	587011	WHO/PHARM/88.537 Appendix 10	Replaces No 377011
Emetine hydrochloride	187134	WHO/PHARM/88.537 Appendix 11	
Neostigmine metilsulfate	187135	WHO/PHARM/88.537 Appendix 12	
Propranolol hydrochloride	187139	WHO/PHARM/88.537 Appendix 13	

LIST OF AVAILABLE INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES

1989

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 65 73)

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	172054
Caffeine	100 mg	181102
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

	<u>Package size</u>	<u>Control Number</u>
Dicoumarol	100 mg	178077
Diethylcarbamazine dihydrogen citrate	100 mg	181100
Digitoxin	100 mg	277010
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
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

	<u>Package size</u>	<u>Control Number</u>
Methyldopa	100 mg	179084
Methyltestosterone	100 mg	167023
Meticillin sodium	200 mg	274024
Metronidazole	100 mg	183118
Nafcillin sodium	200 mg	272025
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
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	283029
Prednisolone acetate	100 mg	167030
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

	<u>Package size</u>	<u>Control Number</u>
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 1988-1989 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.

Ampicillin trihydrate, Control No 274003

Initial analytical report: WHO/PHARM/75.485, Appendix 6

Examination year:	1974	1978	1981	1982	1984	1989
KF, %	13.9	-	13.9	13.5	13.3	-
TGA, %	-	-	-	-	-	13.9
HPLC, %	-	0.3	0.6	0.3	0.9	0.3
Assay, % (mercurimetric)	-	-	-	-	98.6	-
Degradation products, % (mercurimetric)	-	-	-	-	0.9	-
Assay, % (penicillinase)	98.5	-	99.0	-	-	-
PSA, %	1.0	-	-	-	-	-
pH, 0.25% solution	5.1	-	5.1	5.1	5.1	-

Cloxacillin sodium. Control No 274005

Initial analytical report WHO/PHARM/75.485, Appendix 7

Examination year:	1974	1978	1979	1982	1984	1989
KF, %	4.2	-	4.0	3.8	4.0	-
TGA, %	-	-	-	-	-	4.2
HPLC,	-	0.5	-	0.9	1.0	0.6
Assay, % (mercurimetric)	-	-	-	-	98.9	-
Degradation products, % (mercurimetric)	-	-	-	-	0.7	-
Assay, % (alkalimetric)	100.2	-	99.1	100.2	-	-
pH, 2 % solution	-	-	-	5.9	5.9	-
pH, 10% solution	-	-	-	6.2	-	-

Dicloxacillin Sodium. Control No 174071

Initial analytical report: WHO/PHARM/75.485, Appendix 5

Examination year:	1974	1982	1984	1989
KF, %	3.8	3.9	3.8	-
TGA, %	-	-	-	3.9
HPLC, %	-	0.3	0.4	0.3
Assay, % (mercurimetric)	-	-	99.5	-
Degradation products, % (mercurimetric)	-	-	0.6	-
Assay, % (alkalimetric)	99.5	99.4	-	-
pH, 1% solution	5.6	5.9	5.8	-

APPENDIX 5

INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES - PROJECT LIST 1989

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

Carbamazepine (*)

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-hydra-
zino-2-methylalanine (impurity in Carbidopa)

Levonorgestrel

Levothyroxine sodium (*)

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

Oxytetracycline dihydrate (*)

Oxytetracycline hydrochloride (*)

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 1989-1990

p-Acetamidobenzalazine (*)

Ampicillin (*)

Anhydrotetracycline hydrochloride (*)

Bupivacaine hydrochloride (*)

Prednisolone (*)

Prednisolone acetate (*)

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

APPENDIX 6

AMPICILLIN SODIUM

Control No 388002

Analytical Report

INTENDED USE

The stock of the current batch of the International Chemical Reference Substance for ampicillin sodium, Control No 274002, is depleted and has to be replaced.

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

Note: According to the monograph the International Chemical Reference Substance for Ampicillin is used as reference substance in the assay of ampicillin sodium.

MATERIAL

About 200 g of the sample (manufacturers batch No AS 49) were received at the WHO Centre in October 1988. The material is being stored protected from light and moisture 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 (388002). The spectrum is concordant with the spectrum obtained from the ICRS, Control No 274002.

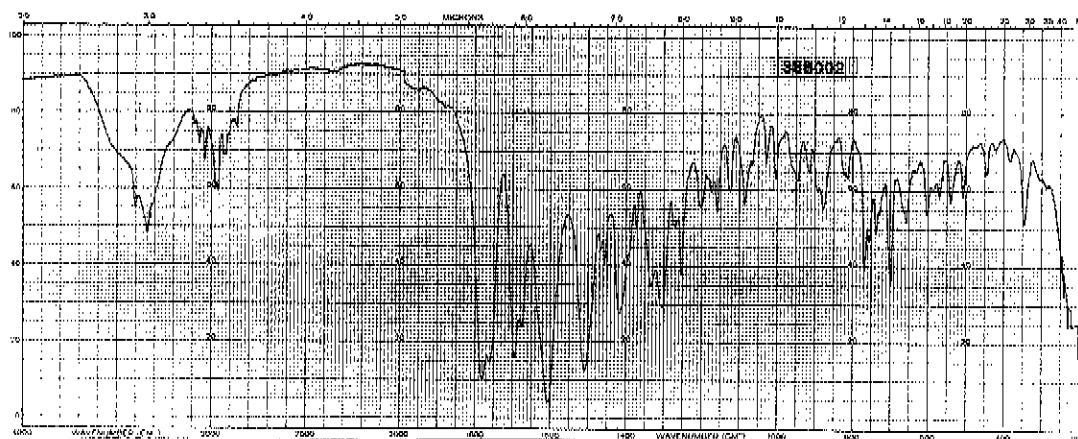


Figure 1. IR-spectrum of 1.2 mg of ampicillin sodium, Control No 388002 in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 580.

Specific optical rotation: $[\alpha]_D^{20} = + 261.7^\circ$ ($n = 8$). Determined in water at a concentration of 5 mg/ml. The result is calculated with reference to the dried substance.

UV-spectrum

A UV-spectrum in phosphate buffer pH 6.9 is given in Figure 2.

λ max in phosphate buffer = 256.5, 261 and 267.5.

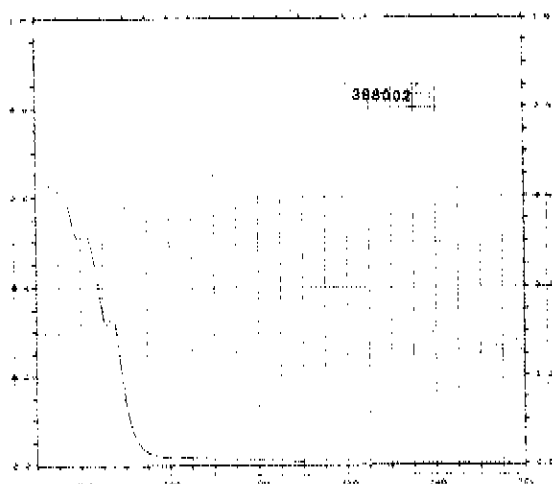


Figure 2. UV-spectrum of ampicillin sodium,

ASSAY

Titrimetric assay: 96.0% ($n = 5$), s rel % = 0.33

Determined by mercurimetric titration with 0.02 M mercuric nitrate according to Ph. Eur 2nd Ed. The result is calculated with reference to the dried substance.

Thermogravimetric analysis: When the substance was heated to 195° C a loss of 0.5% of weight was observed (n = 3).

Instrument: Perkin-Elmer TGA 7 Thermogravimetric analyzer.
Sample weight: 5 mg
Heating rate: 10° C/minute
Decomposition temperature: 205° C

Water: 0.5% determined by Karl Fischer titration.

PURITY

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 3.

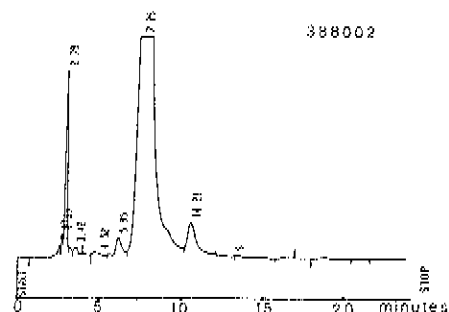


Figure 3. Chromatogram of ampicillin sodium, Control No 388002.

The following conditions were used:

Eluent: Acetonitrile/Phosphate buffer pH 6.8 (12:88)
Column: Spherisorb S5C8
Detector: Shimadzu SPD-2A operated at 225 nm
Pump: Waters 600 Multisolvent Delivery System operated at a flow rate of 1 ml/min
Integrator: Hewlett Packard 3390 A . Attenuation: 4
Sample: 1 mg/ml dissolved in the eluent. 20 µl corresponding to 20 µg were injected.

A comparison was made with ICRS, Control No 274002 which contained about 3.5% impurities and EPCRS Lot 1 which contained about 0.4% 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. An isogram is given in Figure 4.

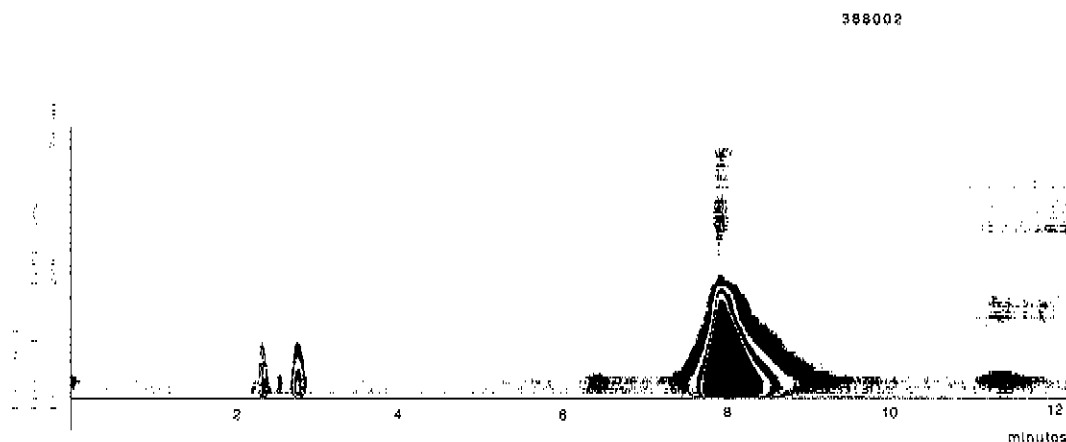


Figure 4. Isogram of ampicillin sodium, Control No 388002.

Sensitivity: 0.02

As seen from the figure ampicillin sodium has an absorbance maximum at 200 nm. Five impurities were detected eluting after 2.2, 2.3, 2.5, 6.2 and 11 minutes with absorbance maxima at 200 nm. They were all visible at 225 nm used in the purity method described above.

DATA GIVEN BY THE MANUFACTURER

Identification: Passes test

Assigned potency: 90.5% free acid which corresponds to 96.2% ampicillin sodium.

STABILITY

No special stability studies were performed as we have good experience of the stability of this substance from earlier batches. Ampicillin sodium, Control No 274002 showed no tendency of degradation when stored for 10 years protected from light and moisture at +5° C at our Centre.

CONCLUSION

Ampicillin sodium, Control No 388002, can be considered suitable as International Chemical Reference Substance for the intended purpose.

DEXAMETHASONE

Control No 388008

Analytical Report

INTENDED USE

The stock of the current batch of the International Chemical Reference Substance for dexamethasone, Control No 279008, is depleted and has to be replaced.

The monograph for dexamethasone in the International Pharmacopeia 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 (blue tetrazolium).

MATERIAL

About 50 g of the sample (manufacturers batch No L-574, 385-000F053) were received at the WHO Centre in September 1987. The material is being stored protected from light and moisture 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 (388008). The spectrum is concordant with the spectrum obtained from the ICRS, Control No 279008.

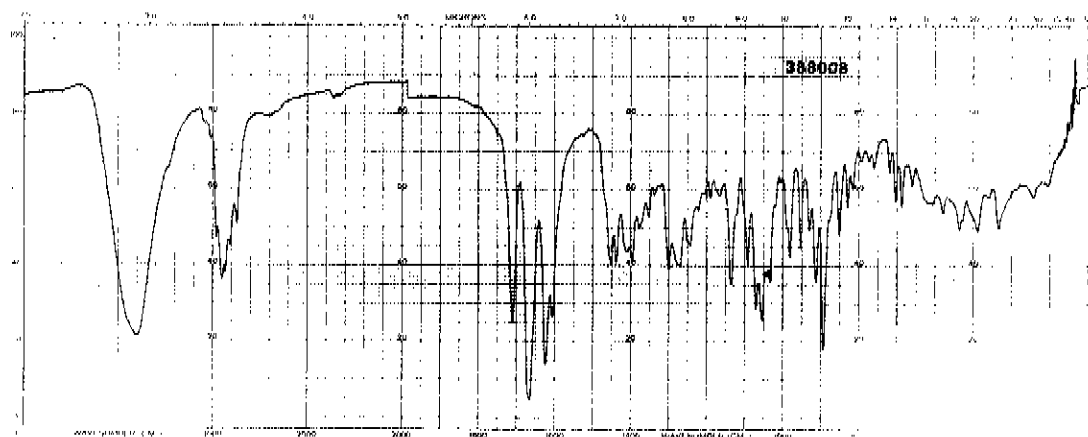


Figure 1. IR-spectrum of 1.3 mg of dexamethasone, Control No 388008 in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 580.

Specific optical rotation: $[\alpha]_D^{20^\circ\text{C}} = +76.6^\circ$ (n = 3). Determined in dioxan at a concentration of 10 mg/ml. The result is calculated with reference to the dried substance.

UV-spectrum

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

λ max in methanol = 239 nm

E (1%, 1 cm) = 391 (n = 4)

The result is calculated with reference to the dried substance.

The absorbance of a 10 $\mu\text{g/ml}$ solution was 0.40.

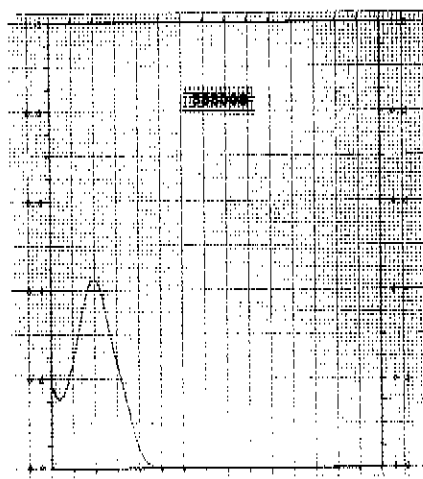


Figure 2. UV-spectrum of dexamethasone, Control No 388008 10.6 $\mu\text{g/ml}$ in methanol.

ASSAY

Spectrophotometric assay: 99.9% (n = 4), s rel % = 0.8 determined according to Ph. Int. 3rd Ed. Vol 2. The ICRS, Control No 279008 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 0.15% was observed.

Instrument:	Perkin-Elmer TGA 7 Thermogravimetric analyzer.
Sample weight:	14 mg
Heating rate:	10° C/minute
Decomposition temperature:	268° C

Loss on drying

0.16% (100° C, vacuo) (n = 3)

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.

According to Cohen, Analytical Profiles of Drug Substances, Vol. 2, two crystal forms of dexamethasone have been observed. Of the two forms form B is the usually observed one. The DSC curves of the proposed ICRS, Control No 388008 and the previous ICRS, Control No 279008 were run from 40° C to 285° C using a heating rate of 10 ° per minute. Instrument: Perkin-Elmer DSC 7 Differential Scanning Calorimeter.

The thermograms did not show any other thermal events than the melting endotherm. The melting point (onset) and the peak maxima are given in the following table:

	Melting point (onset)	Peak maximum
ICRS, Control No 388008	264 °	271 °
ICRS, Control No 279008	260 °	267 °

The two reference materials are probably of the same crystalline form (cp also IR).

Thin-layer chromatography

The total amount of impurities was estimated to about 0.9%.

The following thin-layer chromatographic system was used:

- System 1: According to 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: 100 µg of dexamethasone 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 day-light, and evaluation by densitometry at 525 nm.

A chromatogram is shown in Figure 3.

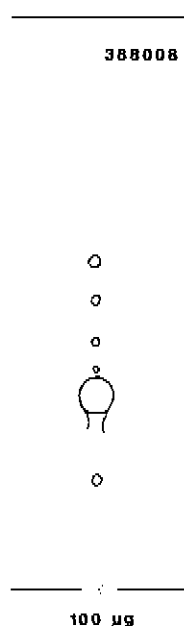


Figure 3. Thin-layer chromatogram of dexamethasone, Control No 388008. Visualization by spraying with blue tetrazolium.

Two secondary spots with $R_f = 0.38$ and $R_f = 0.57$ were detected visually at 254 nm. When evaluating by densitometry at 240 nm five secondary spots were detected their total amount was estimated to 0.8%. The detection limit of this system was about 0.1 µg (0.1%) when scanned at 240 nm.

R_f (dexamethasone) = 0.34

After spraying five secondary spots were detected, they were estimated to 0.9% after scanning at 525 nm.

A comparison was made with ICRS, Control No 279008. It showed the same impurity pattern i.e 0.8 - 0.9%. The EPCRS showed about 0.8% impurities.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 1.0%. A chromatogram is shown in Figure 4.

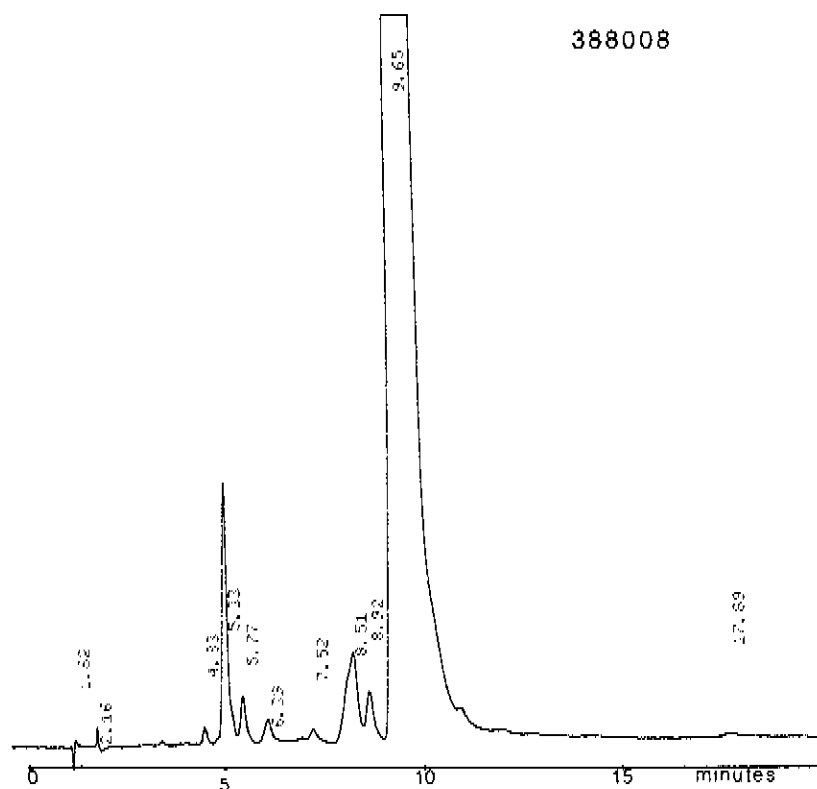


Figure 4. Chromatogram of dexamethasone, Control No 388008.

The following conditions were used:

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

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

Detector: Varian UV 200 operated at 240 nm

Pump: Varian 5560 operated at a flow rate of 1 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 ICRS, Control No 279008 which also contained 1% impurities, and with the USP reference substance Lot G which contained about 0.8% impurities.

DATA GIVEN BY THE MANUFACTURER

Ultraviolet absorption A (1%, 1 cm) 239 nm = 391, dried basis
Assay (HPLC) 99.7% reversed phase
TLC Major spot Rf = 0.3. Unknown minor Rf = 0.45
Specific optical rotation +76.2 °
LOD 0.14%
IR conforms
Phase solubility analysis: 99.4% pure

STABILITY

No special stability studies were performed as we have good experience of the stability of this substance from earlier batches. Dexamethasone, Control No 279008 showed no tendency of degradation when stored for 9 years at +5° C at the Centre.

CONCLUSION

Dexamethasone, Control No 388008, can be considered suitable as International Chemical Reference Substance for the intended purpose. The content of dexamethasone when used in the spectrophotometric (blue tetrazolium) assay is taken to be 100% calculated with reference to the dried substance which corresponds to 99.8% calculated on the "as is" basis.

APPENDIX 8

DEXAMETHASONE ACETATE

(MONOHYDRATE)

Control No 288009

Analytical Report

INTENDED USE

The stock of the current batch of the International Chemical Reference Substance for dexamethasone acetate, Control No 168009, is depleted and has to be replaced.

The monograph for dexamethasone acetate 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 (blue tetrazolium).

MATERIAL

About 50 g of the sample (manufacturers batch No L-573, 274-0003G006) were received at the WHO Centre in september 1987. The material is being stored protected from light and moisture in tightly closed containers at + 5° C.

ANALYTICAL DATA

Description: A white, odourless powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum in KBr is given in Figure 1 (288009A). The spectrum is neither concordant with the spectrum obtained from a sample from Ph Eur, which is also a monohydrate, nor with the ICRS, Control No 168009, which is the anhydrous form. For identification the substance had to be recrystallized in 99% ethanol which gave identical spectra for the sample from Ph Eur (recrystallized) and for ICRS 168009 (recrystallized), see Figure 2.

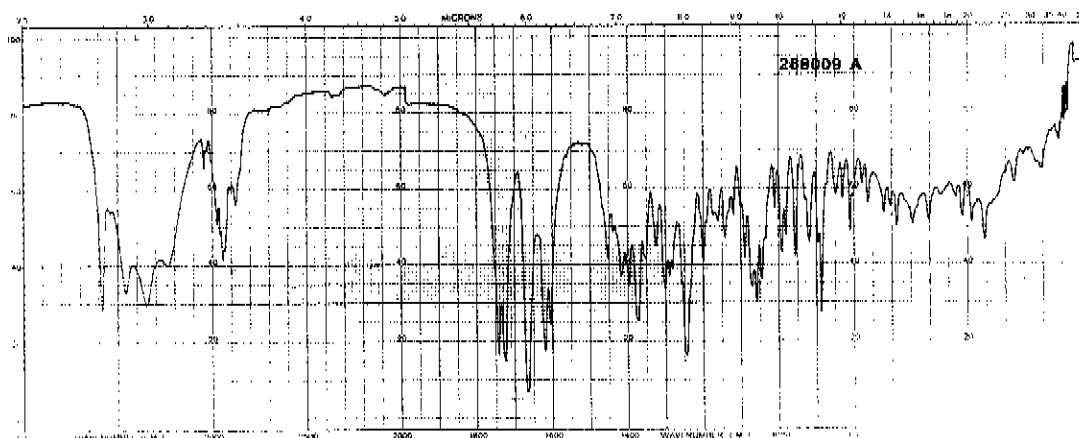


Figure 1. IR-spectrum of 1.2 mg of dexamethasone acetate (monohydrate) 288009A in 300 mg KBr recorded against a KBr disc. Instrument: Perkin-Elmer 580.

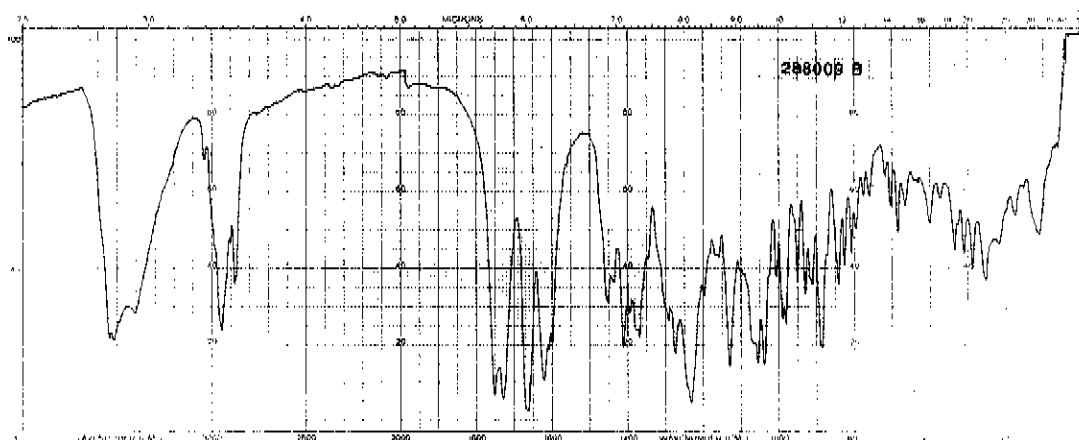


Figure 2. IR-spectrum of 1.1 mg of dexamethasone acetate (monohydrate) recrystallized in 99% ethanol, 288009B in 300 mg KBr recorded against a KBr disc. Instrument: Perkin-Elmer 580.

Specific optical rotation: $[\alpha]_D^{20^\circ} = +86.7^\circ$ ($n=8$). Determined in dioxan at a concentration of 10 mg/ml. The result is calculated with reference to the dried substance.

UV-spectrum

A UV-spectrum in ethanol 99.5% is given in Figure 3.

λ max in ethanol = 238 nm

E (1%, 1 cm) = 347 ($n = 5$), s rel % = 1.3

The result is calculated with reference to the dried substance.

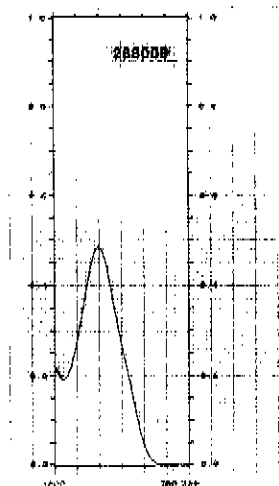


Figure 3. UV-spectrum of dexamethasone acetate, Control No 288009, 14 µg/ml in ethanol.

ASSAY

Spectrophotometric assay: 99.6% (n=6), s rel % = 1.5 determined according to Ph. Int. 3rd Ed. Vol 2. A sample of dexamethasone acetate from Ph Eur (containing 3.6% water) was used as reference and regarded as 100%. The result is calculated with reference to the dried substance. This corresponds to 96.0% dexamethasone acetate when calculated on "as is" basis.

Thermogravimetric analysis: When the substance was heated to 160° C a loss of 3.6% of weight was observed.

Instrument:	Perkin-Elmer TGA 7 Thermogravimetric analyzer.
Sample weight:	3 mg
Heating rate:	10° C/minute
Melting temperature:	216° C

The former dexamethasone acetate, Control No 168009, only lost 0.03% of weight, as it is the anhydrous form.

Loss on drying: 3.8% (100° C, reduced pressure).

PURITY

Thin layer chromatography

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

The following thin-layer chromatographic system was used:

System:	According to the International Pharmacopoeia 3rd Ed, Vol 2.
Thin-layer:	Silica gel 60, F-254 (Merck)
Eluent:	Dichloromethane : ether : methanol : water (77:15:8:1.2)
Sample:	200 µg of dexamethasone acetate were applied.

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 day-light, and evaluation by densitometry at 525 nm.

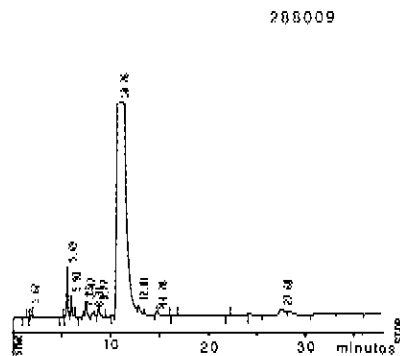
Two secondary spots with $R_f=0.33$ and $R_f=0.58$ were detected. When evaluating by densitometry at 240 nm their total amount was estimated to 0.3%. The detection limit of this system was about 0.1 µg (0.05%) when scanned at 240 nm. R_f (dexamethasone acetate) = 0.46.

After spraying with blue tetrazolium two secondary spots were detected, estimated to about 0.3% after scanning at 525 nm.

A comparison was made with ICRS, Control No 168009. It contained two impurities estimated to 1%.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.7%. The same results were obtained with both a straight phase system and a reversed phase system. Chromatograms are shown in Figure 4 (straight phase) and Figure 5 (reversed phase).



The following conditions were used:

Eluent: Hexane/Dichloromethane/Methanol/Water (72.5+24.0+3.4+0.1) (straight phase)
Eluent: Acetonitrile/Water (47+53) (reversed phase)
Column: Spherisorb S5W, silica (straight phase)
Column: Spheri-5 RP-18, Brownlee (reversed phase)
Detector: Shimadzu SPD-2A operated at 240 nm
Pump: Waters 600 Multisolute Delivery System operated at a flow rate of 1.8 ml/min (straight phase) 1.0 ml/min (reversed phase).
Integrator: Hewlett Packard 3390 A Attenuation: 4
Sample: 1 mg/ml dissolvent in the eluent. 20 µl corresponding to 20 µg were injected.

A comparison was made with ICRS, Control No 168009 which contained about 2% impurities (straight phase)

DATA GIVEN BY THE MANUFACTURER

UV A 1%, 1 cm, 239 nm = 357
Assay HPLC 100.2% w/w, 99.7% by area (reversed phase)
TLC about 0.1%
LOD 3.93%
Specific rotation +86.6 °
IR conforms
PSA 99.0%

STABILITY

No special stability studies were performed as we have good experience of the stability of this substance from earlier batches. Dexamethasone acetate, Control No 168009, showed only a slight tendency of degradation when stored for 16 years at + 5° C at our Centre.

CONCLUSION

Dexamethasone acetate, Control No 288009, can be considered suitable as International Chemical Reference Substance for the intended purpose. The content of dexamethasone acetate when used in the spectrophotometric (blue tetrazolium) assay is taken to be 99.6%, calculated with reference to the dried substance, which corresponds to 96.0% calculated on the "as is" basis.

APPENDIX 9

4-EPIANHROTETRACYCLINE HYDROCHLORIDE

Control No 288097

Analytical Report

INTENDED USE

The stock of the current batch of the International Chemical Reference Substance for 4-epianhydrotetracycline hydrochloride, Control No 180097, is depleted and has to be replaced.

The monograph for tetracycline hydrochloride in the International Pharmacopeia 3rd Ed. Vol 2 requires a reference substance for 4-epianhydrotetracycline hydrochloride to be used in the thin-layer chromatographic test for related substances.

MATERIAL

About 25 g of the sample (manufacturers batch No 11863B-60) were received at the WHO Centre in March 1988. The material is being stored protected from light and moisture in tightly closed containers at + 5° C.

ANALYTICAL DATA

Description: Orange-yellow powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (288097). The spectrum is concordant with the spectrum obtained from the ICRS Control No 180097.

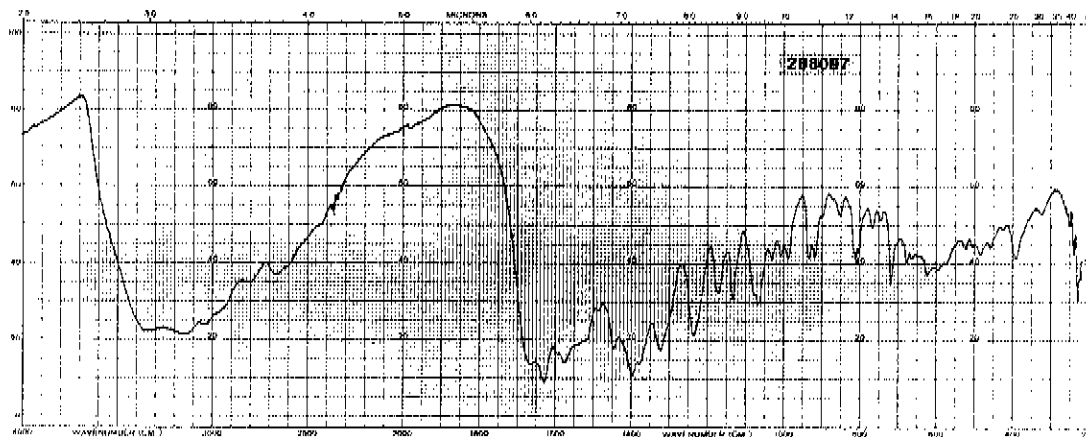


Figure 1. IR-spectrum of 0.9 mg of 4-epianhydrotetracycline hydrochloride, Control No 288097 in 300 mg KBr recorded against a KBr disc. Instrument: Perkin-Elmer 580.

Ultraviolet - visible spectrum

UV - and visible spectra in 0.01 M hydrochloric acid in Figure 2 A and B.

λ max in 0.01 M hydrochloric acid = 223 nm, 273 nm and 429 nm.

E (1%, 1 cm) = 479, 917 and 153 ($n=2$)

The result is calculated with reference to the anhydrous substance.

The absorbance of a 45 μ g/ml solution was 0.61 at 429 nm.

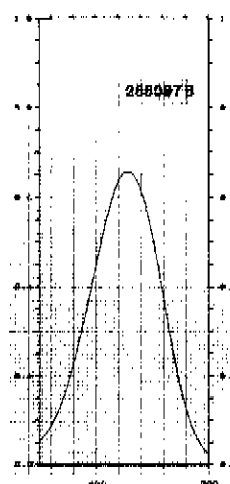
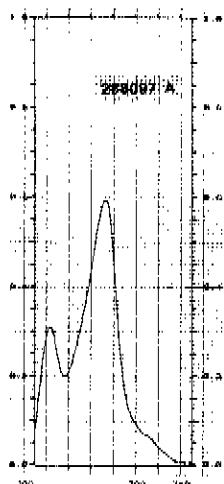


Figure 2 A and B. UV-spectrum (A), visible spectrum (B) of 4-epianhydrotetracycline hydrochloride, Control No 288097, in 0.01 M hydrochloric acid.

ASSAY

Assay: See collaborating laboratories and manufacturer.

Thermogravimetric analysis: When the substance was heated to 180° C a loss of 5.4% of weight was observed.

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

Water: 5.4% determined by Karl Fischer titration.

PURITY

Thin-layer chromatography

The principal spot in the chromatogram obtained with the International Chemical Reference Substance, Control No 288097, corresponded in position, intensity and appearance to the main spot in the chromatogram obtained with ICRS, Control No 180097.

The following thin-layer chromatographic system was used:

- System: According to the International Pharmacopoeia 3rd Ed. Vol 2, with minor modifications.
- Thin-layer: Cellulose "Avicel" (Merck) impregnated with a solution of disodium edetate (0.1 mol/l) VS adjusted to pH = 7.0 with sodium hydroxide (80 g/l) TS. The plate was allowed to develop for a distance of about 18 cm and dried at 50° C for 30 minutes.
- Eluent: Ethyl acetate : acetone : water (60:30:6).
- Sample: 5 and 10 µg of 4-epianhydrotetracycline hydrochloride were applied. After application the plate was sprayed very finely and uniformly with a 5% solution of trimethylpyridine TS until traces of humidity appeared (about 4 ml on a readymade plate). It is very important that the trimethylpyridine is stored at 4° C and that it is sprayed immediately while still cold, as it is only miscible with water at about 4° C. After developing and drying in air the plate was shortly exposed to the vapour of ammonia (260 g/l) before examination.
- Visualization: UV-light of 365 nm, evaluation by densitometry at 365 nm with a Desaga Densitometer CD 60.
- Result: One extra spot corresponding to anhydrotetracycline hydrochloride with R_f about 0.9 was detected by visual inspection. When evaluating by densitometry at 365 nm it was estimated to about 5-6%, however this figure is probably too high due to on-plate epimerization. R_f for 4-epianhydrotetracycline hydrochloride is about 0.2.

At a loading of 0.05 µg, which is the amount prescribed in the monograph for tetracycline hydrochloride, no secondary spot was visible.

High performance liquid chromatography

Two different liquid chromatographic systems were tested. System 1 according to USP with some modifications (another column).

One major impurity was found. It was estimated to about 3.9% by peak area measurement and identified as anhydrotetracycline. A chromatogram is shown in Figure 3.

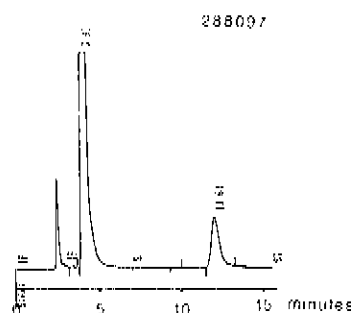


Figure 3. Chromatogram of 4-epianhydrotetracycline hydrochloride, Control No 288097.

The following conditions were used:

Eluent: 0.1 M ammonium oxalate : 0.2 M dibasic ammonium phosphate : dimethylformamide (65+5+30), pH =7.6.

Column: Vydac 218 TP 54 (250 mm x 4.6 mm)

Detector: Shimadzu SPD-2A operated at 273 nm.

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

Integrator: Hewlett Packard 3390 A. Attenuation: 5.

Sample: 0.1 mg/ml dissolved in the eluent. 20 µl corresponding to 2 µg were injected. It is important to inject freshly prepared solutions as rapid epimerization takes place.

A comparison was made with ICRS, Control No 180097 which contained about 4.2% anhydrotetracycline hydrochloride and with EPCRS-I, which contained about 4.4% anhydrotetracycline hydrochloride.

System 2 developed at the WHO Centre:

One major impurity was found. It was estimated to about 4.7% by peak area measurement and identified as anhydrotetracycline hydrochloride. Two chromatograms showing results from eluents with phosphate buffers of different pH are shown in Figure 4 A (pH = 2.4) and 4 B (pH = 7.4).

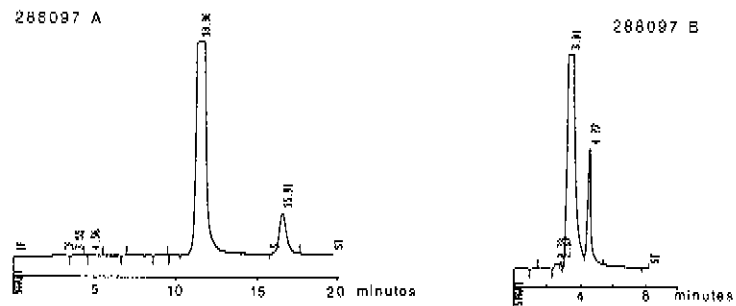


Figure 4 A and B. Chromatograms of 4-epianhydrotetracycline hydrochloride, Control No 288097.

The following conditions were used:

Eluent: Acetonitrile : Phosphate buffer pH = 2.4 or 7.4 (20:80)

Column: Vydac 218 TP 54 (250 mm x 4.6 mm)

Detector: Shimadzu SPD-2A operated at 271 nm.

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

Integrator: Hewlett Packard 3390 A.

Attenuation: 6

Sample: 0.1 mg/ml dissolved in the eluent. 20 μ l corresponding to 2 μ g were injected. It is important to inject freshly prepared solutions as rapid epimerization takes place.

A comparison was made with ICRS, Control No 180097, which contained about 4.5% anhydro-tetracycline hydrochloride.

Chromatograms showing the selectivity and retention order for other tetracyclines is shown in Figure 5 A (System 1) and 5 B (System 2 A, pH 2.4).

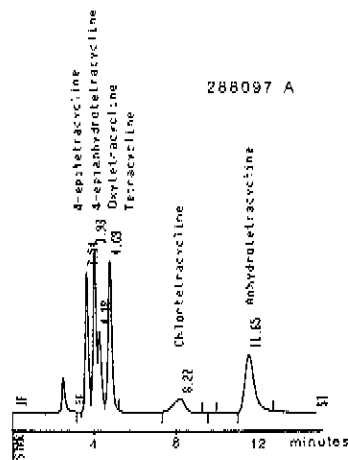


Figure 5 A. Chromatogram of 4-epitetraacycline, 4-epianhydrotetraacycline, oxytetraacycline, tetracycline, chlortetraacycline and anhydrotetraacycline (pH 7.6).

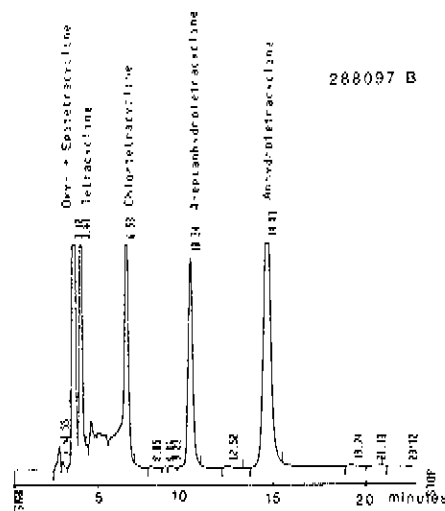


Figure 5 B. Chromatogram of 4-epitetraacycline together with oxytetraacycline, tetracycline, chlortetraacycline, 4-epianhydrotetraacycline and anhydrotetraacycline. pH of phosphate buffer = 2.4.

DATA GIVEN BY COLLABORATING LABORATORIES

USP

Water: 5.0% Karl Fischer titration

Assay: 99.6% potentiometric titration

HPLC: 98.6% 1.5% anhydrotetraacycline

TLC: No impurities detected (on-plate epimerization to anhydrotetraacycline).

DATA GIVEN BY THE MANUFACTURER

HPLC + Titration Potency 99.9%.

STABILITY

No special stability studies were performed as we have good experience of the stability of this substance from earlier batches. 4-epianhydrotetracycline hydrochloride, Control No 180097 showed only a slight tendency of degradation when stored for 5 years at + 5° C at our Centre.

CONCLUSION

4-Epianhydrotetracycline hydrochloride, Control No 288097, can be considered suitable as International Chemical Reference Substance for the intended purpose.

FOLIC ACID

Control No 388019

Analytical Report

INTENDED USE

The stock of the current batch of the International Chemical Reference Substance for folic acid, Control No 277019, is depleted and has to be replaced.

The monograph for folic acid in the International Pharmacopeia 3rd Ed. Vol 2 requires a reference substance to be used in the thin-layer chromatographic test for identity and in the spectrophotometric assay.

MATERIAL

About 100 g of the sample (manufacturers batch No one 004) were received at the WHO Centre in March 1988. The material is being stored protected from light in tightly closed containers at + 5° C.

ANALYTICAL DATA

Description: A yellowish - orange, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (388019). The spectrum is concordant with the spectrum obtained from the USP reference substance Lot K.

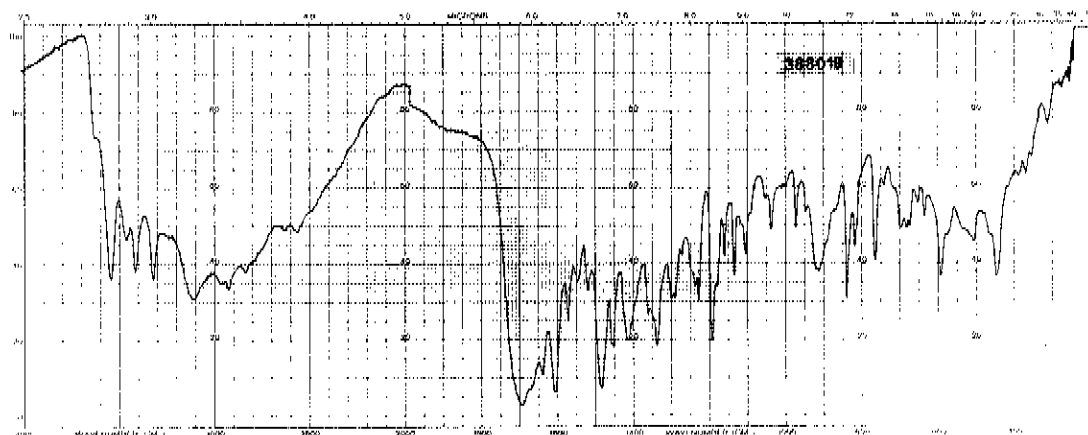


Figure 1. IR-spectrum of 0.9 mg of folic acid, Control No 388019, in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 580.

Specific optical rotation: $[\alpha]_D^{20^\circ C} = + 20^\circ$ (n=3). Determined in 0.1 M sodium hydroxide at a concentration of 5 mg/ml. The result is calculated with reference to the anhydrous substance.

UV-spectrum

A UV-spectrum in 0.1 M sodium hydroxide is given in Figure 2.

λ max in 0.1 M sodium hydroxide = 256, 283 and 365 nm

E (1%, 1 cm) = 577, 570 and 200 (n=4).

The results are calculated with reference to the anhydrous substance.

The absorbances of a 15 μ g/ml solution were 0.82, 0.80 and 0.28 respectively at 256, 283 and 365 nm. The values were calculated on the "as is" basis.

The ratio between the absorbances at 256 nm to that of 365 nm is 2.88.

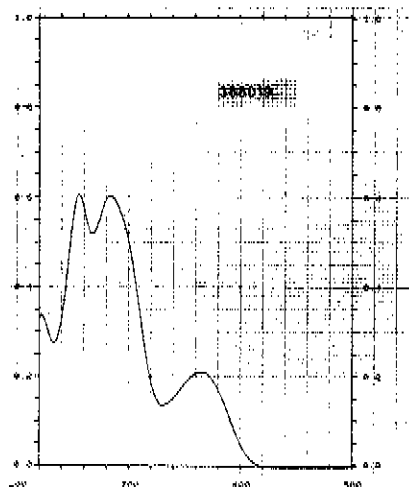


Figure 2. UV-spectrum of folic acid, Control No 388019, 10.6 μ g/ml in 0.1 M sodium hydroxide.

ASSAY

Spectrophotometric assay: 100.0% (n=3), s rel % = 0.3 determined according to Ph. Int. 3rd Ed. Vol 2, but the dilutions were modified according to the same method in Ph. Eur. 2nd Ed. USP reference substance Lot K was used as reference and regarded as 100%. The result is calculated with reference to the anhydrous substance.

Thermogravimetric analysis: When the substance was heated to 230° C a loss of weight estimated to 7.7% was observed (n=3).

Instrument: Perkin Elmer TGA 7 Thermogravimetric analyzer.
Sample weight: 4 mg
Heating rate: 10° C/minute

Water: 7.7% (n=5) determined by Karl Fischer titration. It was not easy to determine water by this method as the substance is difficult to dissolve. Methanol was used here, and several determinations with small amounts of sample were performed.

PURITY

Thin layer chromatography

The following thin-layer chromatographic systems were used:

System 1: According to the International Pharmacopeia 3rd Ed, Vol 2.
Thin-layer: Silica gel 60, F-254 (Merck)
Eluent: n-propanol: ethanol 95%: ammonia (269 g/l) (2+1+2)
Sample: 25 µg of folic acid were applied. The sample was dissolved in a mixture of 1 volume of ammonia (260 g/l) and 9 volumes of methanol.
Visualization: UV-light of 365 nm.
Result: Only one impurity with $R_f=0.6$ was found. R_f (folic acid)=0.55.

System 2: According to Ph. Eur. 2nd Ed.
Thin-layer: Silica gel 60, F-254 (Merck)
Eluent: n-propanol: ethanol 95%: ammonia (260 g/l) (20+60+20)
Sample: 10 and 25 µg of folic acid were applied. The sample was dissolved as described under system 1.
Visualization: UV-light of 365 nm.
Result: Two faint secondary spots with $R_f=0.09$ and 0.45 were detected.
 R_f (folic acid) = 0.18.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.1%. A chromatogram is shown in Figure 3.

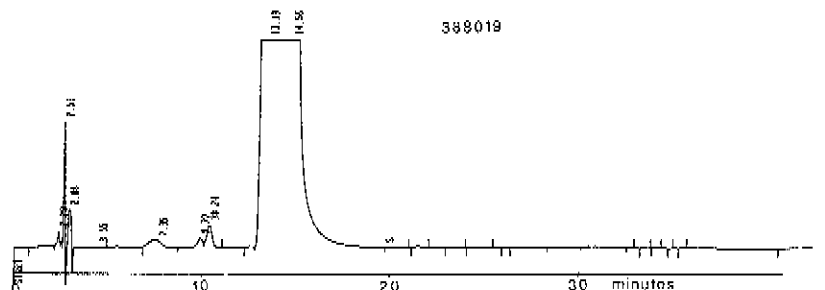


Figure 3. Chromatogram of folic acid, Control No 388019.

The following conditions were used:

Eluent: Dissolve 35.1 g of sodium perchlorate monohydrate, 1.4 g of monobasic potassium phosphate and 7.0 ml of 1 M sodium hydroxide in 900 ml of water. Add 70 ml of methanol adjust pH to 7.2 with 1 M sodium hydroxide and dilute with water to 1000 ml.

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

Detector: Shimadzu SPD-2A operated at 254 nm.

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

Integrator: Hewlett Packard 3390 A

Attenuation: 4

Sample: 1 mg/ml dissolved in an aqueous solution containing 2 ml ammonia and 1 g sodium perchlorate per 100 ml. 20 μ l corresponding to 20 μ g were injected.

DATA GIVEN BY THE MANUFACTURER

Yellowish orange crystalline powder

Free pteridines 0.01%

Free amine 0.09%

Water 7.7%

Assay (HPLC-USP) 100.0%

Residue on ignition 0.02%

STABILITY

No special stability studies were performed as we have good experience of the stability of this substance from earlier batches. Folic acid, Control No 277019, showed no tendency of degradation when stored for 8 years at + 5° C at the Centre.

CONCLUSION

Folic acid, Control No 388019, can be considered suitable as International Chemical Reference Substance for the intended purpose. The content of folic acid when used in the spectrophotometric assay is taken to be 100.0% calculated with reference to the anhydrous substance, which corresponds to 92.3% calculated on the "as is" basis.

SODIUM CROMOGLICATE

Control No 188140

Analytical Report

This reference substance has been evaluated as a collaboration between the WHO Centre in Stockholm and the National Biological Standards Laboratory, Canberra, Australia. Results from NSBL are given under collaborating laboratories at the end of this report.

INTENDED USE

The monograph for sodium cromoglicate in the International Pharmacopeia 3rd Ed. Vol 3 requires a reference substance to be used in the infrared spectrophotometric and thin-layer chromatographic tests for identity.

MATERIAL

About 100 g of the sample (manufacturers batch No 11011 881755P) were received at the WHO Centre in March 1988. The material is being stored protected from light in tightly closed containers at + 5° C.

ANALYTICAL DATA

Description: A fine white powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (188140). The spectrum is concordant with the spectrum obtained from the Nordic Pharmacopoeia Standard Preparation (1973).

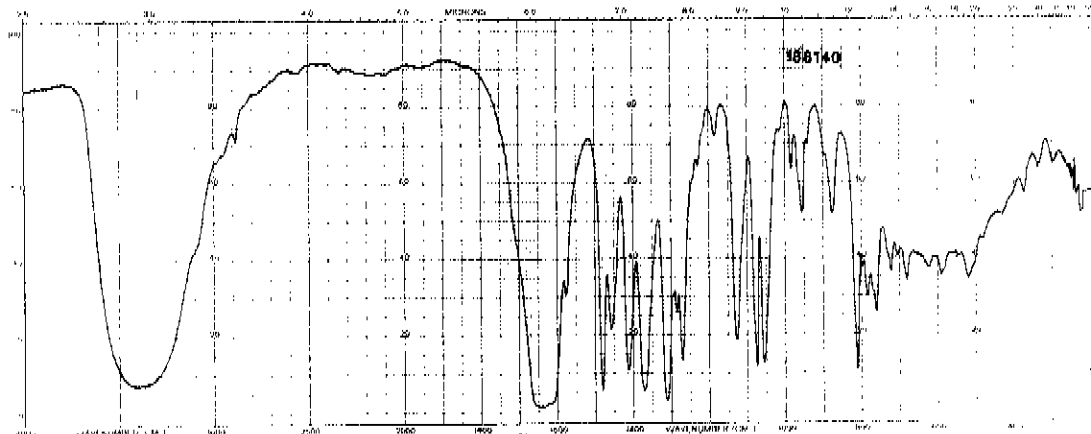


Figure 1. IR-spectrum of 1.2 mg of sodium cromoglicate, Control No 188140, in 300 mg KBr disc. Instrument: Perkin-Elmer 580.

UV-spectrum

A UV-spectrum in 0.1 M phosphate buffer pH 7.4 is given in Figure 2.

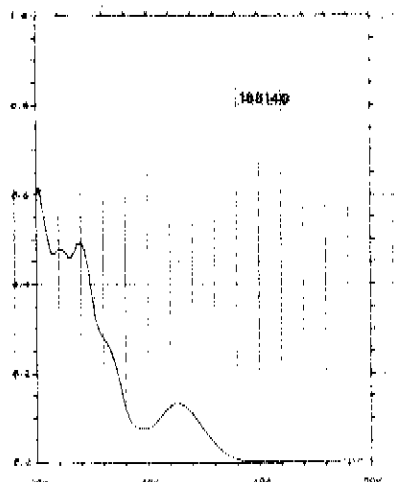


Figure 2. UV-spectrum of sodium cromoglicate Control No 188140. 9 µg/ml in phosphate buffer pH 7.4.

The UV-maxima and E-values, calculated with reference to the anhydrous substance are given in Table 1.

Table 1

λ max, nm	221	238	326
E (1%, 1 cm)	578	600	160
n	5	5	5
s rel %	1.4	1.1	0.7

The content of water was determined to be 7.2% by Karl Fischer titration.

ASSAY

Titrimetric assay: 100.6% (n=5), s rel % = 0.33 determined by nonaqueous potentiometric titration according to Ph Int 3rd Ed. Vol 3.

The result is calculated with reference to the anhydrous substance.

Thermogravimetric analysis: When the substance was heated to 105° C a loss of 8.1% in weight was observed. A loss of 8.6% occurred on further heating to 160° C.

Instrument: Perkin Elmer TGA 7 Thermogravimetric analyzer.
Sample weight: 5 mg
Heating rate: 10° C/minute
Decomposition temperature: 241-242° C

Loss on drying:

5.8% (105° C) (n=3). The substance is hygroscopic, this is the reason why NSBL (6.6%) and manufacturer (4.7%) reports different values for loss on drying. See also stability studies.

Water: 7.2% 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

No secondary spots were observed.

The main spot in the chromatogram obtained with the International Chemical Reference Substance, Control No 188140, corresponded in position, intensity and appearance to the main spot obtained with the Nordic Pharmacopoeia Standard Preparation (1973).

The following thin-layer chromatographic system was used:

According to the International Pharmacopoeia 3rd Ed. Vol 3.

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

Eluent: Chloroform : methanol : glacial acetic acid (9:9:2)

Sample: 200 µg of sodium cromoglicate dissolved in methanol were applied.

Visualization: UV-light of 254 nm, evaluation by densitometry at 254 nm with a Desaga Densitometer CD 60.

Result: No extra spots were detected. The detection limit of this system was about 0.1 µg which corresponds to 0.05% of 200 µg.

Rf (sodium cromoglicate) = 0.26

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.5%. A chromatogram is shown in Figure 3.

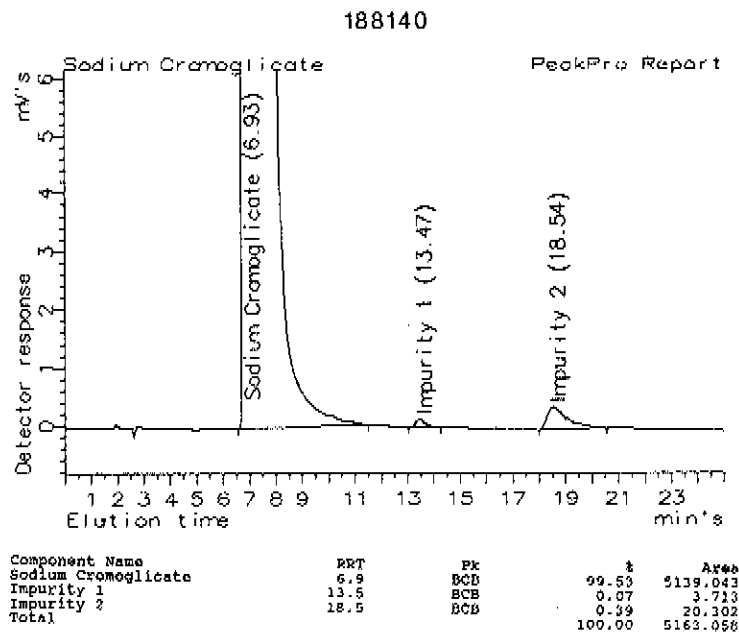


Figure 3. Chromatogram of sodium cromoglicate, Control No 188140.

The following conditions were used:

Eluent: Acetonitrile/Water containing 0.001 M TBAH-sulfate and 1.1% acetic acid.

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

Detector: Varian UV 200 operated at 254 nm

Pump: Varian 5500 operated at a flow rate of 1 ml/min

Integrator: Peak Pro 270 Attenuation:1

Sample: 1 mg/ml dissolved in the eluent. 10 µl corresponding to 10 µg were injected.

The system is in principle the same as published in Journal of Chromatography, 173,89-100 (1979).

The system described under collaborating laboratories was also tested and we found no impurities when using it.

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 20 μ l. An isogram is given in Figure 4.

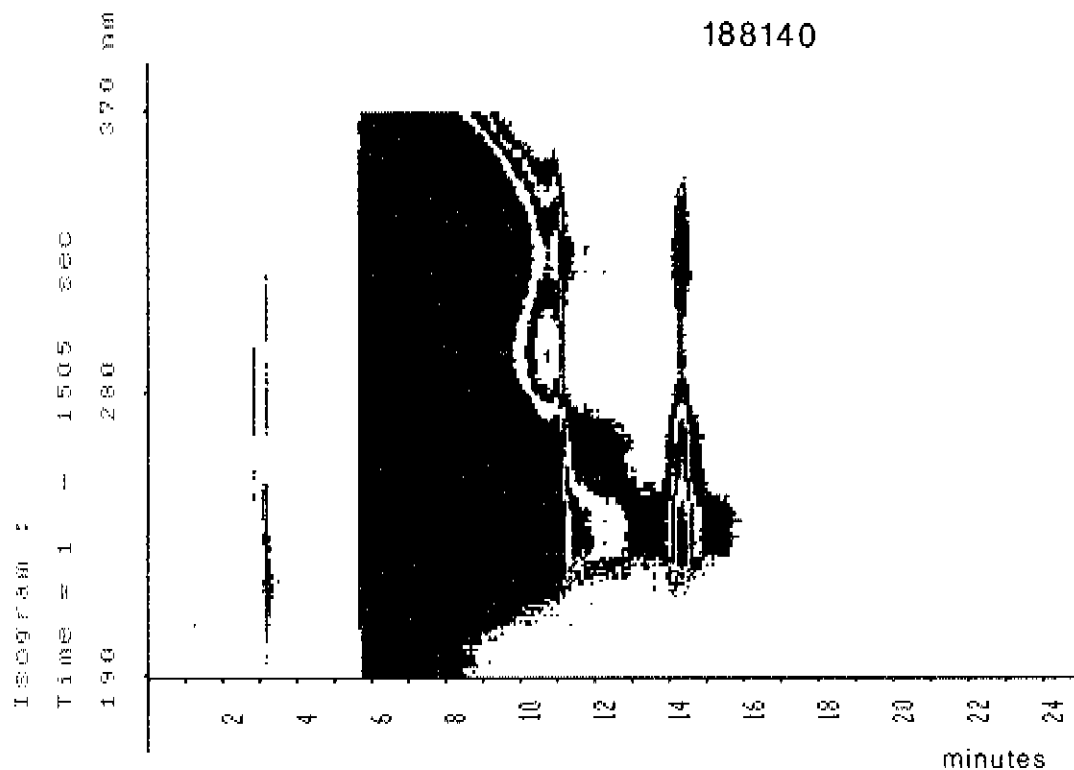


Figure 4. Isogram of sodium cromoglicate, Control No 188140.
Sensitivity: 0.001

As seen from the figure sodium cromoglicate has an absorbance maximum at 229 nm. Two impurities were detected eluting after 11 and 14 minutes with absorbance maxima at 230 and 233 nm. They were both visible at 254 nm used in the purity method described above.

DATA GIVEN BY COLLABORATING LABORATORIES

NBSL

Infrared spectrum

Two infrared spectra were recorded. The Nujol Mull and ATR were recorded on a Perkin Elmer 683 Infrared spectrophotometer. The spectra are concordant with spectra obtained from Clarke's Isolation and Identification of Drugs, and with existing NBSL spectra of sodium cromoglycate.

The nujol mull spectrum is of poor quality and comprises broad bands with very strong water bands. The material was difficult to grind uniformly and was prone to absorb water rapidly due to its hygroscopic nature.

Ultra-Violet Spectrum

The identification test procedure (B) of the BP 1980 was used and two maxima were observed for a 0.0035% w/v solution in phosphate buffer pH 7.4.

peak at 326 nm of absorbance value 0.525
peak at 238 nm of absorbance value 1.950

The $E_{1\text{ cm}}^{1\%}$ for the hygroscopic substance is 150. The substance was determined to contain 6.63% water, therefore as the dry substance an $E_{1\text{ cm}}^{1\%}$ of 160.1 was determined.

GC/MS Analysis: A HP 5988A GC-MS system was used. The sample was mixed with water and then acidified with 0.1 M HCl. The gelatinous precipitate of free acid was filtered and analysed by insertion probe run at 30 sec ramp. Library matching identified the material as cromoglycic acid.

ASSAY

A non-aqueous titration method of the British Pharmacopoeia 1980 was performed. For n=3 determinations using 0.1029 N perchloric acid,

(1) 0.17115 g	92.88%
(2) 0.9411 g	92.76%
(3) 0.18206 g	93.11 %

a mean result of 92.92% was determined.

The loss on drying determined by Karl Fischer was 6.63% for the sample portion taken from the bulk sample. The corrected assay figure as the anhydrous substance is 99.55%.

LOSS ON DRYING

Determined by the procedure of the BP 1980 at 100° C and at a pressure of 5 torr. Two determinations showed a loss of 6.63%. The BP limit shows a loss of not more than 10.0% of its weight.

OXALATE

Test of the BP 80. 0.1036 g of sodium cromoglycate was reacted with 5.0 ml iron salicylate solution in a total volume of 25 ml water and gave an absorbance greater than that obtained by repeating the operation with 0.33 mg oxalic acid.

oxalic acid solution:	0.204
sodium cromoglycate:	0.255

PURITY

Related Substances by TLC

The test procedure of the BP 80 was applied to the sample. No impurity was determined having an intensity greater than that due to a 0.010% (0.10 mg/ml = 1 µg) solution of sodium cromoglycate.

High Performance Liquid Chromatography

No impurities were found.

The following conditions were used:

Eluent:	Water:acetonitrile:glacial acetic acid/88.9 : 10 : 1.1
Column:	Altex 5 µ Ultraspere-ODS, 4.6 mm i d * 25 cm
Detector:	Variable wavelength UV set at 254 nm ETP KORTEC K95, attenuation 0.16
Pump:	ICI LC1500 operated at a flow rate of 2 ml/min
Integrator:	Shimadzu C-R3A Attenuation:3
Sample:	0.1376 mg/ml dissolved in mobile phase 20 L corresponding to 2.75 µg was injected.

DATA GIVEN BY THE MANUFACTURER

Description:	Fine white powder
Solubility:	Complies with BP
Identity:	Complies with BP
Acidity/Alkalinity:	0.05 cm ³ of N/10 NaOH
Lead:	Less than 10 ppm
Oxalate:	0.12% w/w
Related Substances:	Complies with BP
Loss on Drying:	4.7% w/w
Assay:	99.1% w/w

STABILITY

Sodium cromoglicate was exposed to air of different relative humidity at room temperature (about 20° C) for a period of 8 weeks as described in WHO/PHARM/82.509. All samples were unchanged at visual inspection but weight changes were noted as the substance is hygroscopic. The results from the stability study is shown in Figure 5.

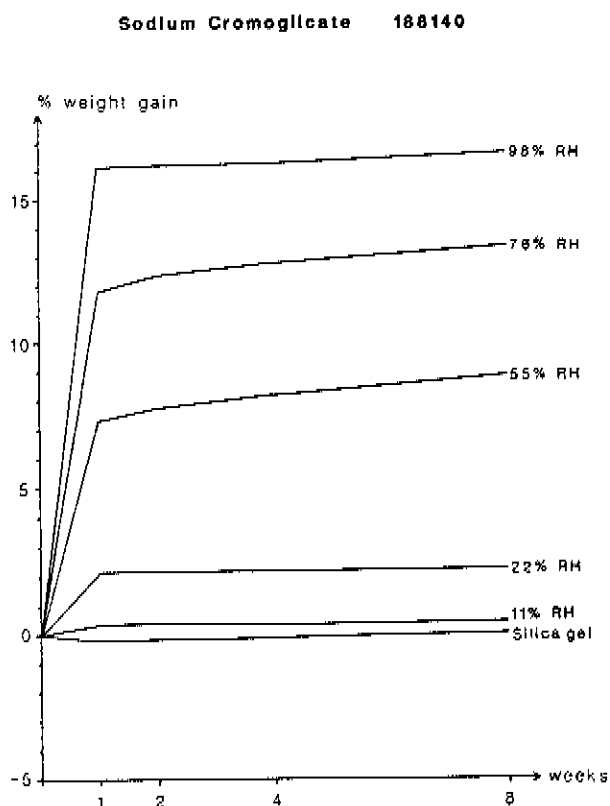


Figure 5. Stability of sodium cromoglicate, Control No 188140 stored at different relative humidity at 20° C.

As can be seen from the figure the substance is hygroscopic. Water is taken up at all humidities from 22% and above. It is recommended to store the substance under dry conditions.

No signs of degradation were observed when the samples were analyzed by the liquid chromatographic method described above.

CONCLUSION

Sodium cromoglicate, Control No 188140, can be considered suitable as International Reference Substance for the intended purpose.



WHO/PHARM/89.544

CORRECTION to APPENDIX 7, page 24

Dexamethasone Control No 388008:

Column: RP-18, Spheri-S (Brownlee) shall be replaced by Spherisorb S5 W, silica.