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## WHO COLLABORATING CENTRE FOR CHEMICAL REFERENCE SUBSTANCES

### Report on the work in 2004

by E. Kagebeck

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 **Mrs. E. Kagebeck**, WHO Collaborating Centre for Chemical Reference Substances, Apoteket AB, Produktion & Laboratorier, Centrallaboratoriet (ACL), Prismavägen 2, SE-141 75 Kungens Kurva, Sweden, along with a copy to be sent to **Dr. P. Vanbel**, Technical Officer, Quality Assurance & Safety: Medicines, World Health Organization, CH-1211 Geneva 27, Switzerland (fax: (+41-22) 791 4730; e-mail: vanbelp@who.int).

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Distribution of reference substances in 2004

During 2004 the total number of International Chemical Reference Substances distributed from the Centre was 890. The most frequently requested substances were in order of demand: Artesunate, Caffeine M.P., Phenacetin M.P., Tetracycline hydrochloride and Vanillin M.P. Detailed figures for the distribution of the individual substances are given in Appendix 1.

Details of the distribution to different WHO Regions are given in Appendix 2. It is observed that 2.3% of the substances went to the African Region, 2.8% to the Region of the Americas, 0.2% to the Eastern Mediterranean Region, 73.8% to the European Region, 6.9% to South-East Asia and 13.9% to the Western Pacific Region.

Distribution of reference spectra in 2004

No reference spectra were distributed during 2004.

Establishment of reference substances in 2004

In accordance with the procedure recommended by the WHO Expert Committee on Specifications for Pharmaceutical Preparations in its [Thirty-second report \(Technical Report Series, No. 823\)](#), six International Chemical Reference Substances were established in 2004. The substances are listed in Appendix 3.

A complete list of all International Chemical Reference Substances available from the Centre in January 2005, with information about package sizes and control numbers for the current batches, is given in Appendix 4.

Establishment of reference spectra in 2004

A complete list of all International Infrared Reference Spectra with the new spectra established since 1993 is given in Appendix 5. No new spectra were established during 2004.

### Work on new and replacement substances completed in 2004

During 2004 work on four new reference substances was performed. The new reference substances are all antiretrovirals, namely Didanosine, Didanosine for System Suitability, Efavirenz and Nevirapine. The analytical reports are given in Appendices 9-12.

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 fifty-three substances were re-examined. The results are given in Appendix 6. Details about the analytical methods used can be obtained from the Centre.

### Work in progress and future work

Work is continuously performed on the substances required to support the monographs in Volume 3, 4 and 5 of *The International Pharmacopoeia*. For the moment fourteen substances of the fifty-eight substances, given in Appendix 7, are in stock. The work is focused on development of ICRS for antiretrovirals.

### Administrative and financial matters

The total cost for running the Centre in 2004 was estimated to US\$ 649 000. The income from sales of reference substances was US\$ 64 800, the contribution from SIDA was US\$ 274 000 and the contribution received from the WHO headquarters was US\$ 18 100. The deficit of US\$ 292 000 was covered by the support from Apoteket AB.

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 2004.

### Acknowledgements

The Centre is grateful to the laboratories that have contributed to the work during 2004. This year we would like to address our thanks in particular to the Centre for Analytical Science, Health Sciences Authority, Singapore; Dr. J.L. Robert, Luxemburg; Prof. J Hoogmartens; Boehringer Ingelheim and Bristol-Myers Squibb Pharma Co.

APPENDIX 1

**DISTRIBUTION OF INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES  
IN 2004**

<b>ICRS</b>	<b>Items sold</b>
p-Acetamidobenzalazine	1
Acetazolamide	3
Allopurinol	1
Amidotrizoic acid	3
2-Amino-5-nitrothiazole	1
3-Aminopyrazole-4-carboxamide hemisulfate	8
3-Amino-2,4,6-triiodobenzoic acid	1
Amitriptyline hydrochloride	2
Amodiaquine hydrochloride	1
Ampicillin (anhydrous)	2
Ampicillin sodium	9
Ampicillin trihydrate	3
Anhydrotetracycline hydrochloride	3
Artemether	6
Artemisinin	3
Artemotil	1
Artenimol	10
Artesunate	62
Atropine sulfate	1
Azathioprine	3
Beclometasone dipropionate	3
Benzylpenicillin potassium	2
Benzylpenicillin sodium	12
Betamethasone	3
Betamethasone sodium phosphate	2
Betamethasone valerate	2
Bupivacaine hydrochloride	1
Caffeine	3
Calcium folinate	1
Captopril	5
Captopril disulfide	12
Carbamazepine	4
Chloramphenicol	2
Chloramphenicol palmitate	7
Chloramphenicol palmitate (polymorph A)	2
5-Chloro-2-methylaminobenzophenone	1
Chloroquine sulfate	3
2-(4-Chloro-3-sulfamoyl-benzoyl)benzoic acid	2
Chlorphenamine hydrogen maleate	1
Chlortalidone	2
Chlortetracycline hydrochloride	2
Cimetidine	1

ICRS	Items sold
Ciprofloxacin hydrochloride	4
Ciprofloxacin by-compound A	11
Ciprofloxacin desfluoro-compound	5
Ciprofloxacin ethylenediamine-compound	22
Ciprofloxacin fluoroquinolonic acid	5
Cisplatin	1
Clomifene citrate	1
Clomifene citrate Z-isomer see Zuclomifene	-
Cloxacillin sodium	1
Colecalciferol	10
Dexamethasone	1
Dexamethasone acetate	4
Dexamethasone phosphoric acid	1
Dexamethasone sodium phosphate	1
Dicloxacillin sodium	1
Dicolinium iodide	1
Digitoxin	1
Digoxin	13
Dopamine hydrochloride	1
Doxorubicin hydrochloride	2
Emetine hydrochloride	1
4-Epianhydrotetracycline hydrochloride	8
4-Epitetracycline hydrochloride	3
Ergocalciferol	2
Ergometrine hydrogen maleate	8
Ergotamine tartrate	12
Erythromycin	3
Erythromycin C	5
Estrone	1
Ethambutol hydrochloride	7
Ethinylestradiol	2
Ethisterone	1
Etocarlide	1
Flucloxacillin sodium	3
Flucytosine	1
Fludrocortisone acetate	1
Fluorouracil	2
Folic acid	22
3-Formylrifamycin	4
Framycetin sulfate	4
Gentamicin sulfate	1
Griseofulvin	1
Haloperidol	1
Hydrochlorothiazide	2
Hydrocortisone	1
Hydrocortisone acetate	5
Hydrocortisone sodium succinate	2
(-)-3-(4-Hydroxy-3-methoxyphenyl)-2-hydrazino-2-methylalanine	8

<b>ICRS</b>	<b>Items sold</b>
(-)-3-(4-Hydroxy-3-methoxyphenyl)-2-methylalanine	7
Ibuprofen	3
Indometacin	1
Isoniazid	7
Kanamycin monosulfate	5
Lanatoside C	3
Leucovorin calcium see Calcium folinate	-
Levodopa	4
Lidocaine hydrochloride	5
Liothyronine sodium	1
Loperamide hydrochloride	2
<i>Melting point reference substances</i>	
Azobenzene	23
Vanillin	50
Benzil	9
Acetanilide	20
Phenacetin	53
Benzanilide	14
Sulfanilamide	20
Sulfapyridine	14
Dicyanodiamide	8
Saccharin	10
Caffeine	60
Phenolphthalein	10
Metazide	1
Methotrexate	1
3- <i>o</i> -Methylcarbidopa see (-)-3-(4-Hydroxy-3-methoxyphenyl)-2-hydrazino-2-methylalanine	-
Methyldopa	1
3- <i>o</i> -Methylmethyldopa see (-)-3-(4-Hydroxy-3-methoxyphenyl)-2-methylalanine	-
Metronidazole	2
Neamine hydrochloride	17
Neomycin B sulfate see Framycetin sulfate	-
Nicotinamide	1
Nicotinic acid	1
Nifurtimox	1
Niridazole	1
Niridazole-chlorethylcarboxamide	4
Norethisterone	0
Oxytetracycline dihydrate	2
Oxytetracycline hydrochloride	2
Paracetamol	47
Prednisolone	1
Prednisolone acetate	1
Propicillin potassium	1
Propranolol hydrochloride	2
Propylthiouracil	1

<b>ICRS</b>	<b>Items sold</b>
Pyridostigmine bromide	1
Retinol acetate (solution à 25000 IU)	1
Riboflavin	2
Rifampicin	4
Rifampicin quinone	3
Streptomycin sulfate	2
Sulfamethoxazole	1
Sulfasalazine	1
Tamoxifen citrate E-isomer	2
Tetracycline hydrochloride	50
Thioacetazone	1
Tolbutamide	5
Trimethoprim	1
Trimethylguanidine sulfate	2
Vitamin A acetate (solution) see Retinol acetate	-
Warfarin	1
Zuclomifene	2

APPENDIX 2

**DISTRIBUTION OF INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES  
TO DIFFERENT WHO REGIONS IN 2004**

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<b>WHO Regions</b>	<b>Number of distributed ICRS</b>
African Region (AFRO)	20
Region of the Americas (AMRO)	25
Eastern Mediterranean Region (EMRO)	2
European Region (EURO)	654
South-East Asia Region (SEARO)	61
Western Pacific Region (WPRO)	123

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APPENDIX 3

**INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES ESTABLISHED IN 2004**

<b>Reference substance</b>	<b>Control number</b>	<b>Analytical report</b>
Artemeter	103225	EDM/QSM/2004.7 Appendix 9
Artemisinin	103222	EDM/QSM/2004.7 Appendix 10
Artemotil	103226	EDM/QSM/2004.7 Appendix 11
Artemimol	103223	EDM/QSM/2004.7 Appendix 12
Artesunate	103224	EDM/QSM/2004.7 Appendix 13
Rifampicin	203151	EDM/QSM/2004.7 Appendix 14

## APPENDIX 4

### **LIST OF AVAILABLE INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES**

**2005**

#### **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 or at the website (see below).

#### **Ordering information**

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

WHO Collaborating Centre for Chemical Reference Substances  
Apoteket AB  
Produktion & Laboratorier  
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 USD 70 per package. An administration charge of USD 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 USD 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: 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 USD 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	10 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
9931411	Efavirenz	100 mg	104229
9930294	Emetine hydrochloride	100 mg	187134
9930197	4-Epianhydrotetracycline hydrochloride	25 mg	288097
9930198	4-Epitetracycline hydrochloride	25 mg	293098
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	150 mg	194186
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	194183
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
9930330	Indometacin	100 mg	178078
9930331	Isoniazid	100 mg	185124
9930332	Kanamycin monosulfate	12 mg	197211
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
9930341	Mebendazole	200 mg	195195

Catalogue number	Reference substances	Package size	Control number
Melting Point Reference Substances			
9930217	Azobenzene (69 °C)	1 g	192168
9930438	Vanillin (83 °C)	1 g	299169
9930222	Benzil (96 °C)	4 g	294170
9930201	Acetanilide (116 °C)	1 g	297171
9930380	Phenacetin (136 °C)	1 g	297172
9930221	Benzanilide (165 °C)	1 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) Neomycin B sulphate <i>see</i> Framycetin sulfate	0.5 mg	193177
9930356	Neostigmine metilsulfate	100 mg	187135
9931412	Nevirapine	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	300152
9930371	Ouabain	100 mg	283026
9930372	Oxacillin sodium	200 mg	382027
9930373	Oxytetracycline dihydrate	200 mg	189142
9930374	Oxytetracycline hydrochloride	200 mg	189141
9930376	Papaverine hydrochloride	100 mg	185127
9930377	Paracetamol	100 mg	195198
9930378	Paromomycin sulfate	75 mg	195197

<b>Catalogue number</b>	<b>Reference substances</b>	<b>Package size</b>	<b>Control number</b>
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
9930407	Retinol acetate (solution)	5 caps (*)	898038
9930408	Riboflavin	250 mg	382035
9930409	Rifampicin	300 mg	191151
9930410	Rifampicin quinone	200 mg	202148
9930412	Sodium amidotrizoate	100 mg	198221
9930413	Sodium cromoglicate	100 mg	188140
9930415	Spectinomycin hydrochloride	200 mg	193176
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 citrate <i>E</i> -isomer	10 mg	196209
9930427	Testosterone enantate	200 mg	194192
9930428	Testosterone propionate	100 mg	167036
9930429	Tetracycline hydrochloride	200 mg	180095
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

<b>Catalogue number</b>	<b>Reference substances</b>	<b>Package size</b>	<b>Control number</b>
9930433	Toluene-2-sulfonamide	100 mg	196204
9930434	Trimethadione	200 mg	185125
9930435	Trimethoprim	100 mg	179093
9930440	Vincristine sulfate Vitamin A acetate (solution) <i>see</i> Retinol acetate (solution)	9.7 mg/vial	193181
9930439	Warfarin	100 mg	168041
9930260	Zuclomifene	50 mg	187137

(\*) About 8 mg in 230 mg oil per capsule

\* \* \*

APPENDIX 5

**LIST OF AVAILABLE INTERNATIONAL INFRARED REFERENCE SPECTRA**

**2005**

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

The current price is USD 5 for a single spectrum and USD 200 for a set of 50 spectra, including a hardcover binder. The binder can be ordered separately for USD 10. An administrative charge of USD 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  
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: 2 98 40-6  
IBAN: SE 65 9500 0099 6026 0029 8406

Our invoice number must be quoted when payment is made.

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

Aceclidine salicylate  
Acetazolamide  
Allopurinol  
Amiloride hydrochloride  
Amitriptyline hydrochloride  
Ampicillin trihydrate

Beclometasone dipropionate  
Benzylpenicillin potassium  
Biperiden  
Biperiden hydrochloride  
Bupivacaine hydrochloride

Caffeine (anhydrous)  
Calcium folinate  
Carbidopa  
Chlorphenamine hydrogen maleate  
Clofazimine  
Cloxacillin sodium  
Colchicine  
Cytarabine

Dexamethasone  
Dexamethasone acetate, monohydrate  
Dextromethorphan hydrobromide  
Diazepam  
Dicolinium iodide  
Dicoumarol  
Diethylcarbamazine dihydrogen citrate  
Diphenoxylate hydrochloride

Erythromycin ethylsuccinate  
Erythromycin stearate  
Etacrynic acid  
Ethionamide  
Ethosuximide

Furosemide

Gallamine triethiodide  
Glibenclamide

Haloperidol  
Hydrochlorothiazide

Ibuprofen  
Imipramine hydrochloride  
Indometacin  
Isoniazid

Lidocaine  
Lidocaine hydrochloride  
Lindane

Metronidazole  
Miconazole nitrate

Niclosamide  
Nicotinamide  
Noscapine

Oxamniquine

Papaverine hydrochloride  
Phenobarbital  
Phenoxymethylpenicillin calcium  
Phenytoin  
Primaquine phosphate  
Propylthiouracil  
Protionamide  
Pyrimethamine

Salbutamol  
Salbutamol sulfate  
Sulfadimidine  
Sulfadoxine  
Sulfamethoxazole  
Sulfamethoxyipyridazine

Tiabendazole  
Trihexyphenidyl hydrochloride  
Trimethoprim

Valproic acid  
Verapamil hydrochloride

\* \* \*

APPENDIX 6**STABILITY TESTING – ANALYTICAL REPORT**

The stability on storage of the International Chemical Reference Substances is monitored by regular re-examination of the substances held in stock at the Centre. The results obtained for the substances re-examined in 2004 are summarized below. For comparison results obtained at 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.

Acetazolamide, Control No 186128

Initial analytical report: WHO/PHARM/87.532, Appendix 6

Examination year:	1986	1996	2004
IR	conforms	conforms	-
TLC, %	no impurities	-	-
HPLC, %	< 0.05	0.2	<0.05
TGA, %	-	0.1	
LOD, %	0.2	-	0.1
Assay, potentiometric, %	99.7	100.6	-

Amidotrizoic acid, Control No 196205

Initial analytical report: WHO/PHARM/96.587, Appendix 9.

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Examination year:	1996	2004
IR	conforms	-
TLC, %	< 0.2	-
HPLC, %	< 0.03	<0.03
TGA, %	5.6	5.6
UV ( $A_{1\text{cm}}^{1\%}$ )	567 (238 nm)	-
Assay, spectrophotometric, %	100.0	-

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3-Amino-2,4,6-triiodobenzoic acid, Control No 196206

Initial analytical report: WHO/PHARM/96.587, Appendix 10.

---

Examination year:	1996	2004
IR	conforms	-
TLC, %	< 0.5	-
HPLC, %	0.8	0.8
TGA, %	3.4	-
KF, %	3.8	3.5
UV ( $A_{1\text{cm}}^{1\%}$ )	626 (231 nm)	-
Residual solvents (GC), %	< 0.1	-

---

Amphotericin B, Control No 191153

Initial analytical report: WHO/PHARM/92.558, Appendix 7

Examination year:	1991	1996	2004
IR	conforms	-	-
TLC, %	one secondary spot	-	-
HPLC, %	2.2 (306 nm)	3 (306 nm)	3.3 (306 nm)
LOD, %	-	-	4.6
Content of tetraenes	-	-	4.1

Anhydrotetracycline hydrochloride, Control No 180096

Initial analytical report: WHO/PHARM/81.508, Appendix 5

Examination year:	1980	1985	1994	2000	2004
IR	conforms	-	-	conforms	-
TLC, %	one secondary spot	one secondary spot	-	-	-
HPLC, %	1.4	1.5	1.3	1.7	1.4
TGA, %	-	-	2.2	-	-
Water (KF), %	-	2.4	-	3.1	2.8
LOD, %	1.2	-	-	-	-
Assay, potentiometric, %	93.9	-	-	-	-

Beclometasone dipropionate, Control No 192175

Initial analytical report: WHO/PHARM/93.564, Appendix 9

---

Examination year:	1992	1999	2004
IR	conforms	-	-
HPLC, %	1.5	1.3	1.3
TGA, %	0.3	0.3	-
LOD, %	0.3	-	0.5

---

Betamethasone, Control No 183113

Initial analytical report: WHO/PHARM/84.513, Appendix 7

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Examination year:	1983	1990	1999	2004
IR	conforms	-	-	conforms
TLC	1.5	1.0	-	-
HPLC, %	1.2	1.3	1.1	1.5
TGA, %	-	<0.1	<0.1	0.2
LOD, %	0.2	-	-	-

---

Betamethasone sodium phosphate, Control No 196203

Initial analytical report: WHO/PHARM/96.587, Appendix 11

Examination year:	1996	2004
IR	conforms	-
HPLC, %	1.9	1.9
TGA, %	9.7	7.7
KF, %	8.2	-
Residual solvents (GC), %	0.3	-

Caffeine, Control No 181102

Initial analytical report: WHO/PHARM/82.509, Appendix 7

Examination year:	1981	1994	2004
IR	conforms	-	conforms
TLC, %	no impurities	-	-
HPLC, %	0.1	< 0.1	< 0.05
TGA, %	-	< 0.1	-
LOD, %	< 0.1	-	< 0.1
Assay, potentiometric, %	99.5	-	-
UV ( $A_{1\text{cm}}^{1\%}$ )	489 (273 nm)	-	-

Carbenicillin monosodium, Control No 383043

Initial analytical report: WHO/PHARM/84.513, Appendix 8

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Examination year:	1983	1990	2004
IR	conforms	-	conforms
HPLC, %	3.8	3.2	3.3
TGA, %	-	4.8	-
KF, %	4.9	-	4.8
Assay, titrimetric, %	96.4	96.8	-

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Chlorphenamine hydrogen maleate, Control No 182109

Initial analytical report: WHO/PHARM/83.510, Appendix 5

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Examination year:	1982	1994	1999	2004
IR	conforms	-	-	conforms
HPLC, %	< 0.1	0.1	0.1	< 0.1
TGA, %	-	< 0.1	< 0.1	< 0.1

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Chlortetracycline hydrochloride, Control No 187138

Initial analytical report: WHO/PHARM/88.537, Appendix 7

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Examination year:	1987	1993	2000	2004
IR	conforms	-	conforms	-
TLC, %	no impurities	-	-	-
HPLC, %	0.5	1.5	0.6	0.6
TGA, %	0.2	0.2	< 0.1	0.2

---

Ciprofloxacin by-compound A, Control No 198220

Initial analytical report: WHO/PHARM/98.603, Appendix 10.

Examination year:	1998	2004
IR	conforms	-
TLC, %	0.9	-
HPLC, %	0.9	1.8
LOD, %	3.6	2.0
UV ( $A_{1cm}^{1\%}$ )	750 (264nm)	-

Ciprofloxacin desfluoro-compound, Control No 198219

Initial analytical report: WHO/PHARM/98.603, Appendix 11.

Examination year:	1998	2004
IR	conforms	-
KF%	5.1	-
HPLC, %	0.5	1.2
TGA, %	4.9	4.9
UV ( $A_{1cm}^{1\%}$ )	1231 (277 nm)	-

Ciprofloxacin ethylenediamine-compound, Control No 198218

Initial analytical report: WHO/PHARM/98.603, Appendix 12.

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Examination year:	1998	2004
IR	conforms	-
TLC, %	< 0.1	-
HPLC, %	0.1	0.3
KF, %	0.6	0.2
Residual solvents (GC), %	< 0.1	-
UV ( $A_{1cm}^{1\%}$ )	1456 (272 nm)	-

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Ciprofloxacin fluoroquinolonic acid, Control No 198217

Initial analytical report: WHO/PHARM/98.603, Appendix 13.

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Examination year:	1998	2004
IR	conforms	-
TLC, %	< 0.1	-
HPLC, %	0.9	1.0
TGA, %	< 0.1	< 0.1
UV ( $A_{1cm}^{1\%}$ )	1176 (261nm)	-

---

Ciprofloxacin hydrochloride, Control No 197210

Initial analytical report: WHO/PHARM/97.595, Appendix 11.

Examination year:	1996	2004
IR	conforms	-
TLC, %	<0.1	-
HPLC, %	<0.06	0.1
TGA, %	6.0	6.5
KF, %	6.1	-
Assay, spectrophotometric, %	100.0	-
UV ( $A_{1cm}^{1\%}$ )	1079 (275nm)	-

Cortisone acetate, Control No 167006

Initial analytical report: WHO/PHARM/67.441, Appendix 1

Examination year:	1966	1975	1984	1992	2004
IR	conforms	-	-	-	-
TLC, %	three impurities	two impurities	three impurities	-	-
HPLC, %	-	-	0.3	0.3	0.1
TGA, %	-	-	-	0.1	-
KF, %	-	-	-	0.2	0.2
LOD, %	< 0.1	0.2	-	-	-
UV ( $A_{1cm}^{1\%}$ )	402	404	396	396	-

Dexamethasone sodium phosphate, Control No 192158

Initial analytical report: WHO/PHARM/93.564, Appendix 11

---

Examination year:	1992	1995	2004
IR	conforms	-	-
HPLC, %	1.4	1.6	2.4
TGA, %	8.0	6.9	-
Water (KF), %	4.3	3.2	-
LOD, %		-	6.7
Residual solvents (GC), %	3.7	-	-

---

Digitoxin, Control No 277010

Initial analytical report: WHO/PHARM/78.494, Appendix 7

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Examination year:	1977	1987	1992	1999	2004
IR	conforms	-	-	-	conforms
TLC, %	0.2	-	0.3	-	-
HPLC, %	no impurities	0.1	0.5	0.6	1.0
TGA, %	-	-	0.6	0.4	0.3
LOD, %	0.6	0.6	-	-	-

---

Digoxin, Control No 587011

Initial analytical report: WHO/PHARM/88.537, Appendix 10

Examination year:	1987	1992	1999	2004
IR	conforms	-	-	-
TLC, %	three impurities	three impurities	-	-
HPLC, %	1.4	1.4	1.3	1.5
TGA, %	0.2	0.11	< 0.1	< 0.1
Water (KF), %	0.2	-	-	-

4-Epianhydrotetracycline hydrochloride, Control No 288097

Initial analytical report: WHO/PHARM/89.544, Appendix 9

Examination year:	1988	1994	2000	2004
IR	conforms	-	conforms	-
TLC, %	one secondary spot	-	-	-
HPLC, %	4.7	4.9	5.8	6.6
TGA, %	5.4	5.3	-	-
KF, %	5.4	-	5.4	6.1
Assay, potentiometric, %	99.6	-	-	-

Estrone, Control No 279015

Initial analytical report: WHO/PHARM/79.499, Appendix 6

Examination year:	1979	1984	1993	2004
IR	conforms	-	-	-
TLC	0.2	0.1	-	-
HPLC, %	0.2	0.1	0.1	0.1
TGA, %	-	-	< 0.1	-
LOD, %	0.1	-	-	< 0.1
UV( $A_{1cm}^{1\%}$ )	78 (281 nm)	77 (281 nm)	-	-

Ethambutol hydrochloride, Control No 179081

Initial analytical report: WHO/PHARM/79.499, Appendix 7

Examination year:	1979	1985	1990	1995	2004
IR	conforms	-	-	conforms	-
TLC, %	no impurities	no impurities	no impurities	no impurities	two impurities <LOQ
TGA, %	-	-	-	< 0.1	-
LOD, %	0.1	0.3	0.3	-	< 0.1
Assay, titration, %	100.1	-	-	-	-

Hydrocortisone, Control No 283020

Initial analytical report: WHO/PHARM/84.513, Appendix 11

Examination year:	1983	1989	1994	2000	2004
IR	conforms	-	conforms	conforms	-
TLC, %	0.3 (4 sec spots)	-	three secondary spots	-	-
HPLC, %	0.3	0.6	0.3	0.3	0.3
TGA, %	-	< 0.1	< 0.1	< 0.1	0.1
LOD, %	< 0.1	-	-	-	-
Assay, spectrophotometric, %	99.9	99.7	99.6	-	-

Hydrocortisone acetate, Control No 280021

Initial analytical report: WHO/PHARM/81.508, Appendix 11

Examination year:	1980	1989	1994	2000	2004
IR	conforms	-	-	conforms	-
TLC, %	0.4 (two secondary spots)	-	-	-	-
HPLC, %	<0.5	0.4	0.5	0.4	0.4
TGA, %	-	0.1	< 0.1	< 0.1	< 0.1
LOD, %	0.1	-	-	-	-
Assay, spectrophotometric, %	99.6	99.9	100.4	-	-

Hydrocortisone sodium succinate, Control No 194184

Initial analytical report: WHO/PHARM/95.577, Appendix 14

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Examination year:	1994	2004
IR	conforms	-
TLC, %	2.3	-
HPLC, %	1.9	2.4
TGA, %	3.6	3.3
Assay, spectrophotometric, %	100.0	100.0

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(-)-3-(4-Hydroxy-3-methoxyphenyl)-2-hydrazino-2-methylalanine , Control No 193180  
(3-O-Methylcarbidopa)

Initial analytical report: WHO/PHARM/94.566, Appendix 10.

---

Examination year:	1994	2004
IR	conforms	-
TGA%	3.5	-
LOD%	-	3.7
HPLC, %	98.8	98.2
UV( $A_{1\text{cm}}^{1\%}$ )	123 (282 nm)	-

---

Imipramine hydrochloride, Control No 172064

Initial analytical report: WHO/PHARM/73.475, Appendix 7

Examination year:	1972	1977	1988	1993	1999	2004
IR	conforms	conforms	conforms	conforms	-	-
TLC, %	four impurities	three impurities	three impurities	0.1 (four impurities)	-	-
HPLC, %	-	-	-	0.1	0.1	0.1
TGA, %	-	-	-	< 0.1	< 0.1	< 0.1
LOD, %	< 0.1	< 0.1	< 0.1	-	-	-

Isoniazid, Control No 185124

Initial analytical report: WHO/PHARM/86.527, Appendix 7

Examination year:	1985	1995	2004
IR	conforms	conforms	-
TLC, %	< 0.01	0.1	-
HPLC, %	< 0.05	< 0.05	< 0.03
LOD, %	< 0.1	< 0.1	0.1
Assay, potentiometric, %	100.0	-	-

Lanatoside C, Control No 281022

Initial analytical report: WHO/PHARM/82.509, Appendix 12

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Examination year:	1981	1987	1994	2000	2004
IR	conforms	conforms	-	-	-
TLC, %	five secondary spots	five secondary spots	-	-	-
HPLC, %	0.8	1.0	1.0	1.1	1.1
TGA, %	-	-	7.5	7.4	7.1
LOD, %	7.2	7.2	-	-	-
PSA, %	< 0.6	-	-	-	-

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Levonorgestrel, Control No 194182

Initial analytical report: WHO/PHARM/95.577, Appendix 15

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Examination year:	1994	1999	2004
IR	conforms	-	
TLC, %	0.1	-	
HPLC, %	0.3	0.3	0.3
TGA, %	< 0.1	< 0.1	0.1

---

Liothyronine sodium, Control No 193179

Initial analytical report: WHO/PHARM/94.566, Appendix 11

Examination year:	1993	2004
IR	conforms	-
TLC, %	1.0	-
HPLC, %	0.8	2.5
TGA, %	3.5	3.4

Mebendazole, Control No 195195

Initial analytical report: WHO/PHARM/96.584, Appendix 14

Examination year:	1996	2004
IR	conforms	-
TLC, %	0.5	-
HPLC, %	0.2	0.2
TGA, %	0.1	0.1
UV ( $A_{1cm}^{1\%}$ )	971 (248 nm)	-

Neamine hydrochloride, Control No 193177

Initial analytical report: WHO/PHARM/94.566, Appendix 12.

Examination year:	1993	2004
IR	conforms	-
TGA%	8.6	-
HPLC, %	4.0	0.9

Neomycin B sulfate, Control No 193178

Initial analytical report: WHO/PHARM/94.566, Appendix 13.

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Examination year:	1993	2004
IR	conforms	-
LOD%	10.8	11.0
HPLC, %	13.8	11.0

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Nifurtimox, Control No 194189

Initial analytical report: WHO/PHARM/95.577, Appendix 18

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Examination year:	1994	1999	2004
IR	conforms	-	-
TLC, %	< 0.06	-	-
HPLC, %	0.03	0.06	0.09
TGA, %	< 0.1	< 0.1	< 0.1

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Norethisterone, Control No 186132

Initial analytical report: WHO/PHARM/87.532, Appendix 12

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Examination year:	1986	1995	2000	2004
IR	conforms	conforms	-	-
TLC, %	0.4	0.1	-	-
HPLC, %	0.3	0.2	0.2	0.3
TGA, %	-	< 0.1	< 0.1	< 0.1
LOD, %	< 0.1	-	-	-
UV ( $A_{1cm}^{1\%}$ )	576 (240 nm)	575 (239 nm)	-	-

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Nystatin, Control No 300152

Initial analytical report: EDM/QSM/2001.4, Appendix 2

Examination year:	2000	2001 (Feb)	2001 (Sept)	2002 (March)	2002 (Oct)	2003 (May)	2003 (Oct)	2004 (June)
IR	Conforms	-	-	-				
KF, %	5.6	-	-	-				
TGA, %	5.1	-	-	-				
LOD, %	4.0	-	4.7	-	4.1		3.9	4.0
Assay, microbiologic, IU/mg	5061	4853	4631	4377	4197	3982	4113	4143
UV ratio A291/A305	0.66	-	-	-	0.67	0.65		0.66
UV ratio A319/A305	0.90	-	-	-	0.90	0.90		0.91

Oxytetracycline dihydrate, Control No 189142

Initial analytical report: WHO/PHARM/90.547.509, Appendix 10

Examination year:	1989	1995	2000	2004
IR	conforms	-	-	-
HPLC, %	1.5	1.7	3.3	3.0
TGA, %		7.3	7.2	7.4
KF, %	7.4	7.6	-	-

Piperazine adipate, Control No 197212

Initial analytical report: WHO/PHARM/97.595, Appendix 15.

Examination year:	1997	2004
IR	conforms	-
TLC, %	no impurities	no impurities
TGA, %	< 0.1	< 0.1

Piperazine citrate, Control No 197213

Initial analytical report: WHO/PHARM/97.595, Appendix 16.

Examination year:	1997	2004
IR	conforms	-
TLC, %	no impurities	no impurities
TGA, %	11.1	11.9

Praziquantel, Control No 194191

Initial analytical report: WHO/PHARM/95.577, Appendix 19

Examination year:	1994	1999	2004
IR	conforms	-	-
TLC, %	0.2	-	-
HPLC, %	0.1	0.1	0.1
TGA, %	< 0.1	< 0.1	< 0.1

Prednisolone, Control No 389029

Initial analytical report: WHO/PHARM/90.547, Appendix 11

Examination year:	1989	1994	1999	2004
IR	conforms	-	-	-
TLC	0.2	0.6	-	-
HPLC, %	0.3	0.6	0.3	0.2
TGA, %	0.2	0.4	0.3	0.4
KF, %	0.3	-	-	-
UV ( $A_{1cm}^{1\%}$ )	417 (243 nm)	418 (244 nm)	-	-
Assay, spectrophotometric, %	100.1	100.3	-	-

Prednisone, Control No 167031

Initial analytical report: WHO/PHARM/67.441, Appendix 3

Examination year:	1967	1975	1984	1994	1999	2004
IR	conforms	-	conforms	-	-	-
TLC, %	no impurities	no impurities	two impurities	three impurities	-	-
HPLC, %	-	-	0.7	0.5	0.9	0.6
TGA, %	-	-	-	< 0.1	< 0.1	< 0.1
LOD, %	< 0.1	0.1	< 0.1	-	-	-

Propylthiouracil, Control No 185126

Initial analytical report: WHO/PHARM/86.527, Appendix 10

Examination year:	1985	1999	2004
IR	conforms	-	conforms
HPLC, %	0.4	0.3	0.3
TGA, %	-	< 0.1	0.1
LOD, %	< 0.1	-	-
UV ( $A_{1cm}^{1\%}$ )	950 (275 nm)	-	-

Pyridostigmine bromide, Control No 182110

Initial analytical report: WHO/PHARM/83.510, Appendix 9

Examination year:	1982	1996	1999	2004
IR	conforms	-	conforms	-
HPLC, %	< 0.1	< 0.1	< 0.05	< 0.04
KF, %	-	0.2	0.1	0.2
LOD, %	< 0.1	-	-	-
Assay, potentiometric, %	100.4	-	-	-
UV ( $A_{1cm}^{1\%}$ )	184 (269 nm)	-	-	-

Sulfacetamide, Control No 196200

Initial analytical report: WHO/PHARM/96.587, Appendix 13.

Examination year:	1996	2004
IR	conforms	-
TGA, %	< 0.1	< 0.1
HPLC, %	0.1	0.1

Tamoxifen citrate, Control No 196208

Initial analytical report: WHO/PHARM/96.587, Appendix 14.

Examination year:	1996	2004
IR	conforms	-
TLC, %	0.1	-
HPLC, %	0.3	0.5
CE, %	0.2	-
TGA, %	0.2	-
LOD, %	-	< 0.1
KF, %	0.1	0.1
Assay, Potentiometric %	99.9	-
UV ( $A_{1\text{cm}}^{1\%}$ )	333 (236 nm) 214 (276 nm)	-

Testosterone enantate, Control No 194192

Initial analytical report: WHO/PHARM/95.577, Appendix 21

Examination year:	1994	1999	2004
IR	conforms	-	-
TLC, %	0.1	-	-
HPLC, %	0.2	0.2	0.2
LOD, %	< 0.1	< 0.1	< 0.1

Tetracycline hydrochloride, Control No 180095

Initial analytical report: WHO/PHARM/81.508, Appendix 12

Examination year:	1980	1985	1989	1993	2000	2004
IR	conforms	-	-	-	-	-
TLC, %	one secondary spot	-	-	-	-	-
HPLC, %	1.5	1	0.7	1.0	1.2	1.3
TGA, %	-	-	0.3	0.3	0.1	0.6
Water (KF), %	0.4	0.3	-	-	-	-
LOD, %	0.3	-	-	-	-	-
Assay, potentiometric, %	99.6	-	-	-	-	-

Toluene-2-sulfonamide, Control No 196204

Initial analytical report: WHO/PHARM/96.587, Appendix 16.

Examination year:	1996	2004
IR	conforms	-
HPLC, %	0.6	0.5
KF, %	< 0.1	< 0.1
UV ( $A_{1cm}^{1\%}$ )	479 (282 nm)	-

Trimethadione, Control No 185125

Initial analytical report: WHO/PHARM/86.527, Appendix 11

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Examination year:	1985	1999	2004
IR	conforms	-	conforms
HPLC, %	0.1	0.2	0.5
TGA, %	-	-	-
KF, %	< 0.1	< 0.1	0.2
Assay, titrimetric %	100.1	100.0	100.4

---

## APPENDIX 7

### **INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES - PROJECT LIST 2005**

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  
Indinavir sulfate  
Lamivudine  
Nelfinavir mesilate  
Ritonavir

Saquinavir  
Saquinavir mesilate  
Stavudine  
Zidovudine

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

## APPENDIX 8

### **INTERNATIONAL INFRARED REFERENCE SPECTRA - PROJECT LIST 2005**

The following International Infrared Reference Spectra are required to support specifications in the third edition of *The International Pharmacopoeia*.

#### Volume 3

Diloxanide furoate  
Metoclopramide hydrochloride (\*\*)  
Naloxone hydrochloride (\*\*)  
Nitrofurantoin  
Pyrazinamide (\*\*)  
Spironolactone (\*\*)

#### Volume 4

Disodium edetate  
Ephedrine sulfate  
Iopanoic acid  
Iotroxic acid  
Ketamine hydrochloride  
Norethisterone enantate  
Pentamidine isetionate  
Timolol maleate

#### Volume 5

Benzoyl peroxide  
Ciprofloxacin  
Codeine phosphate  
Colchicine  
Diethyltoluamide  
Diloxanide furoate  
Dinitrogen oxide  
Erythromycin lactobionate  
Isosorbide dinitrate  
Morphine sulfate  
Pethidine hydrochloride  
Phenobarbital  
Proguanil hydrochloride  
Sulfadiazine  
Tropicamide

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

APPENDIX 9

**DIDANOSINE**

Control No 104228

Analytical Report

**Intended use**

The International Chemical Reference Substance for didanosine is intended to be used in the thin layer chromatographic and UV spectrophotometric test for identity according to the monograph in *The International Pharmacopoeia*, draft for discussion, working document QAS/04.067/Rev.2.

**Material**

About 118 g of the sample (manufacturers batch no 3D72380) were received at the WHO Centre in September 2004. The material is being stored in tightly closed containers in a freezer, protected from light.

CAUTION: Didanosine may be harmful if swallowed. It may cause peripheral nervous system damage, pancreatitis, liver toxicity and lactic acidosis. It should be handled with care.

**Analytical data**

Description

A white powder.

**Evidence of chemical structure**

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W104228b).

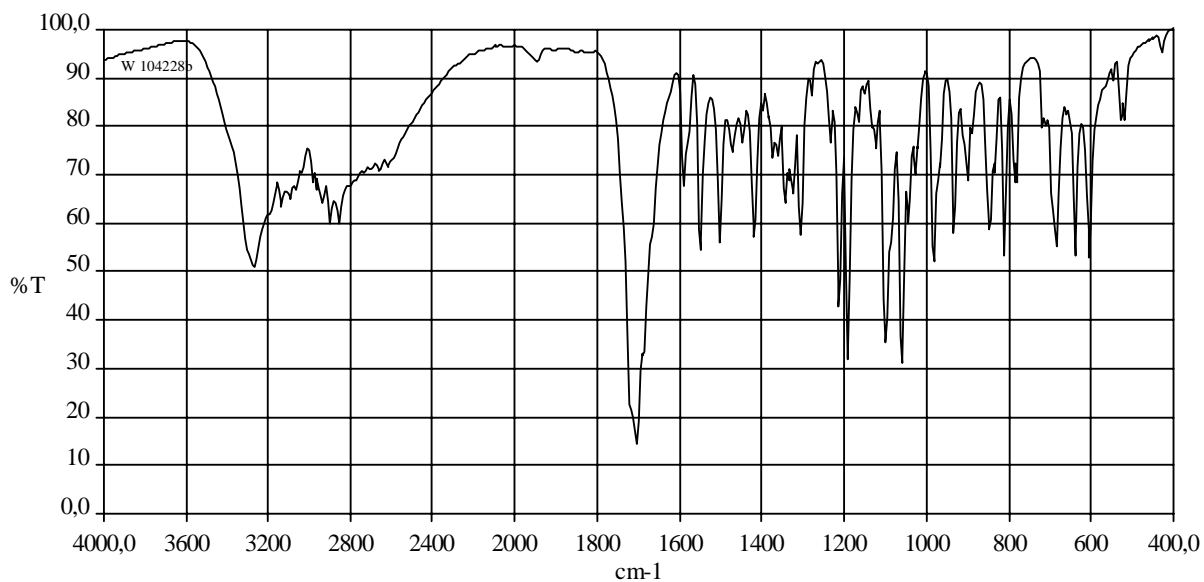


Figure 1. IR-spectrum of 0.7 mg of didanosine Control No 104228 in 300 mg of potassium bromide recorded against a potassium bromide disc.

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 250 nm.

$A_{1cm}^{1\%} = 487$  at 250 nm (n=6, RSD=1.2%)

Calculations were performed with reference to the dried substance.

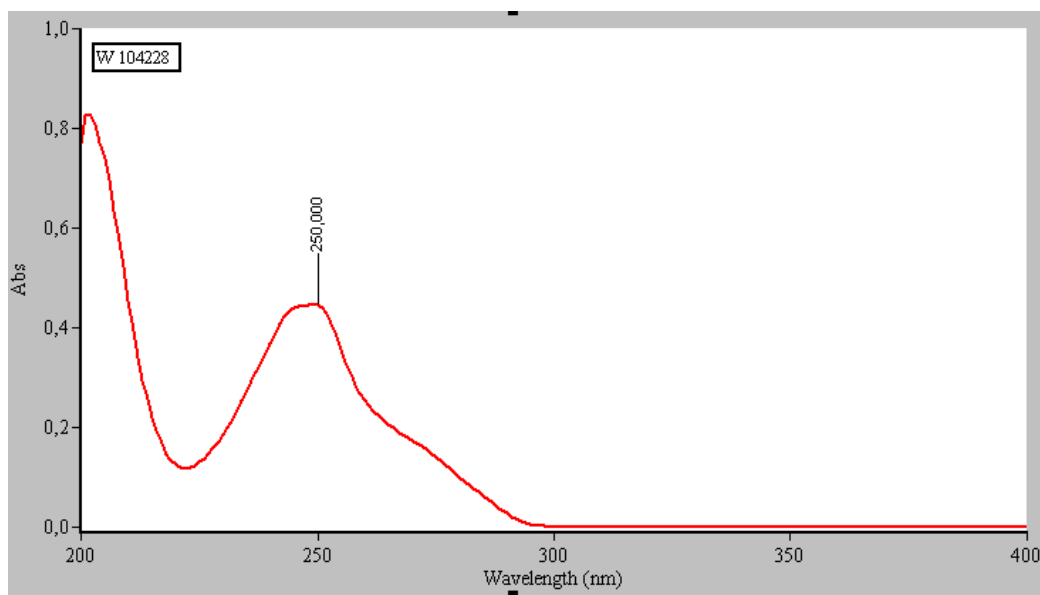


Figure 2. UV-spectrum of didanosine Control No 104228, 10 µg/ml in methanol.

### Thin-layer chromatography

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

## **Assay**

### Potentiometric titration

100.2 % (n=6, RSD=0.2 %) determined on the dried basis by non-aqueous titration according to *The International Pharmacopoeia*, Third Edition, Volume 1.

### Thermogravimetric analysis

When the substance was heated to 130 °C, a loss of 0.2 % (w/w) was observed. (n=6).

Instrument:	Perkin Elmer TGA 7 Thermogravimetric analyzer.
Sample weight:	About 10 mg.
Heating program:	5 °C/min from 20–130°C and then holding at 130 °C for 180 minutes or until the baseline is stable.
Melting point:	About 170 °C.

### Loss on drying

0.4 %, when dried at 130 °C for 3 hour to constant weight.

### Water

0.4 % (n=6) determined by Karl Fischer titration.

### Residual solvents

< 0.1 %. The test included methanol, ethanol, acetone, acetonitrile, dichloromethane, pyridine, chloroform, benzene, trichloroethylene and dioxan. Each of them was estimated to be < 100 ppm.

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

Instrument:	Hewlett Packard 6890
Column:	HP-5 (30 m x 0.53 mm, 2.65 µm film)
Carrier gas:	Helium (5 ml/min)
Split:	1:5
Detector:	FID
Detector temperature:	250 °C
Temperature program:	50 °C for 5 minutes, 10 °C/min and holding at 150 °C for 5 minutes, 50 °C/min and holding at 250 °C for 8 minutes.
Injection:	Head-space HP 7694
Injector temperature:	120 °C
Oven temperature:	80 °C
Vial equilibration:	30 minutes-none shaking

Loop volume: 1.0 ml gas phase

Sample preparation: Blank solution: 1.0 ml dimethyl sulfoxide was diluted to 100.0 ml of Milli-Q water. 1.0 ml of this was diluted to 100.0 ml with Milli-Q water.

Reference solution: 1.0 ml of each of the solvents tested was diluted to 100.0 ml of dimethyl sulfoxide. 1.0 ml of this was diluted to 100.0 ml with Milli-Q water. 1.0 ml was further diluted to 100.0 ml with Milli-Q water.

Sample: 50.0 mg were mixed with 5.0 ml of blank solution in a head-space vial which was then capped.

Spiked sample: 50.0 mg were mixed with 5.0 ml of reference solution in a head-space vial which was then capped.

Reference: 5.0 ml of reference solution in a capped head-space vial.

Blank: 5.0 ml of blank solution in a capped head-space vial.

## Purity

### Thin-layer chromatography

One secondary spots were detected. The amount was estimated to be <LOQ. The following thin-layer chromatographic system was used.

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

Eluent: Dichloromethan : Acetonitrile : Methanol : Ammonia solution (60 : 15 : 22 : 3)

Sample: 100 µg of didanosine dissolved in methanol were applied.

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

$$R_f(x) = 0.3$$

$$R_f(\text{imp A} = \text{Hypoxathine}) = 0.2$$

The detection limit of the system was about 0.1 µg (0.1 %), when scanning at 254 nm.

The spot of didanosine corresponds in position and appearance with another batch of didanosine from Labogen lot 207116.

### High performance liquid chromatography

Imp A was estimated by external standard to 0.1 % . The other impurities were estimated by peak area normalization to about 0.1 % (n=6, RSD=0.0x % for the main peak, RSD=3.6 % calculated on the 0.1 % impurity level (Imp A)).

A chromatogram is shown in Figure 3. Three impurities above the limit of quantification were found. One of them was identified as hypoxanthine (Imp A) (about 3 minutes) and the other one as 2',3'-anhydroinosine (Imp E) (about 7 minutes).

The liquid chromatographic system according to *The International Pharmacopoeia*, draft for discussion, working document QAS/04.067. A chromatogram is shown in Figure 3.

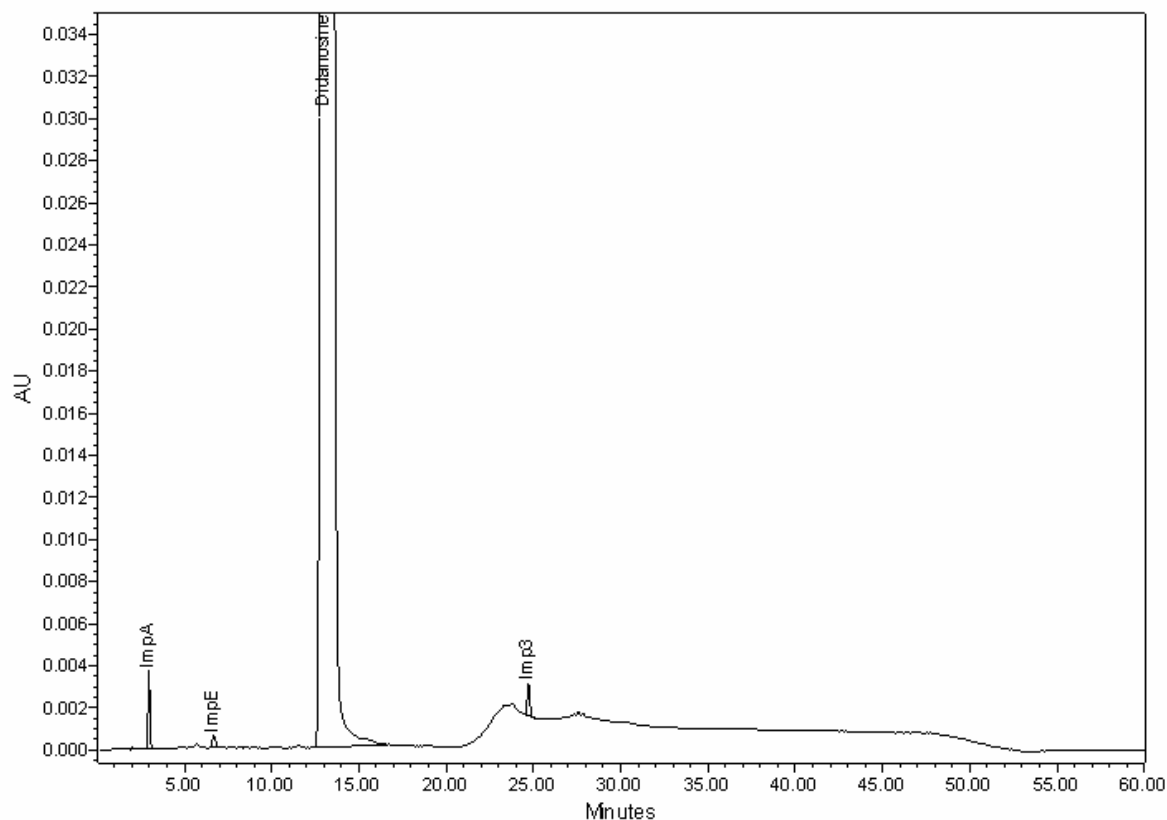


Figure 3. Chromatogram of didanosine Control No 104228 monitored at 254 nm.

The following conditions were used:

Eluent:

A: Methanol

B: 3.85 g ammonium acetate was dissolved in 900 ml. pH was adjusted to 8.0 with ammonia solution and diluted with water to 1000 ml.

Gradient:

% A	% B	Time, minutes	Type
8	92	0-18	isocratic
8→30	92→70	18-25	linear
30	70	25-45	isocratic
30→8	70→92	45-50	linear



APPENDIX 10

**DIDANOSINE FOR SYSTEM SUITABILITY**

Control No 104230

Analytical Report

**Intended use**

The International Chemical Reference Substance for didanosine for system suitability is intended to be used to identify the peaks due to impurities A to F in the liquid chromatographic test of didanosine according to the monograph in *The International Pharmacopoeia*, draft for discussion, working document QAS/04.067/Rev.2.

**Material**

About 12 g of the sample (manufacturers batch no S88D011) were received at the WHO Centre in Centre in September 2004. The material is being stored in tightly closed containers in a freezer, protected from light.

**Analytical data**

Description

A white powder.

High performance liquid chromatography

In the chromatogram obtained with injection of 10 µg didanosine for system suitability, the following peaks were eluted at the following relative retention times with reference to didanosine (retention time = about 13-15):

Impurity A= 0.27

Impurity B= 0.40

Impurity C= 0.46

Impurity D= 0.50

Impurity E= 0.53

Impurity F= 0.78

The resolution factor between the peaks due to impurity C (2'-deoxyinosine) and impurity D (3'-deoxyinosine) was 2.2.

A chromatogram is shown in Figure 1.

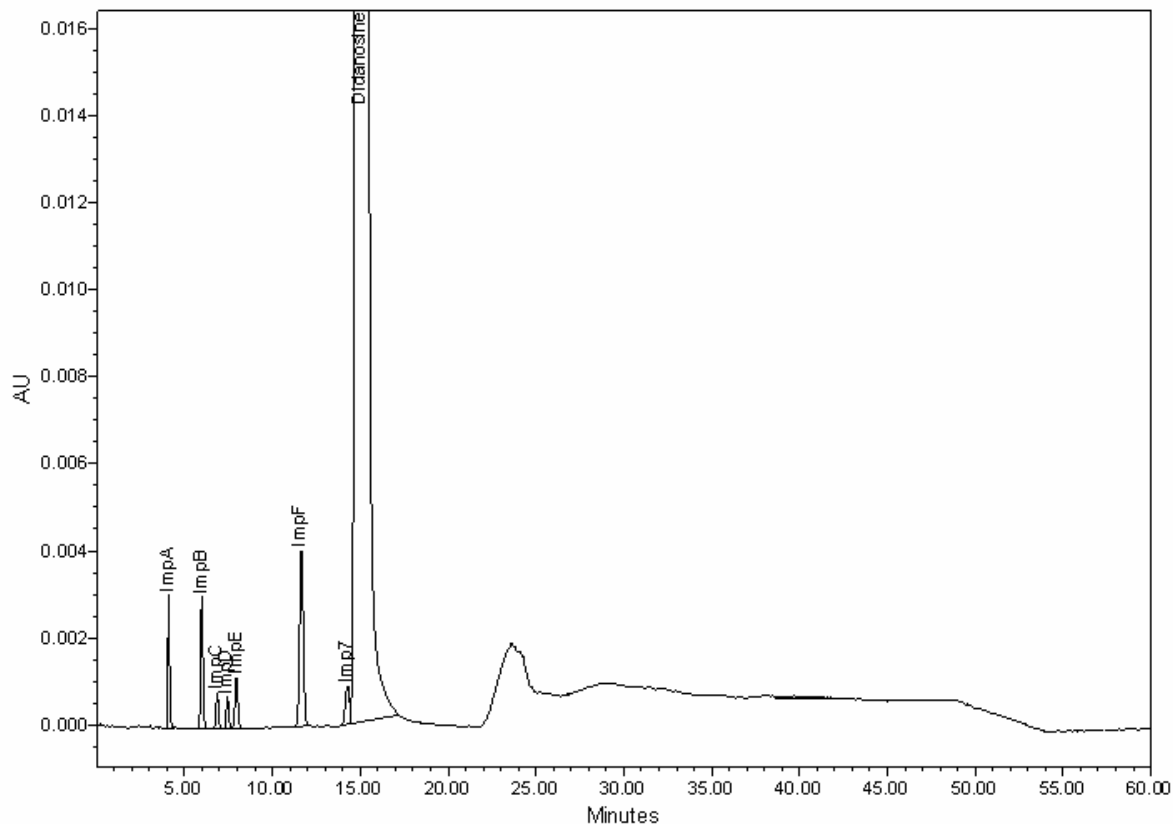


Figure 1. Chromatogram of didanosine for system suitability Control No 104230 monitored at 254 nm.

The following conditions were used:

Eluent:	A: Methanol			
	B: 3.85 g ammonium acetate was dissolved in 900 ml. PH Was adjusted to 8.0 with ammonia solution and diluted with water to 1000 ml.			
Gradient:	% A	% B	Time, minutes	Type
	9	91	0-18	isocratic
	9→30	91→70	18-25	linear
	30	70	25-45	isocratic
	30→8	70→91	45-50	linear
	9	91	50-60	re-equilibr.

Column:	BDS Hypersil C18, 250x4.6 mm, 5 µm
Column temperature:	R.T. (about 20 °C)
Detector, wavelength:	Spectrophotometer, 254 nm
Flow rate:	1.0 ml/min
Injector temperature:	8 °C
Sample:	Dissolve didanosine for system suitability in the eluent to a concentration of 0.5 mg/ml. Inject 20 µl (corresponding to 10 µg).

## **Stability**

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

## **Conclusion**

Didanosine for System Suitability Control No 104230, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 11

**EFAVIRENZ**

Control No 104229

Analytical Report

**Intended use**

The International Chemical Reference Substance for efavirenz is intended to be used in the infrared absorption spectrophotometric test for identity, in the liquid chromatographic test for related substances and in the spectrophotometric assay according to the monograph in *The International Pharmacopoeia*, draft for discussion, working document QAS/04.091.

**Material**

About 140 g of the sample (manufacturers batch no SB706-056) were received at the WHO Centre in October 2004. 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 W104229T).

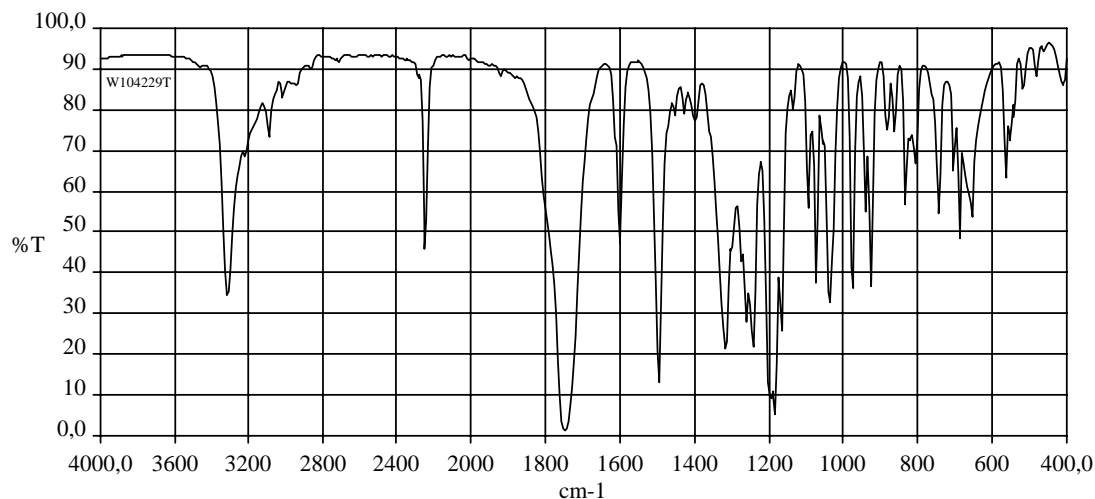


Figure 1. IR-spectrum of 0.9 mg of efavirenz Control No 104229 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

High performance liquid chromatography with mass-spectrometric detection

A spectrum of the main peak (efavirenz) was recorded by atmospheric pressure chemical ionization (APCI) in the positive ion mode. The spectrum shows an  $[M+H]^+$  ion of  $m/z$  316, which supports the identity of efavirenz. The spectrum is given in Figure 2.

Eluent:	Methanol:0,05% trifluoroacetic acid
Column:	Zorbax SB-CN, 4.6x150 mm, 3.5 $\mu$ m particles/ or Direct inlet
Pump:	Waters alliance 2695 operated at a flow rate of 0.2 ml/min
Detector:	Waters ZQ (single quadrupole mass spectrometer)
Operating conditions:	Cone voltage 30 V Source temperature 130 °C
Sample:	Efavirenz was dissolved in the eluent at a concentration of 0.33 mg/ml. 35 $\mu$ l corresponding to 11.6 $\mu$ g were injected.

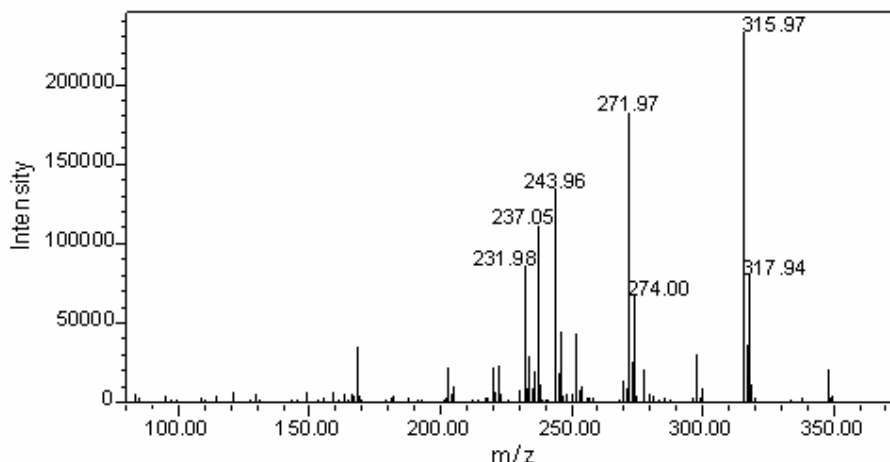


Figure 2. *Positive-ion atmospheric pressure chemical ionization mass spectrum of efavirenz Control No 104229.*

#### UV-spectrum

A UV-spectrum in methanol was recorded on a Varian Cary 5 spectrophotometer. The spectrum is given in Figure 3.

UV-maxima were observed at 293 nm.

$A_{1\text{cm}}^{1\%} = 65,3$  at 293 nm (n=6, RSD=1,0%)

Calculations were performed with reference to the dried substance.

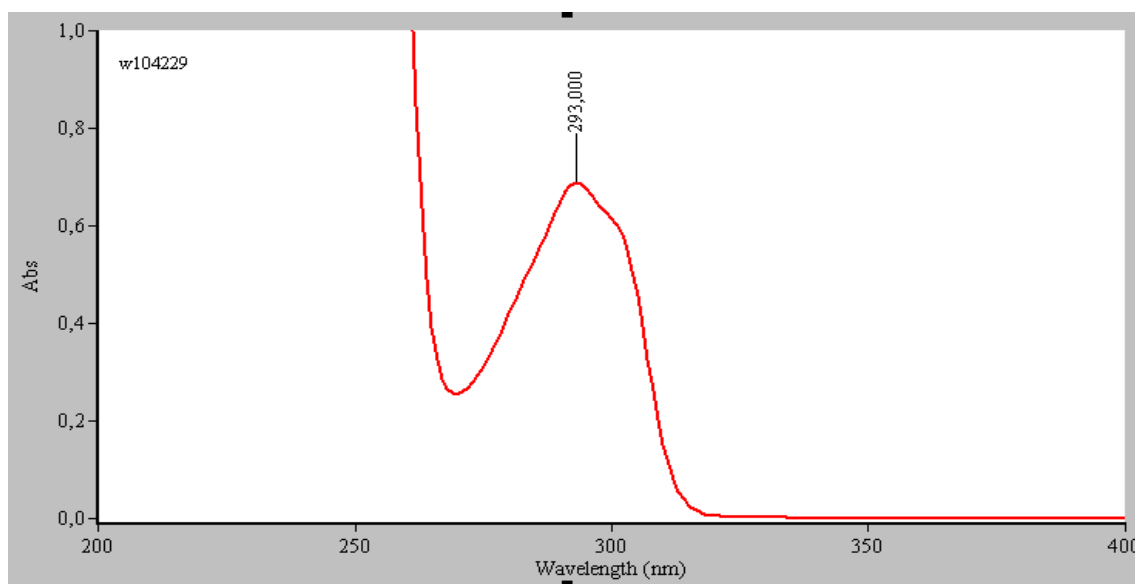


Figure 3. *UV-spectrum of efavirenz Control No 104229, 106 µg/ml in methanol.*

#### Thin-layer chromatography

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

## Assay

### Loss on drying

0.2%, when dried at 105 °C for 4 hour according to the method in the monograph (QAS/04.091).

### Water

<0.1% (n=6) determined by Karl Fischer titration.

### Residual solvents

< 0.1 %. The test included methanol, ethanol, acetone, acetonitrile, dichloromethane, pyridine, chloroform, benzene, trichloroethylene and dioxan. Each of them except pyridine was estimated to be < 100 ppm. Pyridine was estimated to be 110 ppm

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

Instrument:	Hewlett Packard 6890
Column:	HP-5 (30 m x 0.53 mm, 2.65 µm film)
Carrier gas:	Helium (5 ml/min)
Split:	1:5
Detector:	FID
Detector temperature:	250 °C
Temperature program:	50 °C for 5 minutes, 10 °C/min and holding at 150 °C for 5 minutes, 50 °C/min and holding at 250 °C for 8 minutes.
Injection:	Head-space HP 7694
Injector temperature:	120 °C
Oven temperature:	80 °C
Vial equilibration:	30 minutes-none shaking
Loop volume:	1.0 ml gas phase
Sample preparation:	<p><u>Blank solution:</u> 1.0 ml dimethyl sulfoxide was diluted to 100.0 ml of Milli-Q water. 1.0 ml of this was diluted to 100.0 ml with Milli-Q water.</p> <p><u>Reference solution:</u> 1.0 ml of each of the solvents tested was diluted to 100.0 ml of dimethyl sulfoxide. 1.0 ml of this was diluted to 100.0 ml with Milli-Q water. 1.0 ml was further diluted to 100.0 ml with Milli-Q water.</p> <p><u>Sample:</u> 50.0 mg were mixed with 5.0 ml of blank solution in a head-space vial which was then capped.</p> <p><u>Spiked sample:</u> 50.0 mg were mixed with 5.0 ml of reference solution in a head-space vial which was then capped.</p> <p><u>Reference:</u> 5.0 ml of reference solution in a capped head-space vial.</p> <p><u>Blank:</u> 5.0 ml of blank solution in a capped head-space vial.</p>

## Purity

### Thin-layer chromatography

One secondary spot were detected. The amount was estimated to be <LOQ. The following thin-layer chromatographic system was used.

Thin-layer:	Silica gel 60 F-254 (Merck) TLC and HPTLC
Eluent:	Dichloromethane:Isopropanol 9:1
Sample:	100 µg of efavirenz dissolved in dichloromethane were applied.
Visualization:	Scanning at 250 nm with a CAMAG TLC Scanner 3 was performed as well as visualization in day-light.

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

The detection limit of the system was about 0.1 µg (0.1%), when scanning at 250 nm.

### High performance liquid chromatography

The purity was estimated by peak area normalization to about 99.9 % (n=6, RSD=0.0 % for the main peak, RSD=2.8 % calculated on the 0.05 % impurity level).

A chromatogram is shown in Figure 4. 3 impurities above the limit of quantification were found.

The total amount of impurities estimated by peak area normalization was about 0.1%. The liquid chromatographic system used was according to the monograph in *The International Pharmacopoeia*, draft for discussion, working document QAS/04.091. A chromatogram is shown in Figure 4.

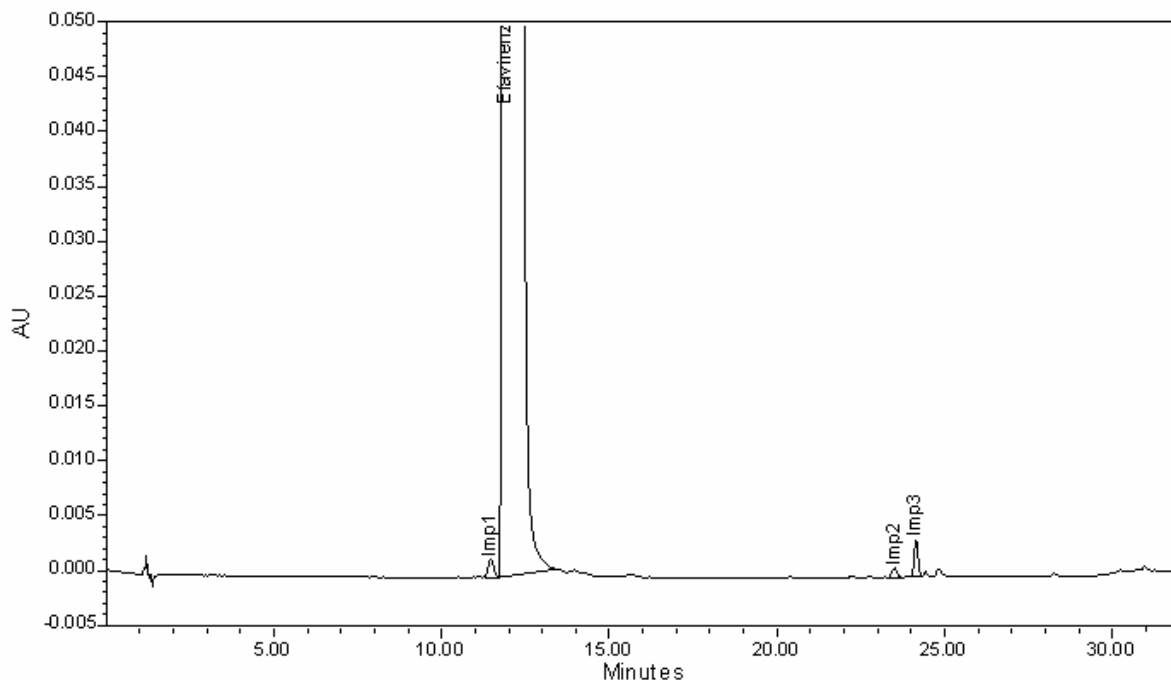


Figure 4. Chromatogram of efavirenz Control No 104229 monitored at 250 nm.

The following conditions were used:

Eluent: A: 90 volumes of a 0.05% trifluoroacetic acid aqueous solution and 10 volumes of methanol  
 B: 10 volumes of a 0.05% trifluoroacetic acid aqueous solution and 90 volumes of methanol

Eluent composition:	<u>% A</u>	<u>% B</u>	<u>Time, minutes</u>	<u>Type</u>
	60	40	0-16	isocratic
	60→50	40→50	16-23	linear
	50→35	50→65	23-28	linear
	35→30	65→70	28-29	linear
	30→20	70→80	29-31	linear
	20	80	31-32	isocratic
	60	40	32-40	re-equilibration

Data collecting time: 32 minutes

Column: Zorbax SB-CN 150x4.6mm 3.5µm

Column temperature: 40 °C

Detector, wavelength: Spectrophotometer, 250 nm

Flow rate:	1.5 ml/min
Injector temperature:	8 °C
Sample preparation:	Dissolve efavirenz in acetonitrile: water 1:1 to a concentration of 1.0 mg/ml. Inject 35 µl (corresponding to 35 µg).
Stability of the sample solution:	The sample is not stable in the eluent. Inject the sample solution immediately after preparation.
Limit of detection:	2 ng (0.005 %) at 250 nm
Limit of quantification:	5 ng (0.015 %) at 250 nm

#### Differential scanning calorimetry

The purity was estimated to 99.3 mol% (n=6, RSD=0.04%) and the melting temperature (T<sub>m</sub>) to 137.7 °C (n=6, RSD=0.05%). The determination was performed on about 2.5 mg using Mettler Toledo DSC 822e with a heating rate of 2 °C per minute.

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

Identification IR:	Conforms.
Assay:	99.5 %
Melting point:	130-136 °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**

Efavirenz, Control No 104229, can be considered suitable as International Chemical Reference Substance for the intended purpose. When used in the spectrophotometric assay according to monograph in *The International Pharmacopoeia*, draft for discussion, working document QAS/04.091 the content of efavirenz is taken to be 100 % calculated with reference to the dried substance which corresponds to 99.8 % on the "as is basis".

APPENDIX 12

**NEVIRAPINE**

Control No 104227

Analytical Report

**Intended use**

The International Chemical Reference Substance for nevirapine is intended to be used in the infrared absorption spectrophotometric test for identity, the liquid chromatographic test for related substances and in the liquid chromatographic assay according to the monograph in *The International Pharmacopoeia*, draft for discussion, working document QAS/03.082/Rev.1.

**Material**

About 200 g of the sample (manufacturers batch no 401369) were received at the WHO Centre in October 2004. The material is being stored in tightly closed containers at + 5 °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 W104227T)

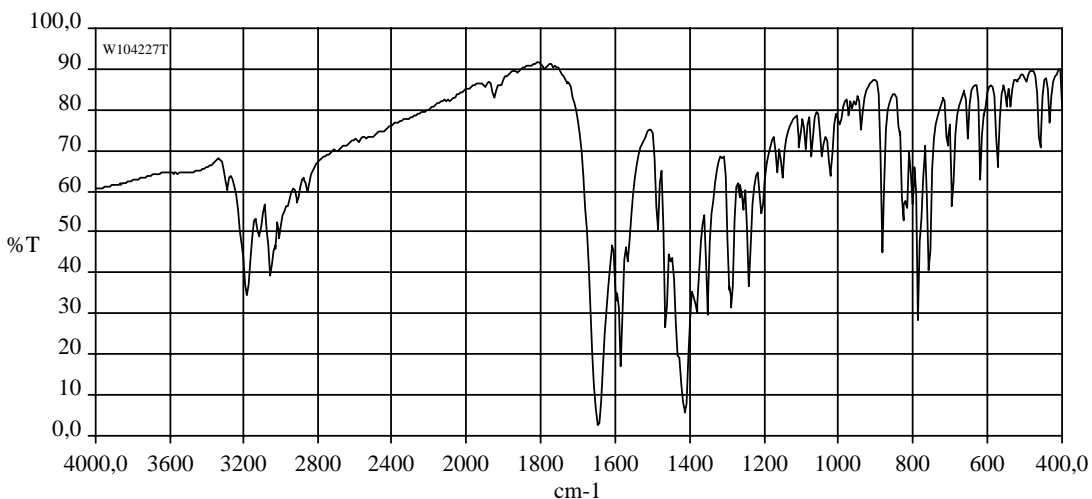


Figure 1. IR-spectrum of 0.8 mg of nevirapine Control No 104227 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

High performance liquid chromatography with mass-spectrometric detection

A spectrum of the main peak was recorded by electrospray (ESI) in the positive ion mode. The spectrum shows an  $[M+H]^+$  ion of  $m/z$  267, which supports the identity of nevirapine. The spectrum is given in Figure 2.

Eluent:	Acetonitrile:Water 1:1
Column:	Direct inlet
Pump:	Waters ZQ syringe pump operated at 10 $\mu$ l/min.
Detector:	Waters ZQ (single quadrupole mass spectrometer)
Operating conditions:	Cone voltage 70 V Source temperature 70 °C
Sample:	Nevirapine was dissolved in the eluent at a concentration of 2.76 $\mu$ g/ml.

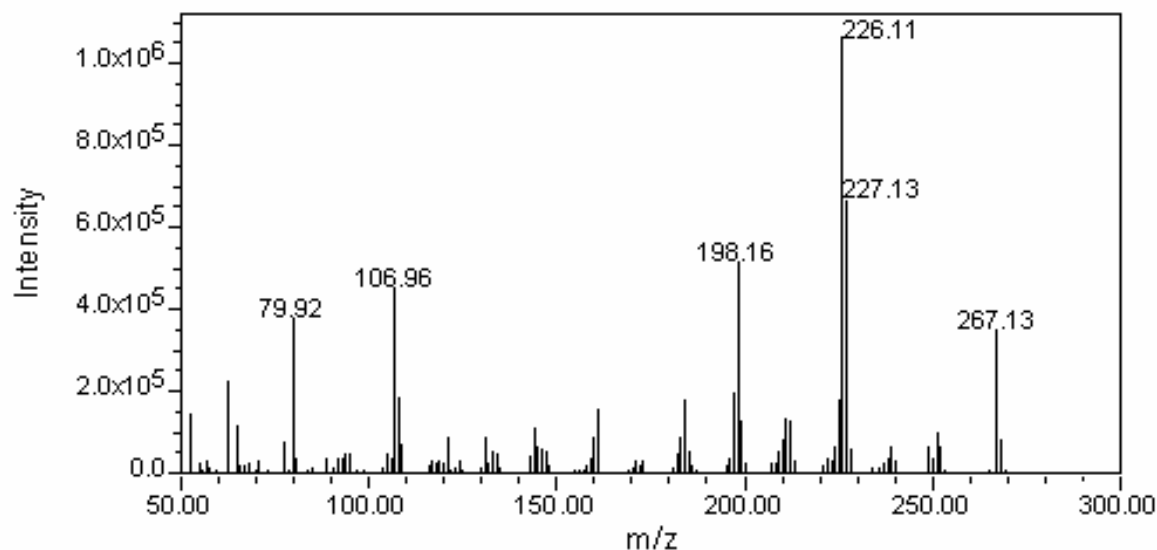


Figure 2. Positive-ion electrospray mass spectrum of nevirapine Control No 104227.

#### Melting point

A melting point determination was performed on a Mettler FP 81 according to *The International Pharmacopoeia*, Third Edition, Volume 1.

Melting point 246.0° C (n=6, RSD=0.03 %), at a heating rate of 1 °C/minute.

#### Thin-layer chromatography

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

#### **Assay**

##### Loss on drying

Less than 0.1% when dried 105 °C to constant weight at ambient pressure.

##### Water

0.2% (n = 6) determined by Karl Fischer titration.

##### Residual solvents

< 0.1 %. The test included methanol, ethanol, acetone, acetonitrile, dichloromethane, pyridine, chloroform, benzene, trichloroethylene and dioxan. Each of them except dichloromethane was estimated to be < 100 ppm. Dichloromethane was estimated to be 135 ppm.

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

Instrument:	Hewlett Packard 6890
Column:	HP-5 (30 m x 0.53 mm, 2.65 µm film)
Carrier gas:	Helium (5 ml/min)
Split:	1:5
Detector:	FID

Detector temperature: 250 °C  
Temperature program: 50 °C for 5 minutes, 10 °C/min and holding at 150 °C for 5 minutes, 50 °C/min and holding at 250 °C for 8 minutes.  
Injection: Head-space HP 7694  
Injector temperature: 120 °C  
Oven temperature: 80 °C  
Vial equilibration: 30 minutes-none shaking  
Loop volume: 1.0 ml gas phase  
Sample preparation: Blank solution: dimethyl sulfoxide.  
Reference solution: 1.0 ml of each of the solvents tested was diluted to 100.0 ml of dimethyl sulfoxide. 1.0 ml of this was diluted to 100.0 ml with dimethyl sulfoxide. 1.0 ml was further diluted to 100.0 ml with dimethyl sulfoxide.  
Sample: 50.0 mg were mixed with 5.0 ml of blank solution in a head-space vial which was then capped.  
Spiked sample: 50.0 mg were mixed with 5.0 ml of reference solution in a head-space vial which was then capped.  
Reference: 5.0 ml of reference solution in a capped head-space vial.  
Blank: 5.0 ml of blank solution in a capped head-space vial.

## Purity

### Thin-layer chromatography

One secondary spot were detected. The amount was estimated to be <0.1%. The following thin-layer chromatographic system was used.

Thin-layer: Silica gel 60 F-254 (Merck) TLC and HPTLC  
Eluent: methylenechloride : acetonitrile : ethylacetate (5 : 3 : 2)  
Sample: 203.4 and 202.8 µg of nevirapine dissolved in methanol were applied.  
Visualization: Scanning at 289 nm with a CAMAG TLC Scanner 3 was performed as well as visualization in day-light.

$$R_f(\text{nevirapine}) = 0.43$$

$$R_f(\text{imp A}) = 0.61$$

The detection limit of the system was about 0.14µg (0.03%), when scanning at 289 nm.

The spot of the impurity corresponds in position and appearance with that of impurity A (see QAS/03.082/Rev.1) lot BIRH 0414 BS, Boehringer Ingelheim.

### High performance liquid chromatography

The purity was estimated by peak area normalization to about 99.9 % (n=6, RSD=0.01 % for the main peak, RSD=4.2 % calculated on the 0.1 % impurity level).

A chromatogram is shown in Figure 3. Two impurities above the limit of quantification were found. One was identified as impurity B (5.4 minutes) and the other was identified as impurity A (10.7 minutes). The purity was estimated by external standard to 99.95 % (w/w).

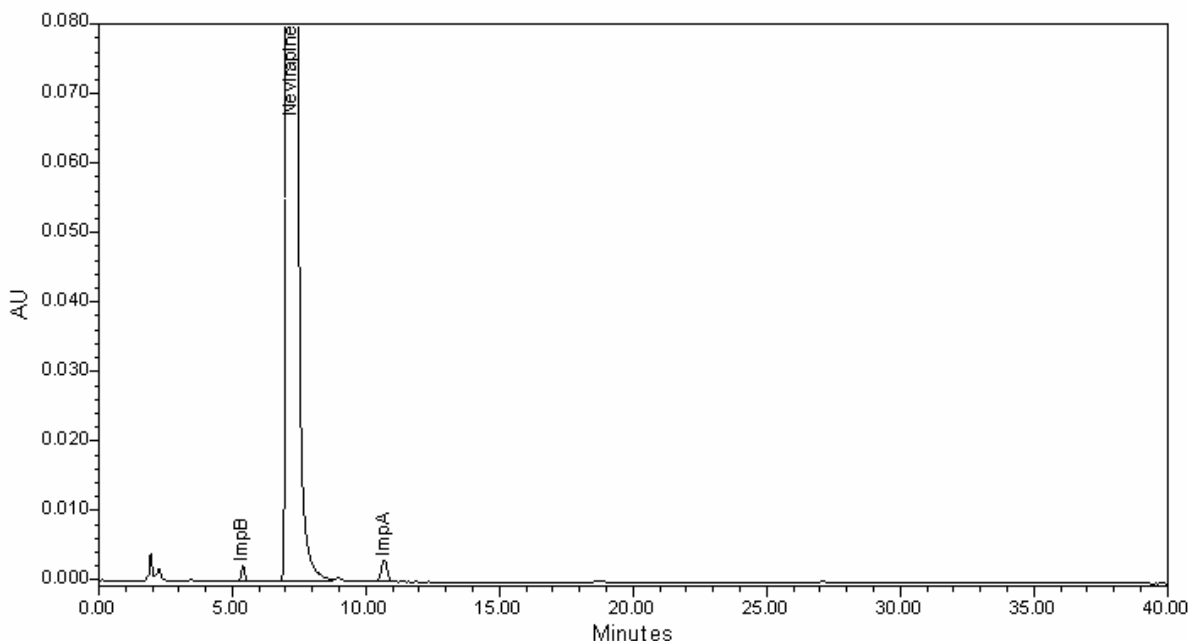


Figure 3. *Chromatogram of nevirapine Control No 104227 monitored at 220 nm.*

The following conditions were used:

Eluent:	A: Acetonitrile			
	B: 25 mM ammoniumdihydrogen phosphate buffer adjusted to pH 5.0 with ammonia ( $\approx 260\text{g/l}$ )			
Eluent composition:	<u>% A</u>	<u>% B</u>	<u>Time, minutes</u>	<u>Type</u>
	20	80	0-30	isocratic
Data collecting time:	30 minutes			
Column:	Discovery RP Amide C16 150x4.6mm 5 $\mu\text{m}^*$			
Column temperature:	35 °C			
Detector, wavelength:	Spectrophotometer, 220 nm			
Flow rate:	1.0 ml/min			
Injector temperature:	8 °C			
Sample preparation:	Dissolve Nevirapine in the eluent to a concentration of 0.24 mg/ml. Inject 50 $\mu\text{l}$ (corresponding to 12 $\mu\text{g}$ ).			

Stability of the sample solution: The sample is not stable in the eluent. Inject the sample solution immediately after preparation.

Limit of detection: 0.5 ng (0.004%) at 220 nm

Limit of quantification: 1.5 ng (0.01%) at 220 nm

\*hexadecylamidylsilyl silicagel

#### Differential scanning calorimetry

The purity was estimated to 100.0 mol% (n=6, RSD=0.01%) and the melting temperature ( $T_m$ ) to 244.8 °C (n=6, RSD=0.03%). The determination was performed on 2 mg using Mettler Toledo DSC 822<sup>e</sup> with a heating rate of 2 °C per minute.

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

Identification IR: Conforms.

Assay: 99.8 %

Melting point: 243.0 °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**

Nevirapine, Control No 104227, can be considered suitable as International Chemical Reference Substance for the intended purpose. When used in the spectrophotometric assay according to *The International Pharmacopoeia*, draft for discussion, working document QAS/03.082/Rev.1., the content of Nevirapine is taken to be 100 % calculated with reference to the dried substance which corresponds to 99.8 % on the "as is basis".

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