

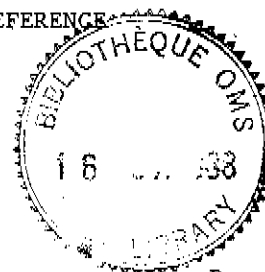


CENTRE COLLABORATEUR OMS POUR LES SUBSTANCES CHIMIQUES DE REFERENCE

Rapport d'activité pour 1987

par M. Westermarck

Table des matières



	<u>Pages</u>
Distribution de substances de référence en 1987	3
Etablissement de substances de référence en 1987	3
Travaux effectués en 1987 sur de nouvelles substances de référence	3
Essais de stabilité	3
Travaux en cours et travaux futurs	4
Questions administratives et financières	4
Remerciements	4
Appendice 1. Distribution de substances chimiques de référence en 1987	5
Appendice 2. Liste des substances chimiques internationales de référence établies en 1987	6
Appendice 3. Liste des substances chimiques internationales de référence disponibles	7
Appendice 4. Essais de stabilité - rapports d'analyse	10
Appendice 5. Substances chimiques internationales de référence - liste prévisionnelle pour 1988	16
Appendice 6. Allopurinol, N° de contrôle 287049	17
Appendice 7. Chlorhydrate de chlortétracycline, N° de contrôle 187138	21

Note : Pour des raisons techniques, les appendices 6 à 13 n'ont été établis qu'en anglais.

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	<u>Pages</u>
Appendice 8. Citrate de clomifène, N° de contrôle 187136	25
Appendice 9. Citrate de clomifène, isomère Z, N° de contrôle 187137	31
Appendice 10. Digoxine, N° de contrôle 587011	37
Appendice 11. Chlorhydrate d'émétine, N° de contrôle 187134	42
Appendice 12. Métilsulfate de néostigmine, N° de contrôle 187135	47
Appendice 13. Chlorhydrate de propranolol, N° de contrôle 187139	52

Distribution de substances de référence en 1987

En 1987, le Centre a distribué à des laboratoires de contrôle pharmaceutique de 40 pays 2013 échantillons de substances chimiques internationales de référence et 28 séries de substances de référence pour la détermination du point de fusion. Ces chiffres représentent une diminution d'environ 11 % par rapport à ceux de 1986. Les cinq substances les plus fréquemment demandées en 1987 ont été, dans l'ordre, l'ampicilline, l'acide folique, la cloxacilline sodique, la propicilline potassique et le trihydrate d'ampicilline. On trouvera à l'appendice 1 le détail de la distribution des diverses substances de référence.

Etablissement de substances de référence en 1987

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 1987 neuf substances chimiques internationales de référence, dont on trouvera la liste à l'appendice 2. Parmi ces substances, le chloramphénicol, le palmitate de chloramphénicol et l'acétate de vitamine A sont des lots de remplacement, les lots précédents ayant été épuisés en 1987.

On trouvera à l'appendice 3 une liste complète de toutes les substances chimiques internationales de référence détenues par le Centre en janvier 1988, 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 au cours du premier semestre de 1988.

Travaux effectués en 1987 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 1987, l'analyse des nouvelles substances de référence suivantes, destinées à accompagner le volume 3 de la Pharmacopée internationale, a été réalisée : chlorhydrate de chlortétracycline, citrate de clomifène, citrate de clomifène (isomère 2), chlorhydrate d'émétine, métilsulfate de néostigmine et chlorhydrate de propranolol. Les rapports d'analyse pour ces substances figurent aux appendices 7, 8, 9, 11, 12 et 13 respectivement. Toutes ces substances ont été jugées satisfaisantes pour l'usage auquel elles sont destinées et il est par conséquent proposé de les adopter comme substances chimiques internationales de référence.

Les deux lots suivants de substances chimiques internationales de référence ont été épuisés et ont été remplacés par de nouveaux lots en 1987. Le lot N° 172049 d'allopurinol a été remplacé par le lot N° 287049 et le lot N° 377011 de digoxine a été remplacé par le lot N° 587011. Les résultats de l'analyse de ces lots figurent aux appendices 6 et 10.

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 1987-1988, le réexamen a porté sur 12 substances.

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.

En 1987, l'analyse thermogravimétrique a été adoptée en remplacement ou en complément de l'essai de perte à la dessiccation.

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 42 nouvelles substances de référence, dont six sont déjà à l'étude. D'anciens lots devront également être remplacés en raison de l'épuisement des stocks. Actuellement, huit substances doivent être remplacées en 1988-1989, 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 1987, 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. En 1987, le Centre a notamment reçu une aide appréciable du Laboratoire national d'Etalons biologiques d'Australie. Le Centre espère que la préparation des nouvelles substances de référence pour le volume 3 de la Pharmacopée internationale sera ainsi facilitée.

Questions administratives et financières

La situation financière du Centre reste mauvaise. Le coût de fonctionnement total du Centre en 1987 a été estimé à US \$307 300. Le revenu provenant des ventes de substances de référence aux laboratoires industriels a été d'environ US \$31 000 et la contribution du Siège de l'OMS de US \$16 000, ce qui laisse un déficit de US \$260 300. 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.

En février 1987, le prix des substances a été porté à 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

Comme les années précédentes, le Centre souhaite exprimer ses plus sincères remerciements au Dr C. A. Johnson, Directeur scientifique et Secrétaire de la Commission de la Pharmacopée britannique et membre du Tableau consultatif OMS d'experts de la Pharmacopée internationale et des Préparations pharmaceutiques, pour l'intérêt indéfectible qu'il a manifesté pour notre travail et pour l'aide extrêmement précieuse qu'il a apportée au Centre en le conseillant sur diverses questions concernant l'établissement de nouvelles substances de référence. Le Centre voudrait 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. Nos remerciements vont en particulier à Andard-Mount Company, Wembley, Angleterre, à Boehringer Ingelheim GmbH, République fédérale d'Allemagne, à Farmitalia Carlo Erba, Milan, Italie, à Hoffman-La Roche, Bâle, Suisse, à Imperial Chemical Industries (ICI), Macclesfield, Angleterre, à Merrel Dow Pharmaceuticals Inc., Cincinnati, Ohio, Etats-Unis d'Amérique, et à la Wellcome Foundation, Dartford, Angleterre.

DISTRIBUTION DE SUBSTANCES CHIMIQUES DE REFERENCE EN 1987

Acéclidine, salicylate d'	1	échantillon(s)	Folique, acide	84	échantillon(s)
p-Acétamidobenzalazine	2	"	Furoséide	21	"
Acétazolamide	-	"	Griséofulvine	23	"
Allopurinol	-	"	Halopéridol	9	"
Amino-2 nitro-5 thiazole	-	"	Hydrochlorothiazide	16	"
Amino-3 pyrazole carboxamide-4, hémisulfate d'	12	"	Hydrocortisone	22	"
Amitryptiline, chlorhydrate d'	11	"	Hydrocortisone, acétate d'	14	"
Ampicilline	101	"	(-)-(Hydroxy-4 méthoxy-3 phényl)-3 méthyl-2 alanine	1	"
Ampicilline sodique	36	"	Ibuprofène	11	"
Ampicilline, trihydrate d'	48	"	Imipramine, chlorhydrate d'	15	"
Anhydrotétracycline, chlorhydrate d'	19	"	Indométacine	10	"
Atropine, sulfate d'	16	"	o-Iodohippurique, acide	5	"
Azathioprine	3	"	Isoniazide	8	"
Benzazoli, chlorhydrate de	5	"	Lanatoside C	14	"
Benzobarbital	5	"	Lévodopa	12	"
Benzylamine, sulfate de	2	"	Lidocaïne	14	"
Benzylpénicilline potassique	19	"	Lidocaïne, chlorhydrate de	20	"
Benzylpénicilline sodique	28	"	Méfénamique, acide	15	"
Béphénium, hydroxynaphtoate de	1	"	Métazide	4	"
Bétaméthasone	14	"	Méthasqualone	9	"
Bétanidine, sulfate de	1	"	Méthylidopa	6	"
NN'-bis(xylol 2,3) anthranilamide	4	"	Méthyltestostérone	19	"
Bupivacaïne, chlorhydrate de	14	"	Méticilline sodique	19	"
Caféine	12	"	Métronidazole	21	"
Carbénicilline monosodique	14	"	Nafcilline sodique	9	"
Chloramphénicol	45	"	Nicotinamide	20	"
Chloramphénicol, palmitate de	4	"	Nicotinique, acide	15	"
Chloramphénicol, palmitate de (forme A)	7	"	Niridazole	-	"
Chloro-5 méthylamino-2 benzophénone	7	"	Niridazole-chloréthylcarboxamide	-	"
(Chloro-4 sulfamoyl-3 benzoyl)-2 benzoïque, acide	1	"	Noréthistérone	2	"
Chlorphénamine, hydrogénomaléate de	8	"	Noréthistérone, acétate de	4	"
Chlorpromazine, chlorhydrate de	14	"	Oxabaïne	22	"
Chlortalidone	7	"	Oxacilline sodique	46	"
Cloxacilline sodique	53	"	Papavérine, chlorhydrate de	11	"
Cortisone, acétate de	17	"	Phénéticilline potassique	9	"
Dapsone	11	"	Phénoxyéthylpénicilline	26	"
Désoxycortone, acétate de	9	"	Phénoxyéthylpénicilline calcique	3	"
Dexaméthasone	20	"	Phénoxyéthylpénicilline potassique	36	"
Dexaméthasone, acétate de	8	"	Phénytoïne	12	"
Diazépam	32	"	Prednisolone	35	"
Diazoxide	2	"	Prednisolone, acétate de	15	"
Dicloxacilline sodique	46	"	Prednisone	18	"
Dicolinium, iodure de	1	"	Prednisone, acétate de	11	"
Dicoumarol	7	"	Procaine, chlorhydrate de	20	"
Diéthylcarbamazine, dihydro- généocitrate de	7	"	Procabazine, chlorhydrate de	1	"
Digitoxine	26	"	Progestérone	18	"
Digoxine	-	"	Propicilline potassique	49	"
Epi-4 anhydrotétracycline, chlorhydrate d'	40	"	Propylthiouracil	6	"
Epi-4 tétracycline, sel d'ammonium de l'	21	"	Pyridostigmine, bromure de	4	"
Ergométrine, hydrogénomaléate d'	12	"	Réserpine	2	"
Ergotamine, tartrate d'	32	"	Riboflavine	38	"
Estradiol, benzoate d'	9	"	Sulfaméthoxazole	19	"
Estrone	11	"	Sulfaméthoxyypyridazine	8	"
Etacrynique, acide	5	"	Sulfanilamide	12	"
Ethambutol, chlorhydrate d'	12	"	Testostérone, propionate de	24	"
Ethinylestradiol	23	"	Tétracycline, chlorhydrate de	34	"
Ethistérone	13	"	Thioacétazone	6	"
Ethosuximide	3	"	Thiodianiline-4,4'	1	"
Etocarlide	4	"	Tolbutamide	4	"
Flucytosine	3	"	Tolnaftate	10	"
Fluorouracil	9	"	Triméthadione	7	"
Fluphénazine, chlorhydrate de	23	"	Triméthoprime	28	"
Fluphénazine, décanate de (dichlorhydrate)	19	"	Triméthylguanidine, sulfate de	4	"
Fluphénazine, énantate de (dichlorhydrate)	7	"	Tubocurarine, chlorure de	10	"
			Vitamine A, acétate de (solution à 25 000 UI)	27	"
			Warfarine	11	"
					2 013 échantillons

Substances de référence pour le point de fusion : 28 séries de 13 substances (environ 4 g de chaque).

LISTE DES SUBSTANCES CHIMIQUES INTERNATIONALES DE REFERENCE ETABLIES EN 1987

Substance de référence	N° de contrôle	Rapport d'analyse	Remarques
Acétazolamide	186128	WHO/PHARM/87.532 Appendice 6	
Amino-2 nitro-5 thiazole	186131	WHO/PHARM/87.532 Appendice 7	
Chloramphénicol	486004	WHO/PHARM/87.532 Appendice 8	Remplace le N° 379004
Chloramphénicol, palmitate de	286072	WHO/PHARM/87.532 Appendice 9	Remplace le N° 175072
Niridazole	186129	WHO/PHARM/87.532 Appendice 10	
Niridazole-chloréthyl-carboxamide	186130	WHO/PHARM/87.532 Appendice 11	
Noréthistérone	186132	WHO/PHARM/87.532 Appendice 12	
Résérpine	186133	WHO/PHARM/87.532 Appendice 13	
Vitamine A, acétate de	686038	WHO/PHARM/87.532 Appendice 14	Remplace le N° 581038

LISTE DES SUBSTANCES CHIMIQUES INTERNATIONALES DE REFERENCE DISPONIBLES

1988

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 proposées 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)

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

Substances de référence	Conditionnement	Numéro de contrôle du lot actuel
Acéclidine, salicylate d'	100 mg	172048
p-Acétamidobenzalazine	100 mg	171042
Acétazolamide	100 mg	186128
Allopurinol	100 mg	287049
Amino-2 nitro-5 thiazole	25 mg	186131
Amino-3 pyrazole carboxamide-4, hémisulfate d'	100 mg	172050
Amtryptiline, chlorhydrate d'	100 mg	181101
Ampicilline	200 mg	274001
Ampicilline sodique	200 mg	274002
Ampicilline, trihydrate d'	200 mg	274003
Anhydrotétracycline, chlorhydrate d'	25 mg	180098
Atropine, sulfate d'	100 mg	183111
Azathioprine	100 mg	172060
Benzazol, chlorhydrate de	100 mg	173056
Benzobarbital	100 mg	172051
Benzylamine, sulfate de	100 mg	172052
Benzylpénicilline potassique	200 mg	180099
Benzylpénicilline sodique	200 mg	280047
Béphénium, hydroxynaphtoate de	100 mg	183112
Bétaméthasone	100 mg	183113
Bétanidine, sulfate de	100 mg	172053
NN'-bis (xylyl-2,3) anthranilamide	50 mg	173067
Bupivacaine, chlorhydrate de	100 mg	172054
Caféine	100 mg	181102
Carbénicilline monosodique	200 mg	383043
Chloramphénicol	200 mg	488004
Chloramphénicol, palmitate de	1 g	286072
Chloramphénicol, palmitate de (forme A)	200 mg	175073
Chloro-5 méthylamino-2 benzophénone	100 mg	172061
(Chloro-4 sulfamoyl-3 benzoyl)-2 benzoinique, acide	50 mg	181106
Chlorphénamine, hydrogénomaléate de	100 mg	182109
Chlorpromazine, chlorhydrate de	100 mg	178080
Chlortalidone	100 mg	183114
Chlortétracycline, chlorhydrate de	200 mg	187138
Clomifène, citrate de	100 mg	187136
Clomifène, citrate de (isomère Z) (Zuclomifène)	50 mg	187137
Cloxacilline sodique	200 mg	274005
Cortisone, acétate de	100 mg	167006
Dapsone	100 mg	183115
Désoxycortone, acétate de	100 mg	167007
Dexaméthasone	100 mg	278008
Dexaméthasone, acétate de	100 mg	168009
Diazépam	100 mg	172062
Diazoxide	100 mg	181103
Dicloxacilline sodique	200 mg	174071
Dicolinium, iodure de	100 mg	172055
Dicoumarol	100 mg	178077
Diéthylcarbamazine, dihydrogénomaléate de	100 mg	181100
Digitoxine	100 mg	277010
Digoxine	100 mg	587011
Emétine, chlorhydrate d'	100 mg	187134
Epi-4 anhydrotétracycline, chlorhydrate d'	25 mg	180097
Epi-4 tétracycline, sel d'ammonium de 1'	25 mg	180098
Ergométrine, hydrogénomaléate d'	50 mg	277012
Ergotamine, tartrate d'	50 mg	385013
Estradiol, benzoate d'	100 mg	167014
Estrone	100 mg	279015
Etacrynique, acide	100 mg	281058
Ethambutol, chlorhydrate d'	100 mg	178081
Ethinylestradiol	100 mg	167016
Ethistérone	100 mg	167017
Ethoximide	100 mg	178088
Etocarlide	100 mg	172057
Flucytosine	100 mg	184121
Fluorouracil	100 mg	184122
Fluphénazine, chlorhydrate de	100 mg	178076
Fluphénazine, décanoate de (dichlorhydrate)	100 mg	182107
Fluphénazine, énantate de (dichlorhydrate)	100 mg	182108
Folique, acide	100 mg	277019
Furoséide	100 mg	171044
Griséofulvine	200 mg	280040
Halopéridol	100 mg	172063
Hydrochlorothiazide	100 mg	178087

Substances de référence	Conditionnement	Numéro de contrôle du lot actuel
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	178079
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
Méthaqualone	100 mg	173069
Méthyl-dopa	100 mg	179084
Méthyltestostérone	100 mg	167023
Méticilline sodique	200 mg	274024
Métronidazole	100 mg	183118
Nafcilline sodique	200 mg	272025
Néostigmine, méthylsulfate 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
Ouabaïne	100 mg	283026
Oxacilline sodique	200 mg	382027
Papavérine, chlorhydrate de	100 mg	185127
Phénéticilline potassique	200 mg	187028
Phénoxy-méthylpénicilline	200 mg	179082
Phénoxy-méthylpénicilline calcique	200 mg	179083
Phénoxy-méthylpénicilline potassique	200 mg	176075
Phénytoïne	100 mg	179089
Prednisolone	100 mg	283029
Prednisolone, acétate de	100 mg	167030
Pradnison	100 mg	167031
Prednison, acétate de	100 mg	169032
Procaine, chlorhydrate de	100 mg	193119
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
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
Sulfaméthoxypyridazine	100 mg	178079
Sulfanilamide	100 mg	179094
Testostérone, propionate de	100 mg	167036
Tétracycline, chlorhydrate de	200 mg	180095
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

* Par 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 1987-1988 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 aire % sauf indication contraire; lorsqu'elle est obtenue par DSC et par DTA, elle est exprimée en mole %, et par PSA 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.

Dapsone, N° de contrôle 183115

Premier rapport d'analyse : WHO/PHARM/84.513, appendice 10

Année d'examen	1983	1988
HPLC, %	1,2	1,9 (1,1) ¹⁾
IR	conforme	-
LOD, %	0,1	-
TGA, %		<0,1
TLC	2-4 taches secondaires	3-4 taches secondaires ²⁾
Titrage, % (volumétrique)	99,9	100,0

- 1) La substance d'origine a été recristallisée et la valeur 1,1 a été obtenue par HPLC après recristallisation.
- 2) Système A (selon Ph Int, ed. III, Vol. 2) : environ 1,2 %
Système B (selon le premier rapport d'analyse) : environ 1,1 %.

Fluphénazine, chlorhydrate de, N° de contrôle 176076

Premier rapport d'analyse : WHO/PHARM/77.491, annexe 5

Année d'examen	1976	1988
HPLC, %	0,5	0,4
IR	conforme	conforme
LOD, %	0	-
TGA, %	-	0
TLC	2 taches secondaires	A : 3 taches secondaires (une très faible) ¹⁾ B : 4 taches secondaires (une très faible) ²⁾
Absorption UV, 260 nm	0,65	0,65

1) Système A selon le premier rapport d'analyse.

2) Système B selon la monographie de la Ph Int, ed. III, Vol. 2 (Substances apparentées).

Griséofulvine, N° de contrôle 280040

Premier rapport d'analyse : WHO/PHARM/81.508, appendice 10

Année d'examen	1980	1988
HPLC, %	0,6 (236 nm) 0,9 (291 nm)	0,6 (236 nm) 0,9 (291 nm)
IR	conforme	conforme
LOD, %	0,1	-
TGA, %	-	0
TLC	1 tache secondaire	1 tache secondaire
Absorption UV, 291 nm	0,68	0,68
Titration, % (spectrophotométrique)	100,0	99,9

Halopéridol, N° de contrôle 172063

Premier rapport d'analyse : WHO/PHARM/73.475, appendice 6

Année d'examen	1972	1977	1979	1988
DSC, mole %	0,36 ^{*)}	-	-	0,1
DTA, mole %				0,1
IR	conforme	-	-	conforme
LOD, %	0,3	0	0	-
TGA, %				0
TLC, système A	3 taches secondaires	1 tache secondaire	3 taches secondaires	2-3 taches secondaires très faibles ¹⁾
système B				Pas de taches secondaires ²⁾
Absorption UV, 245 nm	0,53	0,54	0,52	0,53
Titration, % (potentiométrique)	99,6	99,9	99,8	-

*) Evaluation manuelle.

1) Système A : selon le premier rapport d'analyse.

2) Système B : selon la monographie de la Ph Int, ed. III, Vol. 2 (une seule tache secondaire après application d'une solution datant de 24 heures).

Imipramine, chlorhydrate d', N° de contrôle 172064

Premier rapport d'analyse : WHO/PHARM/73.475, appendice 7

Année d'examen	1972	1977	1983	1988
DSC, mole %	0,22 ^{*)}	-	-	0,19
DTA, mole %			0,56	0,53
IR	conforme	conforme	-	conforme
LOD, %	0	0,05	0	0,08
TLC	4 taches secondaires	3 taches secondaires	3 taches secondaires	3 taches secondaires
Titration, % (potentiométrique)	99,9	99,1	99,1	99,2

*) Evaluation manuelle.

Lidocaïne, N° de contrôle 181104

Premier rapport d'analyse : WHO/PHARM/82.509, appendice 13

Année d'examen	1981	1988
DTA, mole %	0,3	0,4
HPLC, %	<0,1	-
IR	conforme	conforme
LOD, %	0	0,1
TLC	pas de taches secondaires	pas de taches secondaires
Titrage, % (potentiométrique)	99,6	99,8

Lidocaïne, chlorhydrate de, N° de contrôle 181105

Premier rapport d'analyse : WHO/PHARM/82.509, appendice 14

Année d'examen	1981	1988
DSC, mole %	-	0,3
DTA, mole %	0,8	0,8
IR	conforme	conforme
KF, %	6,4	6,2
TLC	pas de taches secondaires	pas de taches secondaires
Titrage, % (potentiométrique)	100,2	100,0

Méthyltestostérone, N° de contrôle 167023

Premier rapport d'analyse : WHO/PHARM/420.64, appendice 3

Année d'examen	1964	1975	1980	1984	1988
DTA, mole %	-	-	0,5	0,6	0,6
HPLC, %	-	-	-	0,2	0,2
IR	conforme	conforme	-	-	conforme
LOD, %	0,3	1,2	0,3	0,8	1,2
Absorption UV, 241 nm	0,54	0,54	0,54	0,54	0,54
Titrage, % (spectrophotométrique)	-	-	-	100	100

Appendice 4Oxacilline sodique, N° de contrôle 382027

Premier rapport d'analyse : WHO/PHARM/83.510, appendice 8

Année d'examen	1982	1984	1987
pH, solution à 1 %	5,7	5,6	-
KF (eau), %	4,3	4,5	4,1
HPLC, %	1,1	1,0	1,3
Produits de dégradation, % (par mercurimétrie)	-	0,3	0,3
Titration, % (alcalimétrique)	100,0	-	-
Titration, % (mercurimétrique)	-	99,4	99,4
PSA, %	0,9	-	-

Phénoxyméthylpénicilline potassique, N° de contrôle 176075

Premier rapport d'analyse : WHO/PHARM/77.491, appendice 6

Année d'examen	1976	1978	1984	1987
pH, solution à 0,5 %	6,0	-	5,9	-
LOD, %	0,1	-	0,1	-
TGA, %	-	-	-	0,3
HPLC, %	-	0,7	0,5	1,4
Produits de dégradation, % (par mercurimétrie)	-	-	0,2	0,2
Titration, % (pénicillinase)	99,6	-	-	-
Titration, % (mercurimétrique)	-	-	100,0	100,0
PSA, %	0,5	-	-	-

Propicilline potassique, N° de contrôle 274034

Premier rapport d'analyse : WHO/PHARM/420.64, appendice 8

Année d'examen	1964	1975	1982	1984	1987
pH, solution à 10 %	5,9	-	-	-	-
pH, solution à 1,0 %	-	-	5,3	5,3	-
KF (eau), %	0,3	-	0,3	0,4	-
TGA, %	-	-	-	-	0,35
HPLC, %	-	0,4	1,0	0,8	0,5
Produits de dégradation, % (par mercurimétrie)	-	-	-	0,8	0,8
Titration, % (pénicillinase)	98,3	-	97,4	-	-
Titration, % (mercurimétrique)	-	-	-	98,2	98,3

Testostérone, propionate de, N° de contrôle 167036

Premier rapport d'analyse : WHO/PHARM/420.64, appendice 4

Année d'examen	1964	1975	1982	1984	1988
DTA, mole %	-	-	0,2	0,2	0,3
HPLC, %	-	-	-	0,8	0,9
IR	conforme	-	-	conforme	conforme
LOD, %	0,1	0,1	0	-	-
TGA, %	-	-	-	-	0,1
TLC	Pas de taches secondaires	Pas de taches secondaires	Pas de taches secondaires	-	Pas de taches secondaires
Absorption UV, 240 nm	0,506	0,499	0,503	0,487	0,488
Titration, % (spectrophotométrique)	-	-	-	100	100

SUBSTANCES CHIMIQUES INTERNATIONALES DE REFERENCE

LISTE PREVISIONNELLE POUR 1988

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)
Béclométasone, 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 rifampicine 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	Sodium, cromoglicat de
(impureté de la carbidopa)	Spectinomycine, chlorhydrate de
Lévonorgestrel	Sulfacétamide
Lévothyroxine sodique (*)	Sulfasalazine
Liothyronine (*)	Testostérone, énantate de
(impureté de la lévothyroxine sodique)	Vincristine, sulfate de

Remplacements

Les substances chimiques internationales ci-dessous devront être remplacées par de nouveaux lots en 1988-1989 :

p-Acétamidobenzalazine (*)
 Anhydrotétracycline, chlorhydrate d' (*)
 Dexaméthasone (*)
 Dexaméthasone, acécate d' (*)
 Epi-4 anhydrotétracycline, chlorhydrate d' (*)
 Folique, acide (*)
 Prednisolone (*)
 Prednisolone, acétate de (*)

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

APPENDIX 6

ALLOPURINOL

Control No 287049

The stock of the current batch of the International Chemical Reference Substance for allopurinol, Control No 172049, is depleted and has to be replaced.

The monograph for allopurinol in the International Pharmacopoeia Ed III, Vol 2 requires a reference substance to be used in the infrared spectrophotometric test for identity.

MATERIAL

About 100 g of the sample (manufacturers batch No QA-0499) were received at the WHO Centre in April 1986. The material is being stored, protected from light, in a tightly closed container at +5 °C.

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (No. 287049). The spectrum is concordant with the spectrum obtained from the ICRS Control No. 172049.

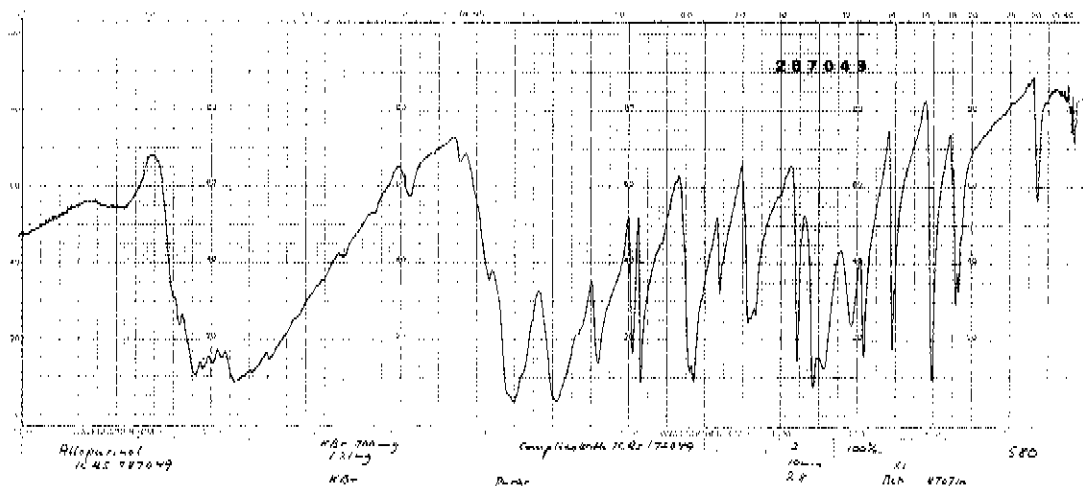


Figure 1. IR-spectrum of 1.2 mg of allopurinol in 300 mg KBr recorded against a KBr disc. Instrument: Perkin Elmer 580.

UV-spectrum

A UV-spectrum in 0.1 M hydrochloric acid is given in Figure 2.

λ max in 0.1 M hydrochloric acid = 250 nm.

E (1%, 1 cm) = 562 (n= 3).

The absorbance of a 10 µg/ml solution was 0.56.

The ratio of the absorbance of a 1-cm layer at 231 nm to that at 250 nm was 0.57.

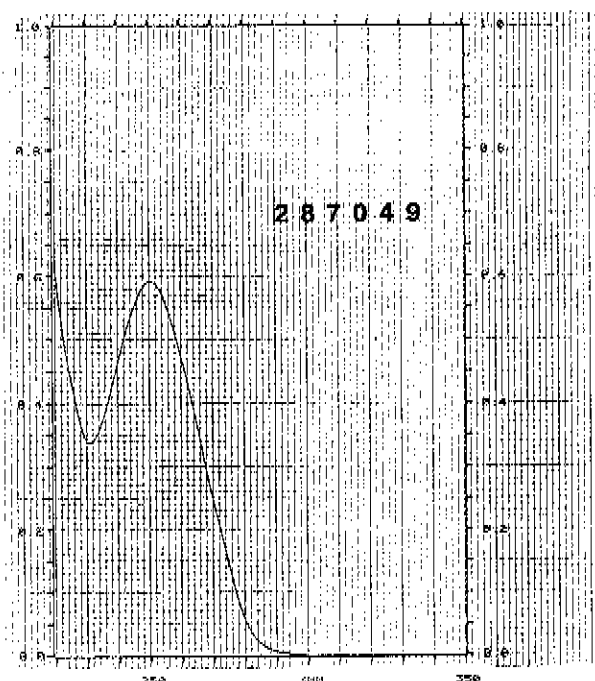


Figure 2. UV-spectrum of allopurinol 10.0 µg/ml in 0.1 M hydrochloric acid.

ASSAY

Thermogravimetric analysis

0% loss in weight.

Titrimetric assay

101.6% (n= 4), determined by differential potentiometric titration with tetrabutylammonium hydroxide (0.1 mol/l).

Using sodium methoxide (0.1 mol/l) according to the monograph or a potentiometric titration values that are too high were obtained.

Since other laboratories have reported similar problems with the assay method, it is suggested that the monograph's high content limit may be disregarded.

PURITY

Total solid impurities

1) Differential thermal analysis (DTA): It was not possible to estimate the purity by this method as the substance melts with decomposition at about 390 °C.

Thin-layer chromatography

The following thin-layer chromatographic system was used:

Thin-layer: Cellulosa F-254 (Merck)

Eluent: 1-butanol saturated with ammonia (100 g/l)

Sample: 250 µg of allopurinol and 0.5 µg of 3-aminopyrazole-4-carboxamide hemisulfate were applied.

Visualization: UV-light of 254 nm.

Result: No secondary spots were observed. The minimum detectable quantity of 3-aminopyrazole-4-carboxamide hemisulfate was 0.5 µg which corresponds to 0.2%.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.02%. A chromatogram is shown in Figure 3. The only impurity observed elutes at about 4.20 minutes and is identical to 3-aminopyrazole-4-carboxamide hemisulfate.

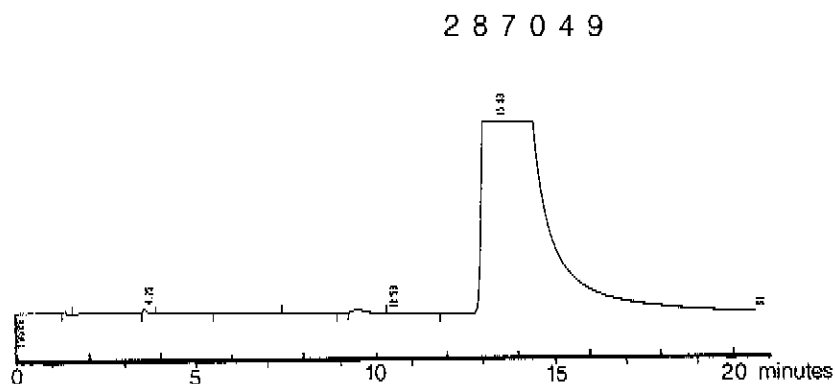


Figure 3. A chromatogram of allopurinol Control No. 287049.

The previous batch of allopurinol (ICRS 172049) as well as the USP reference substance (Lot G) showed the same degree of purity.

The following conditions were used:

Eluent: Monobasic ammonium phosphate buffer (0.05 M)
Column: RP-18, Spheri-5 (Brownlee)
Detector: Shimadzu SPD-2A operated at 254 nm
Pump: Waters 600 operated at a flow rate of 1.5 ml/min
Integrator: Hewlett Packard 3390 A Attenuation: 4
Sample: 0.5 mg/ml dissolved in 0.1 M sodium hydroxide and eluent (1 + 9)
20 µl corresponding to 10 µg were injected.

DIODE-ARRAY DETECTION

The chromatogram was also evaluated with a LKB 2140 Rapid Diode Array Detector. The same chromatographic system as described above was used, except for the injection volume that was increased to 100 µl and the concentration to 1 mg/ml to get maximum sensitivity. An isogram is given in Figure 4.

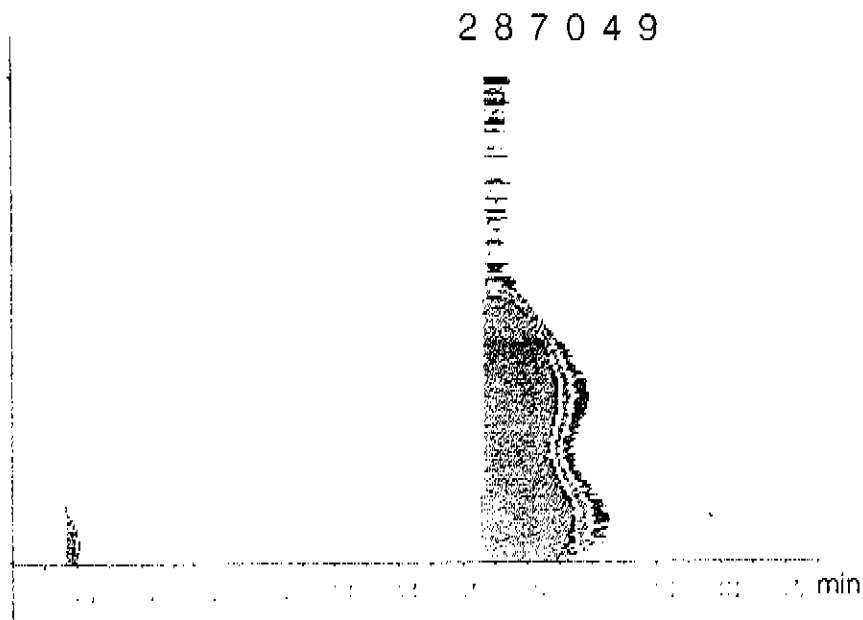


Figure 4. Isogram of allopurinol, Control No 287049. Sensitivity: 0.002

As seen from the figure no impurities are detected. The spot eluting at 2 minutes originates from the blank.

STABILITY

No special stability studies were performed as we have good experience of the stability of this substance from the earlier batch. Allopurinol, Control No 172049 showed no tendency of degradation when stored for 13 years at +5 °C at the Centre.

DATA GIVEN BY THE MANUFACTURER

Description:	a white, crystalline powder
Identification:	positive
Colour & clarity of solution:	satisfactory
Light absorption:	satisfactory
Related substances:	satisfactory
Loss of drying:	0.1%
Sulfated ash:	nil
Assay:	99.9%, calculated with reference to the dried substance
Additional assay (HPLC):	100.0%, calculated by total peak area

CONCLUSION

Allopurinol Control No 287049 can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 7

CHLORTETRACYCLINE HYDROCHLORIDE

Control No 187138

The monograph for chlortetracycline hydrochloride in the International Pharmacopoeia Ed. III, Vol 3 requires a reference substance to be used in the thin-layer chromatographic test for identity. The reference substance will also be used in the thin-layer chromatographic identity tests in the monographs for oxytetracycline hydrochloride, oxytetracycline dihydrate and tetracycline hydrochloride as well as in the test for related substances in the monograph for tetracycline hydrochloride.

MATERIAL

About 200 g of the sample (manufacturers batch NE 0476) were received at the WHO Centre in March 1986. The material is being stored protected from light in tightly closed containers at +5 °C.

ANALYTICAL DATA

Description: A yellow, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (no 187138). The spectrum is concordant with the spectrum obtained from the Eur Ph CRS Batch I.

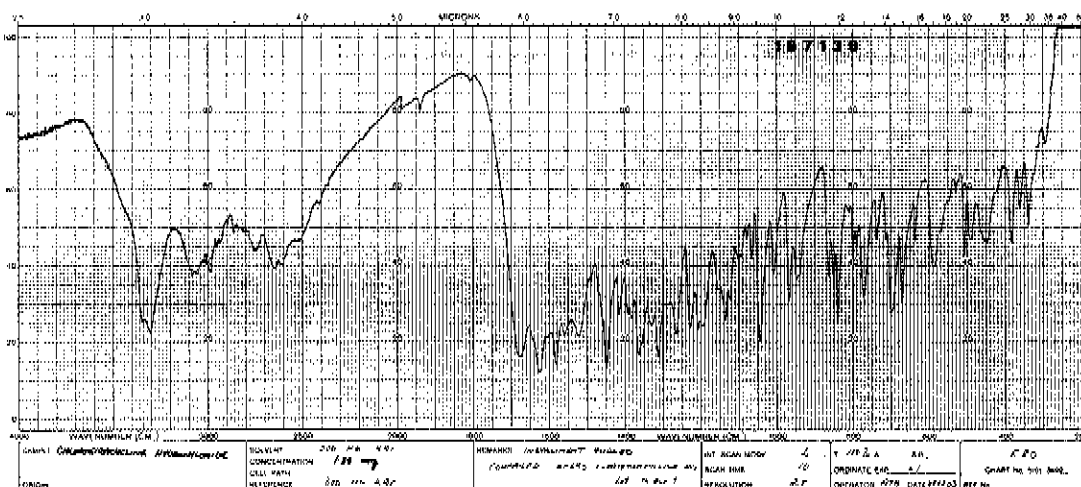


Figure 1. IR-spectrum of 1.4 mg of chlortetracycline hydrochloride in 300 mg KBr recorded against a KBr disc.
Instrument: Perkin Elmer 580.

Specific optical rotation: -242° ($n=4$). Determined in water at a concentration of 5 mg/ml.

UV-spectrum

A UV-spectrum in 0.01 M HCl is given in Figure 2.
 λ max in 0.01M HCl = 369 nm and 266 nm
 E (1%, 1 cm) = 216 (n= 6) and 356 (n= 6) respectively.

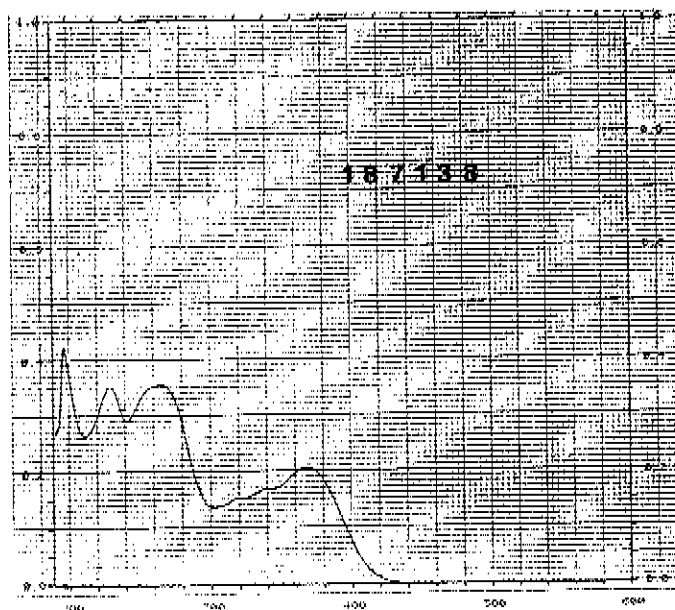


Figure 2. UV-spectrum of chlortetracycline hydrochloride 10 µg/ml in 0.01 M HCl.

Absorption in the ultraviolet region according to Ph.Int:

A 10 µg/ml solution in 0.5 M sulfuric acid gave an absorbance of 0.73 at 273 nm.

ASSAY

Thermogravimetric analysis: 0.15% loss in weight.

Microbiological assay: 100.1%, corresponding to 1001 IE/mg, determined microbiologically. The assay was performed using a conventional two-dose agar diffusion technique. To prepare the solutions of the sample and reference material a sterile phosphate buffer, pH 4.5, was used. Test organism: *Bacillus subtilis* (ATCC 6633). Chlortetracycline hydrochloride EPCRS (Lot 1) was used as reference standard, and taken to be 100%.

PURITY

Thin-layer chromatography

The following thin-layer chromatographic system according to Ph.Int was used:

Thin-layer: Homemade plate of kieselguhr G, containing glycerol, 0.1 M EDTA and with pH adjusted to 7.

Eluent: Chloroform: ethyl acetate:acetone (2:2:1). 200 ml of this mixture was shaken with 25 ml of 0.1 M EDTA with pH adjusted to 7. The organic phase is used as eluent.

Sample: 0.5 µg of chlortetracycline hydrochloride of different origin were applied.
References: 0.5 µg of each oxytetracycline hydrochloride proposed ICRS, tetracycline hydrochloride ICRS 180095 and chlortetracycline hydrochloride proposed ICRS applied as a mixture.

Visualization: Expose the plate to vapour of ammonia and examine in UV-light of 365 nm.

Result: The thin-layer plate is shown in figure 3.

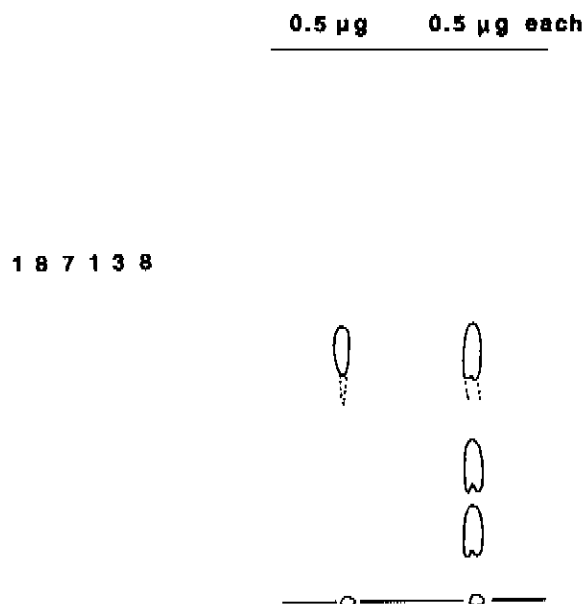


Figure 3. Thin-layer chromatogram of chlortetracycline hydrochloride Control No 187138, tetracycline hydrochloride and oxytetracycline hydrochloride.

As seen from the figure the three substances separate from each other with the following Rf-values:

Chlortetracycline hydrochloride= 0.49
Tetracycline hydrochloride= 0.29
Oxytetracycline hydrochloride= 0.16

All batches of chlortetracycline hydrochloride showed a Rf of 0.49.

High performance liquid chromatography

A chromatogram is shown in figure 4. The main peak, chlortetracycline hydrochloride, elutes at 6.0 min. The peak at 3.6 min was identified against a reference standard as tetracycline hydrochloride and estimated by peak area measurement to about 0.5%. The peak at 4.2 min was unidentified and constituted about 9%. Several chromatographic conditions were tested in order to improve the baseline separation. However, the same pattern was observed in all systems tested and also for samples of chlortetracycline hydrochloride of different origin. Data given by the manufacturer declares 0.6% of tetracycline and negligible amounts of other impurities. If the peak at 4.2 min. and the sloping baseline are caused by degradation during analysis, an equilibrium between two forms of chlortetracycline, or are caused by other factors, remains to be elucidated.

Chlortetracycline hydrochloride in solution is rapidly degraded, thus solutions should be freshly prepared prior to use.

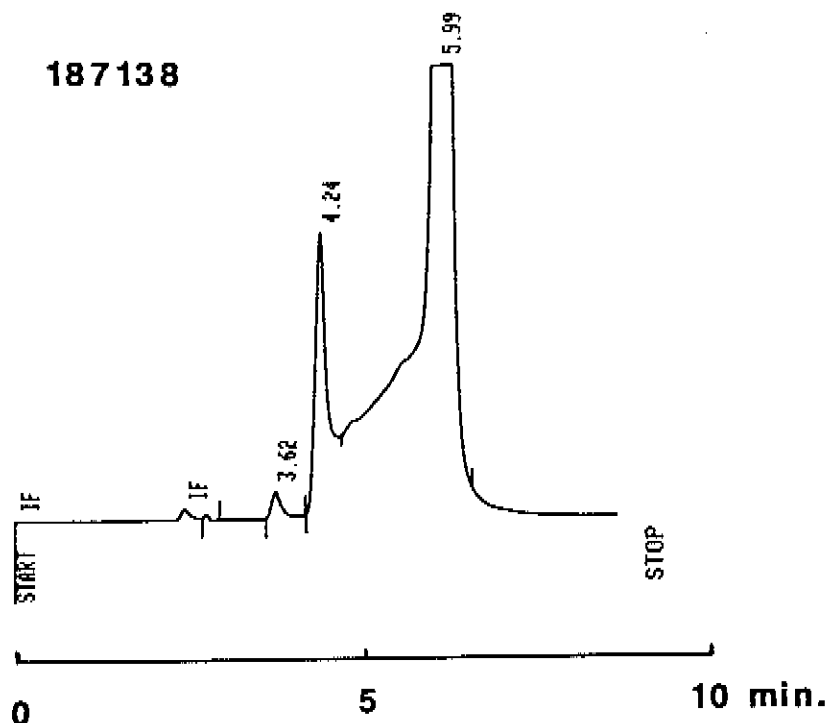


Figure 4. Chromatogram of Chlortetracycline hydrochloride Control No 187138 eluted with 20% acetonitrile: 80% phosphate buffer (0.1 M) pH 2.1.

The following conditions were used:

Eluent: Acetonitrile/Phosphate buffer (0.1 M) pH 2.1 (20:80)
Column: RP-18, Vydac 218TP54
Detector: Shimadzu SPD-2A operated at 270 nm
Pump: Waters 600 operated at a flow rate of 1 ml/min
Integrator: Hewlett Packard 3390A
Attenuation: 4
Sample: 0.1 mg/ml dissolved in the eluent. 20 μ l corresponding to 2 μ g were injected.

STABILITY

Chlortetracycline hydrochloride 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. The samples stored at a relative humidity of 55% and 75% picked up moisture and increased 0.3% in weight. The sample stored at 95% relative humidity increased 4.2% in weight. No signs of degradation were observed when the samples were analyzed by the liquid chromatographic method described above.

CONCLUSION

Chlortetracycline hydrochloride, Control No 187138 can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 8

C L O M I F E N E C I T R A T E

Control No 187136

The monograph for clomifene citrate in the International Pharmacopoeia Ed III, Vol 3 requires a reference substance to be used in the infrared spectrophotometric test for identity.

MATERIAL

About 100 g of the sample (manufacturers code MDL 5008F) were received at the WHO Centre in August 1983. The material is being stored protected from light in tightly closed containers at +5 °C.

ANALYTICAL DATA

Description: A white powder; odourless.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (No 187136). The spectrum is concordant with the spectrum published in British Pharmacopoeia Infrared Reference Spectra 1980.

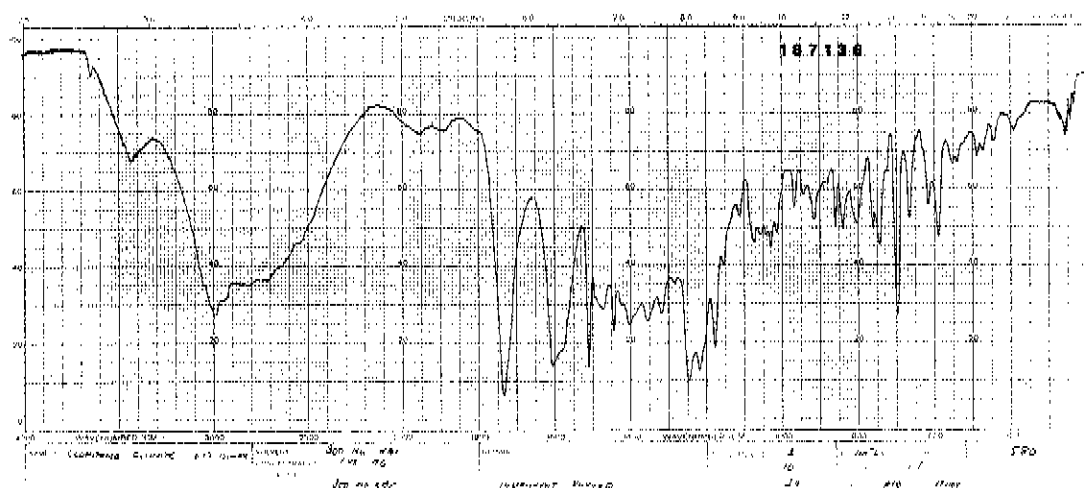


Figure 1. IR-spectrum of 2.1 mg of clomifene citrate in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin Elmer 580.

In the region around 750 cm^{-1} differences can be observed originating from the two isomers E and Z. The ICRS with Control No 187136 is a mixture of 35% Z-isomer and 65% E-isomer. For 100% Z-isomer a more distinct peak is observed at about 740 cm^{-1} .

UV-spectrum

A UV-spectrum in ethanol (750 g/l) was recorded.

λ max in ethanol = 204, 239 and 297 nm
E (1%, 1 cm) = 718, 328 and 192 respectively (n = 2).

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

λ max in 0.1 M HCl = 234 and 290 nm
E (1%, 1 cm) = 317 and 172 respectively (n= 4)

The absorbance of a 25 μ g/ml solution was 0.79 at 234 nm and 0.43 at 290 nm. Clomifene citrate, Control No 187136 consists of a mixture of 35% Z-isomer and 65% E-isomer.

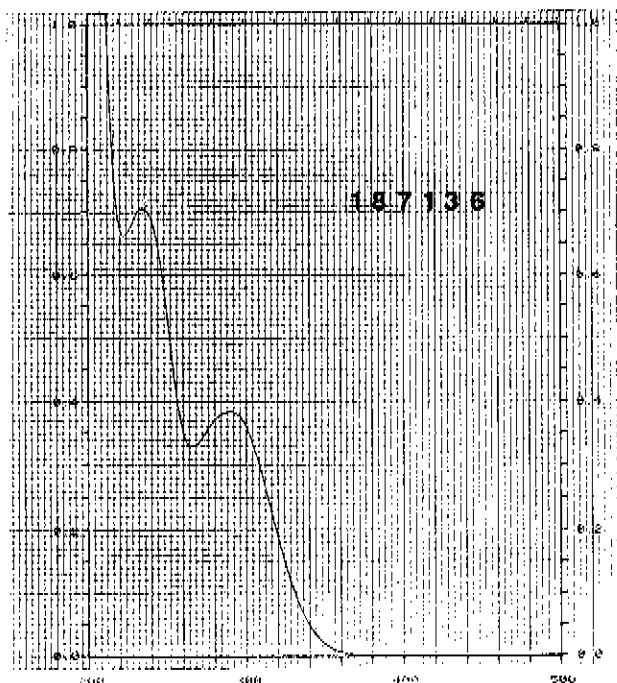


Figure 2. UV-spectrum of clomifene citrate 22 μ g/ml in 0.1 M HCl.

ASSAY

Thermogravimetric analysis

0.3% loss in weight.

Titrimetric assay: 97.6% (n= 11) determined by potentiometric titration according to Ph.Int. Vol 3.

PURITY

Nuclear magnetic resonance (NMR): ^1H NMR spectrum was recorded. The content of Z-isomer was estimated to about 33%.

Total solid impurities

1) Differential thermal analysis (DTA): It was not possible to estimate the purity by this method since in the first place the substance melts with decomposition and secondly that it consists of an isomeric mixture.

Thin-layer chromatography

The following thin-layer chromatographic systems were used:

System I (according to Ph.Int. Vol 3):

Thin-layer: Silica gel 60, F-254 (Merck)
Eluent: Chloroform:methanol:water (90:10:1)
Sample: 200 µg of clomifene citrate (E + Z) and Z-isomer dissolved in chloroform:ethanol (3:1) were applied
Visualization: UV-light of 254 nm.

Result: No extra spots were observed. The thin-layer system did not separate the two isomers sufficiently to estimate the content of Z-isomer. R_f (E-isomer) = 0.32 and R_f (Z-isomer) = 0.28. A more accurate estimation is performed by high-performance liquid chromatography.

System II:

Thin-layer: Silica gel 60, F-254 (Merck)
Eluent: Toluene:triethylamine (90:10)
Sample: About 100 µg were applied as base: Clomifene citrate was dissolved in 0.1 M HCl, 1 M NaOH was added and an extraction to chloroform was performed.
Visualization: UV-light of 254 nm and scanning at 254 and 302 (where the base has its maximum).

Result: By visual inspection at UV-light of 254 nm no extra spots were observed. By scanning, very faint traces of impurities eluting after the main spot were observed. Their total amount was roughly estimated to much less than 0.1%. This system did not separate the two isomers.

High performance liquid chromatography

The content of Z-isomer and additional impurities was determined with two different liquid chromatographic systems.

System I. Straight phase

Determination of Z-isomer: The content of Z-isomer was estimated to 35% (n= 3) by peak area measurement. A chromatogram is shown in Figure 3.

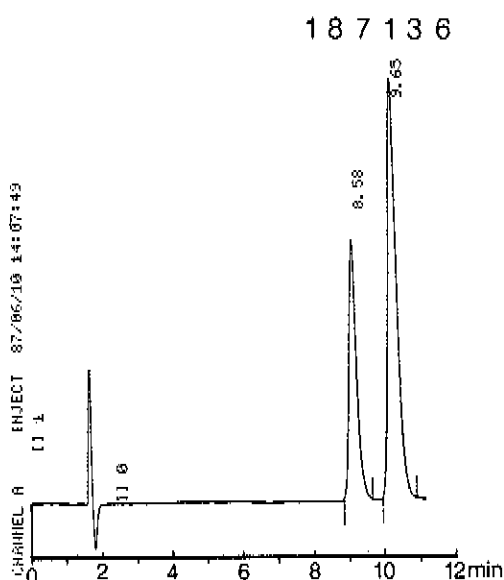


Figure 3. Chromatogram of clomifene citrate (E + Z), Control No 187136.

As seen from the figure the Z-isomer elutes first at about 8.5 minutes. The identity of the two isomers were established by comparison with clomifene citrate Z-isomer, Control No 187137 (proposed ICRS) and BPCRS for the Z and E isomers respectively.

Additional impurities: About 0.3% estimated by peak area measurement. They elute after the main peak and a chromatogram is shown in Figure 4.

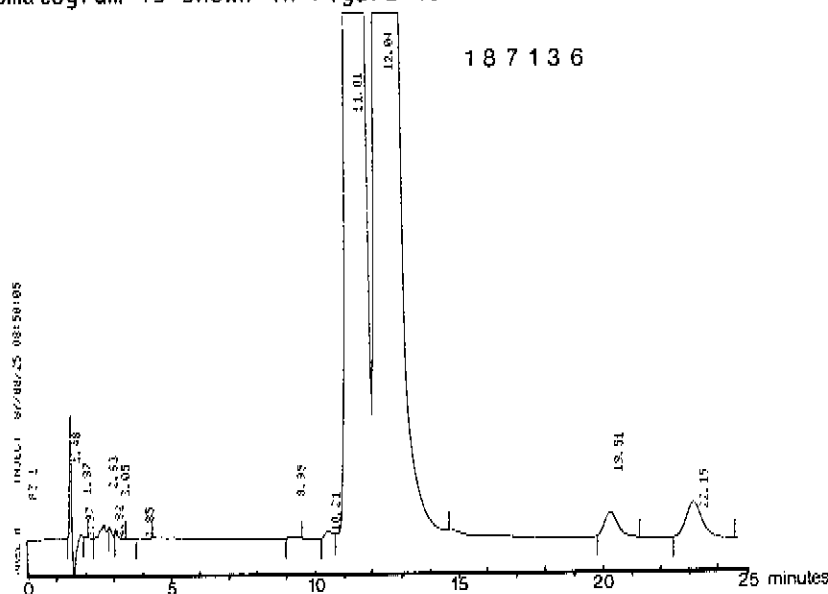


Figure 4. Chromatogram of clomifene citrate (E + Z), Control No 187136 and impurities.

The following conditions were used:

Eluent: Hexane containing 0.2% triethylamine:ethyl acetate (85:15). Minor changes in the content of triethylamine affects the retention time considerably.
Column: Spherisorb Silica S 5W
Detector: Varian UV 200 operated at 302 nm where the base has a maximum.
Pump: Varian 5500 operated at a flow rate of 2 ml/min
Integrator: Varian 4270 Attenuation: 8
Sample: 120 mg of clomifene citrate were dissolved in 25 ml 0.1 M HCl and 5 ml of 1 M NaOH were added. Extraction was performed with four quantities, each of 2 ml, of ethanolfree chloroform (Merck, stabilized with 2-methyl-2-butene). The combined chloroform extracts were washed with about 5 ml of water. The chloroform phase was finally dried over anhydrous sodium sulfate. Chloroform was added to produce 10 ml final solution. 10 µl of this solution, containing about 10 mg/ml of clomifene citrate base, was injected.

For quantitation of the Z-isomer a solution of about 0.20 mg/ml clomifene citrate base was injected. This was necessary in order to obtain baseline separation.

System 2. Reversed phase

Determination of Z-isomer: The content of Z-isomer was estimated to 34.5% (n= 5). The determination was performed against external standards of Z-isomer (Control No 187137 and BPCRS 959). A chromatogram is shown in Figure 5.

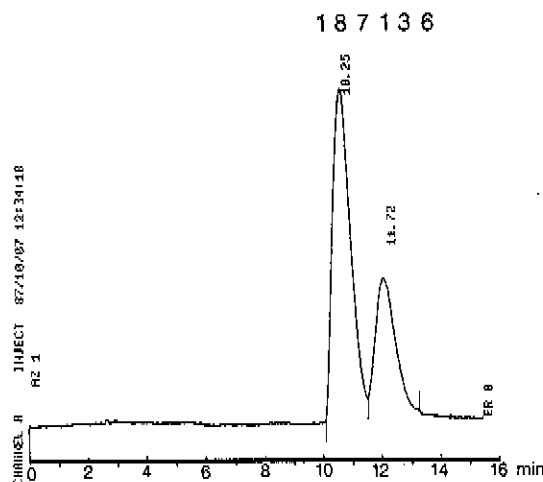


Figure 5. Chromatogram of clomifene citrate (E + Z), Control No 187136.

In this system the Z-isomer elutes at about 11.7 minutes. The identity of the different isomers were established with reference substances as described above.

Additional impurities: About 0.3% estimated by peak area measurement. They elute before the main peak. A chromatogram is shown in Figure 6.

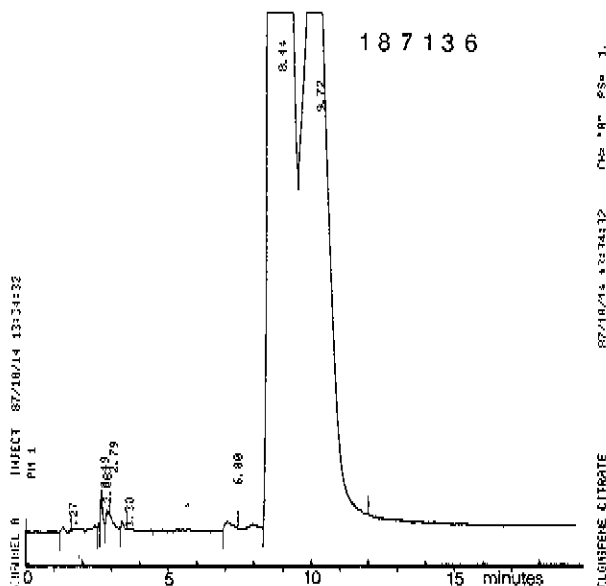


Figure 6. Chromatogram of clomifene citrate (E + Z), Control No 187136 and impurities.

Eluent: Methanol:tetrahydrofuran:triethylamine (80 + 20 + 0.001). Minor changes in the content of triethylamine affects the retention time considerably.
 Column: Spherisorb S 5 ODS 2
 Detector: Varian UV 200 operated at 254 nm
 Pump: Varian 5500 operated at a flow rate of 1 ml/min
 Integrator: Varian 4270 Attenuation: 2
 Sample: 0.1 mg/ml dissolved in the eluent. For quantitation of the Z-isomer a solution of about 0.02 mg/ml was injected.

DIODE-ARRAY DETECTION

The chromatogram was also evaluated with a LKB 2140 Rapid Diode Array Detector. The same chromatographic system as described above under system 1 was used, except for the injection volume that was increased to 100 μ l. An isogram is given in Figure 7.

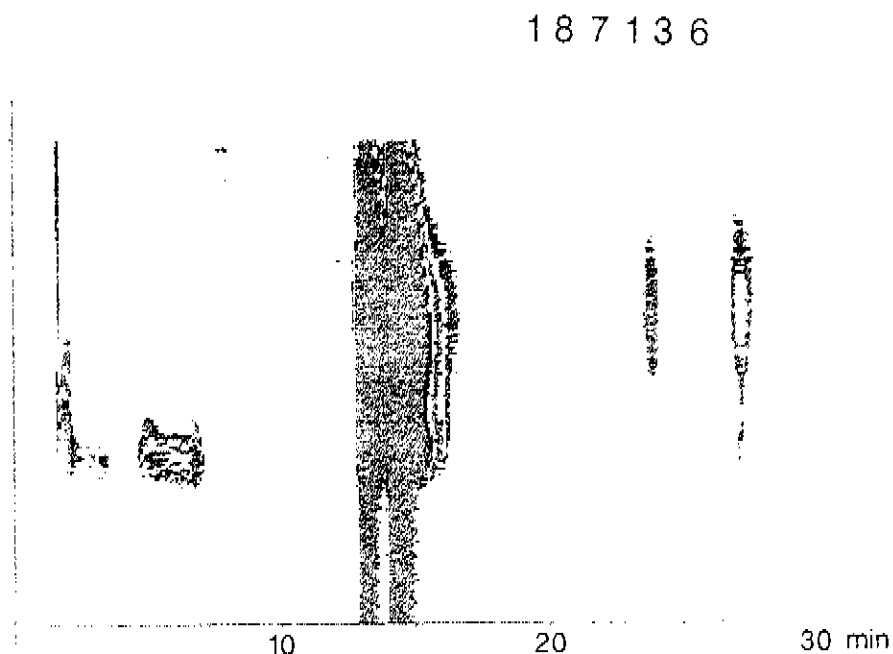


Figure 7. Isogram of clomifene citrate (E + Z), Control No 187136 when chromatographed according to system 1. Sensitivity: 0.002.

As seen from the figure the major impurities eluting at about 22 and 25 minutes exhibit UV-maxima at 295 and 300 nm, respectively. They are all detectable at 302 nm which is the wavelength chosen in the method described above under system 1.

STABILITY

Clomifene citrate was exposed to air of different relative humidity at 20 °C in a thermostated incubator (Termaks) for a period of 9 weeks as described in WHO/PHARM/82.509. All samples were unchanged at visual inspection. The samples stored at 75% relative humidity or above picked up moisture. The increase in weight was determined to 2.3% by thermogravimetric analysis. No signs of degradation were observed when the samples were analyzed by the liquid chromatographic method 2 described above.

CONCLUSION

Clomifene citrate Control No 187136 can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 9

C L O M I F E N E C I T R A T E Z - I S O M E R

Control No 187137

The monograph for clomifene citrate in the International Pharmacopoeia Ed III, Vol 3 requires a reference substance for clomifene citrate Z-isomer to determine the content of Z-isomer in clomifene citrate.

MATERIAL

About 100 g of the sample (manufacturers batch No c-87458) were received at the WHO Centre in August 1983. The material is being stored protected from light in tightly closed containers at +5 °C.

ANALYTICAL DATA

Description: A white powder; odourless; non-homogenous.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (No 187137). The spectrum is concordant with a spectrum obtained with BPCRS 959 Z-isomer.

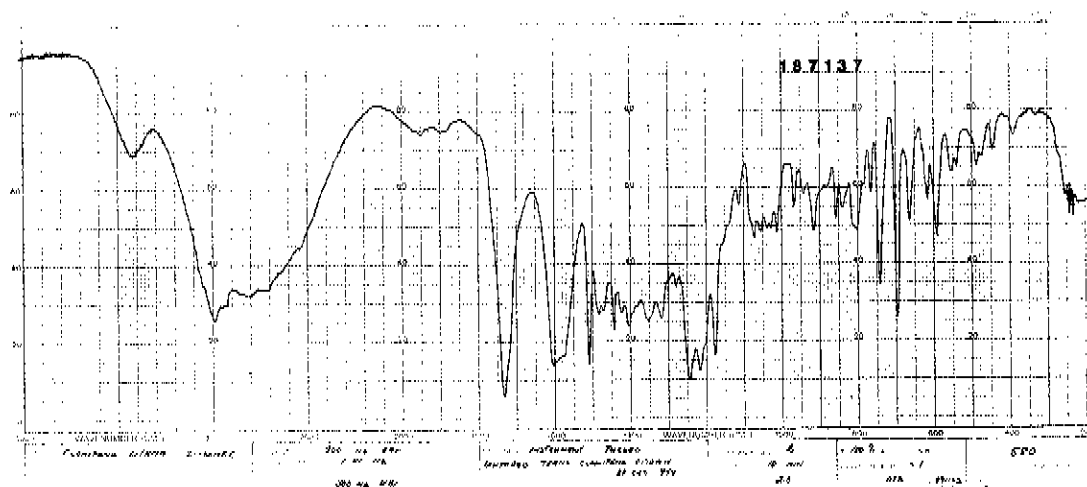


Figure 1. IR-spectrum of 2.1 mg of clomifene citrate Z-isomer in 300 mg KBr recorded against a KBr disc. Instrument: Perkin Elmer 580.

In the region around 750 cm^{-1} differences can be observed originating from the two isomers E and Z. For the Z-isomer a more distinct peak is observed at about 740 cm^{-1} .

UV-spectrum

A UV-spectrum in ethanol (750 g/l) was recorded.

λ max in ethanol = 203, 229 and 298 nm.
E (1%, 1 cm) = 715, 331 and 207 respectively (n= 1).

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

λ max in 0.1 M HCl = 203 and 286 nm
 E (1%, 1 cm) = 680 and 190 respectively (n= 4)

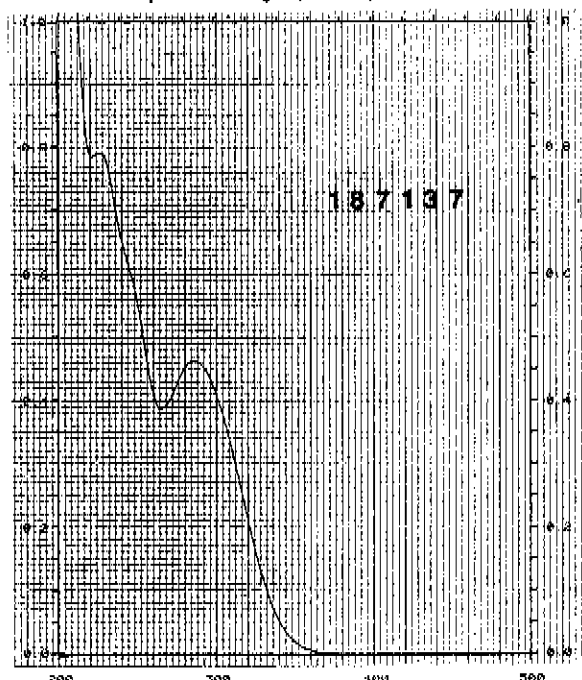


Figure 2. UV-spectrum of clomifene citrate Z-isomer 24.4 $\mu\text{g/ml}$ in 0.1 M HCl.

ASSAY

Thermogravimetric analysis

0.2% loss in weight.

Titrimetric assay: 99.2% (n= 11) determined by potentiometric titration according to Ph.Int. Vol 3.

PURITY

Total solid impurities

1) Differential thermal analysis (DTA): It was not possible to estimate the purity by this method as the substance melts with decomposition.

Thin-layer chromatography

The following thin-layer chromatographic systems were used:

System I, according to Ph.Int. Vol 3:

Thin-layer: Silica gel 60, F-254 (Merck)
 Eluent: Chloroform:methanol:water (90:10:1)
 Sample: 200 μg of clomifene citrate (E + Z) and Z-isomer were applied
 Visualization: UV-light of 254 nm.

Result: No extra spots were observed. The thin-layer system did not separate the two isomers sufficiently to estimate the content of E-isomer. R_f (E-isomer) = 0.32 and R_f (Z-isomer) = 0.28. A more accurate estimation is performed by high-performance liquid chromatography.

System II:

Thin-layer: Silica gel 60, F-254 (Merck)
Eluent: Toluene:triethylamine (90:10)
Sample: About 100 ug were applied as base; Clomifene citrate was dissolved in 0.1 M HCl, 1 M NaOH was added followed by extraction to chloroform.
Visualization: UV-light of 254 nm and scanning at 254 and 302 (where the base has its maximum).

Result: By visual inspection at UV-light of 254 nm no extra spots were observed. By scanning, very faint traces of impurities with higher and lower Rf-values than Clomifene (Z) were detected. Their total amount was roughly estimated to much less than 0.1%. This system did not separate the two isomers.

High performance liquid chromatography

The content of E-isomer and additional impurities was determined with two different liquid chromatographic systems.

System 1. Straight phase

Determination of E-isomer: The content of E-isomer was estimated to 1.7% (n= 3) by peak area measurement. A chromatogram is shown in Figure 3.

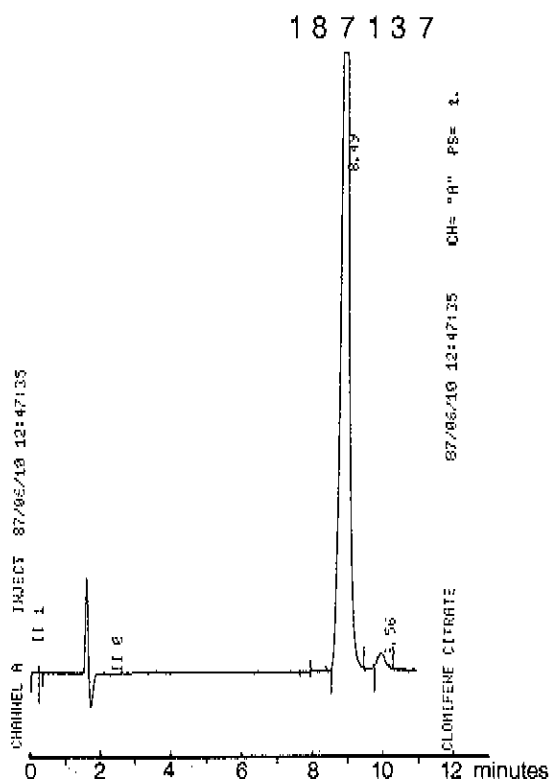


Figure 3. Chromatogram of clomifene citrate Z-isomer, Control No 187137.

As seen from the figure the Z-isomer elutes first, at about 8.5 minutes. The identity of the different isomers was established by comparison with the BPCRS for the Z and E isomers respectively.

Additional impurities: About 0.3% estimated by peak area measurement. The impurities were eluted at about 10 and 19 minutes. A chromatogram is shown in Figure 4.

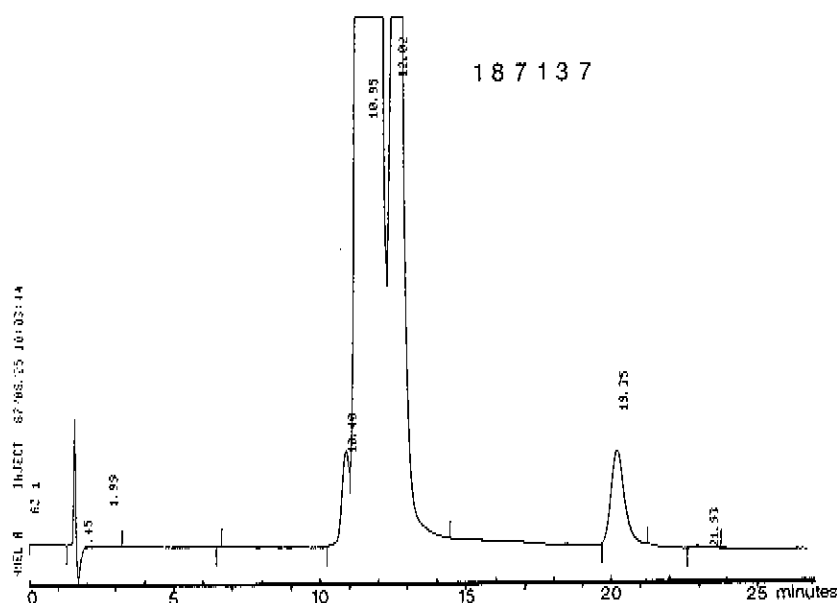


Figure 4. Chromatogram of clomifene citrate Z-isomer, Control No 187137 and impurities.

The following conditions were used:

Eluent: Hexane containing 0.2% triethylamine:ethyl acetate (85:15). Minor changes in the content of triethylamine affects the retention times considerably.
Column: Spherisorb Silica S 5W
Detector: Varian UV 200 operated at 302 nm where the base has a maximum.
Pump: Varian 5500 operated at a flow rate of 2 ml/min
Integrator: Varian 4270 Attenuation: 8
Sample: 120 mg of clomifene citrate Z-isomer were dissolved in 25 ml 0.1 M HCl and 5 ml of 1 M NaOH were added. Extraction was performed with four quantities, each of 2 ml, of ethanolfree chloroform (Merck, stabilized with 2-methyl-2-butene). The combined chloroform extracts were washed with about 5 ml of water. The chloroform phase was finally dried over anhydrous sodium sulfate. Chloroform was added to produce 10 ml of final solution. 10 μ l of this solution containing about 10 mg/ml of clomifene Z-isomer base, was injected.

For quantitation of the E-isomer a solution of about 0.20 mg/ml clomifene base was injected. This was necessary in order to obtain baseline separation.

System 2. Reversed phase

Determination of E-isomer: The content of E-isomer was estimated to about 2% by peak area measurement.

Additional impurities: About 0.2% estimated by peak area measurement. They elute before the main peak. A chromatogram is shown in Figure 5.

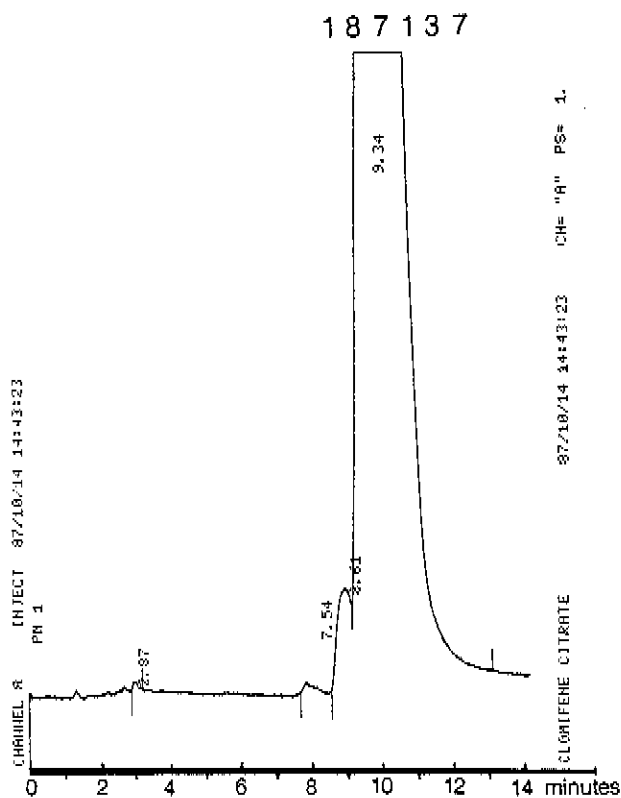


Figure 5. Chromatogram of clomifene citrate Z-isomer, Control No 187137 and impurities.

Eluent: Methanol:tetrahydrofuran:triethylamine (80 + 20 + 0.001). Minor changes in the content of triethylamine affects the retention times considerably.
Column: Spherisorb S 5 ODS 2
Detector: Varian UV 200 operated at 254 nm
Pump: Varian 5500 operated at a flow rate of 1 ml/min
Integrator: Varian 4270 Attenuation: 2
Sample: 0.1 mg/ml dissolved in the eluent.

DIODE-ARRAY DETECTION

The chromatogram was also evaluated with a LKB 2140 Rapid Diode Array Detector. The same chromatographic system as described above under system 1 was used, except for the injection volume that was increased to 100 μ l. An isogram is given in Figure 6.

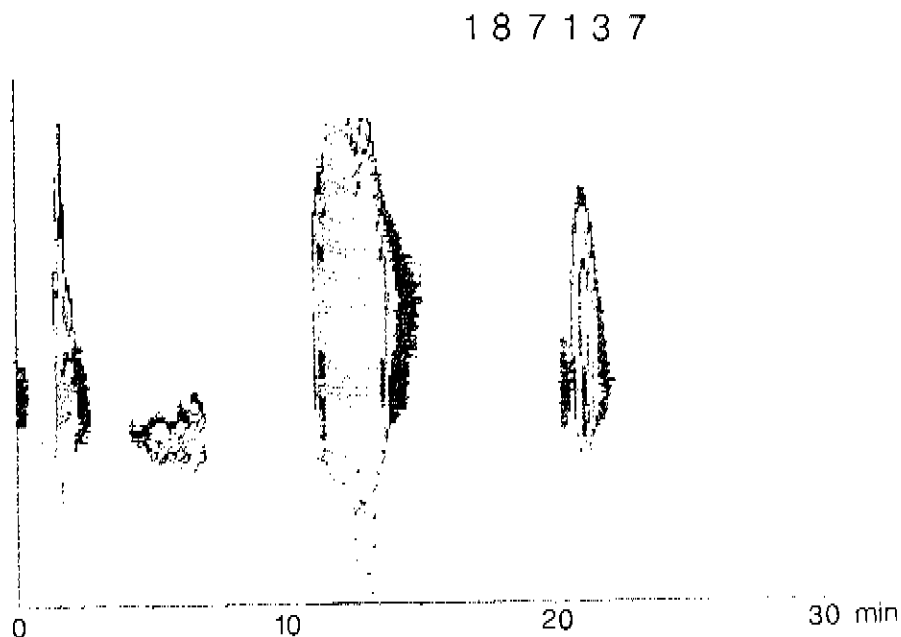


Figure 6. Isogram of clomifene citrate Z-isomer, Control No 187137 when chromatographed according to system 1. Sensitivity: 0.002.

As seen from the figure the major impurities eluting after about 11 and 21 minutes exhibits UV-maxima at 301 and 295 nm, respectively. This means that they are all visible at 302 nm which was chosen in the method described above under system 1.

STABILITY

Clomifene citrate Z-isomer No 187137 was exposed to air of different relative humidity at 20 °C in a thermostated incubator (Termaks) for a period of 9 weeks as described in WHO/PHARM/82.509. All samples remained unchanged at visual inspection. The samples stored at 75% relative humidity or above picked up moisture. The increase in weight was determined to 0.7% by thermogravimetric analysis. No signs of degradation were observed when the samples were analyzed by the liquid chromatographic method 2, described above.

CONCLUSION

Clomifene citrate Z-isomer Control No 187137 can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 10

D I G O X I N

Control No 587011

The monograph for digoxin in the International Pharmacopoeia Ed. III, Vol 2 requires a reference substance to be used in the infrared spectrophotometric identity test, in the thin-layer chromatographic test for identity and in the spectrophotometric assay.

MATERIAL

About 50 g of the sample (manufacturers batch no 170161) were received at the WHO Centre in August 1987. The material is being stored, protected from light, in a tightly closed container at +5 °C.

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (No. 587011). The spectrum is concordant with the spectra obtained from the ICRS Control No 377011 and the EPCRS (lot 3).

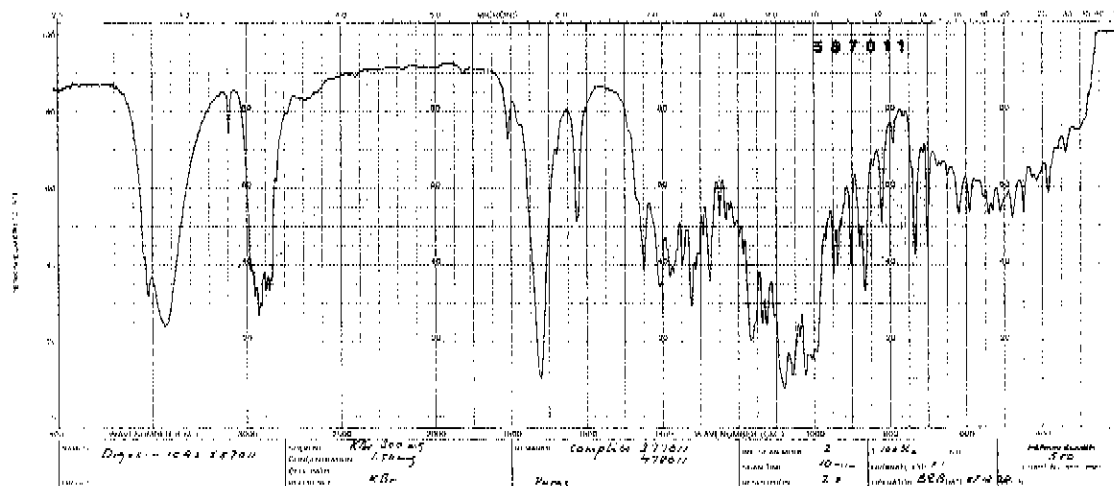


Figure 1. IR-spectrum of 1.5 mg of digoxin in 300 mg KBr recorded against a KBr disc. Instrument: Perkin-Elmer 580.

UV spectrum

A UV-spectrum in ethanol (about 750 g/l) is given in Figure 2.
 λ max in ethanol = 219 nm. E (1%, 1 cm) = 198.

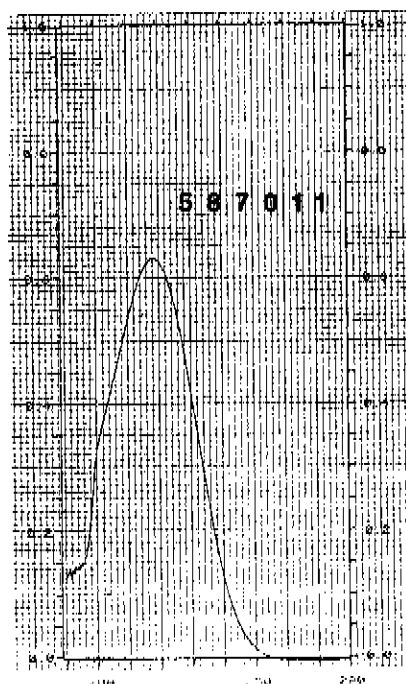


Figure 2. UV-spectrum of digoxin 30 µg/ml in ethanol.

Specific optical rotation: $[\alpha]_{546}^{20} = +14.0^{\circ}$ (n= 2), determined in a 0.10 g/ml solution in pyridine R.

ASSAY

Thermogravimetric analysis

0.15% loss in weight.

Water

0.16% determined by Karl Fischer titration.

Colorimetric assay:

The colorimetric assay described in the European Pharmacopoeia Ed II, Part II, was used. The EPCRS for digoxin (No. 3) was used as standard and regarded as 100%. The result was calculated with reference to the dried substance. Result: 99.65% (n= 6, rel. dev. 1.1%).

PURITY

Thin layer chromatography

The following thin-layer chromatographic systems were used:

I According to the International Pharmacopoeia Ed III, Vol. 2

Thin-layer: Kieselguhr R1, impregnated with a mixture of 10 volumes of formamide R and 90 volumes of acetone R.

Eluent: Xylene R: ethylmethylketone R: formamide R (50 + 50 + 4)

Sample: 5 and 50 µg of digoxin were applied. The sample was dissolved in equal volumes of methanol R and chloroform R.

Visualization: After heating at 115 °C for 20 minutes, spray with a mixture of 15 volumes of a solution of 25 g trichloroacetic acid R in 100 ml of ethanol (about 750 g/l) TS and 1 volume of a freshly prepared 30 mg/ml solution of tosylchloramide sodium R. Heat again for 5 minutes at 115 °C and examine in daylight and in UV-light at 365 nm.

Rf (digoxin) = 0.17
Rf (gitoxin) = 0.30
Rf (digitoxin) = 0.63

Result: One very weak spot was detected before the main spot when applying the amount prescribed in the monograph. When applying 50 µg three extra spots were noted, of which one corresponds to gitoxin.

The same system was also carried out using kieselguhr F-254 (Merck). The separation was improved but the detection limit remained unchanged and about 0.1 µg.

II According to K. Florey; Analytical Profiles, Vol. 9 (1980)

Thin-layer: Silica gel 60, F-254 (Merck)
Eluent: Methylene Chloride R: Methanol R (90 + 10). The plate was developed twice.
Sample: 5 and 50 µg of digoxin were applied (same as system I).
Visualization: After spraying with 20% phosphoric acid R solution and heating at 105 °C for 15 minutes, examination in UV-light at 365 nm.

Result: When 50 µg were applied, two distinct and six faint spots were detected. Two of them had Rf-values corresponding to digitoxin (very weak) and gitoxin. When 5 µg were applied three very faint spots were visible. The detection limit of this system was about 0.05 µg (0.1%).

However, none of the spots was more intense than the reference spot (digitoxin), of which 0.25 µg was applied.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 1.4%. A chromatogram is shown in Figure 3. The main impurities eluted at about 4 and 11.5 minutes corresponding to digoxigenin-bisdigitoxoside and gitoxin, respectively. Very faint traces of digitoxin, eluted at about 22 minutes.

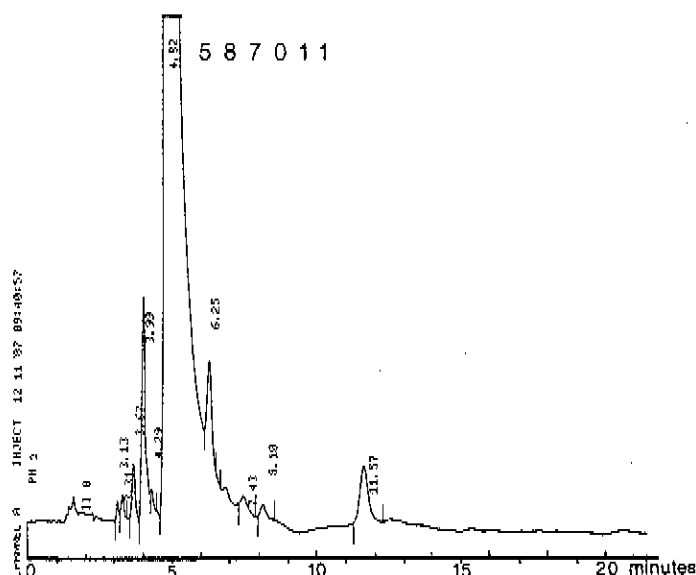


Figure 3. A chromatogram of digoxin, Control No. 587011.

The previous batch of digoxin (ICRS 377011) as well as the EPCRS (Lot 3) showed a higher degree of purity (about 0.6% and 0.4% impurities, respectively).

The following conditions were used:

Eluent: Acetonitrile/Methanol/Water (30 + 30 + 40)
Detector: Varian UV 200 operated 220 nm.
Pump: Varian 5560 operated at a flow rate of 1.0 ml/min
Integrator: Varian 4270 Attenuation: 4
Sample: 1.0 mg/ml dissolved in the eluent by placing in an ultrasonic bath.
10 μ l corresponding to 10 μ g were injected.

DIODE-ARRAY DETECTION

The chromatogram was also evaluated with a LKB 2140 Rapid Diode Array Detector. The same chromatographic system as described above was used, except for the injection volume that was increased to 100 μ l to get maximum sensitivity. An isogram is given in Figure 4.

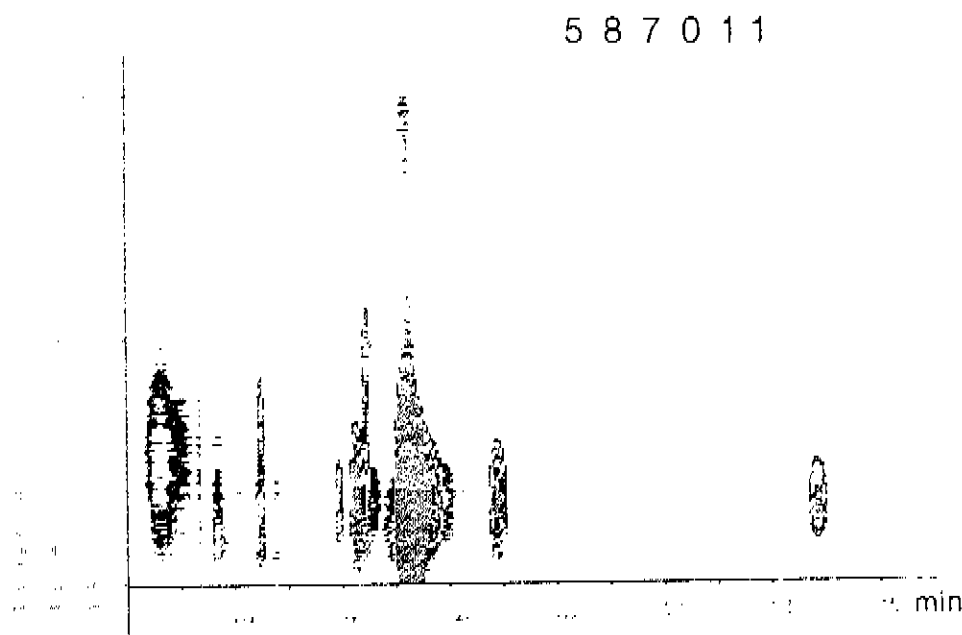


Figure 4. Isogram of digoxin, Control No 587011. Sensitivity: 0.002.

As seen from the figure six impurities were detected. All of them, as well as the main peak, have their maximum at 220 nm. The peaks eluting between 6 and 8 minutes are unknown. The peaks eluting before 2 minutes originate from the blank.

STABILITY

No special stability studies were performed as we have good experience of the stability of this substance from earlier batches. Digoxin ICRS 377011 showed no tendency of degradation when stored for 10 years at +5 °C at the Centre.

DATA GIVEN BY THE MANUFACTURER

HPLC	98.3%
Gitoxin	< 1%
Digitoxin	< 1%
Other glycosides	< 1%
Each impurity	< 3.0%

CONCLUSION

Digoxin Control No 587011 can be considered suitable as International Chemical Reference Substance for the intended purpose with an estimated content of 99.7% $C_{41}H_{64}O_{14}$ (digoxin) determined by colorimetric spectrophotometry and calculated with reference to the dried substance.

EMETINE HYDROCHLORIDE

Control No 187134

The monograph for emetine hydrochloride in the International Pharmacopoeia Ed III, Vol 3 requires a reference substance to be used in the infrared spectrophotometric and thin-layer chromatographic tests for identity.

MATERIAL

About 50 g of the sample (manufacturers batch No S-38/178) were received at the WHO Centre in March 1987. The material is being stored protected from light in tightly closed containers at +5 °C.

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (No 187134). The spectrum is concordant with the spectrum obtained from the EPCRS reference substance.

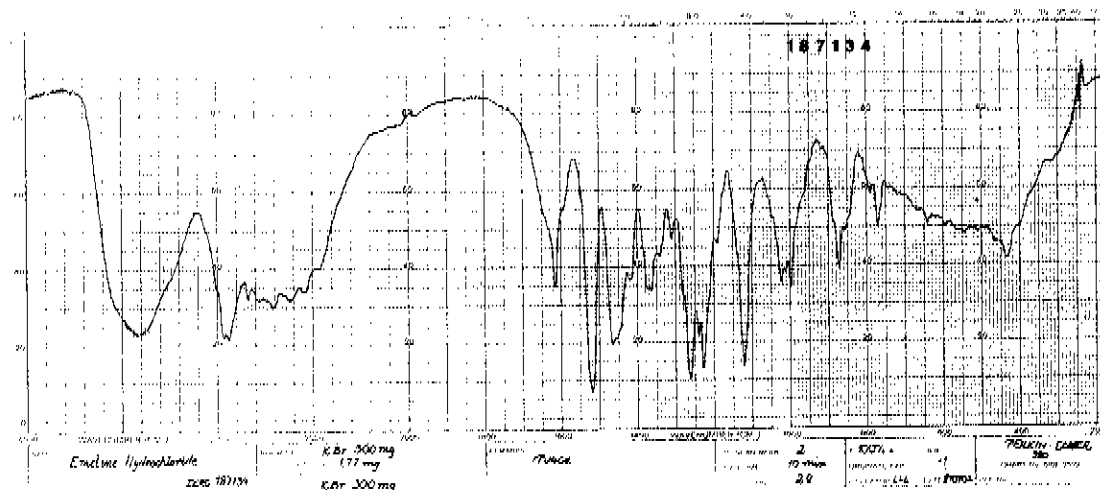


Figure 1. IR-spectrum of 1.77 mg of emetine hydrochloride in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin Elmer 580.

Melting range: 235-243 °C, with decomposition, determined by the capillary method of Ph.Int. Ed III.

Specific optical rotation: $[\alpha]_D^{20} = +17.2^\circ$ (n = 7). Determined in water at a concentration of 50 mg/ml.

The calculations were performed on the dried substance.

UV-spectrum

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

λ max in ethanol = 231 nm and 284 nm.
E (1%, 1 cm) = 291 (n= 4) and 131 (n= 4) respectively.

The determinations were performed with reference to the dried substance.

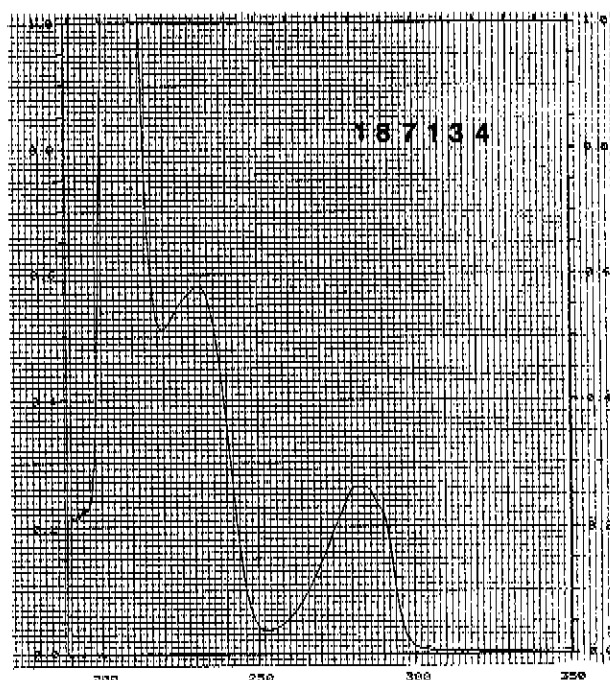


Figure 2. UV-spectrum of emetine hydrochloride 24 μ g/ml in ethanol.

ASSAY

Thermogravimetric analysis

16.8% loss in weight.

Titrimetric assay

99.8% (n= 3) determined by potentiometric titration with 0.1 M perchloric acid according to Ph.Int. Ed III, Vol 3. The titration was performed on substance dried for 2 hours at 130 °C.

PURITY

Total solid impurities

1) Differential thermal analysis (DTA): It was not possible to estimate the purity by this method as the substance melts with decomposition.

Thin-layer chromatography

The following thin-layer chromatographic system was used:

Thin-layer: Silica gel 60 (Merck)

Eluent: Chloroform:ethylene glycol monomethyl ether:methanol:water:diethylamine
(100:20:5:2: 0.5).

Sample: 5 and 100 μ g of emetine hydrochloride were applied. The samples were dissolved in methanol:ammonia conc. (99:1).

Visualization: Spraying with iodine/chloroform TS followed by heating at 60 °C for 15 minutes and examination in UV-light of 365 nm.

R_f (emetine hydrochloride) = 0.7

Rf (cephaeline hydrochloride) = 0.2
Rf (isometine hydrobromide) = 0.5.
The detection limit for cephaeline hydrochloride was 0.15 μg (0.15%).

Result: Four very weak traces were observed, roughly estimated to 0.2% - 0.4%. None of them was identical to cephaeline hydrochloride. However, one of the spots was identical to isoemetine hydrobromide and estimated to 0.2%.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.4%. A chromatogram is shown in Figure 3.

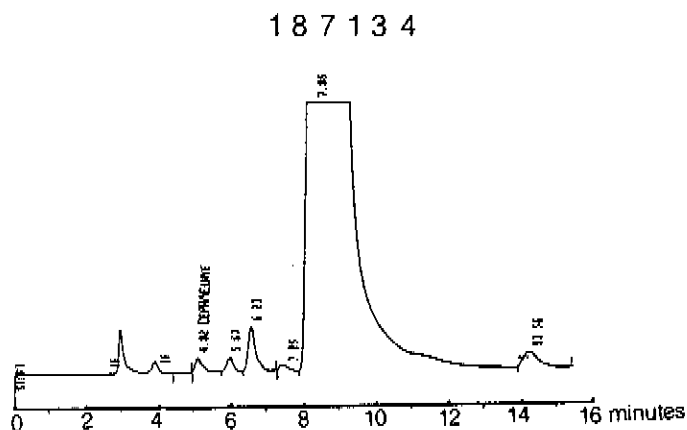


Figure 3. Chromatogram of emetine hydrochloride, Control No 187134.

The following conditions were used:

Eluent: Acetonitrile/Phosphate buffer pH 2 (15:85)
Column: Vydac 218 TP 54 (300 A)
Detector: Shimadzu SPD 2A operated at 230 nm
Pump: Waters 600 operated at a flow rate of 1 ml/min
Integrator: Hewlett Packard 3390A Attenuation: 4
Sample: 1 mg/ml dissolved in the eluent
20 μl corresponding to 20 μg were injected.

Five weak impurity peaks were observed. The peak eluting after 4.8 minutes corresponds to cephaeline which was estimated to 0.04% by peak area measurement. Isoemetine elutes after 8.2 minutes and was subsequently not separated from Emetine Hydrochloride in this system.

DIODE-ARRAY DETECTION

The chromatogram was also evaluated with a LKB 2140 Rapid Diode Array Detector. The same chromatographic system as described above was used, except for the injection volume that was increased to 100 μl to get maximum sensitivity. An isogram is given in Figure 4.

187134

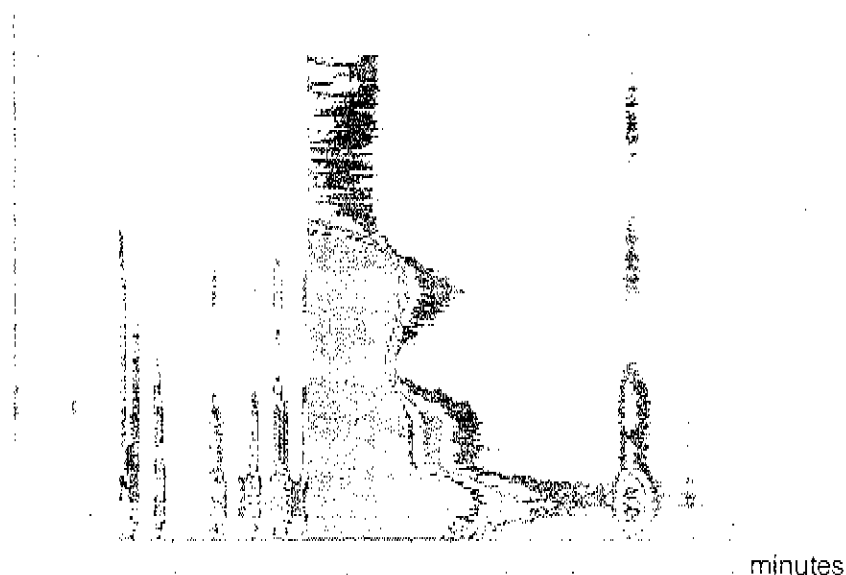


Figure 4. Isogram of emetine hydrochloride, Control No 187134. Sensitivity: 0.002.

As seen from the figure the major impurities, as well as emetine hydrochloride, have their maxima at 205, 230 and 280 nm. The impurities were estimated to 0.4% when the isogram was evaluated by the Nelson chromatography data system at 230 and 280 nm. In Figure 5 chromatograms from the different wavelengths are given.

187134

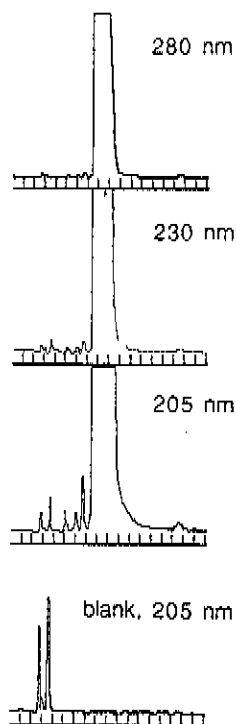


Figure 5. Chromatograms of emetine hydrochloride, Control No 187134 at 280, 230 and 205 nm.

STABILITY

Emetine hydrochloride was exposed to air of different relative humidity at 20 °C in a thermostated incubator (Termaks) for a period of 8 weeks as described in WHO/PHARM/82.509. All samples remained unchanged at visual inspection. Weight changes were noted in all the samples. This is illustrated in Figure 6.

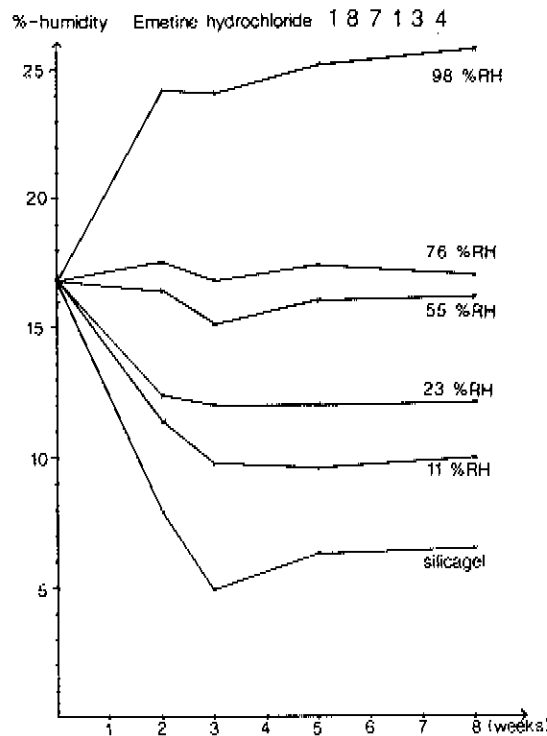


Figure 6. Stability of emetine hydrochloride, Control No 187134 stored at different relative humidity at 20 °C.

As can be seen from the figure, emetine hydrochloride does not form stable hydrates. Water losses take place below 54% relative humidity and water is taken up at higher humidities. It is recommended to store the substance in a desiccator at a relative humidity of about 75%. If stored under other conditions it is important to check the content of water before use.

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

ANALYSIS PERFORMED BY THE MANUFACTURER

Description:	White, crystalline powder; odourless, taste bitter
Identity:	Positive (two tests)
Loss on drying at 105 °C:	16.86%
Acidity (in 100 mg):	0.15 ml N/50 NaOH
Ash:	0.02%
Cephaeline:	Conforms to USPXXI
Assay:	99.08% (on dried substance)

CONCLUSION

Emetine hydrochloride, Control No 187134 can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 12

NEOSTIGMINE METILSULFATE

Control No 187135

The monograph for neostigmine metilsulfate in the International Pharmacopoeia Ed III, Vol 3 requires a reference substance to be used in the infrared spectrophotometric and in the thin-layer chromatographic test for identity.

MATERIAL

About 200 g of the sample (manufacturers batch No 0501007) were received at the WHO Centre in March 1985. The material is being stored protected from light in tightly closed containers at +5 °C.

ANALYTICAL DATA

Description: A white, crystalline powder; odourless.

EVIDENCE OF CHEMICAL STRUCTUREInfrared spectrum

An infrared spectrum is given in Figure 1 (No 187135). The spectrum is concordant with the spectrum obtained from the BP reference substance Batch 1060.

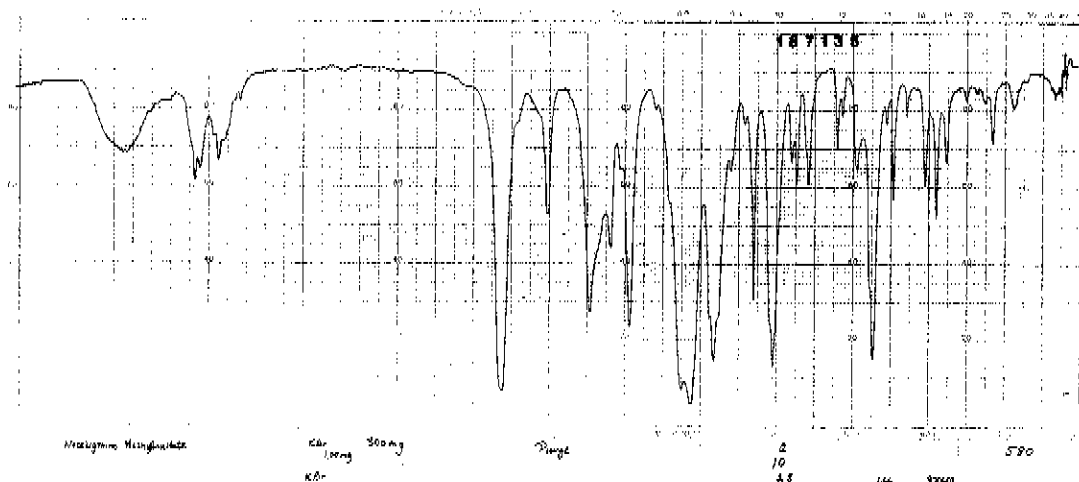


Figure 1. IR-spectrum of 1.0 mg of neostigmine metilsulfate in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin Elmer 580.

UV-spectrum

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

λ max in ethanol = 261 nm
E (1%, 1 cm) = 17 (n= 4)

The absorbance of a 250 µg/ml solution was 0.42. The same result was obtained for USP reference substance Lot G and BP reference substance Batch 1060.

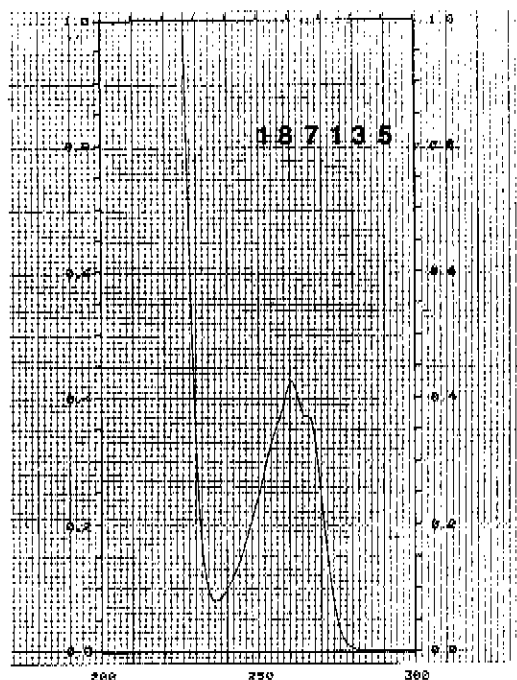


Figure 2. UV-spectrum of neostigmine metilsulfate 255 µg/ml in ethanol.

ASSAY

Loss on drying

0.05% (105 °C) (n= 2)

Thermogravimetric analysis: 0.15% loss in weight.

Titrimetric assay

98.8% (n= 3) determined according to Ph.Int. Ed III, Vol 3 by semi-micro distillation of dimethylamine formed by hydrolysis and subsequent neutralization with sulfuric acid. Finally titration of the excess of sulfuric acid with sodium hydroxide was performed. The difference between the titrations of a blank and a test sample, treated as above, represents the amount of acid required to neutralize the dimethylamine formed from neostigmine.

PURITY

Total solid impurities

1) Differential thermal analysis (DTA): It was not possible to estimate the purity by this method as the substance melts with decomposition. Melting point about 140 °C.

Thin-layer chromatography

The following thin-layer chromatographic systems were used:

Thin-layer: Silica gel 60, F-254 (Merck)
Eluent: Methanol: 3% sodium chloride in water (4 + 1)
Sample: 1, 2 and 200 µg of neostigmine were applied
Visualization: UV-light of 254 nm and at 260 nm by scanning.
After spraying with a modified solution of ninhydrin (0.3 g ninhydrin, 2 ml glacial acetic acid, 2 g sodium acetate, 5 ml water and the volume adjusted with ethanol to 100 ml) heating in an oven at 130 °C for 5-10 min. After cooling, the plate was sprayed with iodoplatinate TS and observed in day-light. R_f (neostigmine metilsulfate) = 0.36. The detection limit for neostigmine metilsulfate was 1 µg (0.5%), when scanned at 260 nm. By spraying the detection limit was 20 µg (10%).

Result: No extra spots were detected. The same result was obtained for the BPCRS Batch 1060 and USP reference substance Lot G.

The thin-layer chromatographic system described in Ph.Int. Ed III, Vol 3 with water:methanol:diethylamine (67 + 30 + 3) as eluent was tested. However, low Rf-values for neostigmine metilsulfate (0.1) were obtained and not even 20 µg was detected, the sensitivity of which is too low for purity determinations.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.4%. Two chromatograms are shown in Figure 3 A + B. As can be seen from Figure A two impurities are observed eluting after 4.9 and 6.4 minutes.

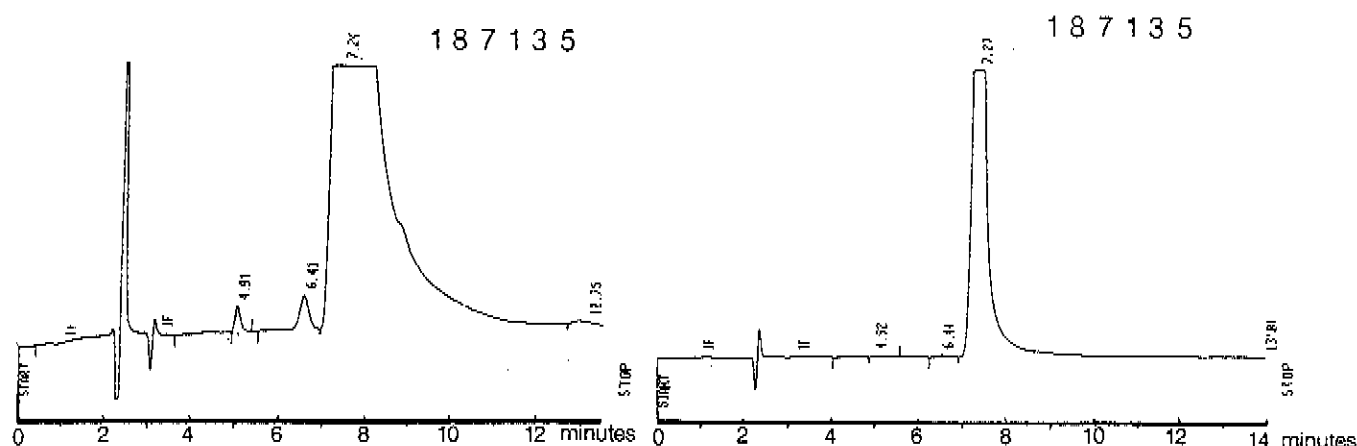


Figure 3 A + B. Chromatograms of neostigmine metilsulfate, Control No 187135. Sensitivity: 4 (A) and 8 (B).

The following conditions were used:

- Eluent: Acetonitrile/Phosphate buffer pH 3 (10:90). The buffer contains 0.01 M sodium heptanesulfonate, 0.01 M sodium dihydrogen phosphate, 0.025 tetrabutylammonium bromide.
The buffer was prepared as follows: Dissolve 2.20 g of sodium heptanesulfonate R, 1.38 g of sodium dihydrogen phosphate R and 0.81 g of tetrabutylammonium bromide R in 900 ml of water, adjust the pH to 3.0 with phosphoric acid conc. and dilute to 1000 ml with water. The column must be conditioned for four hours to get a stable baseline.
- Column: RP-18, Spheri-5.0D-5A (Brownlee)
Detector: Shimadzu SPD-2A operated at 220 nm
Pump: Waters 600 operated at a flow rate of 1 ml/min
Integrator: Hewlett Packard 3392 A Attenuation: 4 and 8
Sample: 1 mg/ml dissolved in the eluent.
20 µl corresponding to 20 µg was injected.

The BPCRS Batch 1060 and the USP reference substance Lot G was also chromatographed in the system as above. They were 99.8% and 99.7% pure, respectively.

DIODE-ARRAY DETECTION

The chromatogram was also evaluated with a LKB 2140 Rapid Diode Array Detector. The same chromatographic system as described above was used except for the injection volume that was increased to 100 µl to get maximum sensitivity. An isogram is given in Figure 4.

187135

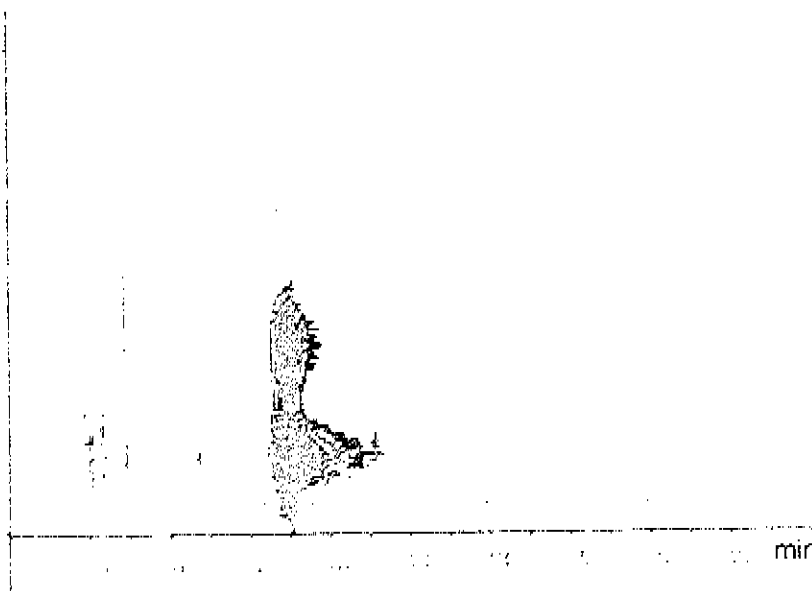


Figure 4. Isogram of neostigmine metilsulfate 187135. Sensitivity: 0.002

The observed impurities had their UV-maxima at 215-220 nm. This means that they were detected by the method described above with the detection wavelength set to 220 nm. The spots eluting at about two minutes originate from the blank.

STABILITY

Neostigmine metilsulfate was exposed to air of different relative humidity at 20 °C in a thermostated incubator (Termaks) for a period of 8 weeks as described in WHO/PHARM/82.509. The samples stored at 75% relative humidity or above, picked up moisture. Neostigmine metilsulfate is hygroscopic and is deliquescent after one weeks exposition to air of relative humidity above 75%. It should be stored in a tightly closed container.

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

Additional data performed by the British Pharmacopoeia Commission Laboratory

Identification by infrared absorption: A spectrum of BCRS 1060 conforms with one from ICRS Control No 187135. The spectra were easily distinguishable from spectra of neostigmine bromide pyridostigmine bromide, physostigmine sulfate and pilocarpine hydrochloride.

Identification by UV absorption: The absorption between 220 nm to 350 nm of a 0.02% w/v solution of ICRS Control No 187135 was measured.
 λ max was 295.5 and 265.5 nm, respectively and A max was 0.574 and 0.486.

Melting range: 147.0-149.5 °C (BP limits 144 to 149 °C)

PURITY

Light absorption: (Content of (3-hydroxyphenyl) trimethylammonium methylsulfate)).
The absorbance of a 0.5% solution at 294 nm was 0.085. Limits > 0.15 = 0.3% (BP).

Related substances (TLC): No secondary spots were detected.

Loss on drying

(105° C): 0.23%

ASSAY

99.16% (titration)

99.6% (according to BP assay of injection)

CONCLUSION

Neostigmine metilsulfate Control No 187135 can be considered suitable as International Chemical Reference Substance for the intended purpose.

PROPRANOLOL HYDROCHLORIDE

Control No 187139

The monograph for propranolol hydrochloride in the International Pharmacopoeia Ed III, Vol 2 requires a reference substance to be used in the infrared spectrophotometric and ultraviolet absorption test for identity.

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

MATERIAL

About 100 g of the sample (manufacturers batch no 1498 A86 P.8274/85) were received at the WHO Centre in March 1986. The material is being stored protected from light in tightly closed containers at +5 °C.

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (No 187139). The spectrum is concordant with the spectrum obtained from the BPCRS Propranolol hydrochloride lot 954.

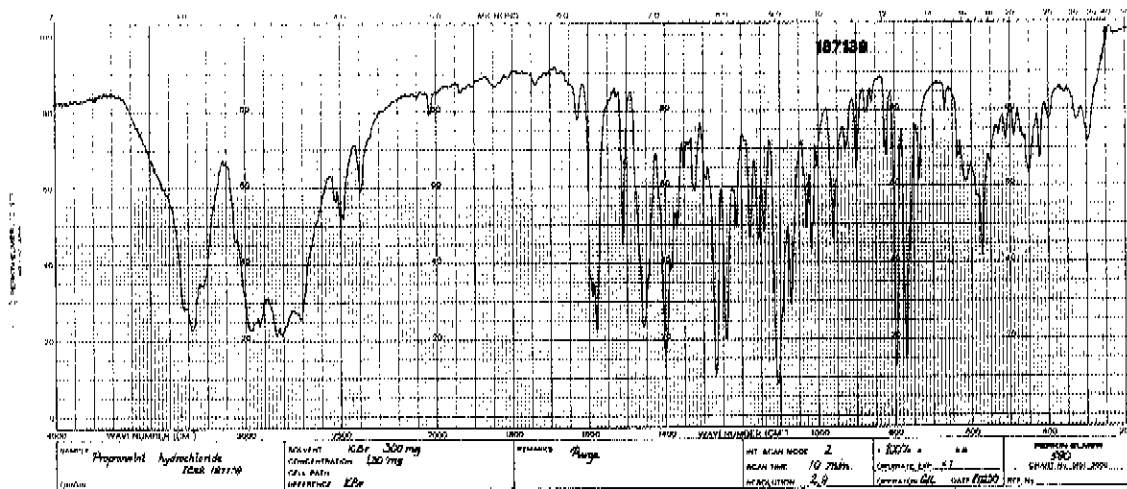


Figure 1. IR-spectrum of 1.30 mg of propranolol hydrochloride in 300 mg KBr recorded against a KBr disc.
Instrument: Perkin Elmer 580.

(*) Infrared spectrum

Two infrared spectra were recorded. A Nujol Mulli and ATR, both of which were recorded on a Perkin Elmer 683 Infrared spectrophotometer. The spectra are concordant with spectra obtained from a British Pharmacopoeia Propranolol reference standard.

(*) UV spectrum

The identification test procedure (B) of the BP 1980 was used and three maxima were observed for a 0.002% w/v solution in methanol.

The measurements were made on a Varian DMS 100 spectrophotometer.

sample		BP 80 (theoretical)	
wavelength (nm)	absorbance	wavelength (nm)	absorbance
290	0.809 (0.2%)**	290	0.84
305	0.462 (0.2%)**	305	0.50
319	0.242 (1.4%)**	319	0.30

** The numbers in brackets are the relative standard deviation for 6 readings.

(*) Melting point

An oil bath/capillary tube system, calibrated against a reference standard of sulfanilamide, was used and the rate of temperature rise adjusted to 2-3 degrees per minute.

Result: 164-165 °C.

(*) GC/MS

GC-MS was carried out using a HP 5988A GC-MS system and a HP-1 Capillary column, 12 x 0.2 mm, with helium as the carrier gas. Mass spectra were obtained with electron impact (EI) under normal conditions.

The sample structure and integrity were confirmed by two different treatments:

Treatment A: A small amount of the sample (<1 mg) was dissolved in dichloromethane (1 ml) and 1 µl injected for analysis.

Result: The total ion chromatogram (TIC) revealed a single peak. The resultant mass spectrum matched up with propranolol in the resident NBSL library and the Pharmaceutical Mass Spectra compilation.

Treatment B: A small amount of the sample (<1 mg) was dissolved in water-ammonia and the solution was extracted with hexane (1 ml). The hexane was evaporated and the residue was dissolved in pyridine. Derivatives were prepared by adding BSTFA to the sample and heating to 90 °C for 20 minutes. The reaction mixture was analysed.

Result: The mass spectrum of the derivatised compound gave fragmentations characteristic of the mono TMS-ether of propranolol.

The sample tested was identified as propranolol and the sample integrity confirmed in the first preparation as the total ion chromatogram did not reveal any other compound in the sample analysed.

(*) ASSAY

Three procedures were used to assess the quantity of propranolol in the sample.

(*) 1) A non-aqueous titration was performed using 0.7 g of material and 1-naphtholbenzein solution as indicator. The method is described in British Pharmacopoeia 1980. The sample was dried at 105 °C for one hour prior to analysis. Assayed content: 99.91%, 99.97%, 99.96%. An RSD of 0.2% was obtained for 3 determinations with a mean result of 99.95%.

(*) 2) UV analysis

A UV spectrum for both a British Pharmacopoeia reference standard (BPRS) and the WHO sample was recorded from 245 to 350 nm on a Varian DMS100 spectrophotometer.

BPRS: 27.75 mg/200 ml water and further diluted 20 ml/100 ml before reading in a 10 mm cell.

WHORS: 57,45 mg/200 ml water and further diluted 20 ml/200 ml before reading in a 10 mm cell.

BPRS: 27.75 µg/ml Abs		WHORS: 28.73 µg/ml Abs	
289 nm	319 nm	289 nm	319 nm
0.543	0.186	0.565	0.194
0.544	0.187	0.565	0.194
0.543	0.187	0.565	0.193
\bar{x} 0.543	0.187	0.565	0.194

The sample content was determined to be:

289 nm: 100.5% (0.1% RSD)

319 nm: 100.2%

(*) 3) HPLC-analysis:

Following the publication in Pharmacopoeial Forum (Jan-Feb 1987) of an evaluation of the USP monograph for propranolol hydrochloride, the raw material sample was examined by the recommended method.

Mobile phase: 0.2% sodium lauryl sulphate, 7.2% 0.15 M H₃PO₄, 36% methanol and 36% acetonitrile.

Column: Altex 5 µ C₁₈, 3.9 mm x 30 cm

Sample solvent: Methanol

Detection

wave length: 290 nm

Sample R_T: 13.5 minutes

A British Pharmacopoeia reference standard (20.21 mg), the WHO sample (20.20 µg) and a manufacturer raw material (20.24 µg) were made up to 100 ml with methanol and 20 µl injected using a fixed volume loop.

Peak areas				\bar{x}	% RSD
BPRS: 109306	110039	109980	110317	109911	0.3
WHORS: 110023	110419	109953	111859	110563	0.7

The content of propranolol was determined to be 100.6% with a % RSD of 0.7.

(*) Loss on drying

0.27 - 0.35 g of material were taken and dried to constant weight at 105° C.

Results: 0.017%, 0.042%, 0.070%, 0.051%
Mean weight loss: 0.045%

PURITY

(*) Acidity

The pH of a 1% w/v solution in water was determined.
pH= 5.13

Total solid impurities

Differential thermal analysis (DTA): About 0.2 mol % (n= 2). The determination was carried out on 2.3 mg using a heating rate of 2 °C per minute.

Melting temperature: 163.1 °C.

Instrument: Mettler TA 2000 system, operated on line with a Hewlett-Packard calculator 9815 A.

Calculation: By the Mettler standard computer program for purity analysis.

(*) Thin-layer chromatography

The system described in the British Pharmacopoeia 1980 monograph for propranolol raw material was used:

Coating substance: Silica gel G

Eluent: Toluene:methanol/90:10 v/v

Sample: 10 µl of each of two solutions in methanol containing:
(1) 10.0% w/v of the substance,
(2) 0.020% w/v of the substance.

Visualization: After drying in air and spraying with a mixture of 0.5 ml of anisaldehyde, 10 ml of glacial acetic acid, 85 ml of methanol and 5 ml of sulfuric acid, heat at 105 °C for fifteen minutes.

Result: The principal spot developed as a pear shaped spot of Rf 0.23. Two very faint spots of at least two orders of magnitude less intense than comparative solution (2) were observed at Rfs 0.33 and 0.45. Solution (2) represents the theoretical impurity limit.

Likely impurities would be propranolol diol, propranolol bio ether and propranolol tert amine.

The TLC appraisal of the sample was confirmed using a modified eluent of methanol: ammonia - 100:1.5. The Rf of the principal spot in the modified system was 0.5 while a faint spot of less than 0.02% remained on the base line.

The chromatogram indicates the raw material supplied is essentially free of impurities when evaluated by this procedure.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.2%. A chromatogram is shown in Figure 2. The main impurity was eluted very close to the main peak at 10.3 minutes.

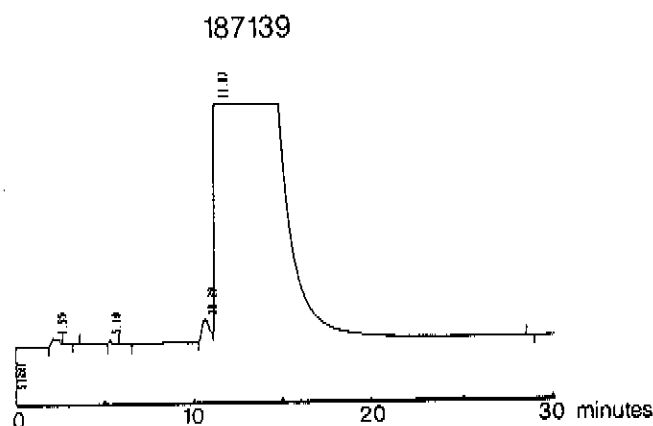


Figure 2. Chromatogram of propranolol hydrochloride, Control No 187139.

The following conditions were used:

Eluent: Acetonitrile/phosphate buffer pH 3.0 (45:55)
Column: RP-18, Spheri-5 (Brownlee)
Detector: Shimadzu operated at 289 nm. Sensitivity: 0.02
Pump: WATERS 600 operated at a flow rate of 1 ml/minute
Integrator: Hewlett Packard 3390 A
Sample: 1 mg/ml dissolved in the eluent.
20 μ l corresponding to 20 μ g were injected.

(*) High Performance Liquid Chromatography

The HPLC procedure used in the sample assay was also used to assess the product purity. The detector sensitivity was increased 8 fold in order to detect the presence of any unknowns.

A second HPLC procedure was used to assess the product purity. The following conditions were used:

Eluent: Methanol, water, acetic acid, with 0.005 M heptane sulfonic acid: 60, 39, 1
Column: Micro BONDAPAC C18 (Waters)
Detector: Perking Elmer variable wavelength, 295 nm, 0.01 AUFS
Pump: WATERS 600 operated at 2 ml/minute
Integrator: Shimadzu CR3A, Attenuation: 0,5
Sample: 10 mg/ml dissolved in water

The system displayed a linear response for variable concentrations and the peak symmetry remained constant.

An impurity at 2.21 minutes was confirmed, with a relative concentration of less than 0.02% w/w.

DIODE-ARRAY DETECTION

The chromatogram was also evaluated with a LKB 2140 Rapid Diode Array Detector. The same chromatographic system as described under figure 2 was used, except for the injection volume that was increased to 100 μ l to get maximum sensitivity. An isogram is given in Figure 3.

187139

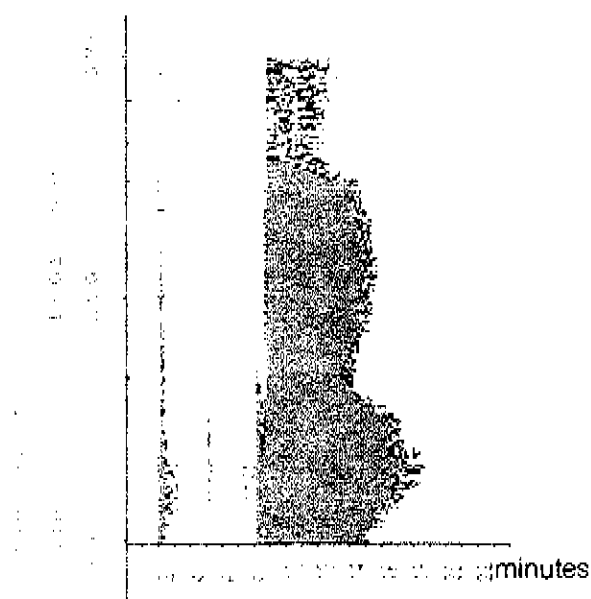


Figure 3. Isogram of propranolol hydrochloride, Control No 187139. Sensitivity: 0.002

As seen from the figure the major impurity eluting after about 8 minutes exhibits UV-maxima at 215 nm and 290 nm. The same maxima were observed for the main peak propranolol hydrochloride. No other impurities were observed at any other wavelength.

(*) Purity - Diode array analysis

The HPLC effluent was further analysed using a Diode Array - LKB 2140 Rapid Spectral Detector with an Olivetti M24 computer.

Spectra were collected at one second intervals from 245 to 340 nm. The spectra were clean.

(*) Purity - gradient HPLC screen

Solvent A: 0,2% (w/v) sodium lauryl sulfate, 7,2% (w/v) 0,15 M H_3PO_4 , 20,8% water, 36% methanol, 36% acetonitrile.

Solvent B: 0,2% (w/v) sodium lauryl sulfate, 7,2% (w/v) 0,15 M H_3PO_4 , 92,8% acetonitrile.

Gradient:	time	sol A%	sol B%	curve
	initial	100	0	-
	10 min	0	100	6

Detection: UV at 290 nm
Sample: 200 μ g/ml methanol

The mobile phase scan indicates that the raw material does not contain any impurities absorbing at 290 nm.

STABILITY

Propranolol hydrochloride, Control No 187139 was exposed to air of different relative humidity at 20 °C in a thermostated incubator (Termaks) for a period of weeks as described in WHO/PHARM/82.509. All samples remained unchanged at visual inspection and no weight changes were noted. No signs of degradation were observed when the samples were analyzed by the liquid chromatographic method described above.

ANALYSIS PERFORMED BY THE MANUFACTURER

Appearance: Fine, white crystalline powder.
Identification: Infrared spectrum conforms with spectrum from standard.
Melting range: 163.1 to 163.6 °C
Strength by determination
of total base: 99.8% w/w
Loss in drying: 0.06% w/w at 105 °C
Sulphated ash: Less than 0.01% w/w
Related impurities: No single impurity exceeds 0.05%

CONCLUSION

Propranolol hydrochloride, Control No 187139 can be considered suitable as International Chemical Reference Substance for the intended purpose.

* * *