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## Centre collaborateur OMS pour les substances chimiques de référence

### Rapport d'activité pour 1992

par M. Westermark

Les substances chimiques internationales de référence nouvellement établies, proposées par le Centre collaborateur OMS pour les substances chimiques de référence sur la base d'essais et d'une caractérisation appropriés, figurent dans le rapport annuel du Centre. Ce rapport est communiqué, entre autres, aux membres du Tableau consultatif d'experts de la Pharmacopée internationale et des Préparations pharmaceutiques, auxquels il est demandé d'examiner soigneusement les propositions ainsi que la documentation jointe concernant les analyses effectuées, et de faire part au Centre de toute réserve ou critique dans les trois mois suivant la réception du document. En de tels cas, le Centre procédera à toutes consultations ou analyses complémentaires nécessaires pour la validation de la substance.

Si aucun commentaire négatif n'est reçu dans les trois mois, les nouvelles substances chimiques internationales de référence proposées peuvent être considérées comme *provisoirement* adoptées. Leur adoption *définitive* fera l'objet d'un examen au cours de la réunion suivante du Comité d'experts.

Prière d'adresser vos remarques à Mme M. Westermark, Centre collaborateur OMS pour les substances chimiques de référence, Apoteksbolaget AB., Centrallaboratoriet, Prismavägen 2, S-10514 Stockholm, Suède.

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Les opinions exprimées dans les documents par des auteurs cités normalement n'engagent que lesdits auteurs.

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Note : Pour des raisons techniques, les appendices 7 à 14 n'ont été établis qu'en anglais.

## Distribution de substances de référence en 1992

En 1992, le Centre a distribué 1125 substances chimiques internationales de référence. Ce chiffre représente une augmentation d'environ 78 % par rapport à celui de 1991. Les six substances les plus fréquemment demandées ont été dans l'ordre l'ampicilline anhydre, la benzylpénicilline sodique, les substances de référence pour le point de fusion, le sulfate d'atropine, le sulfaméthoxazole et l'acide 2-(4-chloro-3-sulfamoyl) benzoïque. On trouvera à l'appendice 1 le détail de la distribution des diverses substances.

En 1992, les substances ont été distribuées dans 32 pays. On trouvera à l'appendice 2 le détail de la distribution par pays. En ce qui concerne la distribution par Région, on observe qu'environ 22 % des substances ont été fournies à la Région africaine, 2 % à la Région des Amériques, 5 % à la Région de la Méditerranée orientale, 26 % à la Région européenne, 35 % à la Région de l'Asie du Sud-Est et 10 % à la Région du Pacifique occidental.

Par rapport aux années précédentes, on observe une augmentation des demandes émanant de la Région de l'Asie du Sud-Est, de la Région du Pacifique occidental et de la Région africaine. Seize nouveaux pays ont commandé des substances, et huit pays figurant sur les listes des années précédentes ne figurent pas sur la liste de 1992. Cela montre à quel point il est difficile de prévoir les demandes de substances chimiques internationales de référence. Il est probable que certains pays, après avoir passé une commande importante, conservent un stock de substances pendant plusieurs années avant de passer une nouvelle commande.

## Etablissement de substances de référence en 1992

Conformément à la procédure recommandée par le Comité OMS d'experts des Spécifications relatives aux Préparations pharmaceutiques dans son trente-deuxième rapport (OMS, Série de Rapports techniques, N° 823), le Centre a établi en 1992 sept substances chimiques internationales de référence, dont on trouvera la liste à l'appendice 3. Parmi ces substances, l'éthinylestradiol et l'acétate de vitamine A sont des lots de remplacement, les lots précédents ayant été épuisés en 1991.

On trouvera à l'appendice 4 une liste complète de toutes les substances chimiques internationales de référence détenues par le Centre en janvier 1993, avec indication de la quantité de substance contenue dans chaque unité de conditionnement et du numéro de contrôle des lots actuels. Cette liste comprend également huit substances mentionnées ci-dessous, dont on peut prévoir qu'elles seront officiellement adoptées par le Comité d'experts en 1994. Fait nouveau cette année, les substances de référence pour le point de fusion ne sont plus disponibles sous forme de série, mais sont maintenant vendues séparément sous leur propre numéro de contrôle, que l'on trouvera à l'appendice 4.

## Travaux effectués en 1992 sur de nouvelles substances de référence

Le Centre a poursuivi ses travaux en vue de fournir de nouvelles substances de référence qui seront nécessaires pour accompagner les spécifications de la troisième édition de la *Pharmacopée internationale*. En 1992, huit nouvelles substances de référence destinées à accompagner le volume 3 ont été examinées : chlorhydrate d'amodiaquine, bacitracine zinc, dipropionate de béclo méthasone, dexaméthasone-acide phosphorique, phosphate sodique de dexaméthasone, chlorhydrate de dopamine, probénécide et embonate de pyrantel. Les rapports d'analyse pour ces substances figurent aux appendices 7 à 14. Ces substances sont jugées satisfaisantes en vue de leur adoption comme substances chimiques internationales de référence.

## Essais de stabilité

Le Centre a poursuivi ses examens périodiques de la stabilité des substances chimiques internationales de référence existantes. En 1992, 13 substances ont été réexaminées. On trouvera les résultats de ce réexamen à l'appendice 5. On peut obtenir auprès du Centre des détails concernant les méthodes utilisées.

### Travaux en cours et travaux futurs

Les travaux sur les substances nécessaires pour accompagner les monographies du volume 3 de la *Pharmacopée internationale* se poursuivent. Actuellement, le Centre procède à l'étude de 9 des 21 substances énumérées à l'appendice 6.

En 1992, le Centre a commencé à diffuser davantage d'informations sur les substances chimiques internationales de référence aux laboratoires participant au contrôle analytique des produits pharmaceutiques. La Fédération internationale de l'Industrie du Médicament (FIIM) a fourni au Centre les adresses des associations membres. Le Centre a commencé à préparer une fiche d'information concernant le Centre lui-même et les substances chimiques internationales de référence, qui sera distribuée lors des conférences et/ou aux personnes intéressées. Cette fiche sera prête au cours de l'année 1993. Le Centre a également participé au Congrès de la FIP à Lyon, avec une conférence sur "le rôle du Centre collaborateur pour les substances chimiques internationales de référence dans la lutte contre les contrefaçons de produits pharmaceutiques".

### Questions administratives et financières

Le coût de fonctionnement total du Centre en 1992 a été estimé à US \$563 600. Le revenu provenant des ventes de substances de référence a été d'environ US \$46 040 et la contribution du Siège de l'OMS de US \$16 000. Cela laisse un déficit de US \$501 560, couvert grâce au soutien de la Corporation nationale des Pharmacies suédoises.

Le prix des substances reste fixé à US \$40 par paquet, et des frais d'expédition et de manutention s'élevant à US \$10 sont ajoutés à chaque commande.

### Remerciements

Le Centre désire exprimer ses remerciements aux laboratoires qui ont contribué à ses travaux en 1992, à savoir le Laboratoire de la Pharmacopée européenne à Strasbourg (France) et le National Biological Standards Laboratory (actuellement Therapeutic Goods Administration Laboratories), Canberra (Australie).

Le Centre désire également remercier les laboratoires pharmaceutiques qui lui ont fourni des substances pour examen et qui ont participé aux travaux d'analyse. Cette année, ses remerciements vont en particulier à A/S Apotekernes Lab., Oslo (Norvège); Fluka Chemie AG, Buchs (Suisse); Glaxo, Greenford (Angleterre); Merck, Sharp et Dohme Ltd, Rahway, NJ (Etats-Unis d'Amérique); Parke Davis, Morris Plains, NJ (Etats-Unis d'Amérique); Pfizer, Groton, NY (Etats-Unis d'Amérique) et Upjohn, Kalamazoo, MI (Etats-Unis d'Amérique).

## DISTRIBUTION DE SUBSTANCES CHIMIQUES DE REFERENCE EN 1992

Acéclidine, salicylate	5 échantillon(s)	Digitoxine	10 échantillon(s)
p-Acétamidobenzazazine	10 "	Digoxine	9 "
Acétazolamide	22 "	Emétine, chlorhydrate	2 "
Allopurinol	5 "	4-Epianhydrotétracycline, chlorhydrate	23 "
2-Amino 5-nitrothiazole	1 "	4-Epitétracycline, sel d'ammonium	9 "
3-Aminopyrazole-4-carboxamide, hémisulfate	10 "	Ergocalciférol	2 "
Amitryptiline, chlorhydrate	9 "	Ergométrine, hydrogénomaléate	4 "
Amphotéricine B	2 "	Ergotamine, tartrate	12 "
Ampicilline (anhydre)	41 "	Erythromycine	6 "
Ampicilline sodique	15 "	Estradiol, benzoate	3 "
Ampicilline, trihydrate	20 "	Estrone	23 "
Anhydrotétracycline, chlorhydrate	24 "	Etacrynique, acide	- "
Atropine, sulfate	28 "	Ethambutol, chlorhydrate	10 "
Azathioprine	2 "	Ethinylestradiol	1 "
Bendazol, chlorhydrate	- "	Ethistérone	1 "
Benzobarbital	2 "	Ethosuximide	2 "
Benzylamine, sulfate	11 "	Étocarlide	- "
Benzylpénicilline potassique	6 "	3-Formylrifamycine	2 "
Benzylpénicilline sodique	39 "	Flucytosine	1 "
Bépnium, hydroxynaphtoate	1 "	Fluorouracil	3 "
Bétaméthasone	17 "	Fluphénazine, décanoate (dichlorhydrate)	2 "
Bétaméthasone, valérate	- "	Fluphénazine, énantate (dichlorhydrate)	1 "
Bétanidine, sulfate	- "	Fluphénazine, chlorhydrate	11 "
NN'-bis(2,3-xylyl)anthranilamide	1 "	Folique, acide	14 "
Bupivacaïne, chlorhydrate	- "	Furosémide	4 "
Caféine	11 "	Griséofulvine	12 "
Carbamazépine	7 "	Halopéridol	5 "
Carbénicilline monosodique	6 "	Hydrochlorothiazide	5 "
Chloramphénicol	16 "	Hydrocortisone	15 "
Chloramphénicol, palmitate	4 "	Hydrocortisone, acétate	14 "
Chloramphénicol, palmitate (forme A)	7 "	(-)-3-(4-Hydroxy-3-méthoxyphényl) -2-méthylalanine	1 "
5-Chloro-2-méthylaminobenzophénone	1 "	Ibuprofène	12 "
2-(4-Chloro-3-sulfamoylbenzoyl) benzoïque, acide	27 "	Imipramine, chlorhydrate	9 "
Chlorphénamine, hydrogénomaléate	20 "	Indométacine	7 "
Chlorpromazine, chlorhydrate	13 "	o-Iodohippurique, acide	- "
Chlortalidone	3 "	Isoniazide	5 "
Chlortétracycline, chlorhydrate	7 "	Lanatoside C	6 "
Cimétidine	2 "	Lévodopa	6 "
Clomifène, citrate	5 "	Lévothyroxine sodique	- "
Clomifène, citrate (isomère Z) (voir zuclofène)		Lidocaine	2 "
Cloxacilline sodique	25 "	Lidocaïne, chlorhydrate	2 "
Colécalciférol	6 "	Méfénamique, acide	3 "
Cortisone, acétate	6 "	Métazide	1 "
Dapsone	6 "	Méthqualone	2 "
Désoxycortone, acétate	5 "	Méthylidopa	11 "
Dexaméthasone	10 "	Méthyltestostérone	8 "
Dexaméthasone, acétate	3 "	Méticilline sodique	- "
Diazépam	11 "	Métronidazole	9 "
Diazoxide	2 "	Nafcilline sodique	1 "
Dicloxacilline sodique	3 "	Néostigmine, métilsulfate	2 "
Dicolinium, iodure	- "	Nicotinamide	10 "
Dicoumarol	- "	Nicotinique, acide	7 "
Diéthylcarbamazine, dihydrogénocitrate	1 "		

## Appendice 1

Niridazole	2 échantillon(s)	Pyridostigmine, bromure	1 échantillon(s)
Niridazole-chloréthylcarboxamide	-	Résérpine	5 "
Noréthistérone	7 "	Rétinol, acétate	
Noréthistérone, acétate	10 "	(solution à 25 000 UI)	15 "
Nystatine	8 "	Riboflavine	7 "
Ouabaïne	1 "	Rifampicine	8 "
Oxacilline sodique	2 "	Rifampicine quinone	3 "
Oxytétracycline, chlorhydrate	14 "	Sodium, cromoglicate	1 "
Oxytétracycline, dihydrate	4 "	Sulfaméthoxazole	27 "
Papavérine, chlorhydrate	3 "	Sulfaméthoxyypyridazine	9 "
Phénéticilline potassique	3 "	Sulfanilamide	9 "
Phénoxyméthylpénicilline	13 "	Sulfasalazine	1 "
Phénoxyméthylpénicilline calcique	1 "	Testostérone, propionate	6 "
Phénoxyméthylpénicilline potassique	11 "	Tétracycline, chlorhydrate	25 "
Phénytoïne	5 "	Thioacétazone	6 "
Prednisolone	9 "	4,4'-Thiodianiline	1 "
Prednisolone, acétate	6 "	Thyroxine sodique	
Prednisone	8 "	(voir lévothyroxine sodique)	
Prednisone, acétate	8 "	Tolbutamide	2 "
Probenécide	1 "	Tolnaftate	7 "
Procaïne, chlorhydrate	3 "	Triméthadione	- "
Procarbazine, chlorhydrate	1 "	Triméthoprime	21 "
Progesterone	7 "	Triméthylguanidine, sulfate	1 "
Propicilline potassique	1 "	Tubocurarine, chlorure	- "
Propranolol, chlorhydrate	12 "	Vitamine A, acétate (solution)	
Propylthiouracile	1 "	(voir rétinol, acétate)	
Pyrantel, embonate	1 "	Warfarine	2 "
		Zuclomifène	4 "

Substances de référence pour le point de fusion : 34 séries de 13 substances.

## DISTRIBUTION DE SUBSTANCES CHIMIQUES INTERNATIONALES DE REFERENCE DANS LES DIFFERENTES REGIONS DE L'OMS EN 1992

Régions OMS	Nombre de SCIR distribuées en 1992
<b>Région africaine (AFRO)</b>	
Ghana	151
Ouganda	28
Swaziland	29
Tanzanie	30
Zimbabwe	5
<b>Région des Amériques (AMRO)</b>	
Argentine	1
Cuba	1
Etats-Unis d'Amérique	8
Panama	9
<b>Région de la Méditerranée orientale (EMRO)</b>	
Egypte	6
République arabe syrienne	19
Yémen	36
<b>Région européenne (EURO)</b>	
Allemagne	61
Autriche	8
Belgique	32
Danemark	6
Fédération de Russie	61
Finlande	5
France	15
Norvège	5
Pays-Bas	3
Royaume-Uni	18
Suède	66
Suisse	2
Tchécoslovaquie	5
Turquie	14
<b>Région de l'Asie du Sud-Est (SEARO)</b>	
Inde	339
Indonésie	59
<b>Région du Pacifique occidental (WPRO)</b>	
Malaisie	4
Nouvelle-Zélande	15
Singapour	23
Viet Nam	68

**LISTE DES SUBSTANCES CHIMIQUES INTERNATIONALES  
DE REFERENCE ETABLIES EN 1992**

Substance de référence	N° de contrôle	Rapport d'analyse	Remarques
Amphotéricine B	191153	WHO/PHARM/92.558 Appendice 7	
Erythromycine	191154	WHO/PHARM/92.558 Appendice 8	
Ethinylestradiol	291016	WHO/PHARM/92.558 Appendice 9	Remplace le N° 167016
Nystatine	191152	WHO/PHARM/92.558 Appendice 10	
Rifampicine	191151	WHO/PHARM/92.558 Appendice 11	
Sulfasalazine	191155	WHO/PHARM/92.558 Appendice 12	
Rétinol, acétate (acétate de vitamine A)	791038	WHO/PHARM/92.558 Appendice 13	Remplace le N° 686038

## LISTE DES SUBSTANCES CHIMIQUES INTERNATIONALES DE REFERENCE DISPONIBLES

1993

### Informations générales

Les substances chimiques internationales de référence sont établies conformément à l'avis du Comité OMS d'experts des Spécifications relatives aux Préparations pharmaceutiques. Elles sont fournies principalement pour être utilisées dans des épreuves physiques et chimiques ainsi que dans des dosages décrits dans les spécifications pour le contrôle de la qualité des produits pharmaceutiques publiées dans la *Pharmacopée internationale* ou proposées sous forme de projets de monographies.

Le mode d'emploi et les données analytiques pour l'usage auquel elles sont destinées dans la spécification correspondante de la *Pharmacopée internationale* sont fournis dans les certificats joints aux substances distribuées. Des comptes rendus d'analyse plus détaillés sur ces substances peuvent être obtenus sur demande auprès du Centre collaborateur de l'OMS pour les substances chimiques de référence.

Les substances chimiques internationales de référence peuvent être utilisées également dans des épreuves et des dosages qui ne sont pas décrits dans la *Pharmacopée internationale*. Cependant, dans ce cas, il incombe à l'utilisateur ou à la Commission de la Pharmacopée, ou à toute autre autorité qui a prescrit l'utilisation de ces substances, de vérifier qu'elles conviennent à l'usage qui en est fait.

Il est en général recommandé de conserver les substances à l'abri de la lumière et de l'humidité et de préférence à une température voisine de +5°C. Lorsque des conditions spéciales de stockage sont nécessaires, l'indication en est portée sur l'étiquette ou figure dans la notice jointe aux substances.

La stabilité des substances chimiques internationales de référence conservées au Centre est surveillée par des examens réguliers et, lorsque cela est nécessaire, les substances détériorées sont remplacées par de nouveaux lots. Des listes indiquant les numéros de contrôle des lots en cours sont publiées dans les rapports annuels du Centre et peuvent être obtenues sur demande.

### Commandes de substances

Les commandes de substances chimiques internationales de référence doivent être envoyées à :

Centre collaborateur OMS pour les substances chimiques de référence  
APOTEKSBOLAGET AB  
Centrallaboratoriet  
S-10514 STOCKHOLM

(Télex : 115 53 APOBOL S)  
(Téléfax : + 46 8 740 60 40)

Les substances chimiques internationales de référence sont exclusivement fournies par paquets standards contenant la quantité indiquée sur la liste ci-après.

Substance de référence	Conditionnement	Numéro de contrôle
Acéclidine, salicylate	100 mg	172048
p-Acétamidobenzalazine	100 mg	290042
Acétazolamide	100 mg	186128
Allopurinol	100 mg	287049
2-Amino-5-nitrothiazole	25 mg	186131
3-Aminopyrazole-4-carboxamide, hémisulfate	100 mg	172050
Amitryptiline, chlorhydrate	100 mg	181101
Amodiaquine, chlorhydrate	200 mg	192160
Amphotéricine B	400 mg	191153
Ampicilline (anhydre)	200 mg	390001
Ampicilline sodique	200 mg	388002
Ampicilline, trihydrate	200 mg	274003
Anhydrotétracycline, chlorhydrate	25 mg	180096
Atropine, sulfate	100 mg	183111
Azathioprine	100 mg	172060
Bacitracine zinc	200 mg	192174
Béclométasone, dipropionate	200 mg	192175
Bendazol, chlorhydrate	100 mg	173066
Benzobarbital	100 mg	172051
Benzylamine, sulfate	100 mg	172052
Benzylpénicilline potassique	200 mg	180099
Benzylpénicilline sodique	200 mg	280047
Béphénium, hydroxynaphtoate	100 mg	183112
Bétaméthasone	100 mg	183113
Bétaméthasone, valérate	100 mg	190145
Bétanidine, sulfate	100 mg	172053
NN'-bis(2,3-xylyl)anthranilamide	50 mg	173067
Bupivacaïne, chlorhydrate	100 mg	289054
Caféine	100 mg	181102
Carbamazépine	100 mg	189143
Carbénicilline monosodique	200 mg	383043
Chloramphénicol	200 mg	486004
Chloramphénicol, palmitate	1 g	286072
Chloramphénicol, palmitate (forme A)	200 mg	175073
5-Chloro-2-méthylaminobenzophénone	100 mg	172061
2-(4-Chloro-3-sulfamoylbenzoyl)benzoïque, acide	50 mg	181106
Chlorphénamine, hydrogénomaléate	100 mg	182109
Chlorpromazine, chlorhydrate	100 mg	178080
Chlortalidone	100 mg	183114
Chlortétracycline, chlorhydrate	200 mg	187138
Cimétidine	100 mg	190150
Clomifène, citrate	100 mg	187136
Clomifène, citrate (isomère Z) (voir zuclomifène)		
Cloxacilline sodique	200 mg	274005
Colécalciférol (vitamine D <sub>3</sub> )	500 mg	190146
Cortisone, acétate	100 mg	167006
Dapsone	100 mg	183115
Désoxycortone, acétate	100 mg	167007
Dexaméthasone	100 mg	388008
Dexaméthasone, acétate	100 mg	288009
Dexaméthasone - acide phosphorique	100 mg	192161
Dexaméthasone, phosphate sodique	100 mg	192158
Diazépam	100 mg	172062
Diazoxide	100 mg	181103
Dicloxacilline sodique	200 mg	174071
Dicolinium, iodure	100 mg	172055
Dicoumarol	100 mg	178077
Diéthylcarbamazine, dihydrogénocitrate	100 mg	181100

Substance de référence	Conditionnement	Numéro de contrôle
Digitoxine	100 mg	277010
Digoxine	100 mg	587011
Dopamine, chlorhydrate	100 mg	192159
Emétine, chlorhydrate	100 mg	187134
4-Eplanhydrotétracycline, chlorhydrate	25 mg	288097
4-Eptitétracycline, sel d'ammonium	25 mg	180098
Ergocalciférol (vitamine D <sub>2</sub> )	500 mg	190147
Ergométrine, hydrogénomaléate	50 mg	277012
Ergotamine, tartrate	50 mg	385013
Erythromycine	250 mg	191154
Estradiol, benzoate	100 mg	167014
Estrone	100 mg	279015
Etacrynique, acide	100 mg	281056
Ethambutol, chlorhydrate	100 mg	179081
Ethinylestradiol	100 mg	291016
Ethistérone	100 mg	167017
Ethosuximide	100 mg	179088
Etocarlide	100 mg	172057
Flucytosine	100 mg	184121
Fluorouracil	100 mg	184122
Fluphénazine, chlorhydrate	100 mg	176076
Fluphénazine, décanoate (dichlorhydrate)	100 mg	182107
Fluphénazine, énantate (dichlorhydrate)	100 mg	182108
Folique, acide	100 mg	388019
3-Formyltrifamycine	200 mg	190149
Furosémide	100 mg	171044
Griséofulvine	200 mg	280040
Halopéridol	100 mg	172063
Hydrochlorothiazide	100 mg	179087
Hydrocortisone	100 mg	283020
Hydrocortisone, acétate	100 mg	280021
(-)-3-(4-Hydroxy-3-méthoxyphényl)-2-méthylalanine	25 mg	179085
Ibuprofène	100 mg	183117
Imipramine, chlorhydrate	100 mg	172064
Indométacine	100 mg	178078
o-Iodohippurique, acide	100 mg	171045
Isoniazide	100 mg	185124
Lanatoside C	100 mg	281022
Lévodopa	100 mg	172065
Lévothyroxine sodique	100 mg	189144
Lidocaïne	100 mg	181104
Lidocaïne, chlorhydrate	100 mg	181105
Méfénamique, acide	100 mg	173068
Métazide	100 mg	172058
Méthaqualone	100 mg	173069
Méthildopa	100 mg	179084
Méthyltestostérone	100 mg	167023
Méticilline sodique	200 mg	274024
Métronidazole	100 mg	183118
Nafcilline sodique	200 mg	272025
Néostigmine, métilsulfate	100 mg	187135
Nicotinamide	100 mg	179090
Nicotinique, acide	100 mg	179091
Niridazole	200 mg	186129
Niridazole-chloréthylcarboxamide	25 mg	186130
Noréthistérone	100 mg	186132
Noréthistérone, acétate	100 mg	185123

Substance de référence	Conditionnement	Numéro de contrôle
Nystatine	200 mg	191152
Ouabaïne	100 mg	283026
Oxacilline sodique	200 mg	382027
Oxytétracycline, chlorhydrate	200 mg	189141
Oxytétracycline, dihydrate	200 mg	189142
Papavérine, chlorhydrate	100 mg	185127
Phénéticilline potassique	200 mg	167028
Phénoxyméthylpénicilline	200 mg	179082
Phénoxyméthylpénicilline calcique	200 mg	179083
Phénoxyméthylpénicilline potassique	200 mg	176075
Phénytoïne	100 mg	179089
Prednisolone	100 mg	389029
Prednisolone, acétate	100 mg	289030
Prednisone	100 mg	167031
Prednisone, acétate	100 mg	169032
Probénécide	100 mg	192156
Procaïne, chlorhydrate	100 mg	183119
Procarbazine, chlorhydrate	100 mg	184120
Progesterone	100 mg	167033
Propicilline potassique	200 mg	274034
Propranolol, chlorhydrate	100 mg	187139
Propylthiouracile	100 mg	185126
Pyrantel, embonate	500 mg	192157
Pyridostigmine, bromure	100 mg	182110
Résérpine	100 mg	186133
Rétinol, acétate (solution)	5 capsules*	791038
Riboflavine	250 mg	382035
Rifampicine	200 mg	191151
Rifampicine quinone	200 mg	190148
Sodium, cromoglicat	100 mg	188140
<i>Substances de référence pour le point de fusion (série de 13 substances)</i>	<i>Ne sont plus disponibles sous forme de série</i>	
<i>Substances de référence individuelles pour le point de fusion</i>		
Azobenzène (69°C)	4 g	192168
Vanilline (83°C)	4 g	192169
Benzile (96°C)	4 g	192170
Acétanilide (116°C)	4 g	192171
Phénacétine (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
Saccharine (229°C)	4 g	192165
Caféine (237°C)	4 g	192166
Phénolphtaléine (263°C)	4 g	192167
Sulfaméthoxazole	100 mg	179092
Sulfaméthoxypyridazine	100 mg	178079
Sulfanilamide	100 mg	179094
Sulfasalazine	100 mg	191155
Testostérone, propionate	100 mg	167036
Tétracycline, chlorhydrate	200 mg	180095
Thioacétazone	100 mg	171046
4,4'-Thiodianiline	50 mg	183116
Thyroxine sodique (voir lévothyroxine sodique)		
Tolbutamide	100 mg	179086

\* Par capsule, environ 9 mg dans 250 mg d'huile.

Substance de référence	Conditionnement	Numéro de contrôle
Tolnaftate	100 mg	176074
Triméthadione	200 mg	185125
Triméthoprim	100 mg	179093
Triméthylguanidine, sulfate	100 mg	172059
Tubocurarine, chlorure	100 mg	170037
Vitamine A, acétate (solution) (voir rétinol, acétate)		
Warfarine	100 mg	168041
Zuclomifène	50 mg	187137

## ESSAIS DE STABILITE

La stabilité des substances chimiques internationales de référence pendant leur stockage est surveillée par un réexamen périodique des substances détenues par le Centre. Les résultats obtenus pour les substances réexaminées en 1991 sont résumés ci-après. A titre comparatif, on a aussi indiqué les résultats obtenus lors des réexamens précédents. Les substances ont été conservées dans des récipients étanches à +5°C et sous une humidité relative d'environ 30 %. Dans les tableaux, on a adopté les abréviations suivantes :

DSC	Calorimétrie différentielle
DTA	Analyse thermique différentielle
HPLC	Chromatographie liquide à haute performance
IR	Spectrophotométrie infrarouge
KF	Méthode de Karl Fischer pour la détermination de la teneur en eau
LOD	Perte à la dessiccation
TLC	Chromatographie en couche mince
PSA	Analyse de solubilité par phases
TGA	Analyse thermogravimétrique

La valeur estimée des impuretés solides totales, obtenue par HPLC et TLC, est exprimée en aire %, sauf indication contraire; lorsqu'elle est obtenue par DSC et par DTA, elle est exprimée en mole %, et par PSA en poids %. Les pertes de poids, mesurées par LOD et TGA, sont exprimées en poids %. Les valeurs obtenues par titrage sont calculées par rapport à la substance desséchée ou anhydre, sauf indication contraire.

Pour plus de détails sur les méthodes d'analyse utilisées, on peut s'adresser au Centre.

**Ampicilline trihydrate, N° de contrôle 274003**

Premier rapport d'analyse : WHO/PHARM/75.485, appendice 6

	Année d'examen						
	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
Titrage, % (mercurimétrique)	-	-	-	-	98,6	-	98,8
Produits de dégradation, % (mercurimétrique)	-	-	-	-	0,9	-	0,7
Titrage, % (pénicilline)	98,5	-	99,0	-	-	-	-
Titrage PSA, %	1,0	-	-	-	-	-	-
pH, solution à 0,25 %	5,1	-	5,1	5,1	5,1	-	-

**Chloramphénicol, N° de contrôle 486004**

Premier rapport d'analyse : WHO/PHARM/87.532, appendice 8

	Année d'examen	
	1986	1992
IR	conforme	-
TLC, %	0,2	-
HPLC, %	0,2	0,3
TGA, %	-	<0,1
LOD, %	0,05	-
Titrage, % (spectrophotométrique)	99,8	100,0
DTA, %	0,2	-
DSC, %	-	0,3

**Chloramphénicol palmitate, N° de contrôle 286072**

Premier rapport d'analyse : WHO/PHARM/87.532, appendice 9

	Année d'examen	
	1986	1992
IR	conforme	conforme
TLC	2 taches secondaires	-
HPLC, %	3,1	3,1
TGA, %	0,1	<0,1
KF, %	0,2	<0,1
LOD, %	<0,1	-
Titration, % (spectrophotométrique)	100,2	100,0
DTA, %	2,7	-
DSC, %	2,7	2,2

**Chloramphénicol, palmitate (forme A), N° de contrôle 175073**

Premier rapport d'analyse : WHO/PHARM/75.485, appendice 10

	Année d'examen	
	1974	1992
IR	conforme	conforme
TLC	2 taches secondaires	-
HPLC, %	-	environ 2
TGA, %	-	<0,1
KF, %	-	<0,1
LOD, %	0,26	-
Titration, % (titrimétrique)	100,9	-
Titration, % (spectrophotométrique)	-	100,0
DSC, %	0,6	0,7
PSA, %	environ 0,5	-

**Cortisone acétate, N° de contrôle 167006**

Premier rapport d'analyse : WHO/PHARM/67.441, appendice 1

	Année d'examen			
	1966	1975	1984	1992
IR	conforme	-	-	-
TLC	3 taches secondaires	2 taches secondaires	3 taches secondaires	-
HPLC, %	-	-	0,3	0,3
TGA, %	-	-	-	0,1
KF, %	-	-	-	0,2
LOD, %	<0,1	0,2	-	-

**Digitoxine, N° de contrôle 277010**

Premier rapport d'analyse : WHO/PHARM/78.494, appendice 7

	Année d'examen		
	1977	1987	1992
IR	conforme	-	-
TLC, %	0,2	<0,1	0,3
HPLC, %	<0,1	0,1	0,5
TGA, %	-	-	0,6
LOD, %	0,6	0,6	-
Titrage, % (spectrophotométrique)	99,7	100,7	100,7

**Digoxine, N° de contrôle 587011**

Premier rapport d'analyse : WHO/PHARM/88.537, appendice 10

	Année d'examen	
	1987	1992
IR	conforme	-
TLC, %	3 taches secondaires	3 taches secondaires
HPLC, %	1,4	1,4
TGA, %	0,15	0,13
KF, %	0,16	-
Titrage, % (spectrophotométrique)	99,7	99,8

**4-Epitétracycline (sel d'ammonium), N° de contrôle 180098**

Premier rapport d'analyse : WHO/PHARM/81.508, appendice 9

	Année d'examen		
	1980	1985	1992
IR	conforme	-	-
TLC	2 taches secondaires	-	-
HPLC, %	0,7	0,4	0,4
TGA, %	-	-	environ 4
KF, %	-	3,9	4,0
LOD, %	0,15	-	3,1

**Fluorouracil, N° de contrôle 184122**

Premier rapport d'analyse : WHO/PHARM/85.517, appendice 8

	Année d'examen	
	1984	1992
IR	conforme	-
TLC	0 tache secondaire	-
HPLC, %	0,03	0,04
TGA, %	-	<0,1 %
LOD, %	<0,1	-
Titrage, % (titrimétrique)	100,9	-

**Furosémide, N° de contrôle 171044**

Premier rapport d'analyse : WHO/PHARM/71.464, appendice 6

	Année d'examen			
	1971	1976	1985	1992
IR	conforme	-	conforme	-
TLC	0 tache secondaire	1 tache secondaire	0 tache secondaire	-
HPLC, %	-	-	-	0,03
TGA, %	-	-	-	0,1
LOD, %	0,1	<0,1	0,2	-
Titrage, % (titrimétrique)	99,4	100,1	-	-
PSA, %	<0,5	-	-	-

**(-)-3-(4-Hydroxy-3-méthoxyphényl)-2-méthylalanine, N° de contrôle 179085**

Premier rapport d'analyse : WHO/PHARM/80.504, appendice 9

	Année d'examen	
	1979	1992
IR	conforme	-
TLC, %	1 tache secondaire	1 tache secondaire
TGA, %	-	7,6
KF, %	7,1	-
Titration, % (titrimétrique)	99,7	-

**Méthildopa, N° de contrôle 179084**

Premier rapport d'analyse : WHO/PHARM/80.504, appendice 10

	Année d'examen		
	1979	1984	1992
IR	conforme	-	-
TLC, %	0,2	1 tache secondaire	0,1
HPLC, %	<0,2	0,2	0,1
TGA, %	-	-	11,7
KF, %	11,5	11,5	-
Titration, % (titrimétrique)	99,7	-	-

**Phénytoïne, N° de contrôle 179089**

Premier rapport d'analyse : WHO/PHARM/80.504, appendice 13

	Année d'examen		
	1979	1985	1992
IR	conforme	-	-
HPLC, %	0,02	-	0,02
TGA, %	-	-	0,1
KF, %	-	-	0,1
LOD, %	<0,1	0,2	-
Titration, % (titrimétrique)	100,1	-	-
DTA, %	0,2	0,3	-
DSC, %	-	-	<0,1

## SUBSTANCES CHIMIQUES INTERNATIONALES DE REFERENCE

## LISTE PREVISIONNELLE POUR 1993

Les substances chimiques internationales de référence ci-après sont nécessaires pour accompagner les spécifications qui figurent dans la troisième édition de la *Pharmacopée internationale* :

## Volume 3

Calcium, folinate (*)	Néamine (*)
Doxorubicine, chlorhydrate	(impureté du sulfate de néomycine)
Fludrocortisone, acétate	Néomycine B, sulfate (*)
Gentamicine, sulfate (*)	(impureté du sulfate de néomycine)
Hydrocortisone sodique, succinate	Nifurtimox
(-)-3-(4-Hydroxy-3-méthoxyphényl)- 2-hydrazino-2-méthylalanine (*)	Noroxymorphone, chlorhydrate (*)
(impureté de la carbidopa)	(impureté du chlorhydrate de naloxone)
Lévonorgestrel	Paromomycine, sulfate
Liothyronine	Praziquantel
(impureté de la lévothyroxine sodique)	Prednisolone sodique, phosphate
Lopéramide, chlorhydrate (*)	Spectinomycine, chlorhydrate (*)
Méthotrexate	Sulfacétamide
	Testostérone, énantate
	Vincristine, sulfate (*)

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(\*) Indique que des travaux sont en cours au Centre sur cette substance.

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.

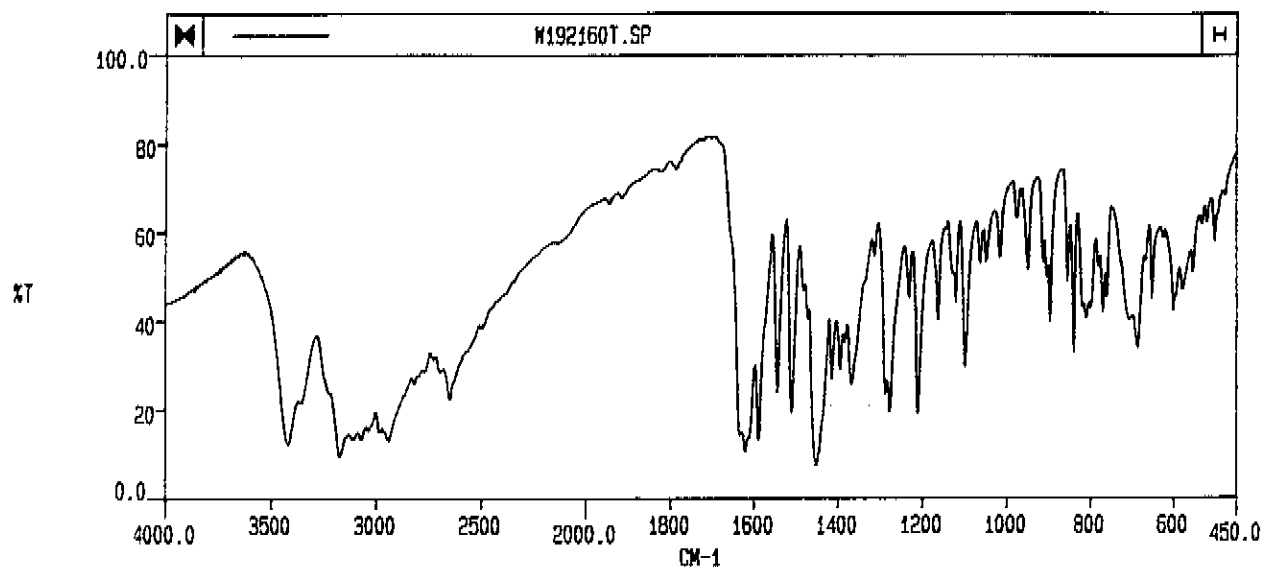


Figure 1. *IR-spectrum of 1.25 mg of amodiaquine hydrochloride Control No 192160 in 300 mg KBr recorded against a KBr disc.*  
Instrument: Perkin-Elmer 1600 FTIR.

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.

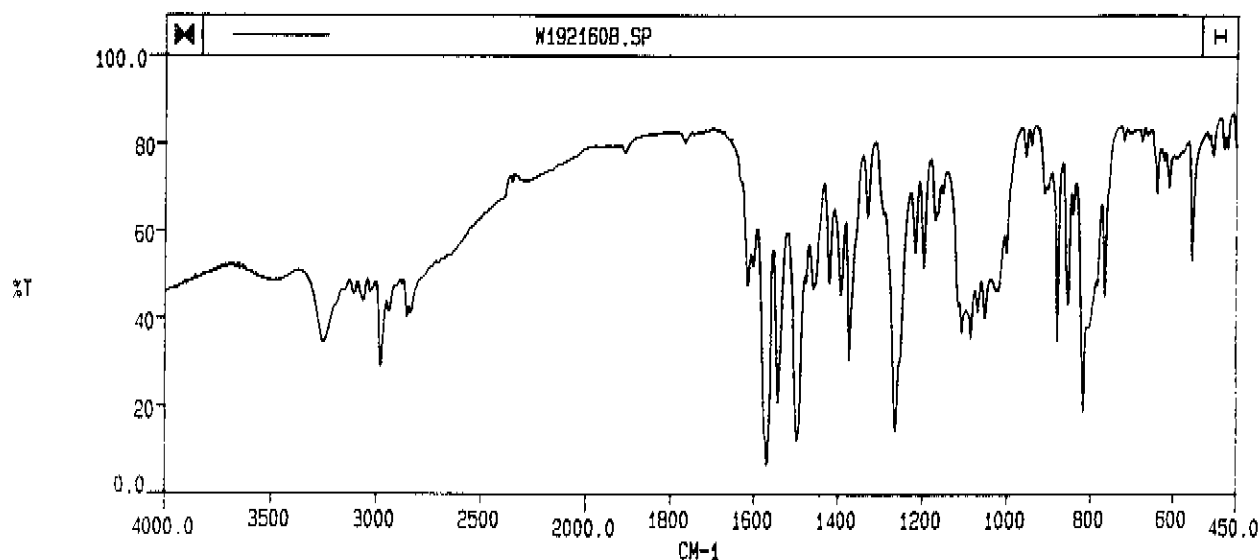


Figure 2. *IR-spectrum of 2 mg of amodiaquine base in 300 mg KBr recorded against a KBr disc.*

(\*)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.

$\lambda$  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.

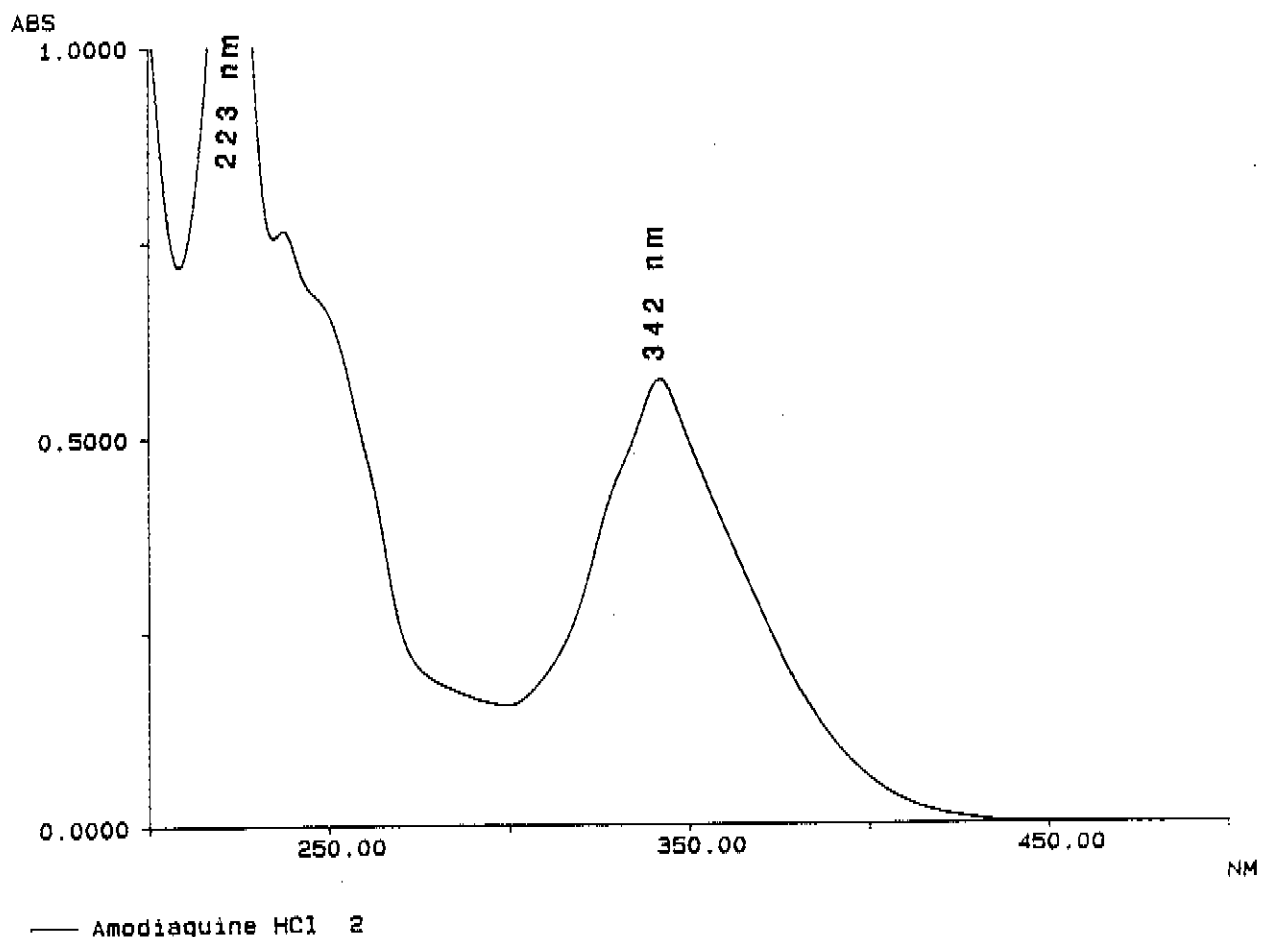


Figure 3. UV-spectrum of amodiaquine hydrochloride Control No 192160 15.2  $\mu$ g/ml in 0.1M HCl.

(\*)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<sub>f</sub> (amodiaquine hydrochloride) = 0.65. One of the impurities was identified as 4-(7-chloro-4-quinolyl-amino)phenol hydrochloride with R<sub>f</sub> = 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.

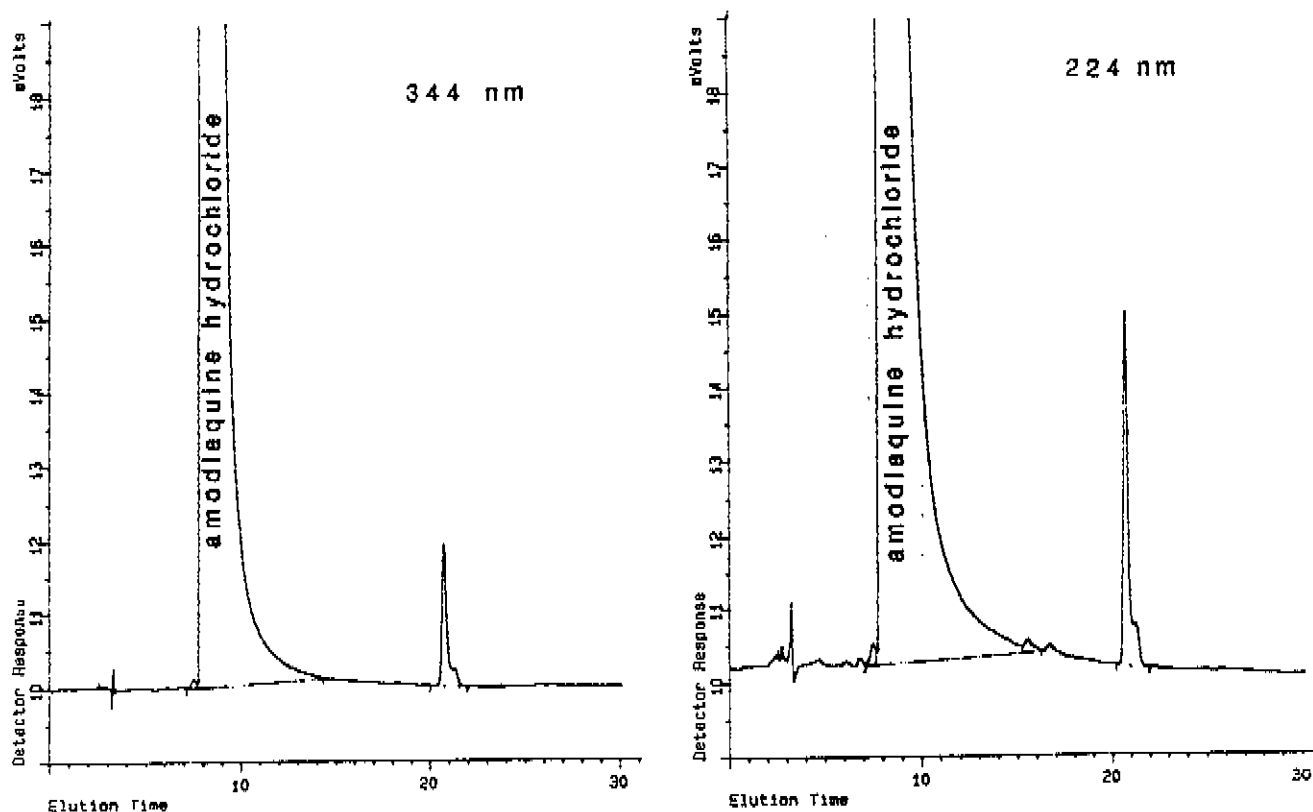


Figure 4. Chromatogram of amodiaquine hydrochloride Control No 192160 monitored at 344 nm and 224 nm.

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  $\mu$ 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  $\mu$ l corresponding to 20  $\mu$ 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  $\mu$ 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  $\text{KH}_2\text{PO}_4$  (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-quinolyamino)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.

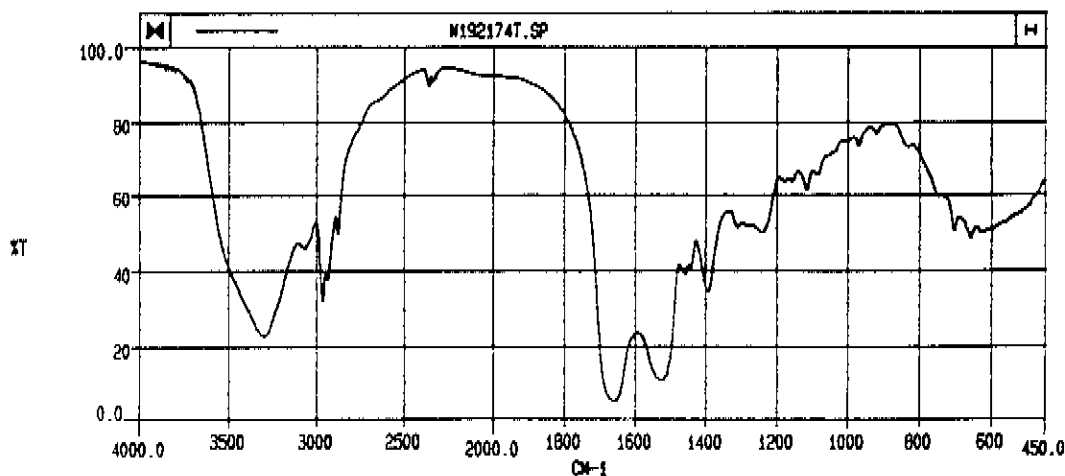


Figure 1. IR-spectrum of 1.4 mg of bacitracin zinc Control No 192174 in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 1600 FTIR.

### UV-spectrum

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

$\lambda$  max in water is 254 nm.

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

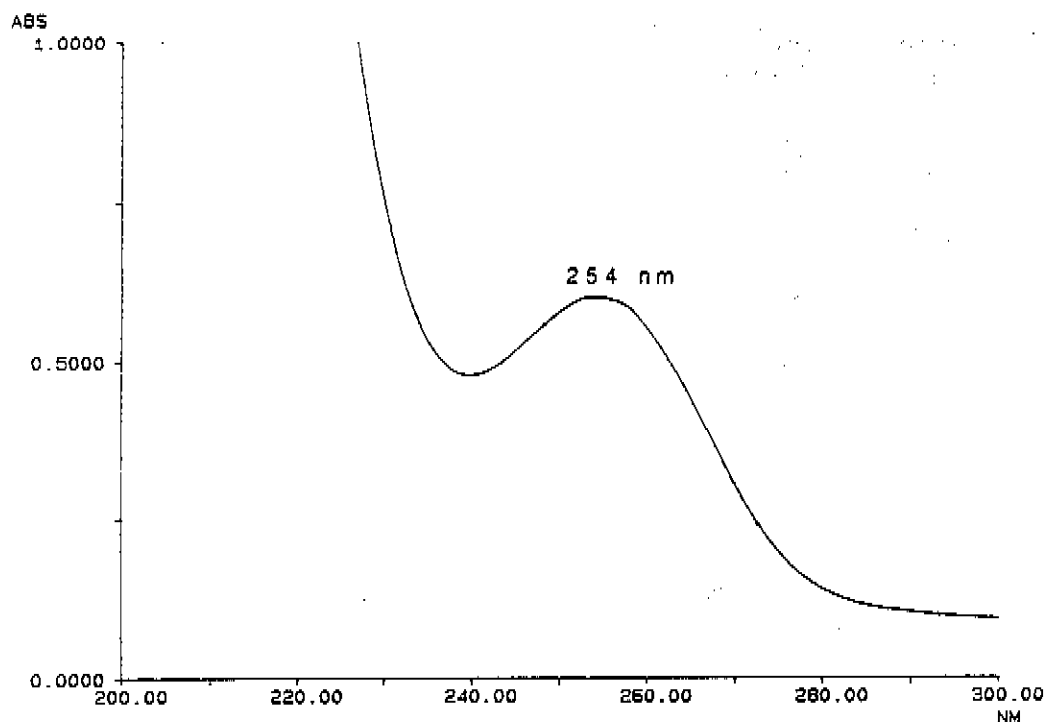


Figure 2. UV-spectrum of bacitracin zinc Control No 192174 (215 µg/ml).

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.

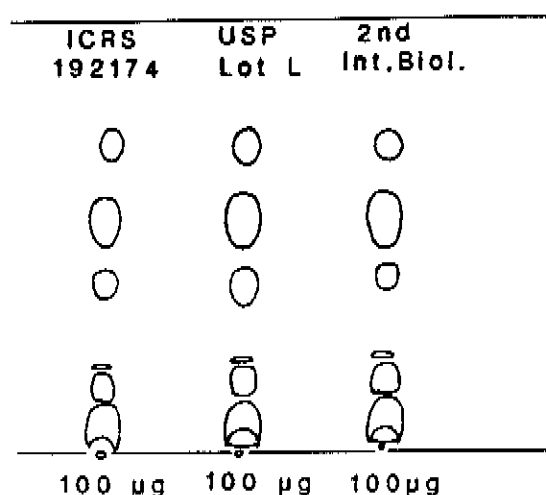


Figure 3. *Thin-layer chromatogram showing the zones obtained for bacitracin zinc Control No 192174, USP lot L and the 2nd International Biological Standard.*

#### 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.

R<sub>f</sub> (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%.

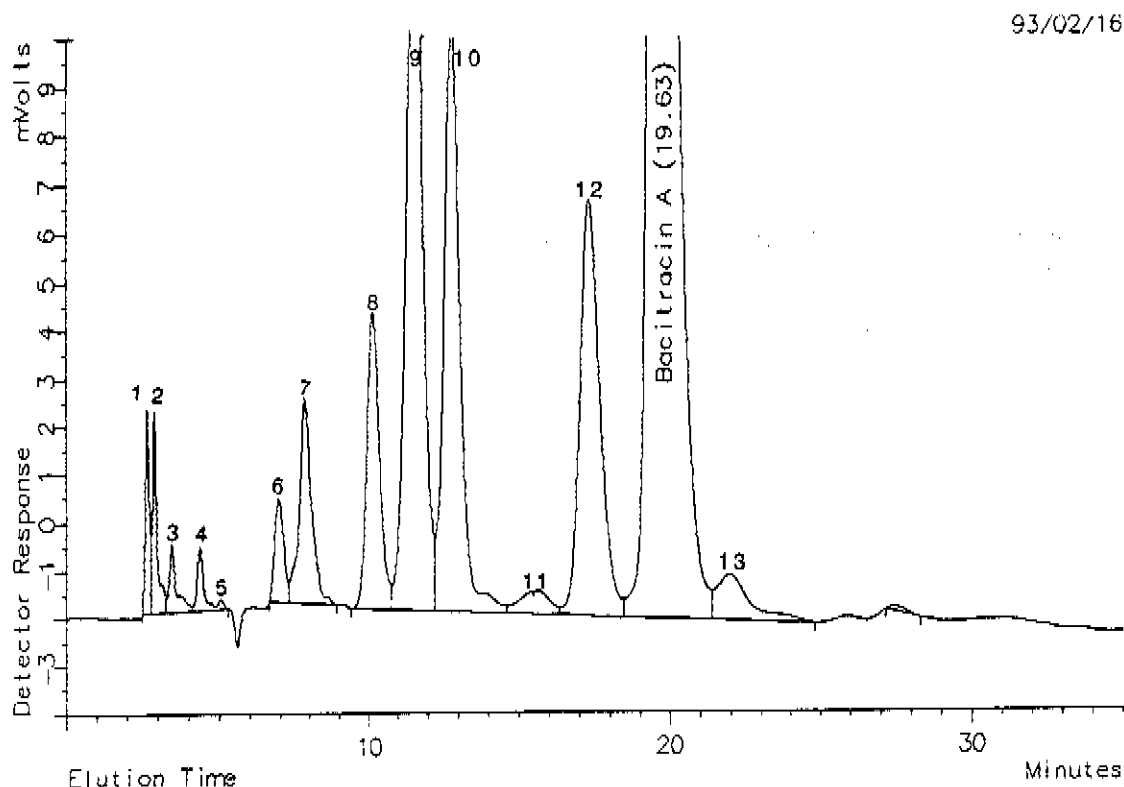


Figure 4. Chromatogram of bacitracin zinc, Control No 192174 chromatographed at 210 nm with eluent 1.

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.

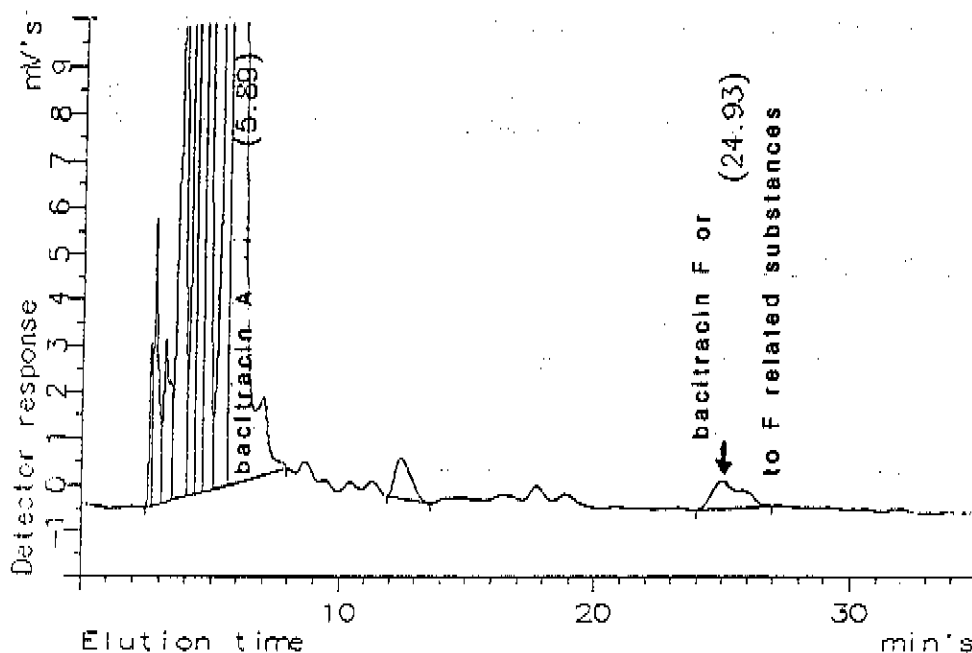


Figure 5. Chromatogram of bacitracin zinc, Control No 192174 chromatographed at 210 nm with eluent 2.

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.

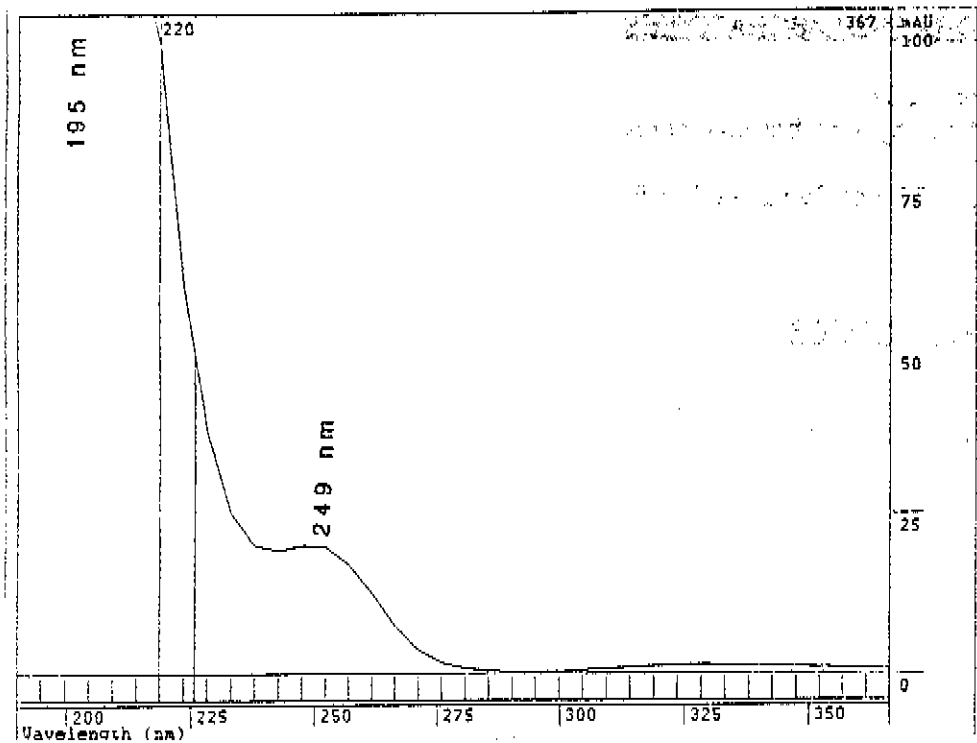


Figure 6. UV-spectrum for bacitracin A in eluent 1.

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.

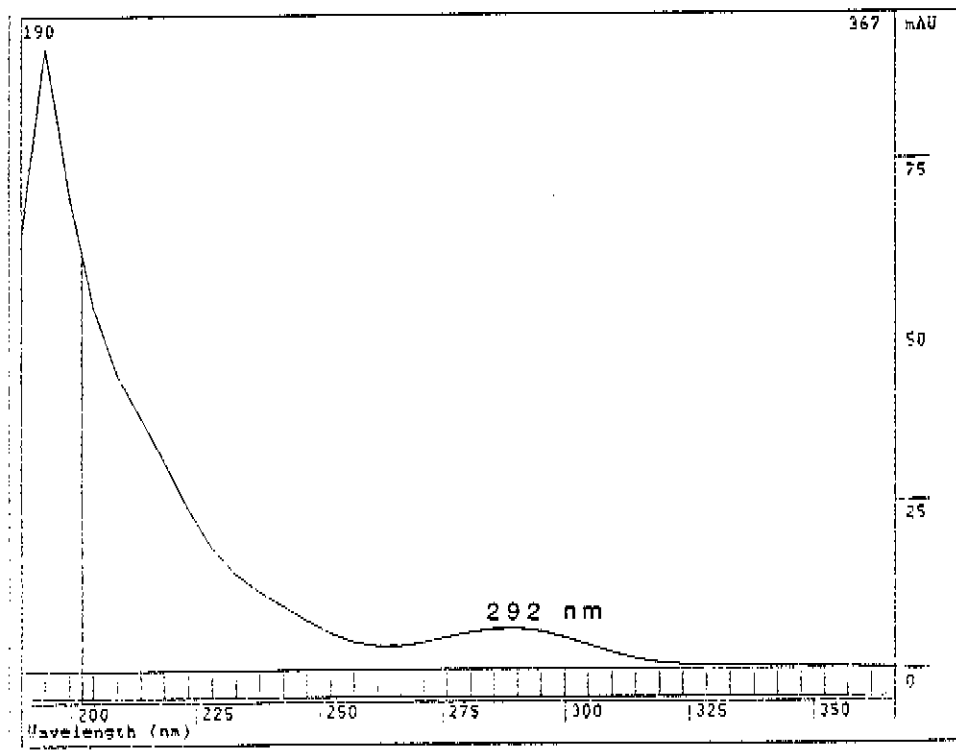


Figure 7. UV-spectrum for bacitracin F (or to F related substances) in eluent 2.

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.

APPENDIX 9**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 STRUCTUREInfrared 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.

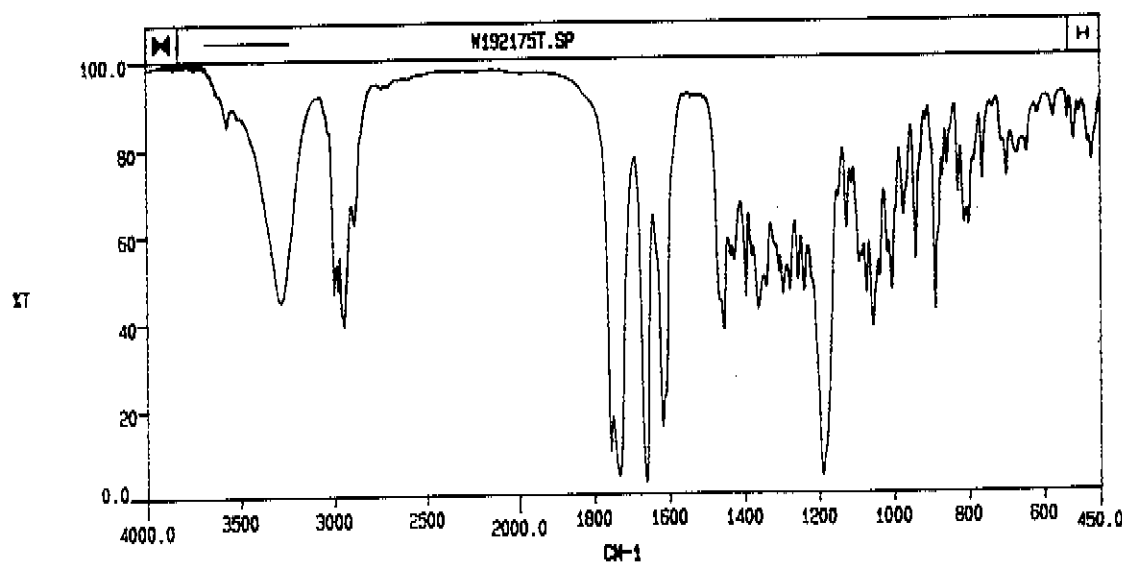


Figure 1. IR-spectrum of 1.45 mg of beclometasone dipropionate Control No 192175 in 300 mg KBr recorded against a KBr disc.

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.

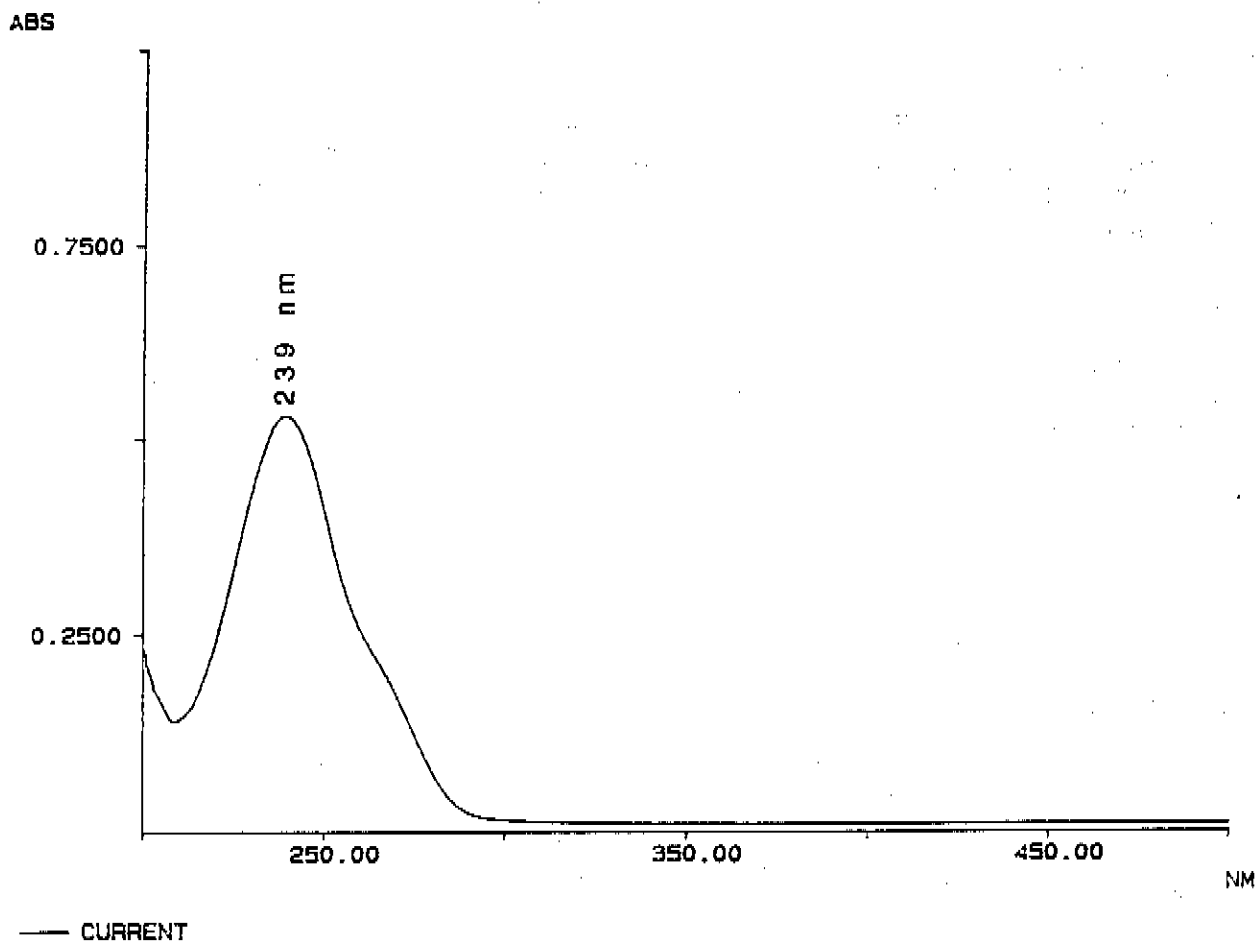


Figure 2. UV-spectrum of beclometasone dipropionate Control No 192175 18 µg/ml in methanol.

## 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.

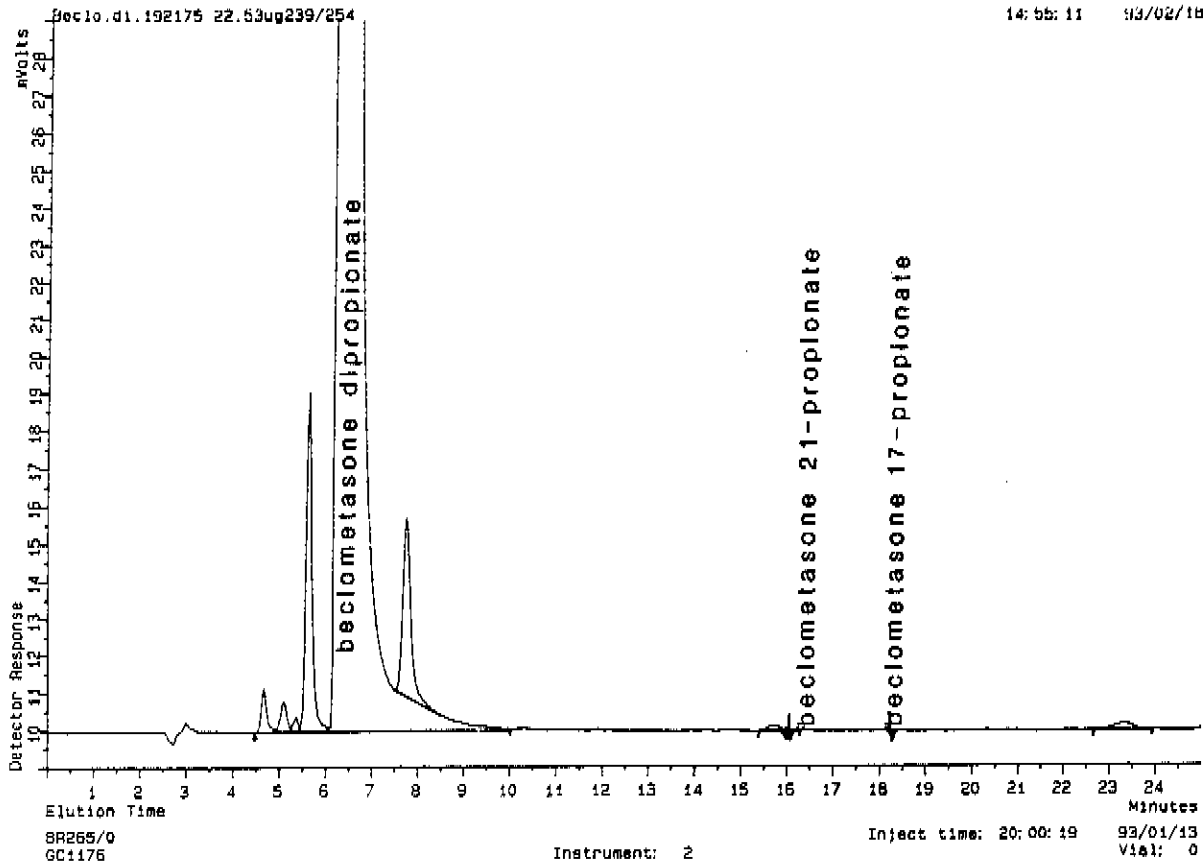


Figure 3. Chromatogram of beclometasone dipropionate, Control No 192175.

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  $C_{28}H_{37}ClO_7$  (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.

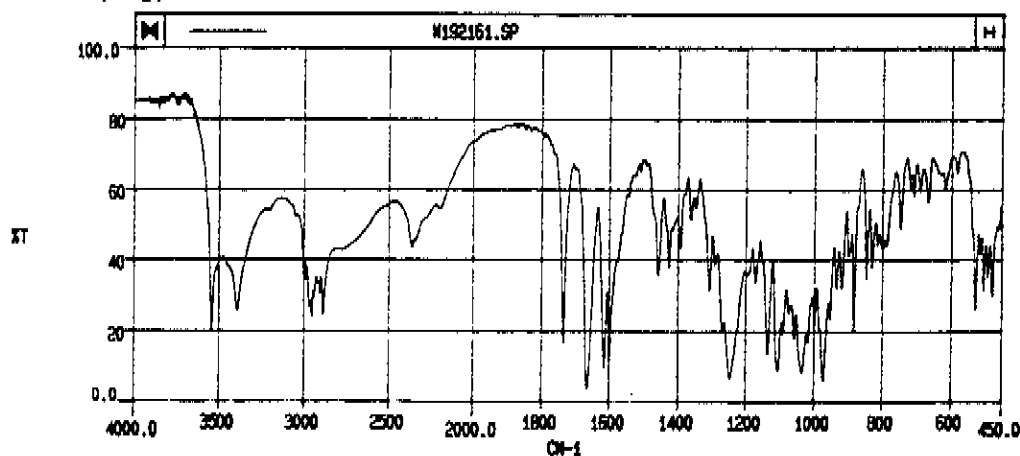


Figure 1. IR-spectrum of 1.4 mg of dexamethasone phosphoric acid Control No 192161 in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 1600 FTIR.

The spectra of dexamethasone sodium phosphate, dexamethasone phosphoric acid and dexamethasone, respectively, differ significantly for example at  $1000\text{ cm}^{-1}$  and  $1100\text{ cm}^{-1}$ . 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.

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

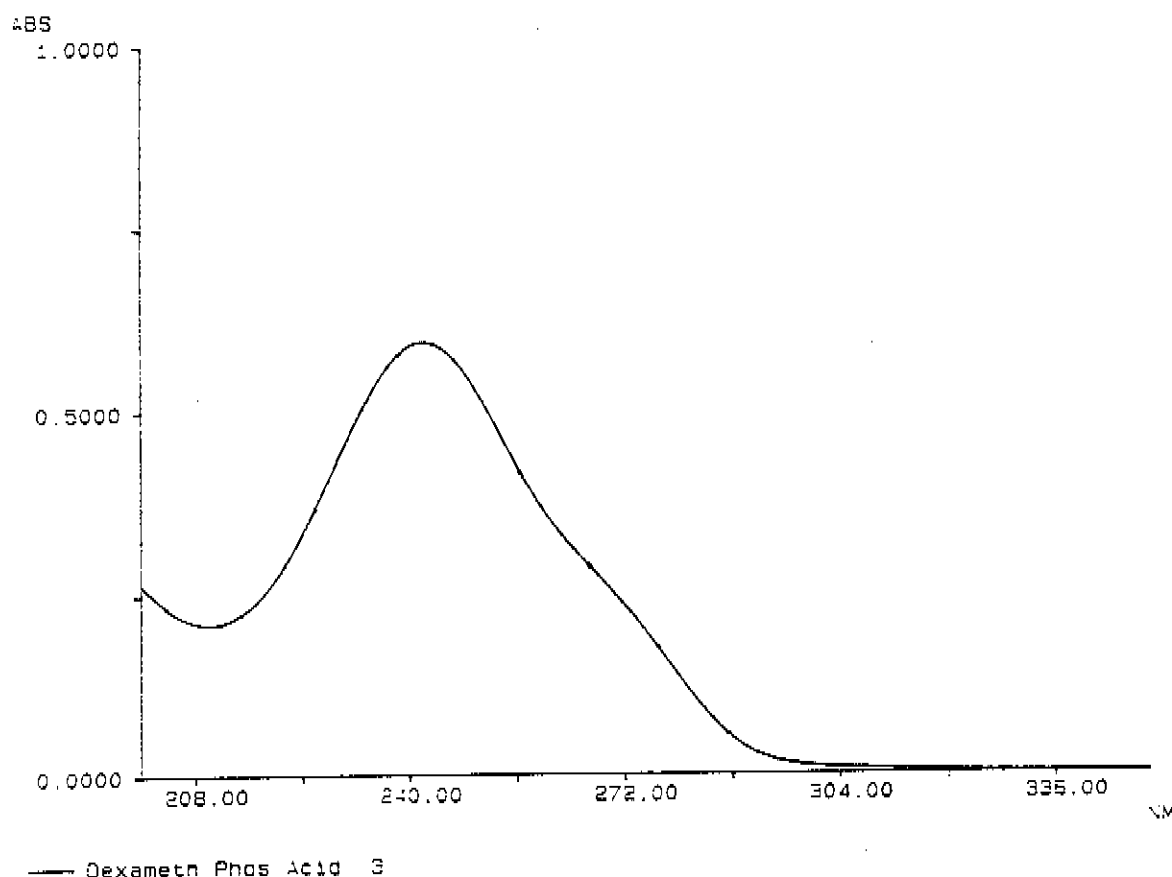


Figure 2. UV-spectrum of dexamethasone phosphoric acid Control No 192161 17.8  $\mu\text{g/ml}$  in water.

### 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 I which was regarded as 100%.

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

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

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%).

R<sub>f</sub> (dexamethasone sodium phosphate) = 0.5

R<sub>f</sub> (dexamethasone phosphoric acid) = 0.5

R<sub>f</sub> (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.

R<sub>f</sub> (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.

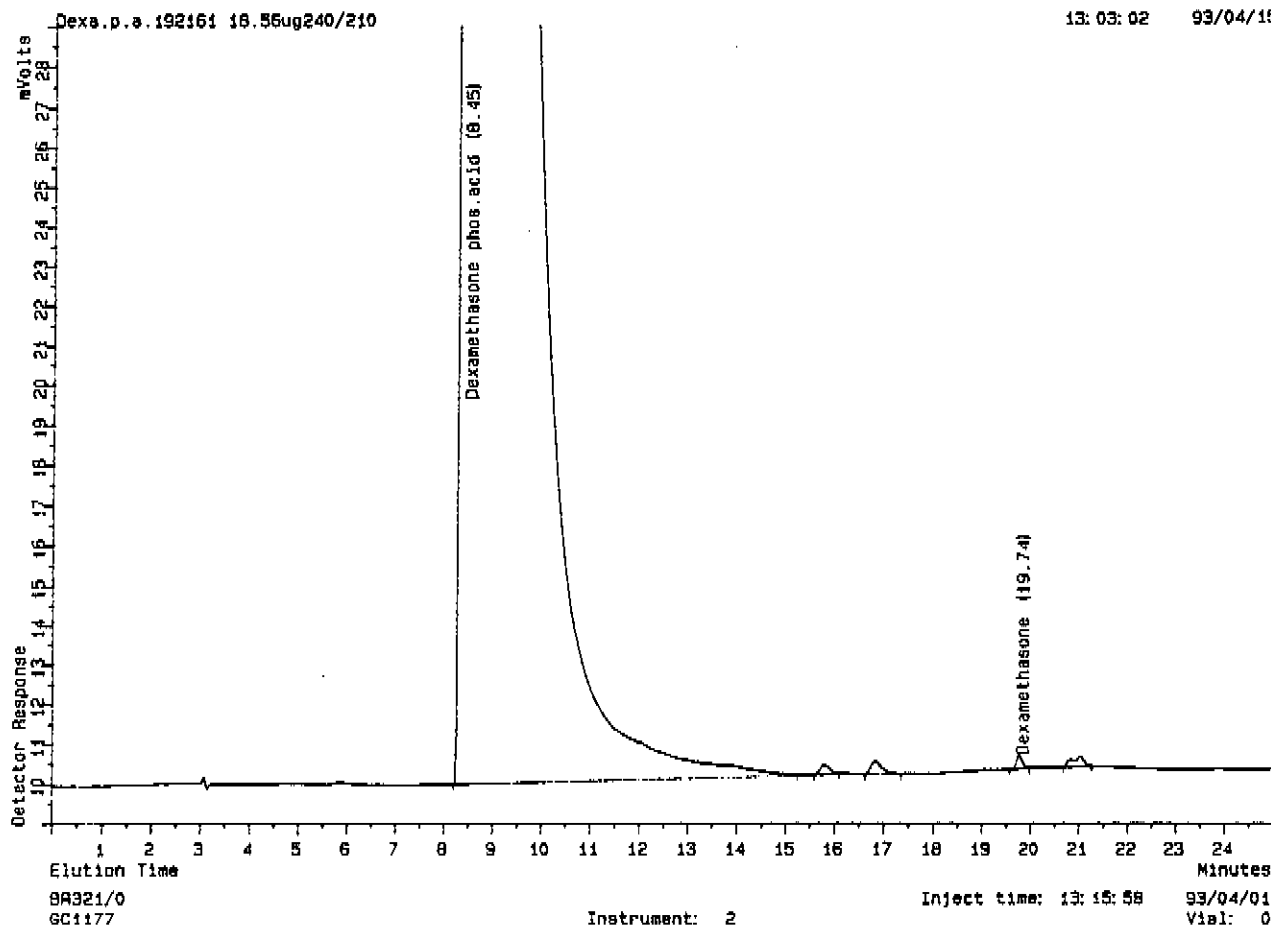


Figure 3. Chromatogram of dexamethasone phosphoric acid, Control No 192161.

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 µl corresponding to 20 µg were injected.

The detection limit for dexamethasone phosphoric acid is approximately 0.1 µg/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.

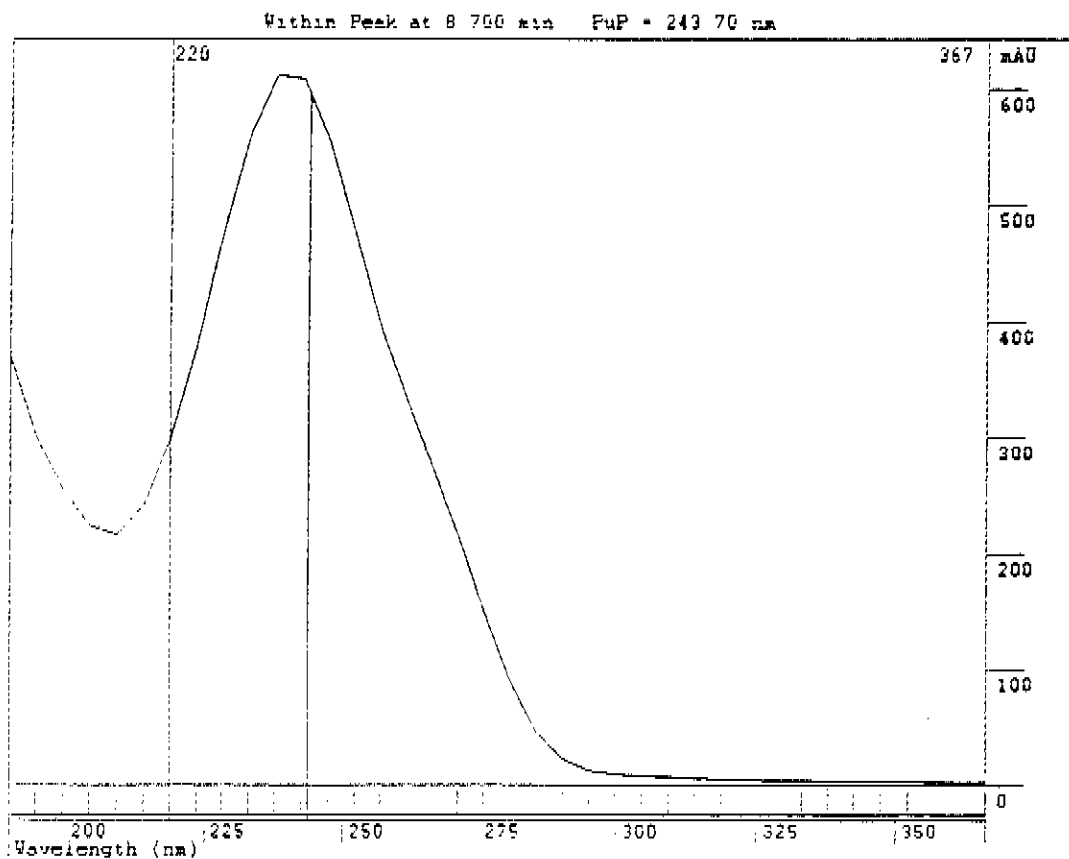


Figure 4. UV-spectrum of dexamethasone phosphoric acid recorded in the eluent.

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 EPCRS Lot no 1.

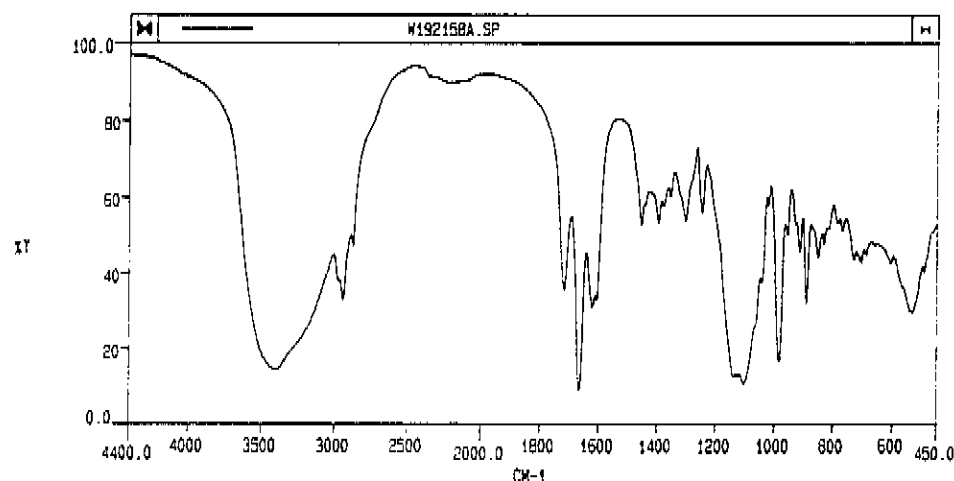


Figure 1. IR-spectrum of 1.38 mg of dexamethasone sodium phosphate Control No 192158 in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 1600 FTIR.

The spectra for dexamethasone sodium phosphate, dexamethasone phosphoric acid and dexamethasone, respectively, differ significantly for example at  $1000\text{ cm}^{-1}$  and  $1100\text{ cm}^{-1}$ . 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.

$A(1\%, 1\text{ cm}) = 291-296$  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.

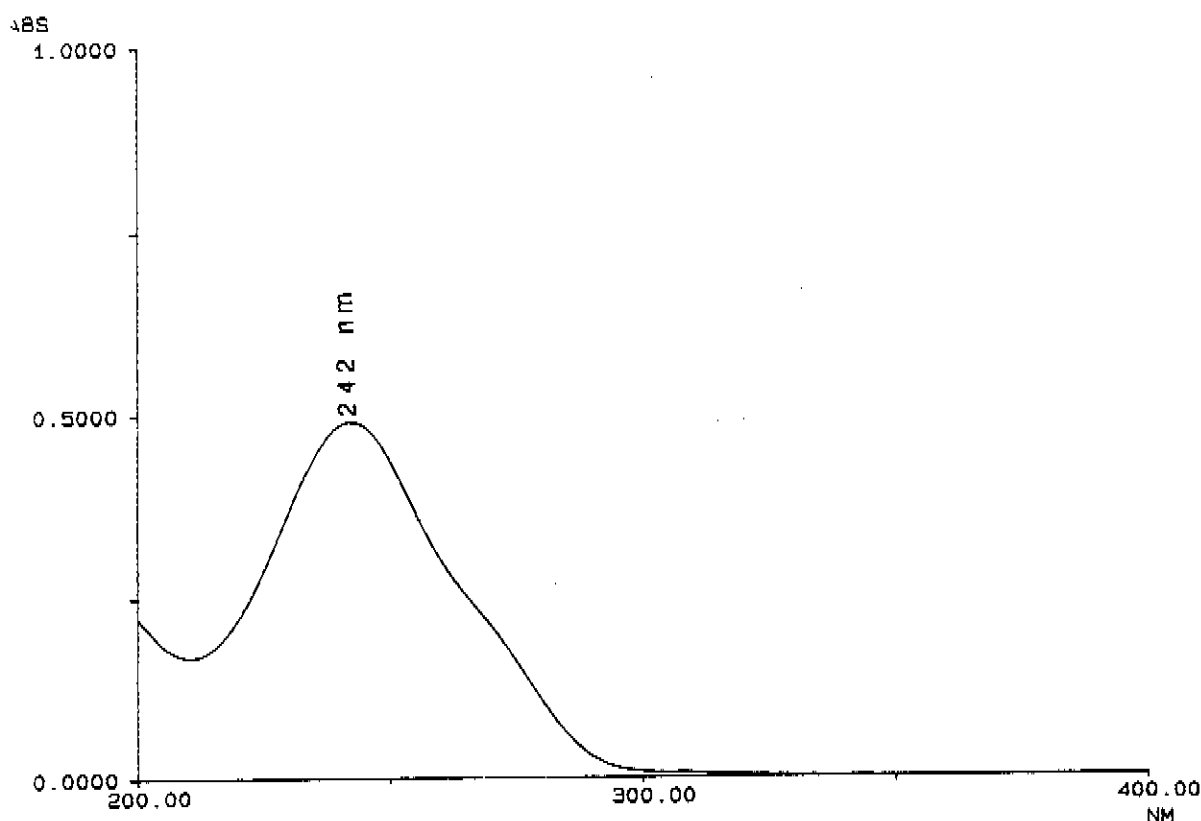


Figure 2. *UV-spectrum of dexamethasone sodium phosphate Control No 192158 18 $\mu\text{g/ml}$  in water.*

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 recommend 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 0.1 µg (0.1%) when scanned at 363 nm.

R<sub>f</sub> (dexamethasone sodium phosphate) = 0.5

R<sub>f</sub> (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.

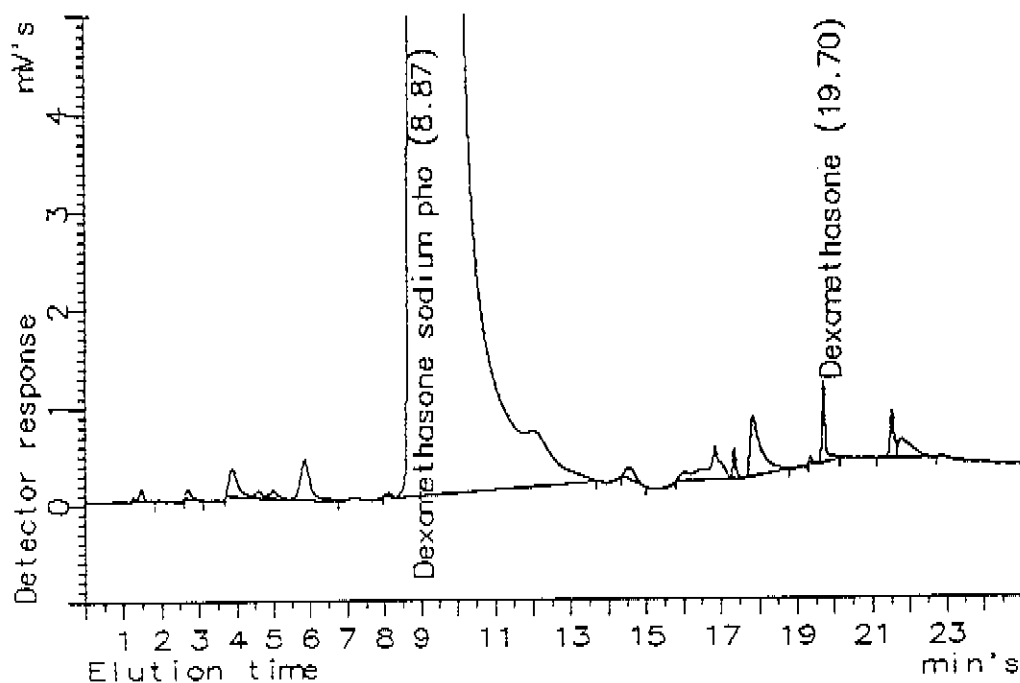


Figure 3. *Chromatogram of dexamethasone sodium phosphate, Control No 192158.*

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  $\mu$ l corresponding to 10  $\mu$ g were injected.

The detection limit for dexamethasone sodium phosphate was approximately 0.5  $\mu$ 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%.

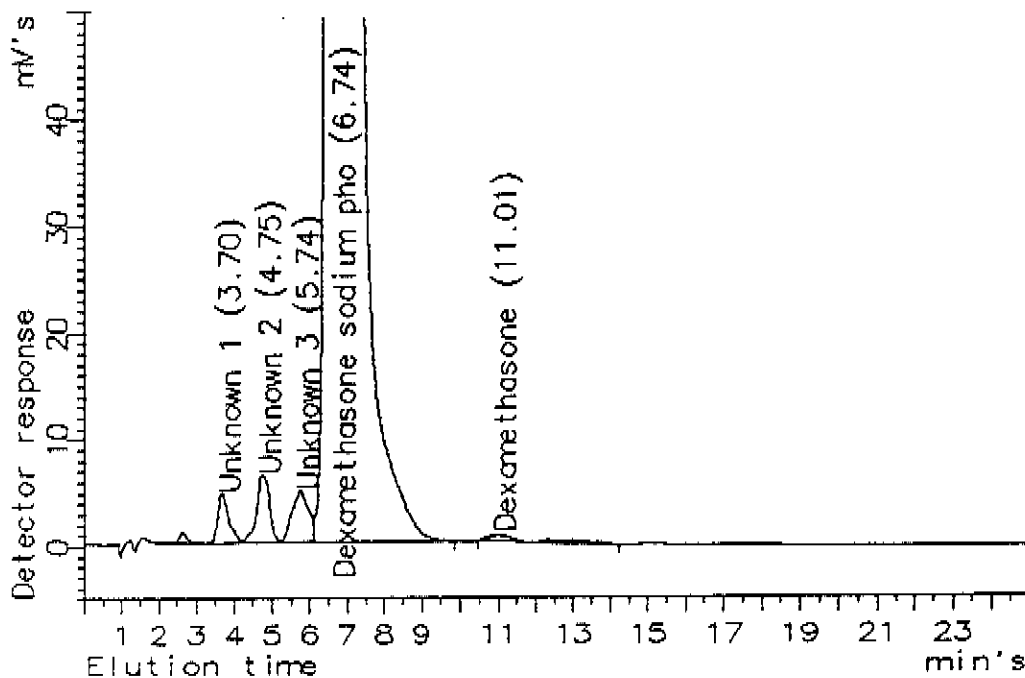


Figure 4. Chromatogram of dexamethasone sodium phosphate, Control No 192158.

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  $\mu$ l corresponding to 20  $\mu$ 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.

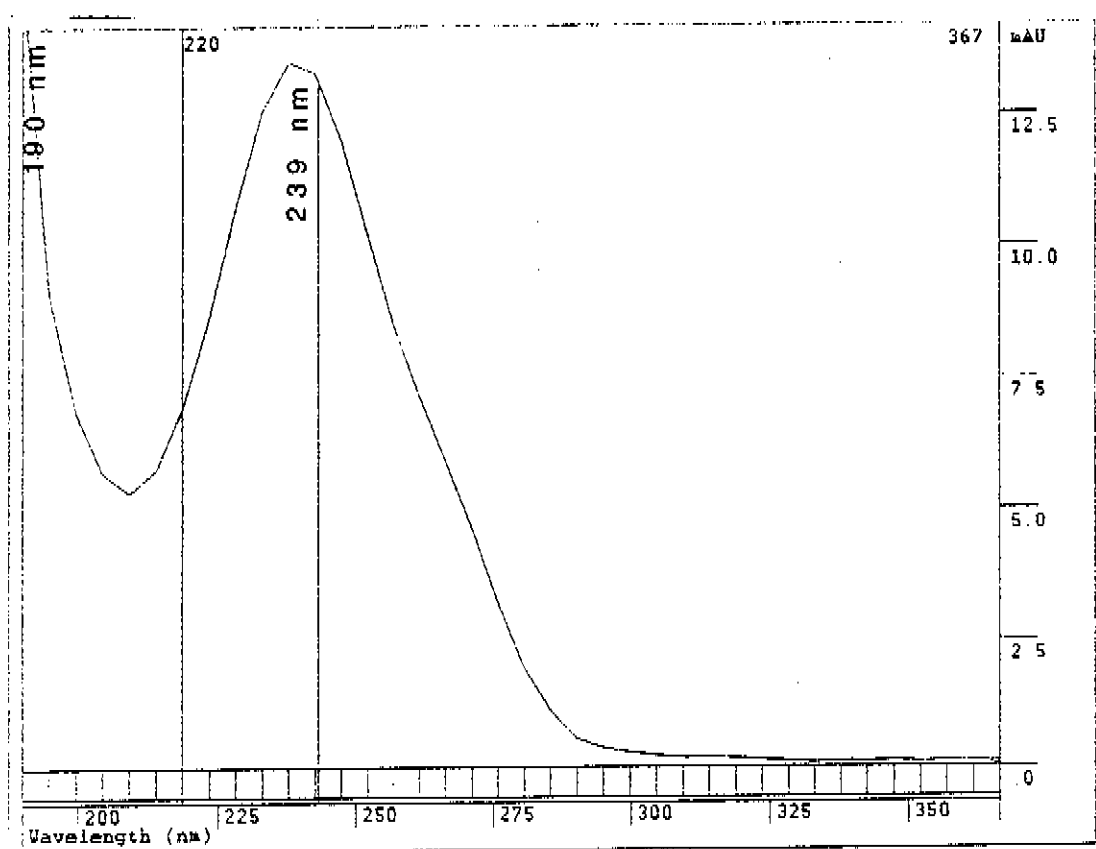


Figure 5. UV-spectrum of dexamethasone sodium phosphate recorded in the eluent.

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 STRUCTUREInfrared 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.

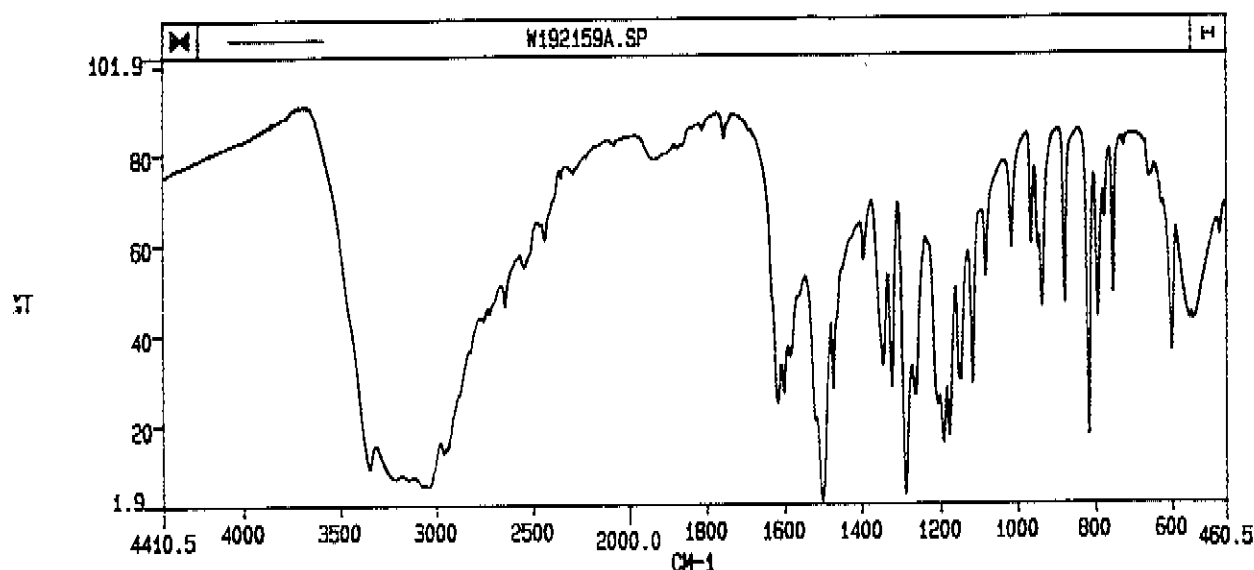


Figure 1. IR-spectrum of 1.13 mg of dopamine hydrochloride Control No 192159 in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 1600 FTIR.

UV-spectrum

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

$\lambda$  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%.

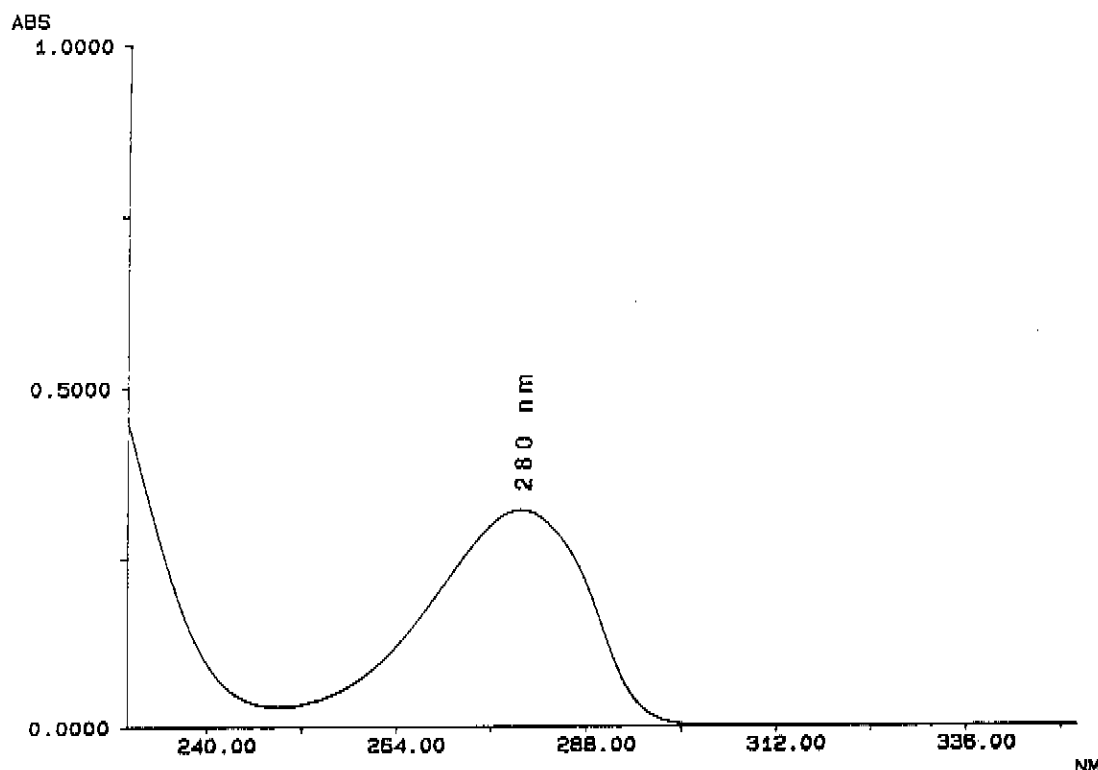


Figure 2. UV-spectrum of probenecid Control No 192159 20 ug/ml in 0.1M HCl.

**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<sub>f</sub> (dopamine hydrochloride) = 0.4.

#### High performance liquid chromatography

No impurities were detected.

A chromatogram is shown in Figure 3.

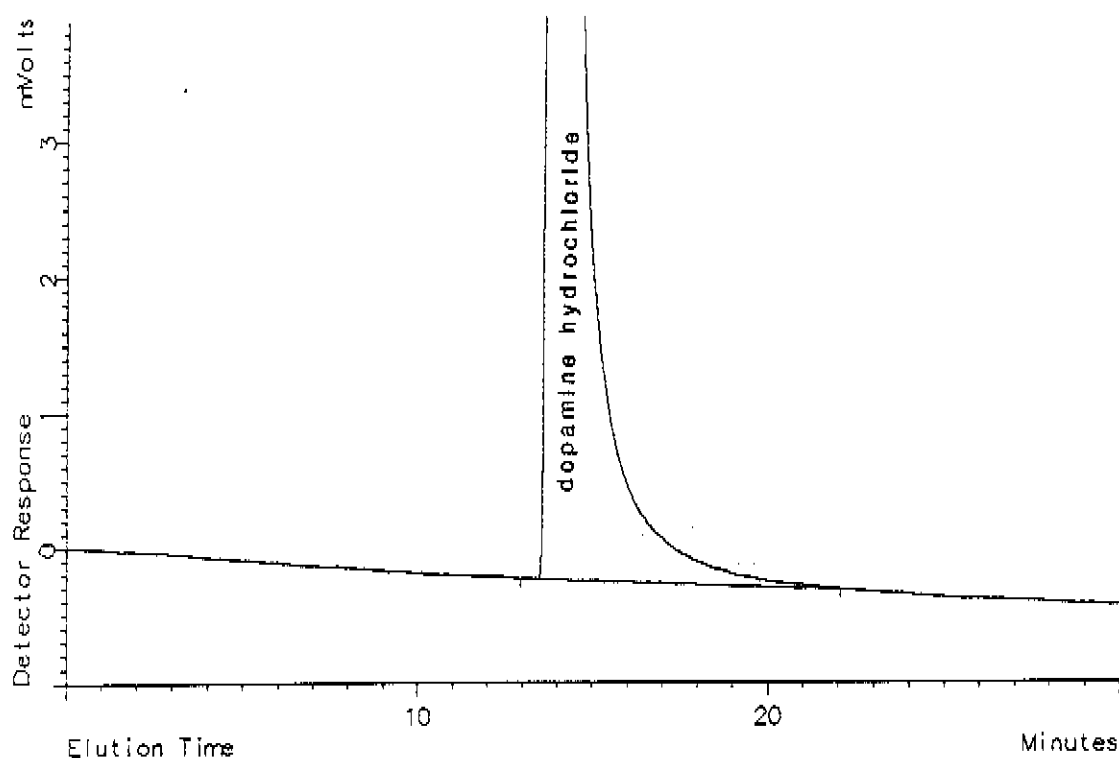


Figure 3. Chromatogram of dopamine hydrochloride Control No 192159 monitored at 280 nm.

The following conditions were used:

Eluent: Acetonitrile: 0.04 M KH<sub>2</sub>PO<sub>4</sub> buffer (30:70) containing 0.01M sodium lauryl sulfate.  
pH = 5.0

Column: RP-18, 5  $\mu$ 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  $\mu$ l corresponding to 10  $\mu$ 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.

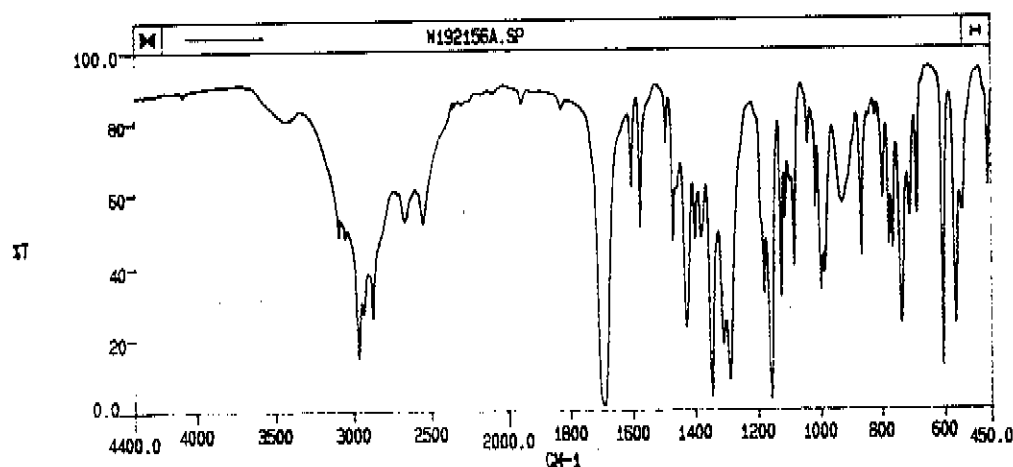


Figure 1. IR-spectrum of 1.25 mg of probenecid Control No 192156 in 300 mg KBr recorded against a KBr disc.

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.

$\lambda$  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%.

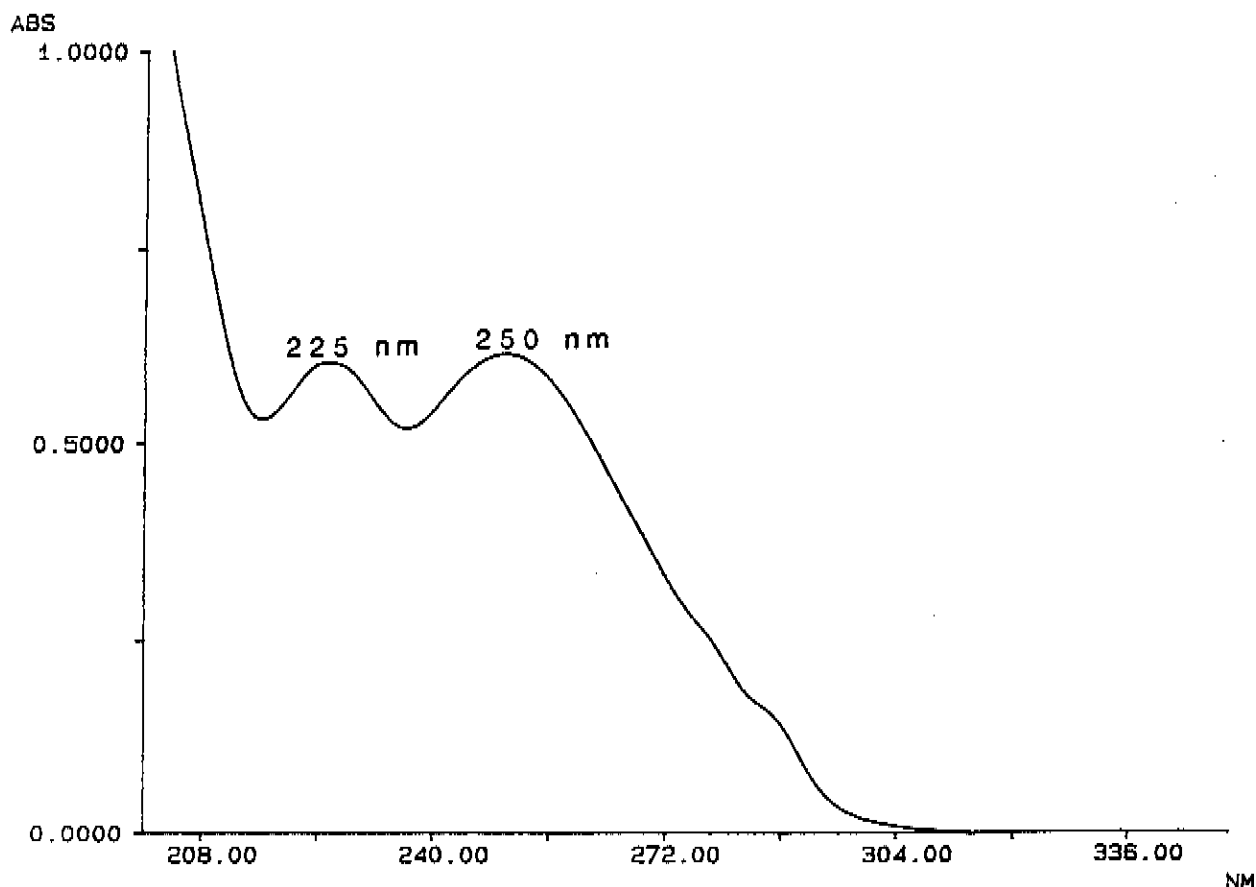


Figure 2. UV-spectrum of probenecid Control No 192156 18 ug/ml in ethanol/0.1M HCl.

(\*)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<sub>M</sub>)

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<sub>f</sub> (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 Rf= 0.59. An impurity was observed at Rf= 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.

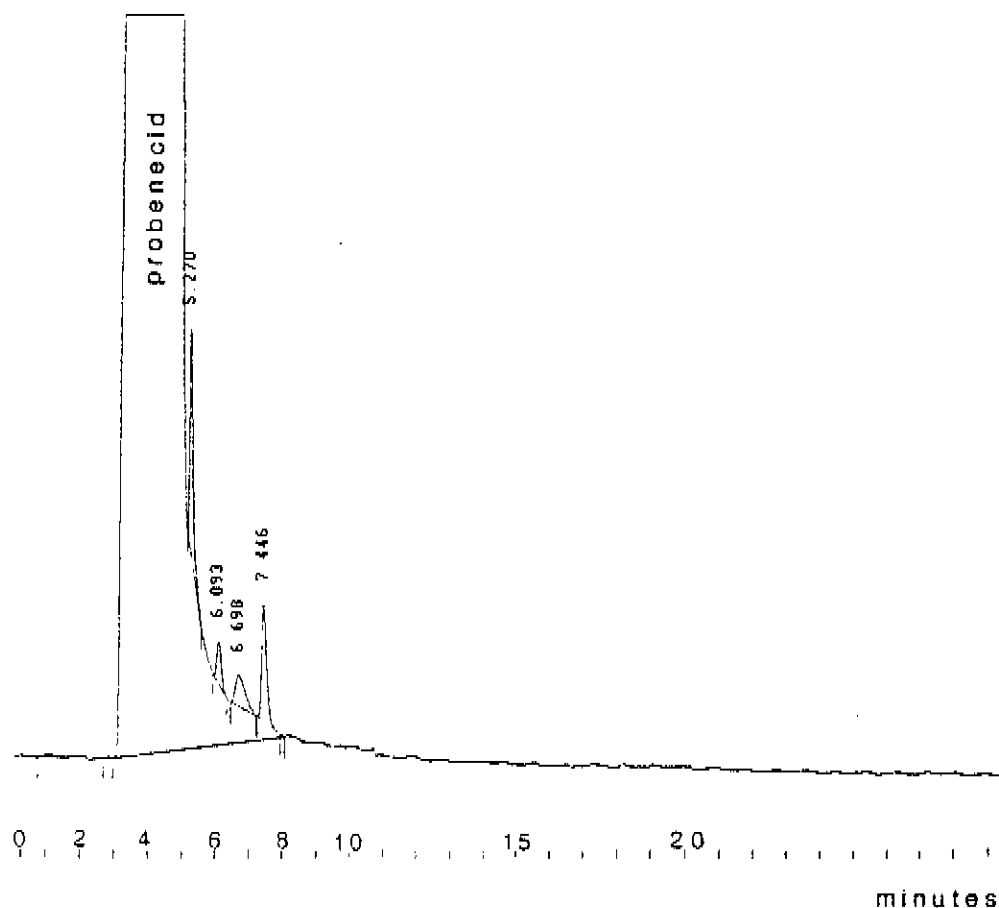


Figure 3. Chromatogram of probenecid Control No 192156 monitored at 239 nm.

The following conditions were used:

- Eluent: Methanol/water (90:10)  
Column: RP-18, 5  $\mu$ 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  $\mu$ l corresponding to 10  $\mu$ 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  $\mu$ 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  $\mu$ l corresponding to 50  $\mu$ 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.

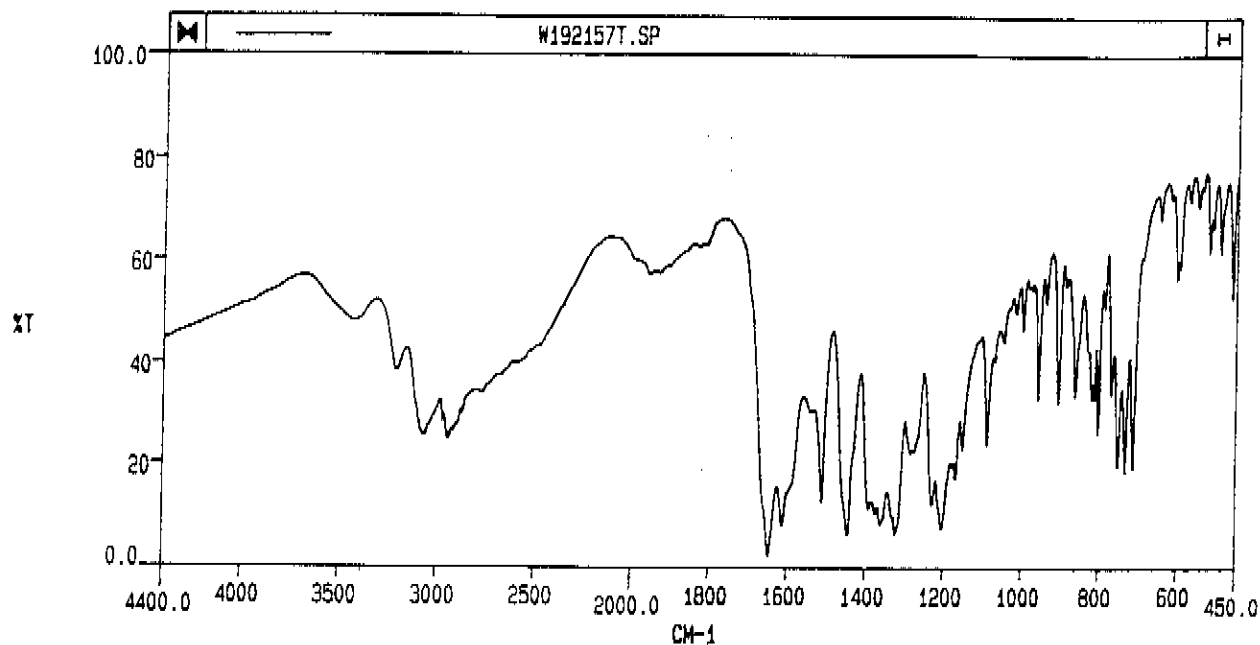


Figure 1. IR-spectrum of 1.42 mg of pyrantel embonate Control No 192157 in 300 mg KBr recorded against a KBr disc.

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%)

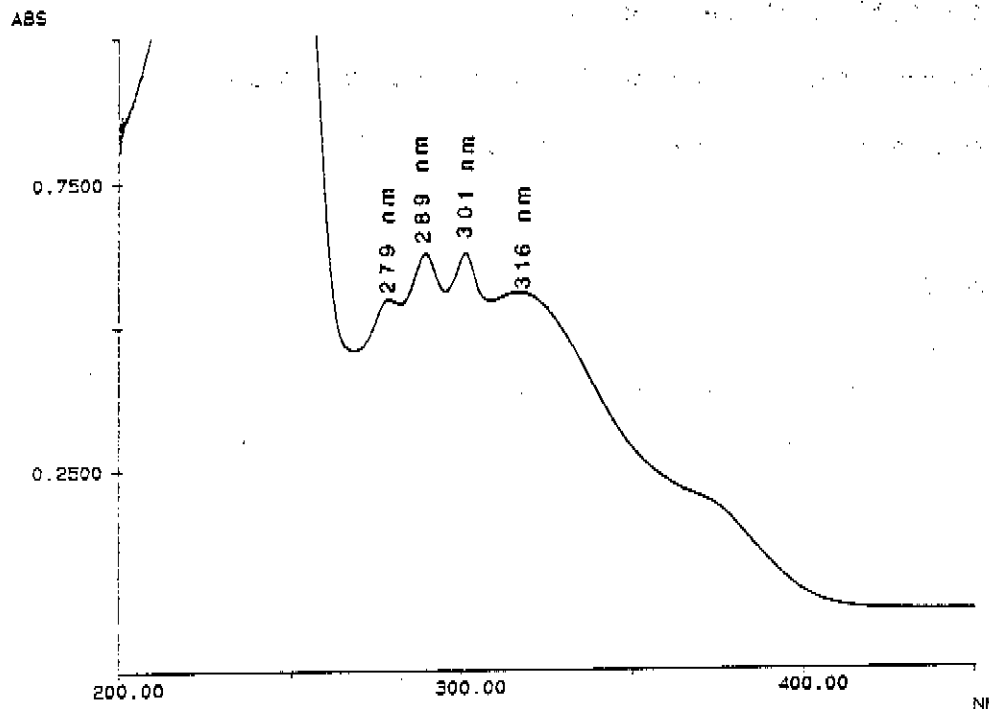


Figure 2. UV-spectrum of pyrantel embonate Control No 192157 16.5 ug/ml in methanol.

(\*) 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.

R<sub>f</sub> (pyrantel embonate) = 0.35

R<sub>f</sub> (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 R<sub>f</sub> = 0.22. It was estimated to be present at less than 0.1%. However the system exhibits poor separation and the R<sub>f</sub> of pyrantel is too low.

R<sub>f</sub> (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.

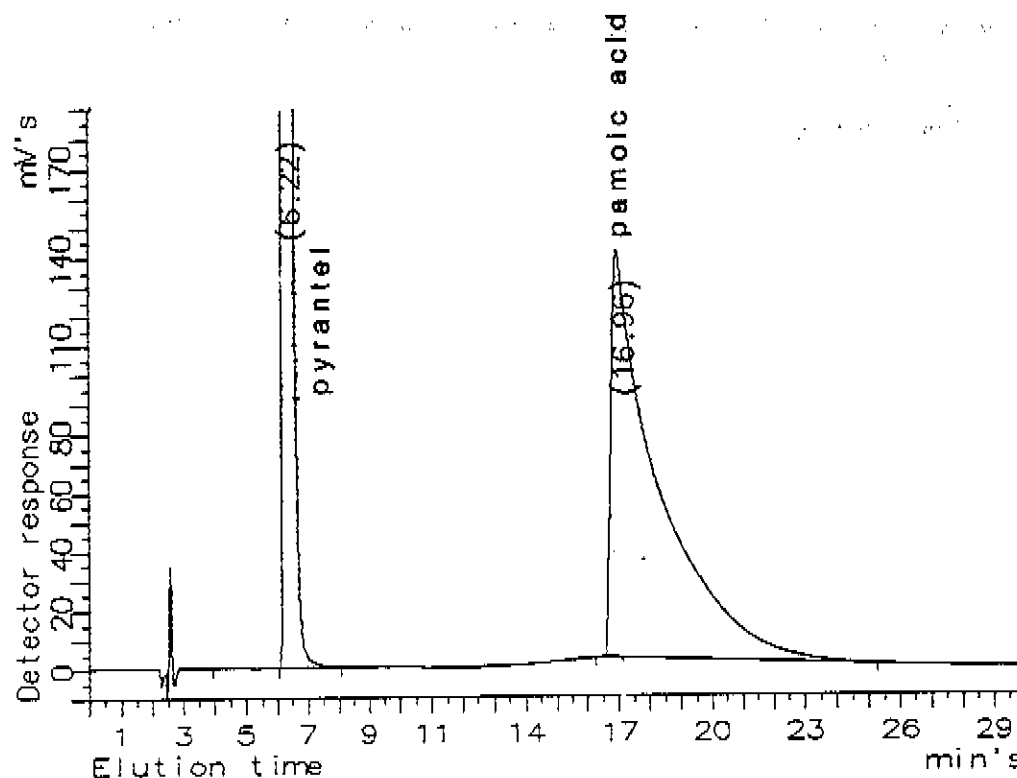


Figure 3. Chromatogram of pyrantel embonate Control No 192157 (reversed phase).

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 1ml/min

Integrator: PeakPro (Beckman)

Sample: 1 mg/ml dissolved in 0.1 ml DMSO and adjusted to 1ml 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.

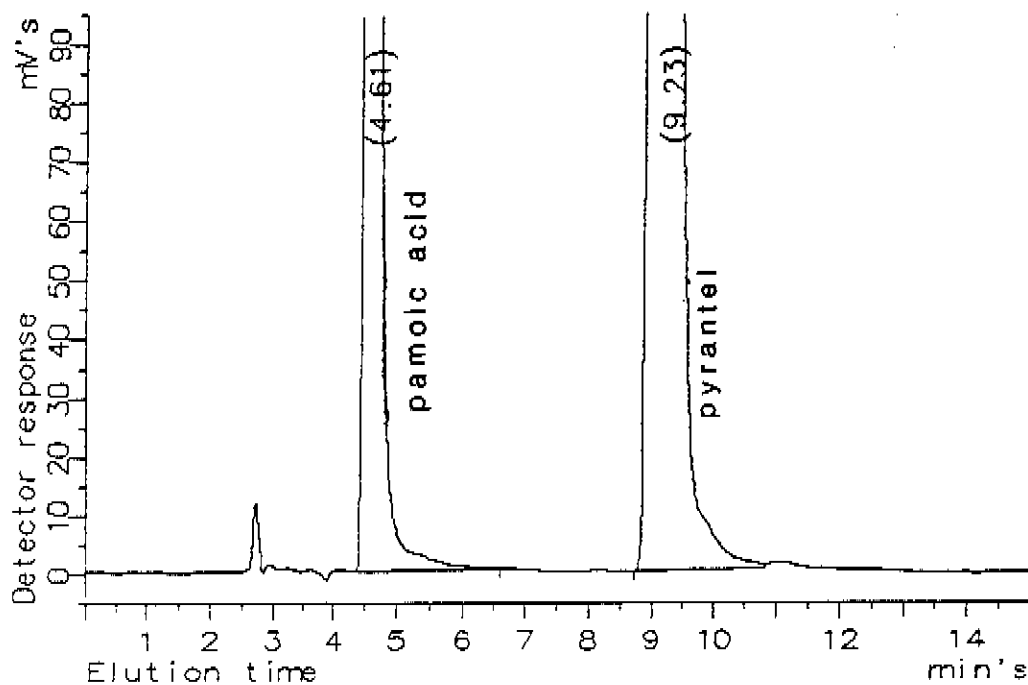


Figure 4. Chromatogram of pyrantel embonate Control No 192157 (straight phase).

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  $\mu$ l corresponding to 10  $\mu$ 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.

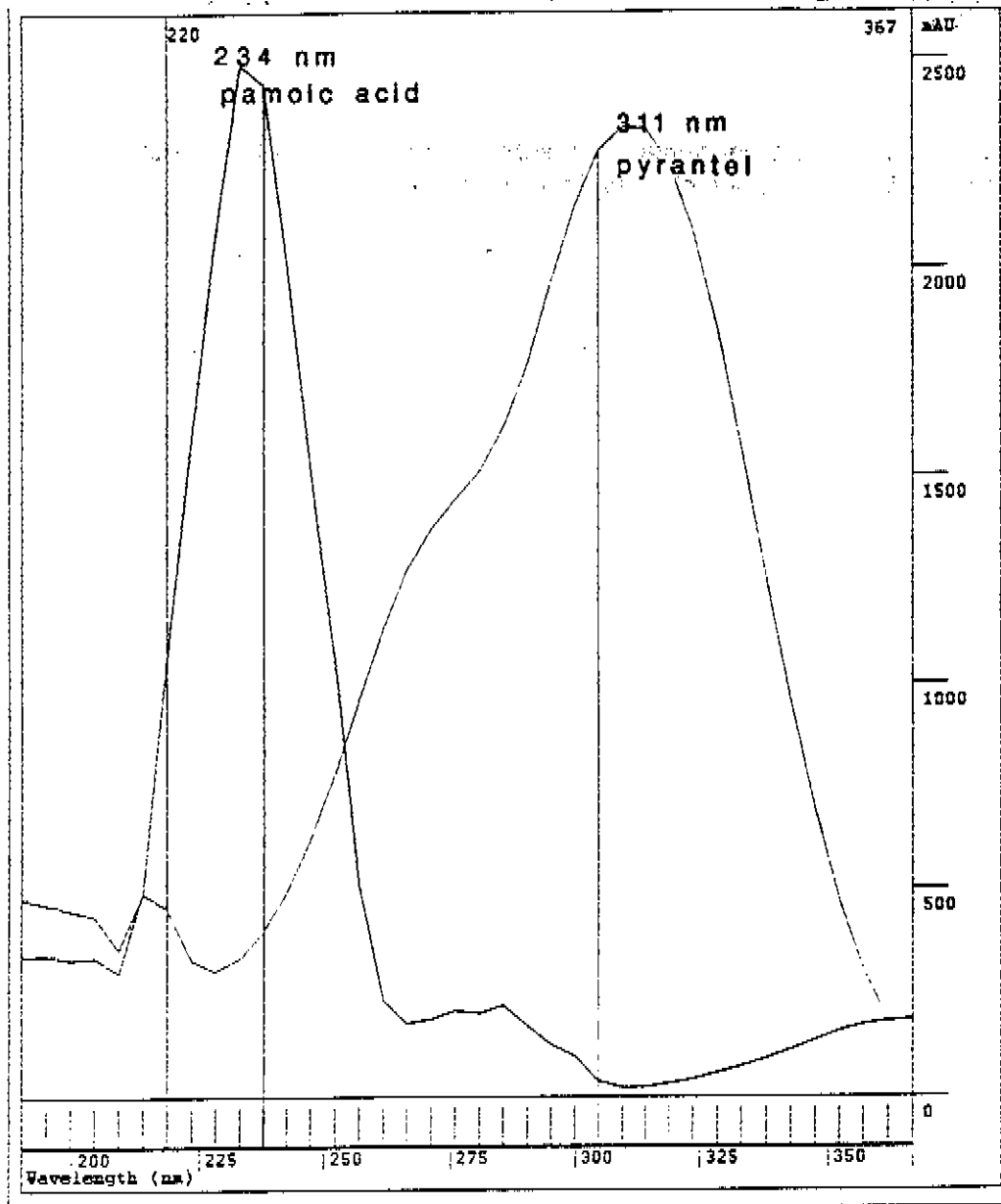


Figure 5. UV-spectra for pyrantel and pamoic acid recorded by the diode array detector.

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.

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