



WHO COLLABORATING CENTRE FOR CHEMICAL REFERENCE SUBSTANCES

Report on the work in 1992

by M. Westermark

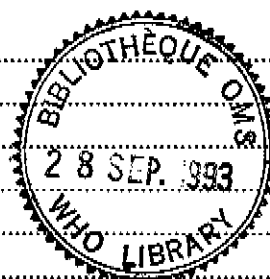
Newly established International Chemical Reference Substances, proposed by the WHO Collaborating Centre for Chemical Reference Substances on the basis of adequate testing and characterization, are included in the Centre's annual report. The report is circulated, *inter alia*, to members of the WHO Expert Advisory Panel on the International Pharmacopoeia and Pharmaceutical Preparations, who are requested to consider the proposals carefully together with the attached analytical documentation, and to notify the Centre of any reservations or adverse comments within three months of the date of mailing. In these cases the Centre will proceed with any consultations or additional analyses necessary for the validation.

If no adverse comments are received within the three-month period, the proposed new International Chemical Reference Substances may be considered *provisionally* adopted. It will be considered for *final* adoption during the subsequent meeting of the Expert Committee.

Kindly address your comments to Mrs M. Westermark, WHO Collaborating Centre for Chemical Reference Substances, Apoteksbolaget AB., Centrallaboratoriet, Prismavägen 2, S-10514 Stockholm, Sweden.

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Distribution of reference substances in 1992

During 1992 the total number of International Chemical Reference Substances distributed from the Centre was 1125. Compared to the figures for 1991 this corresponds to an increase of about 78 per cent. The six most frequently requested substances were in order of demand Ampicillin anhydrous, Benzylpenicillin sodium, Melting Point Reference Substances, Atropine sulfate, Sulfamethoxazole and 2-(4-Chloro-3-sulfamoyl)-benzoic acid. Detailed figures for the distribution of the individual substances are given in Appendix 1.

The substances were distributed to 32 different countries during 1992. Details of the distribution are given in Appendix 2. Considering the distribution to different regions it is observed that approximately 22% went to the African Region, 2% to the Americas, 5% to the Eastern Mediterranean, 26% to Europe, 35% to South East Asia and 10% to the Western Pacific Region.

Compared to last years figures there is an increase in the request from South East Asia, Western Pacific and the African Region. Sixteen new countries have ordered and eight from last years list have disappeared. This shows how difficult it is to foresee the requests for ICRS. Some countries probably place a large order and keep a stock of ICRS for several years before they place a new order.

Establishment of reference substances in 1992

In accordance with the procedure recommended by the WHO Expert Committee on Specifications for Pharmaceutical Preparations in its Thirty-second report (Technical Report Series No 823), 7 International Chemical Reference Substances were established in 1992. The substances are listed in Appendix 3 to this report. Ethinylestradiol and Vitamin A acetate are replacement batches as the former stocks were depleted during 1991.

A complete list of all International Chemical Reference Substances available from the Centre in January 1993, with information about package sizes and control numbers for the current batches is given in Appendix 4 to this report. The list also includes 8 substances mentioned below which are expected to be formally adopted by the Expert Committee in 1994. New this year is also that the Melting Point Reference Substances are no longer available as a set. They are now sold as separate substances with their own control numbers, given in this report in Appendix 4 on page 11.

Work on new reference substances completed in 1992

Work is being continued on new reference substances required to support specifications in the third edition of the International Pharmacopoeia. During 1992 eight new reference substances for volume 3 were examined. They are Amodiaquine hydrochloride, Bacitracin zinc, Beclometasone dipropionate, Dexamethasone phosphoric acid, Dexamethasone sodium phosphate, Dopamine hydrochloride, Probenecid and Pyrantel embonate. The analytical reports are given in Appendices 7-14. These substances are considered suitable for adoption as International Chemical Reference Substances.

Stability testing

The regular stability monitoring of existing International Chemical Reference Substances was continued. This year thirteen substances were re-examined. The results are given in Appendix 5. Details about the analytical methods used can be obtained from the Centre.

Work in progress and future work

Work is continuously performed on the substances required to support the monographs in Volume 3 of the International Pharmacopoeia. For the moment work on nine of the 21 substances, given in Appendix 6, is in progress at the Centre.

During 1992, the Centre started to disseminate more information on International Chemical Reference Substances to laboratories involved in analytical control of pharmaceuticals. The International Federation of Manufacturers Associations (IFPMA) helped to locate addresses of Manufacturers Member Associations. The Centre started the preparation of an information card concerning the Centre to be distributed at conferences and/or to persons for information purposes of the existence of International Chemical Reference Substances. This card will be ready for distribution during 1993. The Centre also participated at the FIP Congress in Lyon giving a lecture with the title "The role of the Chemical Reference Substances Centre in combating counterfeit pharmaceuticals".

Administrative and financial matters

The total cost for running the Centre in 1992 was estimated at 563.600 US\$. The income from sales of reference substances was about 46.040 US\$ and the contribution received from the WHO headquarters was 16.000 US\$. This leaves a deficit of 501.560 US\$, covered by the support from the National Corporation of Swedish Pharmacies.

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

Acknowledgements

The Centre is grateful to the laboratories that have contributed to the work during 1992. This year we want to address our thanks to the European Pharmacopoeia Laboratory in Strasbourg, France and the National Biological Standards Laboratory (now Therapeutic Goods Administration Laboratories), Canberra, Australia.

The Centre is also very grateful to the pharmaceutical companies who have provided candidate materials and participated in the analytical testing. This year we want to give a special thanks to A/S Aphotekarnes Lab., Oslo, Norway; Fluka Chemie AG, Buchs, Switzerland; Glaxo, Greenford, England; Merck, Sharp and Dohme Ltd, Rahway, NJ, USA; Parke Davis, Morris Plains, NJ, USA; Pfizer, Groton, NY, USA and Upjohn, Kalamazoo, MI, USA.

APPENDIX 1

DISTRIBUTION OF CHEMICAL REFERENCE SUBSTANCES IN 1992

Aceclidine salicylate	5 items	Cortisone acetate	6 items
p-Acetamidobenzalazine	10 "	Dapsone	6 "
Acetazolamide	22 "	Desoxycortone acetate	5 "
Allopurinol	5 "	Dexamethasone	10 "
2-Amino-5-nitrothiazole	1 "	Dexamethasone acetate	3 "
3-Aminopyrazole-4-carboxamide hemisulfate	10 "	Diazepam	11 "
Amitriptyline hydrochloride	9 "	Diazoxide	2 "
Amphotericin B	2 "	Dicloxacillin sodium	3 "
Ampicillin (anhydrous)	41 "	Dicolinium iodide	-- "
Ampicillin sodium	15 "	Dicoumarol	-- "
Ampicillin trihydrate	20 "	Diethylcarbamazine dihydrogen citrate	1 "
Anhydrotetracycline hydrochloride	24 "	Digitoxin	10 "
Atropine sulfate	28 "	Digoxin	9 "
Azathioprine	2 "	NN' -Di-(2,3-xylyl)anthranilamide	1 "
Bendazol hydrochloride	-- "	Emetine hydrochloride	2 "
Benzobarbital	2 "	4-Epianhydrotetracycline hydrochloride	23 "
Benzylamine sulfate	11 "	4-Epitetracycline ammonium salt	9 "
Benzylpenicillin potassium	6 "	Ergocalciferol	2 "
Benzylpenicillin sodium	39 "	Ergometrine hydrogen maleate	4 "
Bephenium hydroxynaphthoate	1 "	Ergotamine tartrate	12 "
Betamethasone	17 "	Erythromycin	6 "
Betamethasone valerate	-- "	Estradiol benzoate	3 "
Betanidine sulfate	-- "	Estrone	23 "
Bupivacaine hydrochloride	-- "	Etacrynic acid	-- "
Caffeine	11 "	Ethambutol hydrochloride	10 "
Carbamazepine	7 "	Ethinylestradiol	1 "
Carbenicillin monosodium	6 "	Ethisterone	1 "
Chloramphenicol	16 "	Ethosuximide	2 "
Chloramphenicol palmitate	4 "	Etocarlide	-- "
Chloramphenicol palmitate (Polymorph A)	7 "	3-Formylrifamycin	2 "
5-Chloro-2-methylamino-benzophenone	1 "	Flucytosine	1 "
2-(4-Chloro-3-sulfamoyl-benzoyl)benzoic acid	27 "	Fluorouracil	3 "
Chlorphenamine hydrogen maleate	20 "	Fluphenazine decanoate dihydrochloride	2 "
Chlorpromazine hydrochloride	13 "	Fluphenazine enantate dihydrochloride	1 "
Chlortalidone	3 "	Fluphenazine hydrochloride	11 "
Chlortetracycline hydrochloride	7 "	Folic acid	14 "
Cimetidine	2 "	Furosemide	4 "
Clomifene citrate	5 "	Griseofulvin	12 "
Clomifene citrate Z-isomer see Zuclomifene		Haloperidol	5 "
Cloxacillin sodium	25 "	Hydrochlorothiazide	5 "
Colecalciferol	6 "	Hydrocortisone	15 "
		Hydrocortisone acetate	14 "

(-)-3-(4-Hydroxy-3-methoxy-phenyl)-2-methylalanine	1 item	Phenytoin	5 items
Ibuprofen	12	Prednisolone	9 "
Imipramine hydrochloride	9 "	Prednisolone acetate	6 "
Indometacin	7 "	Prednisone	8 "
o-Iodohippuric acid	-- "	Prednisone acetate	8 "
Isoniazid	5 "	Probenecid	1 "
Lanatoside C	6 "	Procaine hydrochloride	3 "
Levodopa	6 "	Procarbazine hydrochloride	1 "
Levothyroxine sodium	-- "	Progesterone	7 "
Lidocaine	2 "	Propicillin potassium	1 "
Lidocaine hydrochloride	2 "	Propranolol hydrochloride	12 "
Mefenamic acid	3 "	Propylthiouracil	1 "
Metazide	1 "	Pyrantel embonate	1 "
Methaqualone	2 "	Pyridostigmine bromide	1 "
Methyldopa	11 "	Reserpine	5 "
Methyltestosterone	8 "	Retinol acetate	
Meticillin sodium	-- "	(solution à 25000 IU)	15 "
Metronidazole	9 "	Riboflavin	7 "
Nafcillin sodium	1 "	Rifampicin	8 "
Neostigmine metilsulfate	2 "	Rifampicin quinone	3 "
Nicotinamide	10 "	Sodium cromoglicate	1 "
Nicotinic acid	7 "	Sulfamethoxazole	27 "
Niridazole	2 "	Sulfamethoxypyridazine	9 "
Niridazole-chlorethyl-carboxamide	-- "	Sulfanilamide	9 "
Norethisterone	7 "	Sulfasalazine	1 "
Norethisterone acetate	10 "	Testosterone propionate	6 "
Nystatin	8 "	Tetracycline hydrochloride	25 "
Ouabain	1 "	Thioacetazone	6 "
Oxacillin sodium	2 "	4,4'-Thiodianiline	1 "
Oxytetracycline dihydrate	4 "	Thyroxine sodium	
Oxytetracycline hydrochloride	14 "	see Levothyroxine sodium	
Papaverine hydrochloride	3 "	Tolbutamide	2 "
Phenethicillin potassium	3 "	Tolnaftate	7 "
Phenoxymethylpenicillin	13 "	Trimethadione	-- "
Phenoxymethylpenicillin calcium	1 "	Trimethoprim	21 "
Phenoxymethylpencillin potassium	11 "	Trimethylguanidine sulfate	1 "
		Tubocurarine chloride	-- "
		Vitamin A acetate (solution)	
		see Retinol acetate	
		Warfarin	2 "
		Zuclomifene	4 "

Melting Point Reference Substances 34 x 13 substances.

APPENDIX 2

DISTRIBUTION OF INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES
TO DIFFERENT WHO REGIONS IN 1992

<i>WHO Regions</i>	<i>Number of ICRS distributed in 1992</i>
African Region (AFRO)	
Ghana	151
Swaziland	29
Tanzania	30
Uganda	28
Zimbabwe	5
Region of the Americas (AMRO)	
Argentina	1
Cuba	1
Panama	9
United States of America	8
Eastern Mediterranean Region (EMRO)	
Egypt	6
Syrian Arab Republic	19
Yemen	36
European Region (EURO)	
Austria	8
Belgium	32
Czechoslovakia	5
Denmark	6
Finland	5
France	15
Germany	61
Netherlands	3
Norway	5
Russian Federation	61
Sweden	66
Switzerland	2
Turkey	14
United Kingdom	18
South-East Asia Region (SEARO)	
India	339
Indonesia	59
Western Pacific Region (WPRO)	
Malaysia	4
New Zealand	15
Singapore	23
Vietnam	68

APPENDIX 3

INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES ESTABLISHED IN 1992

Reference Substance	Control Number	Analytical Report	Remarks
Amphotericin B	191153	WHO/PHARM/92.558 Appendix 7	
Erythromycin	191154	WHO/PHARM/92.558 Appendix 8	
Ethinylestradiol	291016	WHO/PHARM/92.558 Appendix 9	Replaces No 167016
Nystatin	191152	WHO/PHARM/92.558 Appendix 10	
Rifampicin	191151	WHO/PHARM/92.558 Appendix 11	
Sulfasalazine	191155	WHO/PHARM/92.558 Appendix 12	
Retinol acetate (Vitamin A acetate)	791038	WHO/PHARM/92.558 Appendix 13	Replaces No 686038

LIST OF AVAILABLE INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES

1993

General information

International Chemical Reference Substances are established on 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.

Directions for use and the analytical data required for the tests specified in *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.

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 the use of these substances.

It is generally recommended that the substances 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 stored at the Collaborating Centre is monitored by regular re-examination, and any deteriorated materials are replaced by new batches as 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 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)
(Fax: + 46 8 740 60 40)

International Chemical Reference Substances are supplied only in the standard packages 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	290042
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
Amodiaquine hydrochloride	200 mg	192160
Amphotericin B	400 mg	191153
Ampicillin (anhydrous)	200 mg	390001
Ampicillin sodium	200 mg	388002
Ampicillin trihydrate	200 mg	274003
Anhydrotetracycline hydrochloride	25 mg	180096
Atropine sulfate	100 mg	183111
Azathioprine	100 mg	172060
Bacitracin zinc	200 mg	192174
Beclometasone dipropionate	200 mg	192175
Bendazol hydrochloride	100 mg	173066
Benzobarbital	100 mg	172051
Benzylamine sulfate	100 mg	172052
Benzympenicillin potassium	200 mg	180099
Benzympenicillin sodium	200 mg	280047
Bephenium hydroxynaphthoate	100 mg	183112
Betamethasone	100 mg	183113
Betamethasone valerate	100 mg	190145
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
Cimetidine	100mg	190150
Clomifene citrate	100 mg	187136
Clomifene citrate Z-isomer see Zuclomifene		
Cloxacillin sodium	200 mg	274005
Colecalciferol (Vitamin D ₃)	500mg	190146
Cortisone acetate	100 mg	167006
Dapsone	100 mg	183115
Desoxycortone acetate	100 mg	167007
Dexamethasone	100 mg	388008
Dexamethasone acetate	100 mg	288009
Dexamethasone phosphoric acid	100 mg	192161

	<u>Package size</u>	<u>Control Number</u>
Dexamethasone sodium phosphate	100 mg	192158
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
Digoxin	100 mg	587011
NN'-Di-(2,3-xylyl)anthranilamide	50 mg	173067
Dopamine hydrochloride	100 mg	192159
Emetine hydrochloride	100 mg	187134
4-Epianhydrotetracycline hydrochloride	25 mg	288097
4-Epitetracycline ammonium salt	25 mg	180098
Ergocalciferol (Vitamin D ₂)	500mg	190147
Ergometrine hydrogen maleate	50 mg	277012
Ergotamine tartrate	50 mg	385013
Erythromycin	250 mg	191154
Estradiol benzoate	100 mg	167014
Estrone	100 mg	279015
Etacrynic acid	100 mg	281056
Ethambutol hydrochloride	100 mg	179081
Ethinylestradiol	100 mg	291016
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
3-Formylrifamycin	200 mg	190149
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	100 mg	189144
Lidocaine	100 mg	181104
Lidocaine hydrochloride	100 mg	181105

	<u>Package size</u>	<u>Control Number</u>
Mefenamic acid	100 mg	173068
<i>Melting Point Reference Substances</i> (set of 13 substances)		
<i>No longer available</i>		
Individual Melting Point Reference Substances		
Azobenzene (69 °C)	4 g	192168
Vanillin (83 °C)	4 g	192169
Benzil (96 °C)	4 g	192170
Acetanilide (116 °C)	4 g	192171
Phenacetin (136 °C)	4 g	192172
Benzanilide (165 °C)	4 g	192173
Sulfanilamide (166 °C)	4 g	192162
Sulfapyridine (193 °C)	4 g	192163
Dicyandiamide (210 °C)	4 g	192164
Saccharin (229 °C)	4 g	192165
Caffeine (237 °C)	4 g	192166
Phenolphthalein (263 °C)	4 g	192167
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
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
Nystatin	200 mg	191152
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
Probenecid	100 mg	192156
Procaine hydrochloride	100 mg	183119
Procarbazine hydrochloride	100 mg	184120
Progesterone	100 mg	167033
Propicillin potassium	200 mg	274034

	<u>Package size</u>	<u>Control Number</u>
Propranolol hydrochloride	100 mg	187139
Propylthiouracil	100 mg	185126
Pyrantel embonate	500 mg	192157
Pyridostigmine bromide	100 mg	182110
Reserpine	100 mg	186133
Retinol acetate (solution)	5 caps. (*)	791038
Riboflavin	250 mg	382035
Rifampicin	200 mg	191151
Rifampicin quinone	200 mg	190148
Sodium cromoglicate	100 mg	188140
Sulfamethoxazole	100 mg	179092
Sulfamethoxypyridazine	100 mg	178079
Sulfanilamide	100 mg	179094
Sulfasalazine	100 mg	191155
Testosterone propionate	100 mg	167036
Tetracycline hydrochloride	200 mg	180095
Thioacetazone	100 mg	171046
4,4'-Thiodianiline	50 mg	183116
Thyroxine sodium see Levothyroxine sodium		
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) see Retinol acetate		
Warfarin	100 mg	168041
Zuclomifene	50 mg	187137

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

APPENDIX 5

STABILITY TESTING

The stability on storage of the International Chemical Reference Substances is monitored by regular re-examination of the substances held in stock at the Centre. The results obtained for the substances re-examined in 1992 are summarized below. For comparison results obtained at earlier occasions are included in the summaries. The substances have been stored in tightly closed containers at +5 °C and in a relative humidity of about 30%. 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 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 of weight) are expressed as weight per cent (w/w %). Assay values are calculated with reference to the dried or the anhydrous substance unless otherwise stated.

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	1992
KF, %	13.9	-	13.9	13.5	13.3	-	-
TGA, %	-	-	-	-	-	13.9	13.8
HPLC, %	-	0.3	0.6	0.3	0.9	0.3	0.3
Assay, % (mercurimetric)	-	-	-	-	98.6	-	98.8
Degradation products, % (mercurimetric)	-	-	-	-	0.9	-	0.7
Assay, % (penicillinase)	98.5	-	99.0	-	-	-	-
PSA, %	1.0	-	-	-	-	-	-
pH, 0.25% solution	5.1	-	5.1	5.1	5.1	-	-

Chloramphenicol, Control No 486004

Initial analytical report: WHO/PHARM/87.532, Appendix 8

Examination year:	1986	1992
IR	conforms	-
TLC, %	0.2	-
HPLC, %	0.2	0.3
TGA, %	-	<0.1
LOD, %	0.05	-
Assay, % (spectrophotometric)	99.8	100.0
DTA, %	0.2	-
DSC, %	-	0.3

Chloramphenicol palmitate, Control No 286072

Initial analytical report: WHO/PHARM/87.532, Appendix 9

Examination year:	1986	1992
IR	conforms	conforms
TLC	two secondary spots	-
HPLC, %	3.1	3.1
TGA, %	0.1	<0.1
KF, %	0.2	<0.1
LOD, %	<0.1	-
Assay, % (spectrophotometric)	100.2	100.0
DTA, %	2.7	-
DSC, %	2.7	2.2

Chloramphenicol palmitate (polymorph A). Control No 175073

Initial analytical report: WHO/PHARM/75.485, Appendix 10

Examination year:	1974	1992
IR	conforms	conforms
TLC	two secondary spots	-
HPLC, %	-	about 2
TGA, %	-	<0.1
KF, %	-	<0.1
LOD, %	0.26	-
Assay, titration, %	100.9	-
Assay, % (spectrophotometric)	-	100.0
DSC, %	0.6	0.7
PSA, %	about 0.5	-

Cortisone acetate. Control No 167006

Initial analytical report: WHO/PHARM/67.441, Appendix 1

Examination year:	1966	1975	1984	1992
IR	conforms	-	-	-
TLC	three sec. spots	two sec. spots	three sec. spots	-
HPLC, %	-	-	0.3	0.3
TGA, %	-	-	-	0.1
KF, %	-	-	-	0.2
LOD, %	<0.1	0.2	-	-

Digitoxin, Control No 277010

Initial analytical report: WHO/PHARM/78.494, Appendix 7

Examination year:	1977	1987	1992
IR	conforms	-	-
TLC, %	0.2	<0.1	0.3
HPLC, %	<0.1	0.1	0.5
TGA, %	-	-	0.6
LOD, %	0.6	0.6	-
Assay, % (spectrophotometric)	99.7	100.7	100.7

Digoxin, Control No 587011

Initial analytical report WHO/PHARM/88.537, Appendix 10

Examination year:	1987	1992
IR	conforms	-
TLC, %	three sec. spots	three sec. spots
HPLC, %	1.4	1.4
TGA, %	0.15	0.13
KF, %	0.16	-
Assay, % (spectrophotometric)	99.7	99.8

4-Epitetracycline (ammonium salt). Control No 180098

Initial analytical report: WHO/PHARM/81.508, Appendix 9

Examination year:	1980	1985	1992
IR	conforms	-	-
TLC	two sec.	-	-
HPLC, %	0.7	0.4	0.4
TGA, %	-	-	about 4
KF, %	-	3.9	4.0
LOD, %	0.15	-	3.1

Fluorouracil. Control No 184122

Initial analytical report: WHO/PHARM/85.517, Appendix 8

Examination year:	1984	1992
IR	conforms	-
TLC	no sec. spots	-
HPLC, %	0.03	0.04
TGA, %	-	<0.1%
LOD, %	<0.1	-
Assay, titration, %	100.9	-

Furosemide, Control No 171044

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

Examination year:	1971	1976	1985	1992
IR	conforms	-	conforms	-
TLC	no sec. spots	one sec. spot	no sec. spots	-
HPLC, %	-	-	-	0.03
TGA, %	-	-	-	0.1
LOD, %	0.1	<0.1	0.2	-
Assay, titration, %	99.4	100.1	-	-
PSA, %	<0.5	-	-	-

(-)-3-(4-Hydroxy-3-methoxyphenyl)-2-methylalanine, Control No 179085

Initial analytical report: WHO/PHARM/80.504, Appendix 9

Examination year:	1979	1992
IR	conforms	-
TLC, %	one sec. spot	one sec. spot
TGA, %	-	7.6
KF, %	7.1	-
Assay, titration	99.7	-

Methyldopa. Control No 179084

Initial analytical report: WHO/PHARM/80.504, Appendix 10

Examination year:	1979	1984	1992
IR	conforms	-	-
TLC, %	0.2	one sec. spot	0.1
HPLC, %	<0.2	0.2	0.1
TGA, %	-	-	11.7
KF, %	11.5	11.5	-
Assay, titration, %	99.7	-	-

Phenytoin. Control No 179089

Initial analytical report: WHO/PHARM/80.504, Appendix 13

Examination year:	1979	1985	1992
IR	conforms	-	-
HPLC, %	0.02	-	0.02
TGA, %	-	-	0.1
KF, %	-	-	0.1
LOD, %	<0.1	0.2	-
Assay, titration, %	100.1	-	-
DTA, %	0.2	0.3	-
DSC, %	-	-	<0.1

APPENDIX 6

INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES - PROJECT LIST 1993

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

Volume 3

Calcium folinate (*)	Neomycin B sulfate (*)
Doxorubicin hydrochloride	(impurity in Neomycin sulfate)
Fludrocortisone acetate	Nifurtimox
Gentamicin sulfate (*)	Noroxymorphone hydrochloride (*)
Hydrocortisone sodium succinate	(impurity in Naloxone hydrochloride)
(-)-3-(4-Hydroxy-3-methoxyphenyl)-2-hydra- zino-2-methylalanine (impurity in Carbidopa) (*)	Paromomycin sulfate
Levonorgestrel	Praziquantel
Liothyronine	Prednisolone sodium phosphate
(impurity in Levothyroxine sodium)	Spectinomycin hydrochloride (*)
Loperamide hydrochloride (*)	Sulfacetamide
Methotrexate	Testosterone enantate
Neamine (*)	Vincristine sulfate (*)
(impurity in Neomycin sulfate)	

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

APPENDIX 7

AMODIAQUINE HYDROCHLORIDE

Control No 192160

Analytical Report

INTENDED USE

The monograph for Amodiaquine hydrochloride in the International Pharmacopoeia 3rd Ed. Vol 2 requires a reference substance for amodiaquine hydrochloride to be used in the infrared spectrophotometric identity test. Further the monograph for Amodiaquine in volume 3 requires a reference substance for amodiaquine hydrochloride to be used in the infrared spectrophotometric and in the thin-layer chromatographic tests for identity, as well as in the spectrophotometric assay.

MATERIAL

About 100 g of the sample (manufacturers lot no 18381 R) were received at the WHO Centre in October 1989. The material is being stored in tightly closed containers at + 5 °C, protected from light.

This reference substance has been evaluated in collaboration between the WHO Centre in Stockholm and the National Biological Standards Laboratory, Canberra, Australia. Results reported by the NSBL are indicated with an asterisk (*).

ANALYTICAL DATA

Description: A yellow, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum of amodiaquine hydrochloride is given in Figure 1 (Control No 192160). The spectrum is concordant with the spectra of the USP reference standard Lot G and the BP CRS Lot 1585.

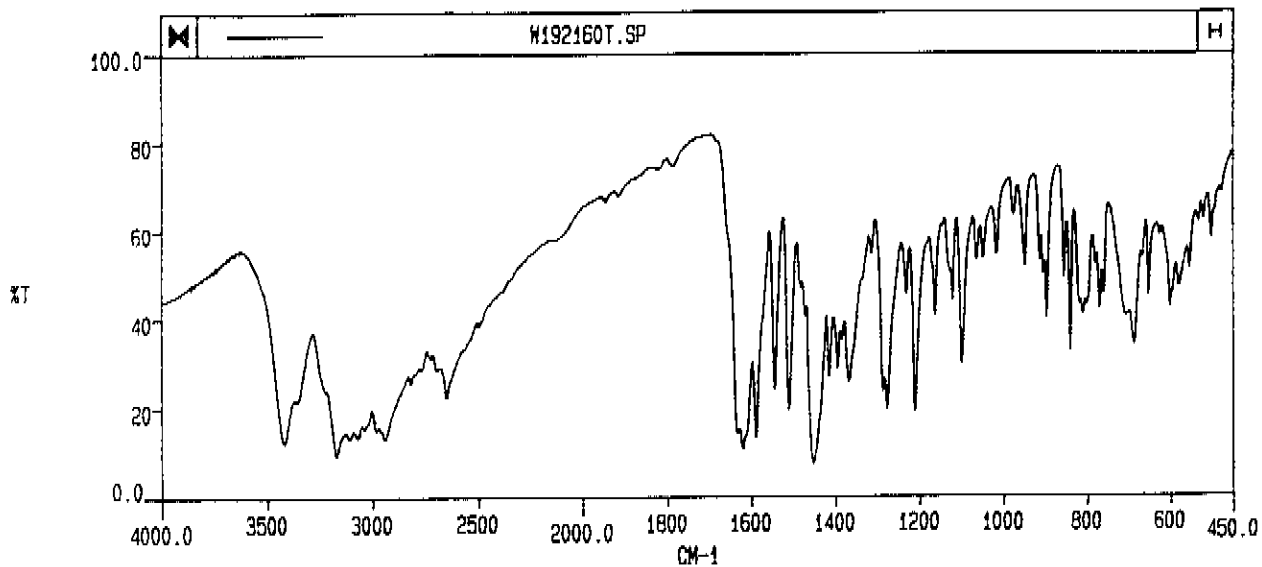


Figure 1. IR-spectrum of 1.25 mg of amodiaquine hydrochloride Control No 192160 in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 1600 FTIR.

The spectrum obtained from the free base of amodiaquine hydrochloride is given in Figure 2. The spectrum is concordant with the spectrum of amodiaquine published in AOAC (1972). The base was prepared by dissolving 20 mg of amodiaquine hydrochloride, ICRS in 10 ml of water in a separator. 1 ml of ammonium hydroxide was added, and extraction with 25 ml of chloroform was performed. The chloroform extract was evaporated and dried at 105 °C.

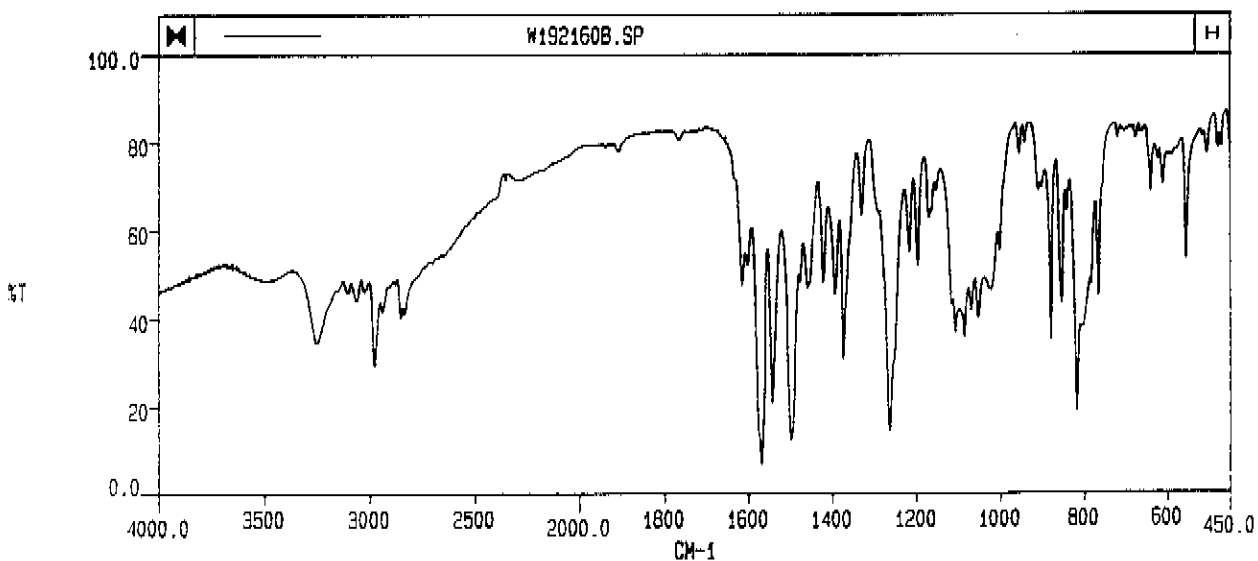


Figure 2. IR-spectrum of 2 mg of amodiaquine base in 300 mg KBr recorded against a KBr disc.

(*)Infrared spectrum

An infrared spectrum of the material, using ATR (attenuated total reflexion) was recorded on a Perkin-Elmer 683 Infrared Spectrophotometer. The spectrum was concordant with the spectrum obtained from the current TGAL reference spectrum.

UV-spectrum

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

λ max in 0.1 M HCl is 223 nm and 342 nm.

A (1%, 1 cm) = 410 at 342 nm (n= 6, RSD= 0.2%)

The result is calculated on the anhydrous substance.

The USP reference standard Lot G has an A-value of 411 at 342 nm and BP CRS Lot 1585 has an A-value of 410 at 342 nm.

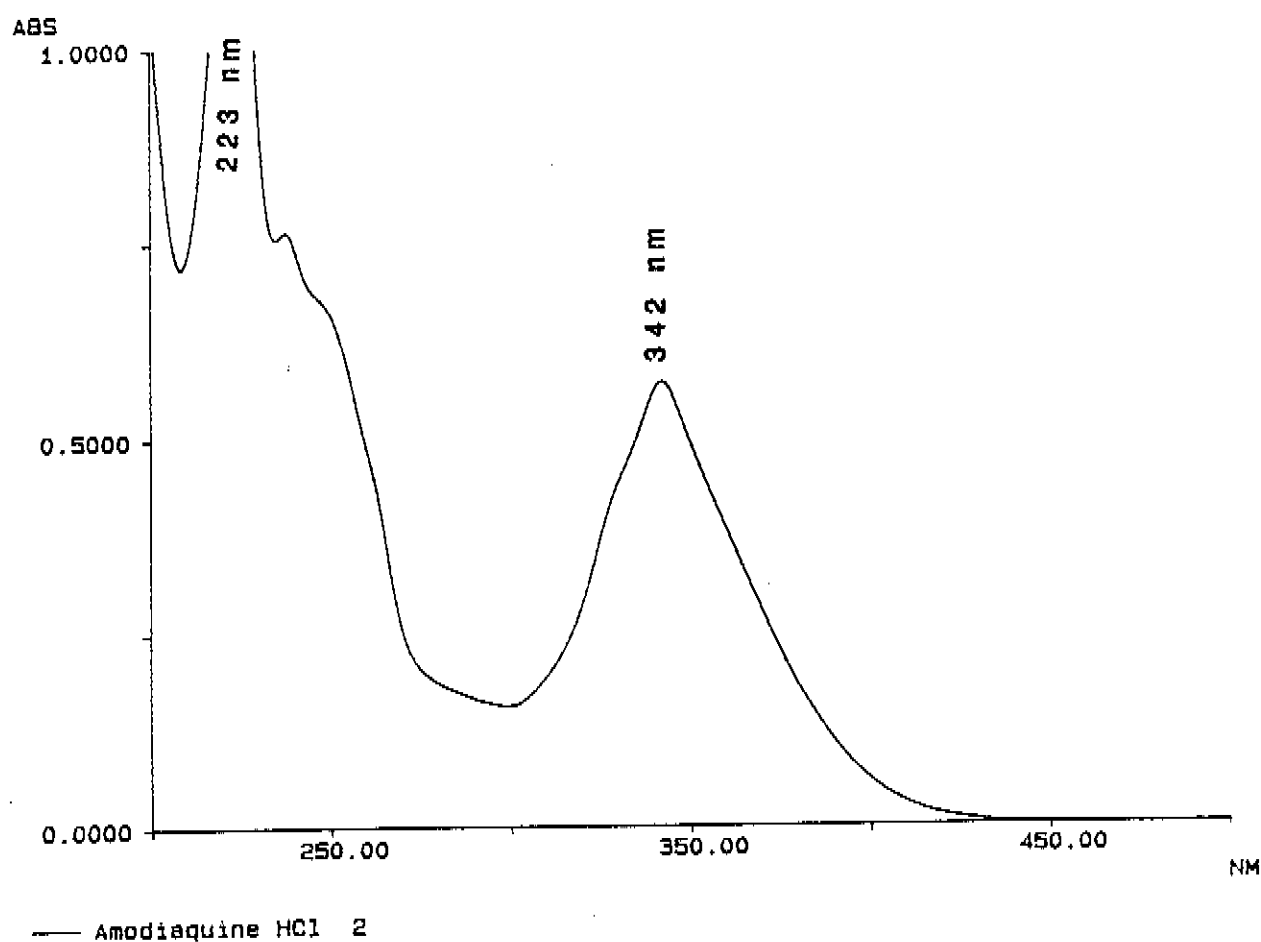


Figure 3. UV-spectrum of amodiaquine hydrochloride Control No 192160 15.2 μ g/ml in 0.1M HCl.

(*)UV-spectrum

A UV-spectrum in 0.1 M HCl was recorded.

UV-maxima were observed at 201 nm, 223 nm, 237 nm and 342 nm.

A (1%, 1 cm) = 407 at 342 nm (n= 2). The result is calculated on the anhydrous substance.

(*)Mass spectrometry

Examined as amodiaquine base using electron ionisation (EI). The mass spectrum was concordant with a reference spectrum of amodiaquine (Wiley reference spectral data base).

ASSAY

Spectrophotometric assay: 99.9% when determined against the BP CRS lot 1585 according to the method described above under UV-spectrum. The BP CRS was found to be the purer substance when examined by chromatographic methods.

(*)Titrimetric assay: 100.7% when determined by non-aqueous titration according to BP 1988.

Thermogravimetric analysis: When heating the substance to 150 °C a loss of weight of 7.9% was observed.

Instrument: Perkin Elmer TGA 7 Thermogravimetric analyzer.
Sample weight: 3 mg
Heating rate: 5 °C
Melting point: about 150 °C

(*)Water content: 8.2% (n= 5) RSD= 0.8% when determined by Karl Fischer titration.

Water: 8.0% (n= 2) determined by Karl Fischer titration.

PURITY

Thin-layer chromatography

The total amount of impurities was estimated to about 0.6 %.

The following thin-layer chromatographic system according to the International Pharmacopoeia 3rd Ed. Vol 3 was used.

Thin-layer: Silica gel 60 F-254 and Silica gel 60 HPTLC (Merck)
Eluent : Chloroform saturated with ammonia : dehydrated ethanol (99%) (90:10)
Sample: 150 µg of amodiaquine hydrochloride were applied.
The sample was dissolved in chloroform.
Visualization: Evaluation under UV-light of 254 nm and scanning by densitometry at 254 nm, 228 nm and 340 nm with a Desaga CD 60 Scanner.

Three secondary spots were detected visually at 254 nm. When evaluated by densitometry four secondary spots were detected. The total amount was estimated to about 0.6% at 228 nm and 254 nm. The detection limit of the system was about 0.2 µg (0.15%) at 254 nm.

R_f (amodiaquine hydrochloride) = 0.65. One of the impurities was identified as 4-(7-chloro-4-quinolyl-amino)phenol hydrochloride with R_f = 0.3.

In the USP reference standard Lot G about 1% impurities were detected. The amount of impurities found in BP CRS lot 1585 was 0.2%.

(*)Thin-layer chromatography

The total amount of impurities was estimated to less than 0.5%.

The following thin-layer chromatographic system used was according to BP 88 and USP XXII.

Thin-layer: Silica gel G

Eluent : Chloroform:butane-2-one:diethylamine (50:40:10)

Sample: 200 µg of amodiaquine hydrochloride were applied.

Visualization: Evaluation under UV-light of 254 nm.

The principal spot was observed at $R_f = 0.55$. One impurity was observed at $R_f = 0.09$ and it was estimated to be less than 0.5%.

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 4.

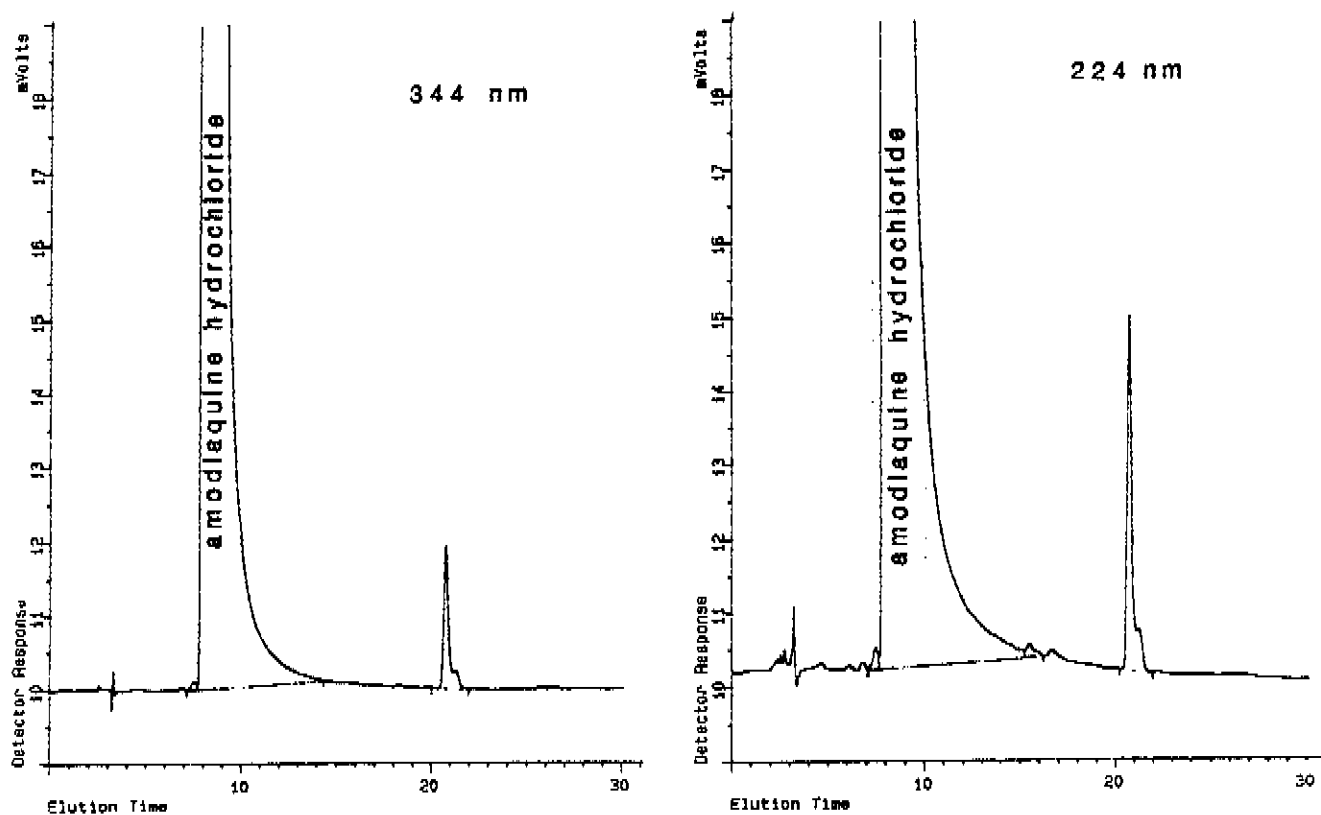


Figure 4. *Chromatogram of amodiaquine hydrochloride Control No 192160 monitored at 344 nm and 224 nm.*

The following conditions were used:

Eluent: Acetonitrile:water containing 1% triethylamine and pH adjusted to 2.8 with phosphoric acid.

The following gradient was used :

<u>Time (minutes)</u>	<u>% Acetonitrile</u>	<u>% Water</u>
0	15	85
25	30	70
27	30	70
28	15	85

Column: RP-18, 5 μ m (Brownlee Labs)

Detector: Varian Polychrom operated at 344 nm and 224 nm.

Pump: Waters 600 operated at a flow rate of 1.0 ml/min.

Integrator: PeakPro (Beckman)

Sample: 1 mg/ml dissolved in the eluent.
20 μ l corresponding to 20 μ g were injected.

When monitored at 344 nm and 228 nm 0.3% impurities were found. The main impurity eluting at about 21 minutes was identified as 4-(7-chloro-4-quinolylamino)phenol hydrochloride and estimated to be 0.23%.

The detection limit for amodiaquine hydrochloride was 0.0002 μ g injected (0.001%).

The USP reference standard lot G was also investigated. It was shown to contain about 0.6% impurities. The BPCRS lot 1585 contained only 0.1% impurities.

(*)High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.4%. One impurity eluting after the main peak was detected. Its UV-spectrum was similar to that of amodiaquine, with maximum at 222 nm.

The following conditions were used:

Eluent: Acetonitrile:0.05 M KH_2PO_4 (15:85)

Column: Spherisorb 5C8 (Phenomenex)

Detector: Diode array (LKB 2140) monitored at 222 nm.

Flow rate: 2ml/min

Diode-array detection

The chromatographic system was also evaluated with a Varian 9065 Polychrom detector. The first chromatographic system described above was used. UV-maxima for amodiaquine hydrochloride and three impurities were found at 224 nm and 344 nm when recorded in the eluent. Both wavelengths can be used for purity determinations, due to higher sensitivity, 224 nm is the first choice.

DATA GIVEN BY THE MANUFACTURER

Identification: Conforms with specified tests

Water: 8.3%

Assay: 99.61% amodiaquine hydrochloride on the anhydrous basis, determined with spectrophotometric method, according to USP.

Purity: < 0.3% 4-hydroxy-7-chloroquinoline
< 0.2% 4-(7-chloro-4-quinolylamino)phenol
4,7-dichloroquinoline not found
4,5-dichloroquinoline not found

Diethylaminomethyl-4-aminophenol: 0.004%

Heavy metals: < 10 ppm

Iron: 4 ppm

pH in 2% solution in water: 4.1

Chromatographic purity: conforms with USP method.

STABILITY

No special stability studies were performed as this substance was found to be resistant to degradation in dry state under the conditions described in WHO/PHARM/86.529. Regular re-examinations of the ICRS will be performed.

CONCLUSION

Amodiaquine hydrochloride, Control No 192160, can be considered suitable as International Chemical Reference Substance for the intended purpose. When calculating results of assays according to the monograph the content of $C_{20}H_{22}ClN_3O$ (amodiaquine hydrochloride) is taken to be 99.9% calculated with reference to the anhydrous substance (corresponding to 91.9% when calculated on an "as is" basis).

BACITRACIN ZINC

Control No 192174

Analytical Report

INTENDED USE

The monographs for Bacitracin and Bacitracin zinc in the International Pharmacopoeia 3rd Ed. Vol 3 require a reference substance for bacitracin zinc to be used in the thin-layer chromatographic identity tests.

MATERIAL

About 334 g of the sample (manufacturers batch no 9332-P887) were received at the WHO Centre in October 1987. The material is being stored in tightly closed containers at + 5 °C, protected from light.

ANALYTICAL DATA

Composition: Bacitracin zinc is a mixture of substances produced by the *licheniformis* group of *Bacillus subtilis*. The main component is bacitracin A.

Description: White to pale brownish yellow powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (Control No 192174). The spectrum is concordant with the spectrum obtained from the spectra of the USP reference standard lot L and the 2nd International Biological Standard.

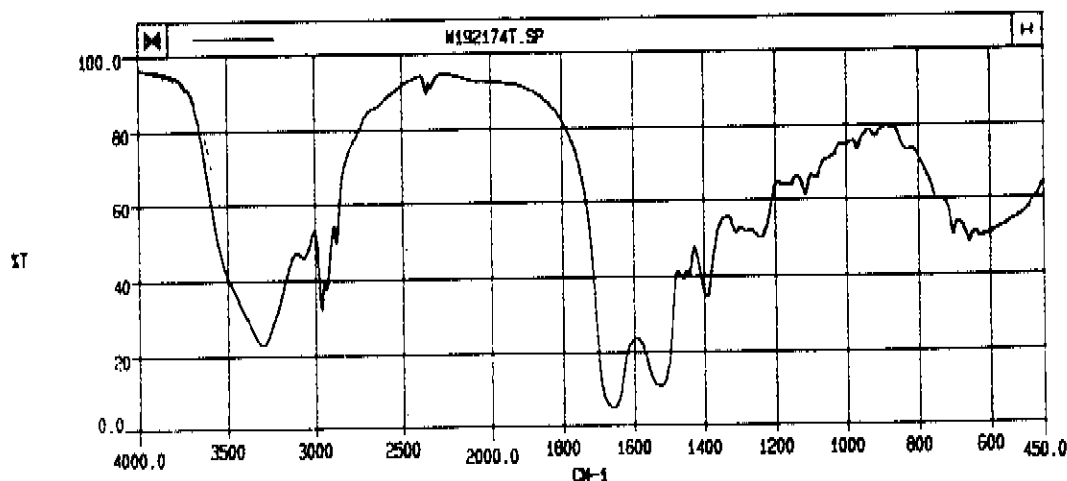


Figure 1. IR-spectrum of 1.4 mg of bacitracin zinc Control No 192174 in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 1600 FTIR.

UV-spectrum

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

λ max in water is 254 nm.

A (1%, 1cm) = 29.3 (n= 5, RSD= 1.4%)

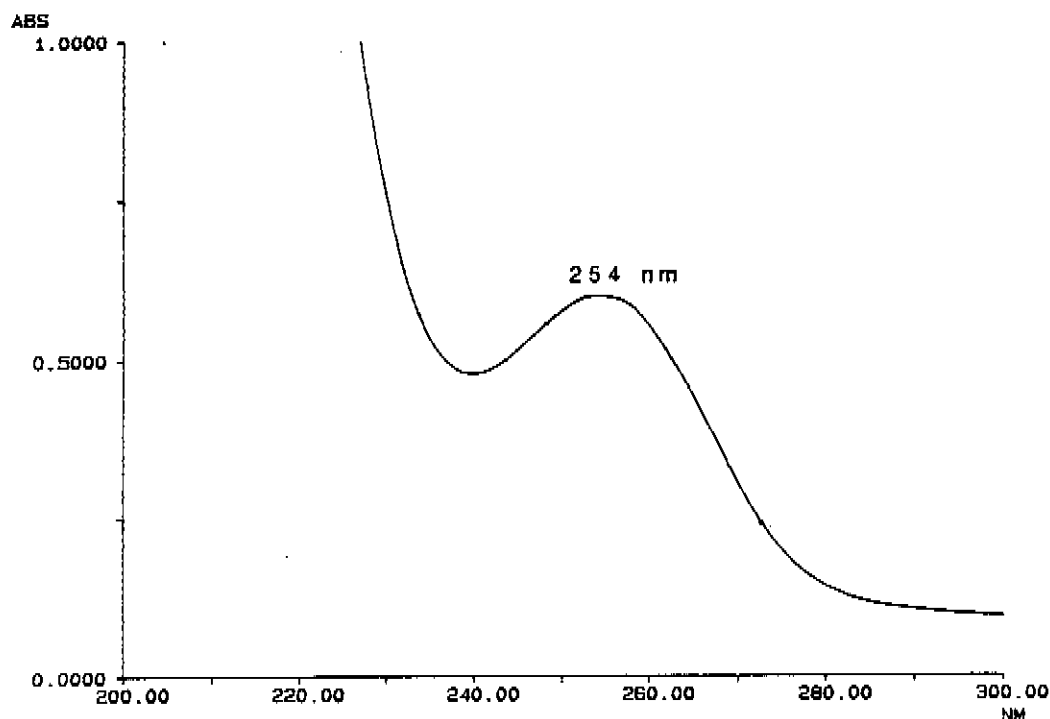


Figure 2. UV-spectrum of bacitracin zinc Control No 192174 (215 µg/ml).

The USP reference standard Lot L has an A -value of 28.1 and the 2nd International Biological Standard has an A -value of 31 when measured at 254 nm. The USP reference substance differed from the two others by giving an opalescent solution, possibly due to some insoluble impurities or just a result of the low solubility of bacitracin zinc in water.

All results are calculated on the dried substances.

Zinc: 4.9% determined by titration with EDTA.

Thin-layer chromatography

The following thin-layer chromatographic system according to EP 2nd Ed. was used. In addition to the main spot five additional spots were observed. The same result was observed for USP reference standard lot L and the 2nd International Biological Standard.

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

Eluent: Water:phenol (25:75). The phenol used was phenol liquefied 90%, Fisher.

Sample: 100 µg of were applied. 5 mg of the substance was dissolved in a mixture of 0.5 ml of hydrochloric acid and 0.5 ml of water. It was heated in a sealed tube at 135 °C for five hours and evaporated to dryness on a waterbath. After application of sample spots, the plate was left for 12 hours in the vapour of the eluent, without having contact with the eluent.

Visualization: The plate was dried at 105 °C and sprayed with ninhydrin solution. Finally it was scanned at 510 nm.

The result is given in Figure 3.

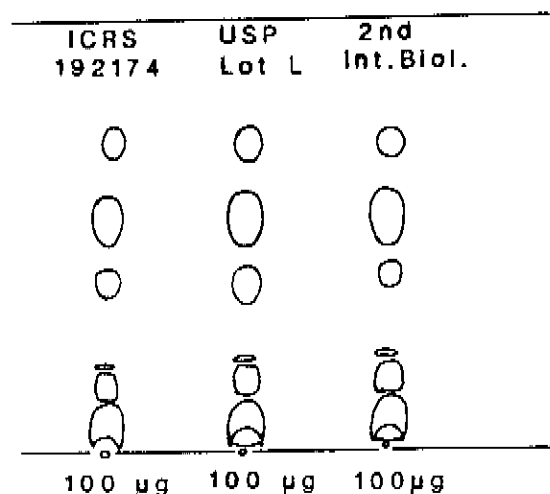


Figure 3. Thin-layer chromatogram showing the zones obtained for bacitracin zinc Control No 192174, USP lot L and the 2nd International Biological Standard.

ASSAY

Microbiological assay: 62 IU/mg. The 2nd Int. Biol. Stand. with a declared content of 74 IU/mg was used as standard.

Thermogravimetric analysis: When the substance was heated to 200 °C a loss of weight of 4.1% (n= 5) was observed.

Instrument: Perkin Elmer TGA 7 Thermogravimetric analyzer.
Sample weight: 3 mg
Heating rate: 10 °C
Melting temperature: about 245 °C (decomposition)

Water: 2.7% determined by Karl Fischer titration.

PURITY

Bacitracin F and related substances: Ratio 0.1. Determined according to Ph. Int. by UV-spectrophotometry. The ratio between the absorbance at 290/252 nm must not exceed 0.15. For the USP reference standard lot L, a ratio of 0.14 was obtained.

Thin-layer chromatography

The following thin-layer chromatographic system according to Ph. Int. Ed. 3 was used. In addition to the main spot four additional spots were observed. The same result and the same Rf-values for the main spots were observed for USP reference standard lot L and for the 2nd International Biological Standard. It is recommended that the liquid chromatographic method given below should be used to obtain a more selective purity method.

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

Eluent: Butanol:water:pyridine:glacial acetic acid:ethanol 95% (60:10:6:15:5)

Sample: 100 µg were applied. The sample was dissolved in a solution containing EDTA (10g/l).

Visualization: The plate was dried at 110 °C for ten minutes and sprayed with ninhydrin solution. Finally it was scanned at 410 nm where the sensitivity was greater than at 510 nm.

Rf (bacitracin) = 0.2

High performance liquid chromatography

The total amount of bacitracin A in the bacitracin complex was found to be about 50% when chromatographed at 210 nm, and estimated by peak area measurement.

A chromatogram is shown in Figure 4. Thirteen further peaks were detected, indicating the complexity of this substance, five of these peaks are present in large amounts, i.e. 3-15%.

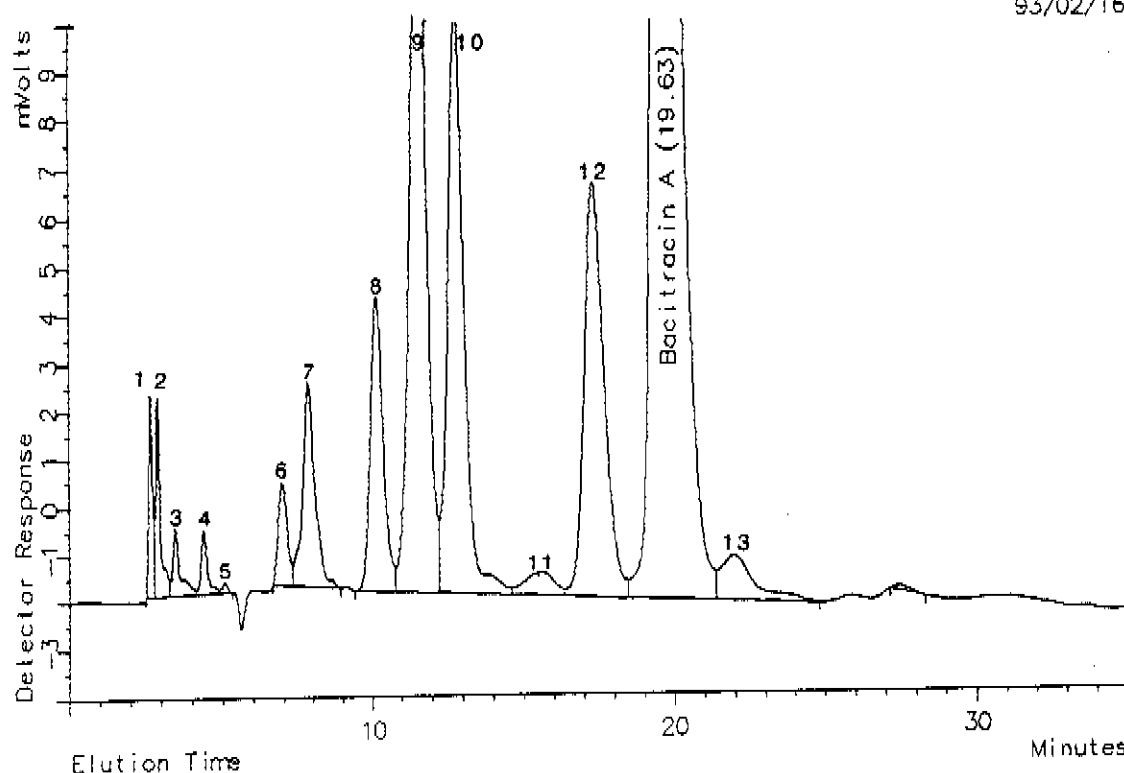


Figure 4. Chromatogram of bacitracin zinc, Control No 192174 chromatographed at 210 nm with eluent 1.

The 2nd International Biological Standard (1964) was also investigated it contained about 70% bacitracin A. The USP reference standard lot L contained about 55% of bacitracin A.

To estimate the potential degradation product bacitracin F, which is more strongly bound to the column, an eluent containing more methanol was used. About 1% of bacitracin F or F related substances were found in the proposed ICRS, compared to 1.6% in the USP lot L and 1.4% in the 2nd Biological standard. A chromatogram of ICRS 192174 is shown in Figure 5.

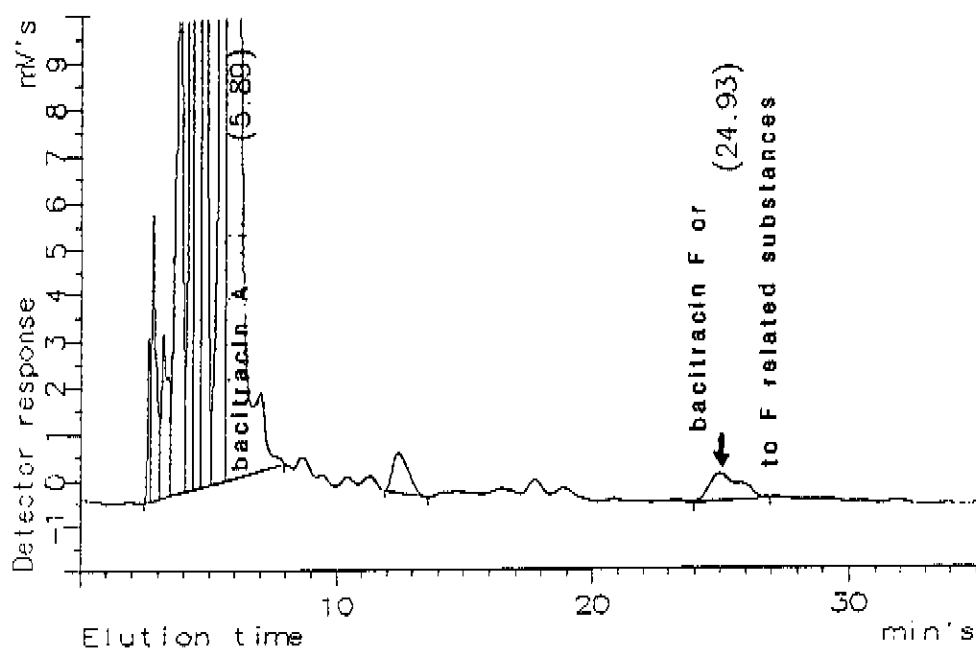


Figure 5. *Chromatogram of bacitracin zinc, Control No 192174 chromatographed at 210 nm with eluent 2.*

The following conditions were used:

Eluent 1: Methanol /sodium phosphate buffer pH 2.0 (51/49) for determination of related substances
Eluent 2: Methanol /sodium phosphate buffer pH 2.0 (59/41) for determination of bacitracin F and F related substances.

Column: Vydac C18 TP 54 (pore size 300 Å, C18 -column)

Detector: Varian UV 200 operated at 210nm (and 254 nm)

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

Integrator: PeakPro (Beckman)

Sample: 1 mg/ml, first dissolved in the buffer of the eluent followed by addition of methanol. 20 µl of the sample was injected.

Diode-array detection

The chromatographic system was also evaluated with a Varian 9065 Polychrom detector. The same chromatographic systems as described above were used. UV-maxima for bacitracin A were found to be at 195 nm and 249 nm. The spectra of the most significant impurities observed when using eluent 1 showed the same UV-maxima. With the exception of the first peak eluting at about 3 minutes (cf Fig. 4) which showed a slightly different spectrum with maxima at 195 nm, 263 nm and 344 nm. The UV-spectrum for bacitracin A in eluent 1 is given in Figure 6.

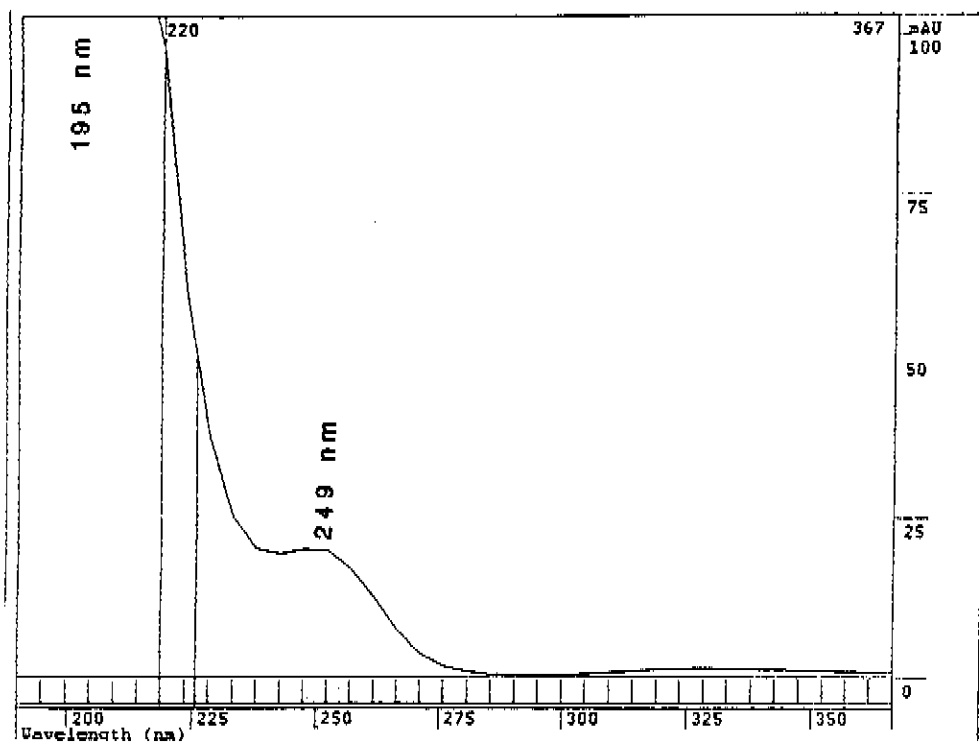


Figure 6. UV-spectrum for bacitracin A in eluent 1.

An attempt was also made to try to identify bacitracin F and its related substances. A spectrum, which is given in Figure 7, was taken for the peak indicated as bacitracin F (cf Fig. 5). It exhibits a different UV-spectrum with a maximum at 292 nm.

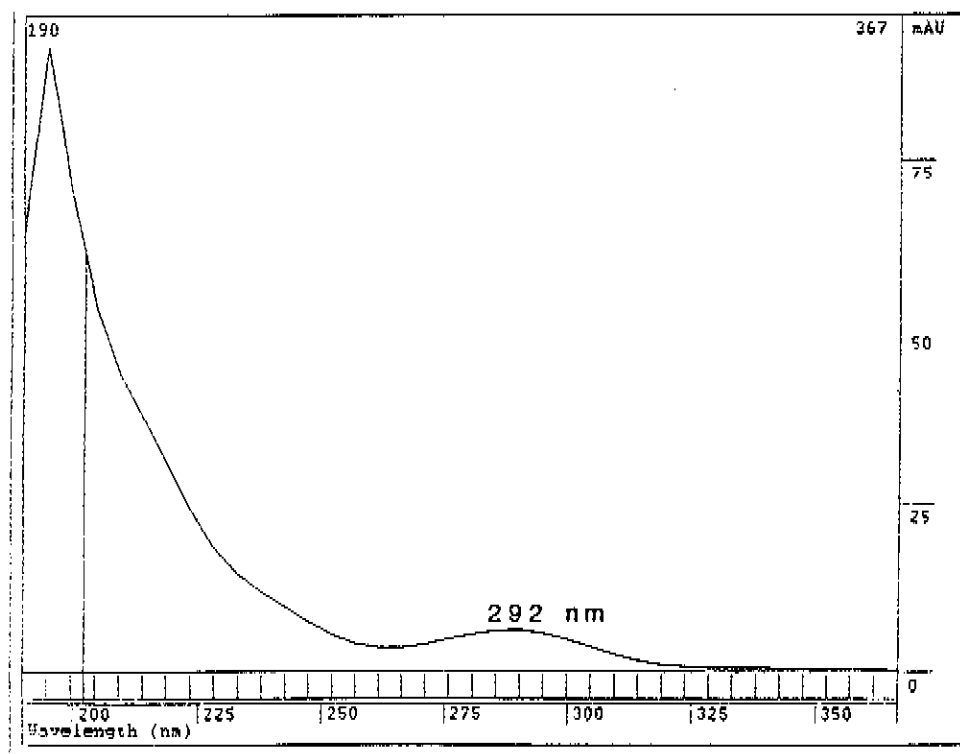


Figure 7. UV-spectrum for bacitracin F (or to F related substances) in eluent 2.

DATA GIVEN BY COLLABORATING LABORATORIES

EPCRS Batch 1 Results from 1987.

Microbiological assay: 63.1 IU/mg (collaborative study)

TLC: Identity complies

Bacitracin F and related substances: Complies 0.105

Zn: 4.9%

LOD: 0.6%

DATA GIVEN BY THE MANUFACTURER

Values reported 1984

Bacitracin F and related substances: 0.09

Zn: 4.8% by AAS

LOD: 3.0%

Assay: 69.2 IU/mg

KF: 2.8%

STABILITY

Stability in dry state:

Bacitracin zinc was exposed to air at different relative humidities at room temperature (about 20 °C) for a period of 8 weeks as described in WHO/PHARM/82.509. Bacitracin zinc is hygroscopic. After one month it had gained between 1.6% to 20% in weight, when stored at 11% RH to 98% RH. The gain in weight may have occurred already after a few days.

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

Stability in solution:

According to Florey Volume 9, Bacitracin F is a degradation product of bacitracin. To identify the retention time of bacitracin F in the liquid chromatographic system used a sample of the proposed ICRS was "stressed" in a buffer solution of pH 11 and stored at 60 °C for at least 24 hours. The main degradation product which elutes at about 24 minutes (Figure 5) accounts for about 7% of the composition after 24 hours. The spectrum of the peak recorded by the diode array detector exhibited a spectrum different to that of bacitracin A, with an absorbance maximum at 292 nm. This indicates that the degradation product is bacitracin F or a related substance.

CONCLUSION

Bacitracin zinc, Control No 192174, can be considered suitable as International Chemical Reference Substance for the intended purpose.

BECLOMETASONE DIPROPIONATE

Control No 192175

Analytical Report

INTENDED USE

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

MATERIAL

About 20 g of the sample (manufacturers batch no 89/26, EPCRS Lot 1) were received at the WHO Centre in October 1990. The material is being stored in tightly closed containers at + 5 °C, protected from light.

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (Control No 192175). The spectrum is concordant with the spectrum of the USP reference standard Lot H.

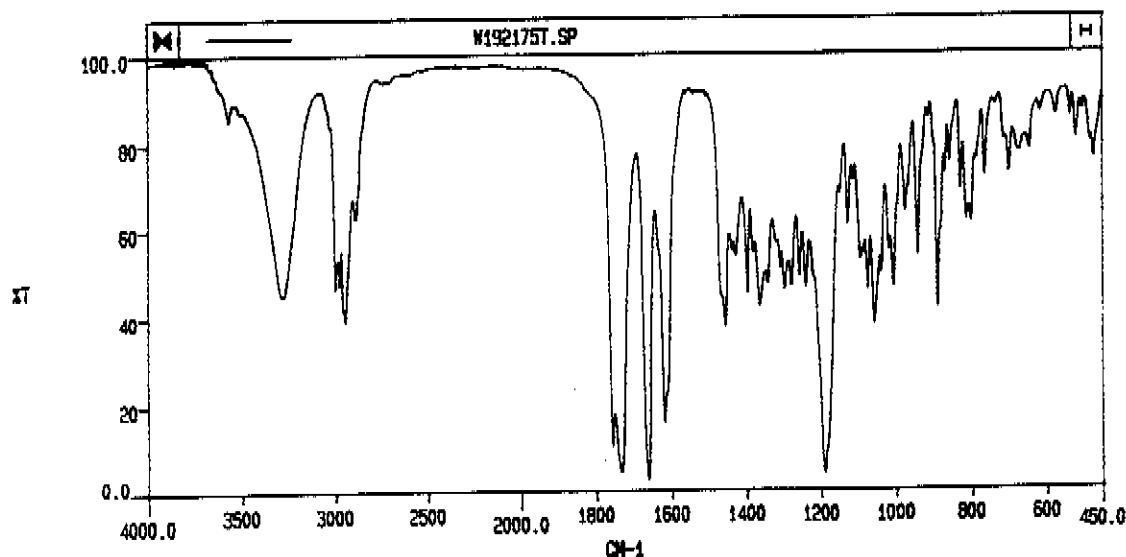


Figure 1. IR-spectrum of 1.45 mg of beclometasone dipropionate Control No 192175 in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 1600 FTIR.

UV-spectrum

A UV-spectrum in methanol was recorded. A maximum was found at 239 nm.

A (1%, 1 cm) = 297 determined at 239 nm (n= 6 RSD= 0.4%).
The result is calculated with reference to the dried substance.
The A-value for the USP reference standard lot H was found to be 296.

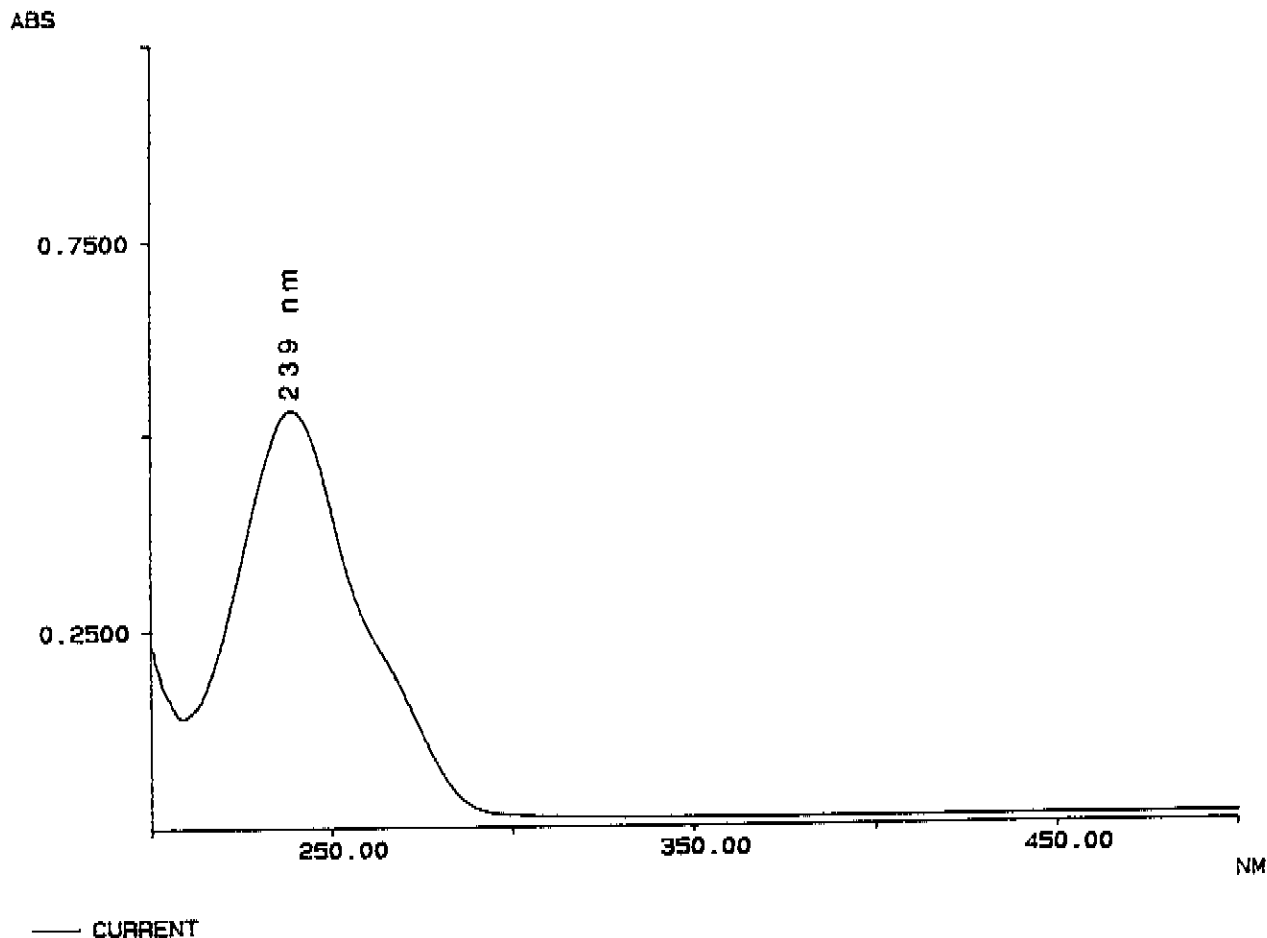


Figure 2. UV-spectrum of beclomethasone dipropionate Control No 192175 18 µg/ml in methanol.

ASSAY

Spectrophotometric assay: 99.8% (n= 7, RSD= 1.3%) calculated with reference to the dried substance. The USP reference standard lot H was used as standard and regarded as 100%. The determination is done by the blue tetrazolium method according to Ph. Int. 3rd Ed. Vol 3.

Thermogravimetric analysis: When the substance was heated to 105 °C a loss of 0.3% of weight was observed.

Instrument:	Perkin Elmer TGA 7 Thermogravimetric analyzer.
Sample weight:	3 mg
Heating rate:	5 °C/min
Decomposition temperature:	about 210 °C

PURITY

Thin-layer chromatography

System 1:

Six secondary spots were found in UV-light by scanning.

The following thin-layer chromatographic system was used according to the International Pharmacopoeia 3rd Edition Vol. 3 page 39 under test for related substances.

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

Eluent: Dichloroethane:Methanol:Water (95:5:0.2)

Sample: 100 µg of beclometasone dipropionate were applied.
The sample was dissolved in chloroform:methanol (9:1).

Visualization: UV-light of 254 nm, evaluation by densitometry at 239 nm and spraying with blue tetrazolium/ethanol TS followed by heating to 105 °C and examination in day-light. Four secondary spots were found in UV-light at 254 nm, when scanned at 239 nm two further spots were observed giving a total number of six spots. After spraying 4 spots were detected.

Rf (beclometasone dipropionate) = 0.25
Rf (beclometasone 17-propionate) = 0.06
Rf (beclometasone 21-propionate) = 0.12

Beclometasone dipropionate USP lot H was also tested, and two secondary spots were found. In BPCRS 1637 five secondary spots were found.

System 2:

The following thin-layer chromatographic system was used according to the the European Pharmacopoeia 2nd Edition page 654 under identification.

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

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

Sample: 100 µg of beclometasone dipropionate were applied.
The sample was dissolved in chloroform:methanol (9:1).

Visualization: UV-light of 254 nm, evaluation by densitometry at 239 nm and spraying with blue tetrazolium/ethanol TS followed by heating to 105 °C and examination in day-light. One secondary spot was found in UV-light at 254 nm, when scanned at 239 nm two further spots were observed giving a total number of three spots. The total amount was estimated to 0.3%. After spraying three spots were detected. For purity determinations system 1 is preferred since it gives better separation. However liquid chromatography is superior for purity determinations.

Rf (beclometasone dipropionate) = 0.6
Rf (beclometasone 17-propionate) = 0.3
Rf (beclometasone 21-propionate) = 0.5

Beclometasone dipropionate USP lot H was also tested, and about 0.1% impurities were found. In BPCRS 1637 about 0.2% impurities were found.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to be about 1.5%. Six extra peaks were observed. None of them corresponded to beclometasone 17- or 21-propionate which eluted at 18.2 and 16.1 minutes respectively.

A chromatogram is shown in Figure 3.

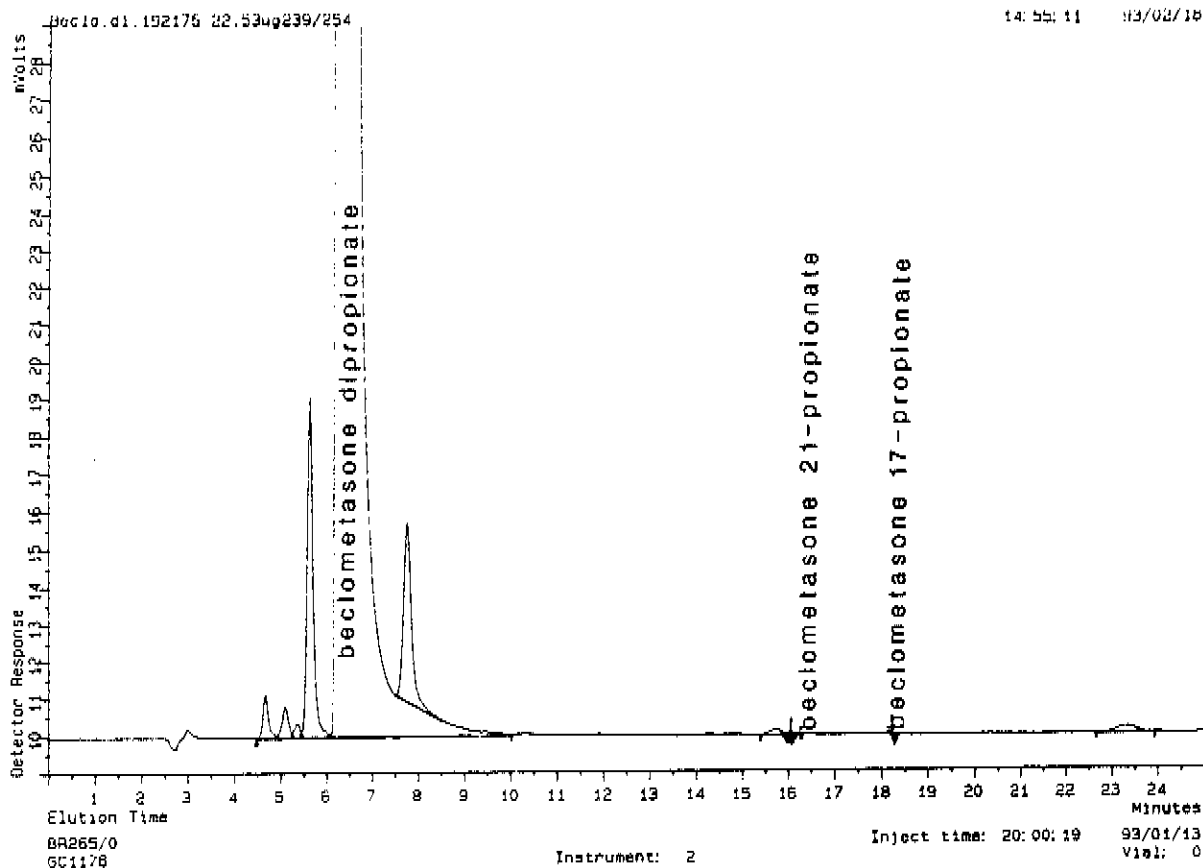


Figure 3. Chromatogram of beclometasone dipropionate, Control No 192175.

The following conditions were used:

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

Column: Spherisorb S5W, silica.

Detector: Varian 9065 Polychrom detector, operated at 239 nm.

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

Integrator: PeakPro (Beckman)

Sample: 1 mg/ml dissolved in the eluent.
20 µl corresponding to 20 µg were injected.

The detection limit for beclometasone dipropionate was about 0.002 µg (0.01%).
A comparison was also made with USP RS lot G which contained about 0.2% impurities and BPCRS 1637 which contained about 1.5% impurities.

Diode-array detection

The chromatographic system described above was also evaluated with a Varian 9065 Polychrom detector. UV-maxima in the eluent were recorded for beclometasone dipropionate and for 4 extra peaks. All UV-maxima were found to be 234-239 nm. This indicates that 239 nm is a suitable detection wavelength for the determination of impurities in beclometasone dipropionate. The major impurity eluting at about 5.6 minutes showed a different UV-spectrum with a second maximum at about 280 nm.

DATA GIVEN BY THE MANUFACTURER

Values reported in 1990.

Specific absorbance 1%, 1 cm at 238 nm	295
Absorbance ratio 238/263 nm	2.36
Related foreign steroids	complies
LOD	0.16%
Melting point	211 °C
Sulfated ash	nil
Specific optical rotation at 25 °C	+92 °
Assay, HPLC, % w/w	99

DATA GIVEN BY COLLABORATING LABORATORIES

The results were reported 1990.

EPCRS

IR: complies with BP standard

TLC: identity complies

Colour reaction: complies

Specific absorbance: 290

HPLC related substances: 0.56% -1.4%

LOD: 0.27%

Optical rotation: + 91.4°

Assay spectrophotometric: 98.3%,102.0%, 98.8% (BPCRS as standard)

DSC: 212.9 °C melting point, uncorrected

PSA: 1.7% (decomposition ?)

STABILITY

No special stability studies were performed as this substance was found to be resistant to degradation in a dry state under conditions described in WHO/PHARM/86.529. Regular re-examinations of the ICRS will be performed.

CONCLUSION

Beclometasone dipropionate, Control No 192175, can be considered suitable as International Chemical Reference Substance for the intended purpose. When calculating results of assays according to the monograph the content of C₂₈H₃₇ClO₇ (beclometasone dipropionate) is taken to be 99.8% calculated with reference to the dried substance (corresponding to 99.5% when calculated on an "as is" basis).

DEXAMETHASONE PHOSPHORIC ACID

Control No 192161

Analytical Report

INTENDED USE

The monograph for Dexamethasone sodium phosphate in the International Pharmacopoeia 3rd Ed. Vol 3 requires a reference substance for dexamethasone sodium phosphate to be used in the thin-layer chromatographic test for identity. Dexamethasone sodium phosphate is a very hygroscopic substance. Therefore, it is recommended that if a reference substance is required, for example for assay purposes, rather to use dexamethasone phosphoric acid ICRS, which is less hygroscopic and easier to handle.

MATERIAL

About 5 g of the sample (manufacturers batch no L-579, 423-000S023) were received at the WHO Centre in March 1993. The material is being stored in tightly closed containers at + 5 °C, protected from light.

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (Control No 192161). The spectrum is concordant with the spectrum obtained with the USP reference standard dexamethasone phosphate acid Lot I and with a test sample of another batch of dexamethasone phosphoric acid from the same manufacturer that supplied the ICRS.

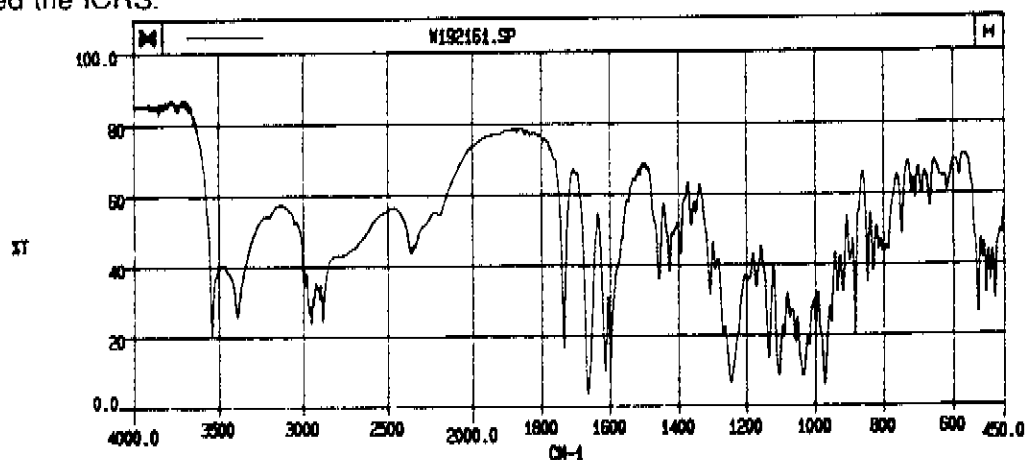


Figure 1. IR-spectrum of 1.4 mg of dexamethasone phosphoric acid Control No 192161 in 300 mg KBr recorded against a KBr disc.
Instrument: Perkin-Elmer 1600 FTIR.

The spectra of dexamethasone sodium phosphate, dexamethasone phosphoric acid and dexamethasone, respectively, differ significantly for example at 1000 cm^{-1} and 1100 cm^{-1} . Thus IR is preferred to TLC, since TLC cannot distinguish between the dexamethasone sodium phosphate and dexamethasone phosphoric acid.

UV-spectrum

The UV-spectrum was recorded in water. A maximum was found at 242 nm.

$A(1\%, 1\text{ cm}) = 336$ ($n=5$, $\text{RSD}=0.5\%$) determined at 242 nm.
The result is calculated with reference to the anhydrous substance.

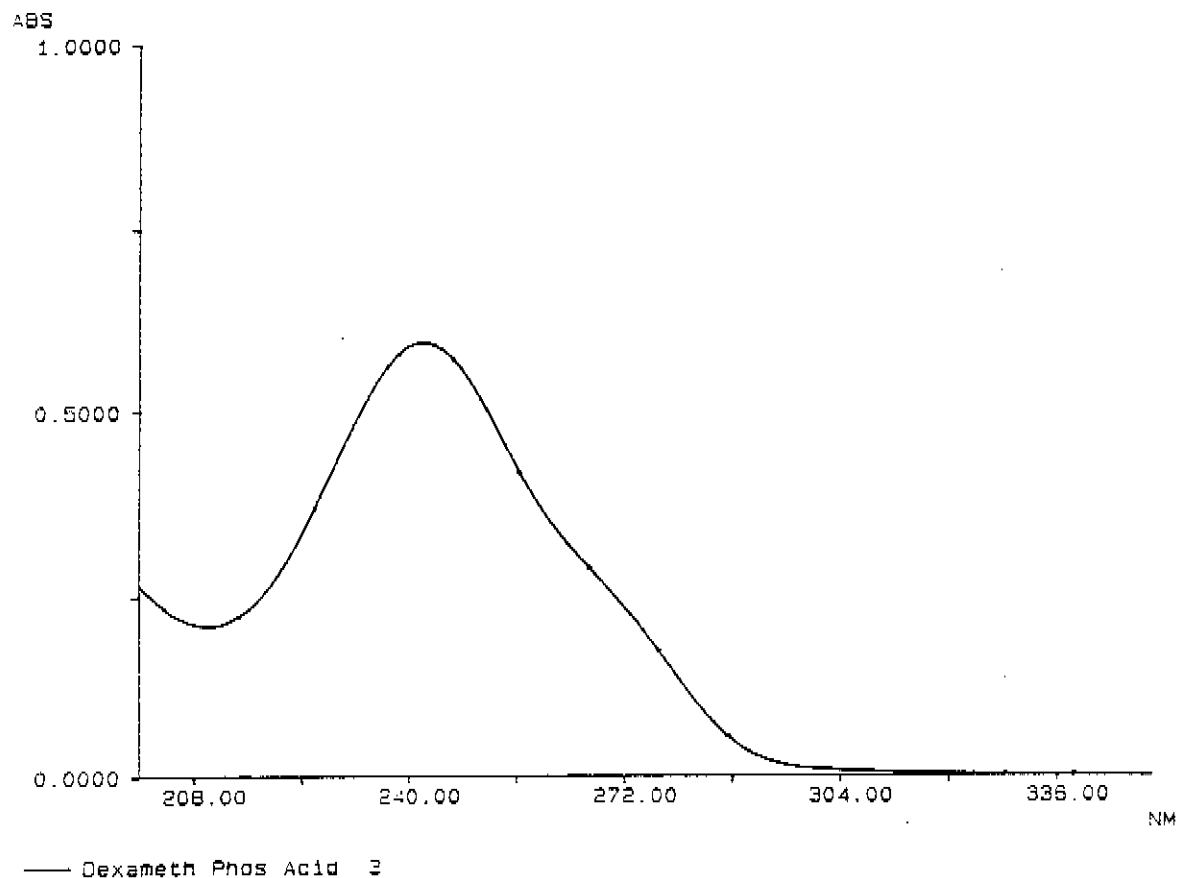


Figure 2. *UV-spectrum of dexamethasone phosphoric acid Control No 192161 17.8 $\mu\text{g}/\text{ml}$ in water.*

ASSAY

Spectrophotometric assay: 100.2% calculated with reference to the anhydrous substance when determined in water against the USP reference standard for dexamethasone phosphate acid Lot 1 which was regarded as 100%.

Thermogravimetric analysis: When the substance was heated to 130 $^{\circ}\text{C}$ a loss of about 1% of weight was observed.

Instrument:	Perkin Elmer TGA 7 Thermogravimetric analyzer.
Sample weight:	1.4 mg
Heating rate:	2 $^{\circ}\text{C}/\text{min}$
Decomposition temperature:	about 190 $^{\circ}\text{C}$

Loss on drying: 1.0% (105 °C, in vacuo for 2 hours)

Ethanol: 0.1% determined by gas chromatography.

Water: 0.7% determined by Karl Fischer titration.

PURITY

Thin-layer chromatography

System 1:

The total amount of impurities was estimated to be approximately 0.1%.

The thin-layer chromatographic system used was according to the International Pharmacopoeia 3rd Edition Vol. 3 page 92 under identity test.

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

Eluent: 1-Butanol:acetic anhydride:water (3:1:1)

Sample: 100 µg of dexamethasone phosphoric acid were applied.
The sample was dissolved in methanol.

Visualization: Spraying with 10% sulphuric acid/ethanol and visualization with ultraviolet light at 365 nm.

One secondary spot was observed close to the starting point both visually at 254 nm and at 365 nm after spraying. No spot corresponding to dexamethasone was observed. It was confirmed by densitometry, at 240 nm and 365 nm. The estimated amount of the impurity was at the detection limit (0.1%).

Rf (dexamethasone sodium phosphate) = 0.5

Rf (dexamethasone phosphoric acid) = 0.5

Rf (dexamethasone) = 0.7

System 2:

The thin-layer chromatographic system used, described in the International Pharmacopoeia 3rd Edition Vol. 3 page 94 in the test for free dexamethasone and other related substances is not considered suitable for purity determinations as it gave rise to elongated spots. No secondary spots were found.

Thin-layer: Silica gel G (Merck).

Eluent: Methanol

Sample: 100 µg of dexamethasone phosphoric acid dissolved in methanol were applied.

Visualization: Spraying with zinc chloride followed by heating for one hour at 125 °C.
Densitometry at 240 nm before spraying and at 365 nm after spraying.

No secondary spots were detected visually after spraying. When evaluated by densitometry, broad peaks were obtained due to the poor selectivity of the system. The liquid chromatographic system described below is considered better for the determination of the content of free dexamethasone.

Rf (dexamethasone sodium phosphate) = 0.7

Rf (dexamethasone phosphoric acid) = 0.7
Rf (dexamethasone) = 0.75

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to be approximately 0.1%. A chromatogram is shown in Figure 3. The peak eluting at 19.7 minutes was identified as dexamethasone and estimated to be about 0.03% by peak area measurement. The same result was obtained when it was estimated against a reference standard for dexamethasone.

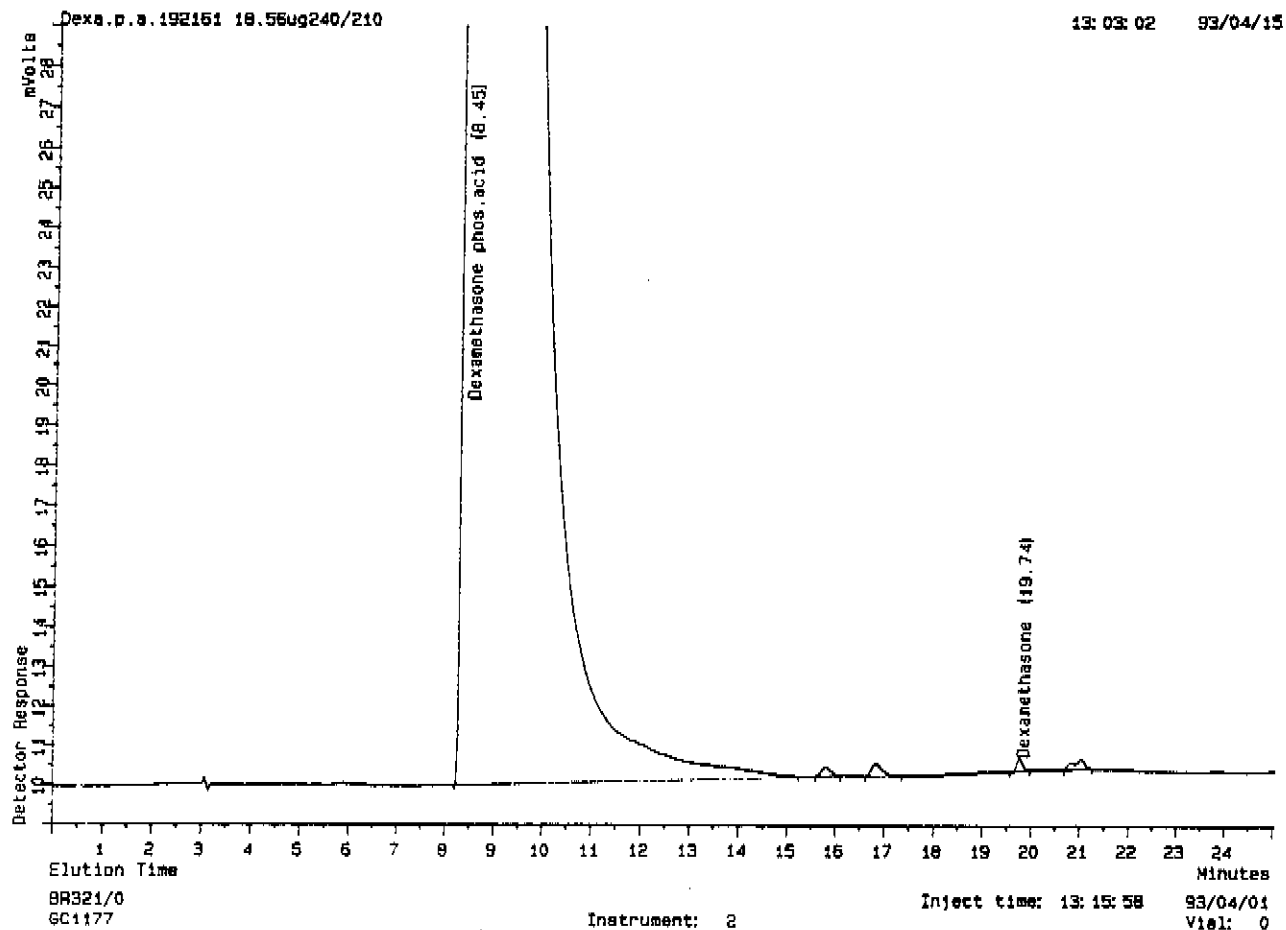


Figure 3. Chromatogram of dexamethasone phosphoric acid, Control No 192151.

The following conditions were used:

Eluent: Acetonitrile: 0.01 M potassium phosphate buffer pH 4.7. To determine the amount of dexamethasone, gradient elution was necessary.

Time, min	% Acetonitrile	% buffer
0	25	75
5	25	75
15	50	50
25	50	50

Column: Spheri -5 OD-5A RP 18, Brownlee Labs

Detector: Varian 9065 Polychrom operated at 239 nm.

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

Integrator: PeakPro (Beckman)

Sample: 1 mg/ml dissolved in the eluent.
20 μ l corresponding to 20 μ g were injected.

The detection limit for dexamethasone phosphoric acid is approximately 0.1 μ g/ml (0.01%).

The USP reference standard lot I was shown to contain 0.1% impurities.

Diode-array detection

The chromatographic system described above was also evaluated with a Varian 9065 Polychrom detector. The same chromatographic system as described above was used. UV-spectra were recorded for dexamethasone phosphoric acid and for 4 other peaks. UV-maxima were found to be at 239 nm for all peaks, indicating that 239 nm is a suitable detection wavelength for the determination of impurities in dexamethasone phosphoric acid. A UV-spectrum for the main peak of dexamethasone phosphoric acid, recorded in the eluent, is given in Figure 4.

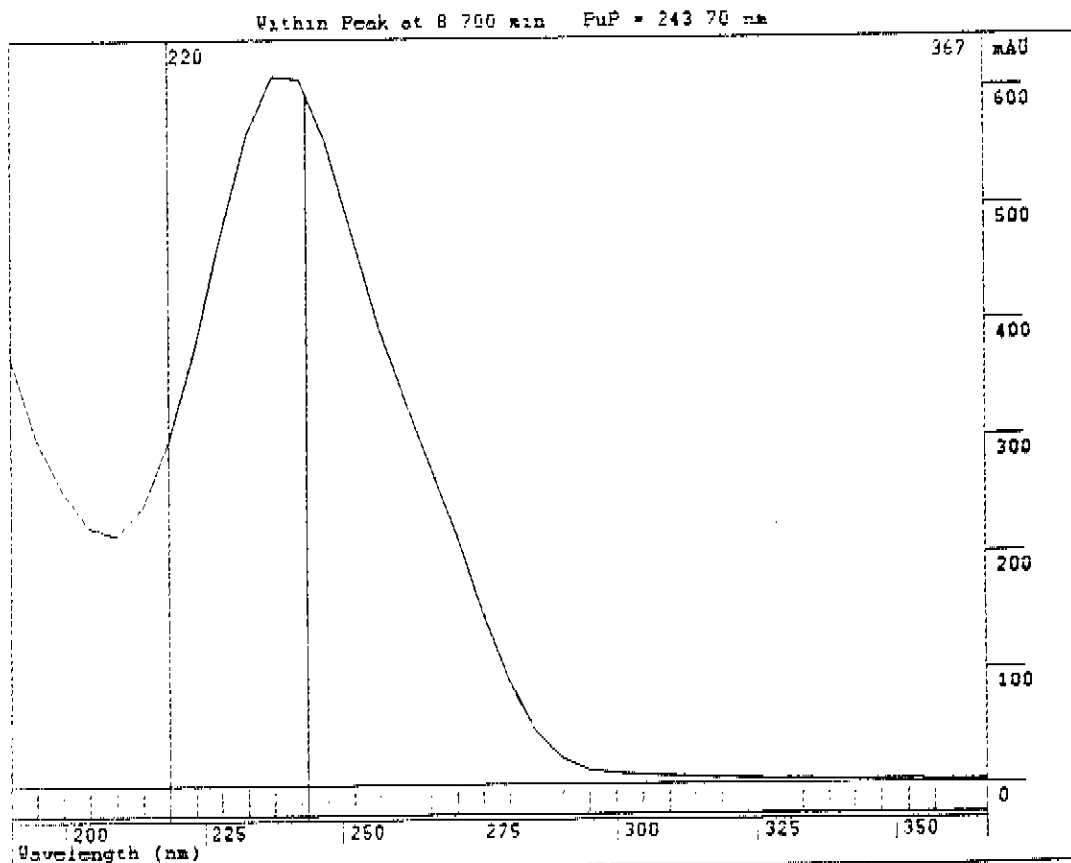


Figure 4. UV-spectrum of dexamethasone phosphoric acid recorded in the eluent.

DATA GIVEN BY THE MANUFACTURER

A (0.1%, 1cm) at 239 nm = 34.2
Water: 0.23%
HPLC: 99.7% by area, purity
HPLC: 101.5%, assay against a standard
Assay: 100.9% potentiometric titration
Free dexamethasone: 0.3% by HPLC
TLC: Single elongated spot
PSA: 99.8%

STABILITY

Dexamethasone phosphoric acid was exposed to air of different relative humidity at room temperature (about 20 °C) for a period of 2 weeks as described in WHO/PHARM/82.509. The substance is hygroscopic. For samples stored between 55% RH to 97% RH an increase in weight between 6-7% was observed. At humidities below 20% RH a loss of about 0.5-1% of weight was observed. When the samples were analysed by the liquid chromatographic method described above, no significant chemical degradation was observed.

CONCLUSION

Dexamethasone phosphoric acid, Control No 192161, can be considered suitable as International Chemical Reference Substance for the intended purpose. When used in assays the content of dexamethasone phosphoric acid is taken to be 100.0% calculated with reference to the dried substance which corresponds to 99.0% calculated on the "as is" basis.

DEXAMETHASONE SODIUM PHOSPHATE

Control No 192158

Analytical Report

INTENDED USE

The monograph for Dexamethasone sodium phosphate in the International Pharmacopoeia 3rd Ed. Vol 3 requires a reference substance for dexamethasone sodium phosphate in the thin-layer chromatographic test for identity. The Centre suggests that infrared spectroscopy should rather be used for identity as this method can distinguish dexamethasone sodium phosphate from dexamethasone phosphoric acid. If a reference substance is needed for assay purposes it is recommended to use dexamethasone phosphoric acid ICRS which is less hygroscopic and easier to handle than dexamethasone sodium phosphate.

MATERIAL

About 100g of the sample (manufacturers batch no 748 AM) were received at the WHO Centre in July 1989. The material is being stored in tightly closed containers at + 5 °C, protected from light.

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (Control No 192158), which is concordant with that of the EPORS Lot no 1.

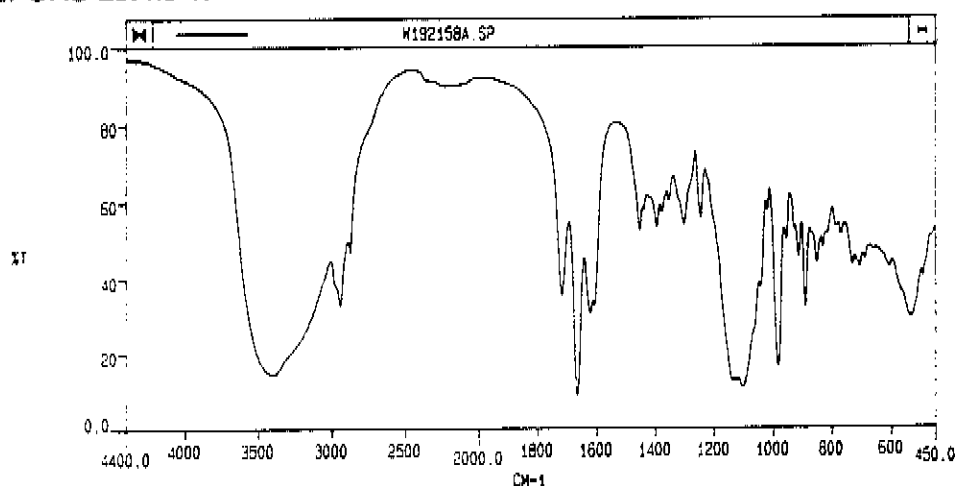


Figure 1. IR-spectrum of 1.38 mg of dexamethasone sodium phosphate Control No 192158 in 300 mg KBr recorded against a KBr disc.
Instrument: Perkin-Elmer 1600 FTIR.

The spectra for dexamethasone sodium phosphate, dexamethasone phosphoric acid and dexamethasone, respectively, differ significantly for example at 1000 cm^{-1} and 1100 cm^{-1} . Thus IR is preferred to TLC since TLC cannot distinguish between the dexamethasone sodium phosphate and dexamethasone phosphoric acid.

UV-spectrum

The UV-spectrum in water was recorded showing a maximum at 242 nm.

$A (1\%, 1\text{ cm}) = 291\text{-}296$ determined at 242 nm with reference to the dried substance. As the substance is hygroscopic it is very important to have reliable values for the content of water and solvents when performing the determination of A. It is recommended that if a reference substance is required for assay purposes, then use dexamethasone phosphoric acid ICRS, which is less hygroscopic and easier to handle.

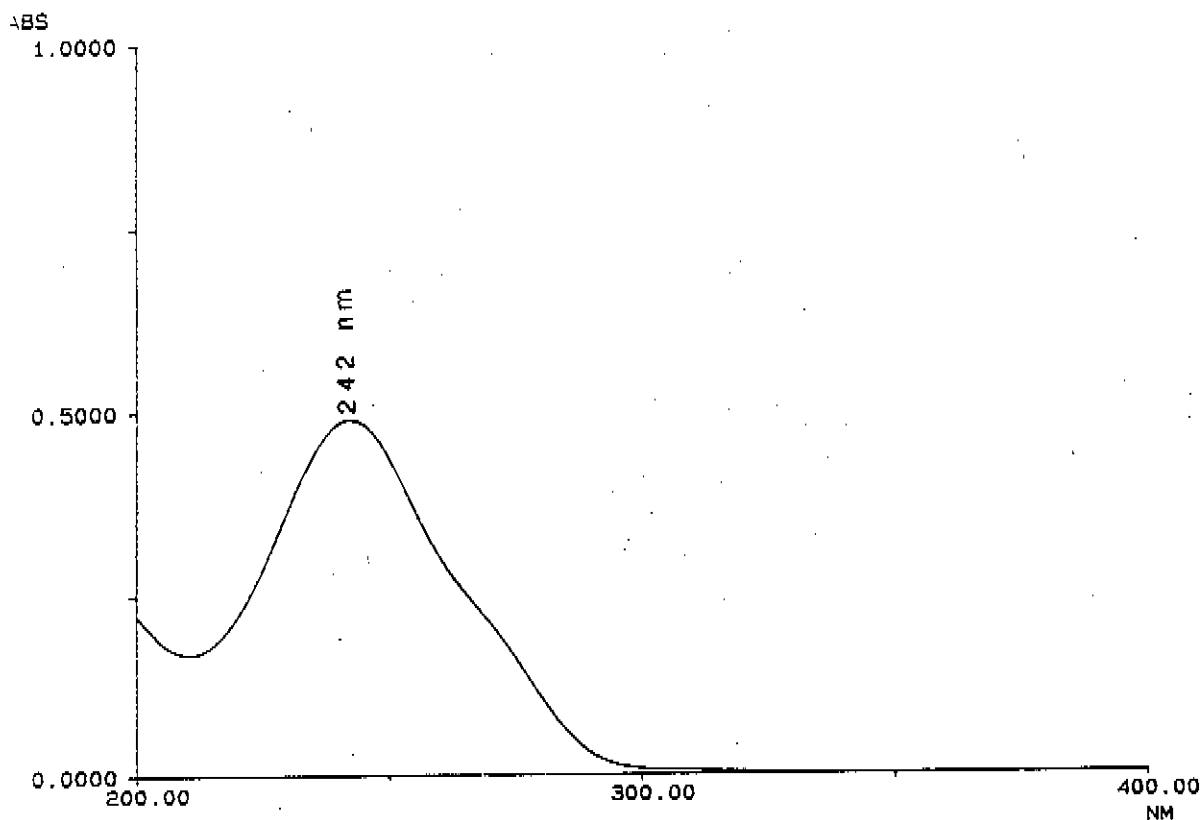


Figure 2. *UV-spectrum of dexamethasone sodium phosphate Control No 192158 18 $\mu\text{g/ml}$ in water.*

Sodium: 8.3%, determined by atomic absorption spectrophotometry.

ASSAY

Spectrophotometric assay: 98-100% calculated with reference to the anhydrous and ethanol-free substance. As the substance is very hygroscopic it is difficult to determine values of water and alcohol, it is recommended to use dexamethasone phosphoric acid ICRS in assays.

Thermogravimetric analysis: When the substance was heated to $190\text{ }^{\circ}\text{C}$ a loss of 8.0-8.5% of weight was observed. It was difficult to perform TG-determinations on this substance as the end-point of the curve was difficult to define.

Instrument: Perkin Elmer TGA 7 Thermogravimetric analyzer.
Sample weight: 8.7 mg
Heating rate: 0.6 °C/min
Decomposition temperature: about 233-235 °C

Loss on drying: 9.7% (105 °C, in vacuo for 25 hours)

Ethanol: 3.7%, determined by gas chromatography.

Water: 4.3%, determined by Karl Fischer titration.

PURITY

Thin-layer chromatography

System 1:

The total amount of impurities was estimated to approximately 0.6%.
The thin-layer chromatographic system used was described in the International Pharmacopoeia 3rd Edition Vol. 3 page 92.

Thin-layer: Silica gel G (Merck).

Eluent: 1-Butanol:acetic anhydride:water (3:1:1)

Sample: 100 µg of dexamethasone sodium phosphate dissolved in methanol were applied.

Visualization: Spraying with 10% sulphuric acid/ethanol and visualization in ultraviolet light at 365 nm.

No secondary spots were detected visually. When evaluated by densitometry, 3 secondary spots were detected estimated to be approximately 0.6% at 363 nm and 0.2% at 230 nm. The principal impurities were close to the starting point. The limit of detection of the system was about 0.1µg (0.1%) when scanned at 363 nm.

Rf (dexamethasone sodium phosphate) = 0.5

Rf (dexamethasone phosphoric acid) = 0.5

Dexamethasone sodium phosphate BPCRS 1281 was also tested and found to contain approximately 0.4% of impurities.

System 2:

The thin-layer chromatographic system of the International Pharmacopoeia 3rd Edition Vol. 3 page 94 was used as described under free dexamethasone and other related substances. This system was not suitable for purity determinations as it gave rise to elongated spots.

Thin-layer: Silica gel G (Merck).

Eluent: Methanol

Sample: 100 µg of dexamethasone sodium phosphate dissolved in methanol were applied.

Visualization: Spraying with zinc chloride followed by heating at 125 °C for one hour. Densitometry at 240 nm before spraying and at 365 nm after spraying.

No secondary spots were detected visually after spraying. When evaluated by densitometry, broad peaks were obtained due to the poor selectivity of the system. To determine free dexamethasone the liquid chromatographic system given below is recommended.

Rf (dexamethasone sodium phosphate) = 0.7

Rf (dexamethasone phosphoric acid) = 0.7

Rf(dexamethasone) = 0.75

Dexamethasone sodium phosphate BPCRS No 1281 and EPCRS Lot 1 were also tested but no secondary spots were found due to the poor selectivity of the system.

High performance liquid chromatography

System 1:

The total amount of impurities was estimated by peak area measurement, to be approximately 1.4%. A chromatogram is shown in Figure 3. The peak eluting at 19-20 minutes was identified as dexamethasone and estimated to be approximately 0.1% by peak area measurement. The same result was obtained using a reference standard for dexamethasone.

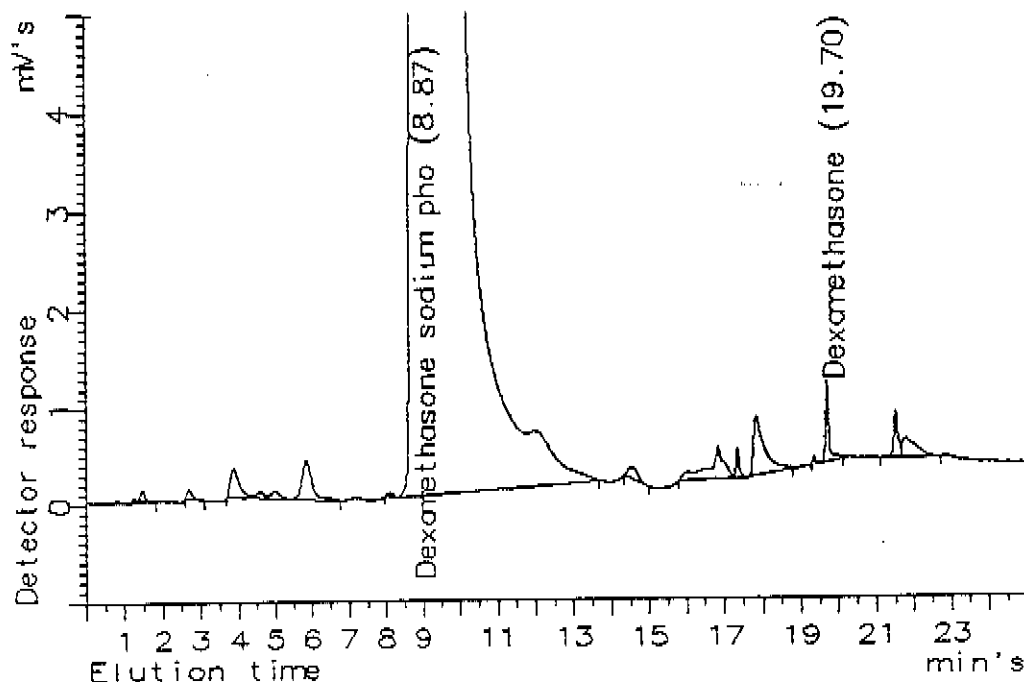


Figure 3. Chromatogram of dexamethasone sodium phosphate, Control No 192158.

The following conditions were used:

Eluent: Acetonitrile:0.01 M potassium phosphate buffer pH 4.7. To determine the amount of dexamethasone gradient elution was used.

<u>Time. min</u>	<u>% Acetonitrile</u>	<u>% buffer</u>
0	20	80
10	20	80
15	50	50
25	50	50

Column: Spheri -5 OD-5A RP 18, Brownlee Labs

Detector: Varian UV-100 operated at 240 nm.

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

Integrator: PeakPro (Beckman)

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

The detection limit for dexamethasone sodium phosphate was approximately 0.5 μ g/ml (0.05%).

A comparison was also made with EPCRS Lot 1 and BPCRS No 1281 which was shown to contain 0.7% and 0.4% impurities respectively.

System 2 :

The total amount of impurities was estimated by peak area measurement to approximately 1.3%.

A chromatogram is shown in Figure 4. The peak eluting at 11 minutes was identified as dexamethasone and estimated to about 0.1%.

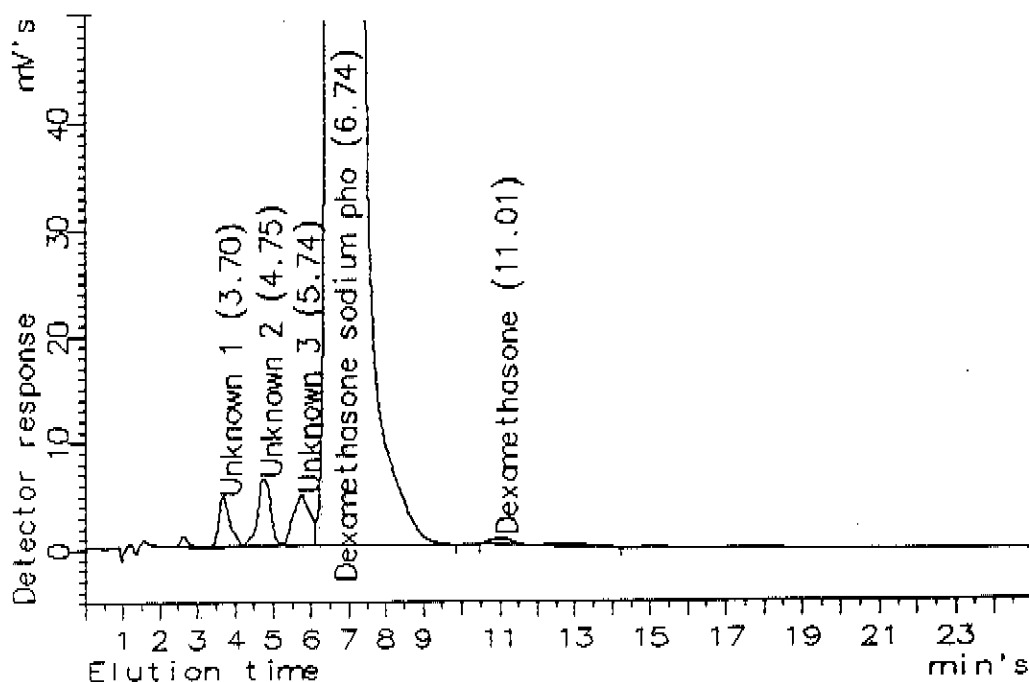


Figure 4. Chromatogram of dexamethasone sodium phosphate, Control No 192158.

The following conditions were used:

Eluent: Methanol:0.75% triethylamine in water pH 5.5 (50:50)

Column: Nova-Pak Phenyl (Waters)

Detector: Lambda Max 481 Waters operated at 254 nm.

Pump: Waters 600 E operated at a flow rate of 1.2 ml/min

Integrator: PeakPro (Beckman)

Sample: 0.1 mg/ml dissolved in the eluent.
20 μ l corresponding to 20 μ g were injected.

Diode-array detection

The chromatographic system described above under system 1 was also evaluated with a Varian 9065 Polychrom detector. UV-spectra were recorded for dexamethasone sodium phosphate and for 12 extra peaks. UV-maxima were found to be between 190-195 nm and 239 nm for all peaks, indicating that 239 nm is a suitable detection wavelength for the determination of impurities in dexamethasone sodium phosphate. The total amount of impurities was estimated to approximately 1.4% at 239 nm. The similar result was obtained at 210 nm. A UV-spectrum for the main peak of dexamethasone sodium phosphate recorded in the eluent is given in Figure 5.

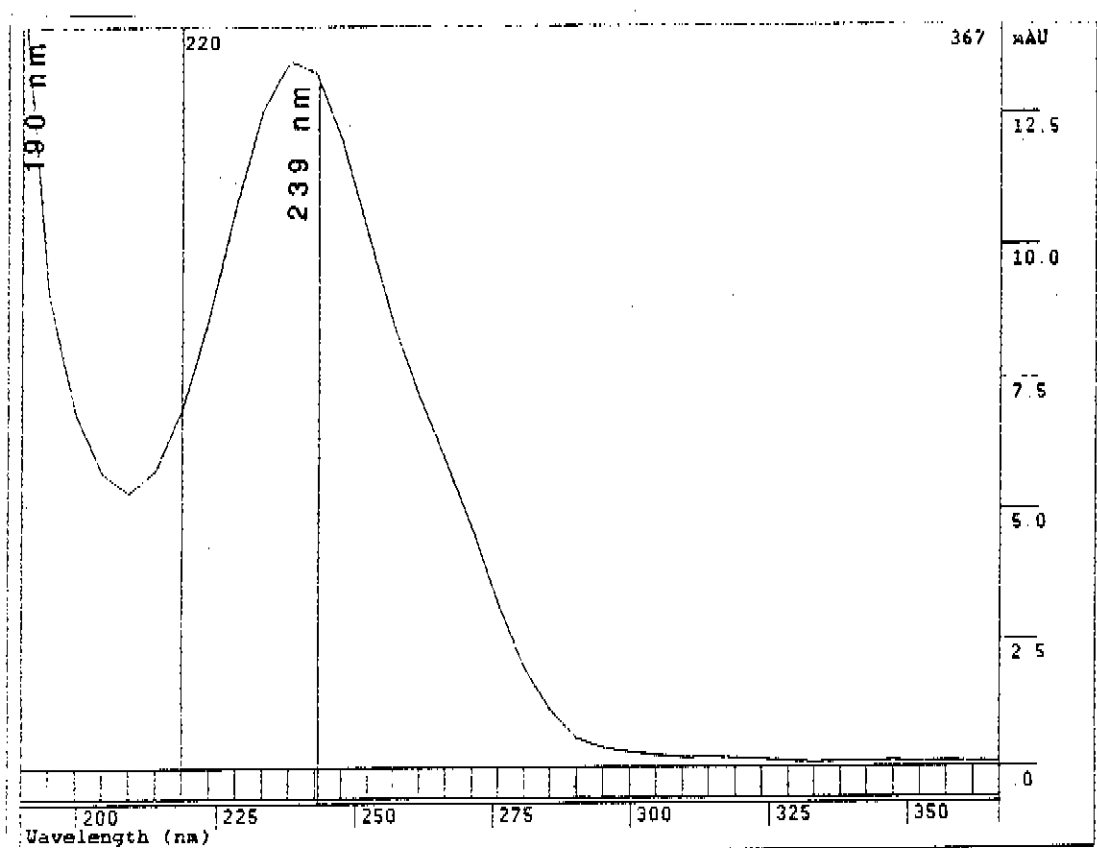


Figure 5. UV-spectrum of dexamethasone sodium phosphate recorded in the eluent.

DATA GIVEN BY THE MANUFACTURER

EP requirements: complies
USP requirements: complies
Assay (USP): 98.8%
Water: 1.3%
Specific rotation: +79 °
pH 8.0
Alcohol: 5.3%

STABILITY

Dexamethasone sodium phosphate was exposed to air at different relative humidities at room temperature (about 20 °C) for a period of 8 weeks as described in WHO/PHARM/82.509. The substance was shown to be very hygroscopic. At the higher humidities a wet cake was formed. For samples stored between 55% RH to 97% RH an increase in weight between 11-29% was found. These changes were already noted after one day. At humidities below 11% RH a loss of weight between 3-6% was observed. Samples that were analysed by the liquid chromatographic method described as system 1, showed no significant chemical degradation.

NB ! Due to the high hygroscopicity of this substance it is considered as only suitable for identification purposes.

CONCLUSION

Dexamethasone sodium phosphate, Control No 192158, can be considered suitable as International Chemical Reference Substance for the intended purpose.

DOPAMINE HYDROCHLORIDE

Control No 192159

Analytical Report

INTENDED USE

The monograph for Dopamine hydrochloride in the International Pharmacopoeia 3rd Ed. Vol 3 requires a reference substance for dopamine hydrochloride to be used for the infrared spectrophotometric test for identity and the thin-layer chromatographic test for purity.

MATERIAL

About 100 g of the sample (manufacturers batch no 296370 290, EPCRS 1) were received at the WHO Centre in October 1990. The material is being stored in tightly closed containers at + 5 °C, protected from light.

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (Control No 192159). The spectrum is concordant with the spectrum of the USP reference standard Lot F-4.

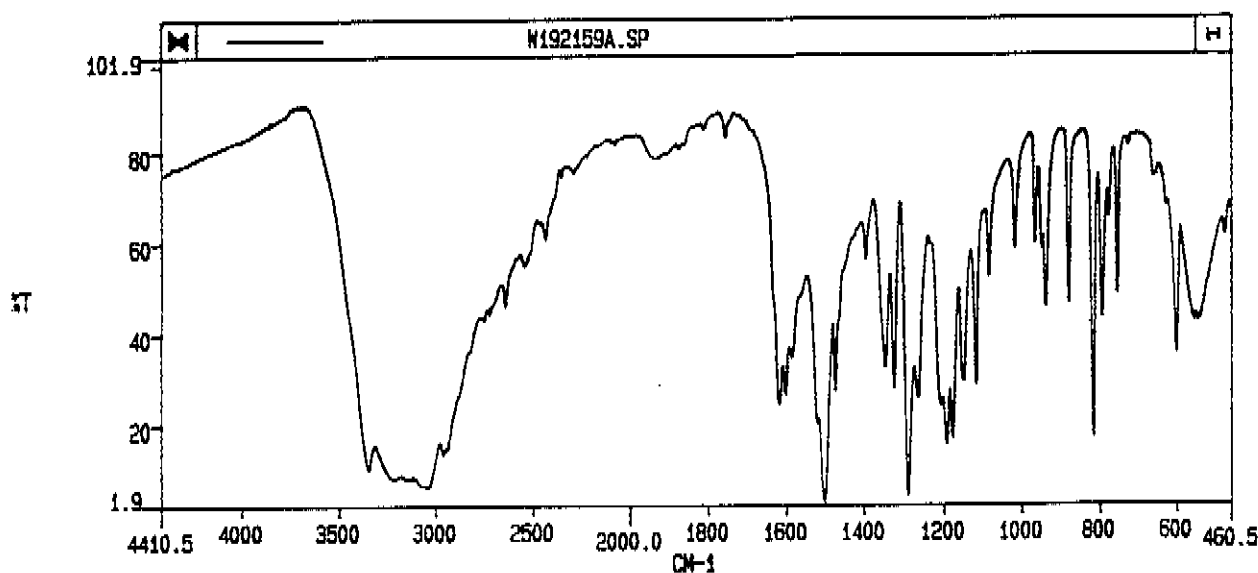


Figure 1. IR-spectrum of 1.13 mg of dopamine hydrochloride Control No 192159 in 300 mg KBr recorded against a KBr disc.
Instrument: Perkin-Elmer 1600 FTIR.

UV-spectrum

The UV-spectrum in 0.1M HCl is given in Figure 2.

λ max in 0.1 M HCl is 280 nm.

A (1%, 1 cm) = 144 at 280 nm (n=6, RSD=0.7%)

When compared to the USP reference standard Lot F-4, for which A also was found to be 144 at 280 nm (n= 6, RSD= 0.5%), the proposed ICRS can be regarded as 100%.

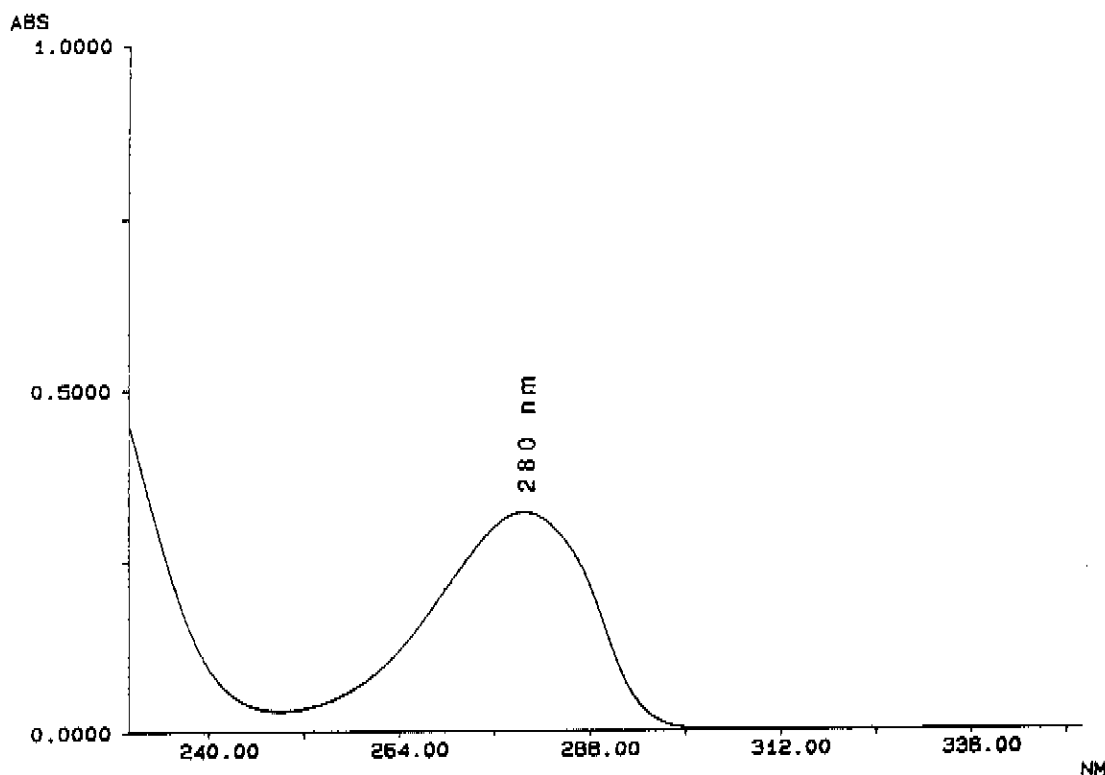


Figure 2. UV-spectrum of probenecid Control No 192159 20 ug/ml in 0.1M HCl.

ASSAY

Spectrophotometric assay: 100.0% when determined against the USP reference substance lot F-4 according to the method described above under UV-spectrum.

Thermogravimetric analysis: When the substance was heated to 200 °C no loss of weight was observed (<0.1%).

Instrument: Perkin Elmer TGA 7 Thermogravimetric analyzer.
Sample weight: 3 mg
Heating rate: 10 °C
Melting point: about 241 °C

PURITY

Thin-layer chromatography

No impurities were detected. The thin-layer chromatographic system used is described in the International Pharmacopoeia 3rd Ed. Vol 3.

Thin-layer: Silica gel 60 G (Merck)

Eluent: Chloroform:methanol: 5M acetic acid (13:9:4)

Sample: 100 µg of dopamine hydrochloride dissolved in methanol were applied.

Visualization: Scanning by densitometry at 280 nm with a Desaga CD 60 Scanner.

Spraying with ferric chloride (50g/l): potassium ferricyanide (50g/l) (2:1) followed by visual examination.

No secondary spots were detected visually at 254 nm. The detection limit of the system was about 0.5µg (0.5%).

R_f (dopamine hydrochloride) = 0.4.

High performance liquid chromatography

No impurities were detected.

A chromatogram is shown in Figure 3.

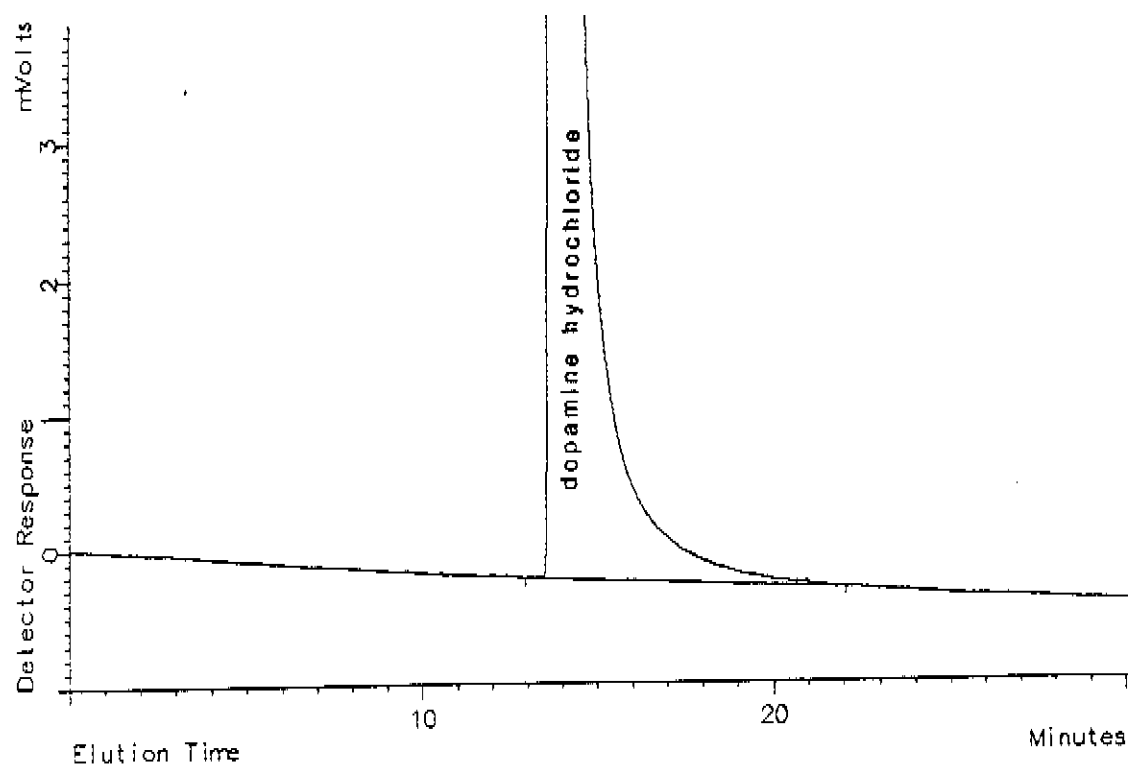


Figure 3. *Chromatogram of dopamine hydrochloride Control No 192159 monitored at 280 nm.*

The following conditions were used:

Eluent: Acetonitrile: 0.04 M KH₂PO₄ buffer (30:70) containing 0.01M sodium lauryl sulfate.
pH = 5.0

Column: RP-18, 5 μ m (Brownlee Labs)
Detector: Varian UV 200 operated at 280 nm.
Pump: Varian 5560 operated at a flow rate of 1.0 ml/min.
Integrator: PeakPro (Beckman)
Sample: 1 mg/ml dissolved in the eluent.
10 μ l corresponding to 10 μ g were injected.

The detection limit for dopamine hydrochloride was 0.005 mg/ml (0.05%).

Diode-array detection

The chromatographic system above was also evaluated with a Varian 9065 Polychrom detector. The same chromatographic system as described above was used. UV-maxima for dopamine hydrochloride were found to be at 200 nm and 280 nm when recorded in the eluent. An impurity peak eluting at 5.6 minutes was found, which UV-maxima were 190 nm and 268 nm. This impurity was estimated to be present at 0.01% using 278 nm as the wavelength of detection. A similar result was obtained at the detection wavelength of 200 nm.

DATA GIVEN BY COLLABORATING LABORATORIES

EPCRS

Infrared: complies

UV: A (1%, 1cm) = 141

Loss on drying: 0.06% (2h, 100-105 °C)

Assay: 100.3%

Liquid chromatography: 99.5% by normalisation at 280 nm.

TLC: No impurities were detected. 4-O-methyldopamine and 3-O-methyldopamine were not found.

STABILITY

No special stability studies were performed as this substance was not suspected to degrade easily. Regular re-examinations of the ICRS will be performed.

CONCLUSION

Dopamine hydrochloride, Control No 192159, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 13

PROBENECID

Control No 192156

Analytical Report

INTENDED USE

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

MATERIAL

About 100 g of the sample (manufacturers batch no 4495 P, EPCRS 1) were received at the WHO Centre in August 1988. The material is being stored in tightly closed containers at + 5 °C, protected from light.

This reference substance has been evaluated in collaboration between the WHO Centre in Stockholm and the National Biological Standards Laboratory, Canberra, Australia. Results reported by the NSBL are indicated with an asterisk (*).

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

The infrared spectrum is given in Figure 1 (Control No 192156). The spectrum is concordant with the spectrum of the USP reference standard Lot H.

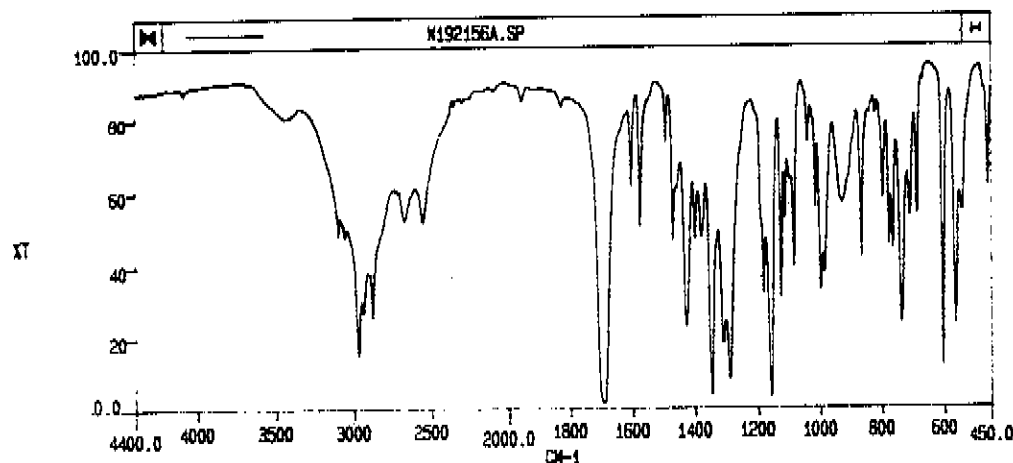


Figure 1. IR-spectrum of 1.25 mg of probenecid Control No 192156 in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 1600 FTIR.

(*)Infrared spectrum

The infrared spectrum of the material, using ATR (attenuated total reflexion) was recorded on a Perkin Elmer 683 Infrared Spectrophotometer. The spectrum was concordant with the BPCRS.

(*)Melting point: 199 °C

UV-spectrum

The UV-spectrum in ethanol/0.1M HCl (9:1) is given in Figure 2.

λ max in ethanol/ 0.1 M HCl are 250 nm and 225 nm.

A (1%, 1 cm) = 337 at 250 nm (n=12, RSD=0.8%)

A (1%, 1 cm) = 320 at 225 nm (n=12, RSD=1.7%)

When compared to the USP reference standard Lot H, for which A was found to be 336 at 250 nm and 314 at 225 nm, the proposed ICRS can be considered as 100.0%.

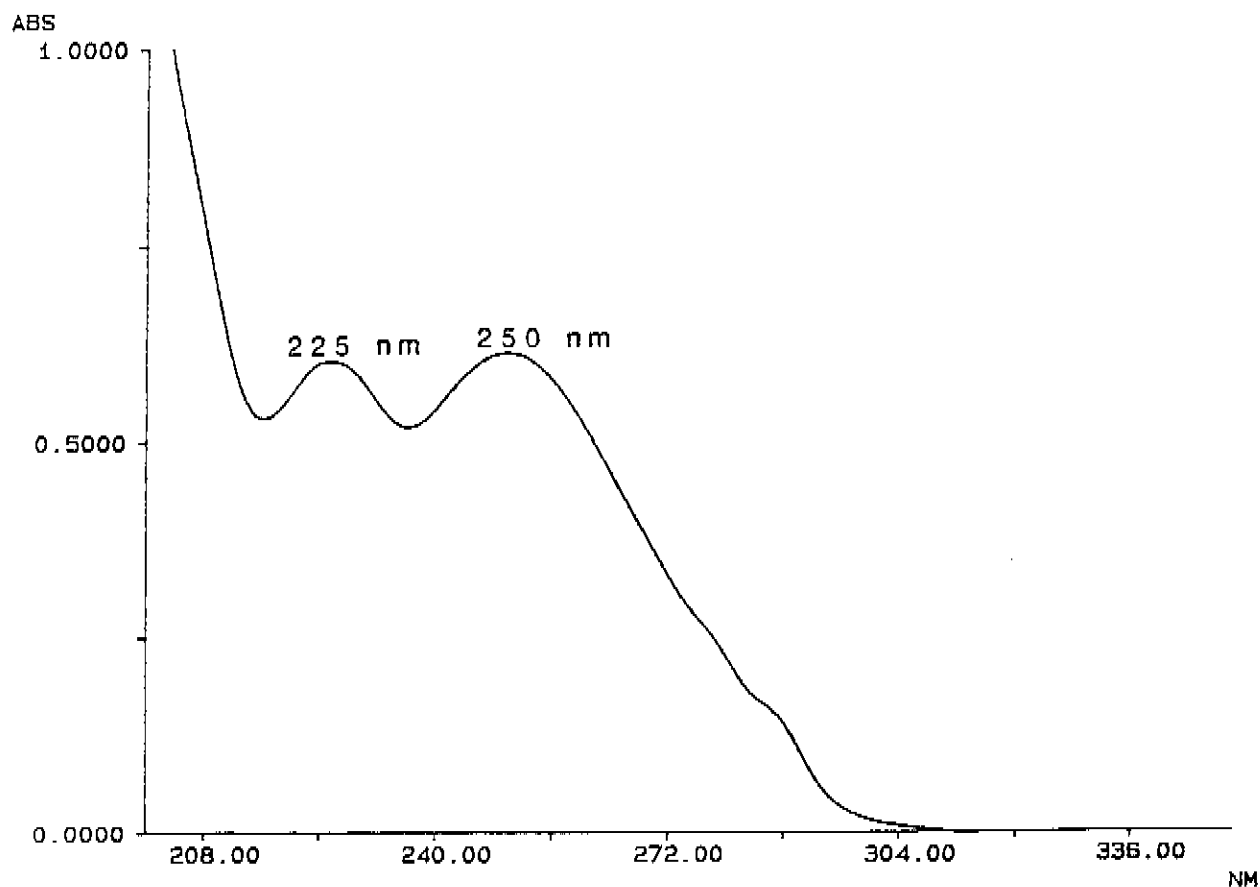


Figure 2. UV-spectrum of probenecid Control No 192156 18 ug/ml in ethanol/0.1M HCl.

(*)UV-spectrum

The identification test procedure of the BP 1988 was used and two maxima were observed for a solution containing approximately 0.001% probenecid in 0.1 M HCl:ethanol (1:9).

A (1%, 1 cm) = 329 at 250 nm (RS= 0.5% n= 4)

A (1%, 1 cm) = 314 at 225 nm (RSD= 1.4% n= 4)

The corresponding A-values for the BP reference substance were 328 at 250 nm and 310 at 225 nm.

ASSAY

Spectrophotometric assay: 100.0% when determined against the USP reference substance lot H according to the method described above under UV-spectrum.

(*)Titrimetric assay: 100.0% (RSD 0.1%) when determined by the method described in the International Pharmacopoeia 3rd Ed. Vol 3.

Thermogravimetric analysis: When the substance was heated to 160 °C no loss of weight was observed (<0.1%).

Instrument: Perkin Elmer TGA 7 Thermogravimetric analyzer.
Sample weight: 3 mg
Heating rate: 5 °C
Melting point: about 199 °C

(*)Loss on drying: No loss was detected when dried to constant weight at 105 °C.

PURITY

Total solid impurities

Differential Scanning Calorimetry (DSC): About 0.05 mol % (n= 4), RSD= 0.03%. The determination was performed on 2 mg using a heating rate of 2 °C per minute.

Melting temperature: 197.6 °C (T_M)

Instrument: Perkin Elmer DSC 7 Differential Scanning Calorimeter.

Thin-layer chromatography

The total amount of impurities was estimated to less than 0.05%.
The following thin-layer chromatographic system used was according to the International Pharmacopoeia 3rd Ed. Vol 3.

Thin-layer: Silica gel 60 HPTLC (Merck)

Eluent: 1-propanol:1M ammonia (15:3)

Sample: 100 µg of probenecid dissolved in ethanol:1M ammonia (9:1) were applied.

Visualization: Evaluation under UV-light of 254 nm and scanning by densitometry at 254 nm with a Desaga CD 60 Scanner.

No secondary spots were detected visually at 254 nm. When evaluated by densitometry one very weak secondary spot was detected in front of the main peak, possibly originating from the solvent. The amount was estimated to be at about the detection limit of the system which was 0.05 µg (0.05%) at 254 nm.

R_f (probenecid) = 0.54-0.60 depending on the amount applied.

(*)Thin-layer chromatography

The amount of impurities was estimated to be approximately 0.1%.

The following thin-layer chromatographic system used is described in the International Pharmacopoeia 3rd Ed. Vol 3.

Thin-layer: Silica gel 60 F254

Eluent: 1-propanol:1M ammonia (15:3)

Sample: 200 µg of probenecid dissolved in ethanol:1M ammonia (9:1) were applied.

Visualization: Evaluation under UV-light at 254 nm.

The principal spot was observed at $R_f = 0.59$. An impurity was observed at $R_f = 0.68$ and was estimated to represent 0.1%. (NB ! A solvent front was observed immediately after the principal spot).

High performance liquid chromatography

The total amount of impurities estimated by peak area measurement was 0.15%.

A chromatogram is shown in Figure 3.

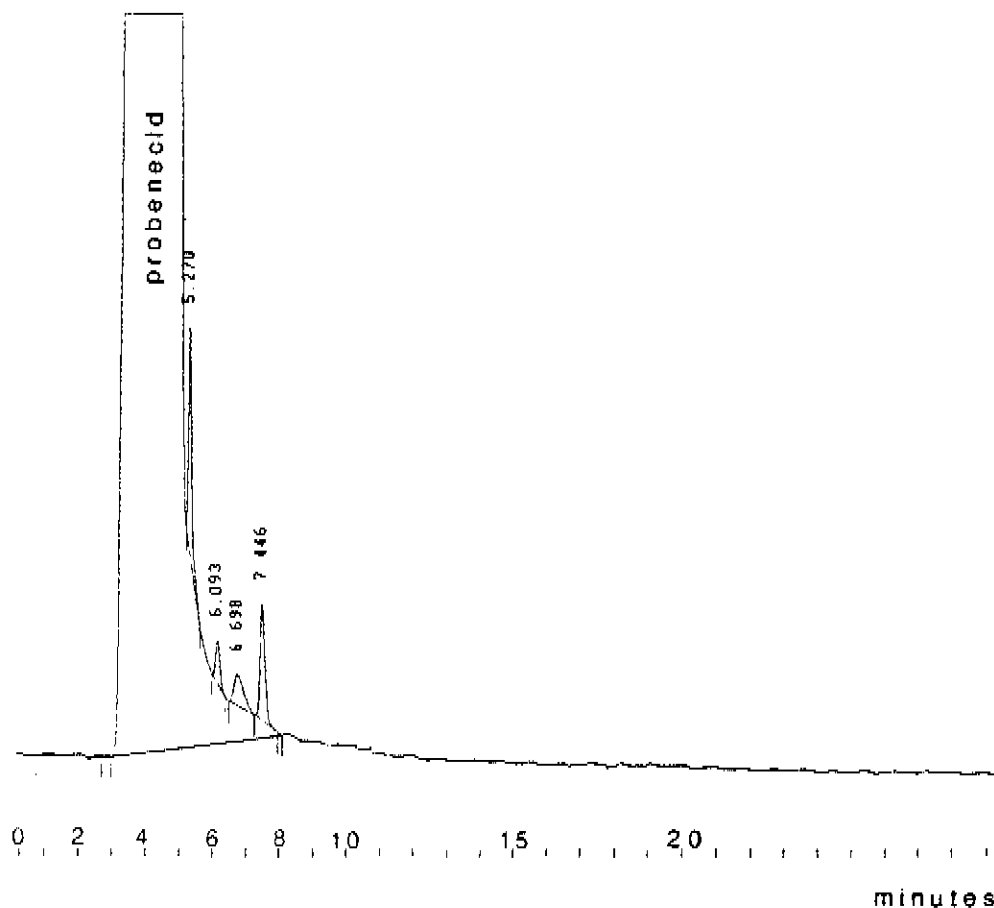


Figure 3. Chromatogram of probenecid Control No 192156 monitored at 239 nm.

The following conditions were used:

Eluent: Methanol/water (90:10)

Column: RP-18, 5 μ m (Brownlee Labs)

Detector: Varian Polychrom operated at 239 nm and 254 nm.

Pump: Waters 600 operated at a flow rate of 0.5 ml/min.

Integrator: PeakPro (Beckman)

Sample: 1 mg/ml dissolved in the eluent. The solution must be freshly prepared.
10 μ l corresponding to 10 μ g were injected.

239 nm is the optimum wavelength for the main peak and the impurities. When monitored at 254 nm 0.08% impurities were found, which is slightly less than at 239 nm where 0.15% were found. The detection limit for probenecid was 0.05 μ g (0.005%).

(*High performance liquid chromatography)

The total amount of impurities estimated by peak area measurement was 0.03%. The sample was examined using the procedure for p-Bis(di-n-propyl)carbonylbenzenesulfonamide in the USP XXI monograph for probenecid.

The following conditions were used:

Eluent: Methanol/water (9:1)

Columns: Waters 10 micron, C18 (3.9x300 mm) coupled to Ultratechsphere 5 micron C18 (4.6 x 250 mm)

Detector: Waters 490 operated at 214, 254 and 280 nm.

Pump: Waters 600 operated at a flow rate of 0.5 ml/min.

Sample: 5 mg/ml dissolved in the eluent.
10 μ l corresponding to 50 μ g were injected

Diode-array detection

The chromatographic system was also evaluated with a Varian 9065 Polychrom detector. The same chromatographic system as described above was used. UV-maxima for probenecid and its four trace impurities were found to be at 200 nm and 239 nm when recorded in the eluent. The wavelength of 239 nm was chosen in the method described above as the best to detect impurities. At 200 nm too much disturbances were observed.

DATA GIVEN BY COLLABORATING LABORATORIES

EPCRS

IR: complies

TLC: complies, no impurities detected

TLC system: Acetic acid/Chloroform/Di-isopropyl ether/toluene (10:15:20:55) Silica Gel GF 254

LOD: no loss was detected

HPLC: 0.1% impurities

DSC: 0.2% impurities

Melting point: 199.1 °C

UV-max: 225 and 250 nm A (1%, 1 cm) 250 nm = 334

NMR: spectrum corresponds to the structure

STABILITY

No special stability studies were performed as this substance was not suspected to degrade easily. Regular re-examinations of the ICRS will be performed.

CONCLUSION

Probenecid, Control No 192156, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 14

PYRANTEL EMBONATE
(PYRANTEL PAMOATE)

Control No 192157

Analytical Report

INTENDED USE

The monograph for Pyrantel embonate in the International Pharmacopoeia 3rd Ed. Vol 3 requires a reference substance of pyrantel embonate to be used in the infrared spectrophotometric test for identity and in the spectrophotometric assay.

MATERIAL

About 100g of the sample (manufacturers batch no 1E214-18QCS corresponding to USP Lot G) were received at the WHO Centre in June 1987. The material is being stored in tightly closed containers at + 5 °C, protected from light.

Pyrantel embonate consists of 34.9% of pyrantel and 64.9% of pamoic acid.

This reference substance has been evaluated in collaboration with the WHO Centre in Stockholm and the National Biological Standards Laboratory, Canberra, Australia. Results reported by the NBSL are indicated with an asterisk (*).

ANALYTICAL DATA

Description: A yellow, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

The infrared spectrum is given in Figure 1 (Control No 192157). The spectrum is concordant with the spectrum of a sample of pyrantel embonate obtained from Sigma and with the spectrum published in Dibbern.

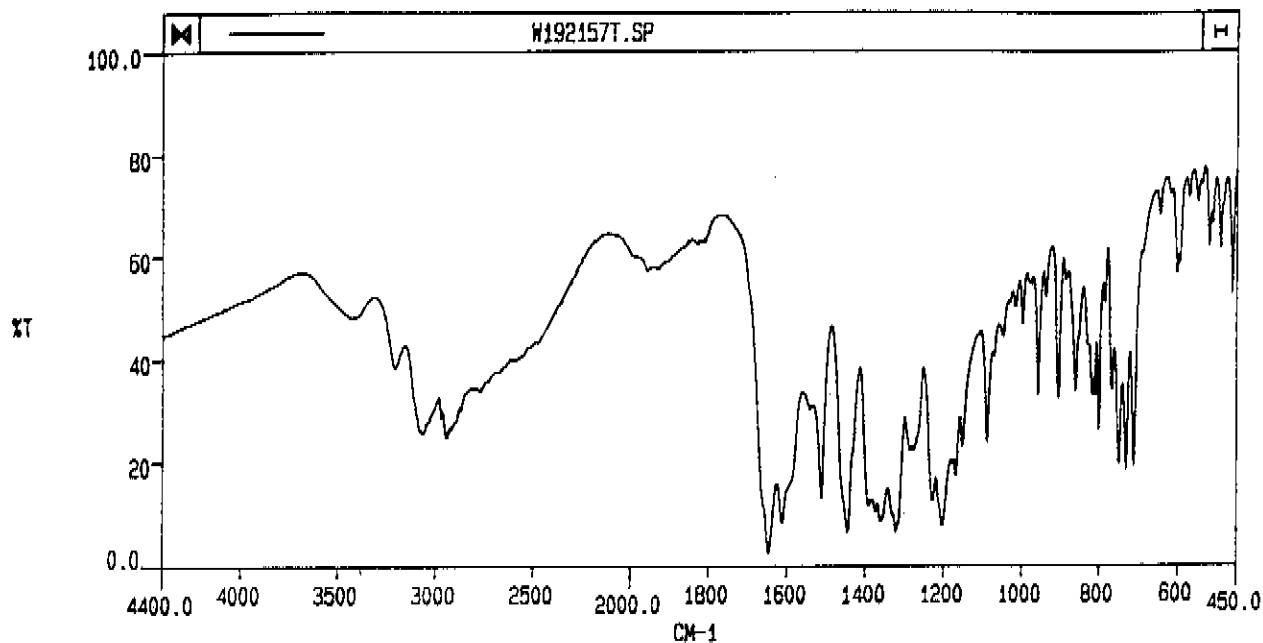


Figure 1. IR-spectrum of 1.42 mg of pyrantel embonate Control No 192157 in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 1600 FTIR.

(*) Infrared spectrum

The infrared spectrum of the material, using ATR (attenuated total reflexion) was recorded on a Perkin Elmer 683 Infrared Spectrophotometer. The spectrum was concordant with a spectrum of the USP pyrantel pamoate standard.

UV-spectrum

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

UV-max were found at 279 nm, 289 nm, 301 nm and 316 nm.

The ratio of the absorbance at 289 to that at 301 is 1.0.

A (1%, 1cm) = 329 at 279 nm (n=5 RSD=0.6%)

A (1%, 1cm) = 379 at 289 nm (n=5 RSD=0.6%)

A (1%, 1cm) = 378 at 301 nm (n=5 RSD=0.5%)

A (1%, 1cm) = 339 at 316 nm (n=5 RSD=0.5%)

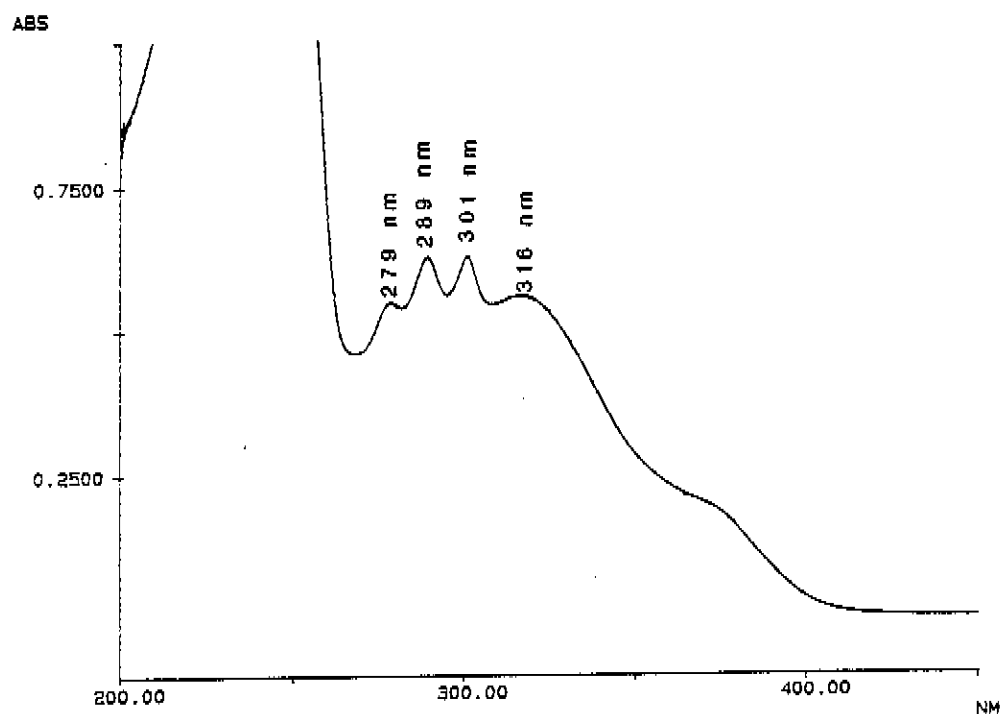


Figure 2. UV-spectrum of pyrantel embonate Control No 192157 16.5 ug/ml in methanol.

(*) UV-spectrum

The identification test procedure B, according to the International Pharmacopoeia 3rd Ed. Vol 3 was used. UV -maxima were observed at 236, 278, 289, 301 and 315 nm for a solution of 13 µg/ml in methanol. The spectrum was concordant to that of a USP Pyrantel pamoate reference standard. The ratio of the absorbance at 289 to that at 301 is 1.0.

A (1%, 1cm) = 365.4 at 289 nm (n= 4 RSD= 0.3%)

A (1%, 1cm) = 362.2 at 301 nm (n= 4 RSD= 0.3%)

The corresponding A-values for a USP Reference standard was 366.1 at 289 nm and 363.0 at 301 nm.

ASSAY

Liquid chromatographic assay: 64.9% of pamoic acid when determined against a sample from Sigma, and 34.9% of pyrantel when determined against a Pfizer house standard with a declared content of 34.4% of pyrantel.

(*)Spectrophotometric assay: 100.6% pyrantel embonate (n= 4, RSD= 0.8%). The material was assayed using the spectrophotometric procedure of the International Pharmacopoeia 3rd Ed. Vol 3. The USP reference standard was used for comparison.

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: 5 mg
Heating rate: 5 °C

Melting point: about 250 °C with decomposition.

(*)Loss on drying: 0.19% when dried for 3 hours at 60 °C under reduced pressure.

Loss on drying: 0.1% when dried to constant weight in vacuum at 60 °C.

PURITY

Thin-layer chromatography

No impurities were observed.

The following thin-layer chromatographic system was used according to Probl. Farm., 4, 73-9.

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

Eluent: Chloroform:methanol:acetic acid (30:7:1)

Sample: 200 µg were applied. The sample was dissolved in chloroform:methanol:ammonia (50:50:5).

Visualization: Visual inspection and at UV 254. Due to tailing peaks it was not meaningful to scan the plate. Pyrantel embonate and pamoic acid were not separated in this system.

Rf (pyrantel embonate) = 0.35

Rf (pamoic acid) = 0.31

(*)Thin-layer chromatography

The total amount of impurities was estimated to be less than 0.1%.

The system described in the International Pharmacopoeia 3rd Ed. Vol 3 was used.

Thin-layer: Silica gel 60 F-254 (0.25 mm layer).

Eluent: Ethyl acetate:methanol:diethylamine(20:5:1.5)

Sample: Two solutions were prepared in a mixture of chloroform:methanol:ammonia conc. (5:5:0.5) 20mg/ml and 0.2 mg/ml. 2000 µg and 20 µg were applied.

Visualization: UV 254 nm and 366 nm.

One secondary spot was detected with Rf= 0.22. It was estimated to be present at less than 0.1%. However the system exhibits poor separation and the Rf of pyrantel is too low.

Rf (pyrantel embonate) = 0.1

High performance liquid chromatography

Two different liquid chromatographic systems were tested one using a reversed phase column and the other a straight phase column. The straight phase column was preferred since in the reversed phase system pamoic acid was strongly adsorbed to the column.

System 1 (reversed phase):

No impurity peaks were detected. The pyrantel peak elutes at about 6 minutes and the pamoic acid peak elutes at about 17 minutes.

A chromatogram is shown in Figure 3.

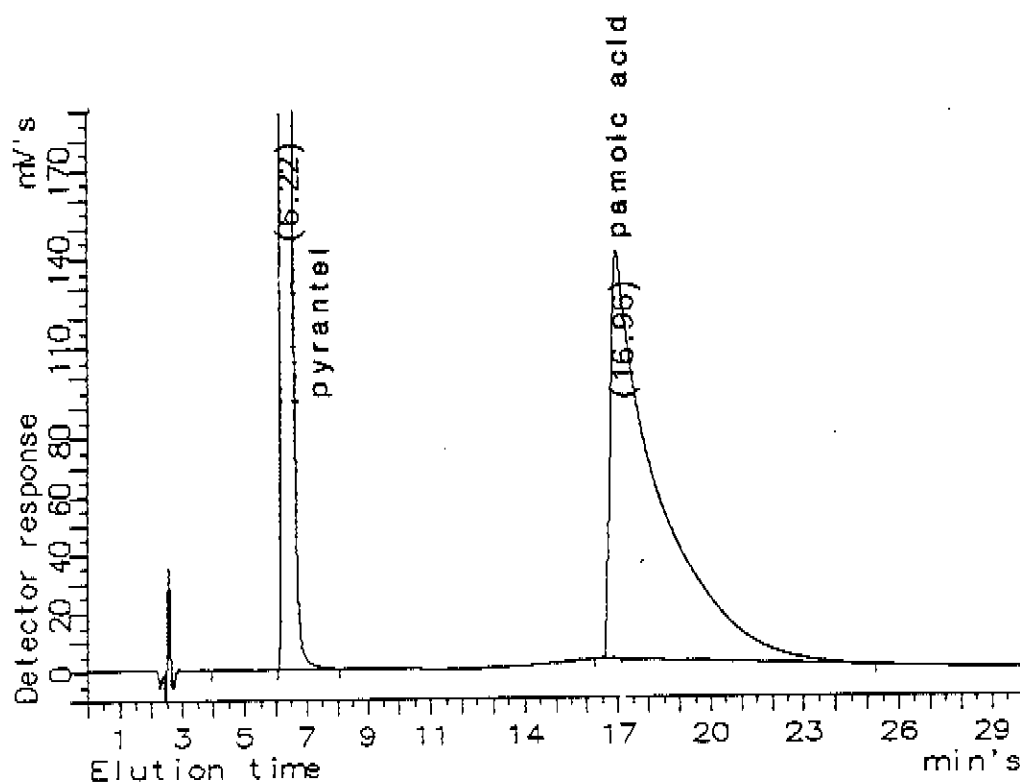


Figure 3. Chromatogram of pyrantel embonate Control No 192157 (reversed phase).

The following conditions were used:

Eluent: Acetonitrile:0.1 M butylamine with pH adjusted to 3.0 with perchloric acid.

The gradient employed is given below.

<u>Time.min</u>	<u>% Acetonitrile</u>	<u>% Aqueous phase</u>
0-10	42	58
10-25	75	25
25-30	42	58

Column: Spheri- 5 OD-5A RP 18 (Brownlee)

Detector: Waters Lambda-Max Model 481 operated at 300 nm

Pump: Waters 600 E operated at 1 ml/min

Integrator: PeakPro (Beckman)

Sample: 1 mg/ml dissolved in 0.1 ml DMSO and adjusted to 1 ml with the eluent.
10 µl corresponding to 10 µg were injected.

System 2 (straight phase):

No impurity peaks were detected. The pamoic acid peak elutes at about 4.6 minutes and the pyrantel peak elutes at about 9 minutes.

A chromatogram is shown in Figure 4.

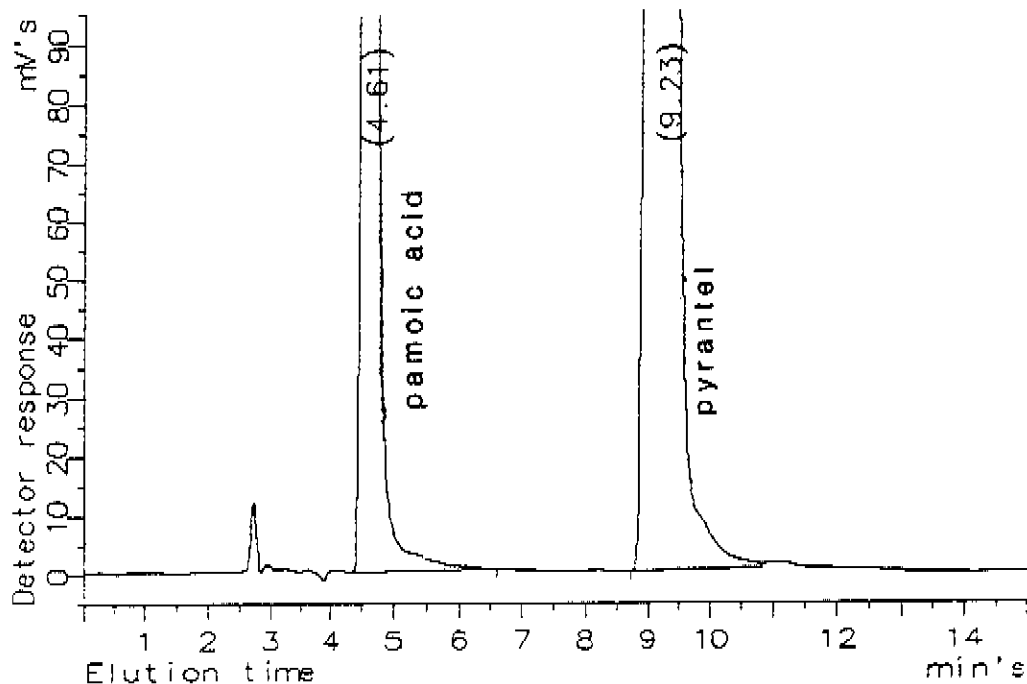


Figure 4. Chromatogram of pyrantel embonate Control No 192157 (straight phase).

The following conditions were used:

Eluent: Acetonitrile:water:6 M acetic acid:diethylamine (94:2.5:2.5:1)

Column: Spherisorb S5W (silica)

Detector: Waters Lambda-Max Model 481 operated at 300 nm

Pump: Waters 600 E operated at 1 ml/min

Integrator: PeakPro (Beckman)

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

Diode-array detection

The chromatographic system was also evaluated with a Varian 9065 Polychrom detector. The same chromatographic system as described under system 2 above was used. UV-spectra in the eluent are

given for the pyrantel peak and the pamoic acid peak in Figure 5.

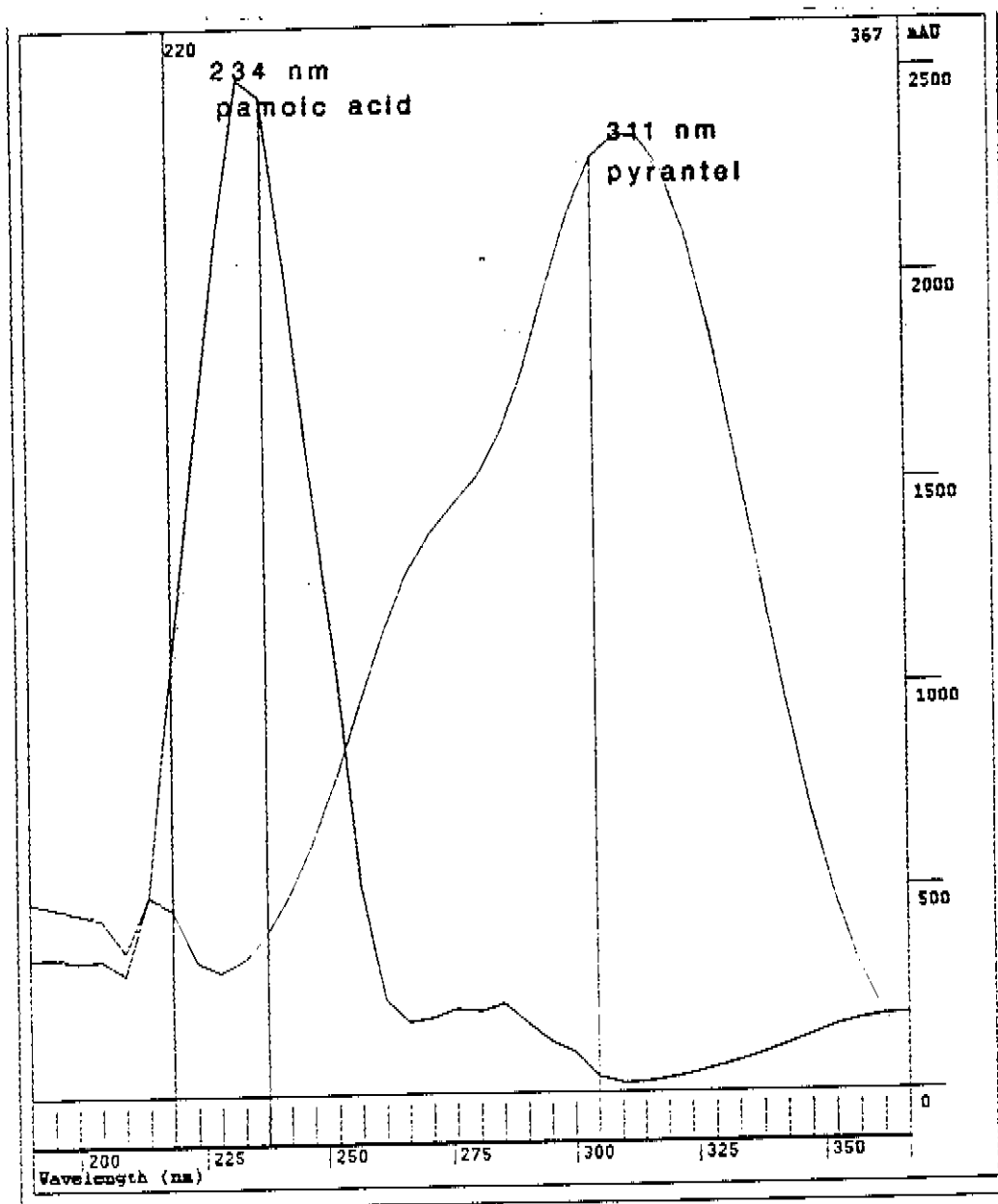


Figure 5. UV-spectra for pyrantel and pamoic acid recorded by the diode array detector.

As the two UV-spectra are quite different it is essential to use reference substances when performing assays of these two components.

DATA GIVEN BY THE MANUFACTURER

- IR KBr: complies
- UV in methanol: complies
- HPLC identity: complies

UV assay (pyrantel base): 34.9% (n= 5)

Pamoic acid by HPLC: 65.0% (n= 3) Column: Zorbax SIL Eluent: Acetonitrile/Water/Acetic acid/
Diethylamine (94:2.5:2.5:1) Wavelength of detection: 288 nm

LOD (60 °C): < 0.01%

Residue on ignition: 0.06%

STABILITY

No special stability studies were performed. The substance was stored for 5 years at +5 °C at the Centre. No sign of degradation has been observed during this time.

CONCLUSION

Pyrantel embonate, Control No 192157, can be considered suitable as International Chemical Reference Substance for the intended purpose. The content of pyrantel embonate when used in the spectrophotometric assay is taken to be 100.0% calculated with reference to the dried substance.
