

## WHO COLLABORATING CENTRE FOR CHEMICAL REFERENCE SUBSTANCES

### Report on the work in 2005

by V. Hjortsberg

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

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

Kindly address your comments to **Ms V. Hjortsberg**, WHO Collaborating Centre for Chemical Reference Substances, Apoteket AB, Produktion & Laboratorier, Farmaci/Centrallaboratoriet (ACL), Prismavägen 2, SE-141 75 Kungens Kurva, Sweden, along with a copy to be sent to **Dr S. Kopp**, Quality Assurance & Safety: Medicines, World Health Organization, CH-1211 Geneva 27, Switzerland; fax: (+41-22) 791 4730; e-mail: [kopps@who.int](mailto:kopps@who.int).

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Distribution of reference substances in 2005

During 2005 the total number of International Chemical Reference Substances distributed from the Centre was 1360. The most frequently requested substances are given in Appendix 1. The five most frequently requested substances were in order of demand: Tetracycline hydrochloride, Artesunate, Caffeine M.P., Phenacetin M.P. and Vanillin M.P.

Details of distribution to the WHO Regions are given in Appendix 2. It is observed that 13.5% of the substances went to the African Region, 0.5% to the Region of the Americas, 0% to the Eastern Mediterranean Region, 83.1% to the European Region, 1.8% to South-East Asia and 1.1% to the Western Pacific Region.

Distribution of reference spectra in 2005

No reference spectra were distributed during 2005.

Establishment of reference substances in 2005

In accordance with the procedure recommended by the WHO Expert Committee on Specifications for Pharmaceutical Preparations in its Thirty-second report (Technical Report Series, No. 823), four International Chemical Reference Substances were established in 2005. The substances are listed in Appendix 3.

A complete list of all International Chemical Reference Substances available from the Centre in January 2006, with information about package sizes and control numbers for the current batches, is given in Appendix 4. An updated list can be found at <http://www.apl.apoteket.se/who>.

#### Reference spectra

A complete list of all International Infrared Reference Spectra with the new spectra established since 1993 is given in Appendix 5.

#### Work on new and replacement substances completed in 2005

During 2005 work on seven new International Chemical Reference Substances was performed. The new reference substances are all antiretrovirals, namely Indinavir, Lamivudine, Nelfinavir mesilate, Ritonavir, Saquinavir mesilate, Stavudine and Zidovudine. The analytical reports are given in Appendices 10-12, 14-16 and 19.

Work on new batches of Erythromycin B with Control No 205186, Gentamicin sulfate with Control No 205183, Nystatin with Control No 405152, Tamoxifen *E*-isomer with Control No 205209 and Tetracycline hydrochloride with Control No 205095 was performed as the former batches were depleted. The analytical reports are given in Appendices 8-9, 13 and 17-18.

The new batches are considered suitable for adoption as International Chemical Reference Substances.

#### Stability testing

The regular stability monitoring of existing International Chemical Reference Substances was continued. This year 27 substances were re-examined. The results are given in Appendix 6.

#### Work in progress and future work

Work is continuously performed on the substances required to support the monographs in Volumes 3, 4 and 5 of *The International Pharmacopoeia*, see Appendix 7. The work in 2006 will focus on development of reference substances for antiretrovirals. The Centre is also planning to develop a thin-layer chromatographic screening test for antimalarials during the year.

#### Administrative and financial matters

The total cost for running the Centre in 2005 was estimated at US\$ 631 900. The income from sales of reference substances was US\$ 95 200, the contribution from SIDA was US\$ 537 000 and the contribution received from the WHO headquarters was US\$ 16 000.

The fee was kept at US\$ 70 per package and the freight and handling charge, added to each order, was kept at US\$ 10 during 2005.

#### Acknowledgements

The Centre is grateful to the laboratories that have contributed to the work during 2005. This year we would like to address our thanks in particular to the Abbott Laboratories, North Chicago, Illinois, USA; APL Research Centre, Aurobindo Pharma Ltd., Hyderabad, India; AstraZeneca, Macclesfield, England; Centre for Analytical Science, Health Sciences Authority, Singapore; Cipla

Ltd., Mumbai, India; F. Hoffmann-La Roche Ltd., Basel, Switzerland and Merck & Co., Inc., West Point, USA.

APPENDIX 1**DISTRIBUTION OF INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES  
IN 2005**

The most frequently requested International Chemical Reference Substances (ICRS) were in order of demand:

<b>ICRS</b>	<b>Items sold</b>
Tetracycline hydrochloride	104
Artesunate	94
Caffeine M.P.	65
Phenacetin M.P.	61
Vanillin M.P.	56
Paracetamol	53
Folic acid	43
Benzympenicillin sodium	42
Oxytetracycline hydrochloride	36
Azobenzene M.P.	33
Oxytetracycline dihydrate	31
Sulfamethoxazole	30
Benzanilide M.P.	26
Acetanilide M.P.	24
Ciprofloxacin ethylenediamine-compound	24
Sulfanilamide M.P.	23
Saccharin M.P.	22
Ciprofloxacin by-compound A	21
Ciprofloxacin desfluoro-compound	19
Ibuprofen	19
Phenolphthalein M.P.	19
Artemimol	17
Artemether	15
Artemisinin	14
Vincristine sulfate	14

APPENDIX 2

**DISTRIBUTION OF INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES  
TO DIFFERENT WHO REGIONS IN 2005**

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<b>WHO Regions</b>	<b>Number of distributed ICRS</b>
African Region (AFRO)	183
Region of the Americas (AMRO)	7
Eastern Mediterranean Region (EMRO)	0
European Region (EURO)	1131
South-East Asia Region (SEARO)	24
Western Pacific Region (WPRO)	15

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APPENDIX 3

**INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES ESTABLISHED IN 2005**

<b>Reference substance</b>	<b>Control number</b>	<b>Analytical report</b>
Didanosine	104228	PSM/QSM/2005.1 Appendix 9
Didanosine for System Suitability	104230	PSM/QSM/2005.1 Appendix 10
Efavirenz	104229	PSM/QSM/2005.1 Appendix 11
Nevirapine	104227	PSM/QSM/2005.1 Appendix 12

## APPENDIX 4

### LIST OF AVAILABLE INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES

2006

#### General information

International Chemical Reference Substances are established upon the advice of the WHO Expert Committee on Specifications for Pharmaceutical Preparations. They are supplied primarily for use in physical and chemical tests and assays described in the specifications for quality control of drugs published in *The International Pharmacopoeia* or proposed in draft monographs. The International Chemical Reference Substances are mainly intended to be used as primary standards to calibrate secondary standards.

Directions for use and required analytical data for the intended use in the relevant specifications of *The International Pharmacopoeia* are given in the certificates enclosed with the substances when distributed.

International Chemical Reference Substances may also be used in tests and assays not described in *The International Pharmacopoeia*. However, the responsibility for assessing the suitability of the substances then rests with the user or with the pharmacopoeia commission or other authority that has prescribed this use.

It is generally recommended that the substances should be stored protected from light and moisture and preferably at a temperature of about +5 °C. When special storage conditions are required, this is stated on the label or in the certificate. It is recommended to the user to purchase only sufficient amount for immediate use.

The stability of the International Chemical Reference Substances kept at the Collaborating Centre is monitored by regular re-examination and any material that has deteriorated is replaced by new batches when necessary. Lists giving control numbers for the current batches are issued in the annual reports from the Centre and new lists may also be obtained on request.

#### Ordering information

Orders for the International Chemical Reference Substances should be sent to:

WHO Collaborating Centre for Chemical Reference Substances  
Apoteket AB  
Produktion & Laboratorier  
Farmaci/Centrallaboratoriet (ACL)  
Prismavägen 2  
SE-141 75 KUNGENS KURVA  
SWEDEN

Fax: + 46 8 740 60 40

E-mail: [who.apl@apoteket.se](mailto:who.apl@apoteket.se)

Website: <http://www.apl.apoteket.se/who>

The current price for the International Chemical Reference Substances (ICRS) is US\$ 70 per package. An administration charge of US\$ 10 is added to each order to cover costs for handling and dispatch by air mail or air parcel post. If dispatch by air freight is wanted the freight costs will amount to about US\$ 200 and these costs have to be paid by the purchaser. Payment should be made according to the invoice. Kindly direct all payments (cheques, bills of exchange, banker's drafts, banker's transfers etc.) to:

**Nordea Bank Sweden, SE-105 71 STOCKHOLM**  
**(Apoteket AB/APL/ACL/WHO)**  
**Swift: NDEASESS**  
**Account no (PG): 2 98 40-6**  
**IBAN: SE 65 9500 0099 6026 0029 8406**

*Our invoice number must be quoted when payment is made.*

If, however, payment in advance is asked for but not allowed according to the regulations of certain countries, **Documentary Remittance (Cash against Documents)** may be used. This means that the invoice is paid at the buyer's bank and against that receipt the parcel is collected at the customs office or, when so agreed, at the bank.

**We regret that we cannot accept payment by letter of credit (L/C).**

Nor can the WHO Centre issue **Certificate of Origin**, as the bulk material for the ICRS originates from different parts of the world. Also the Centre cannot assist in any legalization of such or other documents sometimes asked for, which has to be respected by the purchaser.

On dispatch by air freight, the freight cost is paid directly to the carrier by the purchaser. **In all cases the payment should be net of charge for the WHO Collaborating Centre.**

The administration charge of US\$ 10 covers cost for **handling and dispatch by air mail** (small parcel or air parcel post). If **registered air mail** or **express air mail** is required, an extra charge is added. If safe delivery is possible by means of airmail, it ought to be preferred being a much less expensive way to all parties.

The International Chemical Reference Substances (ICRS) are only supplied in standard packages as indicated in the following list.

<b>Catalogue number</b>	<b>Reference substances</b>	<b>Package size</b>	<b>Control number</b>
9930375	p-Acetamidobenzalazine	25 mg	290042
9930202	Acetazolamide	100 mg	186128
9930204	Allopurinol	100 mg	287049
9930206	Amidotrizoic acid	100 mg	196205
9930191	2-Amino-5-nitrothiazole	25 mg	186131
9930194	3-Aminopyrazole-4-carboxamide hemisulfate	100 mg	172050
9930193	3-Amino-2,4,6-triiodobenzoic acid	100 mg	196206
9930208	Amitriptyline hydrochloride	100 mg	181101
9930209	Amodiaquine hydrochloride	200 mg	192160
9930210	Amphotericin B	400 mg	191153
9930211	Ampicillin (anhydrous)	200 mg	390001
9930212	Ampicillin sodium	200 mg	388002
9930213	Ampicillin trihydrate	200 mg	274003
9930214	Anhydrotetracycline hydrochloride	25 mg	180096
9931408	Artemether	100 mg	103225
9931406	Artemisinin	100 mg	103222
9931407	Artemotil	100 mg	103226
9931410	Artenimol	100 mg	103223
9931409	Artesunate	100 mg	103224
9930215	Atropine sulfate	100 mg	183111
9930216	Azathioprine	100 mg	172060
9930218	Bacitracin zinc	200 mg	192174
9930219	Beclometasone dipropionate	200 mg	192175
9930225	Benzylpenicillin potassium	200 mg	180099
9930226	Benzylpenicillin sodium	200 mg	280047
9930227	Bephenium hydroxynaphthoate	100 mg	183112
9930228	Betamethasone	100 mg	183113
9930229	Betamethasone sodium phosphate	100 mg	196203
9930230	Betamethasone valerate	100 mg	190145
9930233	Bupivacaine hydrochloride	100 mg	289054
9930234	Caffeine	100 mg	181102
9930236	Calcium folinate (Leucovorin calcium)	100 mg	194188
9930237	Captopril	100 mg	197214
9930238	Captopril disulfide	25 mg	198216
9930239	Carbamazepine	100 mg	189143
9930240	Carbenicillin monosodium	200 mg	383043
9930241	Chloramphenicol	200 mg	486004
9930242	Chloramphenicol palmitate	1 g	286072
9930243	Chloramphenicol palmitate (Polymorph A)	200 mg	175073
9930199	5-Chloro-2-methylaminobenzophenone	100 mg	172061
9930245	Chloroquine sulfate	200 mg	195201
9930190	2-(4-Chloro-3-sulfamoylbenzoyl)benzoic acid	50 mg	181106
9930246	Chlorphenamine hydrogen maleate	100 mg	182109
9930247	Chlorpromazine hydrochloride	100 mg	178080
9930248	Chlortalidone	100 mg	183114

<b>Catalogue number</b>	<b>Reference substances</b>	<b>Package size</b>	<b>Control number</b>
9930249	Chlortetracycline hydrochloride	200 mg	187138
9930250	Cimetidine	100 mg	190150
9930256	Ciprofloxacin hydrochloride	400 mg	197210
9930252	Ciprofloxacin by-compound A	20 mg	198220
9930253	Ciprofloxacin desfluoro-compound	20 mg	198219
9930254	Ciprofloxacin ethylenediamine-compound	20 mg	198218
9930255	Ciprofloxacin fluoroquinolonic acid	20 mg	198217
9930258	Cisplatin	100 mg	197207
9930259	Clomifene citrate	100 mg	187136
	Clomifene citrate Z-isomer <i>see</i> Zuclomifene		
9930261	Cloxacillin sodium	200 mg	274005
9930262	Colecalciferol (Vitamin D3)	500 mg	190146
9930263	Cortisone acetate	100 mg	167006
9930265	Dapsone	100 mg	183115
9930266	Desoxycortone acetate	100 mg	167007
9930267	Dexamethasone	100 mg	388008
9930268	Dexamethasone acetate	100 mg	288009
9930269	Dexamethasone phosphoric acid	100 mg	192161
9930270	Dexamethasone sodium phosphate	100 mg	192158
9930282	Diazoxide	100 mg	181103
9930283	Dicloxacillin sodium	200 mg	174071
9930285	Dicoumarol	100 mg	178077
9931413	Didanosine	100 mg	104228
9931414	Didanosine for system suitability	10 mg	104230
9930287	Diethylcarbamazine dihydrogen citrate	100 mg	181100
9930288	Digitoxin	100 mg	277010
9930289	Digoxin	100 mg	587011
9930290	Dopamine hydrochloride	100 mg	192159
9930292	Doxorubicin hydrochloride	100 mg	196202
9930294	Emetine hydrochloride	100 mg	187134
9931411	Efavirenz	100 mg	104229
9930197	4-Epianhydrotetracycline hydrochloride	25 mg	288097
9930295	Ergocalciferol (Vitamin D2)	500 mg	190147
9930296	Ergometrine hydrogen maleate	50 mg	277012
9930297	Ergotamine tartrate	50 mg	385013
9930298	Erythromycin	250 mg	191154
9930299	Erythromycin B	100 mg	205186
9930300	Erythromycin C	25 mg	194187
9930301	Estradiol benzoate	100 mg	167014
9930302	Estrone	100 mg	279015
9930304	Ethambutol hydrochloride	100 mg	179081
9930305	Ethinylestradiol	100 mg	301016
9930306	Ethisterone	100 mg	167017
9930307	Ethosuximide	100 mg	179088

<b>Catalogue number</b>	<b>Reference substances</b>	<b>Package size</b>	<b>Control number</b>
9930309	Flucloxacillin sodium	200 mg	195194
9930310	Flucytosine	100 mg	184121
9930311	Fludrocortisone acetate	200 mg	195199
9930312	Fluorouracil	100 mg	184122
9930313	Fluphenazine decanoate dihydrochloride	100 mg	182107
9930314	Fluphenazine enantate dihydrochloride	100 mg	182108
9930315	Fluphenazine hydrochloride	100 mg	176076
9930316	Folic acid	100 mg	388019
9930195	3-Formylrifamycin	200 mg	202149
9930355	Framycetin sulfate (Neomycin B sulfate)	200 mg	193178
9930318	Furosemide	100 mg	171044
9930319	Gentamicin sulfate	100 mg	205183
9930322	Griseofulvin	200 mg	280040
9930323	Haloperidol	100 mg	172063
9930324	Hydrochlorothiazide	100 mg	179087
9930325	Hydrocortisone	100 mg	283020
9930326	Hydrocortisone acetate	100 mg	280021
9930327	Hydrocortisone sodium succinate	200 mg	194184
9930188	(-)-3-(4-Hydroxy-3-methoxyphenyl)-2-hydrazino-2-methylalanine (3- <i>o</i> -Methylcarbidopa)	25 mg	193180
9930189	(-)-3-(4-Hydroxy-3-methoxyphenyl)-2-methylalanine (3- <i>o</i> -Methylmethyldopa)	25 mg	179085
9930328	Ibuprofen	100 mg	183117
9930329	Imipramine hydrochloride	100 mg	172064
9931415	Indinavir	100 mg	105231
9930330	Indometacin	100 mg	178078
9930331	Isoniazid	100 mg	185124
9930332	Kanamycin monosulfate	12 mg	197211
9931416	Lamivudine	100 mg	105232
9930333	Lanatoside C	100 mg	281022
9930334	Levodopa	100 mg	295065
9930335	Levonorgestrel	200 mg	194182
9930336	Levothyroxine sodium	100 mg	189144
9930337	Lidocaine	100 mg	181104
9930338	Lidocaine hydrochloride	100 mg	181105
9930339	Liothyronine sodium	50 mg	193179
9930340	Loperamide hydrochloride	100 mg	194185

<b>Catalogue number</b>	<b>Reference substances</b>	<b>Package size</b>	<b>Control number</b>
9930341	Mebendazole	200 mg	195195
Melting Point Reference Substances			
9930217	Azobenzene (69 °C)	1 g	192168
9930438	Vanillin (83 °C)	1 g	299169
9930222	Benzil (96 °C)	1 g	294170
9930201	Acetanilide (116 °C)	1 g	297171
9930380	Phenacetin (136 °C)	1 g	297172
9930221	Benzanilide (165 °C)	4 g	192173
9930422	Sulfanilamide (166 °C)	1 g	192162
9930423	Sulfapyridine (193 °C)	4 g	192163
9930286	Dicyanodiamide (210 °C)	1 g	192164
9930411	Saccharin (229 °C)	1 g	192165
9930235	Caffeine (237 °C)	1 g	299166
9930382	Phenolphthalein (263 °C)	1 g	299167
9930345	Methotrexate 3- <i>o</i> -Methylcarbidopa <i>see</i> (-)-3-(4-Hydroxy-3-methoxyphenyl)-2-hydrazino-2-methylalanine 3- <i>o</i> -Methylmethyldopa <i>see</i> (-)-3-(4-Hydroxy-3-methoxyphenyl)-2-methylalanine	100 mg	194193
9930346	Methyldopa	100 mg	179084
9930347	Methyltestosterone	100 mg	167023
9930348	Meticillin sodium	200 mg	274024
9930350	Metronidazole	100 mg	183118
9930351	Nafcillin sodium	200 mg	272025
9930354	Neamine hydrochloride (Neomycin A hydrochloride)	0.5 mg	193177
9931417	Nelfinavir mesilate Neomycin B sulfate <i>see</i> Framycetin sulfate	100 mg	105233
9930356	Neostigmine metilsulfate	100 mg	187135
9931412	Nevirapine anhydrous	100 mg	104227
9930357	Nicotinamide	100 mg	200090
9930358	Nicotinic acid	100 mg	179091
9930359	Nifurtimox	100 mg	194189
9930360	Niridazole	200 mg	186129
9930361	Niridazole-chlorethylcarboxamide	25 mg	186130
9930366	Norethisterone	100 mg	186132
9930367	Norethisterone acetate	100 mg	185123
9930369	Nystatin	200 mg	405152
9930371	Ouabain	100 mg	283026
9930372	Oxacillin sodium	200 mg	382027
9930373	Oxytetracycline dihydrate	200 mg	189142
9930374	Oxytetracycline hydrochloride	200 mg	189141

<b>Catalogue number</b>	<b>Reference substances</b>	<b>Package size</b>	<b>Control number</b>
9930376	Papaverine hydrochloride	100 mg	185127
9930377	Paracetamol	100 mg	195198
9930378	Paromomycin sulfate	75 mg	195197
9930383	Phenoxymethylpenicillin	200 mg	179082
9930384	Phenoxymethylpenicillin calcium	200 mg	179083
9930385	Phenoxymethylpenicillin potassium	200 mg	176075
9930387	Phenytoin	100 mg	179089
9930388	Piperazine adipate	100 mg	197212
9930389	Piperazine citrate	100 mg	197213
9930390	Praziquantel	100 mg	194191
9930391	Prednisolone	100 mg	389029
9930392	Prednisolone acetate	100 mg	289030
9930393	Prednisolone hemisuccinate	200 mg	195196
9930394	Prednisolone sodium phosphate	200 mg	194190
9930395	Prednisone	100 mg	167031
9930396	Prednisone acetate	100 mg	169032
9930397	Probenecid	100 mg	192156
9930398	Procaine hydrochloride	100 mg	183119
9930399	Procarbazine hydrochloride	100 mg	184120
9930400	Progesterone	100 mg	167033
9930402	Propranolol hydrochloride	100 mg	187139
9930403	Propylthiouracil	100 mg	185126
9930404	Pyrantel embonate (Pyrantel pamoate)	500 mg	192157
9930405	Pyridostigmine bromide	100 mg	182110
9930406	Reserpine	100 mg	186133
9930408	Riboflavin	250 mg	382035
9930409	Rifampicin	300 mg	191151
9930410	Rifampicin quinone	200 mg	202148
9931421	Ritonavir	100 mg	105237
9931418	Saquinavir mesilate	100 mg	105234
9930412	Sodium amidotrizoate	100 mg	198221
9930413	Sodium cromoglicate	100 mg	188140
9930415	Spectinomycin hydrochloride	200 mg	193176
9931419	Stavudine	100 mg	105235
9930416	Streptomycin sulfate	100 mg	197215
9930417	Sulfacetamide	100 mg	196200
9930419	Sulfamethoxazole	100 mg	179092
9930420	Sulfamethoxypyridazine	100 mg	178079
9930421	Sulfanilamide	100 mg	179094
9930424	Sulfasalazine	100 mg	191155
9930425	Tamoxifen citrate	100 mg	196208
9930426	Tamoxifen <i>E</i> -isomer	10 mg	205209
9930427	Testosterone enantate	200 mg	194192
9930428	Testosterone propionate	100 mg	167036

<b>Catalogue number</b>	<b>Reference substances</b>	<b>Package size</b>	<b>Control number</b>
9930429	Tetracycline hydrochloride	200 mg	205095
9930430	Thioacetazone	100 mg	171046
9930196	4,4' - Thiodianiline	50 mg	183116
	Thyroxine sodium <i>see</i> Levothyroxine sodium		
9930431	Tolbutamide	100 mg	179086
9930432	Tolnaftate	100 mg	176074
9930433	Toluene-2-sulfonamide	100 mg	196204
9930434	Trimethadione	200 mg	185125
9930435	Trimethoprim	100 mg	179093
9930440	Vincristine sulfate	9.7 mg/vial	193181
9930439	Warfarin	100 mg	168041
9931420	Zidovudine	100 mg	105236
9930260	Zuclomifene	50 mg	187137

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APPENDIX 5

**LIST OF AVAILABLE INTERNATIONAL INFRARED REFERENCE SPECTRA**

**2006**

In addition to International Chemical Reference Substances the WHO Collaborating Centre for Chemical Reference Substances is able to supply 69 International Infrared Reference Spectra.

The current price is US\$ 5 for a single spectrum and US\$ 200 for a set of 50 spectra, including a hardcover binder. The binder can be ordered separately for US\$ 10.

An administrative charge of US\$ 10 is added to each order to cover costs for handling and dispatch by air mail or air parcel post.

Orders should be sent to:

WHO Collaborating Centre for Chemical Reference Substances  
Apoteket AB  
Produktion & Laboratorier  
Farmaci/Centrallaboratoriet (ACL)  
Prismavägen 2  
SE-141 75 KUNGENS KURVA  
SWEDEN

Fax: + 46 8 740 60 40

E-mail: [who.apl@apoteket.se](mailto:who.apl@apoteket.se)

Website: <http://www.apl.apoteket.se/who>

Payment should be made according to the invoice. Kindly direct all payments to:

**Nordea Bank Sweden, SE-105 71 STOCKHOLM**  
**(Apoteket AB/APL/ACL/WHO)**  
**Swift: NDEASESS**  
**Account no (PG): 2 98 40-6**  
**IBAN: SE 65 9500 0099 6026 0029 8406**

Our invoice number must be quoted when payment is made.

The following International Infrared Reference Spectra are available from the Centre:

Aceclidine salicylate	Lidocaine
Acetazolamide	Lidocaine hydrochloride
Allopurinol	Lindane
Amiloride hydrochloride	
Amitriptyline hydrochloride	Metronidazole
Ampicillin trihydrate	Miconazole nitrate
Beclometasone dipropionate	Niclosamide
Benzylpenicillin potassium	Nicotinamide
Biperiden	Noscapine
Biperiden hydrochloride	
Bupivacaine hydrochloride	Oxamniquine
Caffeine (anhydrous)	Papaverine hydrochloride
Calcium folinate	Phenobarbital
Carbidopa	Phenoxymethylpenicillin calcium
Chlorphenamine hydrogen maleate	Phenytoin
Clofazimine	Primaquine phosphate
Cloxacillin sodium	Propylthiouracil
Colchicine	Protionamide
Cytarabine	Pyrimethamine
Dexamethasone	Salbutamol
Dexamethasone acetate, monohydrate	Salbutamol sulfate
Dextromethorphan hydrobromide	Sulfadimidine
Diazepam	Sulfadoxine
Dicolinium iodide	Sulfamethoxazole
Dicoumarol	Sulfamethoxyipyridazine
Diethylcarbamazine dihydrogen citrate	
Diphenoxylate hydrochloride	Tiabendazole
	Trihexyphenidyl hydrochloride
Erythromycin ethylsuccinate	Trimethoprim
Erythromycin stearate	
Etacrynic acid	Valproic acid
Ethionamide	Verapamil hydrochloride
Ethosuximide	
Furosemide	
Gallamine triethiodide	
Glibenclamide	
Haloperidol	
Hydrochlorothiazide	
Ibuprofen	
Imipramine hydrochloride	
Indometacin	
Isoniazid	

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## APPENDIX 6

### STABILITY TESTING – ANALYTICAL REPORT

The stability on storage of the International Chemical Reference Substances is monitored by regular re-examinations of the substances held in stock at the Centre. The results obtained for the substances re-examined in 2005 are summarized below. For comparison, results obtained on earlier occasions are included in the summaries. The substances have been stored in tightly closed containers at +5 °C and in a relative humidity below 30%. The following abbreviations are used in the tables:

CE	Capillary Electrophoresis
DSC	Differential Scanning Calorimetry
DTA	Differential Thermal Analysis
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
IR	Infrared Spectrophotometry
KF	Karl Fischer titration
LC-MS	Liquid Chromatography with Mass Spectrometric Detection
LOD	Loss on Drying
TLC	Thin-layer Chromatography
PSA	Phase Solubility Analysis
TGA	Thermogravimetric Analysis

The estimates of total impurities by HPLC, CE and TLC are expressed as area per cent (area %), if not otherwise stated; by DSC and DTA as mole per cent (mol%) and by PSA as weight per cent (w/w %). LOD and TGA (loss of weight) are expressed as weight per cent (w/w %). Assay values are calculated with reference to the dried or the anhydrous substance unless otherwise stated. More details about the analytical methods used can be obtained from the Centre.

#### Cloxacillin sodium, Control No 274005

Initial analytical report: WHO/PHARM/75.485, Appendix 7

Examination year:	1974	1979	1984	1989	1995	2001	2005
IR	conforms	-	conforms	-	conforms	-	conforms
TLC, %	-	-	-	-	1.0	-	-
HPLC, %	-	0.5	1.0	0.6	1.0	0.3	0.5
TGA, %	-	-	-	4.2	3.9	4.3	4.1
Water (KF), %	4.2	4.0	4.0	-	-	-	-
Assay, mercurimetric, %	100.2	99.1	98.9	-	99.6	-	-

Dicloxacillin sodium, Control No 174071

Initial analytical report: WHO/PHARM/74.478, Appendix 5

Examination year:	1974	1982	1984	1989	1995	2001	2005
IR	conforms	-	-	-	conforms	-	conforms
HPLC, %	-	0.3	0.4	0.3	0.5	0.4	0.3
TGA, %	-	-	-	3.9	3.9	3.9	3.7
Water (KF), %	3.8	3.9	3.8	-	-	-	-
Degradation products, mercurimetric, %	-	-	0.6	-	0.7	-	-
Assay, mercurimetric, %	-	-	99.5	-	-	-	-
Assay, alcalimetric titration, %	99.5	99.4	-	-	-	-	-

Diethylcarbamazine dihydrogen citrate, Control No 181100

Initial analytical report: WHO/PHARM/82.509, Appendix 10

Examination year:	1981	2000	2005
IR	conforms	conforms	-
HPLC, %	-	99.9	100.0
Water (KF), %	0.1	-	-
LOD, %	-	< 0.1	< 0.1

Ergometrine hydrogen maleate, Control No 277012

Initial analytical report: WHO/PHARM/78.494, Appendix 5

---

Examination year:	1977	1982	1989	1993	2000	2005
IR	conforms	-	conforms	-	conforms	-
TLC, %	1 (3 impurities)	3 impurities	0.8 (4 impurities)	-	-	-
HPLC, %	0.8	0.9	-	0.5	0.5	0.4
TGA, %	-	-	0.3	0.5	0.3	0.4
LOD, %	0.8	-	0.3	-	-	-
Assay, titrimetric, %	100.0	99.9	-	-	-	-

---

Ergotamine tartrate, Control No 385013

Initial analytical report: WHO/PHARM/86.527, Appendix 6

---

Examination year:	1985	1993	2001	2005
IR	conforms	-	conforms	conforms
TLC, %	< 0.5	-	-	-
HPLC, %	0.2	1.1	0.4	0.6
TGA, %	-	-	2.8	2.3
LOD, %	2.4	-	-	-

---

Erythromycin C, Control No 194187

Initial analytical report: WHO/PHARM/95.577, Appendix 12

Examination year:	1994	2001	2005
IR	conforms	-	conforms
TLC, %	0.3	-	-
HPLC, %	0.5	0.3	1.0
TGA, %	1.5	0.8	1.1

Flucloxacillin sodium, Control No 195194

Initial analytical report: WHO/PHARM/96.584, Appendix 11

Examination year:	1995	2000	2005
IR	conforms	-	conforms
TLC, %	3 impurities	-	-
HPLC, %	0.7	0.5	0.7
TGA, %	4.4	4.3	4.3

Fludrocortisone acetate, Control No 195199

Initial analytical report: WHO/PHARM/96.584, Appendix 12

---

Examination year:	1995	2000	2005
IR	conforms	-	-
TLC, %	0.3	-	-
HPLC, %	0.6	0.4	0.4
TGA, %	< 0.1	< 0.1	< 0.1
Assay, colorimetric, %	100.3	-	-

---

Folic acid, Control No 388019

Initial analytical report: WHO/PHARM/89.544, Appendix 10

---

Examination year:	1988	1991	1996	2001	2005
IR	conforms	-	-	-	conforms
TLC, %	2 impurities	-	0.2	-	-
HPLC, %	0.1	-	0.1	0.3	0.2
TGA, %	7.7	7.6	7.8	7.5	7.6
Water (KF), %	7.7	-	-	7.1	-
UV ( $A_{1\text{cm}}^{1\%}$ )	577 (256 nm)	-	-	-	-
Assay, spectrophotometric, %	100.0	-	100.3	-	-

---

3-Formylrifamycin, Control No 202149

Initial analytical report: EDM/QSM/2003.6, Appendix 9

Examination year:	2002	2005
IR	conforms	conforms
TLC, %	0.3	-
HPLC, %	0.4	0.4
Water (KF), %	0.4	0.3
Residual solvents (GC), %	6.0 (THF)	-

Griseofulvin, Control No 280040

Initial analytical report: WHO/PHARM/81.508, Appendix 10

Examination year:	1980	1988	1993	2000	2005
IR	conforms	conforms	-	conforms	-
TLC, %	1 impurity	1 impurity	0.4 (1 impurity)	-	-
HPLC, %	0.6*	0.6*, 0.9**	1.1**	0.8**	0.9**
TGA, %	-	< 0.1	< 0.1	< 0.1	< 0.1

\* 236 nm

\*\* 291 nm

Indometacin, Control No 178078

Initial analytical report: WHO/PHARM/79.499, Appendix 8

Examination year:	1978	1985	1995	2000	2005
IR	conforms	-	conforms	-	conforms*
TLC, %	0.1	0.1	< 0.2	-	-
HPLC, %	-	-	< 0.1	< 0.1	< 0.1*
TGA, %	-	-	< 0.1	< 0.1	< 0.1
LOD, %	0.2	< 0.1	-	-	-
Assay, HPLC, %	-	-	-	-	100.0*

\* Analyses performed by the Centre for Analytical Science, Health Sciences Authority, Singapore.

Lidocaine, Control No 181104

Initial analytical report: WHO/PHARM/82.509, Appendix 13

Examination year:	1981	1988	1995	2000	2005
IR	conforms	conforms	-	-	-
TLC, %	< 0.1	< 0.1	0.2	-	-
HPLC, %	< 0.1	-	< 0.1	< 0.02	< 0.05
Water (KF), %	-	-	-	-	< 0.1
LOD, %	< 0.1	0.3	0.3	< 0.1	-
Assay, potentiometric, %	99.6	99.8	-	-	-

Lidocaine hydrochloride, Control No 181105

Initial analytical report: WHO/PHARM/82.509, Appendix 14

Examination year:	1981	1988	1995	2000	2005
IR	conforms	conforms	conforms	-	-
TLC, %	< 0.1	< 0.1	< 0.1	-	-
HPLC, %	< 0.1	-	< 0.1	< 0.03	< 0.03
Water (KF), %	6.4	6.2	6.3	6.3	6.3
Assay, potentiometric, %	100.2	100.0	-	-	-

Loperamide hydrochloride, Control No 194185

Initial analytical report: WHO/PHARM/95.577, Appendix 16

Examination year:	1994	2000	2005
IR	conforms	-	-
TLC, %	< 0.1	-	-
HPLC, %	< 0.1	0.1	0.1
TGA, %	< 0.1	< 0.1	< 0.1

Methyltestosterone, Control No 167023

Initial analytical report: WHO/PHARM/420.64, Appendix 3

Examination year:	1967	1975	1980	1984	1988	1995	2000	2005
IR	conforms	-	-	-	conforms	conforms	-	-
TLC, %	1 impurity	2 imp.	2 imp.	1 imp.	-	0.07	-	-
HPLC, %	-	-	-	0.2	0.2	0.2	0.1	0.2
TGA, %	-	-	-	-	-	1.0	1.0	0.6
LOD, %	0.3	1.2	0.3	0.8	1.2	-	-	-
PSA, %	0.2	-	-	-	-	-	-	-
UV ( $A_{1cm}^{1\%}$ )	541* (242 nm)	-	539*	541*	544*	542* 547**	-	-

\* "As is"

\*\* Calculated with reference to the dried substance

Norethisterone acetate, Control No 185123

Initial analytical report: WHO/PHARM/86.527, Appendix 8

Examination year:	1985	1995	2000	2005
IR	conforms	conforms	-	-
TLC, %	0.4	0.2	-	-
HPLC, %	0.3	0.3	0.3	0.4
TGA, %	-	< 0.1	< 0.1	< 0.1
LOD, %	< 0.1	-	-	-
UV ( $A_{1cm}^{1\%}$ )	516 (241 nm)	515	-	-
Assay, spectrophotometric, %	100.0	99.9	-	-

Oxytetracycline hydrochloride, Control No 189141

Initial analytical report: WHO/PHARM/90.547, Appendix 9

Examination year:	1989	1995	2000	2005
IR	conforms	-	-	-
TLC	identity	identity	-	-
HPLC, %	2	2.1	3.3	3.3
TGA, %	-	0.5	0.6	0.3
Water (KF), %	0.3	0.5	-	-

Paracetamol, Control No 195198

Initial analytical report: WHO/PHARM/96.584, Appendix 15

Examination year:	1995	2000	2005
IR	conforms	-	conforms
TLC, %	< 0.01	-	-
HPLC, %	< 0.03	< 0.04	< 0.04
TGA, %	< 0.1	< 0.1	< 0.1
UV( $A_{1\text{cm}}^{1\%}$ )	695 (257 nm)	-	-

Prednisolone sodium phosphate, Control No 194190

Initial analytical report: WHO/PHARM/95.577, Appendix 20

Examination year:	1994	2000	2005
IR	conforms	-	-
TLC, %	0.8	-	-
HPLC, %	about 1	1.6	1.7
TGA, %	6.1	6.5	6.2
Assay, spectrophotometric, %	99	-	-

Prednisone acetate, Control No 169032

Initial analytical report: WHO/PHARM/70.455, Appendix 4

Examination year:	1969	1975	1984	1994	2000	2005
IR	conforms	-	-	-	-	-
TLC, %	3 impurities	2 imp.	2 imp.	0.6	-	-
HPLC, %	-	-	1.5	0.8	0.5	0.6
TGA, %	-	-	-	0.1	0.1	0.4
LOD, %	0.1	0.3	-	-	-	-
PSA, %	< 0.5	-	-	-	-	-
UV ( $A_{1cm}^{1\%}$ )	372 (238 nm)	371	383	381	-	-

Pyrantel embonate, Control No 192157

Initial analytical report: WHO/PHARM/93.564, Appendix 14

Examination year:	1992	2000	2005
IR	conforms	-	-
TLC, %	< 0.1	-	-
HPLC, %	< 0.1	0.1	0.02
TGA, %	< 0.1	0.1	< 0.1
LOD, %	0.1	-	-
Assay, spectrophotometric, %	100.6	-	-

Rifampicin quinone, Control No 202148

Initial analytical report: EDM/QSM/2003.6, Appendix 10

Examination year:	2002	2005
IR	conforms	conforms
HPLC, %	1.0	1.3
Water (KF), %	0.6	0.7
Residual solvents (GC), %	1.2 (THF)	-

Spectinomycin HCl, Control No 193176

Initial analytical report: WHO/PHARM/94.566, Appendix 14

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Examination year:	1994	2005
IR	conforms	-
TLC, %	no impurities	-
HPLC, %	-	no impurities (ELSD)
TGA, %	18.1	17.9
Water (KF), %	18.4	-

---

Streptomycin sulfate, Control No 197215

Initial analytical report: WHO/PHARM/97.595, Appendix 17.

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Examination year:	1998	2005
IR	conforms	conforms
LC-MS	conforms	-
TLC, %	identity	-
HPLC, %	6.0	6.5
Water (KF), %	-	0.3
CE, %	5.2	-
Microbiological assay, IU/mg	78500	-
Residual solvents (GC), %	< 0.1	-

---

Sulfasalazine, Control No 191155

Initial analytical report: WHO/PHARM/92.558, Appendix 12

Examination year:	1991	2000	2005
IR	conforms	-	conforms
HPLC, %	< 0.1	0.1	0.1
TLC, %	< 0.1	-	-
TGA, %	< 0.1	< 0.1	< 0.1

Warfarin, Control No 168041

Initial analytical report: WHO/PHARM/69.452, Appendix 3

Examination year:	1967	1980	1984	1989	2000	2005
IR	conforms	-	-	conforms	conforms	-
TLC, %	no impurities	no impurities	no impurities	< 0.5	-	-
HPLC, %	-	-	< 0.1	< 0.1	< 0.1	< 0.04
TGA, %	-	-	-	< 0.1	< 0.1	-
LOD, %	< 0.1	< 0.1	-	-	-	< 0.1
PSA, %	< 0.5	-	-	-	-	-
UV ( $A_{1cm}^{1\%}$ )	474	-	-	473	-	-

## APPENDIX 7

### **INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES - PROJECT LIST**

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

#### Volume 3

Noroxymorphone hydrochloride (\*\*)  
(impurity in Naloxone hydrochloride)

#### Volume 4

Dactinomycin  
Iohexol  
Medroxyprogesterone acetate (\*\*)

Pyrazinamide (\*\*)  
Thiopental sodium (\*\*)  
Vinblastine sulfate (\*\*)

#### Volume 5

Albendazole  
Alcuronium chloride  
Amoxicillin trihydrate (\*\*)  
Atenolol  
Atenolol for column validation  
Benznidazole  
9,9'-Bisanthracene-10,10'(9*H*, 9'*H*)-dione  
Chloramphenicol sodium succinate  
Chloramphenicol disodium disuccinate  
Ciclosporin (\*\*)  
Ciclosporin U (\*\*)  
Clindamycin hydrochloride (\*\*)  
Clindamycin phosphate  
Dacarbazine  
Dacarbazine related compound A  
Dacarbazine related compound B  
Dimethyl-2,6-dimethyl-4-(2-nitrophenyl)-  
pyridine-3,5-dicarboxylate  
Dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)-  
pyridine-3,5-dicarboxylate  
1,2-Diphenylethylammonium-3-mercapto-2-  
methylpropanoate (\*\*)

Dithranol  
Doxycycline hyclate (\*\*)  
Econazole nitrate  
6-Epidoxycycline hydrochloride  
Erythromycin ethylsuccinate  
Erythromycin stearate  
Etoposide  
1-Hydroxy-9-anthrone  
Idoxuridine  
Ketoconazole  
Levamisole hydrochloride  
Lincomycin hydrochloride (\*\*)  
Mefloquine hydrochloride  
Metacycline hydrochloride  
DL-methionine  
Metronidazole benzoate  
Nifedipine  
Nonoxinol 9  
Retinol palmitate (\*\*)  
Retinol propionate (\*\*)

#### On-going WHO project relating to specifications for antiretroviral agents

Abacavir sulfate (\*\*)  
Abacavir sulfate for system suitability  
Nevirapine impurity B (\*\*)

(\*\*) Denotes that candidate material is available at the Centre.

APPENDIX 8

**ERYTHROMYCIN B**

Control No 205186

Analytical Report

**Intended use**

The stock of the current batch of the International Chemical Reference Substance for erythromycin B Control No 194186 is depleted and has to be replaced. The International Chemical Reference Substance for erythromycin B is intended to be used in chromatographic purity and identity tests of erythromycin. The monograph for Erythromycin is given in *The International Pharmacopoeia*, Third Edition, Volume 3.

**Material**

About 100 g of the sample (manufacturer's batch no 89-348-BD) were received at the WHO Centre in September 2005. The material is being stored in tightly closed containers at + 5 °C, protected from light.

**Analytical data**

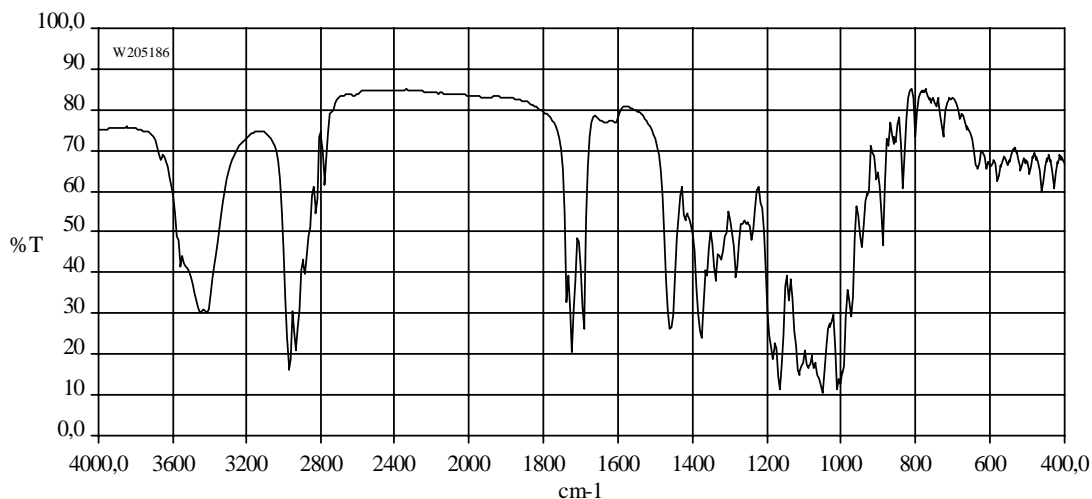
Description

A white powder.

**Evidence of chemical structure**

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W205186). The spectrum is concordant with the spectrum of the previous lot of the International Chemical Reference Substance (ICRS) for erythromycin B with Control No 194186.



**Figure 1.** IR-spectrum of 1.5 mg of Erythromycin B Control No 205186 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

#### Thin-layer chromatography

For the identity of Erythromycin B see results under Purity/Thin-layer chromatography.

#### **Assay**

##### Thermogravimetric analysis

When the substance was heated to 125 °C, a loss of 0.6% (w/w) (n=6, RSD=15.5%) was observed.

##### Water

0.7% (n=4) determined by Karl Fischer titration.

##### Residual solvents

Total content: <0.1%. No peaks were detected.

The test includes Class 1, Class 2 and Class 3 residual solvents according to Ph Eur 5.0 Method 2.4.24 System A.

Reference solutions are corresponding to the following concentrations of each residual solvent in the sample: Benzene (2 ppm), Carbontetrachloride (4 ppm), 1,2-Dichloroethane (5 ppm), 1,1-Dichloroethene (8 ppm), 1,1,1-Trichloroethane (10 ppm), Acetonitrile (410 ppm), Chlorobenzene (360 ppm), Cyclohexane (3880 ppm), 1,2-Dichloroethene (1870 ppm), Methylene chloride (600 ppm), 1,4-Dioxane (380 ppm), Methanol (3000 ppm), Methylcyclohexane (1180 ppm), Tetrahydrofuran (720 ppm), Toluene (890 ppm) and Xylene (2170 ppm).

The content of residual solvents was tested by gas chromatography with the following conditions:

Instrument:	GC Hewlett Packard 6890
Column:	DB-624 (30 m x 0.32 mm, 1.8 µm film)
Carrier gas:	Helium (2.2 ml/min)

Split:	1:2
Detector:	FID
Detector temperature:	250 °C
Temperature program:	40 °C for 20 minutes, 10 °C/min and holding 240 °C for 20 minutes.
Injection:	Headspace HP 7694
Injector temperature:	140 °C
HS-Oven temperature:	105 °C
Vial equilibration:	60 minutes-low shaking
Loop volume:	1.0 ml gas phase

## Purity

### Thin-layer chromatography

Three secondary spots were detected, but in amounts below the limit of quantification. Their total amount was estimated to less than 0.5% visually after spraying. The following thin-layer chromatographic system was used.

Thin-layer:	Silica gel 60 F-254 TLC (Merck)
Eluent:	Diethylether:methanol:ammonium solution 25% (83:15:2)
Sample:	100 µg of erythromycin B dissolved in methanol were applied.
Visualization:	Scanning at 500 nm with a Camag TLC Scanner 3 was performed as well as visualization in day-light, after spraying with anisaldehyde solution and heating the plate at 110 °C for 2 minutes.

$$R_f(\text{erythromycin B}) = 0.2$$

The limit of detection after spraying was about 0.3 µg (0.3%), when scanning at 500 nm.

The spot of erythromycin B corresponds in position and appearance with that of the ICRS lot 194186 of erythromycin B.

### High performance liquid chromatography

The purity was estimated by peak area normalization to 99.8% (n=6, RSD=0.02% for the main peak, RSD=9.4% calculated on the 0.2% impurity level). One impurity above the limit of quantification was detected. A chromatogram is shown in Figure 2.



Sample preparation: Erythromycin B was dissolved in one volume of methanol. Three volumes of phosphate buffer pH 7.0 were added to get a concentration of 10.0 mg/ml. 100 µl (corresponding to 1000 µg) were injected.

Stability of the sample solution: The sample was stable in the dark at 8 °C for at least 24 hours.

Limit of detection: 1 µg (0.1%) at 215 nm

Limit of quantification: 4 µg (0.4%) at 215 nm

#### Diode-array detection

The chromatographic system described above was used to record UV-spectra for the detected peaks. The spectra of the impurity peak as well as the main peak showed UV-maxima below 220 nm. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities.

### **Data given by the manufacturer**

#### Identification

IR: Conforms

NMR: Conforms

MS: Conforms

Impurities, HPLC: 0.3%

#### Residual solvents

Acetone: 0.008%

Moisture by Karl Fischer: 0.8%

Residue on Ignition: 0.02%

Assay, HPLC: 99.1%

### **Stability**

No special stability studies have been performed. It is considered that the substance, based on the experience of the stability of the previous lot, is stable. The previous lot showed no signs of degradation after being stored for 11 years at + 5 °C. Regular re-examinations of this ICRS when stored in the dry state will be performed.

### **Conclusion**

Erythromycin B, Control No 205186, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 9

**GENTAMICIN SULFATE**

Control No 205183

Analytical Report

**Intended use**

The stock of the current batch of the International Chemical Reference Substance of gentamicin sulfate Control No 194183 is depleted and has to be replaced.

The monograph for Gentamicin sulfate in *The International Pharmacopoeia*, Third Edition, Volume 3, requires a reference substance of gentamicin sulfate to be used in the thin-layer chromatographic test for identity.

**Material**

About 150 g of the sample (manufacturer's batch no 01G0642) were received at the WHO Centre in October 2005. The material is being stored in tightly closed containers at + 5 °C, protected from light.

**Analytical data**

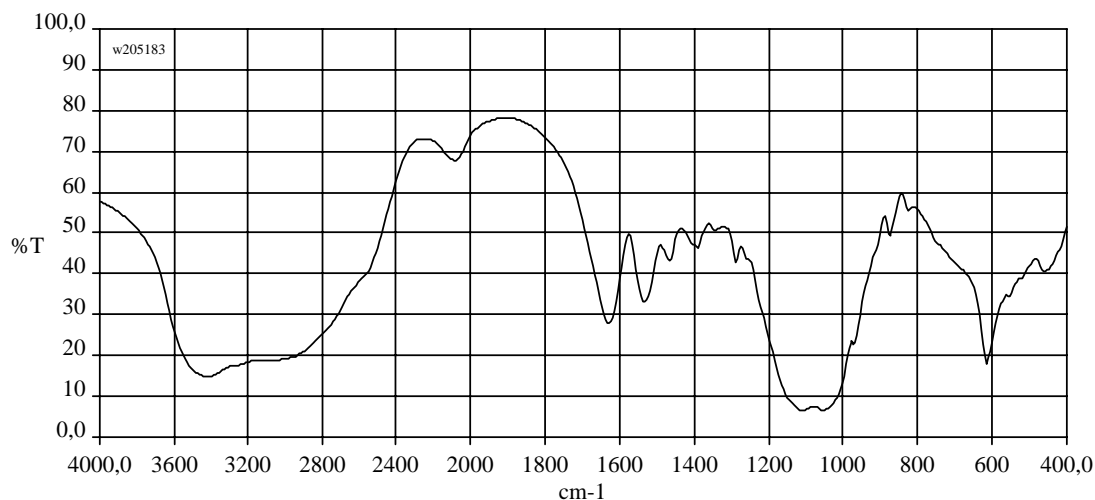
Description

A white powder.

**Evidence of chemical structure**

Infrared spectrum

An infrared spectrum is given in Figure 1 (No w205183). ). The spectrum is concordant with the spectrum of the previous lot of the International Chemical Reference Substance (ICRS) for gentamicin sulfate with Control No 194183.



**Figure 1.** IR-spectrum of 1.6 mg of gentamicin sulfate Control No 205183 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

Specific optical rotation

+110° (limits +107° to +121°) calculated with reference to the anhydrous substance. The determination was performed in water at a concentration of 0.1 g/ml.

Thin-layer chromatography

Three principal spots were detected.

The following thin-layer chromatographic system according to the monograph for Gentamicin sulfate (slight modification) in *The International Pharmacopoeia*, Third Edition, Volume 3 was used.

Thin-layer:	Silica gel 60 F-254 TLC (Merck)
Eluent:	Dichloromethane:methanol:conc. ammonia (1:1:1). Lower layer was used.
Sample:	50 µg of gentamicin sulfate dissolved in water were applied.
Visualization:	Visualization in daylight after spraying with ninhydrin solution (0.02 g/ml of ethanol:glacial acetic acid (5:1))

**Assay**

Microbiological assay

681 IU/mg. The European Pharmacopoeia Chemical Reference Substance (EPCRS) lot 4 of gentamicin sulfate was used as standard. The results are calculated “as is”.

Thermogravimetric analysis

When the substance was heated to 110 °C, a loss of 11.3% (w/w) (n=6, RSD=1.5%) was observed.

Water

11.8% (n=3) determined by Karl Fischer titration.

## Purity

### High performance liquid chromatography

The content of gentamicins was estimated by peak area normalization to 96.1%.

Gentamicin C1: 23.9% (n=6, RSD=0.7%)

Gentamicin C1a: 30.1% (n=6, RSD=0.2%)

Gentamicin C2a: 20.6% (n=6, RSD=0.3%)

Gentamicin C2: 21.5% (n=6, RSD=0.1%)

Four impurities above the limit of quantification were found. A chromatogram is shown in Figure 2.

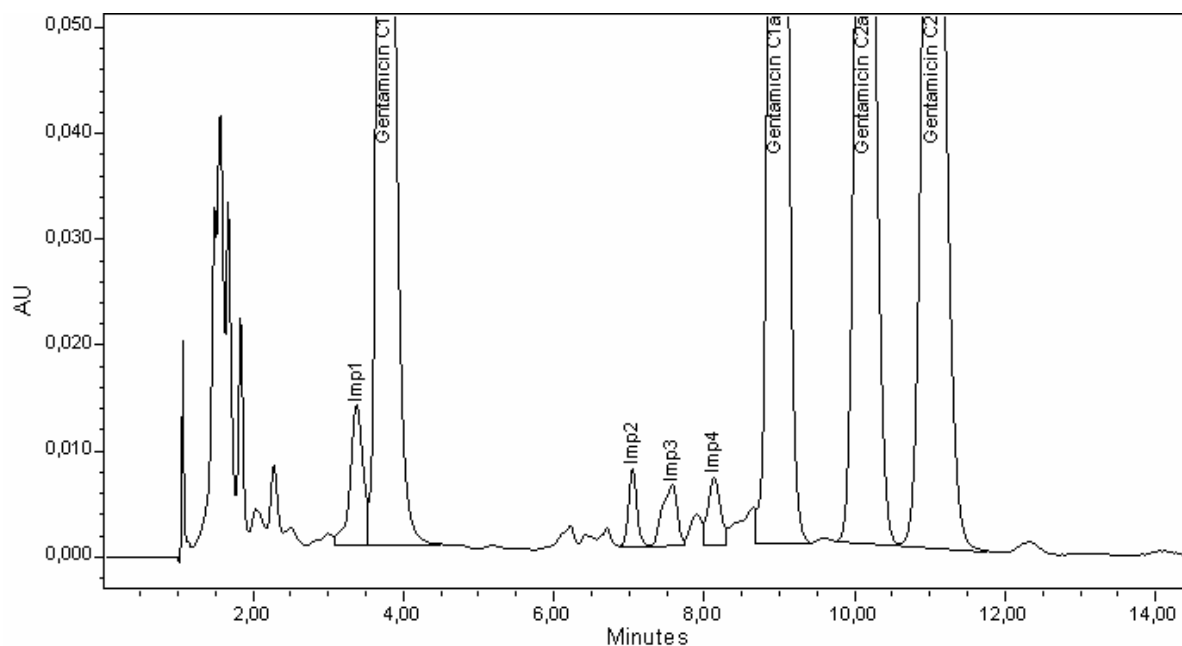


Figure 2. Chromatogram of gentamicin sulfate Control No 205183 monitored at 330 nm.

The following conditions were used:

Eluent: A: Methanol  
B: 10 mM sodium heptanesulfonate and 0.5% phosphoric acid in water

Eluent composition:	<u>% A</u>	<u>% B</u>	<u>Time, minutes</u>	<u>Type</u>
	70	30	0-4.5	isocratic
	70→80	30→20	4.5-5	linear
	80	20	5-14.5	isocratic
	80→70	20→30	14.5-15	linear
	70	30	15-20	re-equilibration

Data collecting time:	14.5 minutes
Column:	Kromasil C18, 4.6 x 150 mm, 5 µm particles
Column temperature:	R.T. (about 20 °C)
Detector, wavelength:	Spectrophotometer, 330 nm
Flow rate:	1.5 ml/min
Injector temperature:	4 °C
Sample preparation:	10 mg of gentamicin sulfate were dissolved in 10.0 ml of water. 5.0 ml of methanol and 4.0 ml of phthalaldehyd reagent were added. The solution was diluted to 25.0 ml with methanol, heated in a water bath at 60 °C for 15 minutes and cooled to room temperature. 20 µl (corresponding to 8 µg) were injected.
Phthalaldehyd reagent:	2.47 g of boric acid were dissolved in 75.0 ml of water. pH was adjusted to 10.4 using a 450 g/l solution of potassium hydroxide and the solution was diluted to 100.0 ml with water.  0.50 g of phthalaldehyde was dissolved in 2.5 ml of methanol. 47.5 ml of the boric acid solution and 1.0 ml of thioglycollic acid were added and pH was adjusted to 10.4 with a 450 g/l solution of potassium hydroxide. The reagent was stored protected from light and used within 3 days.
Stability of the sample solution:	The sample was stable in the dark at 4 °C for at least 2 hours.
Limit of detection:	11 ng (0.1%) at 330 nm
Limit of quantification:	37 ng (0.5%) at 330 nm

### **Data given by collaborating laboratories**

Identification IR:	Conforms
Specific optical rotation:	118°
Residual solvents (GC):	< 0.1% of methanol
Water:	10.6%
Sulphated ash:	0.1%
Sulphate:	33.6%

### **Stability**

Stability studies on the new batch have not been performed. Gentamicin sulfate is hygroscopic. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere. The decomposition is faster at higher temperatures. Regular re-examinations of the ICRS when stored in the dry state will be performed.

## **Conclusion**

Gentamicin sulfate, Control No 205183, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 10

**INDINAVIR**

Control No 105231

Analytical Report

**Intended use**

The monograph for Indinavir sulfate in *The International Pharmacopoeia* requires a reference substance of indinavir to be used in the infrared absorption spectrophotometric and thin-layer chromatographic tests for identity.

**Material**

About 200 g of the sample (manufacturer's batch no L-000735524-002L039) were received at the WHO Centre in May 2005. The material is being stored in tightly closed containers at + 5 °C, protected from light.

**Analytical data**

Description

A white powder.

**Evidence of chemical structure**

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W105231).

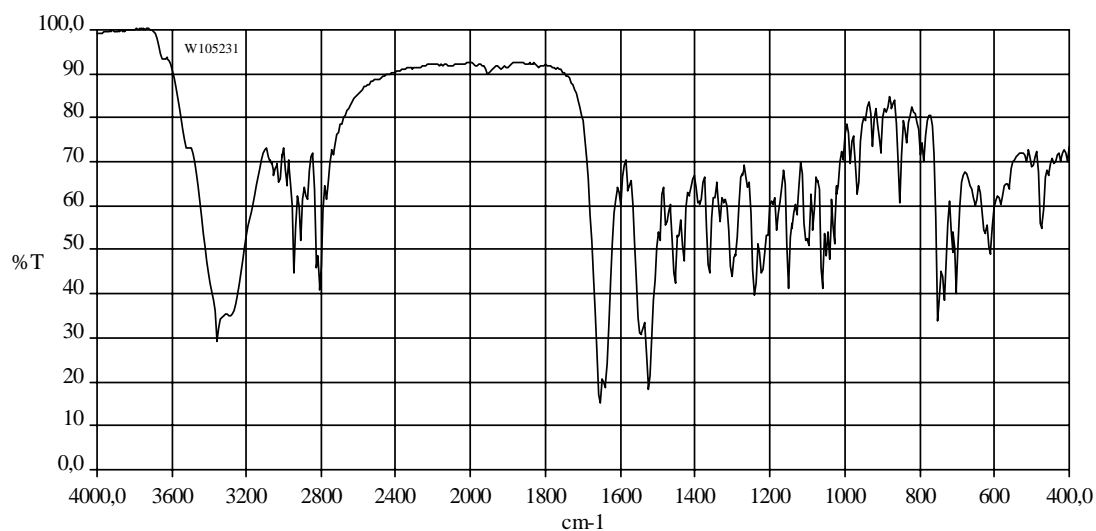


Figure 1. IR-spectrum of 1.5 mg of indinavir Control No 105231 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

#### UV-spectrum

A UV-spectrum in methanol was recorded on a Varian Cary 5 spectrophotometer. The spectrum is given in Figure 2.

A UV-maximum was observed at about 260 nm.

$A_{1\text{cm}}^{1\%} = 60$  at 260 nm (n=6, RSD=0.7%)

Calculations were performed with reference to the dried substance.

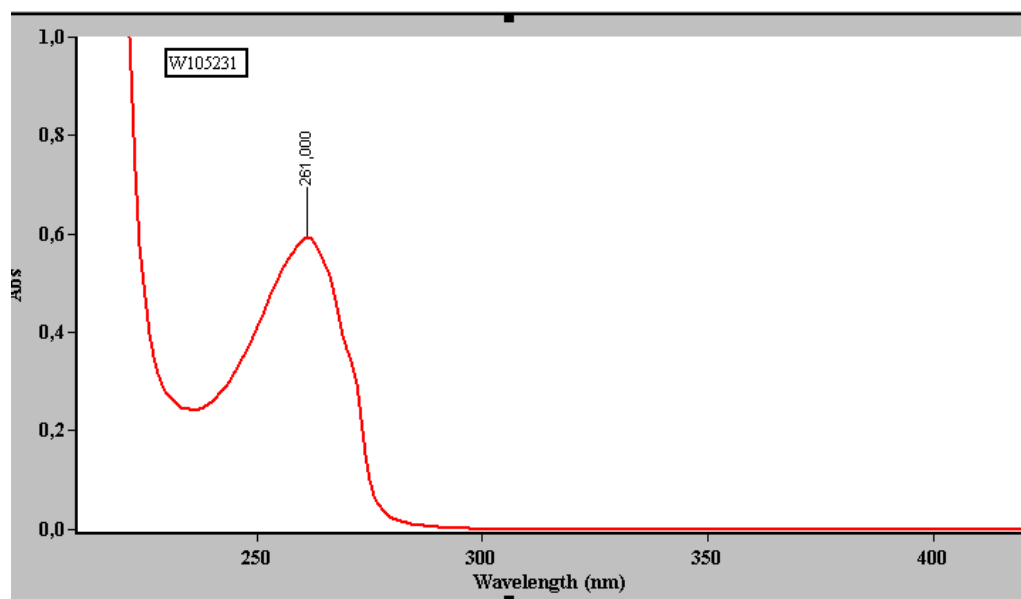


Figure 2. UV-spectrum of indinavir Control No 105231, 101 µg/ml in methanol.

## Assay

### Thermogravimetric analysis

When the substance was heated to 105 °C, a loss of 2.9% (w/w) (n=6, RSD=4.1%) was observed.

### Water

2.9% (n=3) determined by Karl Fischer titration.

### Residual solvents

Total content: < 0.1%. No peaks were detected.

The test includes Class 1, Class 2 and Class 3 residual solvents according to Ph Eur 5.0 Method 2.4.24 System A.

Reference solutions are corresponding to the following concentrations of each residual solvent in the sample: Benzene (2 ppm), Carbontetrachloride (4 ppm), 1,2-Dichloroethane (5 ppm), 1,1-Dichloroethene (8 ppm), 1,1,1-Trichloroethane (10 ppm), Acetonitrile (410 ppm), Chlorobenzene (360 ppm), Cyclohexane (3880 ppm), 1,2-Dichloroethene (1870 ppm), Methylene chloride (600 ppm), 1,4-Dioxane (380 ppm), Methanol (3000 ppm), Methylcyclohexane (1180 ppm), Tetrahydrofuran (720 ppm), Toluene (890 ppm) and Xylene (2170 ppm).

The content of residual solvents was tested by gas chromatography with the following conditions:

Instrument:	GC Hewlett Packard 6890
Column:	DB-624 (30 m x 0.32 mm, 1.8 µm film)
Carrier gas:	Helium (2.2 ml/min)
Split:	1:2
Detector:	FID
Detector temperature:	250 °C
Temperature program:	40 °C for 20 minutes, 10 °C/min and holding 240 °C for 20 minutes.
Injection:	Headspace HP 7694
Injector temperature:	140 °C
HS-Oven temperature:	105 °C
Vial equilibration:	60 minutes-low shaking
Loop volume:	1.0 ml gas phase

## Purity

### Thin-layer chromatography

Three secondary spots below the limit of quantification were detected. The following thin-layer chromatographic system was used.

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

Eluent: Dichloromethane:methanol:ammonium solution 25%  
(83:15:2)

Sample: 450 µg of indinavir dissolved in methanol were applied.

Visualization: Scanning at 254 nm with a CAMAG TLC Scanner 3 was performed.

$$R_f(\text{indinavir}) = 0.6$$

The limit of quantification was about 1 µg (0.2%), when scanning at 254 nm.

#### High performance liquid chromatography

The purity was estimated by peak area normalization to 99.8% (n=6, RSD=0.04% for the main peak, RSD=25.8% calculated on the 0.04% impurity level). 14 impurities above the limit of quantification were detected. A chromatogram is shown in Figure 3.

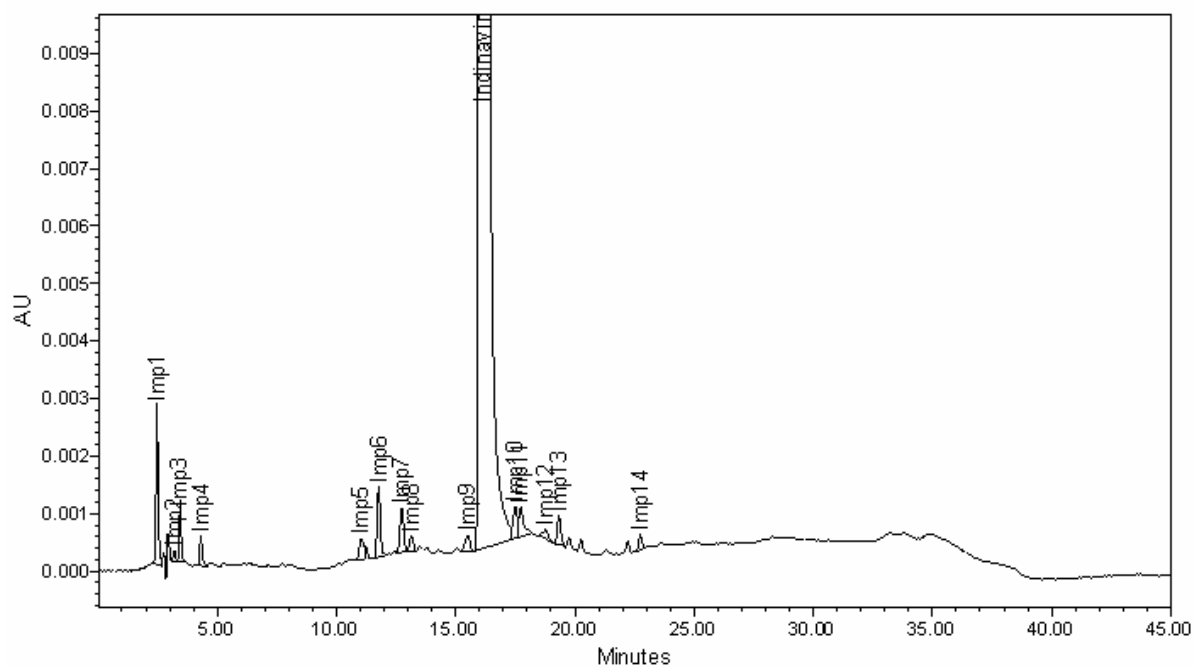


Figure 3. Chromatogram of indinavir Control No 105231 monitored at 220 nm.

The following conditions were used:

Eluent: A: Acetonitrile:phosphate buffer pH 7.5:water (30:5:65)  
B: Acetonitrile:phosphate buffer pH 7.5:water (70:5:25)

1.8 g of disodium hydrogen phosphate, dihydrate, were dissolved in about 50 ml of water. pH was adjusted to 7.5 with 1 M phosphoric acid. The solution was diluted to 100.0 ml with water and filtrated.

Eluent composition:	<u>% A</u>	<u>% B</u>	<u>Time, minutes</u>	<u>Type</u>
	93	7	0-5	isocratic
	93→20	7→80	5-25	linear
	20	80	25-30	isocratic
	20→93	80→7	30-35	linear
	93	7	35-45	re-equilibration

Data collecting time: 30 minutes

Column: BDS Hypersil C18, 250 x 4.6 mm, 5µm

Column temperature: 40 °C

Detector, wavelength: Spectrophotometer, 220 nm

Flow rate: 1.0 ml/min

Injector temperature: 8 °C

Sample preparation: Indinavir was dissolved in mobile phase A and B (50:50) to a concentration of 2.0 mg/ml. 20 µl (corresponding to 40 µg) were injected.

Stability of the sample solution: The sample was stable in the dark at 8 °C for at least 4 hours.

System suitability: A solution of 2 mg/ml of indinavir was prepared according to the "Sample preparation" above. 2 ml of sulfuric acid (190 mg/ml) were added to 2 ml of the indinavir solution. The solution was then heated carefully in a water bath at 80 °C for 60 minutes.

Resolution: Minimum 3.5 between the two principal peaks, with a retention time between 15 and 20.

Stability of the sample solution: The sample was stable in the dark at 8 °C for at least 4 hours.

Limit of detection: 0.5 ng (0.001%) at 220 nm

Limit of quantification: 2 ng (0.005%) at 220 nm

#### Diode-array detection

The chromatographic system described above was used to record UV-spectra for the detected peaks. The spectra of most of the impurity peaks as well as the main peak were similar with UV-maxima at about 260 nm and below 220 nm. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities.

### **Data given by the manufacturer**

Characteristics:	White powder
Identification IR:	Conforms
Assay (HPLC):	100.2%
Total impurities (HPLC):	0.0%
Water (KF):	2.9%
Impurities (TLC):	Single spot
Assigned purity:	97.1% (as is)

### **Stability**

No special stability studies have been performed. Regular re-examinations of this ICRS when stored in the dry state will be performed.

### **Conclusion**

Indinavir, Control No 105231, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 11

**LAMIVUDINE**

Control No 105232

Analytical Report

**Intended use**

The monograph for Lamivudine in *The International Pharmacopoeia* requires a reference substance of lamivudine to be used in the infrared absorption spectrophotometric and thin-layer chromatographic tests for identity.

**Material**

About 100 g of the sample (manufacturer's batch no LA0040105) were received at the WHO Centre in March 2005. The material is being stored in tightly closed containers at + 5 °C, protected from light.

**Analytical data**

Description

A white to off-white powder.

**Evidence of chemical structure**

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W105232T).

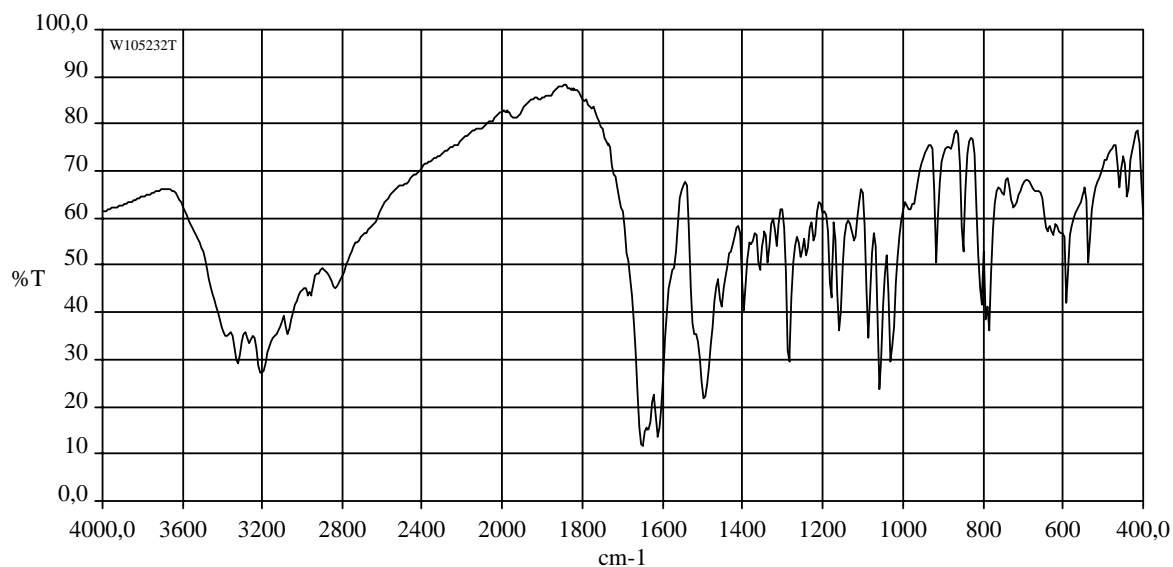


Figure 1. IR-spectrum of 0.8 mg of lamivudine Control No 105232 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

### UV-spectrum

A UV-spectrum in 0.1 M sulfuric acid was recorded on a Varian Cary 5 spectrophotometer. The spectrum is given in Figure 2.

UV-maxima were observed at 206 nm and 280 nm.

$A_{1\text{cm}}^{1\%} = 606$  at 280 nm (n=6, RSD=0.5%)

Calculations were performed with reference to the dried substance.

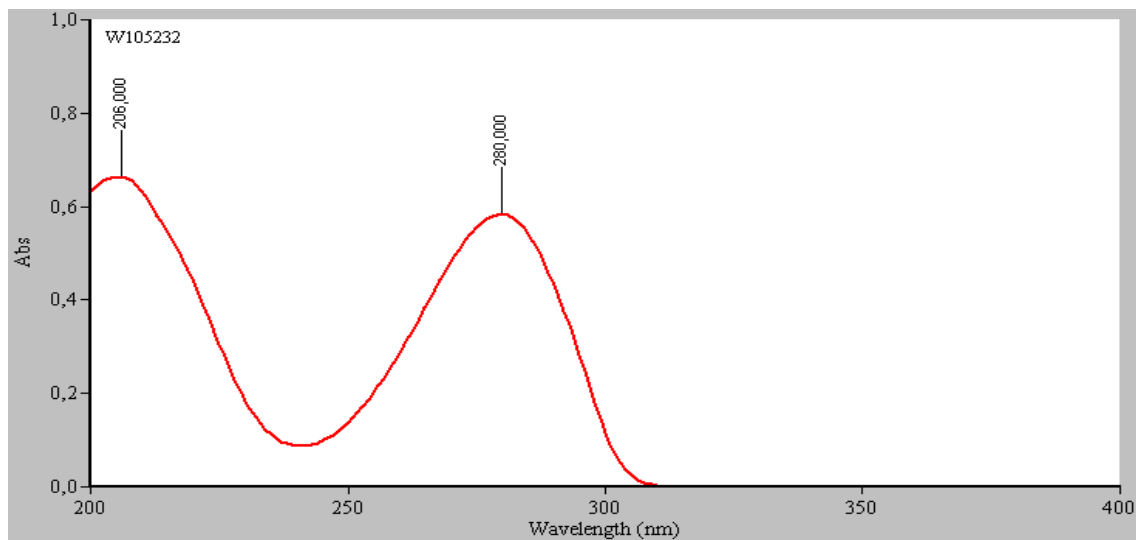


Figure 2. UV-spectrum of lamivudine Control No 105232, 10 µg/ml in 0.1 M sulfuric acid.

## Assay

### Thermogravimetric analysis

When the substance was heated to 120 °C, a loss of < 0.1% (w/w) was observed.

### Water

0.1% (n=3) determined by Karl Fischer titration.

## Purity

### Thin-layer chromatography

No impurities above the limit of quantification were detected. The following thin-layer chromatographic system was used.

Thin-layer:	Silica gel 60 F-254 TLC (Merck)
Eluent:	Dichloromethane: methanol: ammonium solution 25% (69:30:1)
Sample:	100 µg of lamivudine dissolved in methanol were applied.
Visualization:	Scanning at 280 nm with a Camag TLC Scanner 3 was performed.

$$R_f(\text{lamivudine}) = 0.5$$

$$R_f(\text{carboxylic acid}) = 0.3$$

The limit of quantification was about 0.1 µg (0.1%), when scanning at 280 nm.

### High performance liquid chromatography

The purity was estimated to 99.8% at 277 nm. Carboxylic acid and lamivudine diastereomer were estimated by external standard to 0.1% and 0.07% (w/w), respectively. The chiral purity was estimated to 99.9%. A chromatogram is shown in Figure 3.

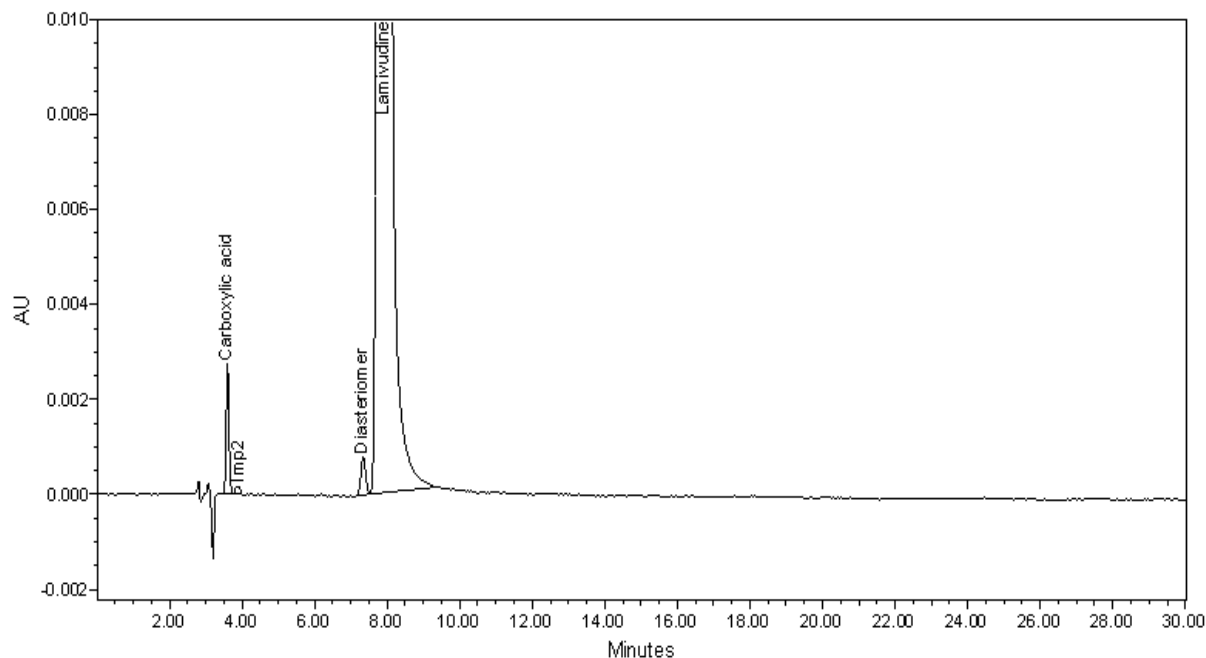


Figure 3. Chromatogram of lamivudine Control No 105232 monitored at 277 nm.

The following conditions were used:

Eluent:	A: Methanol								
	B: 25 mM ammonium acetate buffer pH 3.8								
Eluent composition:	<table border="0"> <thead> <tr> <th><u>% A</u></th> <th><u>% B</u></th> <th><u>Time, minutes</u></th> <th><u>Type</u></th> </tr> </thead> <tbody> <tr> <td>7</td> <td>93</td> <td>0-30</td> <td>isocratic</td> </tr> </tbody> </table>	<u>% A</u>	<u>% B</u>	<u>Time, minutes</u>	<u>Type</u>	7	93	0-30	isocratic
<u>% A</u>	<u>% B</u>	<u>Time, minutes</u>	<u>Type</u>						
7	93	0-30	isocratic						
Data collecting time:	30 minutes								
Column:	BDS Hypersil C18, 250 x 4.6 mm, 5 $\mu$ m								
Column temperature:	35 $^{\circ}$ C								
Detector, wavelength:	Spectrophotometer, 277 nm								
Flow rate:	1.0 ml/min								
Injector temperature:	R.T. (about 20 $^{\circ}$ C)								
Sample preparation:	Lamivudine was dissolved in water to a concentration of 0.13 mg/ml. 40 $\mu$ l (corresponding to about 5 $\mu$ g) were injected.								
Stability of the sample solution:	The sample was stable in the dark for at least 4 hours. One of the impurity peaks increases during the time but the peak is still below the limit of quantification after 4 hours.								
Limit of detection:	0.2 ng (0.004%) at 277 nm								
Limit of quantification:	0.7 ng (0.01%) at 277 nm								

#### Diode-array detection

The chromatographic system described above was used to record UV-spectra for the detected peaks. The spectrum of the main peak showed UV-maxima at about 224 nm and 277 nm. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities.

#### Differential scanning calorimetry

The purity was estimated to 99.9 mol% (n=6, RSD=0.02%) and the melting temperature ( $T_m$ ) to 176.7 °C (n=6, RSD=0.05%). The determination was performed on about 3 mg using Mettler Toledo DSC 822<sup>e</sup> with a heating rate of 2 °C per minute.

### **Data given by the manufacturer**

Description:	White to off-white powder
Identification IR:	Conforms
Water (KF):	0.06%
Residue on ignition:	0.07%
Heavy metals:	< 20 ppm
Related substances (HPLC)	
Carboxylic acid:	0.09%
Lamivudine diastereomer:	0.05%
Salicylic acid:	Not detected
Any other individual impurity:	0.01%
Total amount of impurities:	0.15%
Assay (HPLC):	100.1%
Residual solvents (GC):	0.1% of isopropanol
Melting range:	176.5-177-7 °C

### **Stability**

No special stability studies have been performed. Regular re-examinations of this ICRS when stored in the dry state will be performed.

### **Conclusion**

Lamivudine, Control No 105232, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 12

**NELFINAVIR MESILATE**

Control No 105233

Analytical Report

**Intended use**

The monograph for Nelfinavir mesilate in *The International Pharmacopoeia* requires a reference substance of nelfinavir mesilate to be used in the infrared absorption spectrophotometric and thin-layer chromatographic tests for identity.

**Material**

About 200 g of the sample (manufacturer's batch no BS04121241) were received at the WHO Centre in May 2005. The material is being stored in tightly closed containers at + 5 °C, protected from light.

**Analytical data**

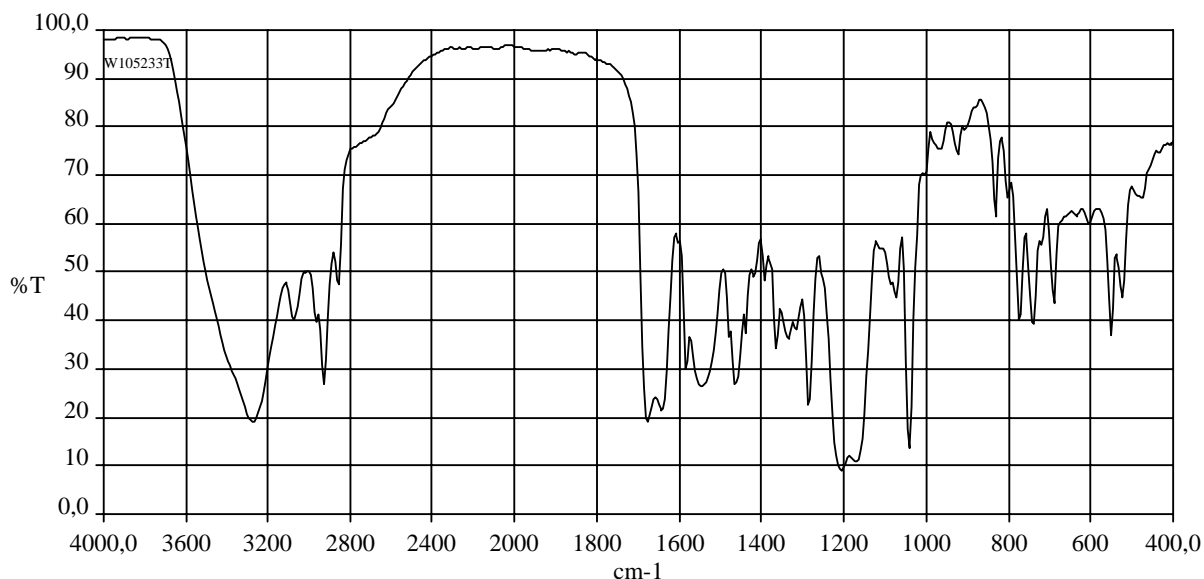
Description

A white powder.

**Evidence of chemical structure**

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W105233T).



**Figure 1.** IR-spectrum of 1.6 mg of nelfinavir mesilate Control No 105233 in 300 mg of potassium bromide recorded against a potassium bromide disc.

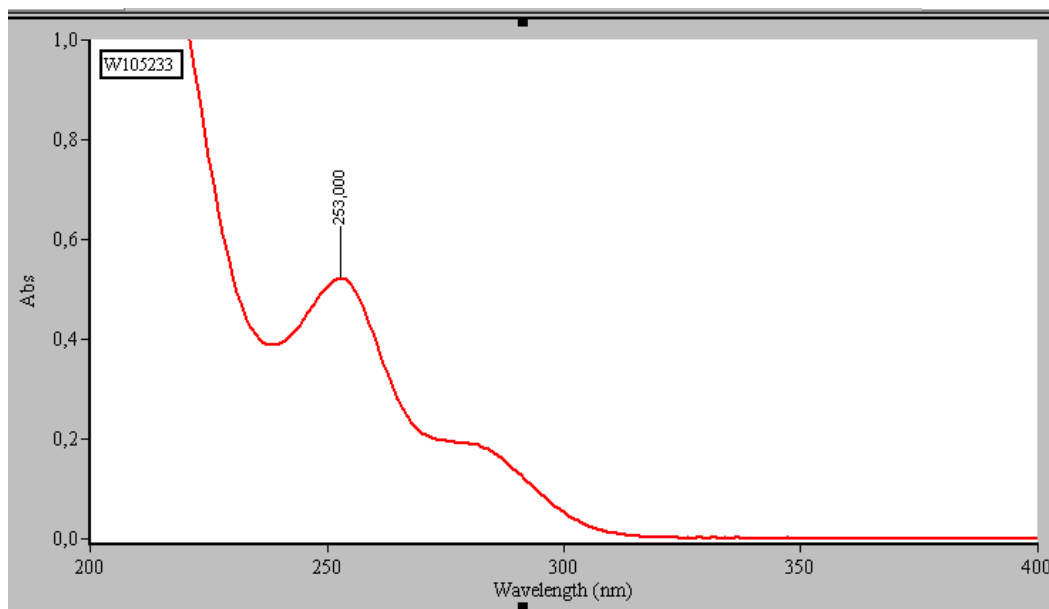
Instrument: Perkin-Elmer Spectrum One.

### UV-spectrum

A UV-spectrum in methanol was recorded on a Varian Cary 5 spectrophotometer. The spectrum is given in Figure 2. A UV-maximum was observed at 253 nm.

$A_{1cm}^{1\%} = 133$  at 253 nm (n=6, RSD=0.8%).

Calculations were performed with reference to the dried substance.



**Figure 2.** UV-spectrum of nelfinavir mesilate Control No 105233, 40 µg/ml in methanol.

## Assay

### Thermogravimetric analysis

When the substance was heated to 110 °C, a loss of 2.6% (w/w) (n=6, RSD=2.3%) was observed.

### Water

1.2% (n=3) determined by Karl Fischer titration.

### Residual solvents

Ethanol: 2.1 %

Total content: 2.1%

No other peaks were detected.

The test includes Class 1, Class 2 and Class 3 residual solvents according to Ph Eur 5.0 Method 2.4.24 System A.

Reference solutions are corresponding to the following concentrations of each residual solvent in the sample: Ethanol (3.2%), Benzene (2 ppm), Carbontetrachloride (4 ppm), 1,2-Dichloroethane (5 ppm), 1,1-Dichloroethene (8 ppm), 1,1,1-Trichloroethane (10 ppm), Acetonitrile (410 ppm), Chlorobenzene (360 ppm), Cyclohexane (3880 ppm), 1,2-Dichloroethene (1870 ppm), Methylene chloride (600 ppm), 1,4-Dioxane (380 ppm), Methanol (3000 ppm), Methylcyclohexane (1180 ppm), Tetrahydrofuran (720 ppm), Toluene (890 ppm) and Xylene (2170 ppm).

The content of residual solvents was tested by gas chromatography with the following conditions:

Instrument:	GC Hewlett Packard 6890
Column:	DB-624 (30 m x 0.32 mm, 1.8 µm film)
Carrier gas:	Helium (2.2 ml/min)
Split:	1:2
Detector:	FID
Detector temperature:	250 °C
Temperature program:	40 °C for 20 minutes, 10 °C/min and holding 240 °C for 20 minutes.
Injection:	Headspace HP 7694
Injector temperature:	140 °C
HS-Oven temperature:	105 °C
Vial equilibration:	60 minutes-low shaking
Loop volume:	1.0 ml gas phase

## Purity

### Thin-layer chromatography

One secondary spot below the limit of quantification was detected. The following thin-layer chromatographic system was used.

Thin-layer: Silica gel 60 F-254 TLC (Merck)  
Eluent: Methanol:dichloromethane (15:85)  
Sample: 200 µg of nelfinavir mesilate dissolved in methanol were applied.  
Visualization: Scanning at 254 nm with a Camag TLC Scanner 3 was performed.

$$R_f(\text{nelfinavir mesilate}) = 0.6$$

The limit of quantification was about 0.6 ng (0.3%), when scanning at 254 nm.

### High performance liquid chromatography

The purity was estimated by peak area normalization to 99.8% (n=6, RSD < 0.01% for the main peak). Three impurities above the limit of quantification were found. A chromatogram is shown in Figure 3.

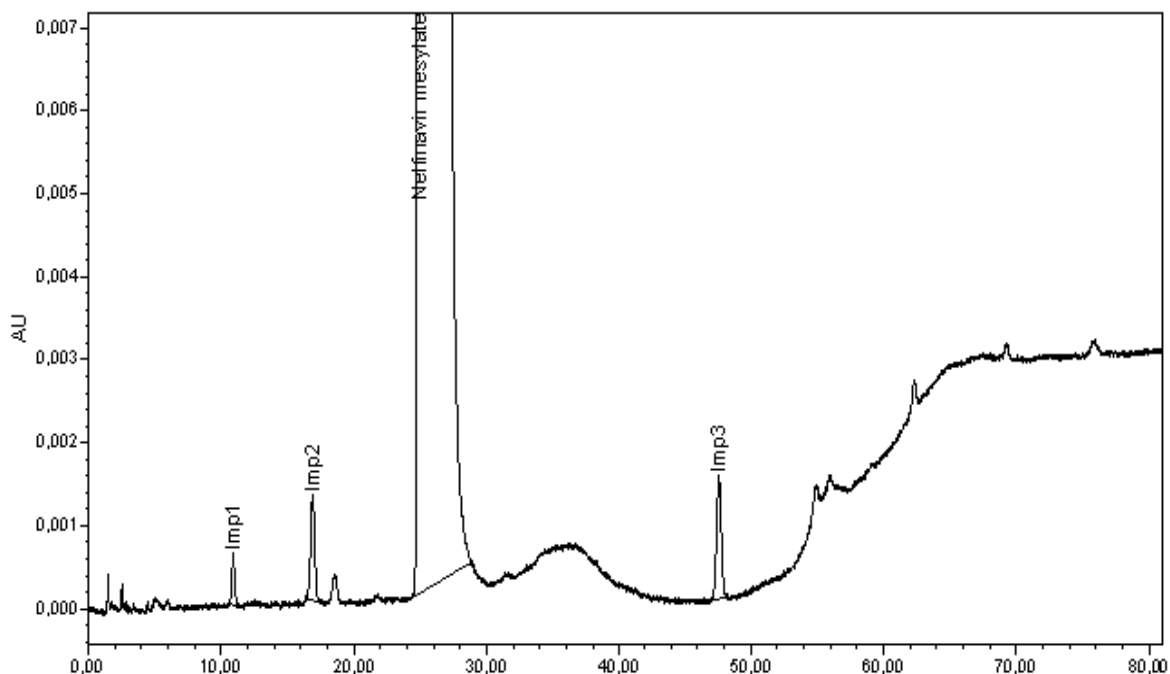


Figure 3. Chromatogram of nelfinavir mesilate Control No 105233 monitored at 225 nm.

The following conditions were used:

Eluent: A: Acetonitrile:Methanol:Phosphate buffer pH 3.4:Water (29:20:28:23)  
 B: Acetonitrile:Methanol:Phosphate buffer pH 3.4 (41:31:28)  
 Buffer: 4.9 g of anhydrous sodium dihydrogen phosphate were dissolved in 800 ml of water. pH was adjusted to 3.4 by adding phosphoric acid. The solution was diluted to 1000 ml with water.

Eluent composition:	<u>% A</u>	<u>% B</u>	<u>Time, minutes</u>	<u>Type</u>
	100	0	0-27	isocratic
	100→0	0→100	27-60	linear
	0	100	60-80	isocratic
	0→100	100→0	80-81	linear
	100	0	81-90	re-equilibration

Data collecting time: 80 minutes

Column: BDS Hypersil C18, 4.6 x 250 mm, 5 µm particles

Column temperature: 35 °C

Detector, wavelength: Spectrophotometer, 225 nm

Flow rate: 1.0 ml/min

Injector temperature: 8 °C

Sample preparation: Nelfinavir mesilate was dissolved in the eluent to a concentration of 2.0 mg/ml. 20 µl (corresponding to 40 µg) were injected.

Stability in the eluent: The sample was stable in the dark at 8 °C for at least 9 hours.

Limit of detection: 7 ng (0.02%) at 225 nm

Limit of quantification: 23 ng (0.06%) at 225 nm

#### Diode-array detection

The chromatographic system described above was used to record UV-spectra for the detected peaks. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities.

### Data given by the manufacturer

Appearance:	Powder
Colour:	White
Identification	
IR:	Conforms
HPLC:	Conforms
Water:	0.3%
Residual solvents ethanol:	2.5%
Sulphated ash:	< 0.1%
Heavy metals.	< 10 ppm
Specific rotation (anhydrous and solvent free substance, 25 °C, methanol):	-113°
Total of all impurities:	0.14%
Assay (anhydrous and solvent free substance):	99.6%

### Stability

No special stability studies have been performed. Regular re-examinations of this ICRS when stored in the dry state will be performed.

### Conclusion

Nelfinavir mesilate, Control No 105233, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 13

**NYSTATIN**

Control No 405152

Analytical Report

**Intended use**

The stock of the current batch of the International Chemical Reference Substance for nystatin Control No 300152 is depleted and has to be replaced.

The monograph for Amphotericin B in *The International Pharmacopoeia*, Third Edition, Volume 3, requires a reference substance of nystatin to be used in the ultraviolet spectrophotometric test for tetraenes.

**Material**

About 200 g of the sample (manufacturer's batch no 5B00322) were received at the WHO Centre in July 2005. The material is being stored in tightly closed containers at - 20 °C, protected from light.

**Analytical data**

Description

A light yellow powder.

**Evidence of chemical structure**

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W405152). The spectrum is concordant with the previous lot of the International Chemical Reference Substance (ICRS) for nystatin with Control No 300152.

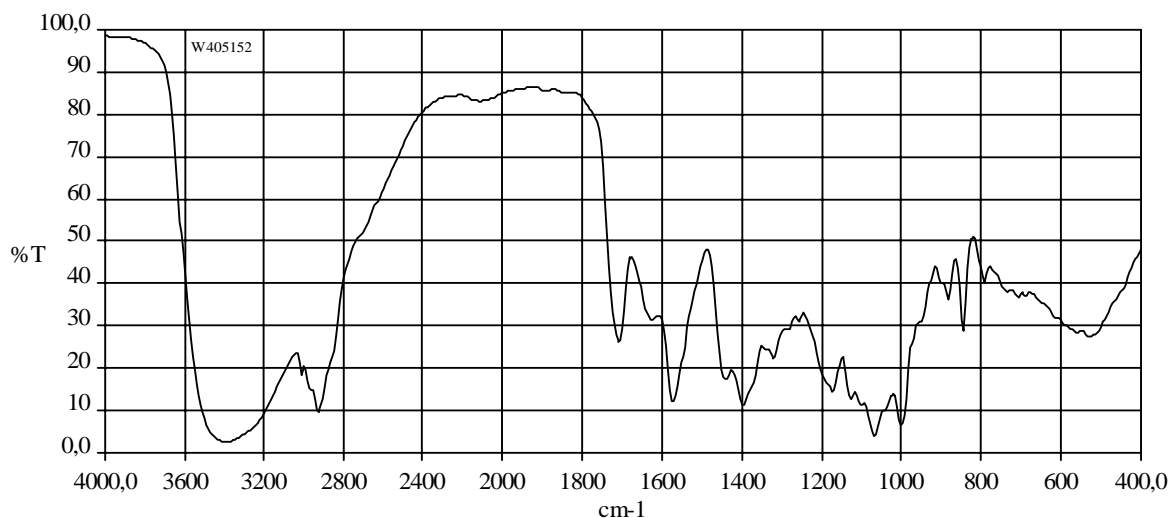


Figure 1. IR-spectrum of 2.4 mg of nystatin Control No 405152 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

#### UV-spectrum

A UV-spectrum in methanol was recorded on a Varian Cary 5 spectrophotometer. The spectrum is given in Figure 2.

$A_{1\text{cm}}^{1\%} = 498$  at 291 nm (n=6, RSD=1.3%)

$A_{1\text{cm}}^{1\%} = 741$  at 305 nm (n=6, RSD=1.2%)

$A_{1\text{cm}}^{1\%} = 680$  at 319 nm (n=6, RSD=1.2%)

The results below are calculated with reference to the dried substance.

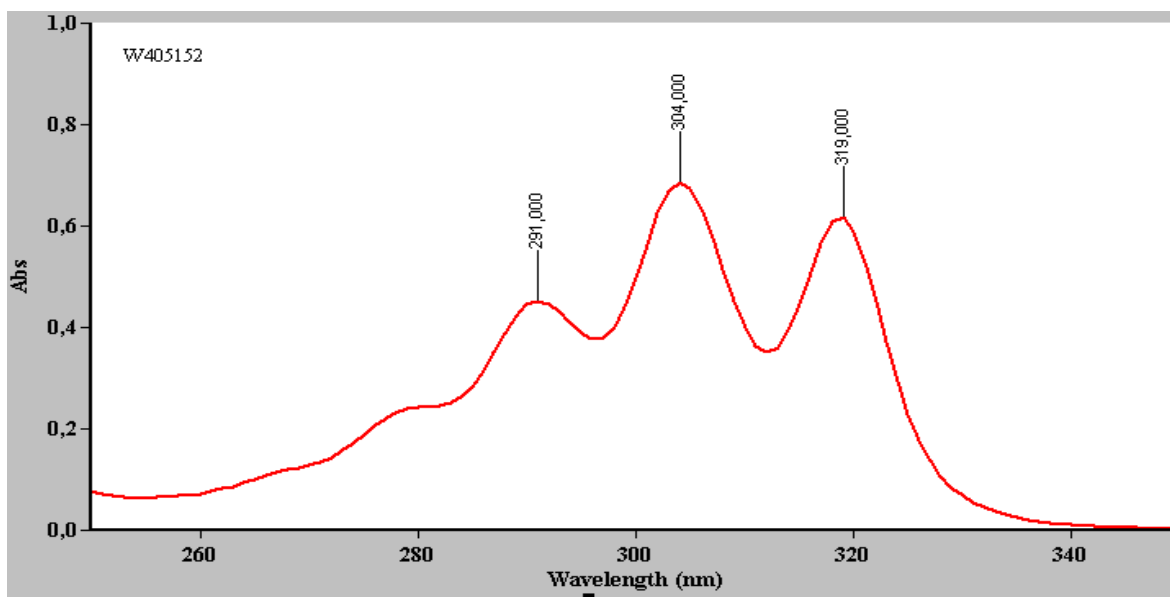


Figure 2. UV-spectrum of nystatin Control No 405152, 10  $\mu\text{g/ml}$  in methanol.

UV-spectrum identity test

The determination was performed according to *The International Pharmacopoeia*, Third Edition, Volume 3.

UV ratio ( $A_{291}/A_{305}$ ) = 0.67 (limits 0.61-0.73)

UV ratio ( $A_{319}/A_{305}$ ) = 0.92 (limits 0.83-0.96)

**Assay**Microbiological assay

5308 IU/mg. The International Biological Standard Nystatin 2<sup>nd</sup> with a declared content of 4855 IU/mg was used as standard. The results are calculated "as is".

Loss on drying

3.8% (n=3), when dried at 60 °C under reduced pressure (< 0.6 kPa).

Residual solvents

Methanol: 0.07%

Acetone: 0.16%

Total content: 0.2%

No other peaks were detected.

The test includes Class 1, Class 2 and Class 3 residual solvents according to Ph Eur 5.0 Method 2.4.24 System A.

Reference solutions are corresponding to the following concentrations of each residual solvent in the sample: Methanol (0.12%), Acetone (0.30%), Benzene (2 ppm), Carbontetrachloride (4 ppm), 1,2-Dichloroethane (5 ppm), 1,1-Dichloroethene (8 ppm), 1,1,1-Trichloroethane (10 ppm), Acetonitrile (410 ppm), Chlorobenzene (360 ppm), Cyclohexane (3880 ppm), 1,2-Dichloroethene (1870 ppm), Methylene chloride (600 ppm), 1,4-Dioxane (380 ppm), Methanol (3000 ppm), Methylcyclohexane (1180 ppm), Tetrahydrofuran (720 ppm), Toluene (890 ppm) and Xylene (2170 ppm).

The content of residual solvents was tested by gas chromatography with the following conditions:

Instrument:	GC Hewlett Packard 6890
Column:	DB-624 (30 m x 0.32 mm, 1.8 µm film)
Carrier gas:	Helium (2.2 ml/min)
Split:	1:2
Detector:	FID
Detector temperature:	250 °C
Temperature program:	40 °C for 20 minutes, 10 °C/min and holding 240 °C for 20 minutes.
Injection:	Headspace HP 7694
Injector temperature:	140 °C
HS-Oven temperature:	105 °C
Vial equilibration:	60 minutes-low shaking
Loop volume:	1.0 ml gas phase

## Purity

### High performance liquid chromatography

The purity was estimated by peak area normalization to about 93.1% (n=6, RSD=0.1% for the main peak, RSD=4.5% calculated on the 1.4% impurity level). Twenty impurities above the limit of quantification were found. A chromatogram is shown in Figure 3.

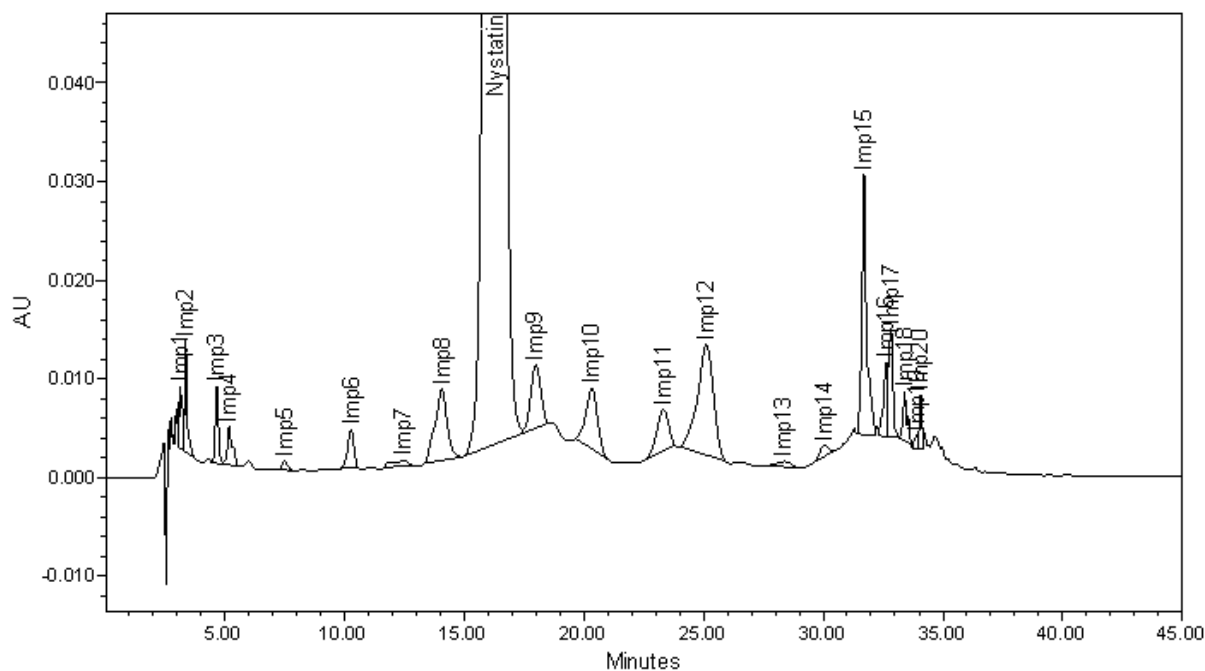


Figure 3. Chromatogram of nystatin Control No 405152 monitored at 305 nm.

The following conditions were used:

Eluent: Buffer: 3.85 g/l ammonium acetate  
 Mobile phase A: Acetonitrile:Buffer (30:70)  
 Mobile phase B: Acetonitrile:Buffer (60:40)

Eluent composition:	<u>% A</u>	<u>% B</u>	<u>Time, minutes</u>	<u>Type</u>
	95	5	0-25	isocratic
	95→0	5→100	25-35	linear
	0	100	35-45	isocratic
	0→95	100→5	45-50	linear
	95	5	50-60	isocratic

Data collecting time: 45 minutes

Column: BDS Hypersil C18, 250 x 4.6 mm, 5 µm

Column temperature: 30 °C

Detector, wavelength:	Spectrophotometer, 305 nm
Flow rate:	1.0 ml/min
Injector temperature:	R.T. (about 20 °C)
Sample preparation:	Nystatin was dissolved in dimethylsulfoxide to a concentration of 0.4 mg/ml. 20 µl (corresponding to 8 µg) were injected.
Stability of the sample solution:	The sample was stable in the dark at R.T. for at least 4 hours.
System suitability:	4 mg of nystatin were dissolved in 5 ml of methanol and diluted to 10.0 ml with water. 2.0 ml of 1 M HCl were added and the solution was kept at R.T. for one hour.  Resolution: Minimum 3.5 between the two principal peaks obtained from the above solution.
Limit of detection:	1 ng (0.01%) at 305 nm
Limit of quantification:	3 ng (0.04%) at 305 nm

### **Data given by the manufacturer**

Identification IR:	Conforms
Identification UV:	Conforms
Microbiological assay:	6332 IU/mg
Loss on drying:	4.3%
UV ratio ( $A_{230}/A_{279}$ ):	1.04
UV ratio ( $A_{291}/A_{305}$ ):	0.67
UV ratio ( $A_{319}/A_{305}$ ):	0.95
Sulphated ash:	1.3%
Heavy metals:	< 20 ppm
Residual solvents (GC)	
Methanol:	0.1%
Acetone:	0.2%

## **Stability**

No special stability studies have been performed. It is considered that the substance, based on the experience of the stability of the previous lot, is not stable. Therefore regular re-examinations of the ICRS will be performed every year. Nystatin is stored in tightly closed containers at - 20 °C, protected from light.

## **Hygroscopicity**

Nystatin is hygroscopic. Even in absence of light, it is gradually degraded on exposure to a humid atmosphere. The decomposition is faster at higher temperatures.

## **Conclusion**

Nystatin, Control No 405152, can be considered suitable as International Chemical Reference Substance for the intended purpose. As the substance is hygroscopic, a determination of moisture content needs to be performed each time prior to the assay.

APPENDIX 14

**RITONAVIR**

Control No 105237

Analytical Report

**Intended use**

The monograph for Ritonavir in *The International Pharmacopoeia* requires a reference substance of ritonavir to be used in the infrared absorption spectrophotometric and thin-layer chromatographic tests for identity.

**Material**

About 200 g of the sample (manufacturer's batch no RS88644TL) were received at the WHO Centre in September 2005. The material is being stored in tightly closed containers at + 5 °C, protected from light.

**Analytical data**

Description

A white powder.

**Evidence of chemical structure**

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W105237).

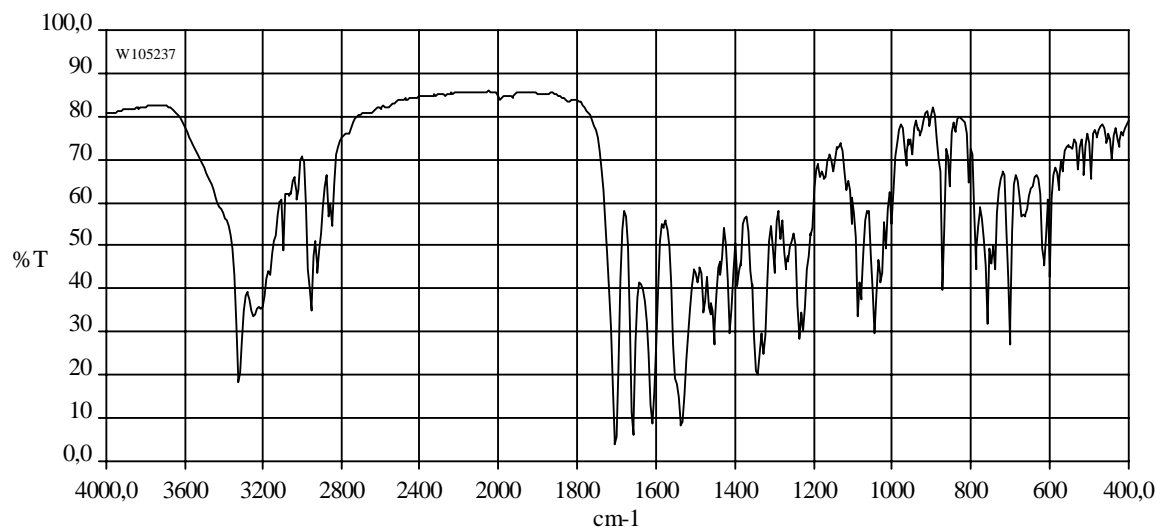


Figure 1. IR-spectrum of 1.2 mg of ritonavir, Control No 105237 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

### UV-spectrum

A UV-spectrum in methanol was recorded on a Varian Cary 5 spectrophotometer. The spectrum is given in Figure 2.

A UV-maximum was observed at about 240 nm.

$A_{1cm}^{1\%} = 127$  at 240 nm (n=6, RSD=1.9%)

Calculations were performed with reference to the dried substance.

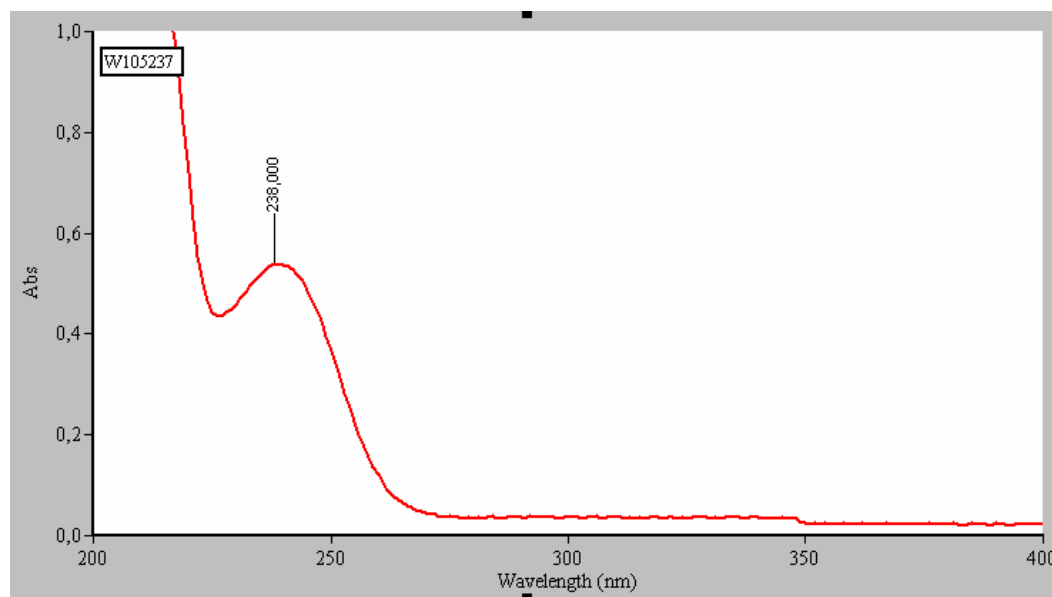


Figure 2. UV-spectrum of ritonavir Control No 105237, 40 µg/ml in methanol.

Specific optical rotation

+10° calculated with reference to the dried substance. The determination was performed in methanol at a concentration of 0.02 g/ml.

**Assay**Potentiometric titration

100.0% (n=3, RSD=0.1%) determined on the dried basis by non-aqueous titration according to *The International Pharmacopoeia*, Third Edition, Volume 1.

Loss on drying

< 0.1%, when dried at 105 °C.

Water

0.1% (n=3) determined by Karl Fischer titration.

Residual solvents

Ethyl acetate: 0.2%

Total content: 0.2%

No other peaks were detected.

The test includes Class 1, Class 2 and Class 3 residual solvents according to Ph Eur 5.0 Method 2.4.24 System A.

Reference solutions are corresponding to the following concentrations of each residual solvent in the sample: Ethyl acetate (2250 ppm), Benzene (2 ppm), Carbontetrachloride (4 ppm), 1,2-Dichloroethane (5 ppm), 1,1-Dichloroethene (8 ppm), 1,1,1-Trichloroethane (10 ppm), Acetonitrile (410 ppm), Chlorobenzene (360 ppm), Cyclohexane (3880 ppm), 1,2-Dichloroethene (1870 ppm), Methylene chloride (600 ppm), 1,4-Dioxane (380 ppm), Methanol (3000 ppm), Methylcyclohexane (1180 ppm), Tetrahydrofuran (720 ppm), Toluene (890 ppm) and Xylene (2170 ppm).

The content of residual solvents was tested by gas chromatography with the following conditions:

Instrument:	GC Hewlett Packard 6890
Column:	DB-624 (30 m x 0.32 mm, 1.8 µm film)
Carrier gas:	Helium (2.2 ml/min)
Split:	1:2
Detector:	FID
Detector temperature:	250 °C
Temperature program:	40 °C for 20 minutes, 10 °C/min and holding 240 °C for 20 minutes.
Injection:	Headspace HP 7694
Injector temperature:	140 °C
HS-Oven temperature:	105 °C

Vial equilibration: 60 minutes-low shaking

Loop volume: 1.0 ml gas phase

## Purity

### Thin-layer chromatography

Two secondary spots below the limit of quantification were detected. The following thin-layer chromatographic system was used.

Thin-layer: Silica gel 60 F-254 (Merck) TLC  
Eluent: Dichloromethane:acetonitrile:methanol (65:20:8)  
Sample: 450 µg of ritonavir dissolved in methanol were applied.  
Visualization: Scanning at 240 nm with a Camag TLC Scanner 3 was performed.

$$R_f(\text{ritonavir}) = 0.4$$

The limit of quantification was about 0.3 µg (0.07%), when scanning at 240 nm.

### High performance liquid chromatography

The purity was estimated by peak area normalization to about 99.7% (n=6, RSD < 0.01% for the main peak, RSD=1.2% calculated on the 0.1% impurity level). Four impurities above the limit of quantification were found. A chromatogram is shown in Figure 3.

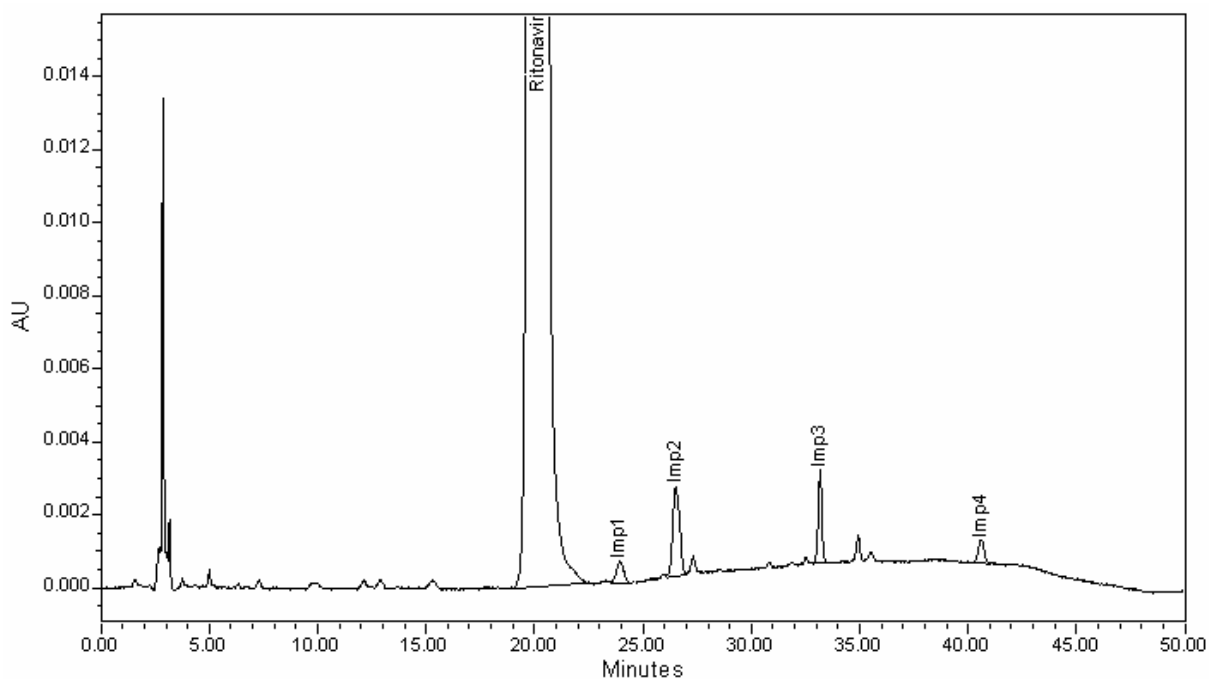


Figure 3. Chromatogram of ritonavir Control No 105237 monitored at 240 nm.

The following conditions were used:

Eluent: A: Acetonitrile:sodium phosphate buffer pH 4.0:water (35:28:37)  
B: Acetonitrile:sodium phosphate buffer pH 4.0:water (70:28:2)

Eluent composition:	<u>% A</u>	<u>% B</u>	<u>Time, minutes</u>	<u>Type</u>
	70	30	0-20	Isocratic
	70→20	30→80	20-30	Linear
	20	80	30-40	Isocratic
	20→70	80→30	40-45	Linear
	70	30	45-50	Re-equilibration

Preparation of buffer: 7.8 g of sodium dihydrogen phosphate dihydrate and 1.88 g of sodium hexanesulfonate were dissolved in 800 ml of water. pH was adjusted to 4.0 by adding 1 M phosphoric acid and the solution was further diluted to 1000 ml with water.

Data collecting time: 40 minutes

Column: BDS Hypersil C18, 250x4.6 mm, 5 µm particles

Column temperature: 35 °C

Detector, wavelength: Spectrophotometer, 240 nm

Flow rate: 1.0 ml/min

Injector temperature: R.T.

Sample preparation: 5.0 mg of ritonavir were dissolved in 1 ml of methanol and were further diluted with 1 ml of water to a concentration of 2.5 mg/ml. 20 µl (corresponding to 50 µg) were injected.

Stability of the sample solution: The sample was stable in the dark at R.T. for at least 5 hours.

Limit of detection: 4 ng (0.008%) at 240 nm

Limit of quantification: 14 ng (0.03%) at 240 nm

#### Diode-array detection

The chromatographic system described above was used to record UV-spectra for the detected peaks. The spectra of the impurity peaks as well as the main peak were similar with UV-maxima at about 240 nm. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities.

## Data given by the manufacturer

### Identification

-IR:	Conforms
-NMR:	Conforms
-MS:	Conforms
Total impurities (HPLC):	0.5%
Impurities (TLC):	No impurity spots were detected
Residual solvents (GC):	0.2% (w/w)
Water:	0.2% (w/w)
Heavy metals:	< 0.002% (w/w)
Residue on ignition:	0.0% (w/w)

## Stability

Stability studies have not been performed. Regular re-examinations of the ICRS when stored in the dry state will be performed.

## Conclusion

Ritonavir, Control No 105237, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 15

**SAQUINAVIR MESILATE**

Control No 105234

Analytical Report

**Intended use**

The monograph for Saquinavir mesilate in *The International Pharmacopoeia* requires a reference substance of saquinavir mesilate to be used in the infrared absorption spectrophotometric and thin-layer chromatographic tests for identity.

**Material**

About 200 g of the sample (manufacturer's batch no BS04050017) were received at the WHO Centre in April 2005. The material is being stored in tightly closed containers at + 5 °C, protected from light.

**Analytical data**

Description

A white powder.

**Evidence of chemical structure**

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W105234T).

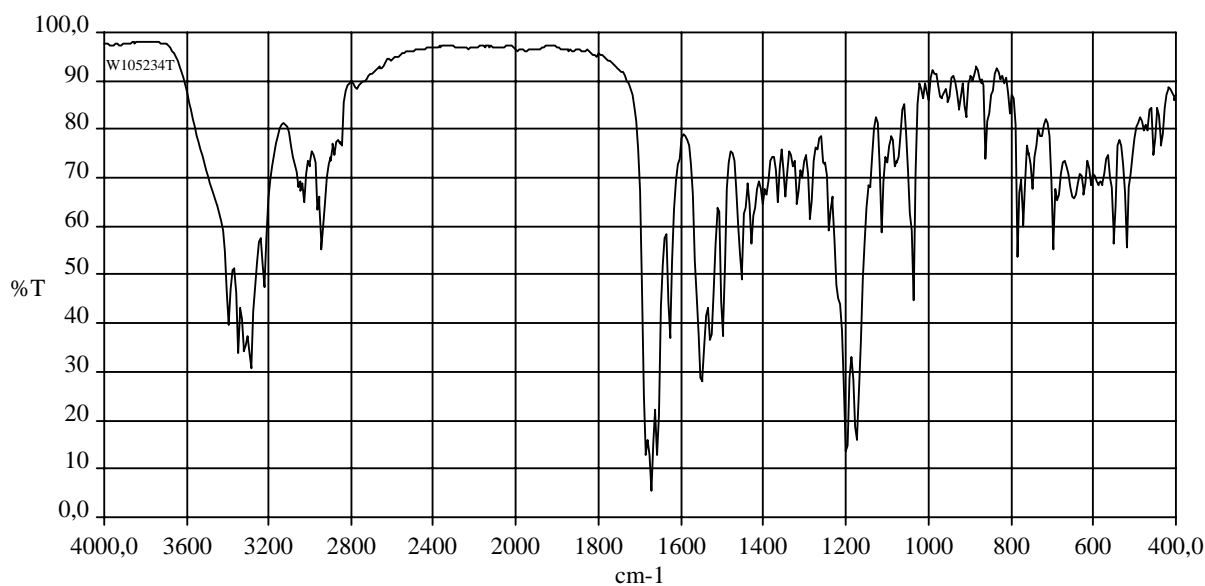


Figure 1. IR-spectrum of 0.8 mg of saquinavir mesilate Control No 105234 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

#### UV-spectrum

A UV-spectrum in methanol was recorded on a Varian Cary 5 spectrophotometer. The spectrum is given in Figure 2.

A UV-maximum was observed at 239 nm.

$A_{1cm}^{1\%} = 600$  at 239 nm (n=6, RSD=0.9%)

Calculations were performed with reference to the dried substance.

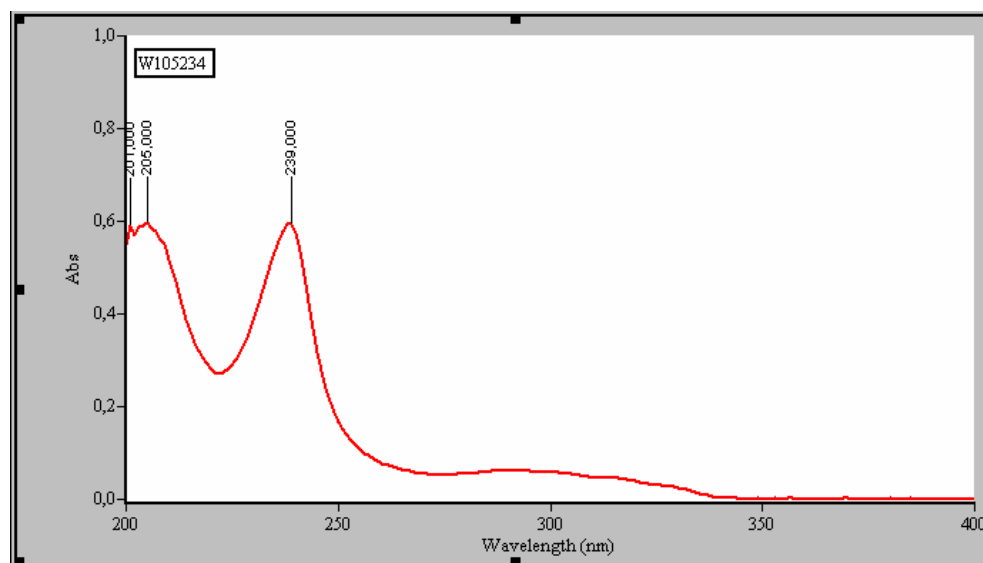


Figure 2. UV-spectrum of saquinavir mesilate Control No 105234, 10 µg/ml in methanol.

## Assay

### Thermogravimetric analysis

When the substance was heated to 105 °C, a loss of 0.4% (w/w) (n=6) was observed.

### Loss on drying

0.3% (n=3), when dried at 105 °C.

### Water

0.5% (n=3) determined by Karl Fischer titration.

### Residual solvents

Total content: < 0.1 %. No peaks were detected.

The test includes Class 1, Class 2 and Class 3 residual solvents according to Ph Eur 5.0 Method 2.4.24 System A.

Reference solutions are corresponding to the following concentrations of each residual solvent in the sample: Benzene (2 ppm), Carbontetrachloride (4 ppm), 1,2-Dichloroethane (5 ppm), 1,1-Dichloroethene (8 ppm), 1,1,1-Trichloroethane (10 ppm), Acetonitrile (410 ppm), Chlorobenzene (360 ppm), Cyclohexane (3880 ppm), 1,2-Dichloroethene (1870 ppm), Methylene chloride (600 ppm), 1,4-Dioxane (380 ppm), Methanol (3000 ppm), Methylcyclohexane (1180 ppm), Tetrahydrofuran (720 ppm), Toluene (890 ppm) and Xylene (2170 ppm).

The content of residual solvents was tested by gas chromatography with the following conditions:

Instrument:	GC Hewlett Packard 6890
Column:	DB-624 (30 m x 0.32 mm, 1.8 µm film)
Carrier gas:	Helium (2.2 ml/min)
Split:	1:2
Detector:	FID
Detector temperature:	250 °C
Temperature program:	40 °C for 20 minutes, 10 °C/min and holding 240 °C for 20 minutes.
Injection:	Headspace HP 7694
Injector temperature:	140 °C
HS-Oven temperature:	105 °C
Vial equilibration:	60 minutes-low shaking
Loop volume:	1.0 ml gas phase

## Purity

### Thin-layer chromatography

Three secondary spots below the limit of quantification were detected. The following thin-layer chromatographic system according to the monograph for Saquinavir mesilate in *The International Pharmacopoeia*, was used.

Thin-layer:	Silica gel 60 F-254 TLC (Merck)
Eluent:	Dichloromethane:2-propanol (4:1)
Sample:	100 µg of saquinavir mesilate dissolved in methanol were applied.
Visualization:	Scanning at 239 nm with a Camag TLC Scanner 3 was performed.

$$R_f(\text{saquinavir mesilate}) = 0.4$$

The limit of quantification was about 0.3 µg (0.3%), when scanning at 239 nm.

### High performance liquid chromatography

The purity was estimated by peak area normalization to 99.9% (n=6, RSD < 0.01% for the main peak). One impurity above the limit of quantification was found. A chromatogram is shown in Figure 3.

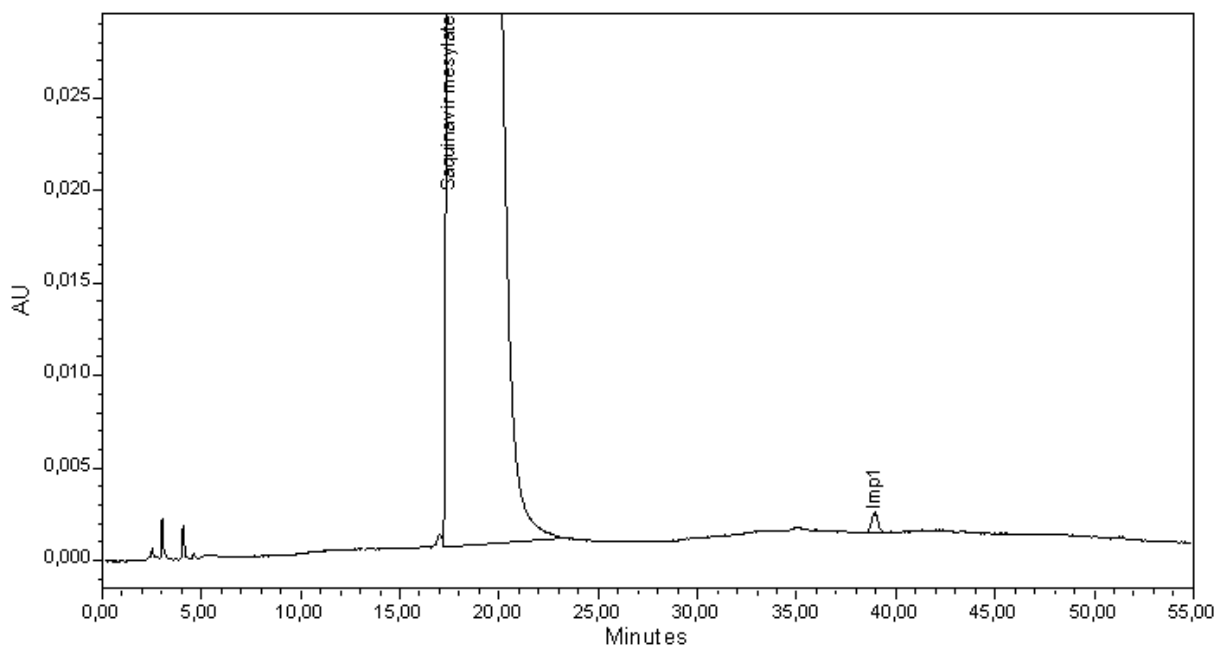


Figure 3. Chromatogram of saquinavir mesilate Control No 105234 monitored at 220 nm.

The following method according to the monograph for Saquinavir mesilate in *The International Pharmacopoeia* was used.

Eluent: A: Acetonitrile:Methanol:Water:Phosphate buffer pH 3.4  
(36:14:35:15)  
B: Acetonitrile:Water:Phosphate buffer pH 3.4  
(70:15:15)

Buffer: 4.9 g of anhydrous sodium dihydrogen phosphate were dissolved in 800 ml of water. pH was adjusted to 3.4 by adding phosphoric acid. The solution was diluted to 1000 ml with water.

Eluent composition:	<u>% A</u>	<u>% B</u>	<u>Time,</u> <u>minutes</u>	<u>Type</u>
	100	0	0-25	isocratic
	100→45	0→55	25-45	linear
	45	55	45-55	isocratic
	45→100	55→0	55-60	linear
	100	0	60-70	re-equilibration

Data collecting time: 55 minutes

Column: BDS Hypersil C18, 4.6 x 250 mm, 5 µm particles

Column temperature: 30 °C

Detector, wavelength: Spectrophotometer, 220 nm

Flow rate: 1.0 ml/min

Injector temperature: 8 °C

Sample preparation: Saquinavir mesilate was dissolved in mobile phase A to a concentration of 0.5 mg/ml. 40 µl (corresponding to 20 µg) were injected.

Stability of the sample solution: The sample was stable in the dark at 8 °C for at least 7 hours.

Limit of detection: 5 ng (0.03%) at 220 nm

Limit of quantification: 16 ng (0.08%) at 220 nm

#### Diode-array detection

The chromatographic system described above was used to record UV-spectra for the detected peaks. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities.

## Data given by the manufacturer

Appearance:	Powder with lumps
Colour:	White
Identification:	Corresponds
Specific rotation (anhydrous and solvent free substance, 436 nm, 20 °C, c=0.5 (m/V) in methanol):	-68.3°
$A_{1\text{cm}}^{1\%}$ at 239 nm:	624
Water:	0.4%
Total all organic impurities:	0.1% (largest impurity 0.10 %).
Assay (anhydrous and solvent-free substance):	99.6%
Methanesulfonic acid (anhydrous and solvent free substance):	12.6%
Residual solvent methanol:	0.01%
Sulphated ash:	< 0.1%
Heavy metals:	< 10 ppm

## Stability

No special stability studies have been performed. Regular re-examinations of this ICRS when stored in the dry state will be performed.

## Conclusion

Saquinavir mesilate, Control No 105234, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 16

**STAVUDINE**

Control No 105235

Analytical Report

**Intended use**

The monograph for Stavudine in *The International Pharmacopoeia* requires a reference substance of stavudine to be used in the infrared absorption spectrophotometric and thin-layer chromatographic tests for identity.

**Material**

About 100 g of the sample (manufacturer's batch number AST050003) were received at the WHO Centre in March 2005. The material is being stored in tightly closed containers at + 5 °C, protected from light.

**Analytical data**

Description

A white crystalline powder.

**Evidence of chemical structure**

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W105235).

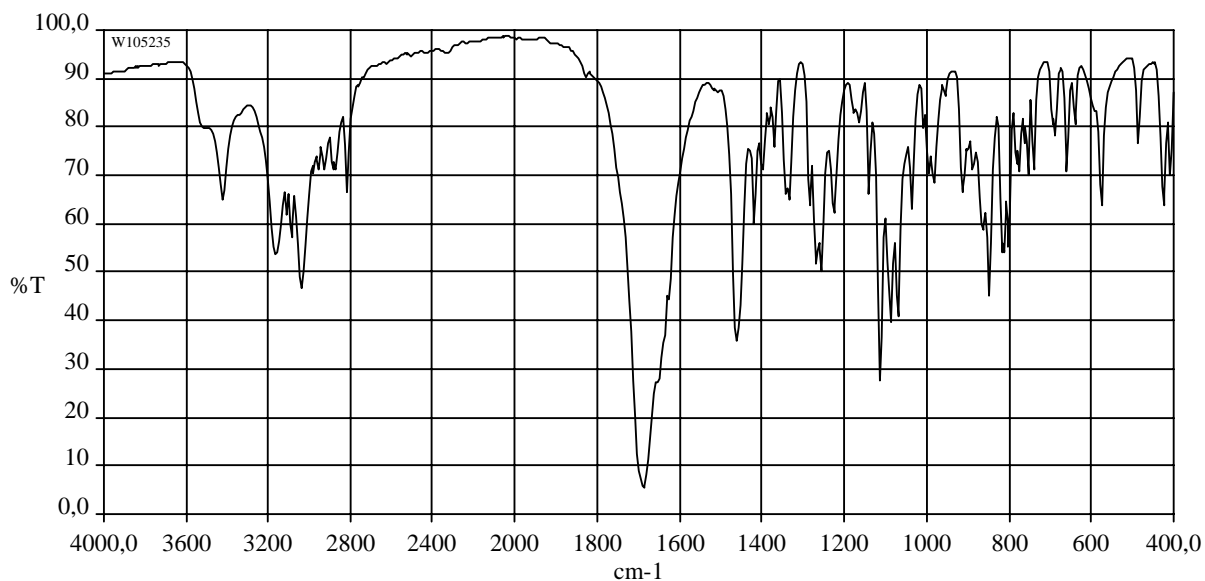


Figure 1. IR-spectrum of 0.6 mg of stavudine Control No 105235 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

#### UV-spectrum

A UV-spectrum was recorded on a Varian Cary 5 spectrophotometer. 10.0 mg of stavudine were dissolved in 20.0 ml of water. 3.0 ml of this solution were diluted with 0.1 M sulfuric acid to a volume of 100.0 ml. The spectrum is given in Figure 2.

A UV-maximum was observed at 266 nm.

$A_{1\text{cm}}^{1\%} = 433$  at 266 nm (n=6, RSD=1.2%)

Calculations were performed with reference to the dried substance.

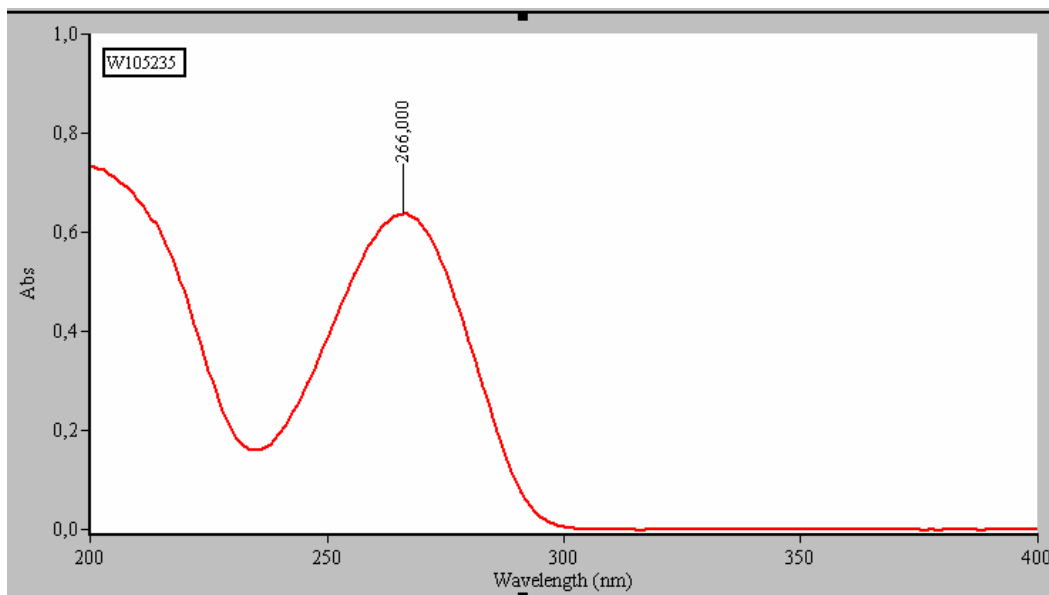


Figure 2. UV-spectrum of stavudine Control No 105235, 15 µg/ml.

## Assay

### Loss on drying

0.2% (n=3), when dried at 105 °C.

### Water

0.3% (n=3) determined by Karl Fischer titration.

### Residual solvents

Total content: < 0.1%. No peaks were detected.

The test includes Class 1, Class 2 and Class 3 residual solvents according to Ph Eur 5.0 Method 2.4.24 System A.

Reference solutions are corresponding to the following concentrations of each residual solvent in the sample: Benzene (2 ppm), Carbontetrachloride (4 ppm), 1,2-Dichloroethane (5 ppm), 1,1-Dichloroethene (8 ppm), 1,1,1-Trichloroethane (10 ppm), Acetonitrile (410 ppm), Chlorobenzene (360 ppm), Cyclohexane (3880 ppm), 1,2-Dichloroethene (1870 ppm), Methylene chloride (600 ppm), 1,4-Dioxane (380 ppm), Methanol (3000 ppm), Methylcyclohexane (1180 ppm), Tetrahydrofuran (720 ppm), Toluene (890 ppm) and Xylene (2170 ppm).

The content of residual solvents was tested by gas chromatography with the following conditions:

Instrument:	GC Hewlett Packard 6890
Column:	DB-624 (30 m x 0.32 mm, 1.8 µm film)
Carrier gas:	Helium (2.2 ml/min)
Split:	1:2
Detector:	FID
Detector temperature:	250 °C
Temperature program:	40 °C for 20 minutes, 10 °C/min and holding 240 °C for 20 minutes.
Injection:	Headspace HP 7694
Injector temperature:	140 °C
HS-Oven temperature:	80 °C
Vial equilibration:	60 minutes-low shaking
Loop volume:	1.0 ml gas phase

## Purity

### High performance liquid chromatography

The purity was estimated by peak area normalization to 99.9% (n=6, RSD < 0.01% for the main peak, RSD=12.9% calculated on the 0.03% impurity level). Two impurities above the limit of quantification were found. A chromatogram is shown in Figure 3.

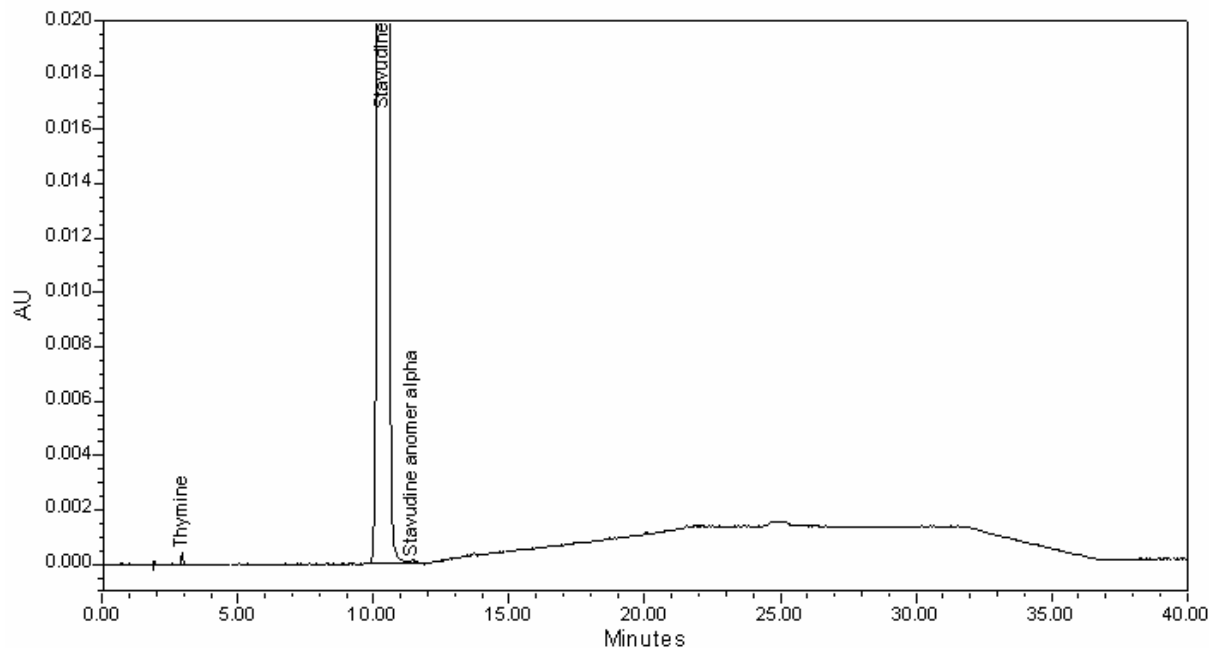


Figure 3. Chromatogram of stavudine Control No 105235 monitored at 254 nm.

The following conditions were used:

Eluent: A: Acetonitrile:0.77 g/l solution of ammonium acetate (35:65)

B: Acetonitrile:0.77 g/l solution of ammonium acetate (1:3)

Eluent composition:	<u>% A</u>	<u>% B</u>	<u>Time, minutes</u>	<u>Type</u>
	100	0	0-10	isocratic
	100→0	0→100	10-20	linear
	0	100	20-30	isocratic
	0→100	100→0	30-35	linear
	100	0	35-40	re-equilibration

Data collecting time: 30 minutes

Column: BDS Hypersil C18, 250x4.6 mm, 5 μm

Column temperature: R.T. (about 20 °C)

Detector, wavelength: Spectrophotometer, 254 nm

Flow rate: 2.0 ml/min  
Injector temperature: 6 °C  
Sample preparation: Stavudine was dissolved in water to a concentration of 0.5 mg/ml. 10 µl (corresponding to 5 µg) were injected.

Stability of the sample solution: The sample was stable in the dark at 8 °C for at least 3 hours.

Limit of detection: 0.2 ng (0.004%) at 254 nm

Limit of quantification: 0.6 ng (0.01%) at 254 nm

#### Diode-array detection

The chromatographic system described above was used to record UV-spectra for the detected peaks. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities.

#### Differential scanning calorimetry

The purity was estimated to 99.7 mol% (n=6, RSD=0.07%) and the melting point ( $T_m$ ) to 166.2 °C (n=6, RSD=0.1%). The determination was performed on about 3 mg using Mettler Toledo DSC 822<sup>e</sup> with a heating rate of 2 °C per minute.

### **Data given by the manufacturer**

Description: A white crystalline powder  
Identification IR: Conforms  
Water (KF): 0.18%  
Residue on ignition: 0.08%  
Heavy metals: < 20 ppm  
Related substances (HPLC)  
Thymine: 0.03%  
Total amount of impurities: 0.04%  
Assay by HPLC (on anhydrous and acetone and isopropanol free basis): 99.5%  
Residual solvents (GC)  
Acetone: Not detected  
Isopropanol: 0.01%

### **Stability**

No special stability studies have been performed. Regular re-examinations of this ICRS when stored in the dry state will be performed.

## **Conclusion**

Stavudine, Control No 105235, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 17

**TAMOXIFEN E-ISOMER**

Control No 205209

Analytical Report

**Intended use**

The stock of the current batch of the International Chemical Reference Substance for tamoxifen citrate *E*-isomer Control No 196209 is depleted and has to be replaced.

The monograph for Tamoxifen citrate in *The International Pharmacopoeia*, Third Edition, Volume 4, requires a reference substance of tamoxifen *E*-isomer to be used in the liquid chromatographic test for related substances.

**Material**

About 10 g of the sample (manufacturer's batch no PH13513/46A) were received at the WHO Centre in October 2005. The material is being stored in tightly closed containers at +5 °C, protected from light.

**Analytical data**

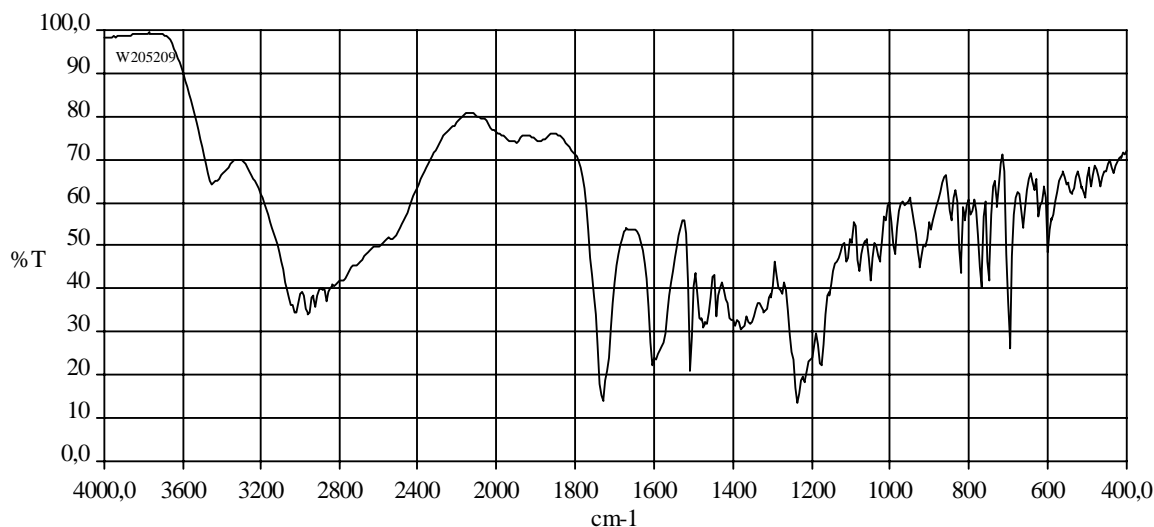
Description

A white powder.

**Evidence of chemical structure**

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W205209).



**Figure 1.** IR-spectrum of 1.7 mg of tamoxifen *E*-isomer Control No 205209 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

### UV-spectrum

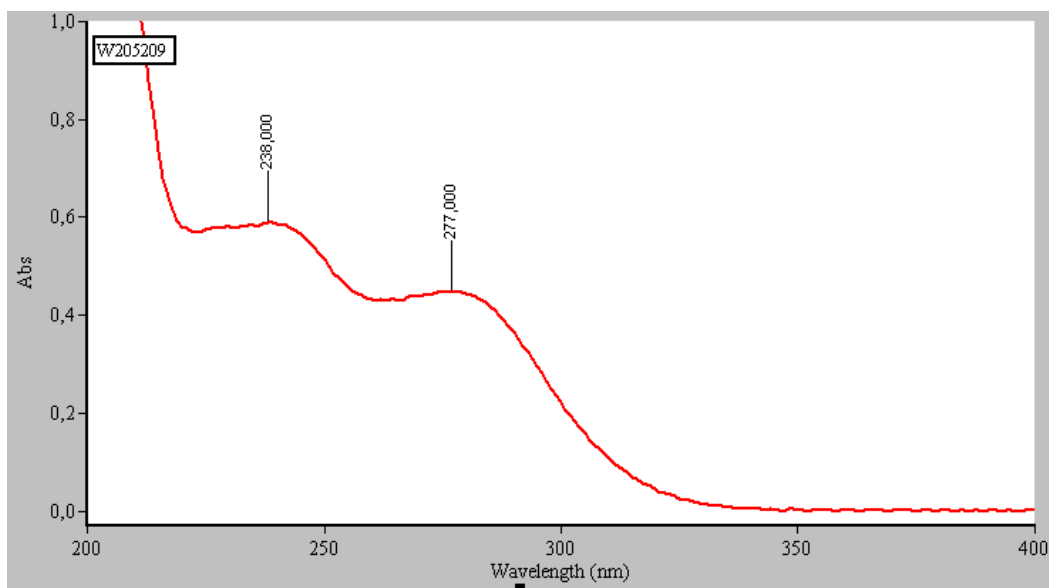
A UV-spectrum in methanol was recorded on a Varian Cary 5 spectrophotometer. The spectrum is given in Figure 2.

UV-maxima were observed at about 240 nm and 277 nm.

$A_{1\text{cm}}^{1\%} = 303$  at 240 nm (n=6, RSD=1.6%)

$A_{1\text{cm}}^{1\%} = 231$  at 277 nm (n=6, RSD=1.2%)

Calculations were performed with reference to the dried substance.



**Figure 2.** UV-spectrum of tamoxifen *E*-isomer Control No 205209, 20 µg/ml in methanol.



Flow rate:	1.0 ml/min
Injector temperature:	8 °C
Sample preparation:	Tamoxifen <i>E</i> -isomer was dissolved in the eluent to a concentration of 1.0 mg/ml, using ultrasonic bath. 20 µl (corresponding to 20 µg) were injected.
Stability of the sample solution:	The sample was stable in the dark at 8 °C for at least 3 hours.
Limit of detection:	3 ng (0.02%) at 240 nm
Limit of quantification:	10 ng (0.05%) at 240 nm

#### Diode-array detection

The chromatographic system described above was used to record UV-spectra for the detected peaks. The spectrum of the main peak showed UV-maxima at about 240 nm and below 220 nm. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities.

#### **Data given by the manufacturer**

Description:	White powder.
Identification by <sup>1</sup> H NMR:	Conforms.
Assigned purity:	97% w/w as received.

#### **Stability**

No special stability studies have been performed. The previous batch of the ICRS showed no signs of degradation after being stored for nine years at +5 °C. Regular re-examinations of this ICRS when stored in the dry state will be performed.

#### **Conclusion**

Tamoxifen *E*-isomer, Control No 205209, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 18

**TETRACYCLINE HYDROCHLORIDE**

Control No 205095

Analytical Report

**Intended use**

The stock of the current batch of the International Chemical Reference Substance for tetracycline hydrochloride Control No 180095 is depleted and has to be replaced.

The monograph for Tetracycline hydrochloride in *The International Pharmacopoeia*, Third Edition, Volume 2, requires a reference substance of tetracycline hydrochloride to be used in the thin-layer chromatographic tests for identity and related substances.

**Material**

About 200 g of the sample (manufacturer's batch no 12704263) were received at the WHO Centre in January 2005. The material is being stored in tightly closed containers at + 5 °C, protected from light.

**Analytical data**

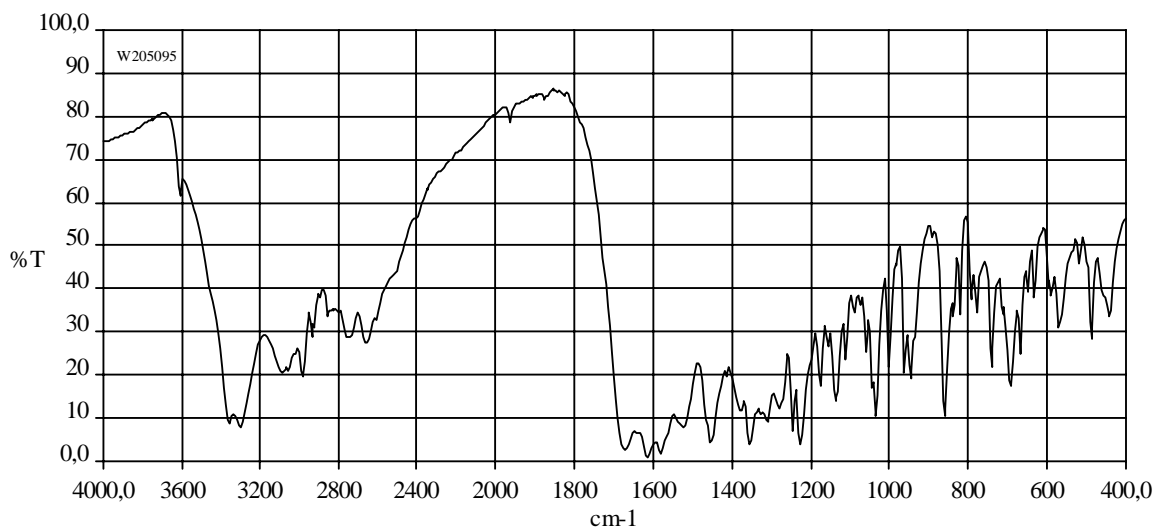
Description

A yellow crystalline powder.

**Evidence of chemical structure**

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W205095). The spectrum is concordant with the spectrum of the previous lot of the International Chemical Reference Substance (ICRS) for tetracycline hydrochloride with Control No 180095.



**Figure 1.** IR-spectrum of 2.2 mg of tetracycline hydrochloride Control No 205095 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

### Assay

#### Potentiometric titration

100.0% (n=6, RSD=0.2%) determined on the dried basis by non-aqueous titration according to *The International Pharmacopoeia*, Third Edition, Volume 2.

#### Thermogravimetric analysis

When the substance was heated to 105 °C, a loss of 0.6% (w/w) (n=6, RSD=6.4%) was observed.

#### Water

0.3% (n=3) determined by Karl Fischer titration.

### Purity

#### Thin-layer chromatography

Two secondary spots were detected. Their total amount was estimated to 2.5%. The following thin-layer chromatographic system was used.

Thin-layer:	Silica gel 60 F-254 TLC (Merck)
Eluent:	Water:methanol:dichloromethane (6:35:59)
Sample:	80 µg of tetracycline hydrochloride dissolved in methanol were applied.
Visualization:	Scanning at 365 nm with a Camag TLC Scanner 3 was performed.

$R_f$ (tetracycline hydrochloride) = 0.2

$R_f$ (4-epitetracycline hydrochloride) = 0.1

The limit of quantification was about 1.2 µg (1.5%), when scanning at 365 nm.

#### High performance liquid chromatography

4-epitetracycline was estimated by external standard to 1.8% (w/w). Other impurities were estimated by peak area normalization to 0.7% at 270 nm (n=6, RSD=0.02% for the main peak). Six impurities above the limit of quantification were found. One of them was identified as 4-epitetracycline hydrochloride (Imp2). A chromatogram is shown in Figure 2.

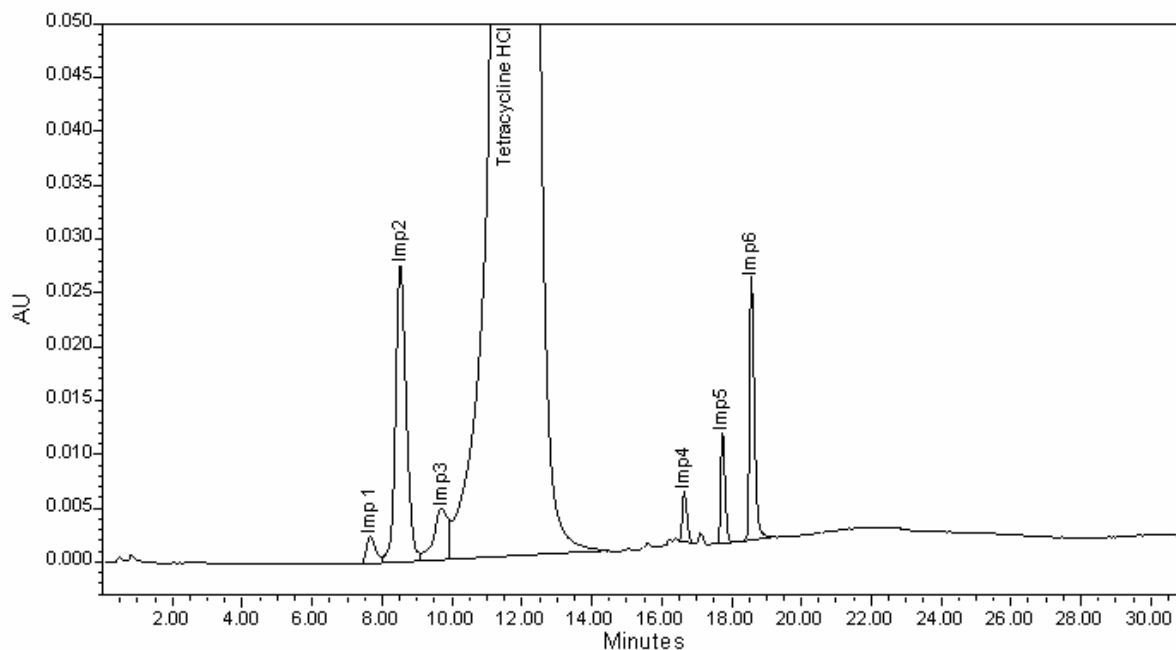


Figure 2. Chromatogram of tetracycline hydrochloride Control No 205095 monitored at 270 nm.

The following conditions were used:

Eluent: A: Acetonitrile

B: 50 mM (sodium) phosphate buffer pH 2.3

Eluent composition:	<u>% A</u>	<u>% B</u>	<u>Time, minutes</u>	<u>Type</u>
	15	85	0-10	isocratic
	15→50	85→50	10-20	linear
	50	50	20-30	isocratic
	50→15	50→85	30-31	linear
	15	85	31-43	re-equilibration

Data collecting time: 30 minutes

Column: Genesis C18, 4.6 x 150 mm, 4 µm particles

Column temperature: R.T. (about 20 °C)

Detector, wavelength:	Spectrophotometer, 270 nm
Flow rate:	1.0 ml/min
Injector temperature:	8 °C
Sample preparation:	Tetracycline hydrochloride was dissolved in the initial eluent to a concentration of 1.0 mg/ml. 20 µl (corresponding to 20 µg) were injected.
Stability in the eluent:	The sample was not stable in the eluent. The sample solution was injected immediately after preparation.
Limit of detection:	3 ng (0.02%) at 270 nm
Limit of quantification:	10 ng (0.05%) at 270 nm

#### Diode-array detection

The chromatographic system described above was used to record UV-spectra for the detected peaks. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities.

### **Stability**

No special stability studies have been performed. The previous lot showed no signs of degradation after being stored for 25 years at + 5 °C. Regular re-examinations of this ICRS when stored in the dry state will be performed.

### **Conclusion**

Tetracycline hydrochloride, Control No 205095, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 19

**ZIDOVUDINE**

Control No 105236

Analytical Report

**Intended use**

The monograph for Zidovudine in *The International Pharmacopoeia* requires a reference substance of zidovudine to be used in the infrared absorption spectrophotometric and thin-layer chromatographic tests for identity as well as in the thin-layer chromatographic test for related substances.

**Material**

About 100 g of the sample (manufacturer's batch no ZD0090904) were received at the WHO Centre in March 2005. The material is being stored in tightly closed containers at + 5 °C, protected from light.

**Analytical data**

Description

A white to yellowish powder.

**Evidence of chemical structure**

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W105236T).

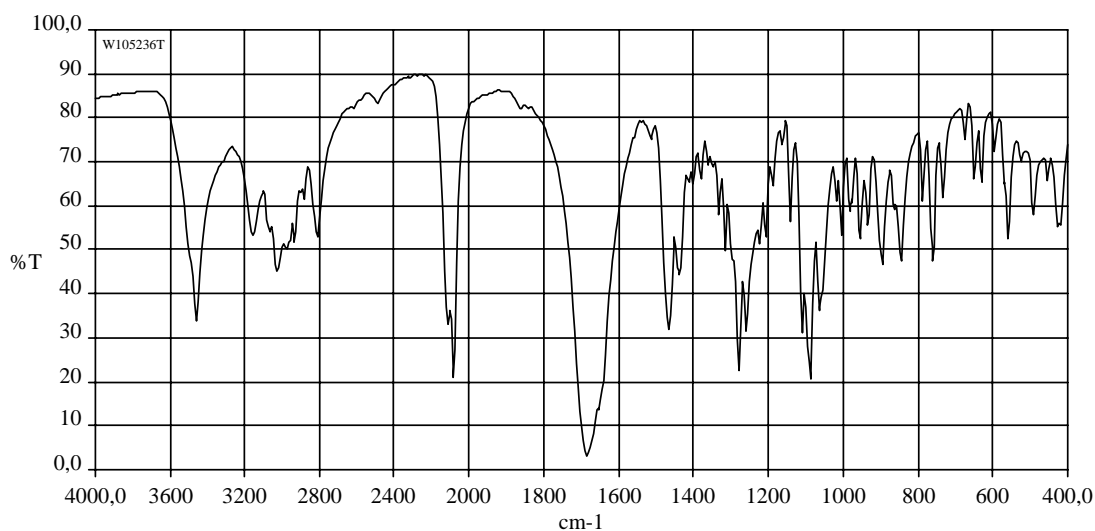


Figure 1. IR-spectrum of 1.1 mg of zidovudine Control No 105236 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

### UV-spectrum

A UV-spectrum was recorded on a Varian Cary 5 spectrophotometer. The spectrum is given in Figure 2.

UV-maxima were observed at 208 nm and at about 266 nm.

$A_{1\text{cm}}^{1\%} = 374$  at 266 nm (n=6, RSD=0.9%)

Calculations were performed with reference to the dried substance.

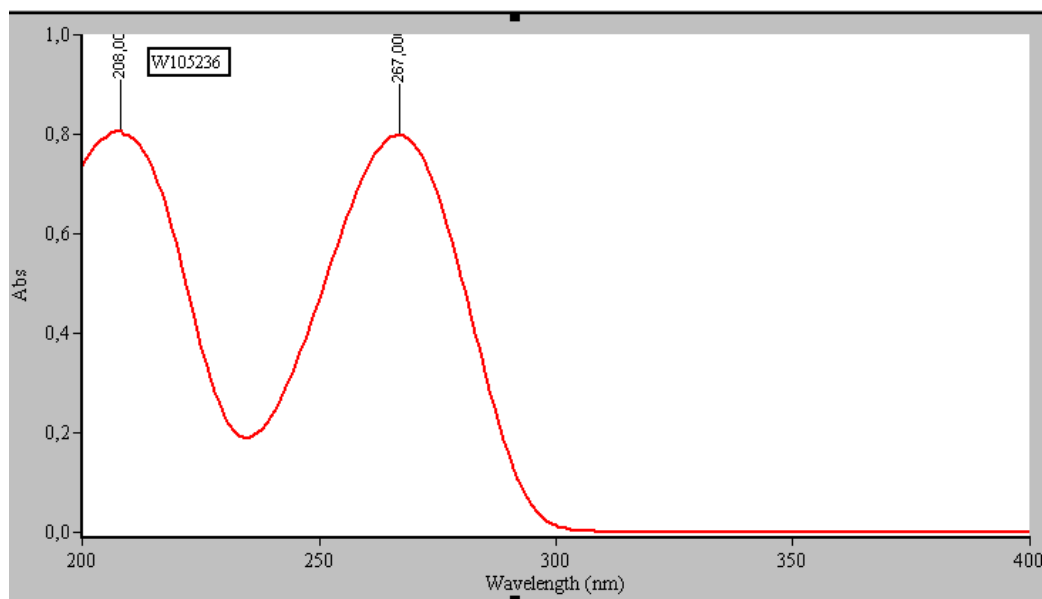


Figure 2. UV-spectrum of zidovudine Control No 105236, 21 µg/ml in methanol:water:0.1 M sulfuric acid (1:4:45).

## Assay

### Thermogravimetric analysis

When the substance was heated to 105 °C, a loss of 0.1% (w/w) (n=6) was observed.

### Water

0.2% (n=3) determined by Karl Fischer titration.

## Purity

### Thin-layer chromatography

Four secondary spots were detected. Their total amount was estimated to 0.2%. One of the impurities was identified as thymidine. The following thin-layer chromatographic system according to the monograph for Zidovudine was used.

Thin-layer:	Silica gel 60 F-254 TLC (Merck)
Eluent:	1,2-dichloroethane:methanol (8:2)
Sample:	100 µg of zidovudine dissolved in methanol were applied.
Visualization:	Scanning at 265 nm with a Camag TLC Scanner 3 was performed.

$$R_f(\text{zidovudine}) = 0.4$$

The limit of quantification was about 0.03 µg (0.03%), when scanning at 265 nm.

### High performance liquid chromatography

The purity was estimated by peak area normalization to 99.5% (n=6, RSD < 0.01% for the main peak). Four impurities above the limit of quantification were found. One of them was identified as thymidine (Imp1). A chromatogram is shown in Figure 3.

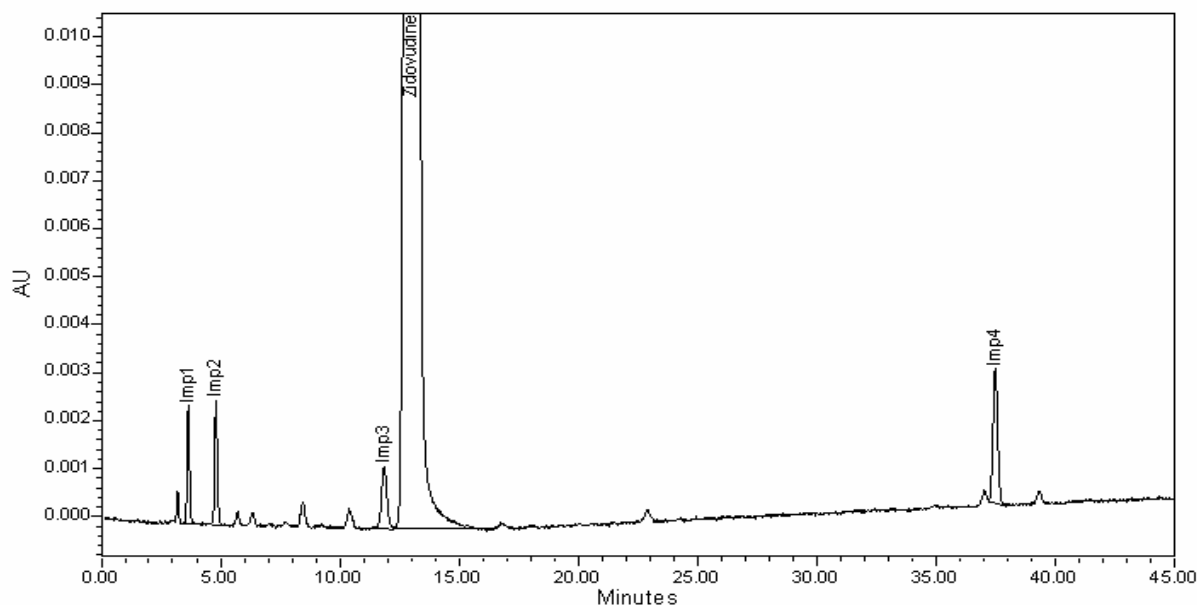


Figure 3. Chromatogram of zidovudine Control No 105236 monitored at 265 nm.

The following conditions were used:

Eluent:                   A: Methanol  
                              B: Water

Gradient:	<u>% A</u>	<u>% B</u>	<u>Time, minutes</u>	<u>Type</u>
	20	80	0-13	isocratic
	20→47	80→53	13-44	linear
	47→20	53→80	44-45	linear
	20	80	45-60	re-equilibration

Data collecting time: 44 minutes

Column: BDS Hypersil C18, 250x4.6 mm, 5 µm

Column temperature: R.T. (about 20 °C)

Detector, wavelength: Spectrophotometer, 265 nm

Flow rate: 1.2 ml/min

Injector temperature: 8 °C

Sample preparation: Zidovudine was dissolved in the eluent by using an ultrasonic bath. The solution was further diluted with the eluent to a concentration of 1.0 mg/ml. 10 µl (corresponding to 10 µg) were injected.

Stability of the sample solution: The sample was stable in the dark at 8 °C for at least 3 hours.

Limit of detection: 2 ng (0.02%) at 265 nm

Limit of quantification: 6 ng (0.06%) at 265 nm

#### Diode-array detection

The chromatographic system described above was used to record UV-spectra for the detected peaks. The spectra of the impurity peaks as well as the main peak were similar with UV-maxima at about 265 nm. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities.

#### Differential scanning calorimetry

The purity was estimated to 99.2 mol% (n=6, RSD=0.02%) and the melting temperature ( $T_m$ ) was estimated to 122.1 °C (n=6, RSD=0.05%). The determination was performed on about 3 mg using Mettler Toledo DSC 822<sup>e</sup> with a heating rate of 2 °C per minute.

## Data given by the manufacturer

Description:	Yellowish powder
Identification IR:	Conforms
Specific rotation (10 mg/ml in alcohol):	+61.9°
Water (KF):	0.16%
Residue on ignition:	0.06%
Related substances (HPLC)	
3'-chloro-3'-deoxythymidine	Not detected
Thymine	0.02%
Related substances (TLC, after spray)	
Any individual impurity:	< 0.5%
Triphenylmethanol:	< 0.5%
Total amount of impurities (HPLC and TLC):	1.19%
Assay (HPLC):	99.9% (anhydrous basis)
Residual solvents (GC):	0.09% of ethylacetate
Melting range:	122.1-124.3 °C

## Stability

No special stability studies have been performed. Regular re-examinations of this ICRS when stored in the dry state will be performed.

## Conclusion

Zidovudine, Control No 105236, can be considered suitable as International Chemical Reference Substance for the intended purpose.

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