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CENTRE COLLABORATEUR OMS POUR LES SUBSTANCES CHIMIQUES DE REFERENCE

Rapport d'activité pour 1988

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Note : Pour des raisons techniques, les appendices 6 à 11 n'ont été établis qu'en anglais.

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Distribution de substances de référence en 1988

En 1988, le Centre a distribué à des laboratoires de contrôle pharmaceutique de 40 pays 1096 échantillons de substances chimiques internationales de référence et 25 séries de substances de référence pour la détermination du point de fusion. Ces chiffres représentent une diminution d'environ 46 % par rapport à ceux de 1987. Les six substances les plus fréquemment demandées en 1988 ont été, dans l'ordre, l'ampicilline, le trihydrate d'ampicilline, le tartrate d'ergotamine, la digoxine et, à égalité, la benzylpénicilline sodique et la prédnisone. On trouvera à l'appendice 1 le détail de la distribution des diverses substances de référence.

Etablissement de substances de référence en 1988

Conformément à la procédure recommandée par le Comité OMS d'experts des Spécifications relatives aux Préparations pharmaceutiques dans son vingt-cinquième rapport (OMS, Série de Rapports techniques, N° 567), le Centre a établi en 1988 huit substances chimiques internationales de référence, dont on trouvera la liste à l'appendice 2. Parmi ces substances, l'allopurinol et la digoxine sont des lots de remplacement, les lots précédents ayant été épuisés en 1988.

On trouvera à l'appendice 3 une liste complète de toutes les substances chimiques internationales de référence détenues par le Centre en janvier 1989, 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 six substances mentionnées ci-dessous, dont on peut prévoir qu'elles seront officiellement adoptées au cours du premier semestre de 1989.

Travaux effectués en 1988 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 1988, l'analyse d'une nouvelle substance de référence destinée à accompagner le volume 3 de la Pharmacopée internationale, le cromoglicatate de sodium, a été réalisée. Le rapport d'analyse pour cette substance figure à l'appendice 11. Cette substance a été jugée satisfaisante pour l'usage auquel elle est destinée et il est par conséquent proposé de l'adopter comme substance chimique internationale de référence.

Les cinq lots suivants de substances chimiques internationales de référence ont été épuisés et ont été remplacés par de nouveaux lots en 1988. Le lot N° 274002 d'ampicilline sodique a été remplacé par le lot N° 388002, le lot N° 279008 de dexaméthasone par le lot N° 388008, le lot N° 168009 d'acétate de dexaméthasone par le lot N° 288009, le lot N° 180097 de chlorhydrate d'épi-4 anhydrotétracycline par le lot N° 288097 et le lot N° 277019 d'acide folique par le lot N° 388019.

Essais de stabilité

Chaque année, un certain nombre de substances chimiques internationales de référence détenues par le Centre sont réexaminées afin de contrôler leur stabilité pendant le stockage. En 1988-1989, le réexamen a porté sur trois pénicillines.

Le choix des méthodes d'analyse à utiliser pour la surveillance de la stabilité exige une mûre réflexion. Il dépend bien entendu de la nature de la substance concernée mais, d'une façon générale, le principe est d'utiliser des méthodes hautement reproductibles et de s'en tenir le plus possible aux mêmes méthodes et dans les mêmes conditions expérimentales pour le réexamen d'une substance de référence que lors de l'analyse initiale. L'influence des erreurs analytiques sera ainsi limitée et on pourra déceler précocement le début d'une éventuelle dégradation de la substance. Il est toutefois judicieux d'examiner de temps à autre les progrès de la chimie analytique et d'introduire de nouvelles méthodes si on les juge plus informatives ou plus commodes.

On trouvera à l'appendice 4 les résultats obtenus lors du réexamen et ceux des examens précédents. On peut obtenir auprès du Centre des détails concernant les méthodes utilisées.

Travaux en cours et travaux futurs

Le Centre poursuit l'établissement de nouvelles substances chimiques de référence. Il manque encore une substance pour accompagner les monographies du volume 2 de la Pharmacopée internationale. Pour le volume 3, il faut encore 40 nouvelles substances de référence, dont 15 sont déjà à l'étude. D'anciens lots devront également être remplacés en raison de l'épuisement des stocks. Actuellement, six substances doivent être remplacées en 1989-1990, mais ce chiffre peut encore augmenter selon les quantités distribuées. Une grande partie de la charge de travail à laquelle le Centre doit faire face vient des demandes de plus en plus nombreuses de réexamen périodique de substances de référence existantes. Certaines substances sont très anciennes et l'augmentation du nombre total de substances de référence entraîne un nouveau surcroît de travail. Les substances de référence que le Centre doit établir sont énumérées à l'appendice 5. Les substances déjà en cours d'examen sont signalées par un astérisque.

En 1988, l'informatisation des activités liées au travail sur les substances de référence s'est poursuivie. Le système utilisé est l'ordinateur personnel IBM XT. Des renseignements à jour sur les commandes de substances en vrac, les protocoles d'analyse, les fiches de travail et le plan de réexamen périodique ont été mis sur ordinateur. L'informatisation des commandes et l'inventaire des stocks de substances existantes sont en cours. La collaboration avec d'autres laboratoires en vue de réduire la charge de travail du Centre de Stockholm a également commencé.

Questions administratives et financières

La situation financière du Centre reste mauvaise. Le coût de fonctionnement total du Centre en 1988 a été estimé à US \$264 300. Le revenu provenant des ventes de substances de référence aux laboratoires industriels a été d'environ US \$28 000 et la contribution du Siège de l'OMS de US \$16 000, ce qui laisse un déficit de US \$220 300. Le Conseil d'administration de l'Association nationale des Pharmacies suédoises a convenu de maintenir au même niveau sa contribution au fonctionnement du Centre, sous réserve que tout soit mis en oeuvre pour diminuer le déficit.

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

Afin de réduire le déficit financier du Centre, il a été demandé aux centres nationaux de recherche de contribuer au travail d'analyse et les bureaux régionaux de l'OMS ont été contactés en vue d'une éventuelle aide financière.

Remerciements

Le Centre désire exprimer ses remerciements aux deux laboratoires qui ont contribué à ses travaux en 1988, le National Biological Standards Laboratory à Canberra, Australie, et le laboratoire de la Pharmacopée européenne à Strasbourg, France. Le Centre désire également exprimer sa plus vive gratitude à toutes les firmes pharmaceutiques qui l'ont aidé en lui fournissant des substances de référence et en participant aux travaux d'analyse. Cette année, nos remerciements vont en particulier à American Cyanamid Company, Pearl River, Etats-Unis d'Amérique, à Beecham Pharmaceuticals, Worthing, Angleterre, à Fisons, Leicestershire, Angleterre, à Merck Sharp & Dohme, Rahway, Etats-Unis d'Amérique, et à Takeda Chemical Industries Ltd, Osaka, Japon.

DISTRIBUTION DE SUBSTANCES CHIMIQUES DE REFERENCE EN 1988

Acéclidine, salicylate d'	1	échantillon(s)	Fluphénazine, énantiote de (dichlorhydrate)	-	échantillon(s)
p-Acétamidobenzalazine	2	"	Folique, acide	3	"
Acétazolamide	1	"	Furoséide	8	"
Allopurinol	13	"	Griséofulvine	18	"
Amino-2 nitro-5 thiazole	-	"	Halopéridol	3	"
Amino-3 pyrazole carboxamide-4, hémisulfate d'	1	"	Hydrochlorothiazide	5	"
Amitryptiline, chlorhydrate d'	5	"	Hydrocortisone	32	"
Ampicilline	72	"	Hydrocortisone, acétate d'	7	"
Ampicilline sodique	21	"	(-)-(Hydroxy-4 méthoxy-3 phényl)-3 méthyl-2 alanine	4	"
Ampicilline, trihydrate d'	52	"	Ibuprofène	6	"
Anhydrotétracycline, chlorhydrate d'	27	"	Imipramine, chlorhydrate d'	4	"
Atropine, sulfate d'	12	"	Indométacine	4	"
Azathioprine	3	"	o-Iodohippurique, acide	1	"
Benzazol, chlorhydrate de	-	"	Isoniazide	5	"
Benzobarbital	-	"	Lanatoside C	-	"
Benzylamine, sulfate de	1	"	Lévodopa	8	"
Benzylpénicilline potassique	8	"	Lidocaïne	4	"
Benzylpénicilline sodique	38	"	Lidocaïne, chlorhydrate de	12	"
Béphénium, hydroxynaphtoate de	-	"	Méfénamique, acide	6	"
Bétaméthasone	4	"	Métazida	1	"
Bétanidine, sulfate de	-	"	Méthacualone	2	"
NN'-bis(xylyl-2,3) anthranilamide	-	"	Méthyl-dopa	6	"
Bupivacaïne, chlorhydrate de	7	"	Méthyltestostérone	-	"
Caféine	4	"	Méticilline sodique	5	"
Carbénicilline monosodique	15	"	Métronidazole	9	"
Chloramphénicol	23	"	Nafcilline sodique	-	"
Chloramphénicol, palmitate de	7	"	Néostigmine, métilsulfate de	-	"
Chloramphénicol, palmitate de (forme A)	6	"	Nicotinamide	11	"
Chloro-5 méthylamino-2 benzophénone	2	"	Nicotinique, acide	11	"
(Chloro-4 sulfamoyl-3 benzoyl)-2 benzoïque, acide	7	"	Niridazole	-	"
Chlorphénamine, hydrogénomaléate de	4	"	Niridazole-chloréthylcarboxamide	-	"
Chlorpromazine, chlorhydrate de	10	"	Noréthistérone	-	"
Chlortalidone	1	"	Noréthistérone, acétate de	2	"
Chlortétracycline, chlorhydrate de	-	"	Ouabaine	-	"
Clomifène, citrate de	-	"	Oxacilline sodique	19	"
Clomifène, citrate de (isomère Z) (Zuelomifène)	-	"	Papavérine, chlorhydrate de	3	"
Cloxacilline sodique	10	"	Phénéticilline potassique	1	"
Cortisone, acétate de	3	"	Phénoxyéthylpénicilline	9	"
Dapsone	4	"	Phénoxyéthylpénicilline calcique	3	"
Désoxycortone, acétate de	2	"	Phénoxyéthylpénicilline potassique	8	"
Dexaméthasone	2	"	Phénytoïne	4	"
Dexaméthasone, acétate de	4	"	Prednisolone	36	"
Diazépam	7	"	Prednisolone, acétate de	4	"
Diazoxide	3	"	Prednisone	38	"
Dicloxacilline sodique	29	"	Prednisone, acétate de	-	"
Dicollinium, iodure de	-	"	Procaine, chlorhydrate de	17	"
Dicoumarol	-	"	Procarbazine, chlorhydrate de	2	"
Diéthylcarbamazine, dihydro- généocitrate de	2	"	Progestérone	3	"
Digitoxine	21	"	Propicilline potassique	13	"
Digoxine	38	"	Propranolol, chlorhydrate de	-	"
Emétine, chlorhydrate d'	1	"	Propylthiouracil	2	"
Epi-4 anhydrotétracycline, chlorhydrate d'	20	"	Pyridostigmine, bromure de	1	"
Epi-4 tétracycline, sel d'ammonium de l'	24	"	Résérpine	-	"
Ergométrine, hydrogénomaléate d'	14	"	Riboflavine	15	"
Ergotamine, tartrate d'	43	"	Sulfaméthoxazole	6	"
Estradiol, benzoate d'	15	"	Sulfaméthoxy-pyridazine	1	"
Estrone	8	"	Sulfanilamide	4	"
Etacrynique, acide	-	"	Testostérone, propionate de	2	"
Ethambutol, chlorhydrate d'	4	"	Tétracycline, chlorhydrate de	17	"
Ethinylestradiol	5	"	Thioacétazone	2	"
Ethistérone	-	"	Thiodianiline-4,4'	2	"
Ethosuximide	1	"	Tolbutamide	5	"
Ebocarlide	-	"	Tolnaftate	6	"
Flucytosine	1	"	Triméthadione	-	"
Fluorouracil	6	"	Triméthoprime	7	"
Fluphénazine, chlorhydrate de	1	"	Triméthylguanidine, sulfate de	-	"
Fluphénazine, décanoate de (dichlorhydrate)	-	"	Tubocurarine, chlorure de	1	"
			Vitamine A, acétate de (solution à 25 000 UI)	34	"
			Warfarine	5	"

Substances de référence pour le point de fusion : 25 séries de 13 substances.

LISTE DES SUBSTANCES CHIMIQUES INTERNATIONALES DE REFERENCE ETABLIES EN 1988

Substance de référence	N° de contrôle	Rapport d'analyse	Remarques
Allopurinol	287049	WHO/PHARM/88.537 Appendice 6	Remplace le N° 172049
Chlortétracycline, chlorhydrate de	187138	WHO/PHARM/88.537 Appendice 7	
Clomifène, citrate de	187136	WHO/PHARM/88.537 Appendice 8	
Clomifène, citrate de (isomère Z) (Zuclomifène)	187137	WHO/PHARM/88.537 Appendice 9	
Digoxine	587011	WHO/PHARM/88.537 Appendice 10	Remplace le N° 377011
Emétine, chlorhydrate d'	187134	WHO/PHARM/88.537 Appendice 11	
Néostigmine, méthilsulfate de	187135	WHO/PHARM/88.537 Appendice 12	
Propranolol, chlorhydrate de	187139	WHO/PHARM/88.537 Appendice 13	

LISTE DES SUBSTANCES CHIMIQUES INTERNATIONALES DE REFERENCE DISPONIBLES

1989

Informations générales

Les substances chimiques internationales de référence sont établies conformément à l'avis du Comité 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 proposés sous forme de projets de monographies.

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.

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 analytiques plus détaillés sur ces substances peuvent être obtenus sur demande auprès du Centre collaborateur de l'OMS pour les substances chimiques de référence.

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-105 14 STOCKHOLM
SUEDE

(Télex : 115 53 APOBOL S)
(Téléfax : + 46 8 740 65 73)

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 d'	100 mg	172048
p-Acétamidobenzalazine	100 mg	171042
Acétazolamide	100 mg	186128
Allopurinol	100 mg	287049
Amino-2 nitro-5 thiazole	100 mg	287049
Amino-3 pyrazole carboxamide-4, hémisulfate d'	25 mg	186131
Amitryptiline, chlorhydrate d'	100 mg	172050
Ampicilline	100 mg	181101
Ampicilline sodique	200 mg	274001
Ampicilline, trihydrate d'	200 mg	388002
Anhydrotétracycline, chlorhydrate d'	200 mg	274003
Atropine, sulfate d'	25 mg	180096
Azathioprine	100 mg	183111
Benzazol, chlorhydrate de	100 mg	172060
Benzobarbital	100 mg	173086
Benzylamine, sulfate de	100 mg	172051
Benzylpénicilline potassique	100 mg	172052
Benzylpénicilline sodique	200 mg	180099
Béphénium, hydroxynaphthoate de	200 mg	280047
Bétaméthasone	100 mg	183112
Bétanidine, sulfate de	100 mg	183113
NN'-bis (xylyl-2,3) anthranilamide	100 mg	172053
Bupivacaine, chlorhydrate de	50 mg	173067
Caféine	100 mg	172054
Carbénicilline monosodique	100 mg	181102
Chloramphénicol	200 mg	383043
Chloramphénicol, palmitate de	200 mg	486004
Chloramphénicol, palmitate de (forme A)	1 g	286072
Chloro-5 méthylamino-2 benzophénone	200 mg	175073
(Chloro-4 sulfamoyl-3 benzoyl)-2 benzoïque, acide	100 mg	172061
Chlorphénamine, hydrogénomaléate de	50 mg	181106
Chlorpromazine, chlorhydrate de	100 mg	182109
Chlortalidone	100 mg	178080
Chlortétracycline, chlorhydrate de	100 mg	183114
Clomifène, citrate de	200 mg	187138
Clomifène, citrate de (isomère Z) (Zuclomifène)	100 mg	187138
Cloxacilline sodique	50 mg	187137
Cortisone, acétate de	200 mg	274005
Dapsone	100 mg	167006
Désoxycortone, acétate de	100 mg	183115
Dexaméthasone	100 mg	187007
Dexaméthasone, acétate de	100 mg	388008
Diazépan	100 mg	288009
Diazoxide	100 mg	172062
Dicloxacilline sodique	100 mg	181103
Dicolinium, iodure de	200 mg	174071
Dicoumarol	100 mg	172055
Diéthylcarbamazine, dihydrogénocitrate de	100 mg	178077
Digitoxine	100 mg	181100
Digoxine	100 mg	277010
Emétine, chlorhydrate d'	100 mg	587011
Epi-4 anhydrotétracycline, chlorhydrate d'	100 mg	187134
Epi-4 tétracycline, sel d'ammonium de 1'	25 mg	288097
Ergométrine, hydrogénomaléate d'	25 mg	180098
Ergotamine, tartrate d'	50 mg	277012
Estradiol, benzoate d'	50 mg	385013
Estrone	100 mg	167014
Etecrinique, acide	100 mg	279015
Ethambutol, chlorhydrate d'	100 mg	281056
Ethinylestradiol	100 mg	178081
Ethistérone	100 mg	187016
Ethosuximide	100 mg	167017
Etocarlide	100 mg	179088
Flucytosine	100 mg	172057
Fluorouracil	100 mg	184121
Fluphénazine, chlorhydrate de	100 mg	184122
Fluphénazine, décanoate de (dichlorhydrate)	100 mg	176076
Fluphénazine, énantiote de (dichlorhydrate)	100 mg	182107
Folique, acide	100 mg	182108
Furozémide	100 mg	388019
Griséofulvine	100 mg	171044
Halopéridol	200 mg	280040
Hydrochlorothiazide	100 mg	172063
	100 mg	179087

Substance de référence	Conditionnement	Numéro de contrôle
Hydrocortisone	100 mg	283020
Hydrocortisone, acétate d'	100 mg	280021
(-)-(Hydroxy-4 méthoxy-3 phényl)-3 méthyl-2 alanine	25 mg	179085
Ibuprofène	100 mg	183117
Imipramine, chlorhydrate d'	100 mg	172064
Indométacine	100 mg	178078
o-Iodochippurique, acide	100 mg	171045
Isoniazide	100 mg	185124
Lanatoside C	100 mg	281022
Lévodopa	100 mg	172058
Lidocaïne	100 mg	181104
Lidocaïne, chlorhydrate de	100 mg	181105
Méfénamique, acide	100 mg	173068
Métazide	100 mg	172058
Méthaquealone	100 mg	173069
Méthildopa	100 mg	179084
Méthyltestostérone	100 mg	167023
Méthicilline sodique	200 mg	274024
Métronidazole	100 mg	183118
Nafcilline sodique	200 mg	272025
Néostigmine, méthilsulfate de	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 de	100 mg	185123
Quabaine	100 mg	283026
Oxacilline sodique	200 mg	382027
Papavérine, chlorhydrate de	100 mg	185127
Phénacéticilline potassique	200 mg	167028
Phénoxyéthylpénicilline	200 mg	179082
Phénoxyéthylpénicilline calcique	200 mg	179083
Phénoxyéthylpénicilline potassique	200 mg	176075
Phénytolne	100 mg	179088
Frednisolone	100 mg	283029
Frednisolone, acétate de	100 mg	167030
Frednisone	100 mg	167031
Frednisone, acétate de	100 mg	169032
Procaine, chlorhydrate de	100 mg	183119
Procarbazine, chlorhydrate de	100 mg	184120
Progestérone	100 mg	167033
Propicilline potassique	200 mg	274034
Propranolol, chlorhydrate de	100 mg	187139
Propylthiouracile	100 mg	185126
Pyridostigmine, bromure de	100 mg	182110
Résérpine	100 mg	186133
Riboflavine	250 mg	382035
Sodium, cromoglicat de	100 mg	188140
Substances de référence pour le point de fusion (série de 13 substances dont la température de fusion va de +69°C à +263°C)	13 x 4 g	
Sulfaméthoxazole	100 mg	179092
Sulfaméthoxyypyridazine	100 mg	178079
Sulfanilamide	100 mg	179094
Testostérone, propionate de	100 mg	167036
Tétracycline, chlorhydrate de	200 mg	180093
Thioacétazone	100 mg	171046
Thiodianiline-4,4'	50 mg	183116
Tolbutamide	100 mg	179086
Tolnaftate	100 mg	176074
Triméthadione	200 mg	185125
Triméthoprime	100 mg	179093
Triméthylguanidine, sulfate de	100 mg	172059
Tubocurarine, chlorure de	100 mg	170037
Vitamine A, acétate de (solution) (Rétinol)	5 capsules	886038
Warfarine	100 mg	168041

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

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 1988-1989 sont résumés ci-dessous. A titre comparatif, on a aussi indiqué les résultats obtenus lors des réexamens précédents. Les substances ont été conservées à +5°C. 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 micro %, 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.

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
KF, %	13,9	-	13,9	13,5	13,3	-
TGA, %	-	-	-	-	-	13,9
HPLC, %	-	0,3	0,6	0,3	0,9	0,3
Titration, % (mercurimétrie)	-	-	-	-	98,6	-
Produits de dégradation, % (par mercurimétrie)	-	-	-	-	0,9	-
Titration, % (pénicillinase)	98,5	-	99,0	-	-	-
PSA, %	1,0	-	-	-	-	-
pH, solution à 0,25 %	5,1	-	5,1	5,1	5,1	-

Cloxacilline sodique, N° de contrôle 274005

Premier rapport d'analyse : WHO/PHARM/75.485, Appendice 7

Année d'examen :	1974	1978	1979	1982	1984	1989
KF, %	4,2	-	4,0	3,8	4,0	-
TGA, %	-	-	-	-	-	4,2
HPLC, %	-	0,5	-	0,9	1,0	0,6
Titrage, % (mercurimétrique)	-	-	-	-	98,9	-
Produits de dégradation, % (par mercurimétrie)	-	-	-	-	0,7	-
Titrage, % (alcalimétrique)	100,2	-	99,1	100,2	-	-
pH, solution à 2 %	-	-	-	5,9	5,9	-
pH, solution à 10 %	-	-	-	6,2	-	-

Dicloxacilline sodique, N° de contrôle 174071

Premier rapport d'analyse : WHO/PHARM/75.485, Appendice 5

Année d'examen :	1974	1982	1984	1989
KF, %	3,8	3,9	3,8	-
TGA, %	-	-	-	3,9
HPLC, %	-	0,3	0,4	0,3
Titration, % (mercurimétrique)	-	-	99,5	-
Produits de dégradation, % (par mercurimétrie)	-	-	0,6	-
Titration, % (alcalimétrique)	99,5	99,4	-	-
pH, solution à 1 %	5,6	5,9	5,8	-

SUBSTANCES CHIMIQUES INTERNATIONALES DE REFERENCE

LISTE PREVISIONNELLE POUR 1989

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 2

Colécalciférol (*)

Volume 3

Amodiaquine, chlorhydrate d'	Lopéramide, chlorhydrate de
Amphotéricine B (*)	Méthotrexate
Bacitracine zinc (*)	Néamine (*) (impureté du sulfate de néomycine)
Béclométhasone, dipropionate de	Néomycine B, sulfate de
Bétaméthasone, valérate de (*)	(impureté du sulfate de néomycine)
Calcium, folinate de	Nifurtimox
Carbamazépine (*)	Noroxymorphone, chlorhydrate de
Cimétidine (*)	(impureté du chlorhydrate de naloxone)
Dexaméthasone sodique, phosphate de	Nystatine
Dopamine, chlorhydrate de	Oxytétracycline, dihydrate d' (*)
Doxorubicine, chlorhydrate de	Oxytétracycline, chlorhydrate d' (*)
Ergocalciférol (*)	Paromomycine, sulfate de
Fludrocortisone, acétate de	Praziquantel
Formyl-3 rifamycine SV (*)	Prednisolone sodique, phosphate de
(impureté de la rifampicine)	Probenécide (*)
Gentamicine, sulfate de	Pyrantel, embonate de (*)
Hydrocortisone sodique, succinate d'	Rifampicine-quinone (*)
(-)-(Hydroxy-4 méthoxy-3 phényl)-3	(impureté de la rifampicine)
hydrazino-2 méthyl-2 alanine	Spectinomycine, chlorhydrate de
(impureté de la Carbidopa)	Sulfacétamide
Lévonorgestrel	Sulfasalazine
Lévothyroxine sodique (*)	Testostérone, énantate de
Liothyronine (*)	Vincristine, sulfate de
(impureté de la lévothyroxine sodique)	

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

Remplacements

Les substances chimiques internationales ci-dessous devront être remplacées par de nouveaux lots en 1989-1990 :

p-Acétamidobenzalazine (*)
Ampicilline (*)
Anhydrotétracycline, chlorhydrate d' (*)
Bupivacaïne, chlorhydrate de (*)
Prednisolone (*)
Prednisolone, acétate de (*)

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

APPENDIX 6

AMPICILLIN SODIUM

Control No 388002

Analytical Report

INTENDED USE

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

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

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

MATERIAL

About 200 g of the sample (manufacturers batch No AS 49) were received at the WHO Centre in October 1988. The material is being stored protected from light and moisture in tightly closed containers at +5° C.

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (388002). The spectrum is concordant with the spectrum obtained from the ICRS, Control No 274002.

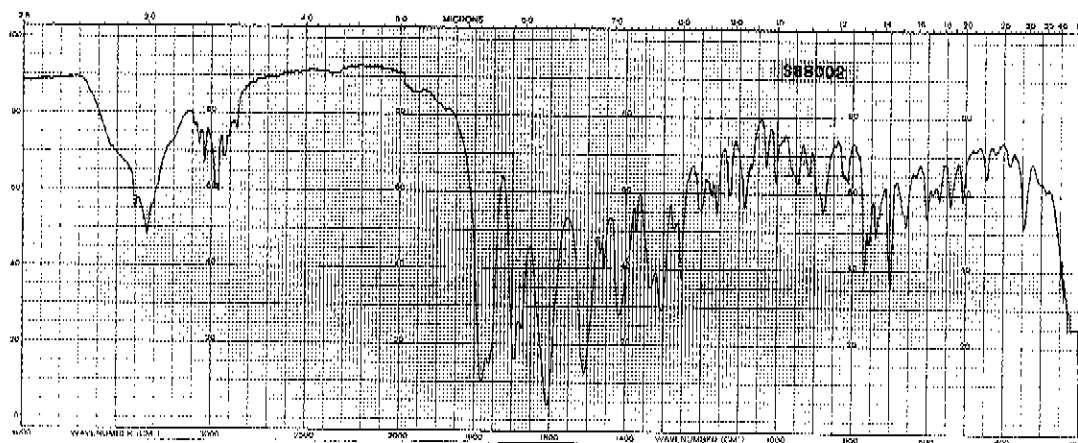


Figure 1. IR-spectrum of 1.2 mg of ampicillin sodium, Control No 388002 in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 580.

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

UV-spectrum

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

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

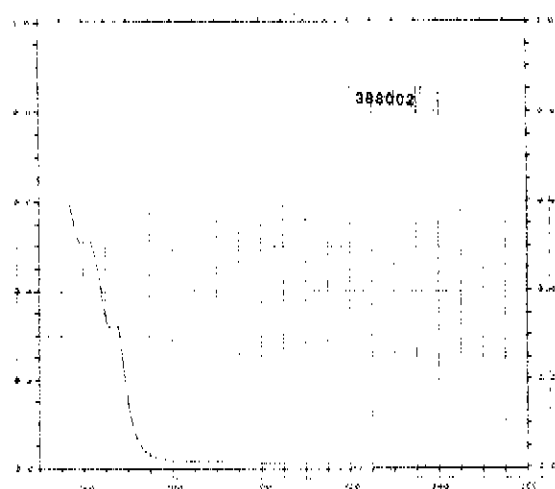


Figure 2. UV-spectrum of ampicillin sodium,

ASSAY

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

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

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

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

Water: 0.5% determined by Karl Fischer titration.

PURITY

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 1.5%. A chromatogram is shown in Figure 3.

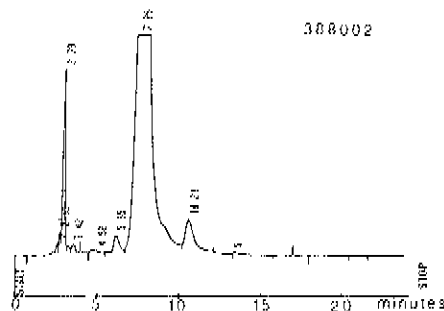


Figure 3. Chromatogram of ampicillin sodium, Control No 388002.

The following conditions were used:

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

A comparison was made with ICRS, Control No 274002 which contained about 3.5% impurities and EPCRS Lot 1 which contained about 0.4% impurities.

Diode-array detection

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

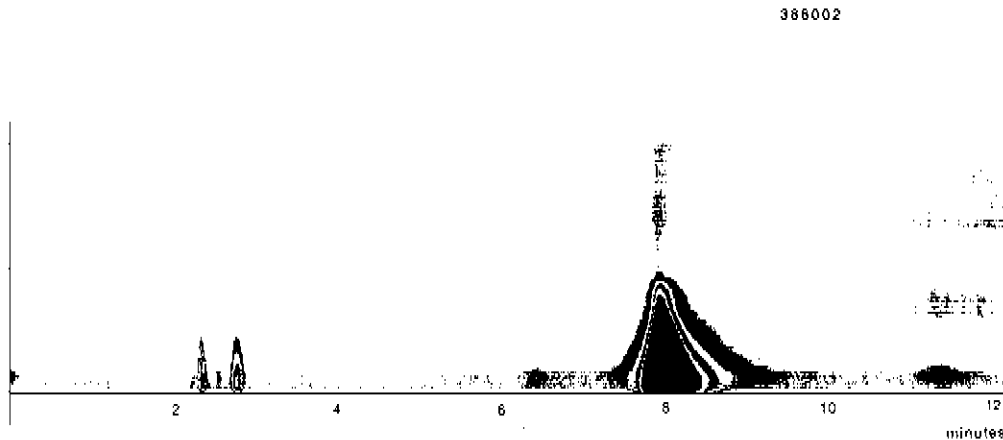


Figure 4. Isogram of ampicillin sodium, Control No 388002.

Sensitivity: 0.02

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

DATA GIVEN BY THE MANUFACTURER

Identification: Passes test

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

STABILITY

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

CONCLUSION

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

DEXAMETHASONE

Control No 388008

Analytical Report

INTENDED USE

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

The monograph for dexamethasone in the International Pharmacopeia 3rd Ed. Vol 2 requires a reference substance to be used in the infrared spectrophotometric and thin-layer chromatographic tests for identity and in the spectrophotometric assay (blue tetrazolium).

MATERIAL

About 50 g of the sample (manufacturers batch No L-574, 385-000F053) were received at the WHO Centre in September 1987. The material is being stored protected from light and moisture in tightly closed containers at +5° C.

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (388008). The spectrum is concordant with the spectrum obtained from the ICRS, Control No 279008.

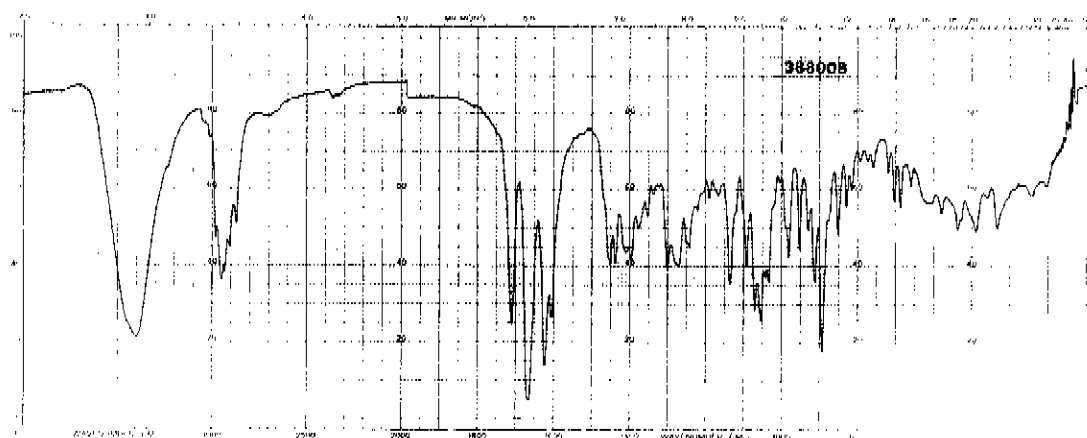


Figure 1. IR-spectrum of 1.3 mg of dexamethasone, Control No 388008 in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 580.

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

UV-spectrum

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

λ max in methanol = 239 nm

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

The result is calculated with reference to the dried substance.

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

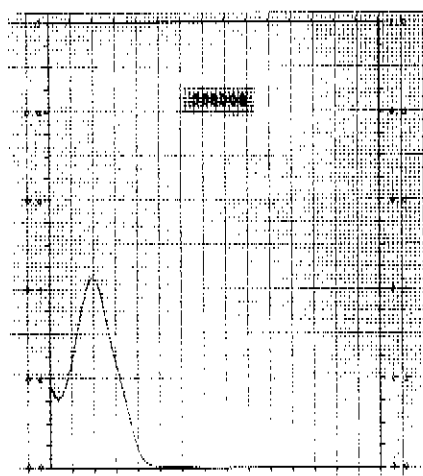


Figure 2. UV-spectrum of dexamethasone, Control No 388008 10.6 $\mu\text{g/ml}$ in methanol.

ASSAY

Spectrophotometric assay: 99.9% (n = 4), s rel % = 0.8 determined according to Ph. Int. 3rd Ed. Vol 2. The ICRS, Control No 279008 was used as reference and regarded as 100%. The result is calculated with reference to the dried substance.

Thermogravimetric analysis: When the substance was heated to 160° C a loss of weight of 0.15% was observed.

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

Loss on drying

0.16% (100° C, vacuo) (n = 3)

PURITY

Total solid impurities

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

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

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

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

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

Thin-layer chromatography

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

The following thin-layer chromatographic system was used:

System 1: According to International Pharmacopeia 3rd Ed, Vol 2.
Thin-layer: Silica gel 60, F-254 (Merck)
Eluent: Dichloromethane:Ether:Methanol:Water (77:15:8:1.2)
Sample: 100 µg of dexamethasone were applied. The plate was developed twice.
Visualization: UV-light of 254 nm, evaluation by densitometry at 240 nm and spraying with blue tetrazolium/ethanol TS followed by heating to 105° C and examination in day-light, and evaluation by densitometry at 525 nm.

A chromatogram is shown in Figure 3.

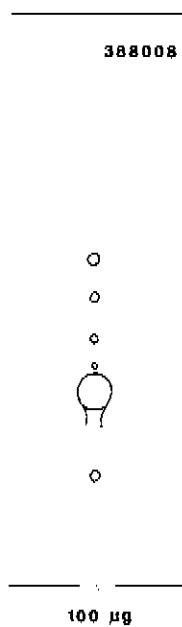


Figure 3. Thin-layer chromatogram of dexamethasone, Control No 388008. Visualization by spraying with blue tetrazolium.

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

R_f (dexamethasone) = 0.34

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

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

High performance liquid chromatography

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

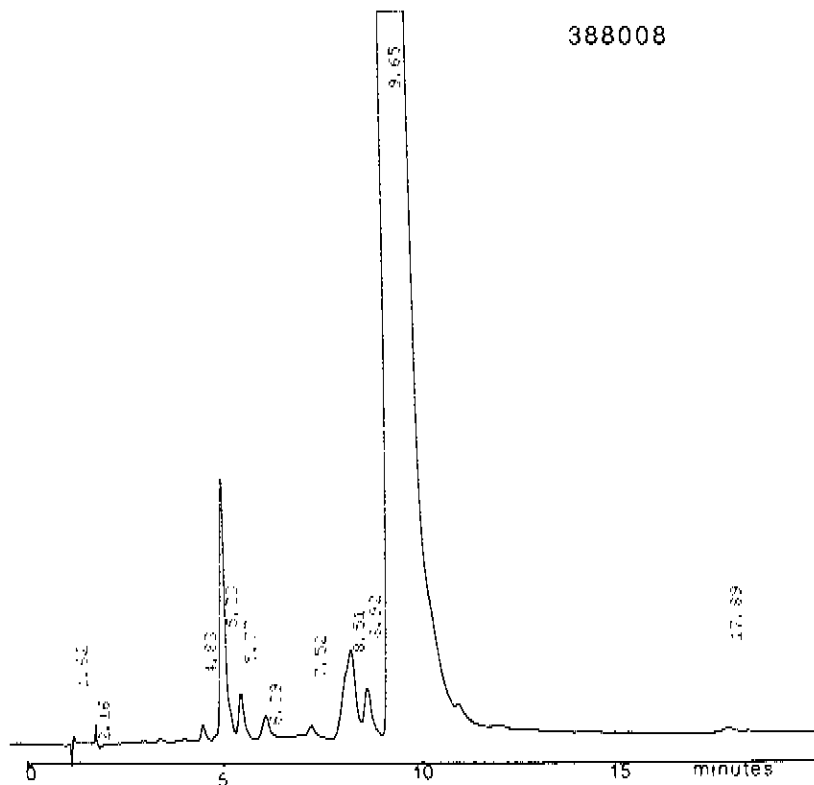


Figure 4. Chromatogram of dexamethasone, Control No 388008.

The following conditions were used:

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

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

Detector: Varian UV 200 operated at 240 nm

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

Integrator: Varian 4270

Attenuation: 1

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

A comparison was made with ICRS, Control No 279008 which also contained 1% impurities, and with the USP reference substance Lot G which contained about 0.8% impurities.

DATA GIVEN BY THE MANUFACTURER

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

STABILITY

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

CONCLUSION

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

APPENDIX 8

DEXAMETHASONE ACETATE

(MONOHYDRATE)

Control No 288009

Analytical Report

INTENDED USE

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

The monograph for dexamethasone acetate in the International Pharmacopeia 3rd Ed. Vol 2 requires a reference substance to be used in the infrared spectrophotometric and thin-layer chromatographic tests for identity and in the spectrophotometric assay (blue tetrazolium).

MATERIAL

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

ANALYTICAL DATA

Description: A white, odourless powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

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

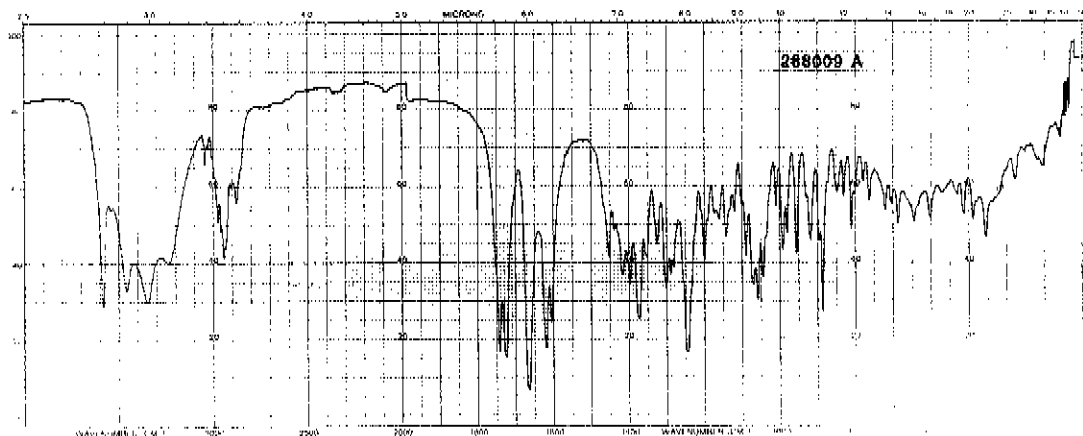


Figure 1. IR-spectrum of 1.2 mg of dexamethasone acetate (monohydrate) 288009A in 300 mg KBr recorded against a KBr disc. Instrument: Perkin-Elmer 580.

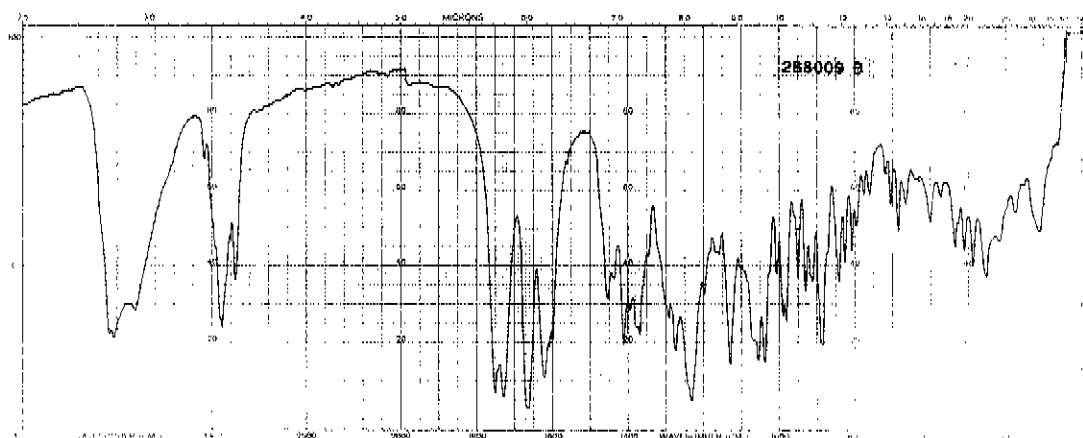


Figure 2. IR-spectrum of 1.1 mg of dexamethasone acetate (monohydrate) recrystallized in 99% ethanol, 288009B in 300 mg KBr recorded against a KBr disc. Instrument: Perkin-Elmer 580.

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

UV-spectrum

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

λ max in ethanol = 238 nm

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

The result is calculated with reference to the dried substance.

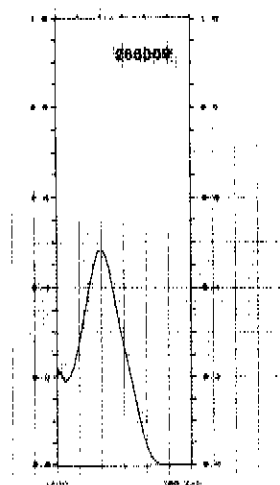


Figure 3. UV-spectrum of dexamethasone acetate, Control No 288009, 14 µg/ml in ethanol.

ASSAY

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

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

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

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

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

PURITY

Thin layer chromatography

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

The following thin-layer chromatographic system was used:

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

The following conditions were used:

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

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

DATA GIVEN BY THE MANUFACTURER

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

STABILITY

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

CONCLUSION

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

APPENDIX 9

4-EPIANHYPOTETRACYCLINE HYDROCHLORIDE

Control No 288097

Analytical Report

INTENDED USE

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

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

MATERIAL

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

ANALYTICAL DATA

Description: Orange-yellow powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

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

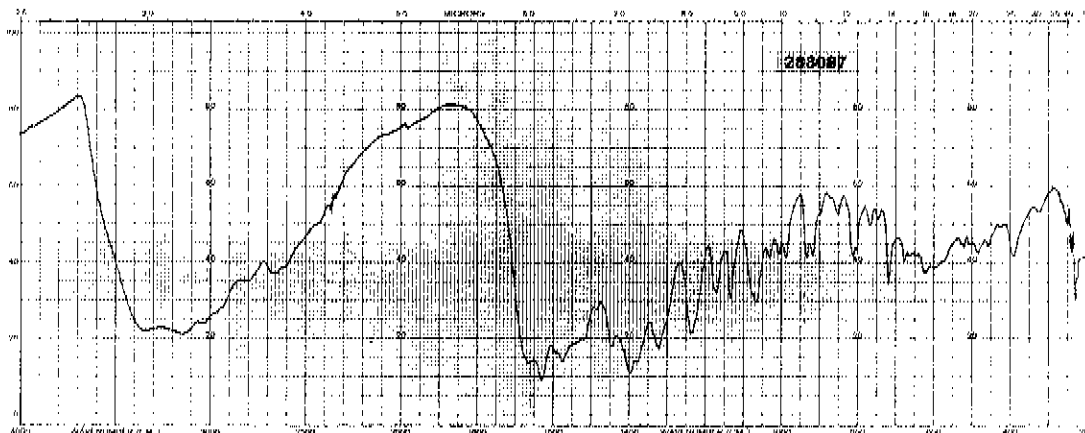


Figure 1. IR-spectrum of 0.9 mg of 4-epianhydrotetracycline hydrochloride, Control No 288097 in 300 mg KBr recorded against a KBr disc. Instrument: Perkin-Elmer 580.

Ultraviolet - visible spectrum

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

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

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

The result is calculated with reference to the anhydrous substance.

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

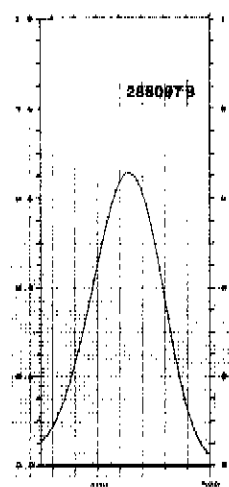
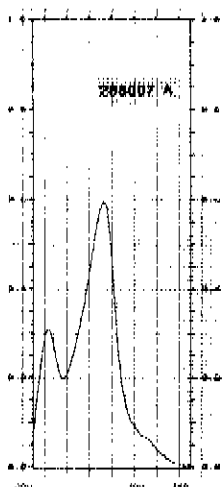


Figure 2 A and B. UV-spectrum (A), visible spectrum (B) of 4-epianhydrotetracycline hydrochloride, Control No 288097, in 0.01 M hydrochloric acid.

ASSAY

Assay: See collaborating laboratories and manufacturer.

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

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

Water: 5.4% determined by Karl Fischer titration.

PURITY

Thin-layer chromatography

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

The following thin-layer chromatographic system was used:

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

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

High performance liquid chromatography

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

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

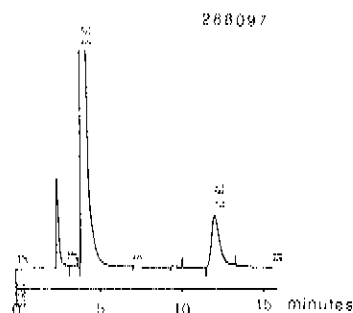


Figure 3. Chromatogram of 4-epianhydrotetracycline hydrochloride, Control No 288097.

The following conditions were used:

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

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

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

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

Integrator: Hewlett Packard 3390 A. Attenuation: 5.

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

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

System 2 developed at the WHO Centre:

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

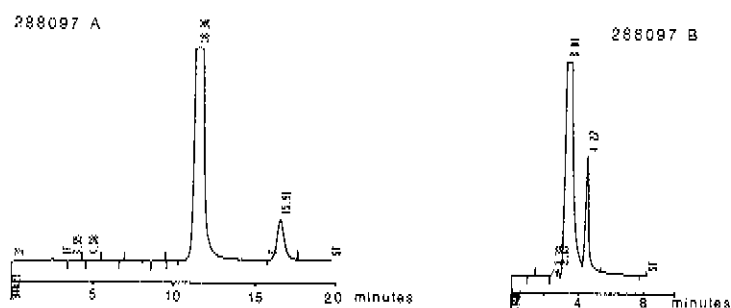


Figure 4 A and B. Chromatograms of 4-epianhydrotetracycline hydrochloride, Control No 288097.

The following conditions were used:

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

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

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

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

Integrator: Hewlett Packard 3390 A.

Attenuation: 6

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

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

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

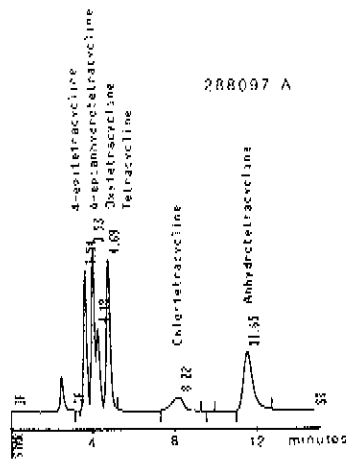


Figure 5 A. Chromatogram of 4-epitetracycline, 4-epianhydrotetracycline, oxytetracycline, tetracycline, chlortetracycline and anhydrotetracycline (pH 7.6).

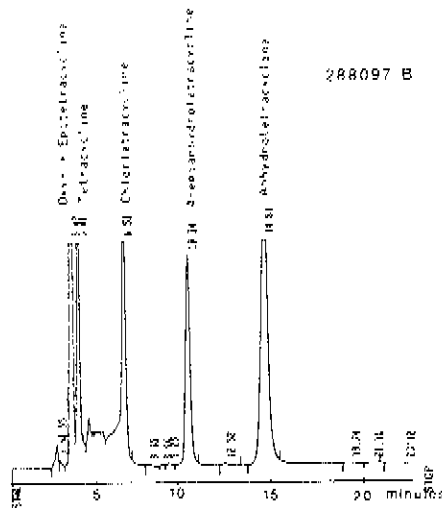


Figure 5 B. Chromatogram of 4-epitetracycline together with oxytetracycline, tetracycline, chlortetracycline, 4-epianhydrotetracycline and anhydrotetracycline. pH of phosphate buffer = 2.4.

DATA GIVEN BY COLLABORATING LABORATORIES

USP

Water: 5.0% Karl Fischer titration

Assay: 99.6% potentiometric titration

HPLC: 98.6% 1.5% anhydrotetracycline

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

DATA GIVEN BY THE MANUFACTURER

HPLC + Titration Potency 99.9%.

STABILITY

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

CONCLUSION

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

FOLIC ACID

Control No 388019

Analytical Report

INTENDED USE

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

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

MATERIAL

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

ANALYTICAL DATA

Description: A yellowish - orange, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

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

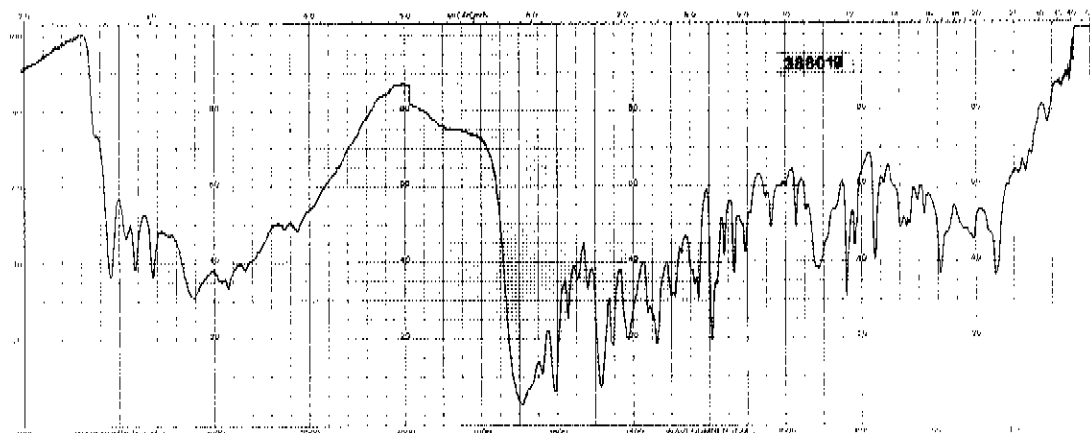


Figure 1. IR-spectrum of 0.9 mg of folic acid, Control No 388019, in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin-Elmer 580.

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

UV-spectrum

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

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

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

The results are calculated with reference to the anhydrous substance.

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

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

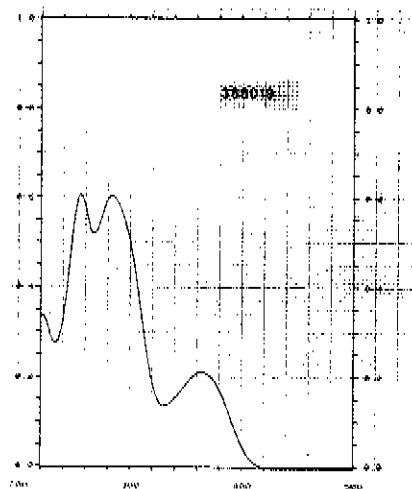


Figure 2. UV-spectrum of folic acid, Control No 388019, 10.6 $\mu\text{g/ml}$ in 0.1 M sodium hydroxide.

ASSAY

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

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

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

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

PURITY

Thin layer chromatography

The following thin-layer chromatographic systems were used:

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

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

Eluent: n-propanol: ethanol 95%: ammonia (269 g/l) (2+1+2)

Sample: 25 µg of folic acid were applied. The sample was dissolved in a mixture of 1 volume of ammonia (260 g/l) and 9 volumes of methanol.

Visualization: UV-light of 365 nm.

Result: Only one impurity with $R_f=0.6$ was found. R_f (folic acid)=0.55.

System 2: According to Ph. Eur. 2nd Ed.

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

Eluent: n-propanol: ethanol 95%: ammonia (260 g/l) (20+60+20)

Sample: 10 and 25 µg of folic acid were applied. The sample was dissolved as described under system 1.

Visualization: UV-light of 365 nm.

Result: Two faint secondary spots with $R_f=0.09$ and 0.45 were detected.
 R_f (folic acid) = 0.18.

High performance liquid chromatography

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

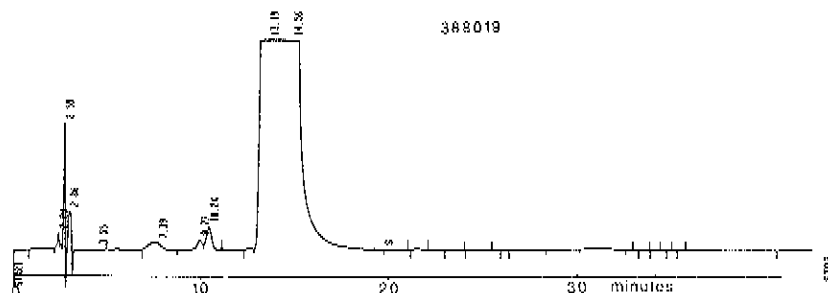


Figure 3. Chromatogram of folic acid, Control No 388019.

The following conditions were used:

- Eluent: Dissolve 35.1 g of sodium perchlorate monohydrate, 1.4 g of monobasic potassium phosphate and 7.0 ml of 1 M sodium hydroxide in 900 ml of water. Add 70 ml of methanol adjust pH to 7.2 with 1 M sodium hydroxide and dilute with water to 1000 ml.
- Column: RP-18, Spheri-5 (Brownlee)
- Detector: Shimadzu SPD-2A operated at 254 nm.
- Pump: Waters 600 Multisolvent Delivery System operated at a flow rate of 1 ml/min.
- Integrator: Hewlett Packard 3390 A
- Attenuation: 4
- Sample: 1 mg/ml dissolved in an aqueous solution containing 2 ml ammonia and 1 g sodium perchlorate per 100 ml. 20 μ l corresponding to 20 μ g were injected.

DATA GIVEN BY THE MANUFACTURER

Yellowish orange crystalline powder
Free pteridines 0.01%
Free amine 0.09%
Water 7.7%
Assay (HPLC-USP) 100.0%
Residue on ignition 0.02%

STABILITY

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

CONCLUSION

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

APPENDIX 11

SODIUM CROMOGLICATE

Control No 188140

Analytical Report

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

INTENDED USE

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

MATERIAL

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

ANALYTICAL DATA

Description: A fine white powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

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

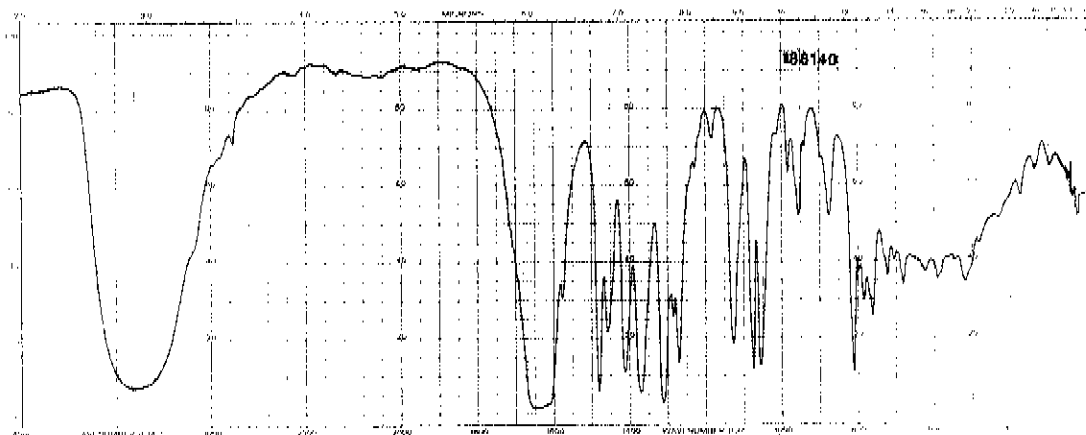


Figure 1. IR-spectrum of 1.2 mg of sodium cromoglicate, Control No 188140, in 300 mg KBr disc. Instrument: Perkin-Elmer 580.

UV-spectrum

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

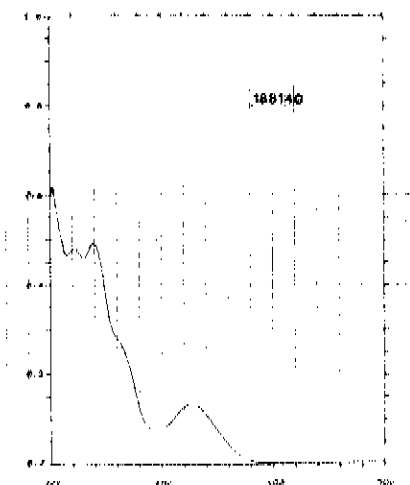


Figure 2. UV-spectrum of sodium cromoglicate Control No 188140. 9 µg/ml in phosphate buffer pH 7.4.

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

Table 1

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

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

ASSAY

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

The result is calculated with reference to the anhydrous substance.

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

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

Loss on drying:

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

Water: 7.2% determined by Karl Fischer titration.

PURITY

Total solid impurities

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

Thin layer chromatography

No secondary spots were observed.

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

The following thin-layer chromatographic system was used:

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

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

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

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

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

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

Rf (sodium cromoglicate) = 0.26

High performance liquid chromatography

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

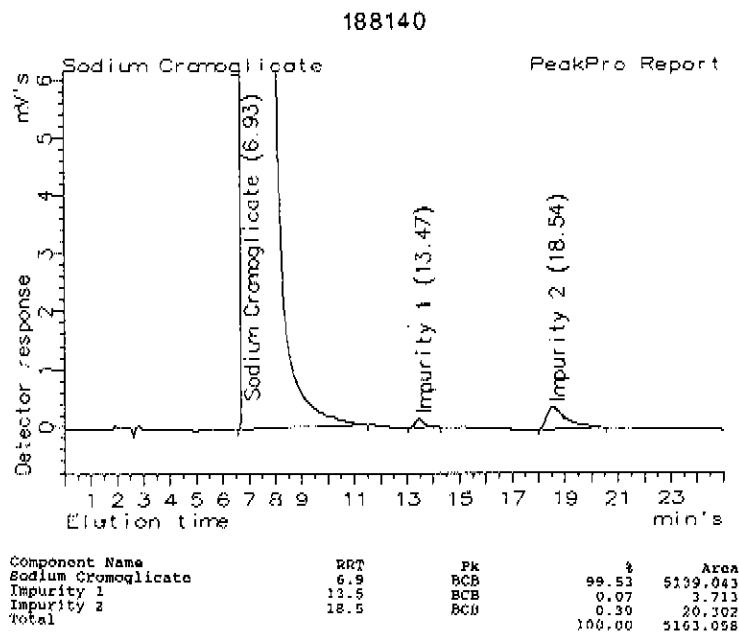


Figure 3. Chromatogram of sodium cromoglicate, Control No 188140.

The following conditions were used:

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

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

Detector: Varian UV 200 operated at 254 nm

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

Integrator: Peak Pro 270 Attenuation:1

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

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

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

Diode-array detection

The chromatogram was also evaluated with a LKB 2140 Rapid Diode Array Detector. The same chromatographic system as described above was used, except for the injection volume that was increased to 20 μ l. An isogram is given in Figure 4.

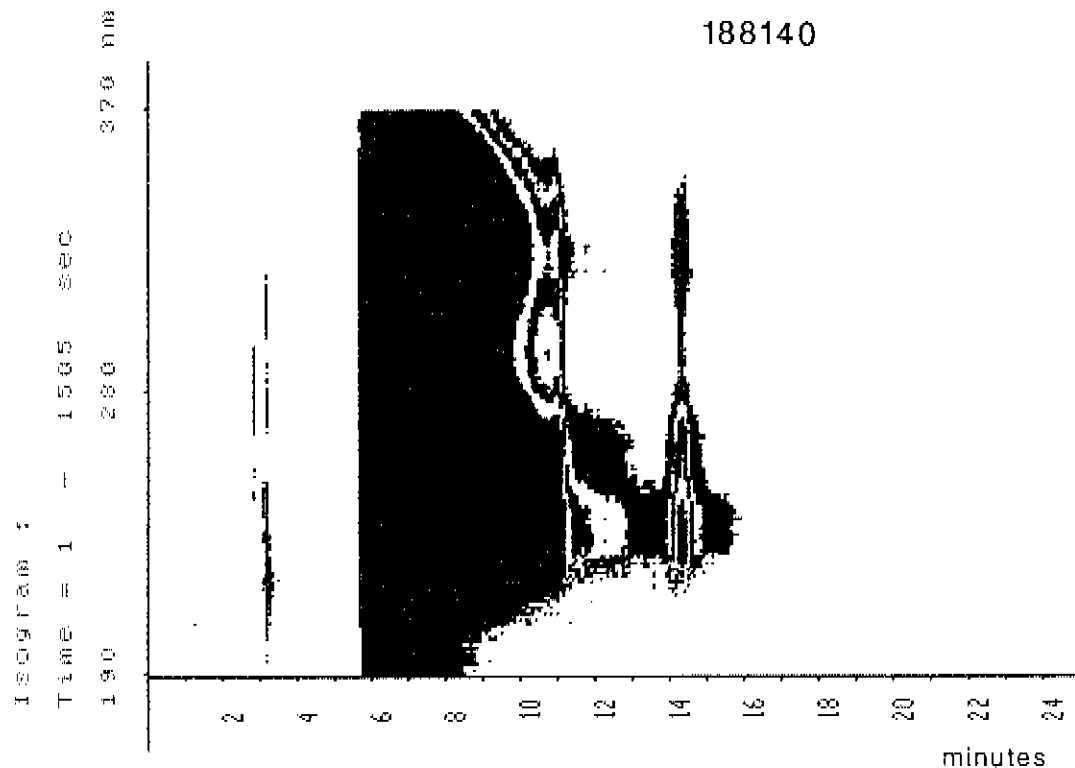


Figure 4. Isogram of sodium cromoglicate, Control No 188140.
Sensitivity: 0.001

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

DATA GIVEN BY COLLABORATING LABORATORIES

NBSL

Infrared spectrum

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

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

Ultra-Violet Spectrum

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

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

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

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

ASSAY

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

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

a mean result of 92.92% was determined.

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

LOSS ON DRYING

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

OXALATE

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

oxalic acid solution:	0.204
sodium cromoglycate:	0.255

PURITY

Related Substances by TLC

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

High Performance Liquid Chromatography

No impurities were found.

The following conditions were used:

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

DATA GIVEN BY THE MANUFACTURER

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

STABILITY

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

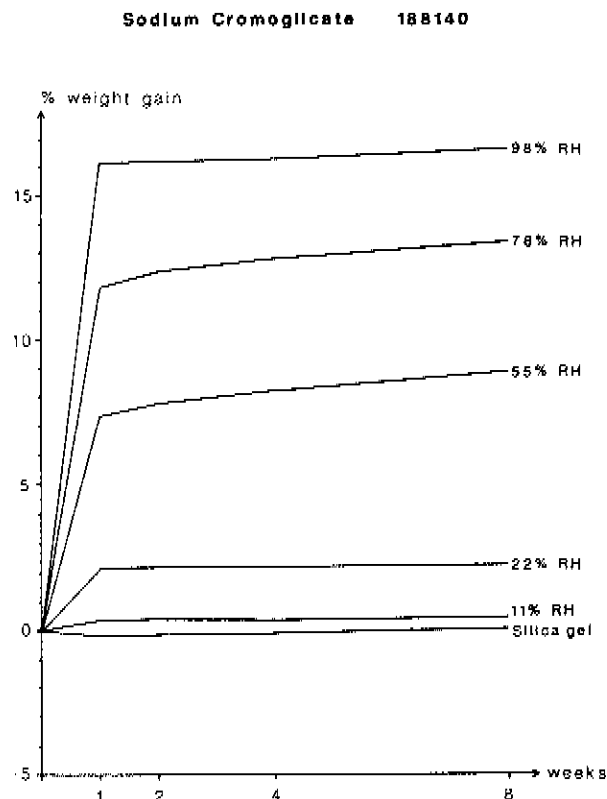


Figure 5. Stability of sodium cromoglicate, Control No 188140 stored at different relative humidity at 20° C.

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

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

CONCLUSION

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