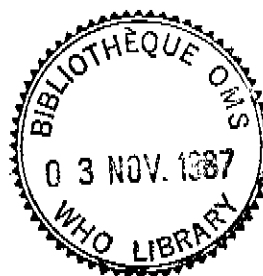




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$\Sigma = 14157$

DISTR. : LIMITED
DISTR. : LIMITEE

WHO/PHARM/87.532

ORIGINAL : ANGLAIS

CENTRE COLLABORATEUR OMS POUR LES SUBSTANCES CHIMIQUES DE REFERENCE

14165

Rapport d'activité pour 1986

par M. Westermark

Table des matières

	<u>Pages</u>
Distribution de substances de référence en 1986	3
Etablissement de substances de référence en 1986	3
Travaux effectués en 1986 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 1986	5
Appendice 2. Liste des substances chimiques internationales de référence établies en 1986	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 1987	15
Appendice 6. Acétazolamide, N° de contrôle 186128	16
Appendice 7. Amino-2 nitro-5 thiazole, N° de contrôle 186131	20
Appendice 8. Chloramphénicol, N° de contrôle 486004	24
Appendice 9. Palmitate de chloramphénicol, N° de contrôle 286072	28

Note : Pour des raisons techniques, les appendices 6 à 16 n'ont été établis qu'en anglais.

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	<u>Pages</u>
Appendice 10. Niridazole, N° de contrôle 186129	35
Appendice 11. Niridazole-chloréthylcarboxamide, N° de contrôle 186130	39
Appendice 12. Noréthistérone, N° de contrôle 186132	44
Appendice 13. Résérpine, N° de contrôle 186133	50
Appendice 14. Acétate de vitamine A, N° de contrôle 686038	56

Distribution de substances de référence en 1986

En 1986, le Centre a distribué à des laboratoires de contrôle pharmaceutique de 46 pays 2239 échantillons de substances chimiques internationales de référence et 25 séries de substances de référence pour la détermination du point de fusion. Ces chiffres représentent une augmentation de 1,4 % par rapport à ceux de 1985. Les cinq substances les plus fréquemment demandées en 1986 ont été, dans l'ordre, le trihydrate d'ampicilline, l'ampicilline sodique, la benzyl-pénicilline sodique, le chlorhydrate de tétracycline et la dicloxacilline sodique. On trouvera à l'appendice 1 le détail de la distribution des diverses substances de référence.

Etablissement de substances de référence en 1986

Conformément à la procédure recommandée par le Comité OMS d'experts des Spécifications relatives aux préparations pharmaceutiques dans son vingt-cinquième rapport (Série de Rapports techniques, N° 567), le Centre a établi en 1986 six substances chimiques internationales de référence, dont on trouvera la liste à l'appendice 2. Parmi ces substances, le tartrate d'ergotamine est un lot de remplacement, le lot précédent ayant été épuisé en 1986.

On trouvera à l'appendice 3 une liste complète de toutes les substances chimiques internationales de référence détenues par le Centre en janvier 1987, 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 9 substances mentionnées ci-dessous, dont on peut prévoir qu'elles seront officiellement adoptées au cours du premier semestre de 1987.

Travaux effectués en 1986 sur de nouvelles substances de référence

Le Centre a poursuivi ses travaux en vue de fournir les nouvelles substances de référence qui seront nécessaires pour accompagner les spécifications de la troisième édition de la Pharmacopée internationale. En 1986, l'analyse des nouvelles substances de référence suivantes a été réalisée : acétazolamide, amino-2 nitro-5 thiazole, niridazole, niridazole-chloréthylcarboxamide, noréthistérone et réserpine. Les rapports d'analyse pour ces substances figurent aux appendices 6, 7, 10, 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 trois lots suivants de substances chimiques internationales de référence ont été épuisés et ont été remplacés par de nouveaux lots en 1986. Le lot N° 379004 de chloramphénicol a été remplacé par le lot N° 486004, le lot N° 175072 de palmitate de chloramphénicol a été remplacé par le lot N° 286072 et le lot N° 581038 d'acétate de vitamine A a été remplacé par le lot N° 686038. Les résultats de l'analyse de ces lots figurent aux appendices 8, 9 et 14.

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 1986, 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.

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 3 substances pour accompagner les monographies du volume 2 de la Pharmacopée internationale. Pour deux d'entre elles, les travaux seront achevés dans le courant de 1987. Pour le volume 3, il faut encore 45 nouvelles substances de référence, dont 11 sont déjà à l'étude. D'anciens lots devront également être remplacés en raison de l'épuisement des stocks. Actuellement, trois substances doivent être remplacées en 1987, 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 1986 a commencé l'informatisation des activités liées au travail sur les substances de référence. 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. On espère qu'elle facilitera la préparation des nouvelles substances de référence devant accompagner le volume 3 de la Pharmacopée internationale.

Questions administratives et financières

La situation financière du Centre reste mauvaise. Le coût de fonctionnement total du Centre en 1986 a été estimé à US \$207 000. Le revenu provenant des ventes de substances de référence aux laboratoires industriels a été d'environ US \$19 000 et la contribution du Siège de l'OMS de US \$16 000, ce qui laisse un déficit de US \$172 000. Le Conseil d'administration de l'Association nationale des Pharmacies suédoises a convenu de maintenir au même niveau sa contribution au fonctionnement du Centre, mais a instamment demandé que tout soit mis en oeuvre pour diminuer le déficit.

En 1986, le prix des substances est resté fixé à US \$25 par paquet. Toutefois, dès janvier 1987, il sera porté à US \$40 par paquet et des frais d'expédition et de manipulation s'élevant à US \$10 seront 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. Cette année, nos remerciements vont en particulier à Ercopharm, Vedbaeck, Danemark, à CIBA-GEIGY AG, Bâle, Suisse, à Farmitalia Carlo Erba, Milan, Italie, à SYNTEX, Palo Alto, Etats-Unis d'Amérique et à l'US Pharmacopial Convention Inc., Etats-Unis d'Amérique.

DISTRIBUTION DE SUBSTANCES CHIMIQUES DE REFERENCE EN 1986

Acéclidine, salicylate d'	4	échantillons	Fluphénazine, décanoate de (dichlorhydrate)	11	échantillons
p-Acétamidobenzalazine	3	"	Fluphénazine, énantate de (dichlorhydrate)	10	"
Allopurinol	2	"	Folique, acide	36	"
Amino-3 pyrazole carboxamide-4, hémissulfate d'	7	"	Furosémide	15	"
Amitryptiline, chlorhydrate d'	24	"	Griséofulvine	22	"
Ampicilline	54	"	Halopéridol	13	"
Ampicilline sodique	82	"	Hydrochlorothiazide	13	"
Ampicilline, trihydrate d'	84	"	Hydrocortisone	39	"
Anhydrotétracycline, chlorhydrate d'	46	"	Hydrocortisone, acétate d' (-)-(Hydroxy-4 méthoxy-3 phényl)-3 méthyl-2 alanine	35	"
Atropine, sulfate d'	24	"	Ibuprofène	3	"
Azathioprine	4	"	Imipramine, chlorhydrate d'	19	"
Benzadol, chlorhydrate de	4	"	Indométacine	12	"
Benzobarbital	8	"	o-Iodohippurique, acide	23	"
Benzylamine, sulfate de	4	"	Isoniazide	4	"
Benzylpénicilline potassique	50	"	Lanatoside C	14	"
Benzylpénicilline sodique	70	"	Lévodopa	17	"
Béphénium, hydroxynaphtoate de	10	"	Lidocaïne	5	"
Bétaméthasone	21	"	Lidocaïne, chlorhydrate de	16	"
Bétanidine, sulfate de	4	"	Méfénamique, acide	29	"
NN'-bis(xylyl 2,3) anthranilamide	4	"	Métazide	4	"
Rupivacaïne, chlorhydrate de	5	"	Méthaquealone	3	"
Caféine	17	"	Méthaldopa	8	"
Carbénicilline monosodique	25	"	Méthyltestostérone	11	"
Chloramphénicol	36	"	Méticilline sodique	10	"
Chloramphénicol, palmitate de	18	"	Métronidazole	16	"
Chloramphénicol, palmitate de (forme A)	40	"	Nafcilline sodique	22	"
Chloro-5 méthylamino-2 benzophénone	6	"	Nicotinamide	7	"
(Chloro-4 sulfamoyl-3 benzoyl)-2 benzoïque, acide	11	"	Nicotinique, acide	30	"
Chlorphénamine, hydrogénomaléate de	8	"	Noréthistérone, acétate de	17	"
Chlorpromazine, chlorhydrate de	21	"	Oxabaïne	5	"
Chlortalidone	5	"	Oxacilline sodique	6	"
Cloxacilline sodique	33	"	Papavérine, chlorhydrate de	39	"
Cortisone, acétate de	26	"	Phénéticilline potassique	6	"
Dapsone	13	"	Phénoxyéthylpénicilline	7	"
Désoxycortone, acétate de	9	"	Phénoxyéthylpénicilline calcique	32	"
Dexaméthasone	36	"	Phénoxyéthylpénicilline potassique	8	"
Dexaméthasone, acétate de	11	"	Phénytoïne	34	"
Diazépam	23	"	Prednisolone	9	"
Diazoxide	6	"	Prednisolone, acétate de	39	"
Dicloxacilline sodique	57	"	Prednisone	15	"
Dicolinium, iodure de	3	"	Prednisone, acétate de	25	"
Dicoumarol	8	"	Procaïne, chlorhydrate de	12	"
Diéthylcarbamazine, dihydro- génocitrate de	3	"	Procarbazine, chlorhydrate de	14	"
Digitoxine	20	"	Progéstérone	7	"
Digoxine	38	"	Propicilline potassique	18	"
Epi-4 anhydrotétracycline, chlorhydrate d'	35	"	Propylthiouracil	19	"
Epi-4 tétracycline, sel d'ammonium de l'	31	"	Pyridostigmine, bromure de	2	"
Ergométrine, hydrogénomaléate d'	17	"	Riboflavine	9	"
Ergotamine, tartrate d'	20	"	Rose Bengale sodique	25	"
Estradiol, benzoate d'	10	"	Sulfaméthoxazole	3	"
Estrone	8	"	Sulfaméthoxy-pyridazine	33	"
Etacrynique, acide	4	"	Sulfanilamide	10	"
Ethambutol, chlorhydrate d'	12	"	Testostérone, propionate de	12	"
Ethinylestradiol	32	"	Tétracycline, chlorhydrate de	16	"
Ethistérone	10	"	Thioacétazone	59	"
Ethosuximide	5	"	Thiodianiline-4,4'	4	"
Etocarlide	3	"	Tolbutamide	7	"
Flucytosine	6	"	Tolnaftate	6	"
Fluorouracil	19	"	Triméthoprime	7	"
Fluphénazine, chlorhydrate de	13	"	Triméthylguanidine, sulfate de	31	"
			Tubocurarine, chlorure de	3	"
			Vitamine A, acétate de (soluté)	3	"
			Warfarine	26	"
				12	"

2 264 échantillons

LISTE DES SUBSTANCES CHIMIQUES INTERNATIONALES DE REFERENCE ETABLIES EN 1986

Substance de référence	N° de contrôle	Rapport d'analyse	Remarques
Ergotamine, tartrate d'	385013	WHO/PHARM/86.527 Appendice 6	Remplace le N° 276013
Isoniazide	185124	WHO/PHARM/86.527 Appendice 7	
Noréthistérone, acétate de	185123	WHO/PHARM/86.527 Appendice 8	
Papavérine, chlorhydrate de	185127	WHO/PHARM/86.527 Appendice 9	
Propyathiouracile	185126	WHO/PHARM/86.527 Appendice 10	
Triméthadione	185125	WHO/PHARM/86.527 Appendice 11	

LISTE DES SUBSTANCES CHIMIQUES INTERNATIONALES DE REFERENCE DISPONIBLES

1987

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
Amino-2 nitro-5 thiazole	25 mg	186131
Allopurinol	100 mg	172049
Amino-3 pyrazole carboxamide-4, hémisulfate d'	100 mg	172050
Amitriptyline, 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	180096
Atropine, sulfate d'	100 mg	183111
Azathioprine	100 mg	172060
Benzazol, chlorhydrate de	100 mg	173066
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
Bupivacaïne, chlorhydrate de	100 mg	172054
Caféine	100 mg	181102
Carbénicilline monosodique	200 mg	383043
Chloramphénicol	200 mg	486004
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 benzoïque, acide	50 mg	181106
Chlorphénamine, hydrogénomaléate de	100 mg	182109
Chlorpromazine, chlorhydrate de	100 mg	178080
Chlortalidone	100 mg	183114
Cloxacilline sodique	200 mg	274005
Cortisone, acétate de	100 mg	167006
Dapsone	100 mg	183115
Désoxycortone, acétate de	100 mg	167007
Dexaméthasone	100 mg	279008
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
Dicomarol	100 mg	178077
Diéthylcarbamazine, dihydrogénocitrate de	100 mg	181100
Digitoxine	100 mg	277010
Digoxine	100 mg	377011
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
Étaacrynique, acide	100 mg	281056
Ethambutol, chlorhydrate d'	100 mg	179081
Ethinylestradiol	100 mg	167016
Ethistérone	100 mg	167017
Ethosuximide	100 mg	179088
Etocarlide	100 mg	172057
Flucytosine	100 mg	184121
Fluorouracil	100 mg	184122
Fluphénazine, chlorhydrate de	100 mg	176076
Fluphénazine, décamoate de (dichlorhydrate)	100 mg	182107
Fluphénazine, énantiote de (dichlorhydrate)	100 mg	182108
Folique, acide	100 mg	277019
Furosémide	100 mg	171044
Griséofulvine	200 mg	280040
Halopéridol	100 mg	172063
Hydrochlorothiazide	100 mg	179087
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

Substances de référence	Conditionnement	Numéro de contrôle du lot actuel
Imipramine, chlorhydrate d'	100 mg	172064
Indométacine	100 mg	178078
o-Iodohippurique, acide	100 mg	171045
Isoniazide	100 mg	185124
Lanatoside C	100 mg	281022
Lévodopa	100 mg	172065
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
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	167028
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
Prednisone	100 mg	167031
Prednisone, acétate de	100 mg	169032
Procaïne, chlorhydrate de	100 mg	183119
Procabazine, chlorhydrate de	100 mg	184120
Progestérone	100 mg	167033
Propicilline potassique	200 mg	274034
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
Sulfaméthoxy pyridazine	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 (soluté)	5 capsules*	686038
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 1986 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 :

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

La valeur estimée des impuretés solides totales, obtenue par HPLC et TLC est exprimée en aire pour cent sauf indication contraire; lorsqu'elle est obtenue par DTA, elle est exprimée en mole pour cent, et par PSA en poids pour cent. 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.

Benzobarbital, N° de contrôle 172051

Premier rapport d'analyse : WHO/PHARM/72.471, appendice 13

Année d'examen	1972	1977	1981	1987
Absorption UV à 250 nm	0,495	0,499	0,495	0,486
TLC	Pas de tache secondaire	Une tache secondaire	2 taches secondaires (< 1 %)	4 à 5 taches secondaires (environ 0,5 %)
IR	Conforme	-	-	Conforme
DTA, %	-	-	1,7	Environ 1
HPLC, %	-	-	-	0,3
Perte à la dessiccation, %	0,4	0,02	0,1	0,1
Titration, % (potentiométrique)	100,0	100,2	-	100,3

Digitoxine, N° de contrôle 277010

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

Année d'examen	1977	1987
TLC	5 légères taches secondaires	Pas de tache secondaire
IR	Conforme	Conforme
HPLC, %	Pas de contaminant	Environ 0,1
Perte à la dessiccation, %	0,6	0,6
Titrage, % (colorimétrique)	99,7	100,7

Hydrochlorothiazide, N° de contrôle 179087

Premier rapport d'analyse : WHO/PHARM/80.504, appendice 8

Année d'examen	1979	1987
IR	Conforme	Conforme
DTA, %	0,4	0,7
HPLC, %	0,4	0,4
Perte à la dessiccation, %	0,0	0,2

Lanatoside C, N° de contrôle 281022

Premier rapport d'analyse : WHO/PHARM/82.509, appendice 12

Année d'examen	1981	1987
TLC	5 taches secondaires	5 taches secondaires
IR	Conforme	Conforme
HPLC, % (0,4 % sous forme de lanatoside B)	0,8	1,0
Perte à la dessiccation, %	7,2	7,2
Titrage, %	99,9	99,9

Nicotinamide, N° de contrôle 179090

Premier rapport d'analyse : WHO/PHARM/80.504, appendice 11

Année d'examen	1979	1987
Absorption UV à 263 nm	0,59	0,59
TLC	2 taches secondaires	2 taches secondaires
DTA, %	Environ 0,1	0,04
IR	Conforme	Conforme
Perte à la dessiccation, %	0,3	0,0
Titrage, % (potentiométrique)	100,0	99,8

Nicotinique, acide, N° de contrôle 179091

Premier rapport d'analyse : WHO/PHARM/80.504, appendice 12

Année d'examen	1979	1987
Absorption UV à 263 nm	0,57	0,59
TLC	Pas de tache secondaire	Pas de tache secondaire
DTA, %	0,1	0,1
IR	Conforme	Conforme
Perte à la dessiccation, %	0,05	Environ 0,2
Titrage, % (potentiométrique)	99,8	99,9

Ouabaïne, N° de contrôle 283026

Premier rapport d'analyse : WHO/PHARM/84.513, appendice 14

Année d'examen	1983	1987
Perte à la dessiccation, %	20,0	19,9
HPLC, %	0,4	0,7
TLC	0,2	0,3
Titrage, % (réaction de Baljet)	100,1	100,0

Prednisolone, N° de contrôle 283029

Premier rapport d'analyse : WHO/PHARM/84.523, appendice 15

Année d'examen	1983	1987
Absorption UV à 263 nm	0,417	0,416
TLC, %	1,6 2 taches secondaires	Environ 2,5 2 taches secondaires
Perte à la dessiccation, %	0,08	-
KF (eau), %	-	0,2
IR	Conforme	Conforme
HPLC, %	1,4	2,1
Titrage, % (spectrophotométrique)	100,0	99,9

Prednisolone, acétate de, N° de contrôle 167030

Premier rapport d'analyse : WHO/PHARM/66.431, appendice 7

Année d'examen	1966	1975	1984	1987
Absorption UV à 242 nm, E (1 %, 1 cm)	382	377	377	376
Perte à la dessiccation, %	0,0	0,2		0,0
TLC	2 taches secondaires	1 tache secondaire	3 taches secondaires	3 taches secondaires
IR	Conforme	-	-	Conforme
HPLC, %	-	-	1,8	2,2
PSA, %	0,5	-	-	-

Riboflavine, N° de contrôle 382035

Premier rapport d'analyse : WHO/PHARM/83.510, appendice 10

Année d'examen	1982	1987
IR	Conforme	Conforme
Perte à la dessiccation, %	0,3	0,4
HPLC, %	<1	0,6
Titrage, % (spectrophotométrique)	99,5	100,0

Sulfaméthoxypyridazine, N° de contrôle 178079

Premier rapport d'analyse : WHO/PHARM/79.499, appendice 11

Année d'examen	1978	1987
IR	Conforme	Conforme
DTA, %	0,2	0,3
HPLC, %	-	0,22
Perte à la dessiccation, %	0,0	0,2
Titrage, % (potentiométrique)	99,8	100,4

Tolbutamide, N° de contrôle 179086

Premier rapport d'analyse : WHO/PHARM/80.504, appendice 16

Année d'examen	1979	1987
TLC, %	Pas de tache secondaire	Pas de tache secondaire
IR	Conforme	Conforme
DTA, %	0,2	0,2
Perte à la dessiccation, %	0,1	0
HPLC, %	0,02	0,01

SUBSTANCES CHIMIQUES INTERNATIONALES DE REFERENCE

LISTE PREVISIONNELLE POUR 1987

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

Chlortétracycline, chlorhydrate de (*)
 Colécalciférol
 Propranolol, chlorhydrate de (*)

Volume 3

Amodiaquine, chlorhydrate d'
 Amphotéricine B (*)
 Bacitracine Zinc
 Béclometasone, dipropionate de
 Bétaméthasone, valérate de
 Calcium, folinate de
 Carbamazépine (*)
 Cimétidine
 Clomifène, citrate de (*)
 Clomifène, citrate de, isomère Z (*)
 Dexaméthasone sodique, phosphate de
 Dopamine, chlorhydrate de
 Doxorubicine, chlorhydrate de
 Émétine, chlorhydrate d' (*)
 Ergocalciférol
 Fludrocortisone, acétate de
 Formyl-3 rifamycine SV
 (impureté de la rifampicine)
 Gentamicine, sulfate de
 Hydrocortisone sodique, succinate d'
 (-)-(Hydroxy-4 méthoxy-3 phényl)-3
 hydrazino-2 méthyl-2 alanine
 (impureté du carbidopa)
 Lévonorgestrel
 Lévothyroxine sodique
 Liothyronine
 (impureté de la lévothyroxine sodique)

Lopéramide, chlorhydrate de
 Méthotrexate
 Néamine (impureté du sulfate de néomycine)
 Néomycine B, sulfate de
 (impureté du sulfate de néomycine)
 Néostigmine, méthilsulfate de (*)
 Nifurtimox
 Noroxymorphone, chlorhydrate de
 (impureté du chlorhydrate de naloxone)
 Nystatine
 Oxytétracycline, dihydrate d' (*)
 Oxytétracycline, chlorhydrate d' (*)
 Paromomycine, sulfate de
 Praziquantel
 Prednisolone sodique, phosphate de
 Probenécide (*)
 Pyrantel, embonate de (*)
 Rifampicine-quinone
 (impureté de la rifampicine)
 Salazosulfapyridine
 Sodium, cromoglicat de
 Spectinomycine, chlorhydrate de
 Sulfacétamide
 Testostérone, énantate de
 Vincristine, sulfate de

Remplacements

Les substances chimiques internationales ci-dessous devront être remplacées par de nouveaux lots en 1987 :

Allopurinol (*)
 Digoxine (*)

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

A C E T A Z O L A M I D E

Control No 186128

Analytical Report

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

MATERIAL

About 100 g of the sample (manufacturers lot no R 6233) were received at the WHO Centre in November 1984. 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 186128). The spectrum is concordant with the spectrum obtained from the USP reference substance Lot I.

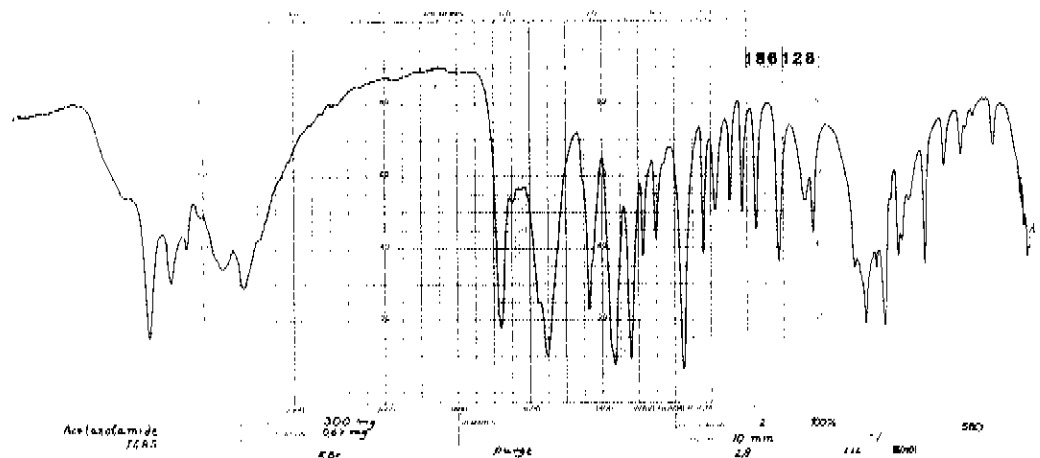


Figure 1. IR-spectrum of 0.7 mg of acetazolamide in 300 mg KBr, recorded against a KBr reference disc. Instrument: Perkin Elmer 580.

Elemental analysis

	C(%)	H(%)	N(%)
Theoretical	21.6	2.7	25.2
Found	21.7	2.7	25.2

The analysis was performed at Mikro Kemi AB, Uppsala.

UV-spectrum

A UV-spectrum in 0.1 M sodium hydroxide is given in Figure 2.
 λ max in 0.1 M sodium hydroxide = 291 nm. $E(1\%, 1 \text{ cm}) = 601$ ($n=8$). Spectra were also recorded in ethanol, methanol and acetonitrile λ max were 264 nm, 264 nm and 263 nm, respectively. However, irregular E-values were obtained due to solubility problems.

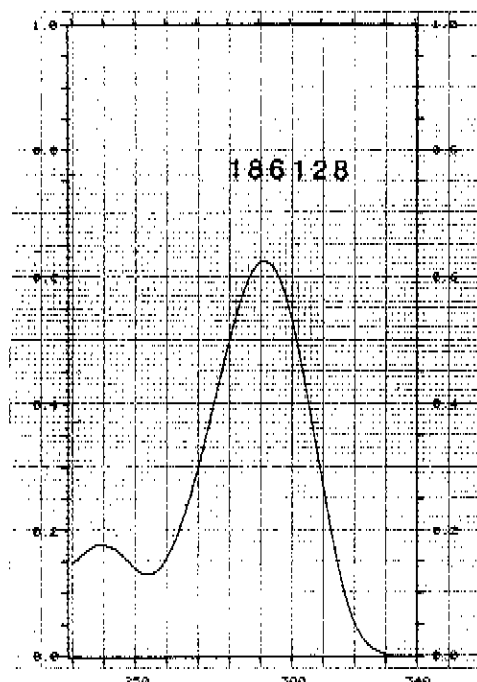


Figure 2. UV-spectrum of acetazolamide 10.4 $\mu\text{g/ml}$ in 0.1 M sodium hydroxide.

ASSAY

99.7% ($n=7$). Determined by potentiometric titration with 0.1 M tetrabutylammonium hydroxide (solution in methanol/toluene) according to Ph. Int. Ed. III, Vol 2.

Loss on drying

0.15% (105 °C)

PURITY

Total solid impurities

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 chromatography systems were used.

I. Thin-layer: Silica gel 60, F-254 (Merck)
Eluent: Ethyl acetate:acetone:chloroform:methanol (5 + 5 + 5 + 1)
Sample: 100 μg of acetazolamide were applied. 20 mg of the substance was dissolved in 0.5 ml of DMF followed by the addition of 1.5 ml of acetone.
Visualization: UV-light at 254 nm.

R_f (acetazolamide) = 0.4

Result: No extra spots were detected. The detection limit for the system was less than 0.1 µg (0.1%). The chromatogram was evaluated using a Zeiss KM3 Chromatogram Spectrophotometer operated in the reflectance mode at 264 nm.

A comparison was made with USP reference substance Lot I which contained two impurities estimated to about 0.5%.

II. This system was used in order to look for hydrazine.

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

Eluent: Acetone:water (98 + 2)

Sample: 1000 µg of acetazolamide were applied. The substance was dissolved as above. As reference 0.1 µg hydrazine hydrate was applied.

Visualization: Spraying with 4-dimethylaminobenzaldehyde and examination in daylight.

Result: No extra spots were detected.

High performance liquid chromatography

No impurities were found. A chromatogram is shown in Figure 3.

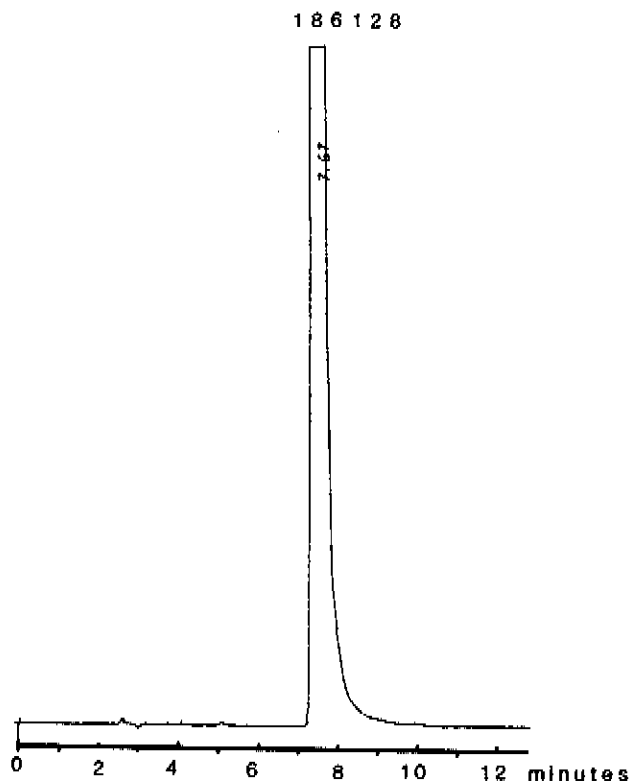


Figure 3. A chromatogram of acetazolamide Control No 186128. In USP reference substance Lot I about 0.9% impurities were found.

The following conditions were used:

Eluent: Acetonitrile/Acetate buffer pH 4.0 (10:90)

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

Detector: Varian UV-200 operated at 254 nm

Pump: Varian 5560 operated at a flow rate of 1.0 ml/min

Integrator: Varian 4270 Attenuation: 1

Sample: 1 mg/ml. First 25 mg were dissolved in 2.5 ml of acetonitrile, thereafter 2.5 ml of eluent were added and the final dilution was performed with eluent. 10 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 which was changed to 100 μ l. An isogram is given in Figure 4.

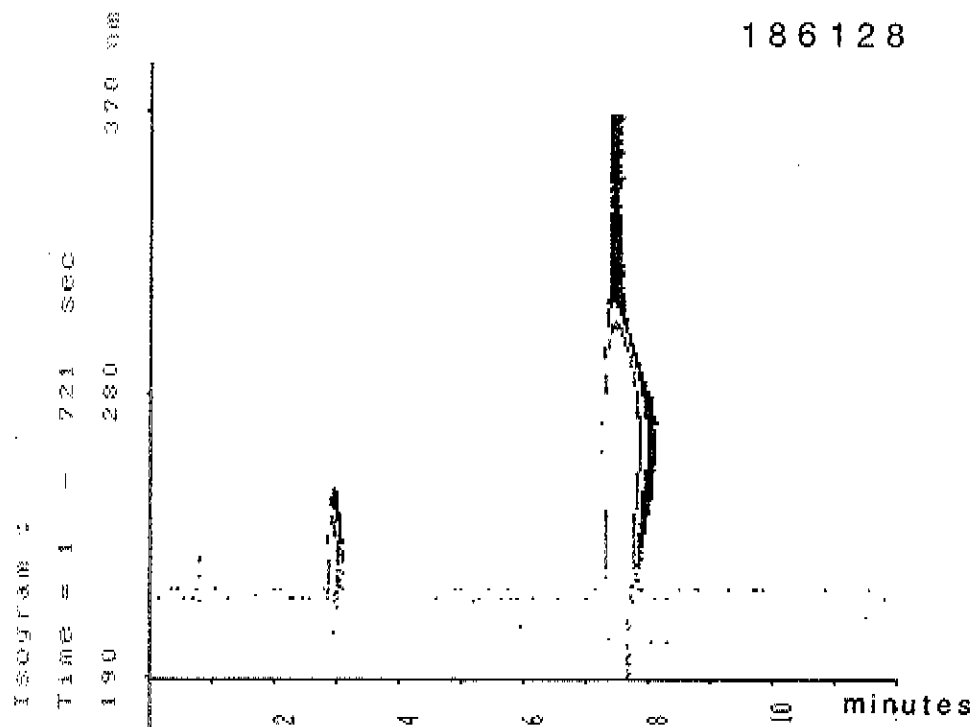


Figure 4. Isogram of acetazolamide Control No 186128. Sensitivity: 0.005

As seen from the figure no impurities are observed at any wavelength. Acetazolamide eluting after about 7.5 minutes has a maximum absorbance at 265 nm. The peak purity was tested by recording spectra at the up- and downslope of the peak. They were all identical with maxima at 265 nm. The small spot at about 3 minutes originates from the blank.

STABILITY

Acetazolamide was exposed to air of different relative humidity at room temperature (about 20°C) for a period of 6 weeks as described in WHO/PHARM/82.509. All samples were unchanged at visual inspection and no weight changes were noted. No signs of degradation were observed when selected samples were analyzed by the liquid chromatographic method described above.

DATA GIVEN BY THE MANUFACTURER

Description	A white crystalline powder, odourless.
Identification	Conforms
Clarity and colour of solution	Conforms
Acidity or alkalinity	Conforms
Sulfate	<0.5 ‰
Heavy metals	<10 ppm
Sulfated ash	0.0%
Assay	99.5%

CONCLUSION

Acetazolamide Control No 186128 can be considered suitable as International Chemical Reference Substance for the intended purpose.

2 - A M I N O - 5 - N I T R O T H I A Z O L E

Control No 186131

Analytical Report

The International Chemical Reference Substance for 2-amino-5-nitrothiazole is intended to be used in the thin-layer chromatographic test for related substances according to the monograph for niridazole in the International Pharmacopoeia, Ed. III, Vol 3.

MATERIAL

About 25 g of the sample (manufacturers batch no Sch-1695-10) were received at the WHO Centre in December 1984. The material is being stored protected from light in a tightly closed container at +5 °C.

ANALYTICAL DATA

Description: A yellow powder, almost odourless.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (no 186131). The spectrum is concordant with a reference spectrum received from the manufacturer.

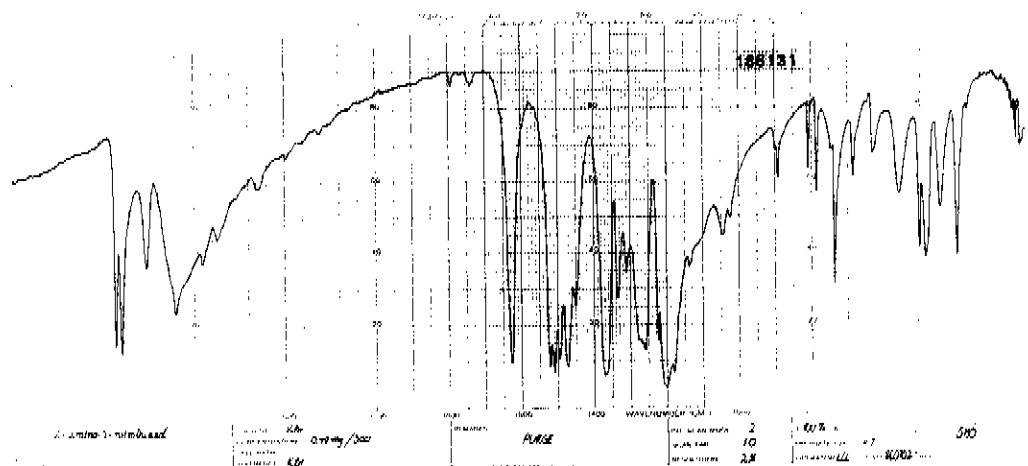


Figure 1. IR-spectrum of 0.5 mg of 2-amino-5-nitrothiazole in 300 mg KBr recorded against a KBr disc. Instrument: Perkin Elmer 580.

Melting temperature

About 195-200 °C with decomposition, determined by the capillary method of Ph. Int. Ed. III. According to Merck Index, Ed 9, it decomposes at 202 °C.

Elemental analysis: The analysis was performed at Mikro Kemi AB, Uppsala.

	C (%)	H (%)	N (%)
Theoretical	24.8	2.1	29.0
Found	24.8	1.9	28.5

UV-spectrum

A UV-spectrum in 0.1% dimethylformamide in ethanol is given in Figure 2. The substance is first dissolved in dimethylformamide and then diluted with ethanol.

λ max in ethanol = 379 nm
E (1%, 1cm) = 1037 (n= 5)

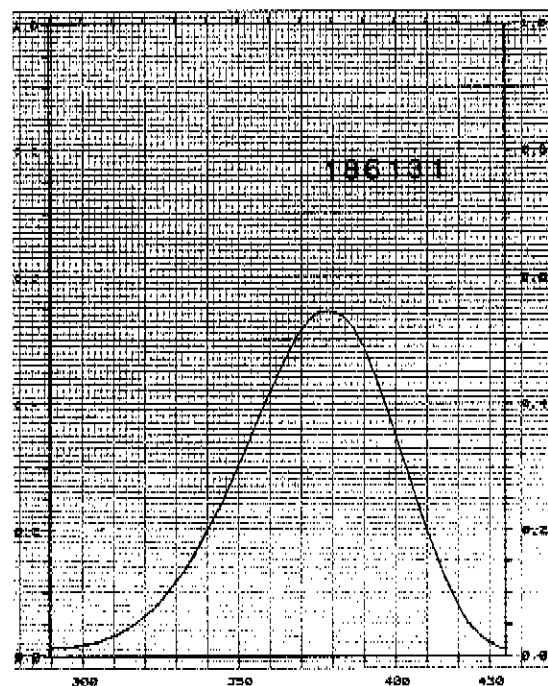


Figure 2. UV-spectrum of 2-amino-5-nitrothiazole 5 μ g/ml in ethanol.

Loss on drying

0.5% (100 °C, reduced pressure)

PURITY

Total solid impurities

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 TLC systems were used.

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

Eluent: Toluene: Acetone: Acetic acid: Isobutanol (12 + 8 + 3 + 8)

Sample: 100 μ g of 2-amino-5-nitrothiazole were applied.

Visualization: UV-light of 254 nm and 365 nm visually and 379 nm by scanning. Rf (niridazole) = 0.48; Rf (2-amino-5-nitrothiazole) = 0.55; Rf (niridazole-chlorethyl-carboxamide) = 0.58. The detection limit for 2-amino-5-nitrothiazole was 0.006 μ g (0.006%) when scanned at 379 nm.

Result: No impurities were found.

The thin-layer chromatographic system described in Ph. Int. Ed, III, Vol. 3 with toluene:acetone (12+8) as eluent was tested but 2-amino-5-nitrothiazole did not separate from niridazole. No impurities were found.

High performance liquid chromatography

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

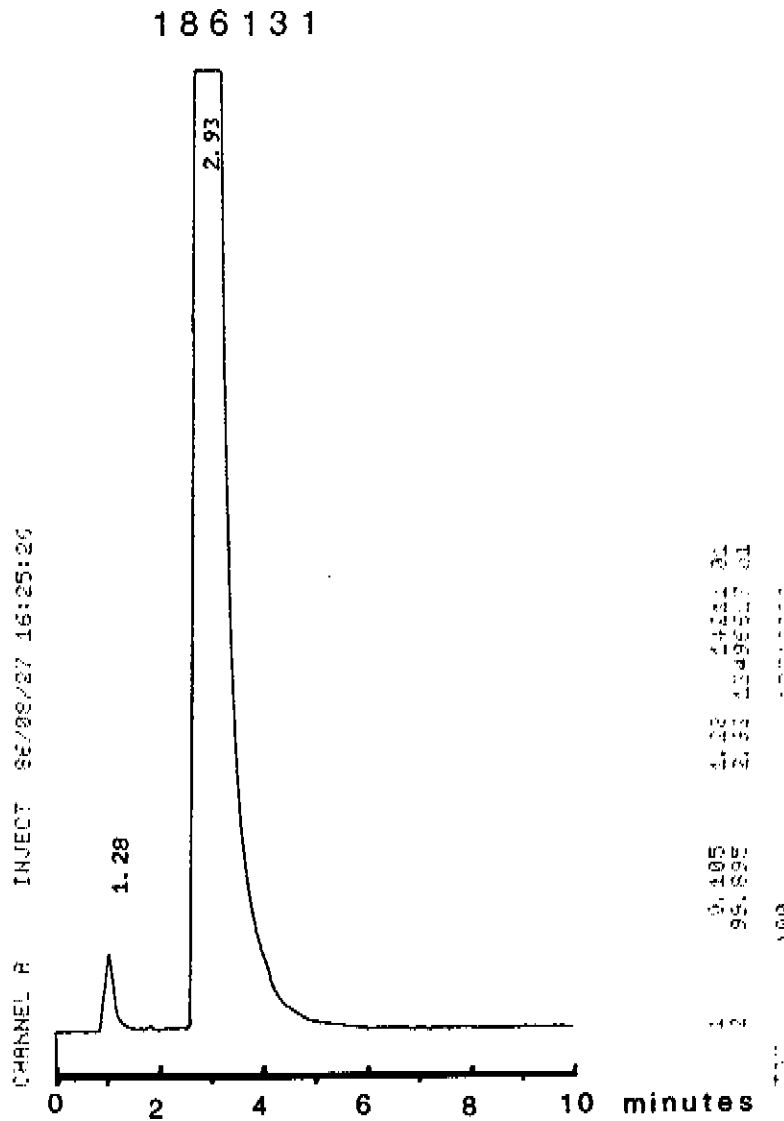


Figure 3. A chromatogram of 2-amino-5-nitrothiazole No 186131.

The following conditions were used:

- Eluent: Acetonitrile / Water (55 + 45)
- Column: RP-18, Spheri-5 (Brownlee)
- Detector: Varian UV 200 operated at 379 nm
- Pump: Varian 5560 operated at a flow rate of 1 ml/min.
- Integrator: Varian 4270 Attenuation: 8
- Sample: 0.6 mg/ml dissolved in acetonitrile with additional adjustment to eluent composition. 10 µl corresponding to 6 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 μ l to get maximum sensitivity. An isogram is given in Figure 4.

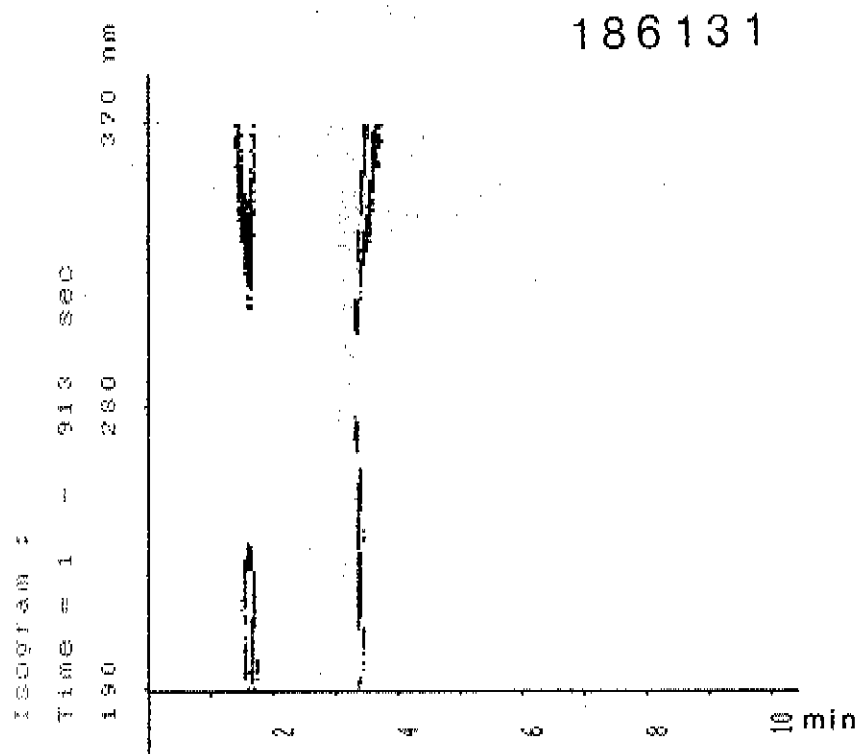


Figure 4. Isogram of 2-amino-5-nitrothiazole, Control No 186131. Sensitivity: 0.005

As seen from the figure the impurity eluting after 1.5 minutes exhibits UV-maxima at 210 nm and 360 nm, respectively. The results from peak area measurements at these wavelengths were compared to the result at 379 nm which is chosen in the method described above. At 379 nm 0.1% impurities were detected compared to 0.1% at 360 nm and 0.2%-0.3% at 210 nm. The higher value at 210 nm is due to the fact that 2-amino-5-nitrothiazole has lower UV-absorption at this wavelength than at 379 nm.

STABILITY

2-Amino-5-nitrothiazole was exposed to air of different relative humidity at room temperature (about 20^o C) for a period of eight weeks as described in WHO/PHARM/82.509. All samples were unchanged at visual inspection and no weight changes were noted. No signs of degradation were observed when selected samples were analyzed by the liquid chromatographic method described above.

DATA GIVEN BY THE MANUFACTURER

IR spectrum	conforms
Proton NMR sepctrum	conforms
Elemental analysis	C (25.16%) H (2.08%) N (28.97%)
Melting point	195-200 ^o C
TLC	more than 95% pure

CONCLUSION

2-Amino-5-nitrothiazole No 186131 can be considered suitable as International Chemical Reference Substance for the intended purpose.

C H L O R A M P H E N I C O L

Control No 486004

Analytical Report

The stock of the current batch of the International Chemical Reference Substance for chloramphenicol, control no 379004, is depleted and has to be replaced. The monograph for chloramphenicol in the International Pharmacopoeia Ed. III, Vol 2 requires a reference substance to be used in the infrared spectrophotometric and thin-layer chromatographic identity tests. The reference substance is also to be used in the spectrophotometric assay.

MATERIAL

About 200 g of the sample (manufacturers lot no AR15A001) were received at the WHO Centre in July 1985. The material is being stored protected from light in tightly closed containers at +5 °C.

ANALYTICAL DATA

Description: A greyish-white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (no 486004). The spectrum is concordant with the spectrum obtained from the ICRS control no 379004.

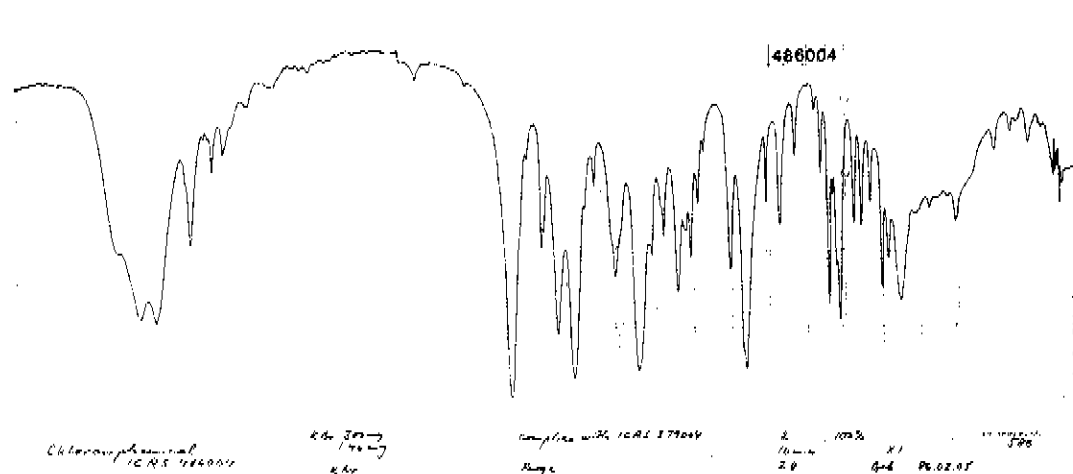


Figure 1. IR-spectrum of 1.46 mg of chloramphenicol in 300 mg KBr recorded against a KBr reference disc. Instrument: Perkin Elmer 580.

UV-spectrum

A UV-spectrum in water is given in Figure 2.
 λ max in water = 277.6 nm
E(1%, 1 cm) = 295 (n= 5)

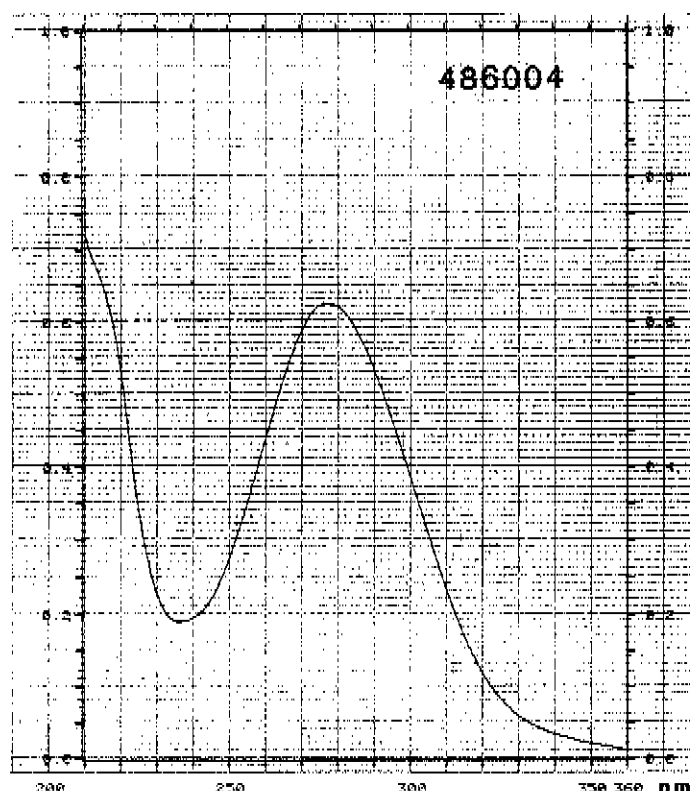


Figure 2. UV-spectrum of chloramphenicol 20 µg/ml in water.

Test for free chlorides

According to the monograph not more than 0.5 mg/g (500 ppm).
Result: Conforms (less than 250 ppm).

Melting range: 151-152 °C, determined by the capillary method of Ph Int III.

Specific optical rotation: $[\alpha]_D^{20} = +20.1^\circ$ (n= 2), determined in a 50 mg/ml solution in dehydrated ethanol R.

ASSAY

The spectrophotometric assay described in the International Pharmacopoeia Volume 3 was used. The ICRS for chloramphenicol (Control No 379004) was used as standard and regarded as 100%. The result is calculated with reference to the dried substance.
Result: 99.8% (n= 5)

Loss on drying

0.05% (105 °C to constant weight).

PURITY

Total solid impurities

1) Differential thermal analysis (DTA): About 0.2 mol % (n= 4). The determination was carried out on about 3 mg using a heating rate of 2 °C per minute. Melting temperature: 150.4 °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 total amount of impurities was estimated to about 0.2%.
The following thin-layer chromatographic systems were used.

Thin-layer: Silica gel 60, F-254 (Merck) and HPTLC, silica gel 60, F-254 (Merck)
Eluent: Chloroform /Methanol (80 + 20)
Sample: 100 µg and 400 µg of chloramphenicol, 50 mg/ml in ethanol (750 g/l).
Visualization: UV-light of 254 nm after heating at 105 °C for 5 minutes. The chromatogram was evaluated using a Zeiss PMQ3/MQ3 Chromatogram Spectrophotometer operated in the reflectance mode at 278 nm.

Result: One very faint secondary spot with $R_f = 0.41$ was noted when 100 µg were applied (the amount prescribed in the monograph). The R_f -value for chloramphenicol was 0.47 and the detection limit for the system was about 0.2 µg. Three very faint secondary spots were noted when 400 µg were applied. They were estimated to about 0.2% when evaluated by densitometry at 278 nm.

The TLC system described above was also applied to a HPTLC-plate. The impurities were about the same but the spots were more distinct and the detection limit was 0.1 µg.

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 3.

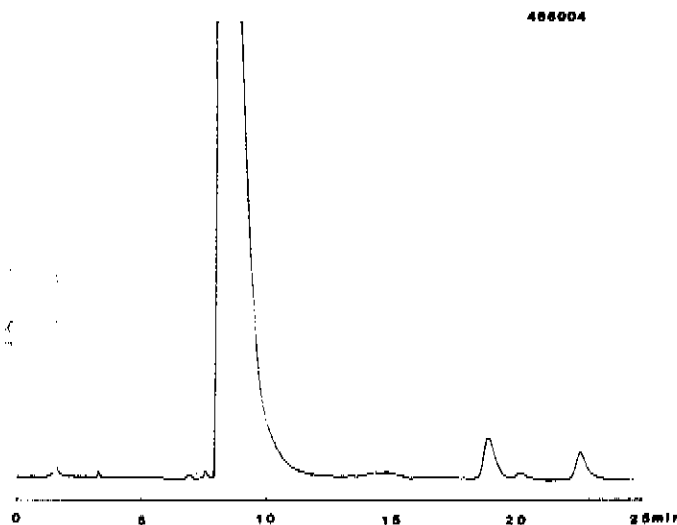


Figure 3. Chromatogram of chloramphenicol Control No. 486004

The following conditions were used:

Eluent: Acetonitrile/Water (30:70)
Column: Spheri 5, RP18, Brownlee (300 x 4.6 mm)
Detector: Varian UV 200 operated at 280 nm
Pump: Varian 5560
Integrator: Varian 4270 Attenuation: 1
Sample: 5 mg/ml dissolved in the eluent.
10 µl corresponding to 50 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 μ l to get maximum sensitivity.

An isogram is given in Figure 4.

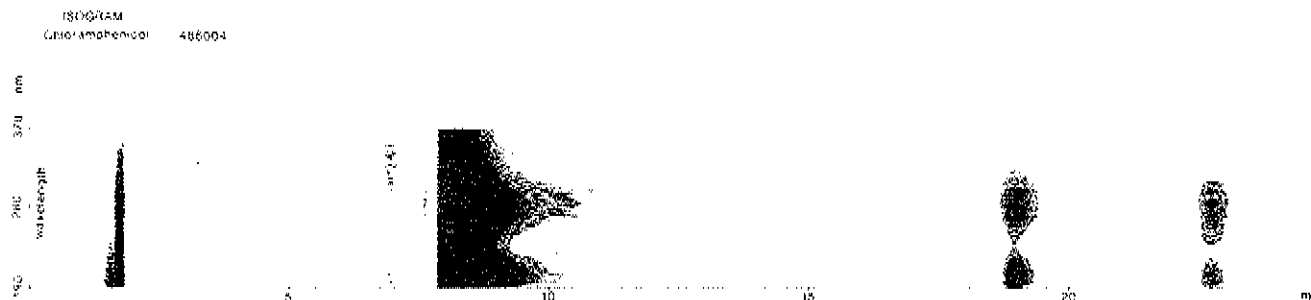


Figure 4. Isogram of chloramphenicol Control No 486004.

As seen from the figure the impurities eluting after 1.5, 18.9 and 22.6 minutes, respectively, are all detected at the same wavelengths i.e. with maxima at 280 nm and 200 nm. Chromatograms recorded simultaneously at these wavelengths were identical.

STABILITY

No special stability studies were performed as we have good experience of the stability of this substance from earlier batches. Chloramphenicol ICRS 370994 showed no tendency of degradation when stored for seven years at +5 $^{\circ}$ C at our Centre.

DATA GIVEN BY THE MANUFACTURER

Description:	White or yellowish white, crystalline powder.
Melting point:	151 $^{\circ}$ C
Optical rotation:	20.21 $^{\circ}$
Loss on drying:	0.06%
Sulfated ash:	0.04%
Chlorides:	<100 ppm
Related compounds:	passes test
Assay	99.8% (spectrophotometrically on dried substance)

CONCLUSION

Chloramphenicol No 486004 can be considered suitable as International Chemical Reference Substance for the intended purpose. On the basis of the results obtained the content of chloramphenicol when used in the spectrophotometric assay is taken to be 99.8% calculated with reference to the dried substance.

CHLORAMPHENICOL PALMITATE

Control No 286072

Analytical Report

The stock of the current batch of the International Chemical Reference substance for chloramphenicol palmitate, Control No 175072 is depleted and has to be replaced. The monograph for chloramphenicol palmitate in the International Pharmacopoeia Ed. III, Vol. 3 requires a reference substance to be used in the thin-layer chromatographic identity test. The reference substance may also be used in the infrared spectrophotometric limit test for polymorph A in chloramphenicol palmitate mixture.

MATERIAL

About 200 g of the sample (manufacturers lot no AR15D014) were received at the WHO Centre in July 1985. The material is being stored protected from light in tightly closed containers at +5 °C.

ANALYTICAL DATA

Description: A white, unctuous powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (no 286072). The spectrum is concordant with the spectrum obtained from the ICRS control no 175072.

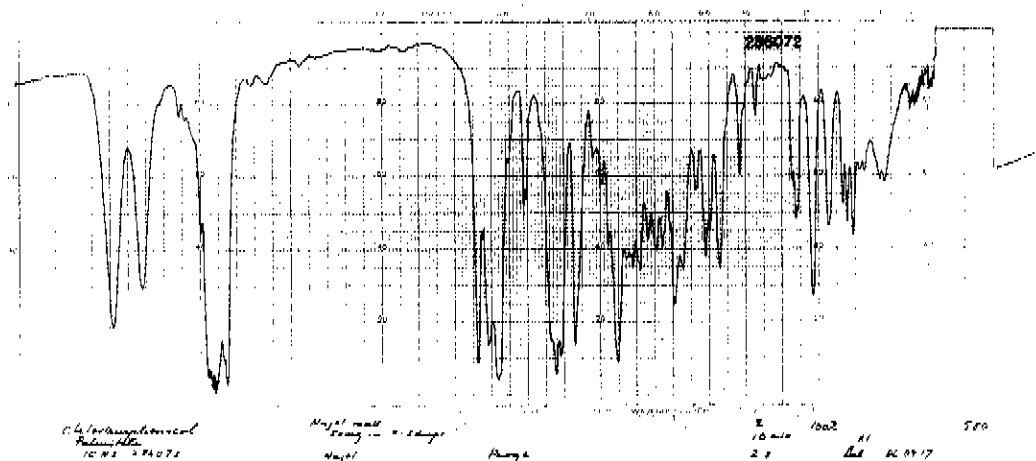


Figure 1. IR-spectrum of about 50 mg of chloramphenicol palmitate in a liquid paraffin mull. Instrument: Perkin Elmer 580.

Note: The International Chemical Reference Substance for chloramphenicol palmitate may also be used in the limit test for biologically inactive chloramphenicol palmitate (polymorph A) in chloramphenicol palmitate mixture. A suitable infrared spectrophotometric method is described in the British Pharmacopoeia 1980, Vol. II, page 688 (B.P. 80).

Polymorph A: Not detected by infrared spectrophotometry in ICRS 286072.

Infrared absorption spectra of mulls prepared as directed in B.P. 80 are shown in Figure 2, a-d.

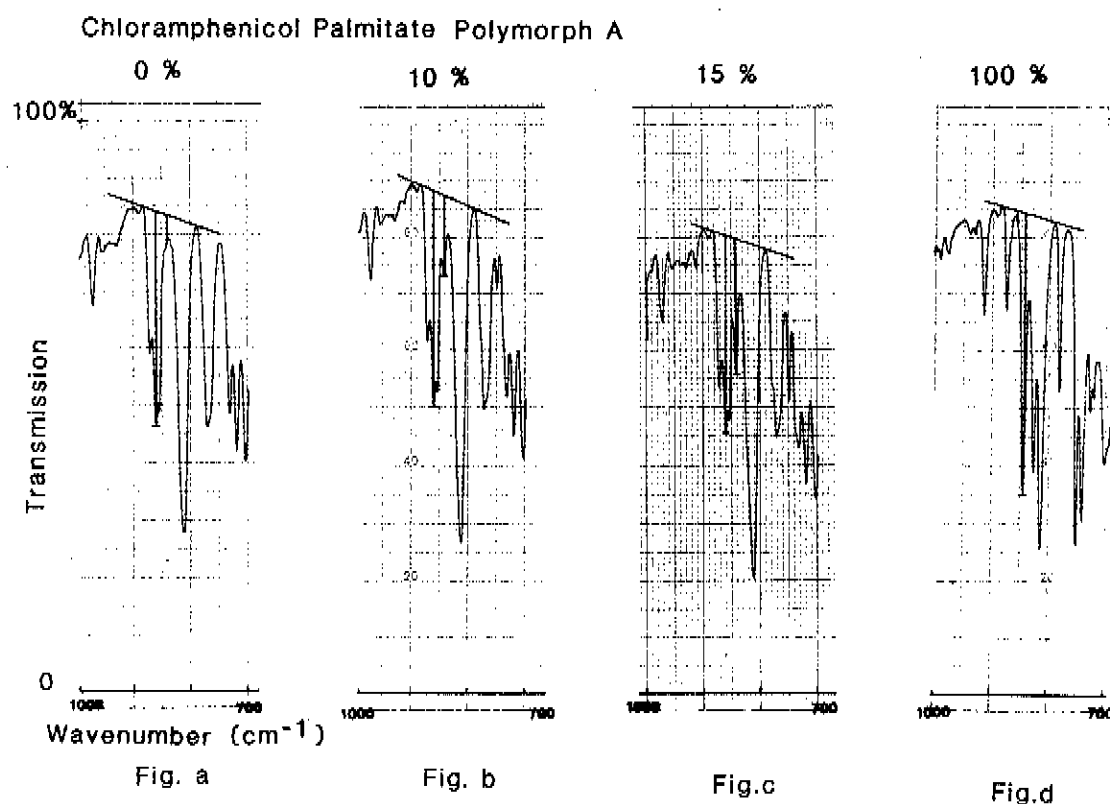


Figure 2. Infrared absorption spectra of chloramphenicol palmitate with increasing amount of chloramphenicol palmitate polymorph A added.

Figure a) shows the spectrum obtained with the International Chemical Reference Substance for chloramphenicol palmitate. The ratio of the peak height at about 858 cm⁻¹ to that at about 840 cm⁻¹ is about 7.6.

Figure b) shows the spectrum obtained with the ICRS for chloramphenicol palmitate to which 10% of ICRS chloramphenicol palmitate (polymorph A) has been added. The ratio is about 2.9.

Figure c) and d) show how the peak at about 840 cm⁻¹ increases with increasing content of chloramphenicol palmitate (polymorph A).

Specific optical rotation: $[\alpha]_D^{20} = +24.3^{\circ}$ (n= 3), determined in a 50 mg/ml solution in dehydrated ethanol.

UV-spectrum

A UV-spectrum in dehydrated ethanol is given in Figure 3.

λ max in dehydrated ethanol = 270.8 nm
E (1%, 1 cm) = 180 (n= 5)

The absorbance of a 30 μ g/ml solution was 0.57.

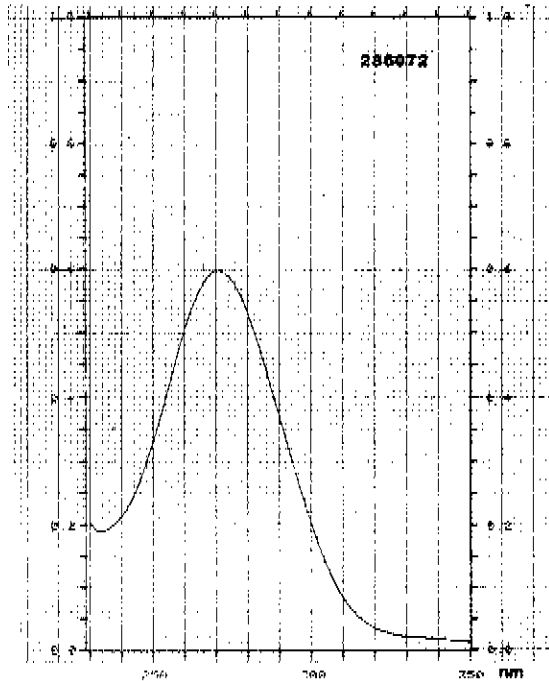


Figure 3. UV-spectrum of chloramphenicol palmitate in dehydrated ethanol, 31 µg/ml.

Thin-layer chromatography: The system described in Ph. Eur., Ed. II where chloramphenicol palmitate is partly hydrolyzed to chloramphenicol and palmitic acid was used. Both hydrolyzed products were found and no other extra spots were observed. See figure 4.

Chlor- amphe- nicol	Palmi- tic acid	Hydrolyzed chlorampheni- col palmitate	Chloram- phenicol palmitate
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Figure 4. Thin-layer chromatogram of hydrolyzed chloramphenicol palmitate.

286072

○ Rf=0.73 ○

Rf=0.39 ○

○

○

○ Rf=0.32

—*—*—*—*

ASSAY

The spectrophotometric assay described in the International Pharmacopoeia, Volume 3 was used. The ICRS for chloramphenicol palmitate (Control No 175072) was used as reference substance and regarded as 100%. The result is calculated with reference to the dried substance. Result: 100.2% (n= 5).

When calculated according to the International Pharmacopoeia, Ed. III, Volume 3, E(1%, 1 cm)=178, the result was 101.2%.

Loss on drying

0% (60 °C to constant weight under reduced pressure over phosphorous pentoxide R).

Water

0.18%, determined by Karl Fischer titration.

Thermogravimetric analysis

0.1% loss in weight.

PURITY

Total solid impurities

1) Differential thermal analysis (DTA): About 2.7 mol % (n= 5). The determination was carried out on 4.3 mg using a heating rate of 2 °C per minute.

Melting temperature: 89.1 °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.

2) Differential scanning calorimetry (DSC): About 2.7 mol % (n= 2). The determination was carried out on 1.6 mg using a heating rate of 2 °C per minute.

Instrument: Perkin Elmer DSC-4

Polymorph A: Not detected in ICRS 286072 by DTA and DSC. The minimum amount that could be detected is about 0.1%.

Free chloramphenicol

Determined according to Ph. Int. Ed. III, Vol. 3.

Result: 0.085 mg/g (85 ppm)

Thin-layer chromatography

The total amount of impurities was estimated to about 2.7% when compared to corresponding impurity references. The following thin-layer chromatographic system was used.

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

Eluent: Chloroform/Methanol/Water (90 + 10 + 1)

Sample: 100 µg of chloramphenicol palmitate (10 mg/ml) in acetone

Visualization: UV-light of 254 nm.

The chromatograms were evaluated using a CAMAG TLC Scanner II with integrator SP 4290 as well as with a Zeiss PMQ 3 Scanner, both operated in the reflectance mode at 271 nm.

Result: Two secondary spots with $R_f = 0.58$ (chloramphenicol palmitate isomer) and $R_f = 0.79$ (chloramphenicol dipalmitate) were noted when 100 μg was applied (the amount prescribed in the monograph). The R_f -value for chloramphenicol palmitate was 0.65 and the detection limit for the system was about 0.5 μg . No further secondary spots were noted when 250 μg were applied. A chromatogram is shown in Figure 5.

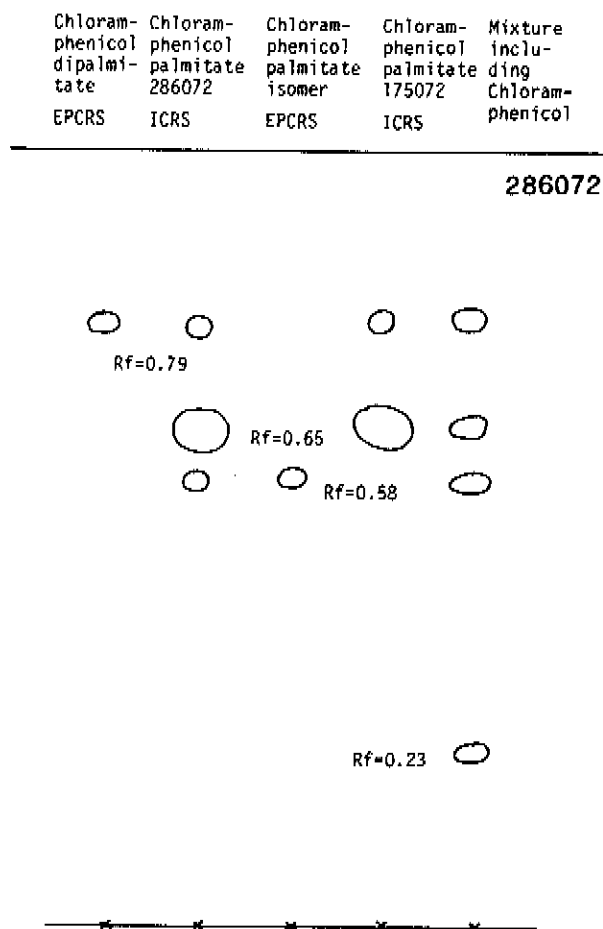


Figure 5. Thin-layer chromatogram of chloramphenicol palmitate, Control No 286072.

In the new ICRS 286072 1.5% of the chloramphenicol palmitate isomer and 1.2% of chloramphenicol dipalmitate were found by comparison with the corresponding EPCRS.

The thin-layer chromatographic system for related substances described in Ph. Eur. Ed. II was also tested. One of the two impurities observed was found to be identical with chloramphenicol palmitate isomer (EPCRS). It was estimated to about 1.5%. $R_f = 0.25$ (corresponding to the spot with $R_f = 0.58$ in the Ph. Int. system). The chromatogram was evaluated using a Zeiss PMQ 3 Scanner operated in the reflectance mode at 271 nm.

High performance liquid chromatography

The total amount of impurities (except chloramphenicol dipalmitate) was estimated by peak area measurements to about 3.1%. A chromatogram is shown in Figure 6.

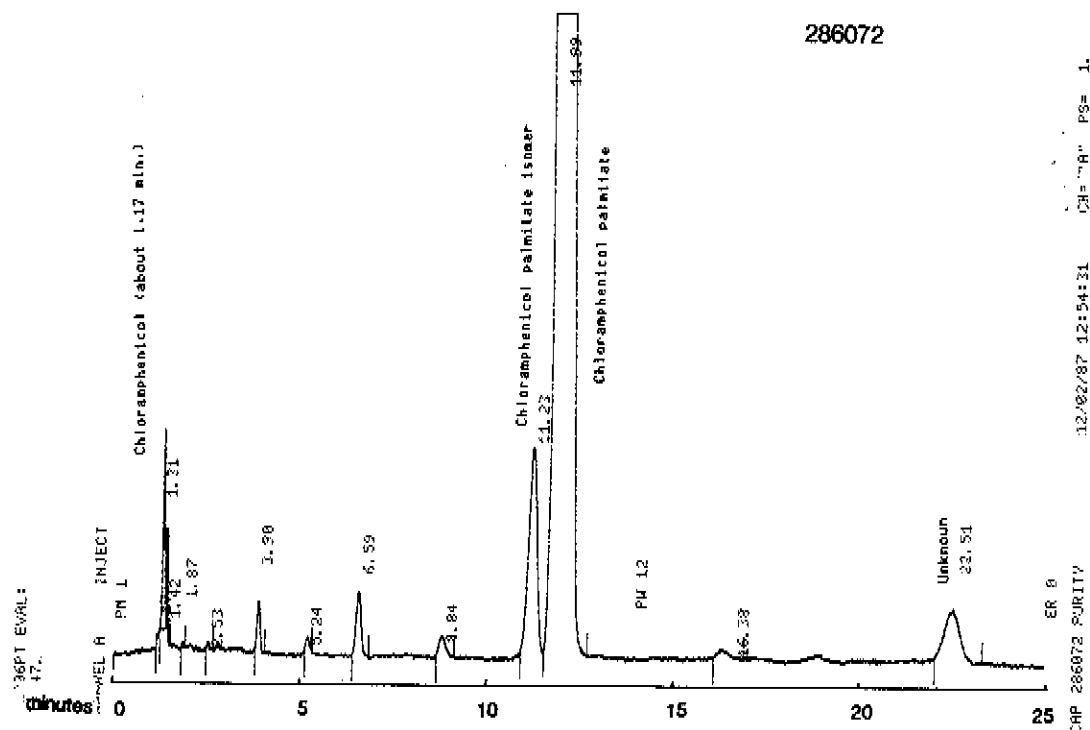


Figure 6. A chromatogram of chloramphenicol palmitate No 286072.

The following conditions were used (according to a modified method from USP XXI):

- Eluent: Acetonitrile/water/glacial acetic acid (86:13.5:0.5)
- Column: Spheri-5, RP-18, Brownlee, (250 x 4.6 mm) 5 μ m particles
- Detector: Varian Vista 2500 operated at 271 nm
- Pump: Varian 5560
- Integrator: Varian 4270 Attenuation: 2
- Sample: 0.52 mg/ml dissolved in the eluent. 10 μ l corresponding to 5.2 μ g were injected

As seen from the figure at least seven impurities are observed. The major impurities are chloramphenicol palmitate isomer eluting after 11 minutes (1.4%) and one unknown eluting after 22 minutes (0.7%). When the former ICRS 175072 was subjected to chromatography in this system the result was about 1.8% impurities compared to 3.1% in ICRS 286072.

However, it was not possible to elute chloramphenicol dipalmitate in the above mentioned system. By changing the eluent to 100% acetonitrile the dipalmitate was eluted after 29-30 minutes and the amount was estimated to about 1.2% against the EPCRS. Chloramphenicol palmitate elutes after 4 minutes with this eluent.

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 7.

286072

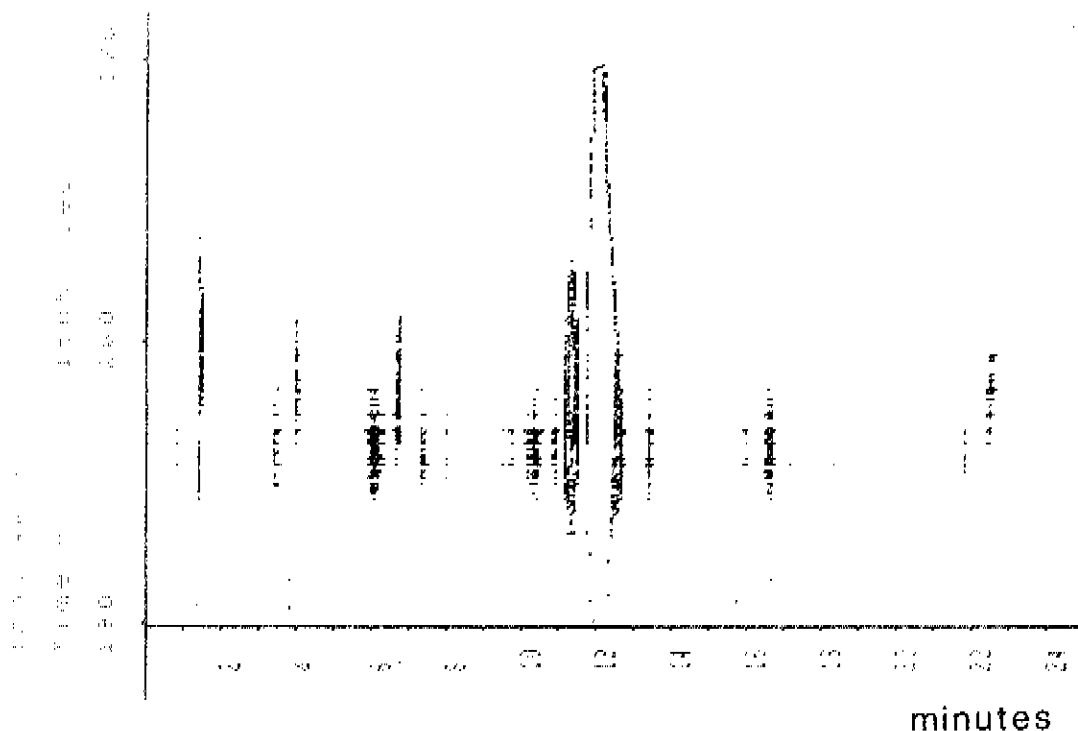


Figure 7. Isogram of chloramphenicol palmitate, Control No 286072. Sensitivity: 0.002

As seen from the figure chloramphenicol palmitate has an absorbance maximum at 271 nm and the observed impurities are all visible at this wavelength.

STABILITY

No special stability studies were performed as we have good experience of the stability of this substance from the earlier batch. Chloramphenicol palmitate ICRS 175072 showed no tendency of degradation when stored for ten years at +5 °C at our Centre.

DATA GIVEN BY THE MANUFACTURER

Description:	White or almost white powder
Melting point:	89.5 °C
Specific optical rotation:	+24.16°
Acidity:	0.24%
(palmitic acid)	
Loss on drying:	0.07%
Sulphated ash:	<0.1%
Free chloramphenicol:	95 ppm
Related compounds:	passes test
Assay:	101.74% (spectrophotometrically on dried substance)
Non Polymorphous "A":	100%
(=Polymorph B)	

CONCLUSION

Chloramphenicol palmitate No 286072 can be considered suitable as International Chemical Reference Substance for the intended purpose.

N I R I D A Z O L E

Control No 186129

Analytical Report

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

MATERIAL

About 200 g of the sample (manufacturers lot Prod. Stand. 85) were received at the WHO Centre in June 1985. The material is being stored protected from light in tightly closed containers at +5 °C.

ANALYTICAL DATA

Description: A yellow, crystalline powder: odourless or almost odourless.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (No 186129). The spectrum is concordant with the reference spectrum from the manufacturer.

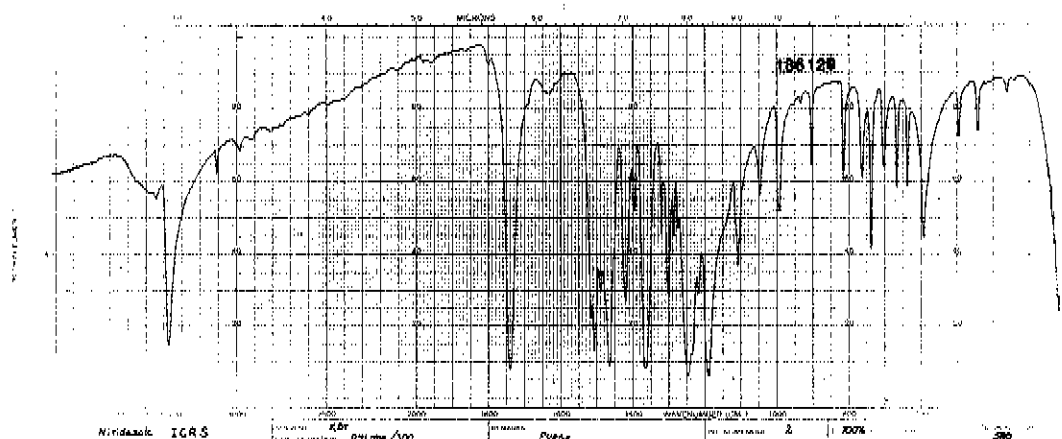


Figure 1. IR-spectrum of 0.4 mg of Niridazole in 300 mg KBr recorded against a KBr disc. Instrument: Perkin Elmer 580.

Melting temperature

About 264 °C with decomposition, determined by the capillary method of Ph. Int. Ed. III.

Elemental analysis

	C (%)	H (%)	N (%)
Theoretical	33.6	2.8	26.2
Found	33.4	2.6	26.0

The analysis was performed at Mikro Kemi AB, Uppsala.

UV-spectrum

A UV-spectrum in 0.1% dimethylformamide in ethanol is given in Figure 2. The substance is first dissolved in dimethylformamide and then diluted with ethanol.

λ max in methanol = 358 nm
E (1%, 1 cm) = 704 (n= 6)
The absorbance of a 10 μ g/ml solution was 0.70

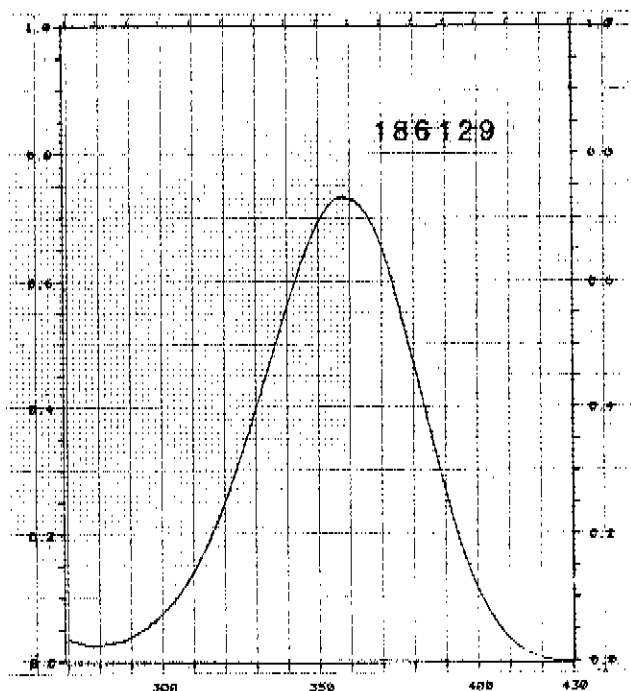


Figure 2. UV-spectrum of niridazole 10.4 μ g/ml

ASSAY

When used in the spectrophotometric assay according to Ph. Int. Ed. III, Vol 3, the content is 100.0% with reference to the dried substance. See results under UV-spectrum.

Loss on drying

0.2% (100 °C, reduced pressure)

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.

2) Phase solubility analysis: About 0.2%. When calculated with the method of least squares with 95 per cent confidence intervals as described in WHO/PHARM/66.431: 0.17 ± 0.34 .

Solvent: Pyridine:Ethanol (750 g/l), 60 + 40

Equilibration: Vibro-mixer at 28.0 °C for 45 hours.

Thin-layer chromatography

The following thin-layer chromatographic systems were used:

Thin-layer: Silica gel 60, F-254 (Merck)
Eluent: Toluene: Acetone: Acetic acid: Isobutanol
(12 + 8 + 3 + 8)
Sample: 100 μ g of Niridazole were applied

Visualization: UV-light of 254 nm and 365 nm visually and by scanning. Rf (niridazole)= 0.48; Rf (2-amino-nitrothiazole)= 0.55; Rf (niridazole-chlorethylcarboxamide)= 0.58. The detection limit for niridazole was 0.01 µg (0.01%) when scanned at 365 nm. By visual detection the detection limit was only 0.1%.

Result: Only one impurity was found and it was estimated to about 0.03%. This impurity was only observed when the chromatogram was evaluated using a Zeiss KM3 Chromatogram Spectrophotometer operated in the reflectance mode at 365 nm. The impurity was identical with niridazole-chlorethylcarboxamide.

The thin-layer chromatographic system described in Ph. Int. Ed. III, Vol 3 with toluene: acetone (12 + 8) as eluent was tested but 2-amino-5-nitrothiazole did not separate from niridazole. Rf (niridazole)= 0.38, Rf (2-amino-5-nitrothiazole)= 0.39 and Rf (niridazole-chlorethylcarboxamide)= 0.48. One weak impurity with Rf= 0.48 was observed.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.1%. A chromatogram is shown in Figure 3. The peak eluting after 5.62 minutes corresponds to niridazole-chlorethylcarboxamide it was estimated to 0.04%. 2-amino-5-nitrothiazole elutes after 2.87 minutes in negligible amounts.

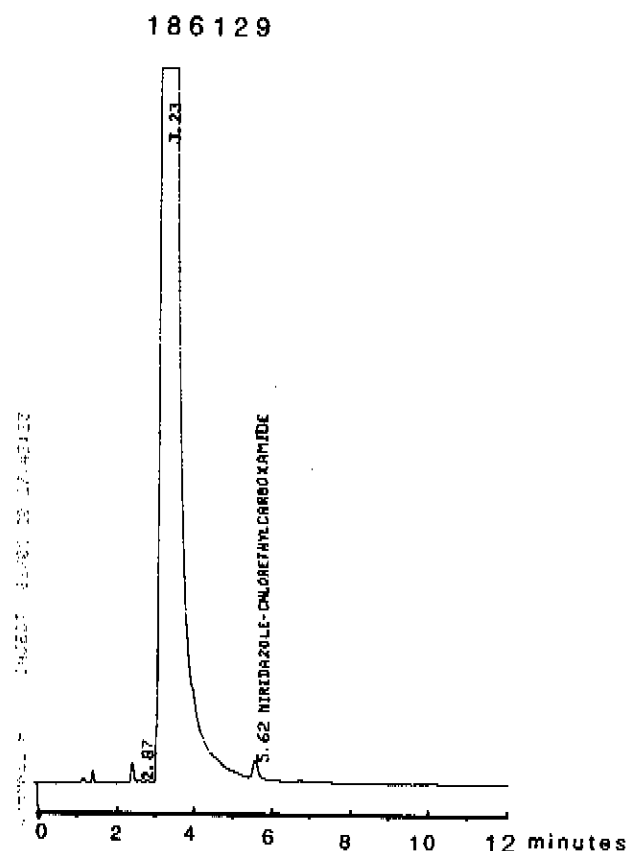


Figure 3. Chromatogram of niridazole No 186129.

The following conditions were used:

Eluent: Acetonitrile/Water (55 + 45)
Column: RP-18, Spheri-5 (Brownlee)
Detector: Varian UV 200 operated at 215 nm.
Pump: Varian 5560 operated at a flow rate of 1 ml/min.
Integrator: Varian 4270 Attenuation: 16
Sample: 1 mg/ml dissolved in the acetonitrile with additional adjustment to eluent composition. 10 µl corresponding to 10 µg were injected.

The two potential impurities were also estimated against corresponding reference substances which gave the same result as the peak area measurements.

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.

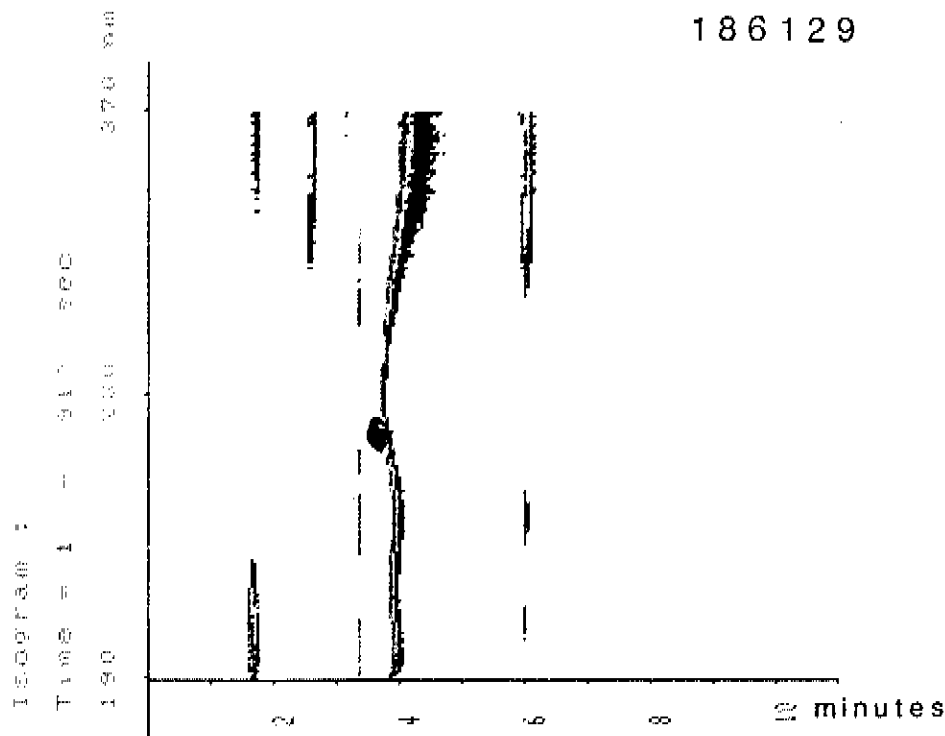


Figure 4. Isogram of niridazole, Control No 186129. Sensitivity: 0.005

As can be seen from the figure the impurities eluting after 1.4, 2.3 and 6.0 minutes, respectively are all visible at the wavelength chosen in the method i.e. 359 nm.

STABILITY

Niridazole 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 and no weight changes were noted. No signs of degradation were observed when samples were analyzed by the liquid chromatographic method described above.

DATA GIVEN BY THE MANUFACTURER

Loss on drying:	<0.05%
Assay:	100.0%
TLC:	on by-product
Total by-products:	0.1%
Sulfate:	<200 ppm
Lead:	<4 ppm
Arsenic:	<2 ppm
Copper:	<6 ppm
Sulfated ash:	<0.05%

CONCLUSION

Niridazole No 186129 can be considered suitable as International Chemical Reference Substance for the intended purpose. The content of niridazole when used in the spectrophotometric assay is taken to be 100.0% calculated with reference to the dried substance.

NIRIDAZOLE -
CHLORETHYL CARBOXAMIDE

Control No 186130

Analytical Report

The International Chemical Reference Substance for niridazole-chlorethylcarboxamide is intended to be used in the thin-layer chromatographic test for related substances according to the monograph for niridazole in the International Pharmacopoeia Ed. III, Vol. 3.

MATERIAL

About 25 g of the sample (manufacturers lot no C-44'132-Ba/II) were received at the WHO Centre in December 1984. The material is being stored protected from light in a tightly closed container at +5 °C.

ANALYTICAL DATA

Description: A light yellow, crystalline powder, odourless.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (No 186130). The spectrum is concordant with a spectrum from the manufacturer.

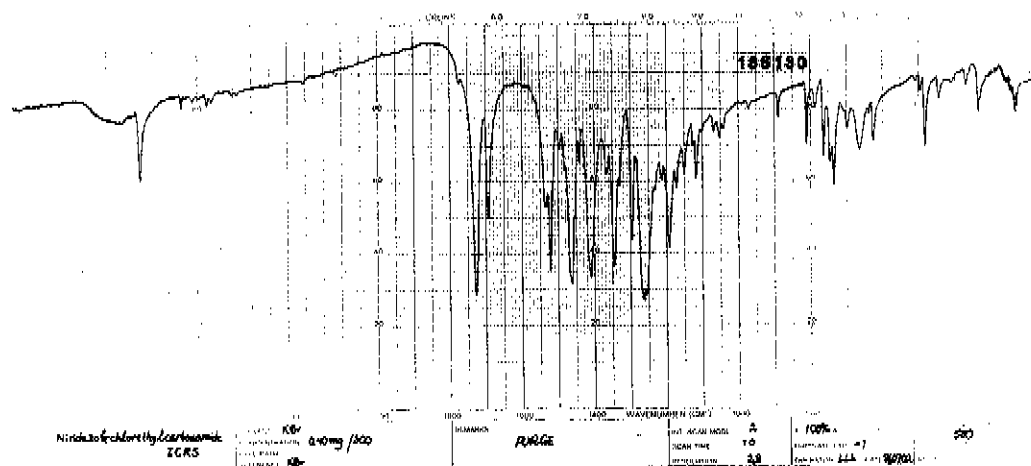


Figure 1. IR-spectrum of 0.4 mg of niridazole-chlorethylcarboxamide in 300 mg KBr recorded against a KBr disc. Instrument: Perkin Elmer 580.

Melting range: About 195.6-196.1 °C determined by the capillary method of Ph. Int. Ed. III.

Elemental analysis

	C (%)	H (%)	N (%)
Theoretical	33.8	3.2	21.9
Found	33.7	3.0	21.8

The analysis was performed at Mikro Kemi AB, Uppsala.

UV-spectrum

A UV-spectrum in 0.1% dimethylformamide in ethanol is given in Figure 2. The substance is first dissolved in dimethylformamide and then diluted with ethanol.

λ max in water = 346 nm
E (1%, 1 cm) = 485 (n= 4)

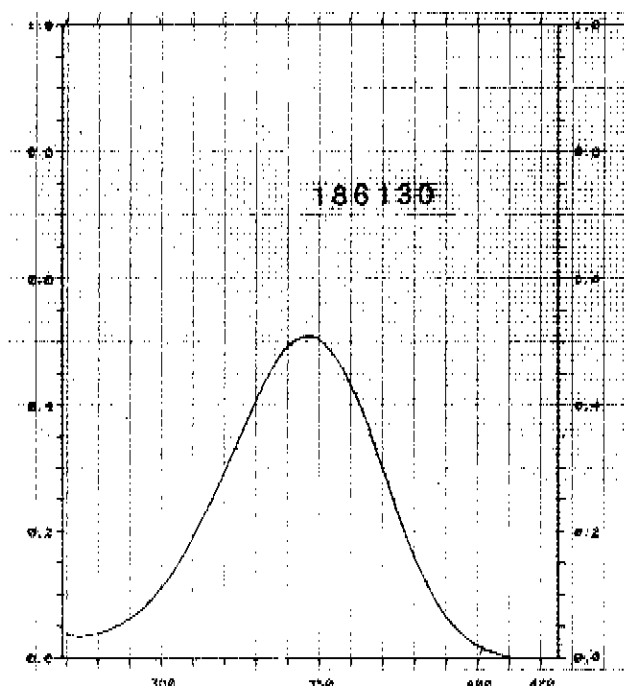


Figure 2. UV-spectrum of niridazole-chlorethylcarboxamide 10.5 $\mu\text{g/ml}$.

Loss on drying

0.1% (100 °C, reduced pressure)

PURITY

Total solid impurities

1) Differential thermal analysis (DTA):

About 0.1 mol per cent (n= 5). The determination was carried out on about 3 mg using a heating rate of 2 °C per minute.

Melting temperature: 195.0 °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 following TLC systems were used:

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

Eluent: Toluene: Acetone: Acetic acid: Isobutanol (12 + 8 + 3 + 8)

Sample: 100 μg of niridazole-chlorethylcarboxamide were applied

Visualization: UV-light of 254 nm and 365 nm visually and at 346 nm by scanning

R_f (niridazole)= 0.48: R_f (2-amino-5-nitrothiazole)= 0.55; R_f (niridazole-chlorethylcarboxamide)= 0.58. The detection limit for niridazole-chlorethylcarboxamide was 0.05 μg (0.05%) when scanned at 346 nm.

Result: Only one impurity was found and it was estimated to about 0.1%. This impurity was only observed when the chromatogram was evaluated using a Zeiss KM3 Chromatogram Spectrophotometer operated in the reflectance mode at 346 nm. The impurity was identical to niridazole.

The thin-layer chromatographic system described in Ph. Int. Ed. III, Vol 3 with toluene:acetone (12 + 8) as eluent was tested but 2-amino-5-nitrothiazole did not separate from niridazole.

Rf (niridazole)= 0.38, Rf (2-amino-5-nitrothiazole)= 0.39 and Rf (niridazole-chlorethylcarboxamide)= 0.48. This system was the best to separate the two impurities from niridazole-chlorethylcarboxamide. The amount of niridazole was estimated to about 0.08% and 2-amino-5-nitrothiazole to about 0.05%.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.1%. A chromatogram is shown in Figure 3. The peak eluting after 2.93 minutes corresponds to 2-amino-5-nitrothiazole, it was estimated to 0.05%. Niridazole elutes after 3.24 minutes and estimated to 0.03%. The same results were obtained by peak area measurements as when compared with external standards of the two impurities.

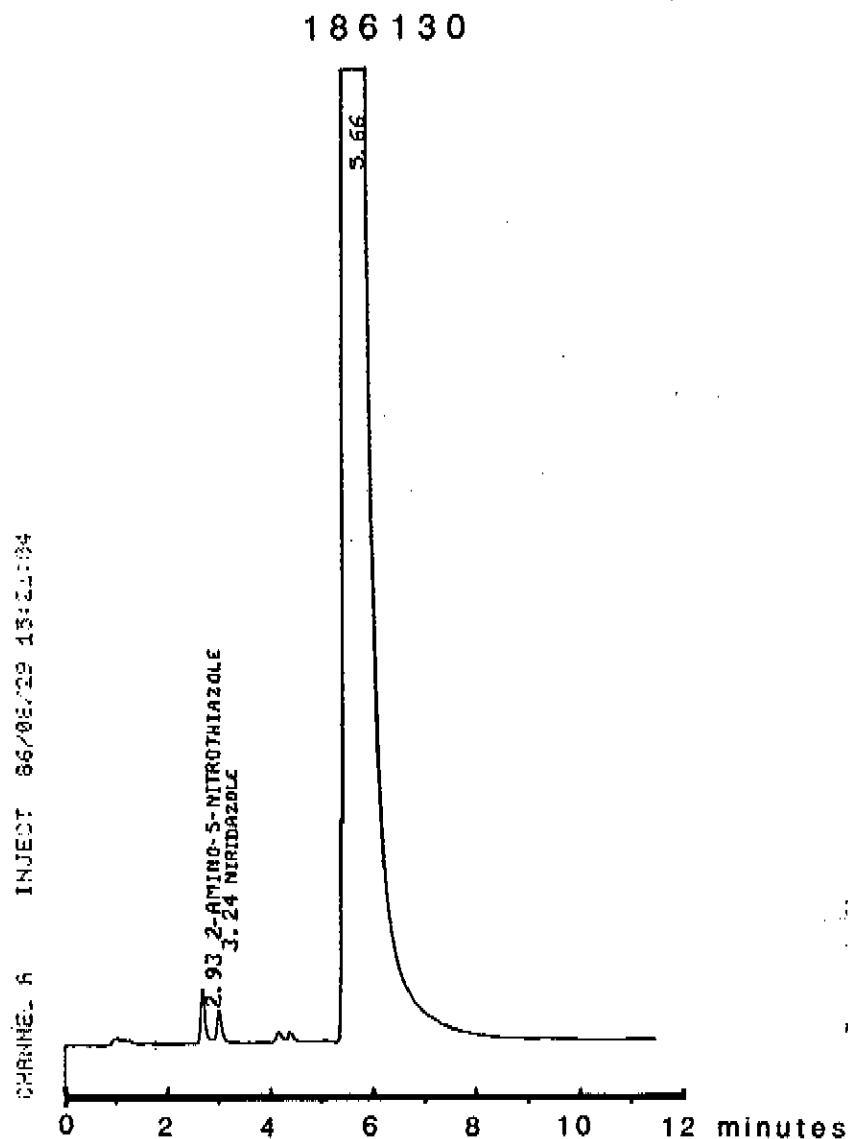


Figure 3. A chromatogram of niridazole-chlorethylcarboxamide No 186130.

The following conditions were used:

Eluent: Acetonitrile/Water (55:45)
Column: RP-18, Spheri-5 (Brownlee)
Detector: Varian UV 200 operated at 346 nm
Pump: Varian 5560 operated at a flow rate of 1 ml/min
Integrator: Varian 4270 Attenuation: 8
Sample: 0.6 mg/ml dissolved in acetonitrile with additional adjustment to eluent composition.
10 μ l corresponding to 6 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.

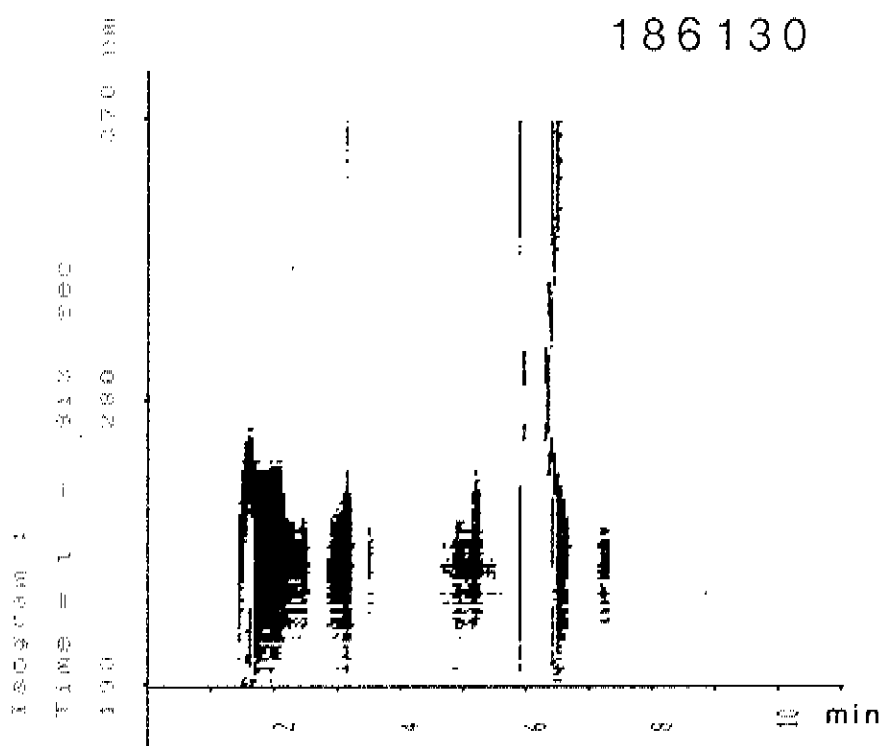


Figure 4. Isogram of niridazole-chlorethylcarboxamide, Control No 186130. Sensitivity: 0.005

As seen from the figure impurities are visible mainly at 227 nm but traces are also observed at higher wavelengths and 346 nm which was chosen in the method described above. The results from peak area measurements at these wavelengths were compared and it was found that at 227 nm 0.5% impurities were detected compared to 0.1% at 346 nm. The difference is mainly due to the fact that the UV-absorption for niridazole-chlorethylcarboxamide is higher at 346 nm.

STABILITY

Niridazole-chlorethylcarboxamide 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 and no weight changes were noted. No signs of degradation were observed when selected samples were analyzed by the liquid chromatographic method described above.

DATA GIVEN BY THE MANUFACTURER

Melting point: 196.0 °C capillary method
196.2 °C Mettler FP 5
IR spectrum: conforms
Proton NMR: conforms
Elemental analysis: C(33.9%) H(3.3%) N(21.96%) O(20.2%) S (9.81%) Cl(11.09%)
TLC: more than 95% pure

CONCLUSION

Niridazole-chlorethylcarboxamide No 186130 can be considered suitable as International Chemical Reference Substance for the intended purpose.

N O R E T H I S T E R O N E

Control No 186132

Analytical Report

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

MATERIAL

About 100 g of the sample (manufacturers batch No 50-0074) were received at the WHO Centre in August 1985. 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 186132). The spectrum is concordant with the spectrum obtained from the USP reference substance Lot H.

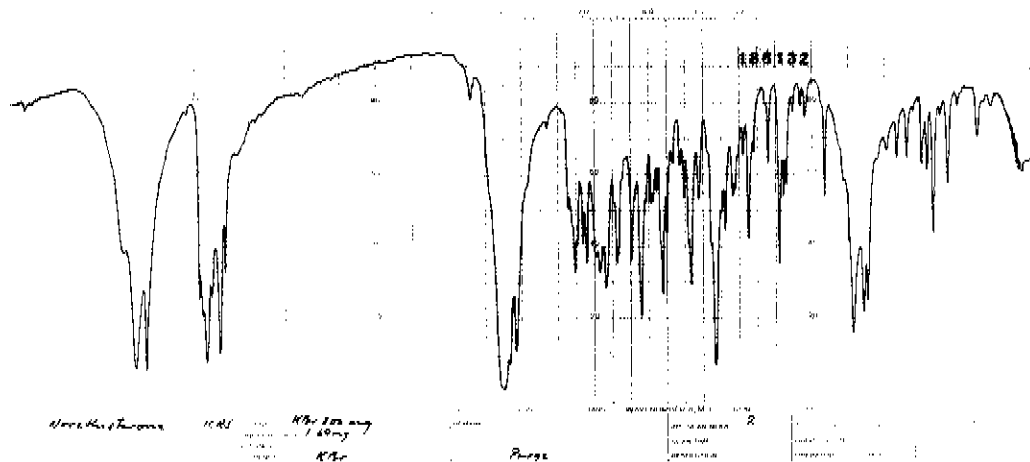


Figure 1. IR-spectrum of 1.7 mg of norethisterone in 300 mg KBr recorded against a KBr reference disc. Instrument: Perkin Elmer 580.

Melting range: 202.5-206.8 °C (n= 3) determined by the capillary method of Ph. Int. Ed. III.

Specific optical rotation: $[\alpha]_D^{20} = -24^\circ$ (n= 3), determined in chloroform at a concentration of 10 mg/ml.

UV-spectrum

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

λ max in ethanol = 240 nm

E(1%, 1 cm) = 576 (n= 5)

The absorbance of a 10 µg/ml solution was 0.58

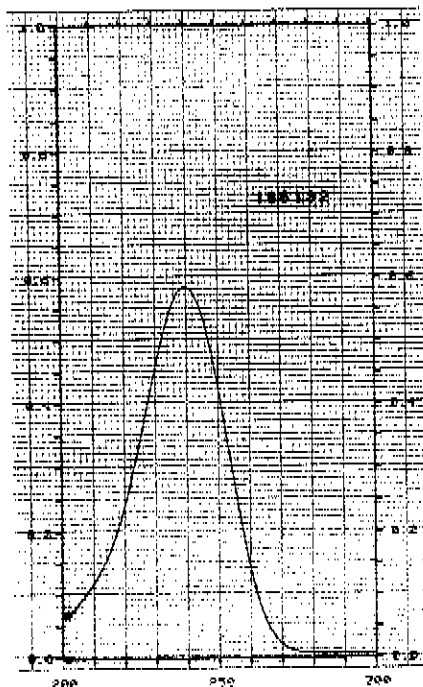


Figure 2. UV-spectrum of norethisterone 10.1 µg/ml in ethanol.

ASSAY

Loss on drying

0% (105 °C) (n= 3)

Spectrophotometric assay: 99.8% (n= 5) determined according to Ph. Int. Ed. III, Vol. 2. USP reference substance Lot H was used as reference and regarded as 100%.

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.

2) Phase solubility analysis: About 0.7%. When calculated with the method of least squares with 95 per cent confidence intervals as described in WHO/PHARM/66.431: 0.7 ± 0.18 .

Solvent: Ethanol (750 g/l)/Water (85 + 15)

Equilibration: Vibro-mixer at 27.0 °C for 120 hours.

Thin-layer chromatography

The following thin-layer chromatographic systems were used.

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

Eluent: Chloroform: methanol (95 + 5)

Sample: 100-200 µg of norethisterone were applied

Visualization: UV-light of 254 nm, scanning at 240 nm and spraying with sulfuric acid/ethanol TS followed by heating to 105 °C and examination in day-light.

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

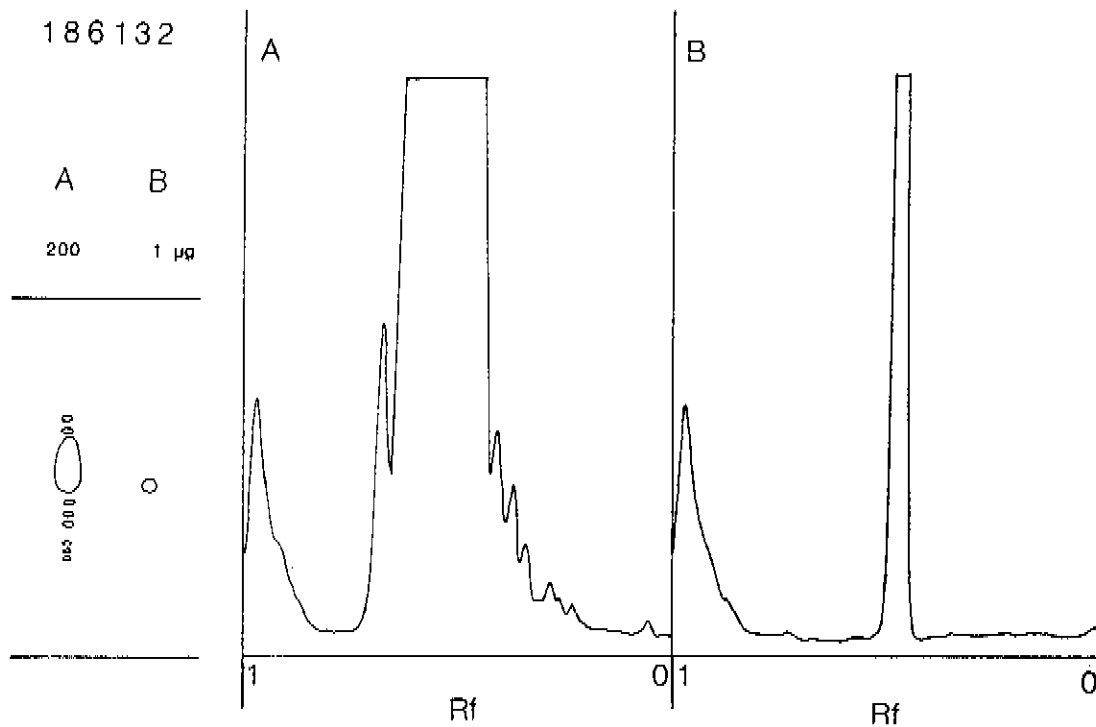


Figure 3. High performance thin-layer chromatogram of norethisterone No 186132.

As seen from the figure two dominating extra spots are observed above the norethisterone spot. Six faint additional spots below the main spot are also observed. The total amount of impurities was roughly estimated to about 0.7% after spraying. When the chromatogram was evaluated using a CAMAG TLC scanner with the scanning wavelength set to 240 nm the total amount of impurities was estimated to 0.4%. The system separates norethisterone acetate but not ethisterone. In the USP reference substance Lot H six weak spots were observed but the total amount of impurities was less than in the proposed International Chemical Reference Substance.

As described in Ph. Int. Ed. III, Vol. 2 an ordinary thin-layer plate was also used. This gave similar results but the impurities were more distinctly separated on the HPTLC-plate. The strongest spots were estimated to 0.3% and 0.2%, respectively, all the others were significantly weaker.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.3%. Two chromatograms are shown in Figure 4 A + B. As seen from the figure, 80% acetonitrile is required to elute the most lipophilic impurities. However, to check the presence of impurities with similar structure to norethisterone a system with 50% acetonitrile was conveniently chosen.

Figure 4 A + B. Chromatogram of norethisterone Control No. 186132 eluted with 50% acetonitrile (A) and 80% acetonitrile (B).

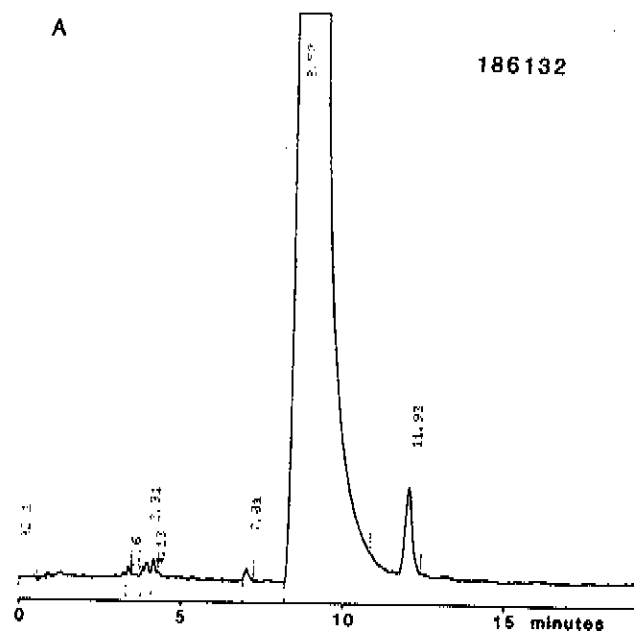


Figure 4 A.

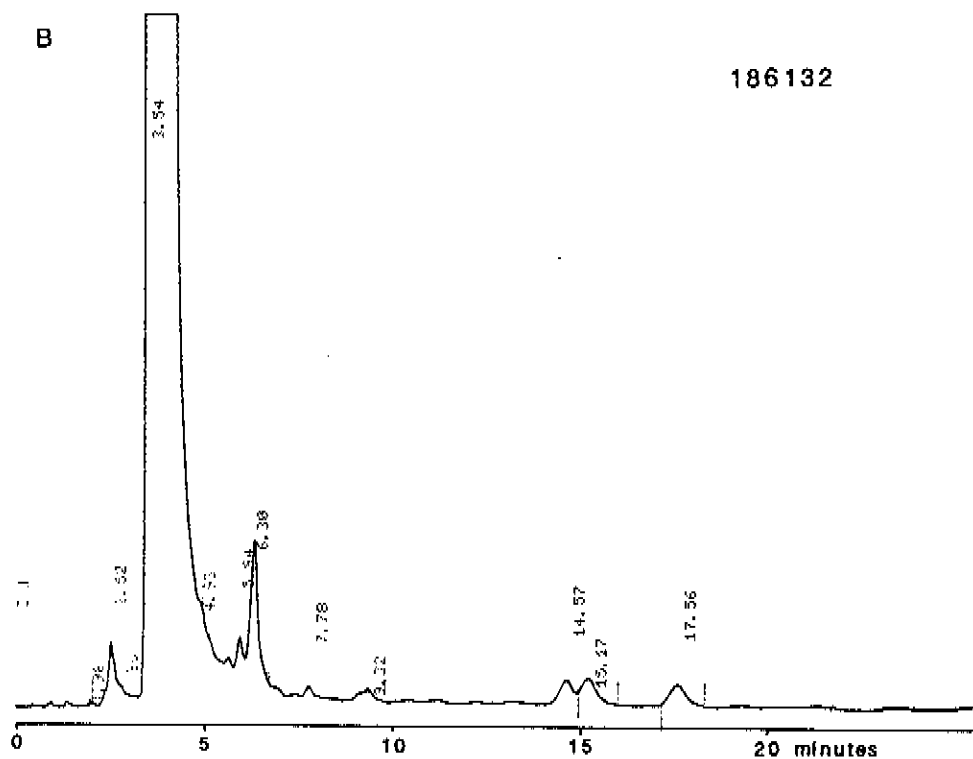


Figure 4 B.

The following conditions were used:

Eluent: Acetonitrile/Water (50:50) and (80:20)
Column: RP18, Spheri-5, (Brownlee)
Detector: Varian UV 200 operated at 240 nm
Pump: Varian 5560 operated at a flow rate of 1 ml/min

Integrator: Varian 4270 Attenuation: 1
Sample: 1 mg/ml dissolved in the eluent.
10 μ l corresponding to 10 μ g were injected.

Ethisterone: The norethisterone sample was spiked with 10% of ethisterone. When running the system with 50% acetonitrile a distinct peak elutes after 10.15 minutes (ethisterone) while norethisterone elutes after 8.5 minutes. However, to detect minor amounts of ethisterone a system with better selectivity is necessary.

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 and the concentration to 2 mg/ml to get maximum sensitivity.

An isogram is given in Figure 5.

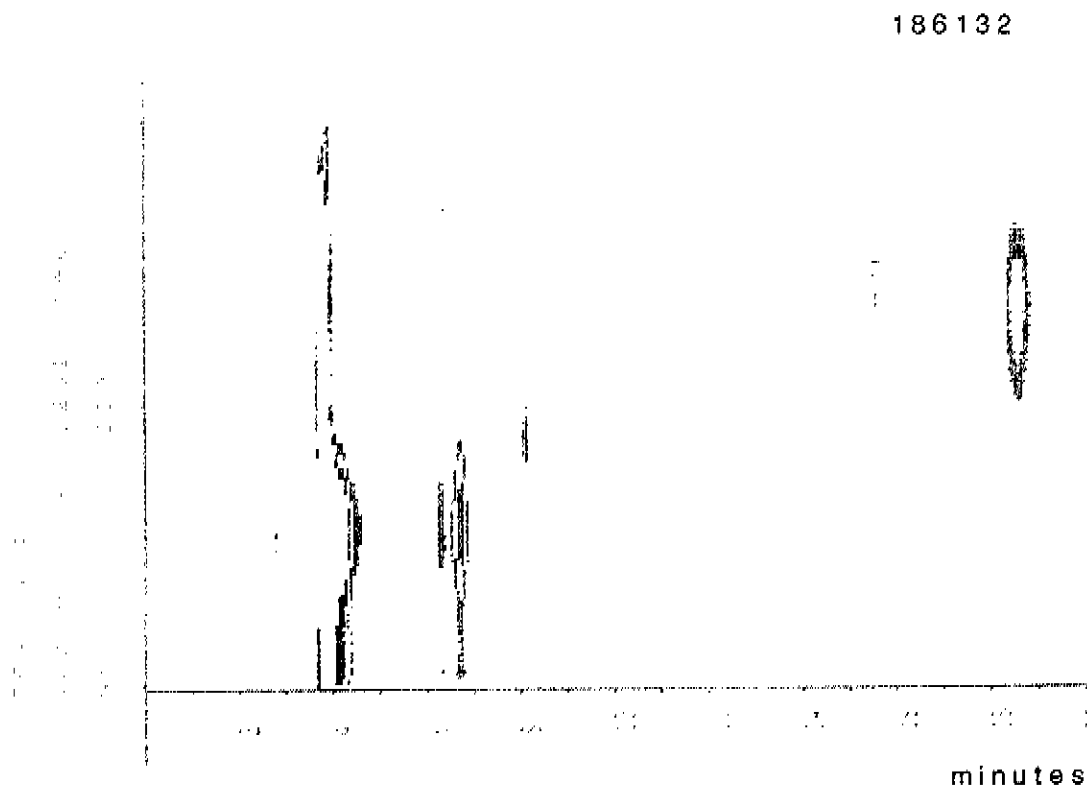


Figure 5. Isogram of norethisterone Control No 186132. Eluent 80% acetonitrile. Sensitivity: 0.004.

As seen from the figure the major impurity eluting after about 6.5 minutes exhibits a UV-maximum at 240 nm. One small impurity with a UV-maximum at 284 nm can also be observed, hidden in the front of the main peak. The impurities eluting at 15.6 and 18.6 minutes have their maxima at 310 - 320 nm. The results from peak area measurements at these wavelengths were compared to the result at 240 nm, which is chosen in the method described above. At 315 nm 17% impurities were detected, compared to 2.3% impurities at 284 nm and 0.26% at 240 nm. The higher values at 315 nm and 284 nm are due to the fact that norethisterone has lower UV-absorption at these wavelengths than at 240 nm. Based on the results from other analytical techniques 240 nm seems to be the best wavelength for the estimation of impurities.

STABILITY

Norethisterone 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 and no weight changes were noted. No signs of degradation were observed when the samples were analyzed by the liquid chromatographic method described above.

CONCLUSION

Norethisterone No 186132 can be considered suitable as International Chemical Reference Substance for the intended purpose. The content of norethisterone when used in the spectrophotometric assay is taken to be 99.8% calculated with reference to the dried substance.

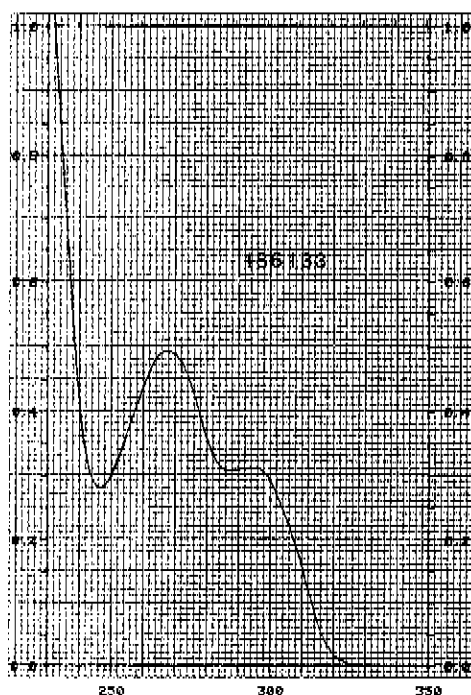


Figure 2. UV-spectrum of reserpine 18 µg/ml in ethanol.

λ max in ethanol = 268 nm and 295 nm

E (1%, 1 cm) was determined at three wavelengths i.e. 247, 268 and 295 nm. The results are given in Table 1.

Table 1

Wavelength	247 nm	268 nm	295 nm
E (1%, 1 cm)	155	273	173
n	4	4	4
s rel %	0.73	0.70	0.70

Oxidation products, absorbance at 388 nm: 0.0, 0.2 mg/ml of reserpine was dissolved in glacial acetic acid. The UV-absorbance was measured at 388 nm.

ASSAY

Thermogravimetric analysis

0.2% loss in weight

Water

0.14% determined by Karl Fischer titration

Colorimetric assay

Determined by the colorimetric assay according to Ph. Int. Ed. III, Vol. 2. The USP reference substance Lot M was used as reference with the content taken to be 100%.

Result:

Content of proposed IGRS	Reference substance	s rel % (n= 5)
99.8%	USP Lot M	0.59

The absorbance of the reference solution was 0.42 for the proposed International Chemical Reference Substance.

Titrimetric assay

99.4% (n= 9), S rel % = 0.59, determined by titration with perchloric acid according to Ph. Int. Ed. III, Vol. 3. The calculations are performed with reference to the anhydrous substance.

PURITY

Thin-layer chromatography

The following thin-layer chromatographic system was used:

Thin-layer: HPTLC, Silica gel 60, F-254 (Merck)
Eluent: Chloroform: diethylamine:cyclohexane (40:10:50)
Sample: 100 µg of reserpine were applied
The following possible impurities were also applied:
1. Reserpic acid
2. Methylreserpate
3. 3,4,5-Trimethoxybenzoic acid
4. 3,4-Dehydroreserpine
5. 3-Isoreserpine

Visualization: UV-light of 254 nm and 365 nm.

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

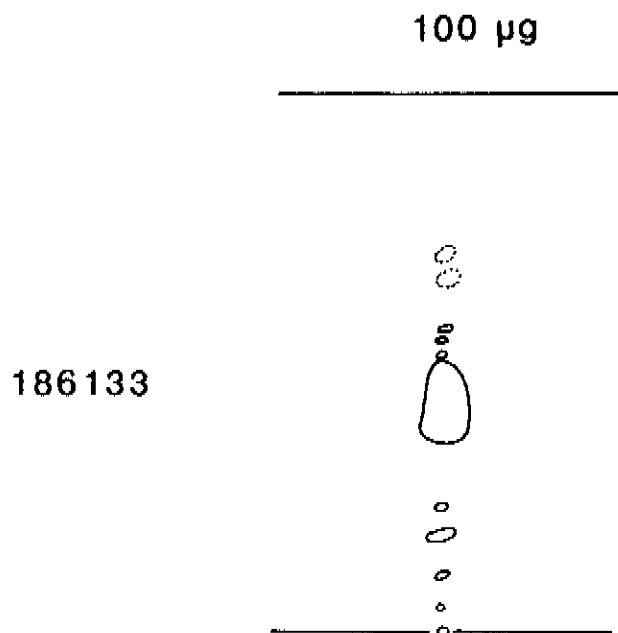


Figure 3. High performance thin-layer chromatogram of reserpine No 186133.

As seen from the figure about ten weak extra spots are observed, five above the main spot and five below. R_f (reserpine) is equal to 0.44. The dominating extra spot has R_f = 0.67 which corresponds to 3,4-dehydroreserpine. Reserpic acid with R_f = 0 as well as 3-isoreserpine with R_f = 0.54 were also detected. 3,4-Dehydroreserpine and 3-isoreserpine were probably formed on the plate or during the run. This results in that the thin-layer chromatographic method is not suitable for a quantitative evaluation. USP reference substance Lot L was also applied to the plate and showed several extra spots as well.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.8%. A chromatogram is shown in Figure 4.

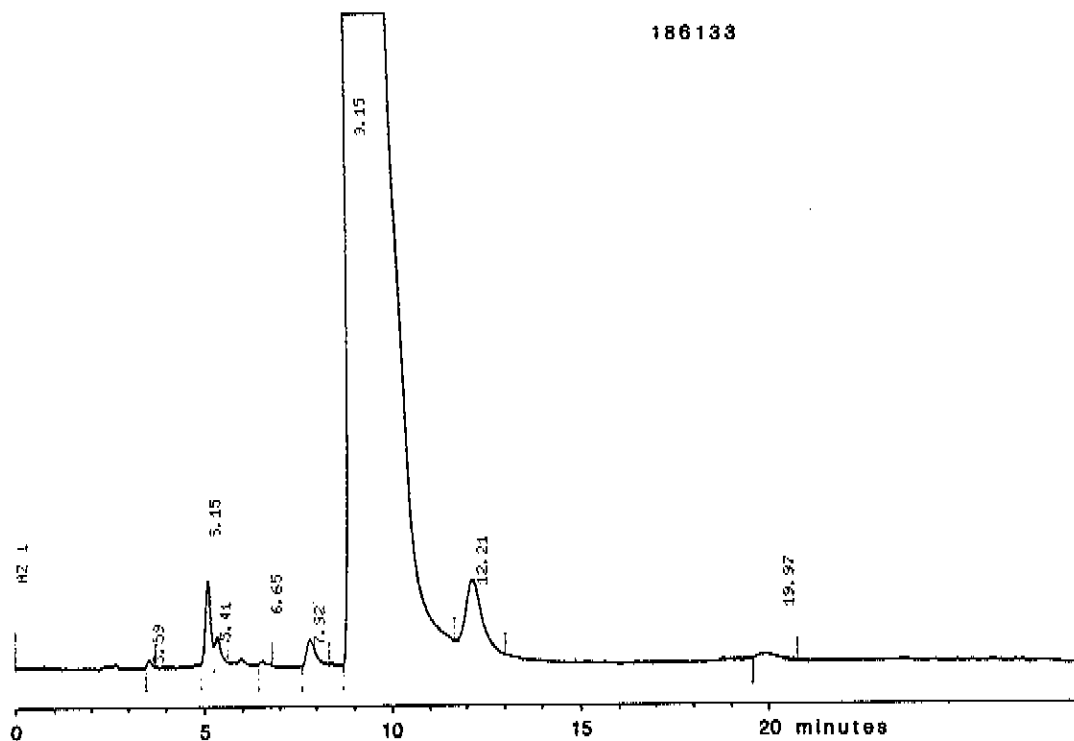


Figure 4. Chromatogram of reserpine Control No. 186133.

The following conditions were used:

Eluent: Acetonitrile/Phosphate pH= 4.5 (35:65)
Column: Vydac 218 TP
Detector: Varian UV 200 operated at 220 and 254 nm
Pump: Varian 5560 operated at a flow rate of 1 ml/min
Integrator: Varian 4270 Attenuation:
Sample: 1 mg/ml dissolved in the eluent.
10 μ l corresponding to 10 μ g were injected.

The International Chemical Reference Substance with Control No. 186133 was spiked with the impurities used in the TLC system. The major impurity determined by peak area measurement was estimated to 0.4%. It elutes after 12.2 min and is identical to 3,4-dehydroreserpine. After 5.1 min one unknown impurity estimated to 0.2% was observed. Reserpine acid elutes after 2.6 min and was estimated to 0.02%. 3-Isoreserpine was not found in the sample. 3,4-Dehydroreserpine was also quantified against an external standard and estimated to 0.5%. In the USP reference substance Lot M about 1% impurities were found. A chromatogram with the potential impurities and degradation products in the proposed International Chemical Reference Substance is shown in Figure 5.

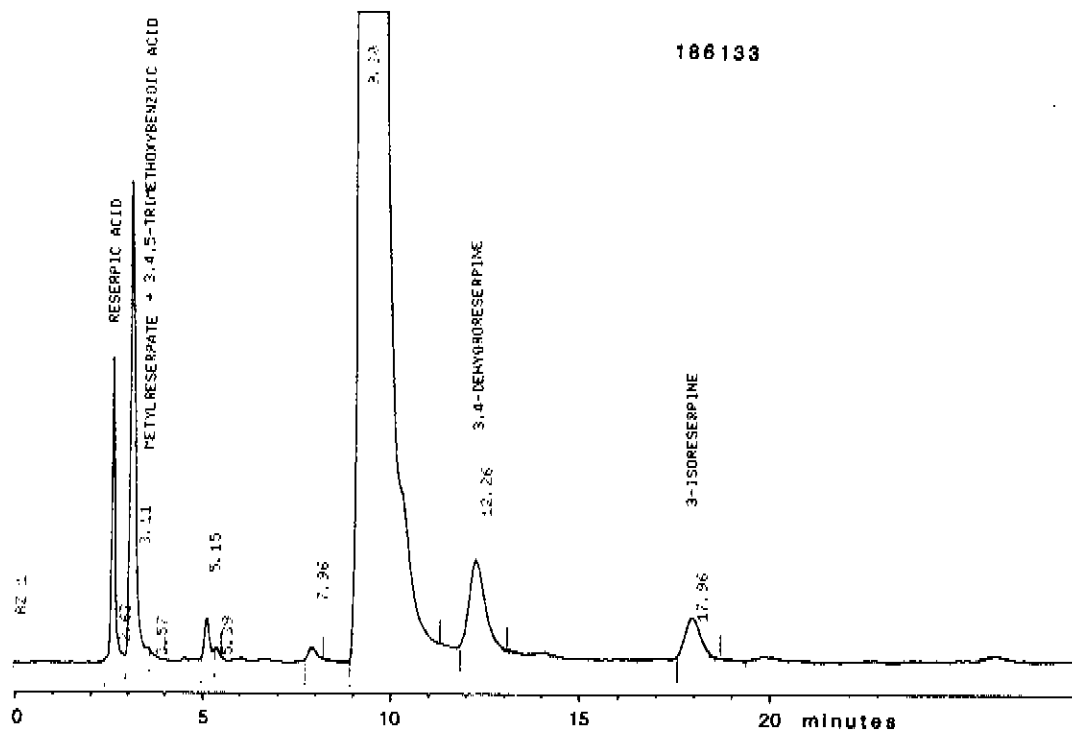


Figure 5. Chromatogram of reserpine spiked with potential impurities and degradation products (1% of each).

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 6.

186133

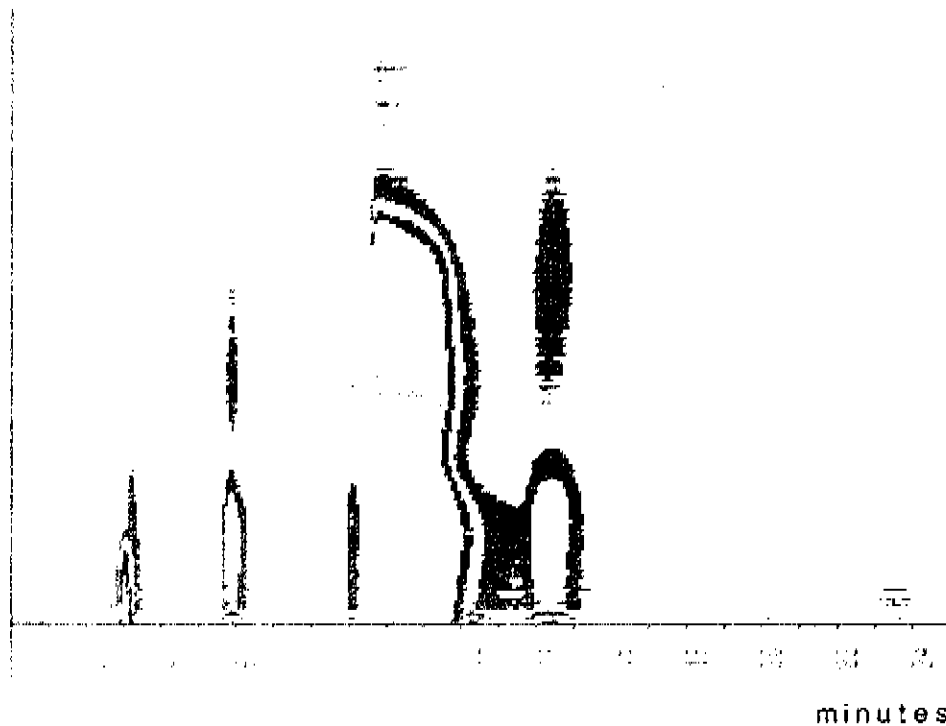


Figure 6. Isogram of reserpine Control No 186133. Sensitivity: 0.01

As can be seen from the figure the major impurities as well as reserpine have their absorbance maximum at 220 nm.

STABILITY

Reserpine 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 and no weight changes were noted. No signs of degradation were observed when selected samples were analyzed by the liquid chromatographic method described above.

DATA GIVEN BY THE MANUFACTURER

Appearance:	Faintly pale, beige powder
Specific rotation:	-129.4° in dioxan
Absorbance 296 nm:	175.9
Absorbance 268 nm:	276.0
Absorbance 247 nm:	154.8
Identity:	IR, TLC
Absorbance 388 nm:	0.022
Loss on drying:	0.0%
Sulfated ash:	0.10%
Thin-layer chromatography:	0.1% of reserpine acid
Additional by-products:	each of them less than 1%
Assay acidimetric:	100.2%
Assay photometric:	100.8%

CONCLUSION

Reserpine No 186133 can be considered suitable as International Chemical Reference Substance for the intended purpose. The content of reserpine when used in the colorimetric assay is taken to be 99.8% calculated with reference to the dried substance, which corresponds to 99.6% calculated on the "as is" basis.

APPENDIX 14

V I T A M I N A A C E T A T E

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

(solution)

Control No 686038

Analytical Report

The International Chemical Reference Substance for vitamin A acetate is intended to be used in validation of UV spectrophotometric assay procedures. The preparation is a solution in cottonseed oil of crystalline all-trans retinol acetate, dispensed in gelatin capsules, each containing approximately 9 mg in 250 mg of oil per capsule.

MATERIAL

The material proposed as a new International Chemical Reference Substance for vitamin A acetate has generously been donated by the U.S. Pharmacopeial Convention Inc. and is of the same batch as the U.S.P. Reference Standard Lot S.

The content of vitamin A acetate in the material has been determined in a collaborative study organized by the USP. The following result was obtained:

34.7 mg vitamin A acetate per g solution (equivalent to 30.3 mg of retinol)

The determination was carried out as a direct spectrophotometric measurement of the absorption in 2-propanol. The value given is an average of the results obtained in 6 different laboratories.

ASSAY

34.4 mg of vitamin A acetate per g of solution (equivalent to 30.0 mg of retinol and to about 100,000 IU of vitamin A per g of solution).

Method: Direct spectrophotometric measurement of the absorption (a) at the maximum at 325 nm of a 100 µg/ml solution in 2-propanol. The result was calculated using the following formula.

$$a (\lambda_{\max}, \text{caps.}) \times 1000$$

$$= \text{mg vitamin A acetate/g}$$

$$a (\lambda_{\max}, \text{theoretical value for vitamin A acetate})$$

Theoretical absorption for vitamin A acetate = 155.7

Duplicate determinations were carried out on 4 capsules (n = 7, relative standard deviation 1%)

In the calculations the following conversion factors were used:

1 mg of vitamin A (retinol) is equivalent to 1.1468 mg of vitamin A acetate.

1 IU of vitamin A is equivalent to 0.000344 mg of vitamin A acetate.

PURITY

Thin-layer chromatography

The following chromatographic systems were used to check the identity of vitamin A acetate.

Thin-layer: Silica gel 60, F-254 (Merck) and HPTLC silica gel 60 F-254 (Merck)
Eluent: Cyclohexane/diethyl ether (4:1)
Sample: 200 µg oil which corresponds to about 7 µg vitamin A acetate.
Visualization: UV-light of 254 nm and 325 nm and iodine vapor.

Result: Rf of vitamin A acetate = 0.4 corresponding to the retention of a crystalline sample. It was not possible to estimate the purity by this method as vitamin A acetate degrades on the plate (checked by two-dimensional TLC), besides the cottonseed oil gave rise to disturbing spots.

ADDITIONAL DATA

Assay, vitamin A acetate HPLC: 101900 IU/g. As a standard USP lot R with a potency of 85600 IU/g was used.

Assay vitamin A acetate USP: 100600 IU/g

Vitamin A acetate crystals prior to encapsulation.

Vitamin A acetate HPLC: 0.08% cis-A acetate
99.77% trans A acetate

The results were obtained through the U.S.P.

CONCLUSION

Vitamin A acetate No 686038 can be considered suitable as International Chemical Reference Substance for the intended purpose. The content of vitamin A acetate is taken to be 34.4 mg per g of solution, corresponding to 30.0 mg of retinol.

* * *