



## WHO COLLABORATING CENTRE FOR CHEMICAL REFERENCE SUBSTANCES

### Report on the work in 2007

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 **Ms E. Kagebeck**, WHO Collaborating Centre for Chemical Reference Substances, Apoteket AB, Produktion & Laboratorier, Farmaci/Centrallaboratorium (ACL), Prismavägen 2, SE-141 75 Kungens Kurva, Sweden, along with a copy to be sent to **Dr S. Kopp**, Quality Assurance Programme, 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 2007

During 2007 the total number of International Chemical Reference Substances distributed from the Centre was 2332. The most frequently requested substances are given in Appendix 1. The six most frequently requested substances were in order of demand: Artesunate, Prednisolone, Artemether, Artemisinin, Efavirenz and Phenacetin M.P.

Details of distribution to the WHO Regions are given in Appendix 2. It is observed that 21.7% of the substances went to the African Region, 2.7% to the Region of the Americas, 1.9% to the Eastern Mediterranean Region, 37.9% to the European Region, 7.8% to South-East Asia and 28.0 % to the Western Pacific Region.

Distribution of reference spectra in 2007

No reference spectra were distributed during 2007.

Establishment of reference substances in 2007

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), seven International Chemical Reference Substances were established in 2007. The substances are listed in Appendix 3.

A complete list of all International Chemical Reference Substances available from the Centre in January 2008, 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 2007

During 2007 work on five new International Chemical Reference Substances was performed. The new reference substances are Abacavir sulfate for system suitability, Amoxicillin trihydrate, Lamivudine for system suitability, Norethisterone enantate and Zidovudine impurity B. The analytical reports are given in Appendices 7-9, 11 and 13.

Work on new batches of Levotyroxine sodium with Control No 207144, and Paracetamole with Control No 207198 was performed as the former batches were almost depleted. The analytical reports are given in Appendices 10 and 12.

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

### Re-examination

The regular stability monitoring of existing International Chemical Reference Substances was continued. This year 55 substances were re-examined. The results are given in Appendix 6.

### Work in progress and future work

The fourth edition of *The International Pharmacopoeia* was published at the end of 2006. Work is continuously performed on the substances required to support the monographs in the Pharmacopoeia. In addition, during 2007 work was initiated to publish Infrared spectra on the WHO website. During 2008 chromatograms from the related substances tests will be published on the WHO website.

### Administrative and financial matters

The total cost for running the Centre in 2007 was estimated to US\$ 720 500. The income from sales of reference substances was US\$ 163 200 and the contribution received from the WHO headquarters was US\$ 16 000. The deficit of US\$ 541 300 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 2007.

### Acknowledgements

The Centre is grateful to the laboratories that have contributed to its work during 2007. This year we would like to address our thanks in particular to the Centre for Analytical Science, Health Sciences Authority, Singapore and GlaxoSmithKline, Durham, Great Britain.

APPENDIX 1

**DISTRIBUTION OF INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES  
IN 2007**

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

| <b>ICRS</b>               | <b>Items sold</b> |
|---------------------------|-------------------|
| Artesunate                | 156               |
| Prednisolone              | 113               |
| Artemether                | 80                |
| Artemisinin               | 63                |
| Efavirenz                 | 60                |
| Phenacetin M.P.           | 60                |
| Nevirapine anhydrous      | 56                |
| Folic acid                | 53                |
| Caffeine M.P.             | 51                |
| Vanillin M.P.             | 49                |
| Lamivudine                | 47                |
| Stavudine                 | 43                |
| Abacavir sulfate          | 42                |
| Nelfinavir mesilate       | 42                |
| Digoxin                   | 40                |
| Ritonavir                 | 36                |
| Zidovudine                | 35                |
| Artemimol                 | 34                |
| Indinavir                 | 34                |
| Phenolphthalein M.P.      | 33                |
| Saquinavir mesilate       | 32                |
| Azobenzene M.P.           | 31                |
| Amodiaquine hydrochloride | 28                |
| Didanosine                | 27                |
| Ethambutol hydrochloride  | 27                |

APPENDIX 2

**DISTRIBUTION OF INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES  
TO DIFFERENT WHO REGIONS IN 2007**

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| <b>WHO Regions</b>                  | <b>Number of distributed ICRS</b> |
|-------------------------------------|-----------------------------------|
| African Region (AFRO)               | 505                               |
| Region of the Americas (AMRO)       | 63                                |
| Eastern Mediterranean Region (EMRO) | 45                                |
| European Region (EURO)              | 884                               |
| South-East Asia Region (SEARO)      | 181                               |
| Western Pacific Region (WPRO)       | 654                               |

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APPENDIX 3

**INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES ESTABLISHED IN 2007**

| <b>Reference substance</b>                | <b>Control number</b> | <b>Analytical report</b>      |
|---|-----------------------|-------------------------------|
| Abacavir sulfate                          | 106238                | PSM/QSM/2007.6<br>Appendix 7  |
| Anhydrotetracycline<br>hydrochloride      | 206096                | PSM/QSM/2007.6<br>Appendix 8  |
| 4-epianhydrotetracycline<br>hydrochloride | 306097                | PSM/QSM/2007.6<br>Appendix 9  |
| 4-epitetracycline hydrochloride           | 306098                | PSM/QSM/2007.6<br>Appendix 10 |
| Medroxyprogesterone acetate               | 106241                | PSM/QSM/2007.6<br>Appendix 11 |
| Nevirapine impurity B                     | 106239                | PSM/QSM/2007.6<br>Appendix 12 |
| Pyrazinamide                              | 106240                | PSM/QSM/2007.6<br>Appendix 13 |

## APPENDIX 4

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

**2008**

#### **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)

Web site: <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**

**We prefer payment by swift**

*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.

|         |   |        |        |
|---------|---|--------|--------|
| 9931422 | Abacavir sulfate                            | 100 mg | 106238 |
| 9931552 | Abacavir sulfate for system suitability     | 10 mg  | 107244 |
| 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 |
| 9931426 | Amoxicillin trihydrate                      | 100 mg | 106242 |
| 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  | 206096 |
| 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 |

|         |   |        |        |
|---------|---|--------|--------|
| 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 |
| 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 |
| 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 |
| 9930294 | Emetine hydrochloride                             | 100 mg | 187134 |
| 9931411 | Efavirenz   | 100 mg | 104229 |
| 9930197 | 4-Epianhydrotetracycline hydrochloride            | 25 mg  | 306097 |
| 9930198 | 4-Epitetracycline hydrochloride                   | 25 mg  | 306098 |
| 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                                    | 25 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 |
| 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<br>(3- <i>o</i> -Methylcarbidopa) | 25 mg  | 193180 |
| 9930189 | (-)-3-(4-Hydroxy-3-methoxyphenyl)-2-methylalanine<br>(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 |
| 9931553 | Lamivudine for system suitability   | 10 mg  | 107246 |
| 9930333 | Lanatoside C  | 100 mg | 281022 |
| 9930334 | Levodopa  | 100 mg | 295065 |
| 9930335 | Levonorgestrel  | 200 mg | 194182 |
| 9930336 | Levothyroxine sodium  | 50 mg  | 207144 |
| 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 |
| 9930454 | Medroxyprogesterone acetate   | 100 mg | 106241 |

## 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) | 1 g | 192173 |

|         |   |        |        |
|---------|---|--------|--------|
| 9930422 | Sulfanilamide (166 °C)  | 1 g    | 192162 |
| 9930423 | Sulfapyridine (193 °C)  | 1 g    | 192163 |
| 9930286 | Dicyanodiamide (210 °C)   | 1 g    | 192164 |
| 9930411 | Saccharin (229 °C)  | 1 g    | 202165 |
| 9930235 | Caffeine (237 °C)   | 1 g    | 299166 |
| 9930382 | Phenolphthalein (263 °C)  | 1 g    | 299167 |
| 9930345 | Methotrexate  | 100 mg | 194193 |
|         | 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            |        |        |
| 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 |
| 9931417 | Nelfinavir mesilate   | 100 mg | 105233 |
|         | Neomycin B sulfate <i>see</i> Framycetin sulfate  |        |        |
| 9930356 | Neostigmine metilsulfate  | 100 mg | 187135 |
| 9931412 | Nevirapine anhydrous  | 100 mg | 104227 |
| 9931423 | Nevirapine impurity B   | 10 mg  | 106239 |
| 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 |
| 9972123 | Norethisterone enantate   | 50 mg  | 107243 |
| 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 |
| 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 |
| 9931424 | Pyrazinamide                                     | 100 mg | 106240 |
| 9930405 | Pyridostigmine bromide                           | 100 mg | 182110 |
| 9930406 | Reserpine  | 100 mg | 186133 |
| 9930408 | Riboflavin                                       | 250 mg | 382035 |
| 9930409 | Rifampicin                                       | 300 mg | 203151 |
| 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 |
| 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 |
| 9930439 | Warfarin   | 100 mg | 168041 |
| 9931420 | Zidovudine                                       | 100 mg | 105236 |
| 9931554 | Zidovudine impurity B                            | 10 mg  | 107247 |
| 9930260 | Zuclomifene                                      | 50 mg  | 187137 |

APPENDIX 5

**LIST OF AVAILABLE INTERNATIONAL INFRARED REFERENCE SPECTRA**

**2008**

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)

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

APPENDIX 6

**RE-EXAMINATION – 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 2007 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.

Acetanilide, Melting Point Reference Substance, Control No 297171

Initial analytical report: WHO/PHARM/97.595, Appendix 9

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| Examination year:           | 1997     | 2003     | 2007     |
|-----------------------------|----------|----------|----------|
| IR                          | conforms | conforms | conforms |
| Capillary melting point, °C | 115.7*   | 115.9    | 115.5    |
| DSC, mol%                   | 0.03     | 0.04     | 0.04     |

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\* Collaborative trial

Amitriptyline hydrochloride, Control No 181101

Initial analytical report: WHO/PHARM/82.509, Appendix 6

| Examination year:     | 1982     | 1985     | 1993  | 2007     |
|-----------------------|----------|----------|-------|----------|
| IR                    | conforms | conforms | -     | conforms |
| TLC, %                | < 0.1    | < 0.1    | -     | -        |
| HPLC, %               | < 0.1    | < 0.1    | < 0.1 | < 0.1    |
| LOD, %                | 0.1      | 0.1      | -     | < 0.1    |
| Water (KF), %         | -        | 0.1      | -     | -        |
| Assay, titrimetric, % | 100.3    | 100.3    | -     | -        |
| PSA, %                | 0.3      | -        | -     | -        |

Amodiaquine hydrochloride, Control No 192160

Initial analytical report: WHO/PHARM/93.564, Appendix 7

| Examination year:      | 1992         | 1997 | 2003 | 2007     |
|------------------------|--------------|------|------|----------|
| IR                     | conforms     | -    | -    | conforms |
| TLC, %                 | 0.6          | -    | -    | -        |
| HPLC, %                | 0.3          | 0.4  | 0.2  | 0.4      |
| TGA, %                 | 7.9          | 7.8  | -    | 7.8      |
| Water (KF), %          | 8.0          | -    | 7.8  | -        |
| UV ( $A_{1cm}^{1\%}$ ) | 410 (342 nm) | -    | -    | -        |

Azathioprine, Control No 172060

Initial analytical report: WHO/PHARM/72.471, Appendix 11

| Examination year: | 1972     | 1979         | 1981         | 1997     | 2003 | 2007     |
|-------------------|----------|--------------|--------------|----------|------|----------|
| IR                | conforms | -            | -            | conforms | -    | conforms |
| TLC, %            | 0.5      | one impurity | one impurity | -        | -    | -        |
| HPLC, %           | -        | -            | -            | 0.5      | 0.7  | 0.7      |
| TGA, %            | -        | -            | -            | 0.6      | -    | -        |
| LOD, %            | 0.7      | 0.7          | -            | -        | 0.7  | 0.6      |

Azobenzene, Melting Point Reference Substance, Control No 192168

Initial analytical report: WHO/PHARM/92.558, Appendix 5

| Examination year:           | 1963   | 1978 | 1991 | 1998 | 2003     | 2007     |
|-----------------------------|--------|------|------|------|----------|----------|
| IR                          | -      | -    | -    | -    | conforms | conforms |
| Capillary melting point, °C | 68.8 * | 69   | 68.7 | 68.9 | 68.9     | -        |
| DSC, mol%                   | -      | -    | 0.1  | 0.1  | 0.1      | 0.1      |

\* Collaborative trial

Benzanilide, Melting Point Reference Substance, Control No 192173

Initial analytical report: WHO/PHARM/92.558, Appendix 5

| Examination year:           | 1963    | 1978  | 1991  | 1998  | 2003     | 2007     |
|-----------------------------|---------|-------|-------|-------|----------|----------|
| IR                          | -       | -     | -     | -     | conforms | conforms |
| Capillary melting point, °C | 164.7 * | 165.0 | 164.5 | 165.0 | 164.9    | 164.9    |

\*Collaborative trial

Benzil, Melting Point Reference Substance, Control No 294170

Initial analytical report: WHO/PHARM/95.577, Appendix 9

| Examination year:           | 1994     | 2003 | 2007     |
|-----------------------------|----------|------|----------|
| IR                          | conforms | -    | conforms |
| Capillary melting point, °C | 95.9     | 95.6 | -        |
| DSC, mol%                   | 0.2      | 0.07 | 0.08     |

Bephenium hydroxynaphthoate, Control No 183112

Initial analytical report: WHO/PHARM/84.513, Appendix 16

| Examination year:      | 1983            | 1998  | 2003     | 2007     |
|------------------------|-----------------|-------|----------|----------|
| IR                     | conforms        | -     | conforms | conforms |
| TLC, %                 | two impurities  | -     | -        | -        |
| HPLC, %                | 0.4             | 0.3   | 0.5      | 0.5      |
| TGA, %                 | -               | < 0.1 | -        | 0.1      |
| LOD, %                 | < 0.1           | -     | < 0.1    | -        |
| Assay, titrimetric, %  | 99.4            | -     | -        | -        |
| UV ( $A_{1cm}^{1\%}$ ) | 140<br>(269 nm) | -     | -        | -        |

Caffeine, Control No 299166

Initial analytical report: WHO/EDM/QSM/2000.6, Appendix 8.

| Examination year:           | 1999     | 2007     |
|-----------------------------|----------|----------|
| IR                          | conforms | conforms |
| Capillary melting point, °C | 237.2*   | 237.4    |

\*Collaborative trial

Calcium folinate (Leucovorin calcium), Control No 194188

Initial analytical report: WHO/PHARM/95.577, Appendix 10.

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| Examination year:            | 1994     | 2007     |
|------------------------------|----------|----------|
| IR                           | conforms | conforms |
| HPLC, %                      | 0.2      | 0.1      |
| TGA, %                       | 13.5     | 13.2     |
| Water (KF), %                | 13.0     | -        |
| Specific optical rotation, ° | +17.8    | -        |

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Captopril, Control No 197214

Initial analytical report: WHO/PHARM/97.595, Appendix 10

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| Examination year:             | 1997            | 2003  | 2007     |
|-------------------------------|-----------------|-------|----------|
| IR                            | conforms        | -     | conforms |
| TLC, %                        | 0.7             | -     | -        |
| HPLC, %                       | 0.5             | 0.4   | 0.4      |
| DSC, mol%                     | 0.5             | -     | -        |
| Loss on drying, %             | 0.1             | < 0.1 | < 0.1    |
| UV ( $A_{1\text{cm}}^{1\%}$ ) | 259<br>(235 nm) | -     | -        |

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Captopril disulfide, Control No 198216

Initial analytical report: WHO/PHARM/98.603, Appendix 9

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| Examination year: | 1998     | 2003  | 2007     |
|-------------------|----------|-------|----------|
| IR                | conforms | -     | conforms |
| TLC, %            | < 0.1    | -     | -        |
| HPLC, %           | < 0.1    | < 0.1 | < 0.2    |
| TGA, %            | < 0.1    | < 0.1 | < 0.1    |

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5-Chloro-2-methylaminobenzophenone, Control No 172061

Initial analytical report: WHO/PHARM/73.475, Appendix 4

| Examination year: | 1972          | 1980          | 1985          | 1991          | 1997     | 2002  | 2007     |
|-------------------|---------------|---------------|---------------|---------------|----------|-------|----------|
| IR                | conforms      | -             | -             | -             | conforms | -     | conforms |
| TLC, %            | no impurities | no impurities | no impurities | no impurities | -        | -     | -        |
| HPLC, %           | -             | -             | -             | 0.8           | 1.0      | 1.0   | 1.0      |
| LOD, %            | 0.1           | -             | -             | -             | -        | < 0.1 | <0.1     |
| Water (KF), %     | -             | -             | -             | -             | 0.3      | -     | -        |
| DTA, mol%         | 0.4           | -             | 0.5           | -             | -        | -     | -        |
| DSC, mol%         | -             | -             | -             | 0.5           | -        | -     | -        |

2-(4-Chloro-3-sulfamoylbenzoyl)benzoic acid, Control No 181106

Initial analytical report: WHO/PHARM/82.509, Appendix 8

| Examination year: | 1981           | 1997  | 2003  | 2007     |
|-------------------|----------------|-------|-------|----------|
| IR                | conforms       | -     | -     | conforms |
| TLC, %            | two impurities | -     | -     | -        |
| HPLC, %           | 0.4            | 0.2   | 0.3   | 0.4      |
| TGA, %            | -              | < 0.1 | -     | 0.1      |
| LOD, %            | 0.2            | -     | < 0.1 | -        |

Chlortalidone, Control No 183114

Initial analytical report: WHO/PHARM/84.513, Appendix 9

| Examination year:      | 1983      | 1997  | 2003 | 2007     |
|------------------------|-----------|-------|------|----------|
| IR                     | conforms  | -     | -    | conforms |
| TLC, %                 | 0.3       | -     | -    | -        |
| HPLC, %                | 0.5       | 0.9   | 1.0  | 0.8      |
| TGA, %                 | -         | < 0.1 | -    | 0.1      |
| LOD, %                 | 0.1       | -     | 0.1  | -        |
| UV ( $A_{1cm}^{1\%}$ ) | 55(284nm) | -     | -    | -        |
| Assay, titrimetric, %  | 99.5      | -     | -    | -        |

Clomifene citrate, Control No 187136

Initial analytical report: WHO/PHARM/88.537, Appendix 8

| Examination year:   | 1987                 | 1997            | 2002            | 2007            |
|---------------------|----------------------|-----------------|-----------------|-----------------|
| IR                  | conforms             | -               | conforms        | conforms        |
| TLC, %              | no impurities        | -               | -               | -               |
| HPLC, %             | 0.3                  | 0.9             | 1.6             | 1.2             |
| Z-isomer by HPLC, % | about 35<br>(area %) | 32.6<br>(w/w %) | 32.4<br>(w/w %) | 31.8<br>(w/w %) |
| Z-isomer by NMR, %  | 33                   | -               | -               | -               |
| TGA, %              | 0.3                  | -               | -               | -               |
| Water (KF), %       | -                    | 0.7             | -               | 0.4             |
| LOD, %              | -                    | -               | 1.2             | -               |

Clomifene citrate Z-isomer, Control No 187137

Initial analytical report: WHO/PHARM/88.537, Appendix 9

| Examination year:   | 1987             | 1997        | 2002         | 2007         |
|---------------------|------------------|-------------|--------------|--------------|
| IR                  | conforms         | -           | conforms     | Conforms     |
| TLC, %              | < 0.1            | -           | -            | -            |
| HPLC, %             | 0.3              | 0.3         | 0.6          | 0.5          |
| E-isomer by HPLC, % | about 2 (area %) | 2.1 (w/w %) | 2.1 (area %) | 2.1 (area %) |
| TGA, %              | 0.2              | -           | -            | -            |
| Water (KF), %       | -                | 0.2         | 0.1          | 0.1          |

Dapsone, Control No 183115

Initial analytical report: WHO/PHARM/84.513, Appendix 10

| Examination year:             | 1983                                      | 1988  | 1997 | 2003     | 2007     |
|-------------------------------|---|-------|------|----------|----------|
| IR                            | conforms                                  | -     | -    | conforms | conforms |
| TLC, %                        | 1.0                                       | 1.0   | -    | -        | -        |
| HPLC, %                       | 1.2                                       | 1.1   | 0.7  | 0.7      | 0.6      |
| TGA, %                        | -   | < 0.1 | 0.1  | -        | 0.1      |
| LOD, %                        | 0.1                                       | -     | -    | < 0.1    | -        |
| UV ( $A_{1\text{cm}}^{1\%}$ ) | 733<br>(265 nm),<br>1191<br>(295<br>nm)** | -     | -    | -        | -        |
| Assay, titrimetric, %         | 99.9                                      | 100.0 | -    | -        | -        |

Desoxycortone acetate, Control No 167007

Initial analytical report: WHO/PHARM/66.431, Appendix 1

| Examination year:          | 1965            | 1975          | 1980 | 1984           | 1997  | 2002     | 2007     |
|----------------------------|-----------------|---------------|------|----------------|-------|----------|----------|
| IR                         | conforms        | -             | -    | -              | -     | conforms | conforms |
| TLC, %                     | no impurities   | no impurities | -    | two impurities | -     | -        | -        |
| HPLC, %                    | -               | -             | -    | 0.2            | 0.7   | 0.9      | 0.7      |
| TGA, %                     | -               | -             | -    | -              | < 0.1 | -        | 0.1      |
| LOD, %                     | < 0.1           | 0.1           | -    | < 0.1          | -     | < 0.1    | -        |
| DTA, mol%                  | -               | -             | 0.7  | 0.7            | -     | -        | -        |
| PSA, %                     | < 1             | -             | -    | -              | -     | -        | -        |
| UV, $A_{1\text{cm}}^{1\%}$ | 456<br>(242 nm) | 452           | -    | 455            | -     | -        | -        |

Diazoxide, Control No 181103

Initial analytical report: WHO/PHARM/82.509, Appendix 9

| Examination year: | 1981     | 1998     | 2003  | 2007     |
|-------------------|----------|----------|-------|----------|
| IR                | conforms | conforms | -     | conforms |
| HPLC, %           | 0.5      | 0.5      | 0.3   | 0.4*     |
| TGA, %            | -        | < 0.1    | -     | < 0.1    |
| LOD, %            | < 0.1    | -        | < 0.1 | -        |

\* The re-examination in 2007 has been partially performed by the Centre for Analytical Science, Health Sciences Authority, Singapore.

Dicoumarol, Control No 178077

Initial analytical report: WHO/PHARM/79.499, Appendix 5

| Examination year:    | 1978          | 1997  | 2002     | 2007     |
|----------------------|---------------|-------|----------|----------|
| IR                   | conforms      | -     | conforms | conforms |
| TLC, %               | no impurities | -     | -        | -        |
| HPLC, %              | < 0.05        | < 0.1 | < 0.02   | < 0.07   |
| TGA, %               | -             | < 0.1 | -        | < 0.1    |
| LOD, %               | < 0.1         | -     | < 0.1    | -        |
| Assay, titrimetric % | 100.0         | -     | -        | -        |

Dicyanodiamide, Melting Point Reference Substance, Control No 192164

Initial analytical report: WHO/PHARM/92.558, Appendix 5

| Examination year:           | 1963    | 1978 | 1991  | 1998  | 2003     | 2007     |
|-----------------------------|---------|------|-------|-------|----------|----------|
| IR                          | -       | -    | -     | -     | conforms | conforms |
| Capillary melting point, °C | 210.2 * | -    | 209.9 | 210.0 | 210.3    | 210.5    |
| DSC, mol%                   | -       | -    | 0.1   | 0.06  | 0.1      | 0.1      |

\* Collaborative trial

Didanosine, Control No 104228

Initial analytical report: PSM/QSM/2005.1. Appendix 9

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| Examination year:            | 2004         | 2007     |
|------------------------------|--------------|----------|
| IR                           | conforms     | conforms |
| TLC, %                       | < 0.3        | -        |
| HPLC, %                      | 0.2          | 0.1      |
| TGA, %                       | 0.2          | 0.4      |
| LOD, %                       | 0.4          | -        |
| Water (KF), %                | 0.4          | -        |
| UV( $A_{1\text{cm}}^{1\%}$ ) | 487 (250 nm) | -        |
| Residual solvents (GC), %    | < 0.1        | -        |

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Didanosine for system suitability, Control No 104230

Initial analytical report: PSM/QSM/2005.1, Appendix 10

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| Examination year:  | 2004        | 2007        |
|--|-------------|-------------|
| HPLC, relative retention with<br>reference to didanosine | Imp. A=0.27 | Imp. A=0.28 |
|  | Imp. B=0.40 | Imp. B=0.40 |
|  | Imp. C=0.46 | Imp. C=0.46 |
|  | Imp. D=0.50 | Imp. D=0.50 |
|  | Imp. E=0.53 | Imp. E=0.53 |
|  | Imp. F=0.78 | Imp. F=0.78 |
| HPLC, resolution (imp. C - imp. D)                       | 2.2         | 2.2         |

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Dopamine hydrochloride, Control No 192159

Initial analytical report: WHO/PHARM/93.564, Appendix 12

| Examination year:            | 1992            | 1998  | 2003  | 2007     |
|------------------------------|-----------------|-------|-------|----------|
| IR                           | conforms        | -     | -     | conforms |
| TLC, %                       | no impurities   | -     | -     | -        |
| HPLC, %                      | < 0.05          | 0.1   | 0.1   | 0.1      |
| TGA, %                       | < 0.1           | < 0.1 | -     | -        |
| LOD, %                       | -               | -     | < 0.1 | <0.1     |
| UV ( $A_{1cm}^{1\%}$ )       | 144<br>(280 nm) | -     | -     | -        |
| Assay, spectrophotometric, % | 100.0           | -     | -     | -        |

Efavirenz, Control No 104229

Initial analytical report: PSM/QSM/2005.1, Appendix 11

| Examination year:         | 2004     | 2007     |
|---------------------------|----------|----------|
| IR                        | conforms | conforms |
| TLC, %                    | < 0.3    | -        |
| HPLC, %                   | 0.1      | 0.1      |
| LOD, %                    | -        | < 0.1    |
| Water (KF), %             | < 0.1    | -        |
| Residual solvents (GC), % | < 0.1    | -        |

Emetine hydrochloride, Control No 187134

Initial analytical report: WHO/PHARM/88.537, Appendix 11

| Examination year:        | 1987     | 1988 | 1990 | 1999     | 2007     |
|--------------------------|----------|------|------|----------|----------|
| IR                       | conforms | -    | -    | conforms | conforms |
| TLC, %                   | 0.2      | -    | -    | -        | -        |
| HPLC, %                  | 0.4      | -    | -    | 0.3      | 0.6      |
| TGA, %                   | 16.8     | 16.2 | 16.1 | 16.5     | -        |
| LOD, %                   | -        | -    | -    | -        | 17.0     |
| Assay, potentiometric, % | 99.8     | -    | -    | -        | -        |

Ergocalciferol, Control No 190147

Initial analytical report: WHO/PHARM/91.555, Appendix 11

| Examination year:             | 1990         | 1995         | 2003     | 2007     |
|-------------------------------|--------------|--------------|----------|----------|
| IR                            | conforms     | conforms     | conforms | conforms |
| TLC, %                        | 0.3          | 0.1          | -        | -        |
| HPLC, %                       | 0.9          | 0.9          | 0.9      | 0.9      |
| TGA, %                        | < 0.1        | < 0.1        | < 0.1    | < 0.1    |
| Assay, spectrophotometric, %  | 100.2        | 100.1        | -        | -        |
| UV ( $A_{1\text{cm}}^{1\%}$ ) | 475 (265 nm) | 476 (265 nm) | -        | -        |

Ethisterone, Control No 167017

Initial analytical report: WHO/PHARM/66.431, Appendix 2

| Examination year:            | 1967             | 1975           | 1984             | 1996  | 2002     | 2007     |
|------------------------------|------------------|----------------|------------------|-------|----------|----------|
| IR                           | conforms         | -              | conforms         | -     | conforms | conforms |
| TLC, %                       | three impurities | two impurities | three impurities | -     | -        | -        |
| HPLC, %                      | -                | -              | 0.5              | 0.5   | 0.5      | 0.7      |
| TGA, %                       | -                | -              | -                | < 0.1 | -        | < 0.1    |
| LOD, %                       | < 0.1            | 0.3            | -                | -     | < 0.1    | -        |
| Assay, titrimetric, %        | 100.6            | -              | -                | -     | -        | -        |
| Assay, spectrophotometric, % | -                | -              | -                | 100.0 | -        | -        |
| PSA, %                       | 0.5              | -              | -                | -     | -        | -        |

Ethosuximide, Control No 179088

Initial analytical report: WHO/PHARM/80.504, Appendix 7

| Examination year:     | 1979          | 1998     | 2003  | 2007     |
|-----------------------|---------------|----------|-------|----------|
| IR                    | conforms      | conforms | -     | conforms |
| TLC                   | no impurities | -        | -     | -        |
| HPLC, %               | -             | 0.1      | 0.1   | 0.1*     |
| Water (KF), %         | < 0.1         | < 0.1    | < 0.1 | < 0.1    |
| Assay, titrimetric, % | 99.5          | -        | -     | -        |

\* The re-examination in 2007 has been partially performed by the Centre for Analytical Science, Health Sciences Authority, Singapore.

Furosemide, Control No 171044

Initial analytical report: WHO/PHARM/71.464, Appendix 5

| Examination year:   | 1971            | 1976            | 1985        | 1992 | 1998 | 2003     | 2007     |
|---------------------|-----------------|-----------------|-------------|------|------|----------|----------|
| IR                  | conforms        | -               | conforms    | -    | -    | conforms | conforms |
| TLC, %              | no impurity     | one impurity    | no impurity | -    | -    | -        | -        |
| HPLC, %             | -               | -               | -           | 0.03 | 0.1  | 0.07     | 0.03     |
| TGA, %              | -               | -               | -           | 0.1  | 0.1  | -        | -        |
| LOD, %              | 0.1             | < 0.1           | 0.2         | -    | -    | < 0.1    | 0.2      |
| UV, $A_{1cm}^{1\%}$ | 110<br>(271 nm) | 108<br>(271 nm) | -           | -    | -    | -        | -        |

Ibuprofen, Control No 183117

Initial analytical report: WHO/PHARM/84.513, Appendix 12

| Examination year:        | 1983     | 1993 | 1998 | 2003  | 2007     |
|--------------------------|----------|------|------|-------|----------|
| IR                       | conforms | -    | -    | -     | conforms |
| TLC, %                   | 0.7      | -    | -    | -     | -        |
| HPLC, %                  | 0.7      | 0.6  | 0.6  | 0.6   | 0.6      |
| Water (KF), %            | -        | -    | 0.15 | < 0.1 | -        |
| LOD, %                   | < 0.1    | -    | -    | -     | < 0.1    |
| Assay, potentiometric, % | 99.5     | -    | -    | -     | -        |

Levodopa, Control No 295065

Initial analytical report: WHO/PHARM/96.584, Appendix 13

| Examination year:             | 1995         | 2002  | 2007     |
|-------------------------------|--------------|-------|----------|
| IR                            | conforms     | -     | conforms |
| TLC, %                        | < 0.5        | -     | -        |
| HPLC, %                       | < 0.1        | < 0.1 | < 0.1    |
| TGA, %                        | < 0.1        | -     | -        |
| LOD, %                        | -            | < 0.1 | < 0.1    |
| UV ( $A_{1\text{cm}}^{1\%}$ ) | 141 (280 nm) | -     | -        |

Meticillin sodium, Control No 274024

Initial analytical report: WHO/PHARM/74.478, Appendix 9

| Examination year:       | 1974     | 1978  | 1984 | 1997 | 2003     | 2007     |
|-------------------------|----------|-------|------|------|----------|----------|
| IR                      | conforms | -     | -    | -    | conforms | conforms |
| HPLC, %                 | -        | < 0.1 | 0.2  | 0.2  | 0.4      | 0.4      |
| TGA, %                  | -        | -     | -    | 4.5  | -        | -        |
| KF, %                   | 4.7      | -     | 4.6  | -    | 4.5      | 4.5      |
| Assay, alcalimetric, %  | 99.9     | -     | -    | -    | -        | -        |
| Assay, mercurimetric, % | -        | -     | 99.5 | -    | -        | -        |

Metronidazole, Control No 183118

Initial analytical report: WHO/PHARM/84.513, Appendix 13

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| Examination year:     | 1983     | 1993 | 1998     | 2003 | 2007     |
|-----------------------|----------|------|----------|------|----------|
| IR                    | conforms | -    | conforms | -    | conforms |
| TLC, %                | 0.1      | -    | -        | -    | -        |
| HPLC, %               | <0.2     | 0.1  | 0.1      | 0.1  | 0.1      |
| TGA, %                | -        | <0.1 | <0.1     | -    | -        |
| LOD, %                | <0.1     | -    | -        | <0.1 | <0.1     |
| Assay, titrimetric, % | 100.1    | -    | -        | -    | -        |

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Neostigmine metilsulfate, Control No 187135

Initial analytical report: WHO/PHARM/88.537, Appendix 12

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| Examination year:      | 1987     | 1989  | 1999     | 2004     | 2007     |
|------------------------|----------|-------|----------|----------|----------|
| IR                     | conforms | -     | conforms | conforms | conforms |
| TLC, %                 | < 0.5    | -     | -        | -        | -        |
| HPLC, %                | 0.4      | -     | 0.3      | 0.2      | 0.4      |
| TGA, %                 | 0.2      | < 0.1 | < 0.1    | -        | -        |
| LOD, %                 | < 0.1    | -     | -        | -        | < 0.1    |
| PSA, %                 |          |       |          |          |          |
| Assay, titrimetric., % | 98.8     | -     | -        | -        | -        |

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Nevirapine, Control No 104227

Initial analytical report: PSM/QSM/2005.1, Appendix 12

| Examination year:         | 2004     | 2007     |
|---------------------------|----------|----------|
| IR                        | conforms | conforms |
| LC-MS                     | conforms | -        |
| TLC, %                    | < 0.1    | -        |
| HPLC, %                   | 0.1      | 0.1      |
| LOD, %                    | < 0.1    | < 0.1    |
| Residual solvents (GC), % | < 0.1    | -        |

Nystatin, Control No 405152

Initial analytical report: PSM/QSM/2006.1, Appendix 13

| Examination year:             | 2005     | April 2006 | November 2006 | June 2007 |
|-------------------------------|----------|------------|---------------|-----------|
| IR                            | conforms | -          | conforms      | -         |
| HPLC, %                       | 6.9      | 6.9        | 7.4           | 7.8       |
| LOD, %                        | 3.8      | 3.9        | 4.0           | 4.0       |
| Assay, microbiological, IU/mg | 5308     | 4600       | 4900          | 4700      |
| UV ratio A291/A305            | 0.67     | -          | 0.65          | -         |
| UV ratio A319/A305            | 0.92     | -          | 0.91          | -         |
| Residual solvents (GC), %     | 0.2      | -          | -             | -         |

Ouabain, Control No 283026

Initial analytical report: WHO/PHARM/84.513, Appendix 14

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| Examination year:      | 1983     | 1987  | 1997 | 2003     | 2007     |
|------------------------|----------|-------|------|----------|----------|
| IR                     | conforms | -     | -    | conforms | conforms |
| TLC, %                 | 0.2      | 0.3   | -    | -        | -        |
| HPLC, %                | 0.4      | 0.7   | 0.6  | 0.6      | 0.5      |
| TGA, %                 | -        | -     | 19.9 | -        | 19.2     |
| LOD, %                 | 20.0     | 19.9  | -    | 19.9     | -        |
| Assay, colorimetric, % | 100.1    | 100.0 | -    | -        | -        |

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Phenacetin, Control No 297172

Initial analytical report: WHO/PHARM/97.595, Appendix 14

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| Examination year:           | 1997     | 1998  | 2003  | 2007     |
|-----------------------------|----------|-------|-------|----------|
| IR                          | conforms | -     | -     | conforms |
| Capillary melting point, °C | 136.0 *  | 136.6 | 136.4 | 136.6    |
| DSC, mol%                   | 0.01     | 0.02  | 0.02  | 0.01     |

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\* Collaborative trial

Phenolphthalein, Melting Point Reference Substance, Control No 299167

Initial analytical report: WHO/EDM/QSM/2000.6, Appendix 10.

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| Examination year:           | 1999     | 2007     |
|-----------------------------|----------|----------|
| IR                          | conforms | conforms |
| DSC, mol%                   | 0.04     | 0.02     |
| Capillary melting point, °C | 263.1*   | 263.2    |

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\*Collaborative trial

Prednisolone hemisuccinate, Control No 195196

Initial analytical report: WHO/PHARM/96.584, Appendix 17

| Examination year:      | 1996     | 2002 | 2007     |
|------------------------|----------|------|----------|
| IR                     | conforms | -    | conforms |
| TLC, %                 | < 0.1    | -    | -        |
| HPLC, %                | 0.8      | 0.7  | 0.9      |
| TGA, %                 | 0.3      | -    | 0.1      |
| LOD, %                 | -        | 0.2  | -        |
| Assay, colorimetric, % | 100.5    | -    | -        |

Procarbazine hydrochloride, Control No 184120

Initial analytical report: WHO/PHARM/85.517, Appendix 9

| Examination year:     | 1984     | 1995     | 2006     |
|-----------------------|----------|----------|----------|
| IR                    | conforms | conforms | conforms |
| HPLC, %               | 0.7      | 0.7      | 0.4      |
| TGA, %                | -        | 0.1      | 0.1      |
| LOD, %                | < 0.1    | -        | -        |
| Assay, titrimetric, % | 99.0     | -        | -        |

Progesterone, Control No 167033

Initial analytical report: WHO/PHARM/67.441, Appendix 4

| Examination year:             | 1967            | 1975          | 1980 | 1984 | 1998     | 2003 | 2007     |
|-------------------------------|-----------------|---------------|------|------|----------|------|----------|
| IR                            | conforms        | -             | -    | -    | conforms | -    | conforms |
| TLC, %                        | no impurities   | no impurities | -    | -    | -        | -    | -        |
| HPLC, %                       | -               | -             | -    | 0.1  | 0.2      | 0.1  | 0.1      |
| TGA, %                        | -               | -             | -    | -    | < 0.1    | -    | 0.1      |
| LOD, %                        | < 0.1           | 0.1           | -    | -    | -        | 0.1  | -        |
| DTA, %                        | -               | -             | 0.1  | 0.1  | -        | -    | -        |
| UV ( $A_{1\text{cm}}^{1\%}$ ) | 545<br>(242 nm) | 544           | -    | 538  | -        | -    | -        |

Riboflavin, Control No 382035

Initial analytical report: WHO/PHARM/83.510, Appendix 10

| Examination year:            | 1982     | 1987     | 1991     | 1996  | 2003     | 2007     |
|------------------------------|----------|----------|----------|-------|----------|----------|
| IR                           | conforms | conforms | conforms | -     | conforms | conforms |
| HPLC, %                      | < 1.0    | 0.6      | 0.7      | 1.2   | 1.1      | 0.9      |
| TGA, %                       | -        | -        | 0.3      | 0.3   | -        | -        |
| LOD, %                       | 0.3      | 0.4      | -        | -     | 0.2      | 0.3      |
| Assay, potentiometric, %     | 99.4     | -        | -        | -     | -        | -        |
| Assay, spectrophotometric, % | 99.7     | 100.0    | 100.2    | 100.3 | -        | -        |

Sodium amidotrizoate, Control No 198221

Initial analytical report: WHO/PHARM/98.603, Appendix 15

| Examination year:             | 1998         | 2003 | 2007     |
|-------------------------------|--------------|------|----------|
| IR                            | conforms     | -    | conforms |
| TLC, %                        | < 0.2        | -    | -        |
| HPLC, %                       | < 0.04       | 0.02 | < 0.03   |
| Water (KF), %                 | 5.7          | -    | 6.5      |
| LOD, %                        | -            | 5.5  | -        |
| Residual solvents (GC), %     | < 0.1        | -    | -        |
| UV ( $A_{1\text{cm}}^{1\%}$ ) | 543 (238 nm) | -    | -        |

Sodium cromoglicate, Control No 188140

Initial analytical report: WHO/PHARM/89.544, Appendix 11

| Examination year:       | 1988     | 1994     | 2003   | 2007     |
|-------------------------|----------|----------|--------|----------|
| IR                      | conforms | conforms | -      | conforms |
| TLC, %                  | < 0.05   | -        | -      | -        |
| HPLC, %                 | 0.5      | 0.1      | < 0.06 | 0.1      |
| LOD, %                  | -        | -        | 7.0    | -        |
| TGA, %                  | 8.1      | 7.9      | -      | 8.1      |
| Water (KF), %           | 7.2      | -        | -      | -        |
| Assay potentiometric, % | 100.6    | -        | -      | -        |

Sulfanilamide, Melting Point Reference Substance, Control No 192162

Initial analytical report: WHO/PHARM/92.558, Appendix 5

| Examination year:           | 1963   | 1978 | 1991  | 1998  | 2003     | 2007     |
|-----------------------------|--------|------|-------|-------|----------|----------|
| IR                          | -      | -    | -     | -     | conforms | conforms |
| Capillary melting point, °C | 165.9* | 166  | 165.8 | 165.9 | 165.8    | 165.7    |
| DSC, mol%                   | -      | -    | 0.15  | 0.10  | 0.06     | 0.05     |

\* Collaborative trial

Sulfanilamide, Control No 179094

Initial analytical report: WHO/PHARM/80.504, Appendix 15

| Examination year:       | 1980          | 1991 | 1998 | 2007     |
|-------------------------|---------------|------|------|----------|
| IR                      | conforms      | -    | -    | conforms |
| TLC                     | no impurities | -    | -    | -        |
| HPLC, %                 | -             | -    | -    | < 0.1    |
| TGA, %                  | -             | -    | -    | -        |
| LOD, %                  | < 0.1         | -    | -    | < 0.1    |
| Assay, potentiometric % | 100.0         | -    | -    | -        |
| DTA, %                  | 99.95         | -    | -    | -        |
| DSA, %                  | -             | 99.9 | 99.9 | -        |

Sulfapyridine, Melting Point Reference Substance, Control No 192163

Initial analytical report: WHO/PHARM/92.558, Appendix 5

| Examination year:           | 1963   | 1978 | 1991  | 1998  | 2003     | 2007     |
|-----------------------------|--------|------|-------|-------|----------|----------|
| IR                          | -      | -    | -     | -     | conforms | conforms |
| Capillary melting point, °C | 192.7* | 193  | 192.3 | 192.4 | 192.3    | 192.5    |
| DSC, mol%                   | -      | -    | 0.08  | 0.08  | 0.04     | 0.06     |

\*Collaborative trial

Testosterone propionate, Control No 167036

Initial analytical report: WHO/PHARM/420.64, Appendix 4

| Examination year: | 1967          | 1975          | 1988          | 1996  | 2002     | 2007     |
|-------------------|---------------|---------------|---------------|-------|----------|----------|
| IR                | conforms      | -             | -             | -     | conforms | conforms |
| TLC, %            | no impurities | no impurities | no impurities | -     | -        | -        |
| HPLC, %           | -             | -             | 0.9           | 1.0   | 1.1      | 1.0      |
| DTA, %            | -             | -             | 0.3           | -     | -        | -        |
| TGA, %            | -             | -             | 0.1           | < 0.1 | -        | -        |
| LOD, %            | 0.1           | 0.1           | -             | -     | 0.1      | < 0.1    |

4,4'-Thiodianiline, Control No 183116

Initial analytical report: WHO/PHARM/84.513, Appendix 17

| Examination year:     | 1983              | 1998     | 2003 | 2007     |
|-----------------------|-------------------|----------|------|----------|
| IR                    | conforms          | conforms | -    | conforms |
| TLC, %                | Identity conforms | 2.0      | -    | -        |
| HPLC, %               | 0.5               | 2.2      | 2.0  | 2.4      |
| TGA, %                | -                 | 0.2      | -    | -        |
| LOD, %                | 0.3               | -        | <0.1 | <0.1     |
| Assay, titrimetric, % | 99.6              | -        | -    | -        |

Tolbutamide, Control No 179086

Initial analytical report: WHO/PHARM/80.504, Appendix 16

| Examination year:        | 1979          | 1987          | 1998   | 2003     | 2007      |
|--------------------------|---------------|---------------|--------|----------|-----------|
| IR                       | conforms      | conforms      | -      | conforms | conforms* |
| TLC, %                   | no impurities | no impurities | -      | -        | -         |
| HPLC, %                  | < 0.1         | < 0.1         | < 0.02 | 0.1      | 0.1*      |
| TGA, %                   | -             | -             | < 0.1  | -        | < 0.1     |
| LOD, %                   | 0.1           | < 0.1         | -      | < 0.1    | -         |
| DTA, mol%                | 0.2           | 0.2           | -      | -        | -         |
| Assay, potentiometric, % | 100.2         | -             | -      | -        | -         |

\* The re-examination in 2007 has been partially performed by the Centre for Analytical Science, Health Sciences Authority, Singapore.

Tolnaftate, Control No 176074

Initial analytical report: WHO/PHARM/76.488, Appendix 8

| Examination year: | 1976          | 1980          | 1998     | 2003   | 2007     |
|-------------------|---------------|---------------|----------|--------|----------|
| IR                | conforms      | -             | conforms | -      | conforms |
| TLC, %            | no impurities | no impurities | -        | -      | -        |
| HPLC, %           | -             | -             | < 0.04   | < 0.03 | < 0.03   |
| LOD, %            | < 0.1         | < 0.1         | < 0.1    | < 0.1  | < 0.1    |
| DTA, mol%         | 0.3           | -             | -        | -      | -        |
| DSC, mol%         | -             | -             | 0.2      | -      | -        |
| PSA, %            | 0.3           | -             | -        | -      | -        |

Vanillin, Control No 299169

Initial analytical report: WHO/PHARM/ QSM/2000.6, Appendix 11.

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|                             |          |          |
|-----------------------------|----------|----------|
| Examination year:           | 1999     | 2007     |
| IR                          | conforms | conforms |
| DSC, mol%                   | 0.02     | 0.04     |
| Capillary melting point, °C | 83.2*    | 82.7     |

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\* Collaborative trial

APPENDIX 7

## **ABACAVIR SULFATE FOR SYSTEM SUITABILITY**

Control No 107244

Analytical Report

### **Intended use**

The monograph for Abacavir sulfate in *The International Pharmacopoeia* requires a reference substance of abacavir sulfate for system suitability to be used in the liquid chromatographic test for related substances.

### **Material**

About 10 g of the sample (manufacturer's batch no G1188-42A) were received at the WHO Centre in February 2007. The material is being stored in tightly closed containers at + 5 °C, protected from light.

### **Analytical data**

#### Description

A creamy white powder.

#### High performance liquid chromatography

When abacavir sulfate for system suitability was injected, the following peaks were eluted at the following relative retention times with reference to abacavir (retention time about 19 minutes):

Impurity B = 1.3

Impurity C = 0.7

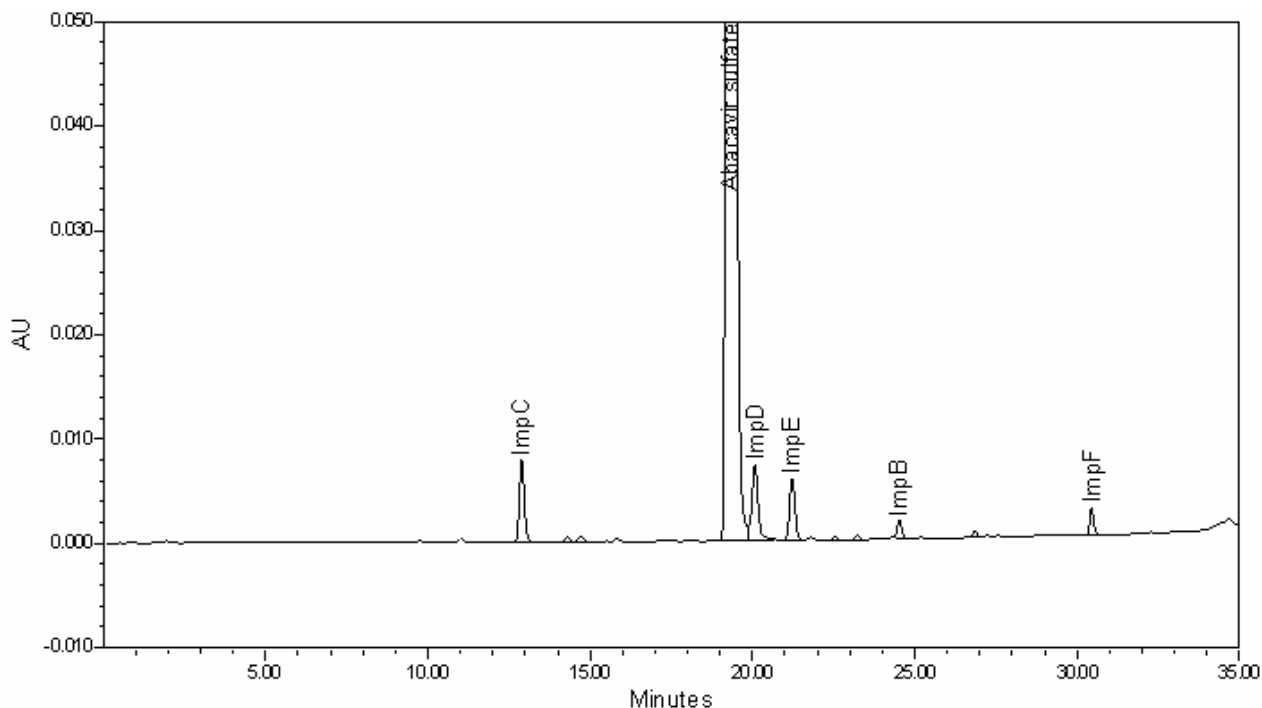
Impurity D = 1.04

Impurity E = 1.10

Impurity F = 1.6

The resolution between abacavir and impurity D was 2.3.

A chromatogram is shown in Figure 1.



**Figure 1.** Chromatogram of abacavir sulfate for system suitability Control No 107244 monitored at 254 nm.

The method used is described in the liquid chromatographic test for related substances according to the monograph for Abacavir sulfate, final draft QAS/05.144/Final, March 2006.

The following conditions were used:

Eluent:                                   A: 0.05 % solution of trifluoroacetic acid  
   B: 85 volumes of methanol and 15 volumes of water  
 N.B: The system was conditioned with the mobile phase for four hours.

| Eluent composition: | <u>% A</u> | <u>% B</u> | <u>Time, minutes</u> | <u>Type</u>      |
|---------------------|------------|------------|----------------------|------------------|
|                     | 95→70      | 5→30       | 0-20                 | linear           |
|                     | 70→10      | 30→90      | 20-35                | linear           |
|                     | 10→95      | 90→5       | 35-40                | linear           |
|                     | 95         | 5          | 40-45                | re-equilibration |

Data collecting time: 35 minutes

Column: Waters Symmetry C18, 4.6 x 150 mm, 5 µm particles

Column temperature: 30 °C

Detector, wavelength: Spectrophotometer, 254 nm

Flow rate: 0.8 ml/min

Injector temperature: 8 °C

|                                     |  |
|-------------------------------------|--|
| Preparation of dissolution solvent: | 1 ml of phosphoric acid (~1440 g/l) was diluted to 1000 ml with water.   |
| Sample preparation:                 | Abacavir sulfate for system suitability was dissolved in the dissolution solvent to a concentration of 0.2 mg/ml. 20 µl (corresponding to 4 µg) were injected. |
| Stability of the sample solution:   | The sample was stable in the dark at 8 °C for at least 5 hours.  |

## **Stability**

Regular re-examinations of this ICRS when stored in the dry state will be performed.

## **Conclusion**

Abacavir sulfate for system suitability, Control No 107244, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 8

**AMOXICILLIN TRIHYDRATE**

Control No 106242

Analytical Report

**Intended use**

The monograph for amoxicillin trihydrate in *The International Pharmacopoeia*, Fourth Edition, requires a reference substance of amoxicillin trihydrate to be used in the infrared absorption spectrophotometric and thin-layer chromatographic tests for identity as well as in chromatographic purity and the spectrophotometric assay.

**Material**

About 250 g of the sample (manufacturer's batch no 99560350) were received at the WHO Centre in April 1999. 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 W106242). The spectrum is concordant with the spectrum of the European Pharmacopoeia Chemical Reference Substance (EPCRS) lot 3 of amoxicillin trihydrate.

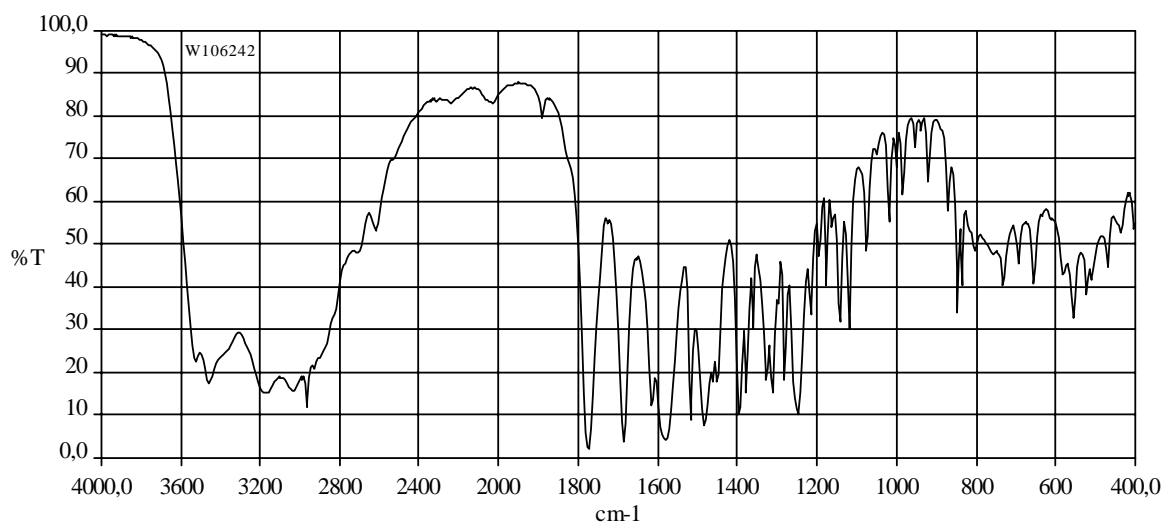


Figure 1. IR-spectrum of 1.3 mg of amoxicillin trihydrate Control No 106242 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

#### Specific optical rotation

+ 301°. The determination was performed in water at a concentration of 2 mg/ml. The result was calculated with reference to the dried substance.

#### Identity test C

2.0 mg amoxicillin trihydrate was placed in a test-tube, moistened with 1 drop of water. 2 ml of sulphuric acid (~1760 g/l) were added and the content was mixed by swirling. The solution was remained colourless. Then it was placed in the water-bath. A yellow colour was developed.

#### pH Value

4.8. The determination was performed in water at a concentration of 2 mg/ml.

#### **Assay**

##### Thermogravimetric analysis

When the substance was heated to 120 °C, a loss of 13.2% (w/w) was observed (n=6, RSD=0.4%).

Instrument: Thermogravimetric analyzer, Mettler Toledo TGA/SDTA 851°.

Sample weight: 4-8 mg.

Heating program: 5 °C/min from 25–120°C and then holding 120 °C for 120 minutes or until the baseline is stable.

##### Water

13.5% (n=3) determined by Karl Fischer titration.

### Residual solvents

Total content was estimated to 0.1% (Acetone: 160 ppm, n-butylacetate: 310 ppm, 2-propanol: 720 ppm).

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

|                       |  |
|-----------------------|--|
| Instrument:           | Hewlett Packard 6890   |
| Column:               | DB-624 (30 m × 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:   | 45 minutes-low shaking   |
| Loop volume:          | 1.0 ml gas phase   |

### **Purity**

#### Thin-layer chromatography

Five secondary spots were detected of which, three were above the quantification limit. Their total amount was estimated to be 1.1%. The following thin-layer chromatographic system was used.

|                |   |
|----------------|---|
| Thin-layer:    | Silica gel 60 F-254 (Merck) TLC   |
| Eluent:        | Ethanol 95%: Dichloromethane: Diethylamine: Tetraethyl ammonium solution 0.1M in methanol/propanol (10:15:2:1)  |
| Sample:        | 300 μg of amoxicillin trihydrate dissolved in 3% ammonia (~260 g/l) in methanol, was applied. The samples were applied within 30 minutes after preparation. |
| Visualization: | Scanning at 235 nm with a CAMAG TLC Scanner 3 was performed.  |

$$R_f(\text{amoxicillin trihydrate}) = 0.5$$

The limit of detection was about 0.6 μg (0.2%), when scanning at 235 nm.

High performance liquid chromatography

The purity was estimated by peak area normalization to 98.8% (n=6, RSD=0.01% for the main peak, RSD=0.3% for the impurity with a value of 0.3%). Nine impurities above the limit of quantification were detected. A chromatogram is shown in Figure 2.

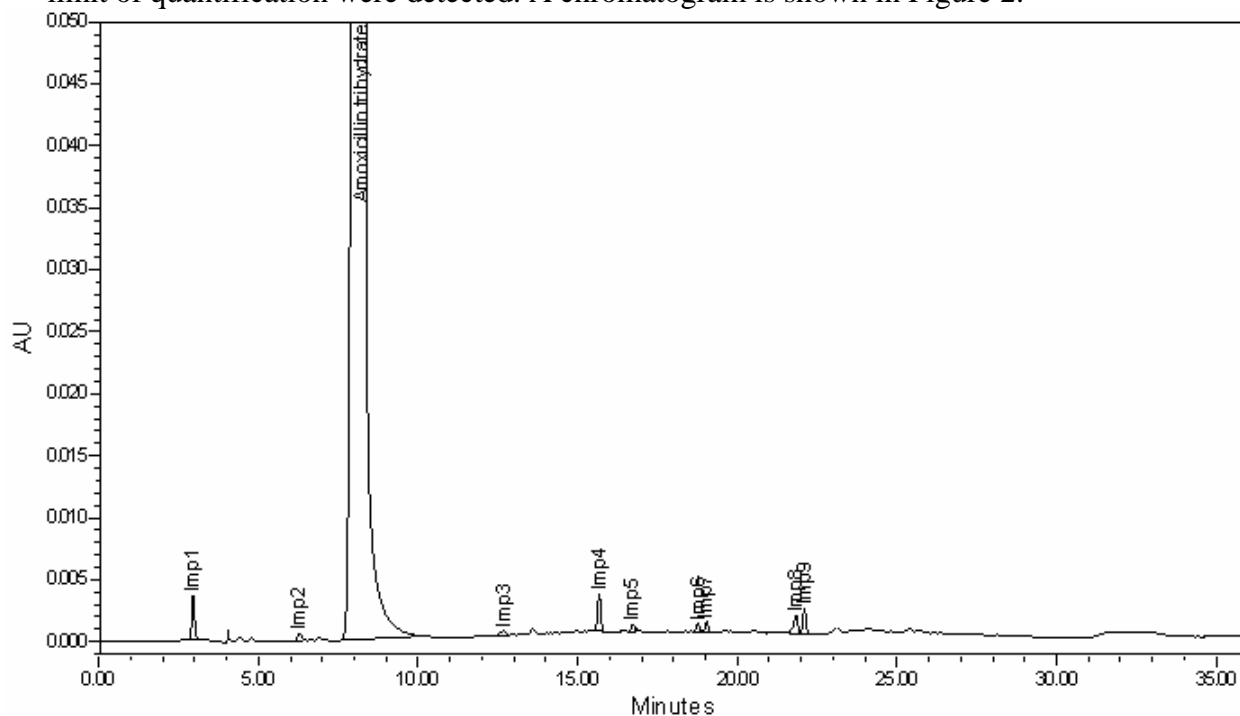


Figure 2. Chromatogram of amoxicillin trihydrate Control No 106242 monitored at 254 nm.

The method used was a slight modification of the liquid chromatographic test for related substances in *The International Pharmacopoeia*, Fourth Edition.

The following conditions were used:

Eluent: A: Acetonitrile:Buffer (1:99)  
B: Acetonitrile:Buffer (20:80)

Buffer: To 250 ml of potassium dihydrogen phosphate (27.2 g/l), 2 M sodium hydroxide was added until a pH of 5.0 was reached. The solution was further diluted with water to a volume of 1000 ml.

| Eluent composition:   | <u>% A</u> | <u>% B</u> | <u>Time, minutes</u> | <u>Type</u>      |
|-----------------------|------------|------------|----------------------|------------------|
|                       | 92         | 8          | 0-7                  | isocratic        |
|                       | 92→0       | 8→100      | 7-25                 | linear           |
|                       | 0          | 100        | 25-35                | isocratic        |
|                       | 0→92       | 100→8      | 35-36                | linear           |
|                       | 92         | 8          | 36-46                | re-equilibration |
| Data collecting time: | 35 minutes |            |                      |                  |

|                                   |  |
|-----------------------------------|--|
| 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 preparation:               | Amoxicillin trihydrate was dissolved in the mobile phase A to a concentration of 1.5 mg/ml. 50 µl was injected (corresponding to 75 µg). |
| Stability of the sample solution: | The sample was stable in the dark at 8 °C for at least 5 hours.  |

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

|                            |           |
|----------------------------|-----------|
| Identification IR:         | Conforms. |
| Assay, HPLC :              | 98.5%     |
| Specific optical rotation: | 302.8°    |
| Water:                     | 13.2%     |

### **Stability**

Regular re-examinations of the ICRS when stored in the dry state will be performed.

### **Conclusion**

Amoxicillin trihydrate, Control No 106242, can be considered suitable as International Chemical Reference Substance for the intended purpose. When used in the spectrophotometric assay according to *The International Pharmacopoeia*, Fourth Edition, the content of amoxicillin trihydrate is taken to be 86.4% calculated with reference to the dried substance (corresponding to 100.0% on the "as is basis").

APPENDIX 9

**LAMIVUDINE FOR SYSTEM SUITABILITY**

Control No 107246

Analytical Report

**Intended use**

The monograph for Lamivudine in *The International Pharmacopoeia*, requires a reference substance of lamivudine for system suitability to be used in the liquid chromatographic test for related substances.

**Material**

About 5 g of the sample (manufacturer's batch no G4141-147D) were received at the WHO Centre in February 2007. The material is being stored in tightly closed containers at + 5 °C, protected from light.

**Analytical data**

Description

A white powder.

High performance liquid chromatography

When lamivudine for system suitability was injected, the following peaks were eluted at the following relative retention times with reference to lamivudine (retention time about 9 minutes):

Impurity A = 0.40

Impurity B = 0.92

The resolution between lamivudine and impurity B was 2.6. A chromatogram is shown in Figure 1.

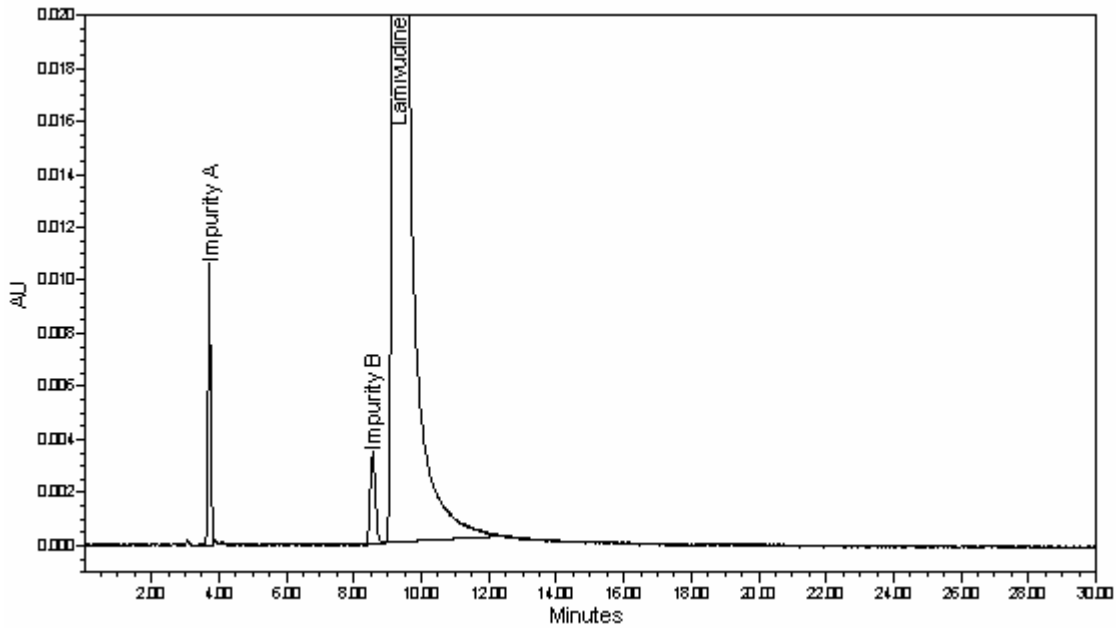


Figure 1. Chromatogram of Lamivudine for system suitability Control No 107246 monitored at 277 nm.

The method used was described in the draft for monograph to The International Pharmacopoeia.

The chromatogram obtained with solutions (3) and (4) were used to identify the peaks due to impurities A, B, E, F and C.

Impurity E = cytosine, impurity F = uracil and impurity C = salicylic acid.

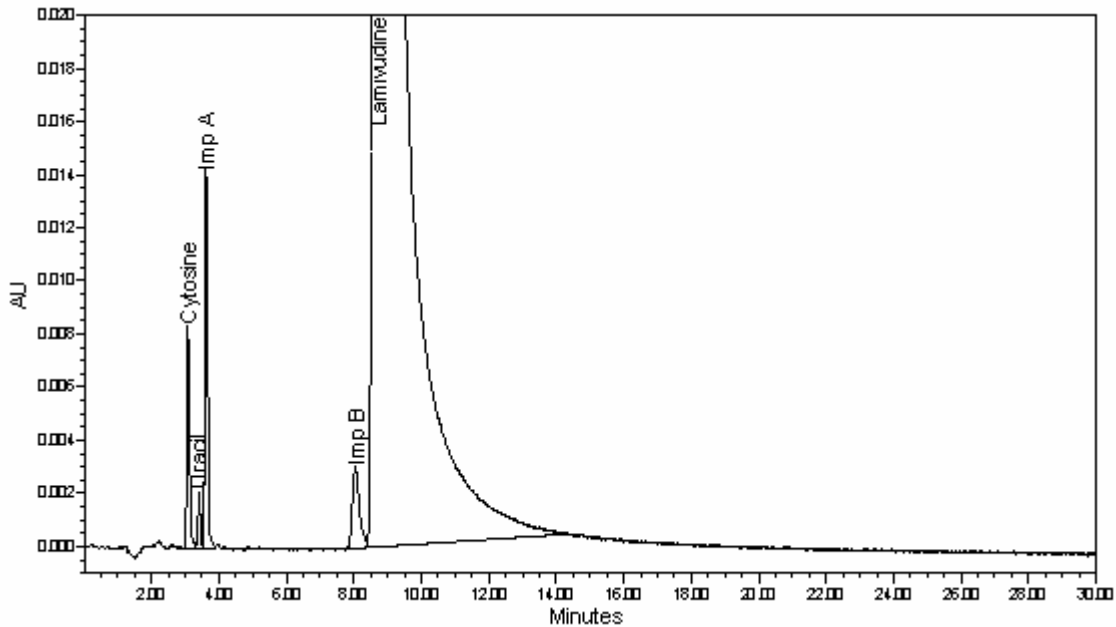


Figure 2. Solution 4: Chromatogram of 5 µg lamivudine for system suitability Control No 107246, 10 ng cytosine and 10 ng uracil, monitored at 277 nm.

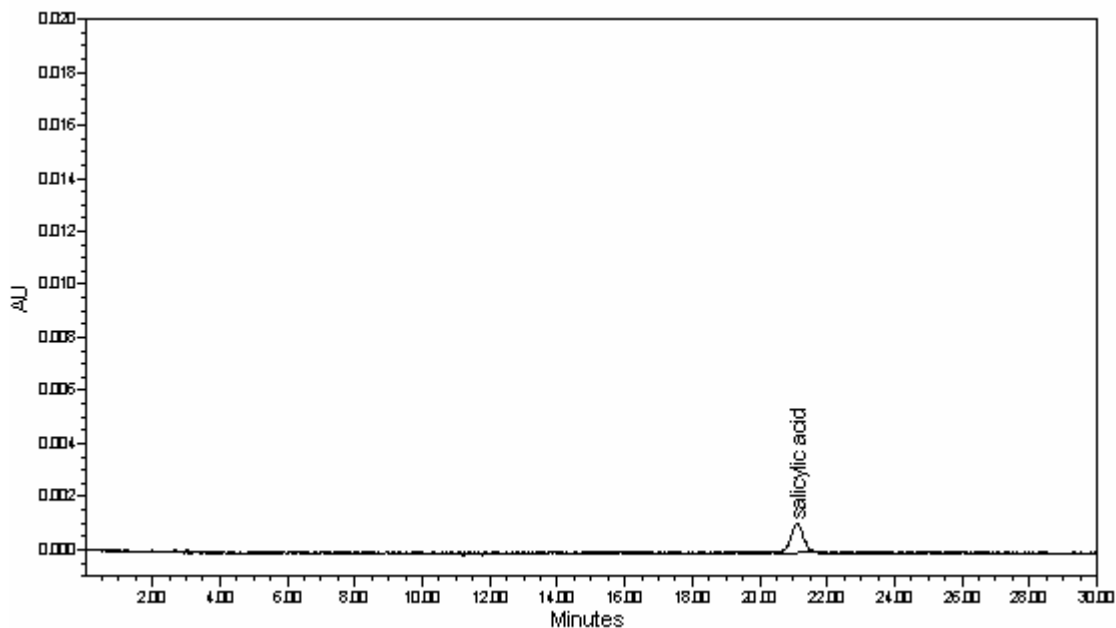


Figure 3. Chromatogram of 50 ng salicylic acid monitored at 277 nm.

Salicylic acid in figure 3 was dissolved to 0.25 mg/ml in the mobile phase, 1.0 ml of the resulting solution was further diluted to 50 ml in the mobile phase. 10 $\mu$ l was injected.

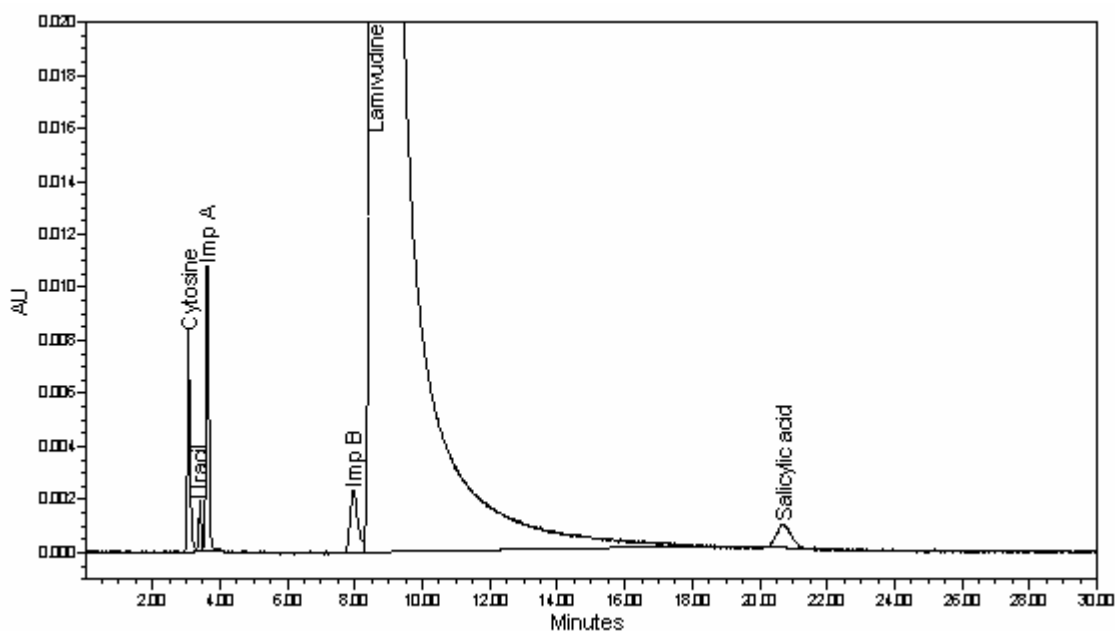


Figure 4. Chromatogram of 5  $\mu$ g lamivudine for system suitability Control No 107246, 5 ng cytosine, 5 ng uracil, and salicylic acid 50 ng monitored at 277 nm.

Preparation: 5 mg cytosine and 5 mg uracil were dissolved in 50 ml of the mobile phase, this solution was diluted 1:10 with mobile phase (CU). 2.5 mg salicylic acid was diluted in 100 ml mobile phase. 1 ml of the CU solution and 2 ml of the salicylic acid solution were added to 5 mg lamivudine for system suitability. This solution was then diluted to 10 ml with mobile phase and 10  $\mu$ l were injected.

Both the CU solution and the salicylic acid was sonicated for about 10 minutes to enhance the dissolution.

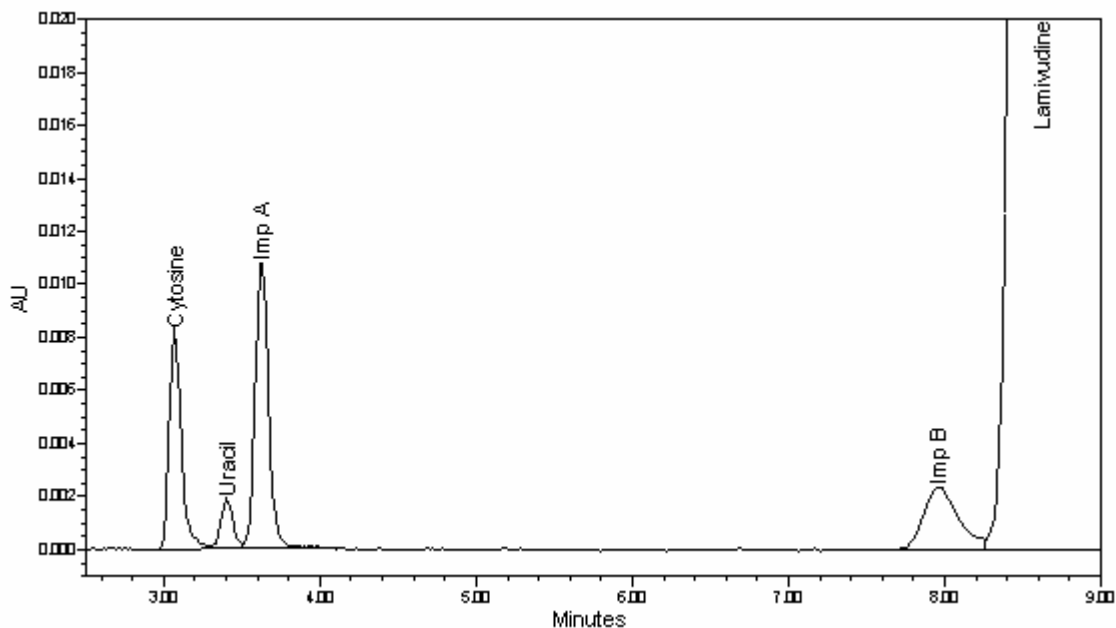


Figure 5. Enlargement of the chromatogram in figure 4.

The following conditions were used:

Eluent: A: Methanol  
B: 1.9 g/l ammonium acetate, adjusted to pH 3.8 with glacial acetic acid

Eluent composition:

| <u>% A</u> | <u>% B</u> | <u>Time, minutes</u> | <u>Type</u> |
|------------|------------|----------------------|-------------|
| 5          | 95         | 0-30                 | isocratic   |

Data collecting time: 30 minutes  
 Column: BDS Hypersil C18, 4.6 x 250 mm, 5 µm particles  
 Column temperature: 35°C  
 Detector, wavelength: Spectrophotometer, 277 nm  
 Flow rate: 1.0 ml/min  
 Injector temperature: 8 °C  
 Sample preparation: Lamivudine for system suitability was dissolved in the mobile phase to a concentration of 0.5 mg/ml. 10 µl (corresponding to 5 µg) was injected.  
 Stability of the sample solution: The sample was stable in the dark at 8 °C for at least 3 hours.

## **Stability**

Regular re-examinations of this ICRS when stored in the dry state will be performed.

## **Conclusion**

Lamivudine for system suitability, Control No 107246, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 10

## LEVOTHYROXINE SODIUM

Control No 207144

Analytical Report

### **Intended use**

The stock of the current batch of the International Chemical Reference Substance (ICRS) of levothyroxine sodium Control No 189144 is depleted and has to be replaced. The monograph for Levothyroxine sodium in *The International Pharmacopoeia*, Fourth Edition, requires a reference substance of levothyroxine sodium to be used in the thin-layer chromatographic test for identity.

### **Material**

About 80 g of the sample (manufacturer's batch no A010873) were received at the WHO Centre in September 2007. The material is being stored in tightly closed containers at + 5 °C, protected from light.

### **Analytical data**

#### Description

A creamy white powder.

### **Evidence of chemical structure**

#### Infrared spectrum

An infrared spectrum is given in Figure 1 (No W207144). The spectrum is concordant with the spectrum of the previous lot of the ICRS of levothyroxine sodium with Control No 189144.

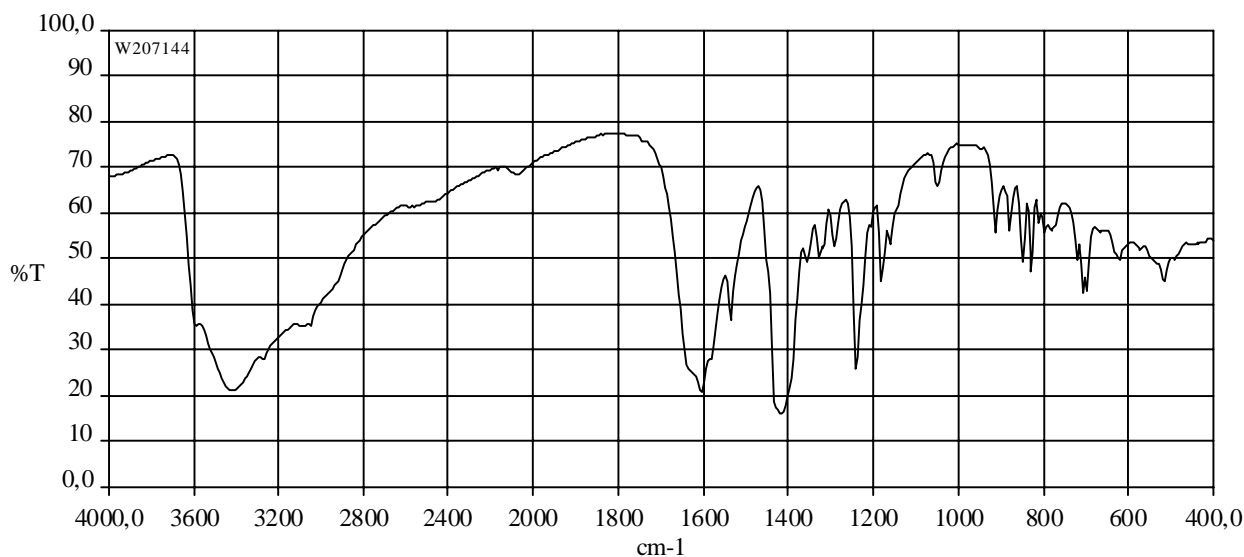


Figure 1. IR-spectrum of 1.6 mg of levothyroxine sodium Control No 207144 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

#### Specific optical rotation

+17°. The determination was performed according to the method in *The International Pharmacopoeia*. The result was calculated with reference to the dried substance.

#### **Assay**

##### Thermogravimetric analysis

When the substance was heated to 140 °C, a loss of 9.4% (w/w) was observed. (n=6, RSD=0.8%).

Instrument: Thermogravimetric analyzer, Mettler Toledo TGA/SDTA 851°.

Sample weight: 3-6 mg.

Heating program: 5 °C/min from 20–140 °C and then holding 140 °C for 120 minutes.

Melting point: 233-237 °C.

##### Water

9.8% (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.

The content of residual solvents was tested by gas chromatography (GC-MS) with the following conditions:

|                       |  |
|-----------------------|--|
| Instrument:           | Gas chromatograph: Agilent 6890                                    |
| Column:               | DB-624 (30 m x 0.32 mm, 1.8 µm film)                               |
| Carrier gas:          | Helium (2.0 ml/min)  |
| Split:                | 1:5  |
| Detector:             | MSD  |
| Detector temperature: | 280 °C   |
| Temperature program:  | 40 °C for 20 minutes, 10 °C/min and holding 240 °C for 20 minutes. |
| Injection:            | Agilent G1888 Headspace sampler                                    |
| Injector temperature: | 140 °C   |
| HS-Oven temperature:  | 80 °C  |
| Vial equilibration:   | 60 minutes-low shaking   |
| Loop volume:          | 1.0 ml gas phase   |

## Purity

### Thin-layer chromatography

Three secondary spots were detected. Their total amount was estimated to be about 0.4%. The following thin-layer chromatographic system was used.

|                |  |
|----------------|--|
| Thin-layer:    | Silica gel 60 F-254 (Merck) TLC  |
| Eluent:        | Ammonia(~260g/l):2-propanol:ethyl acetate (20:35:55)                             |
| Sample:        | 100 µg of levothyroxine sodium dissolved in ammonia:methanol (5:75) were applied |
| Visualization: | Scanning at 225 nm with a CAMAG TLC Scanner 3 was performed                      |

$$R_f(\text{levothyroxine}) = 0.2$$

$$R_f(\text{lithyronine}) = 0.3$$

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

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

The limit of quantification was <0.1 µg (<0.1%), when scanning at 225 nm.

### High performance liquid chromatography

27 impurities above the limit of quantification were detected. One of them was identified as lithyronine (7.7 minutes). Lithyronine was estimated by external standard to 0.2% (w/w), (RSD=0.7%). Other impurities were estimated by peak area normalization to 1.3% at 225 nm. A chromatogram is shown in Figure 2.

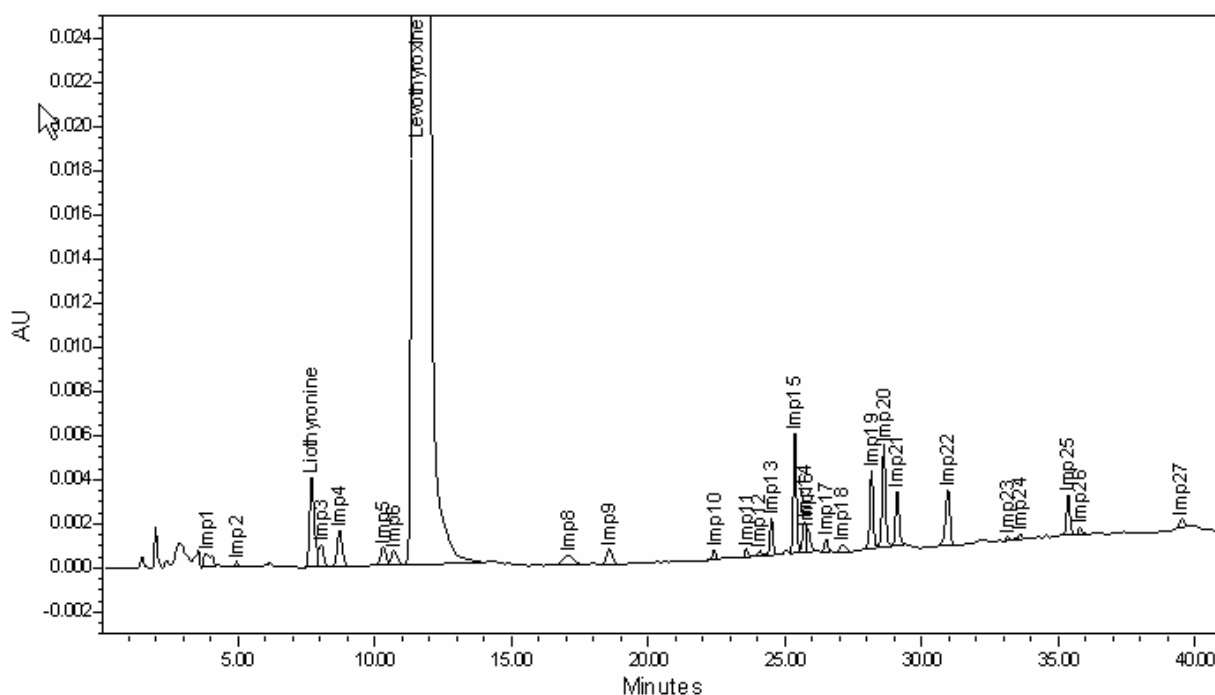


Figure 2. Chromatogram of levothyroxine sodium Control No 207144 monitored at 225 nm.

The following conditions were used:

Eluent: A: Acetonitrile  
B: 1.0 ml concentrated phosphoric acid in 700 ml water.

| Eluent composition: | % A   | % B   | Time, minutes | Type             |
|---------------------|-------|-------|---------------|------------------|
|                     | 32    | 68    | 0-13          | isocratic        |
|                     | 32→70 | 68→30 | 13-32         | linear           |
|                     | 70    | 30    | 32-40         |                  |
|                     | 70→32 | 30→68 | 40-42         | linear           |
|                     | 32    | 68    | 42-52         | re-equilibration |

Data collecting time: 40 minutes  
 Column: Zorbax SB-CN, 4.6 x 250 mm, 5 µm particles  
 Column temperature: R.T. (about 20 °C)  
 Detector, wavelength: Spectrophotometer, 225 nm  
 Flow rate: 1.0 ml/min  
 Injector temperature: 8 °C  
 Solvent: 120 mg sodium hydroxide was dissolved in 150 ml water and then further diluted with 150 ml methanol.

|                                    |  |
|------------------------------------|--|
| Sample preparation:                | Levothyroxine sodium was dissolved in the solvent to a concentration of 0.2 mg/ml. 40 µl (corresponding to 8 µg) was injected.<br><br>Samples have to be protected from light throughout the analyses. |
| Stability of the sample: solution: | The sample is stable in the dark at 8 °C for at least 4 hours.   |
| Limit of detection:                | 1.4 ng (0.01 %) at 225 nm  |
| Limit of quantification:           | 0.4 ng (0.004 %) at 225 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 225 nm. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities.

### **Stability**

No special stability studies were performed. It was considered that this substance, based on the experience of the stability of the previous lot, was stable. The previous lot showed no signs of degradation after being stored for 14 years at + 5 °C. Regular re-examinations of this ICRS when stored in the dry state will be performed.

### **Conclusion**

Levothyroxine sodium, Control No 207144, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 11

**NORETHISTERONE ENANTATE**

Control No 107243

Analytical Report

**Intended use**

The monograph for Norethisterone enantate in *The International Pharmacopoeia*, Fourth Edition, requires a reference substance of norethisterone enantate to be used in the infrared absorption spectrophotometric test for identity.

**Analytical data**

Description

A white, crystalline powder.

**Evidence of chemical structure**

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W107243).

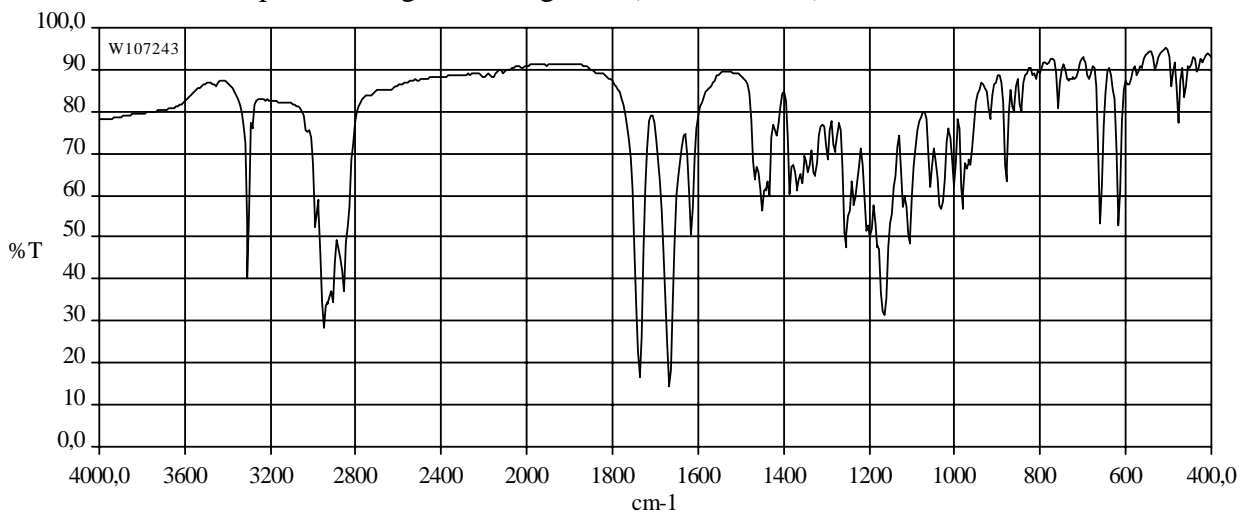


Figure 1. IR-spectrum of 1.3 mg of norethisterone enantate Control No 107243 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

### Mass-spectrometric detection

A spectrum was recorded by atmospheric pressure chemical ionization (APCI) in the positive ion mode. The spectrum shows an  $[M+H]^+$  ion of 411, which supports the identity of norethisterone enantate. The spectrum is given in Figure 2.

Instrument and operating conditions:

Detector: Waters ZQ 2000 (single quadrupole mass spectrometer)

Operating conditions: Corona current: 3  $\mu$ A

Cone voltage: 30 V

Source temperature: 130 °C

Apci probe temperature: 400 °C

Data acquisition: Scan: 50 – 500 Da

Injection by syringe pump: 10  $\mu$ l/min

Sample preparation: Norethisterone enantate was dissolved in methanol to a concentration of 1 mg/ml.

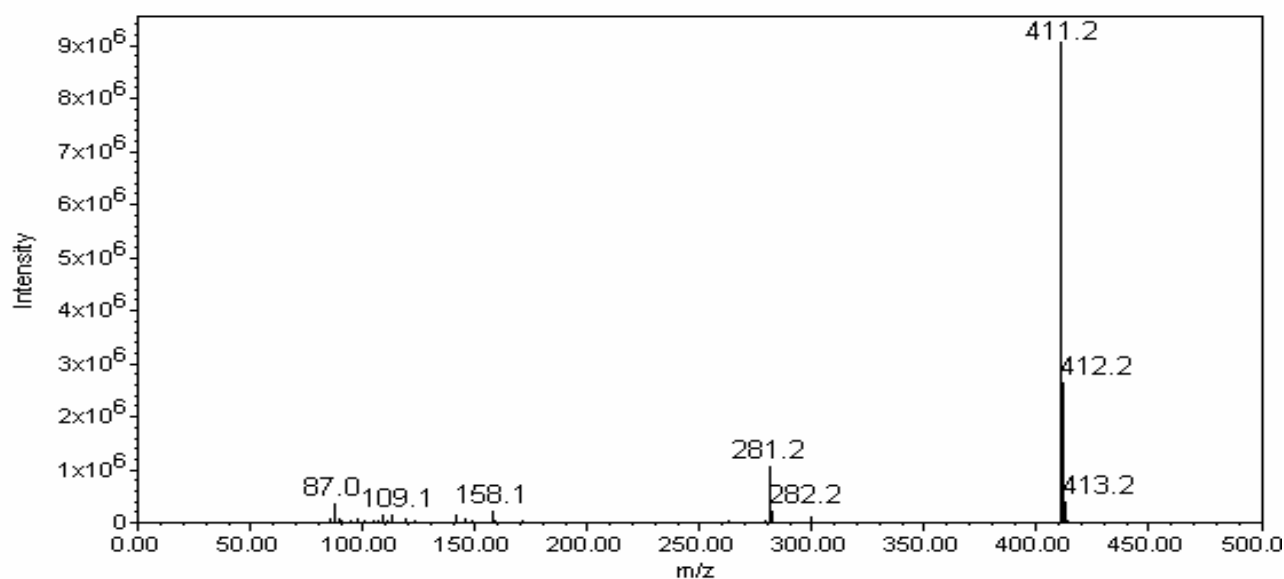


Figure 2. Positive-ion electrospray mass spectrum of norethisterone enantate Control No 107243.

### UV-spectrum

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

UV-maxima was observed at 240 nm.

$A_{1cm}^{1\%} = 437$  at 240 nm (n=6, RSD=0.06%)

Calculations were performed with reference to the dried substance.

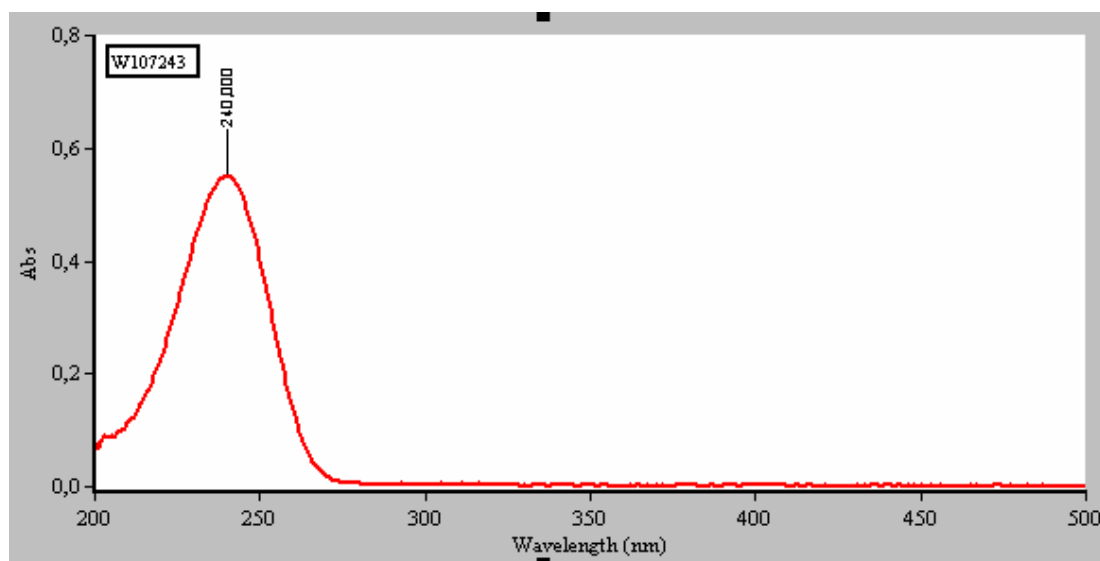


Figure 3. UV-spectrum of norethisterone enantate Control No 107243, 12.5 µg/ml in methanol.

#### Specific optical rotation

- 13°. The determination was performed in chloroform at a concentration of 20 mg/ml. The result was calculated with reference to the dried substance.

#### Identity test C

2.0 mg norethisterone enantate was dissolved in 2 ml ethanol and 1 ml of sulfuric acid (~1760 g/l). The solution became violet, which meets the test described in the monograph for Norethisterone enantate in *The International Pharmacopoeia*, Fourth Edition.

#### **Assay**

##### Water

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

##### Residual solvents

Hexane: 0.65%, 2-methylpentane: 0.02%, 3-methylpentane: 0.4%, methylcyclopentane:0.2%.

The test included residual solvents according to Ph Eur 6.0 Method 2.4.24 System A.

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

|                       |   |
|-----------------------|---|
| Instrument:           | Gas chromatograph: 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

Oven temperature: 105 °C

Vial equilibration: 60 minutes-low shaking

Loop volume: 1.0 ml gas phase

Sample preparation: Blank solution: Milli-Q-water.

Reference solution 1: 1.5 ml of hexane was diluted to 200.0 ml of methanol. 1.0 ml of this was diluted to 20.0 ml with water.

Reference solution 2: 3.0 ml of hexane was diluted to 200.0 ml of methanol. 1.0 ml of this was diluted to 20.0 ml with water.

Reference solution 3: 6.0 ml of hexane was diluted to 200.0 ml of methanol. 1.0 ml of this was diluted to 20.0 ml with water.

Sample solution: 20.0 mg sample was dissolved in DMF and diluted to 20.0 mL with DMF.

Sample: 5.0 ml sample solution + 1.0 ml blank solution in a capped head-space vial.

Reference 1: 5.0 ml DMF + 1.0 ml reference solution 1 in a capped head-space vial.

Reference 2: 5.0 ml DMF + 1.0 ml reference solution 2 in a capped head-space vial.

Reference 3: 5.0 ml DMF + 1.0 ml reference solution 3 in a capped head-space vial.

Blank: 5.0 ml DMF + 1.0 ml blank solution in a capped head-space vial.

#### Free enantiic acid

The amount of free enantiic acid was determined to be < 1.3 mg/g, which meets the limit in the monograph for Norethisterone enantate in *The International Pharmacopoeia*, Fourth Edition

#### **Purity**

##### Thin-layer chromatography

5 secondary spots were detected. Their total amount was estimated to be about 0.3%. The following thin-layer chromatographic system according to the monograph for Norethisterone enantate in *The International Pharmacopoeia*, Fourth Edition was used.

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

Eluent: Cyclohexane:Ethyl acetate (2:1)

Sample: 800 µg of norethisterone enantate dissolved in dichloromethane were applied

Visualization: Scanning at 254 nm with a CAMAG TLC Scanner 3 was performed as well as visualization at 365 nm after spraying with antimony trichloride reagent. The plate was heated at 110 °C for 15 minutes after spraying

$R_f$ (Norethisterone enantate) = 0.30

$R_f$ (Imp1) = 0.0

$R_f$ (Imp2) = 0.06

$R_f$ (Imp3) = 0.13

$R_f$ (Imp4) = 0.19

$R_f$ (Imp5) = 0.39

The limit of detection was about 0.02 µg (0.002%), when scanning at 254 nm.

#### High performance liquid chromatography

The purity was estimated by peak area normalization to 99.7% (n=6, RSD=0.004% for the main peak, RSD=2.0% for the impurity with a value of 0.1%). 9 impurities above the limit of quantification were detected. A chromatogram is shown in Figure 4.

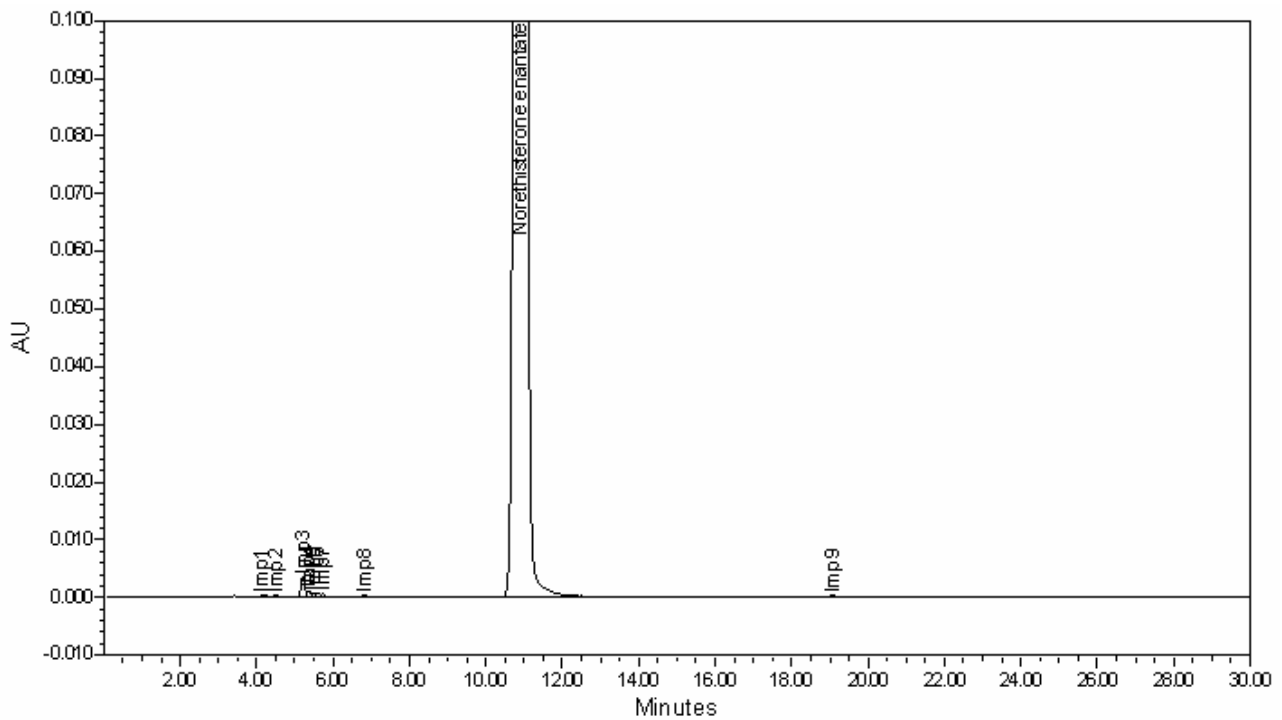


Figure 4. Chromatogram of norethisterone enantate Control No 107243 monitored at 254 nm.

The method used was a slight modification of the liquid chromatographic test for related substances in *The International Pharmacopoeia*, Fourth Edition.

The following conditions were used:

|                                   |   |            |                      |             |
|-----------------------------------|---|------------|----------------------|-------------|
| Eluent:                           | A: Acetonitrile   |            |                      |             |
|                                   | B: Water  |            |                      |             |
| Eluent composition:               | <u>% A</u>  | <u>% B</u> | <u>Time, minutes</u> | <u>Type</u> |
|                                   | 85  | 15         | 0-30                 | isocratic   |
| Data collecting time:             | 30 minutes  |            |                      |             |
| Column:                           | BDS Hypersil C18, 250x4.6 mm, 5 µm  |            |                      |             |
| Column temperature:               | R.T. (about 20 °C)  |            |                      |             |
| Detector, wavelength:             | Spectrophotometer, 240 nm   |            |                      |             |
| Flow rate:                        | 1.0 ml/min  |            |                      |             |
| Injector temperature:             | 8 °C  |            |                      |             |
| Sample preparation:               | Norethisterone enantate was dissolved in the eluent to a concentration of 1.0 mg/ml. 8 µl (corresponding to 8 µg) was injected. |            |                      |             |
| Stability of the sample solution: | The sample was stable in the dark at 8 °C for at least 3 hours.   |            |                      |             |
| Limit of detection:               | 1 ng (0.01%) at 240 nm  |            |                      |             |
| Limit of quantification:          | 2 ng (0.02%) 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.

#### Differential scanning calorimetry

The purity was estimated to 99.6 mol% (n=6, RSD=0.08%). The determination was performed on 1-2 mg using Mettler Toledo DSC 822° with a heating rate of 2 °C per minute.

### **Stability**

Regular re-examinations of the ICRS when stored in the dry state will be performed.

### **Conclusion**

Norethisterone enantate, Control No 107243, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 12

**PARACETAMOL**

Control No 207198

Analytical Report

**Intended use**

The stock of the current batch of the International Chemical Reference Substance of paracetamol Control No 195198 is depleted and has to be replaced. The monograph for Paracetamol in *The International Pharmacopoeia*, Fourth Edition, requires a reference substance of paracetamol to be used in the thin-layer chromatographic test for identity.

**Material**

About 210 g of the sample (manufacturer's batch no 2221760) were received at the WHO Centre in October 2007. 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 W207198). The spectrum is concordant with the spectrum of the previous lot of the International Chemical Reference Substance (ICRS) of paracetamol with Control No 195198.

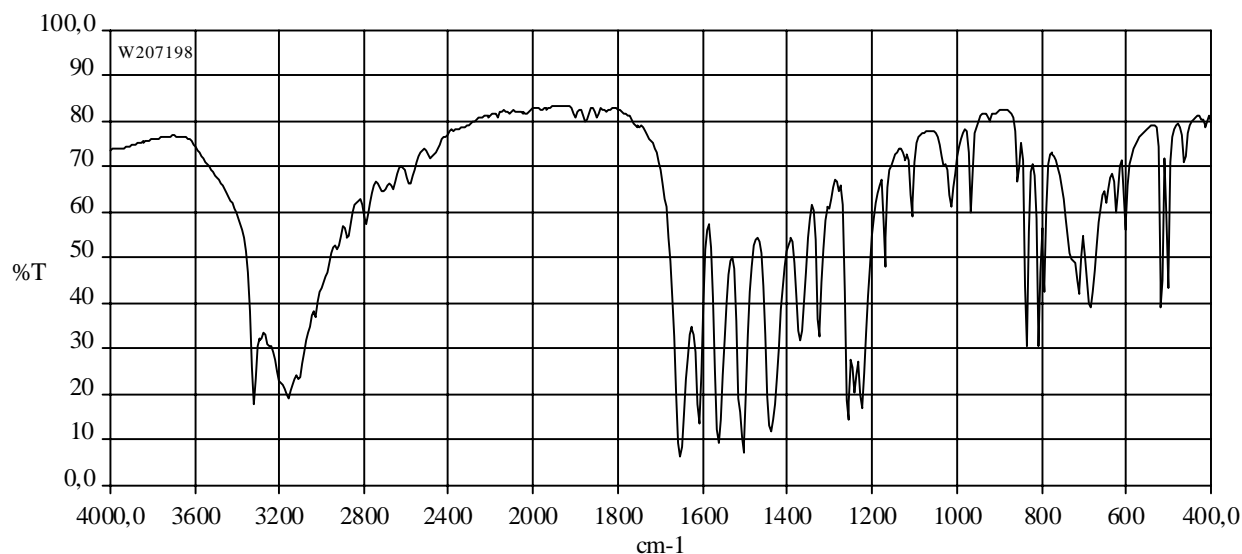


Figure 1. IR-spectrum of 0.6 mg of paracetamol Control No 207198 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

#### UV-spectrum

A UV-spectrum was recorded on a Varian Cary 5 spectrophotometer. Paracetamol was dissolved in methanol to a concentration of 0.5 mg/ml. To 1.0 ml of this solution 0.5 ml hydrochloric acid (0.1 mol/l) was added and then diluted to 100 ml with methanol. Dark volumetric flasks were used. The samples were freshly prepared. The spectrum is given in Figure 2.

UV-maxima were observed at 249 nm.

$A_{1\text{cm}}^{1\%} = 894$  at 249 nm (n=6, RSD=1%)

Calculations were performed with reference to the dried substance.

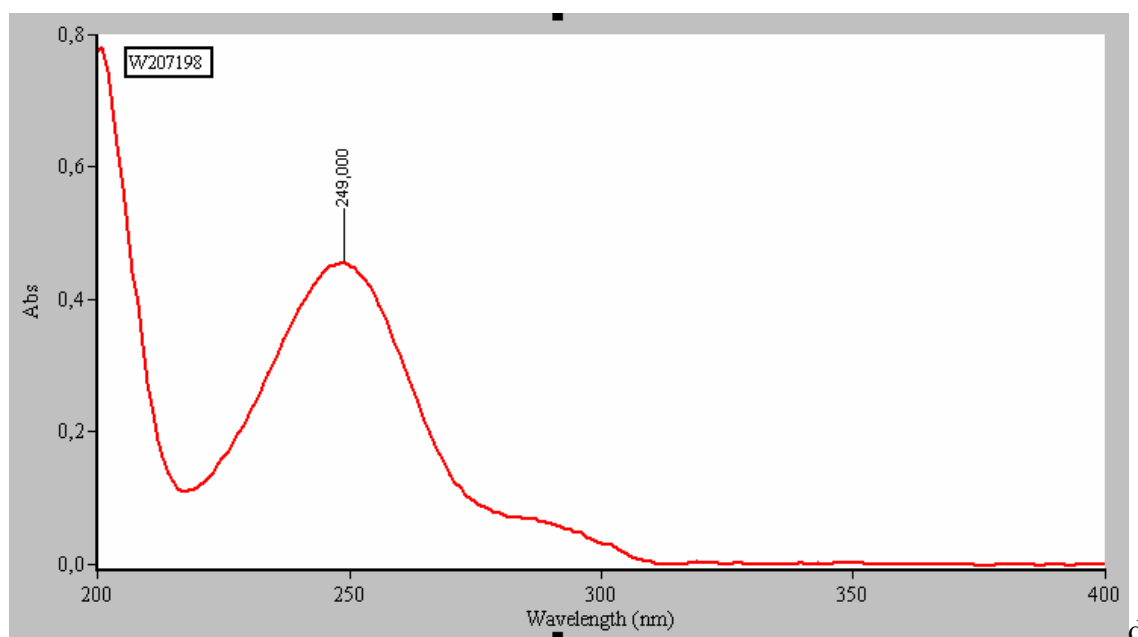


Figure 2. UV-spectrum of paracetamol Control No 207198, 5 µg/ml.

#### Identity test B

About 50 mg of the substance was dissolved in 5.0 ml water. A violet-blue colour was produced when 25 µl of a 25 mg/ml ferric chloride solution in water was added, which meets the test described in the monograph for Paracetamol in *The International Pharmacopoeia*, Fourth Edition.

#### Identity test C

About 50 mg of the substance was dissolved in 0.5 ml hydrochloric acid (70g/l) and boiled gently for 3 minutes. 5.0 ml water was added and cooled. No precipitate was formed. The solution slowly turned violet when potassium dichromate was added, which meets the test described in the monograph for Paracetamol in *The International Pharmacopoeia*, Fourth Edition.

#### 4-Aminophenol

Sample solution: 0.1 g of the substance was dissolved and diluted with 2.0 ml of a mixture of equal volumes of methanol and water. 40 µl of a mixture containing of 10mg/ml sodium nitroprusside dihydrate and 10mg/ml sodium carbonate in water was added. The sample solution was allowed to stand for 30 minutes.

Reference solution: 0.1 g of 4-aminophenol- free paracetamol was dissolved and diluted with 2.0 ml of the mixture of equal volumes of methanol and water. 40 µl of the mixture containing of 10mg/ml sodium nitroprusside dihydrate and 10mg/ml sodium carbonate in water was added. 0.1 ml of a solution containing 0.050 mg/ml of 4-aminophenol in the same solvent mixture was added.

The colour of the reference solution was light green and the sample solution stayed transparent, which meets the test described in the monograph for Paracetamol in *The International Pharmacopoeia*, Fourth Edition.

### **Assay**

#### Thermogravimetric analysis

When the substance was heated to 100 °C, a loss of <0.1% (w/w) was observed.

|                  |   |
|------------------|---|
| Instrument:      | Thermogravimetric analyzer, Mettler Toledo TGA/SDTA 851°.       |
| Sample weight:   | 3-5 mg.   |
| Heating program: | 5 °C/min from 25–100°C and then holding 100 °C for 120 minutes. |
| Melting point:   | 169- 170.5 °C.  |

#### Water

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

### Residual solvents

Total content: <0.1%.

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

The content of residual solvents was tested by gas chromatography (GC-MS) with the following conditions:

|                       |  |
|-----------------------|--|
| Instrument:           | Gas chromatograph: Agilent 6890                                    |
| Column:               | DB-624 (30 m x 0.32 mm, 1.8 µm film)                               |
| Carrier gas:          | Helium (2.0 ml/min)  |
| Split:                | 1:5  |
| Detector:             | MSD  |
| Detector temperature: | 280 °C   |
| Temperature program:  | 40 °C for 20 minutes, 10 °C/min and holding 240 °C for 20 minutes. |
| Injection:            | Agilent G1888 Headspace sampler                                    |
| Injector temperature: | 140 °C   |
| HS-Oven temperature:  | 80 °C  |
| Vial equilibration:   | 60 minutes-low shaking   |
| Loop volume:          | 1.0 ml gas phase   |

### **Purity**

#### Thin-layer chromatography

0 secondary spots were detected. The following thin-layer chromatographic system according to the monograph for Paracetamol in *The International Pharmacopoeia*, Fourth Edition was used.

|                |  |
|----------------|--|
| Thin-layer:    | Silica gel 60 F-254 (Merck) TLC                              |
| Eluent:        | Chloroform: acetone: toluene (65:25:10)                      |
| Sample:        | 200 µg of paracetamol dissolved in ethanol was applied.      |
| Visualization: | Scanning at 254 nm with a CAMAG TLC Scanner 3 was performed. |

$$R_f(\text{paracetamol}) = 0.2$$

The limit of detection was about 0.05 µg (0.02%), when scanning at 254 nm.



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

Limit of detection: 0.2 ng (<0.01 %) at 244 nm

Limit of quantification: 0.7 ng (0.02 %) at 244 nm

\* A purity analysis was also performed at 220 nm, since 4-aminophenol, a possible degradation product, has a UV-maximum at 220 nm and a UV-minimum at about 244 nm.

#### Differential scanning calorimetry

The purity was estimated to 100.0 mol% (n=6). The determination was performed using Mettler Toledo DSC 822<sup>e</sup> with a heating rate of 2 °C per minute.

### **Stability**

No special stability studies were performed. It was considered that this substance, based on the experience of the stability of the previous lot, was stable. The previous lot showed no signs of degradation after being stored for 13 years at + 5 °C. Regular re-examinations of this ICRS when stored in the dry state will be performed.

### **Conclusion**

Paracetamol, Control No 207198, can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 13

**ZIDOVUDINE IMPURITY B**

Control No 107247

Analytical Report

**Intended use**

The monographs for Zidovudine in *The International Pharmacopoeia*, requires a reference substance of zidovudine impurity B to be used in the liquid chromatographic test for related substances.

**Material**

About 5 g of the sample (manufacturer's batch no G4146-156B) was received at the WHO Centre in October 2007. The material is being stored in tightly closed containers at + 5 °C, protected from light.

**Other name**

3'-Chloro-3'deoxythymidine

**Analytical data**

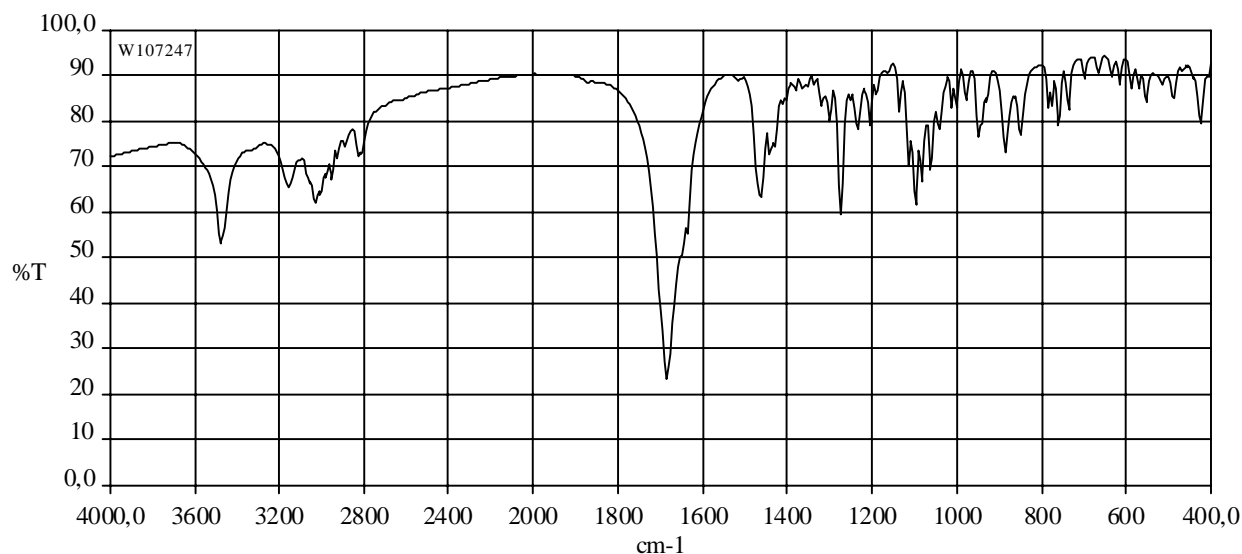
Description

A white powder.

**Evidence of chemical structure**

Infrared spectrum

An infrared spectrum is given in Figure 1 (No W107247).



**Figure 1.** IR-spectrum of 1.0 mg of zidovudine impurity B Control No 107247 in 300 mg of potassium bromide recorded against a potassium bromide disc.

Instrument: Perkin-Elmer Spectrum One.

#### Mass-spectrometric detection

A spectrum was recorded by atmospheric pressure chemical ionization (APCI) in the positive ion mode. The spectrum shows an  $[M+H]^+$  ion of zidovudine impurity B, which supports the identity of zidovudine impurity B. The spectrum is given in Figure 2.

Instrument and operating conditions:

|                       |   |
|-----------------------|---|
| Detector:             | Waters ZQ 2000 (single quadrupole mass spectrometer)  |
| LC-pump:              | Waters Alliance 2695  |
| Operating conditions: | Corona current: 3 $\mu$ A   |
|                       | Cone voltage: 5 V   |
|                       | Source temperature: 130 °C  |
|                       | Apci probe temperature: 300 °C  |
| Data acquisition:     | Scan: 33 – 350 Da   |
| Mobile phase:         | Methanol:Milli-q water (70:30)  |
| Injection volume:     | 20 $\mu$ L  |
| Sample preparation:   | Zidovudine Impurity B was dissolved in methanol:0.1% formic acid (70:30) to a concentration of 0.7 mg/ml. |

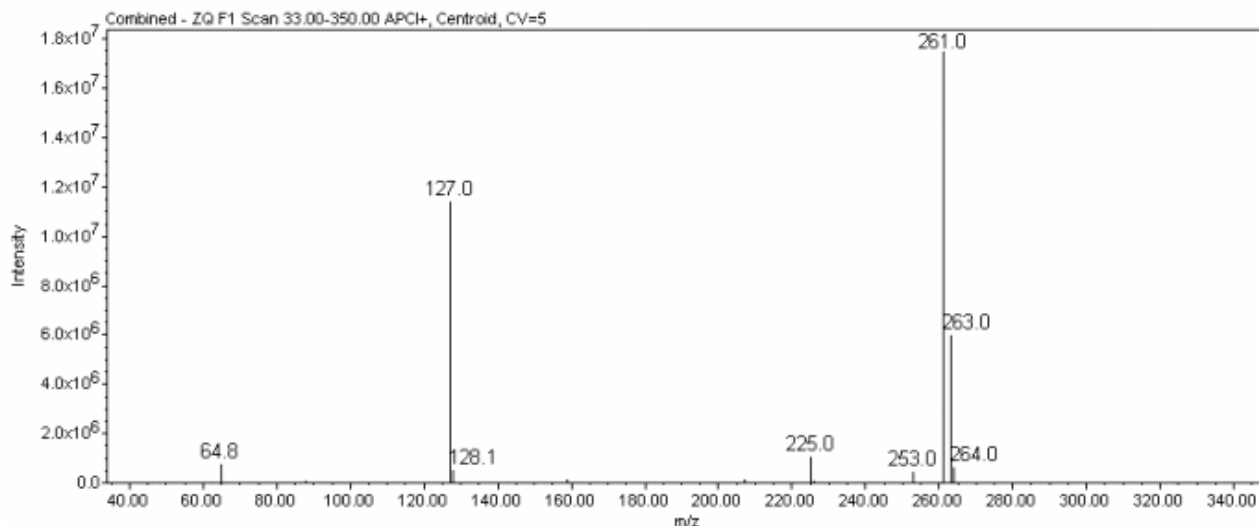


Figure 2. APCI mass spectrum in the positive ion mode of zidovudine impurity B Control No 107247.

## Assay

### Thermogravimetric analysis

When the substance was heated to 190 °C, a loss of 0.2% (w/w) was observed before degradation began. (n=6, RSD=14.1%).

Instrument: Thermogravimetric analyzer, Mettler Toledo TGA/SDTA 851°

Sample weight: 2-7 mg.

Heating program: 5 °C/min from 25–190 °C.

Melting point: 169.0 °C.

### Water

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

## Purity

### Thin-layer chromatography

No secondary spot was detected. The following thin-layer chromatographic system according to the monograph for Zidovudine in *The International Pharmacopoeia*, Fourth Edition was used.

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

Eluent: Dichloromethane : Methanol (9:1)

Sample: 100 µg of zidovudine impurity B dissolved in methanol was applied.

Visualization: Scanning at 265 nm with a CAMAG TLC Scanner 3 was performed as well as visualization in daylight after spraying with a solution containing 0.1 g carbazole in 19 ml of ethanol and 1 ml of sulfuric acid (1760 g/l). The plate was heated for 10 minutes at 120°C.

$R_f$  (zidovudine impurity B) = 0.4

The limit of detection was about 0.02 µg (0.02%), when scanning at 265 nm.

High performance liquid chromatography

The purity was estimated by peak area normalization to 100.0% (n=6, RSD=0.00% for the main peak). No impurities above the limit of quantification were detected.

A chromatogram is shown in Figure 3.

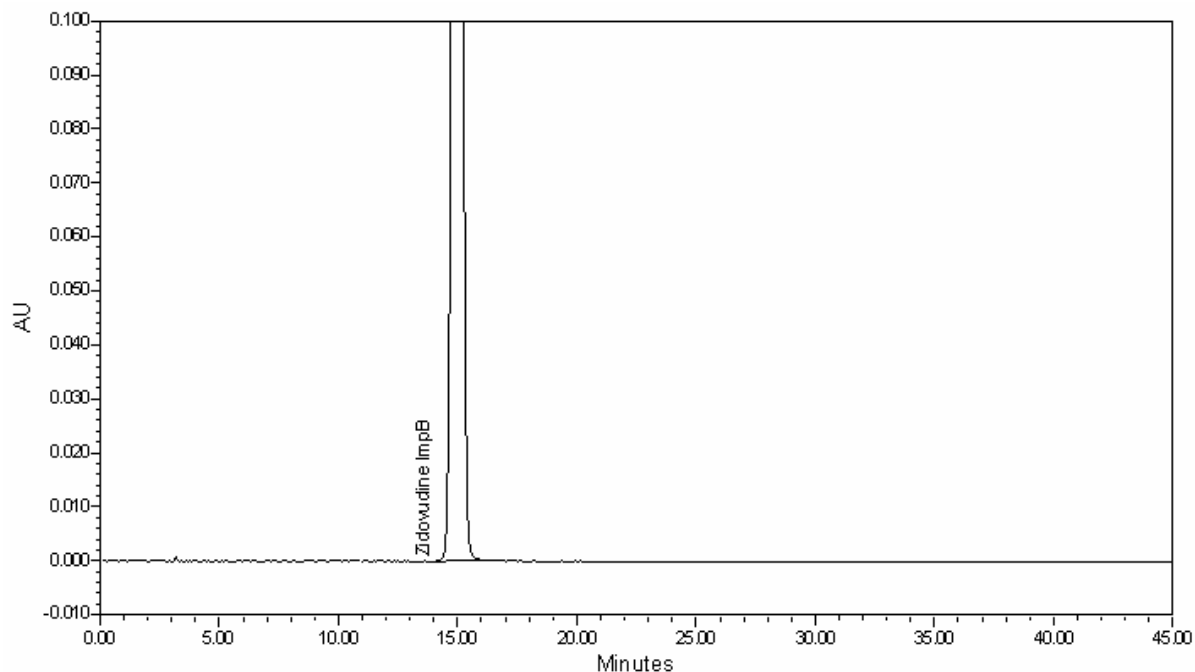


Figure 3. Chromatogram of zidovudine impurity B Control No 107247 monitored at 265 nm.

The method used is described in the liquid chromatographic test for related substances in the monograph for Zidovudine in *The International Pharmacopoeia*.

The following conditions were used:

|                       |                                  |            |                      |             |
|-----------------------|----------------------------------|------------|----------------------|-------------|
| Eluent:               | A: Methanol                      |            |                      |             |
|                       | B: Water                         |            |                      |             |
| Eluent composition:   | <u>% A</u>                       | <u>% B</u> | <u>Time, minutes</u> | <u>Type</u> |
|                       | 20                               | 80         | 0-45                 | isocratic   |
| Data collecting time: | 45 minutes                       |            |                      |             |
| Column:               | BDS Hypersil C18 250x4.6 mm, 5µm |            |                      |             |
| 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 impurity B was dissolved in methanol (2 volumes) and sonicated for 4 minutes and then further diluted with water (8 volumes) to a concentration of 1.0 mg/ml. 10 µl (corresponding to 10 µg) was injected. |
| Stability of the sample solution: | The sample was stable in the dark at 8 °C for at least 3 hours.   |
| Detection limit:                  | 3 ng (0.03 %) at 265 nm   |
| Quantification limit:             | 10 ng (0.10 %) at 265 nm  |

#### Diode-array detection

The chromatographic system described above was used to record UV-spectrum for the detected peak. The spectra of the main peak had a UV-maximum at 266 nm. The main peak was investigated by the peak purity program and showed no signs of co-eluting impurities.

### **Stability**

Regular re-examinations of the ICRS when stored in the dry state will be performed.

### **Conclusion**

Zidovudine impurity B, Control No 107247, can be considered suitable as International Chemical Reference Substance for the intended purpose.

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