



22374

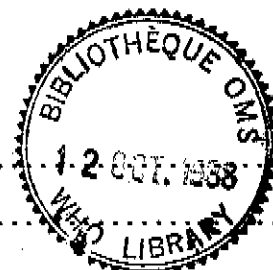
WHO COLLABORATING CENTRE FOR CHEMICAL REFERENCE SUBSTANCES

Report on the work in 1987

by M. Westermark

CONTENTS

	<u>Page</u>
Distribution of reference substances in 1987.....	2
Establishment of reference substances in 1987.....	2
Work on new reference substances completed in 1987.....	2
Stability testing.....	2
Work in progress and future work.....	3
Administrative and financial matters.....	3
Acknowledgements.....	3
Appendix 1. Distribution of chemical reference substances in 1987.....	4
Appendix 2. International Chemical Reference Substances established in 1987.....	6
Appendix 3. List of available International Chemical Reference Substances.....	7
Appendix 4. Stability testing - analytical report.....	11
Appendix 5. International Chemical Reference Substances - Project list 1988.....	16
Appendix 6. Allopurinol, Control No 287049.....	17
Appendix 7. Chlortetracycline hydrochloride, Control No 187138.....	21
Appendix 8. Clomifene citrate, Control No 187136.....	25
Appendix 9. Clomifene citrate Z-isomer, Control No 187137.....	31
Appendix 10. Digoxin, Control No 587011.....	37
Appendix 11. Emetine hydrochloride, Control No 187134.....	42
Appendix 12. Neostigmine metilsulfate, Control No 187135.....	47
Appendix 13. Propranolol hydrochloride, Control No 187139.....	52



The issue of this document does not constitute formal publication. It should not be reviewed, abstracted or quoted without the agreement of the World Health Organization. Authors alone are responsible for views expressed in signed articles.

Ce document ne constitue pas une publication. Il ne doit faire l'objet d'aucun compte rendu ou résumé ni d'aucune citation sans l'autorisation de l'Organisation Mondiale de la Santé. Les opinions exprimées dans les articles signés n'engagent que leurs auteurs.

Distribution of reference substances in 1987

During 1987 the total number of International Chemical Reference Substances distributed from the Centre were 2013 and 28 sets of Melting Point Reference Substances. The substances were distributed to drug control laboratories in 40 different countries. Compared to the figures for 1986 this corresponds to a decrease of about 11 per cent. The five most frequently requested substances during 1987 were in order of demand Ampicillin, Folic Acid, Cloxacillin Sodium, Propicillin Potassium and Ampicillin Trihydrate. Detailed figures for the distribution of the individual substances are given in Appendix 1.

Establishment of reference substances in 1987

In accordance with the procedure recommended by the WHO Expert Committee on Specifications for Pharmaceutical Preparations in its Twenty-fifth report (Technical Report Series No. 567), 9 International Chemical Reference Substances were established in 1987. The substances are listed in Appendix 2 to this report. Chloramphenicol, Chloramphenicol palmitate and Vitamin A acetate are replacement batches as the former stocks were depleted during 1987.

A complete list of all the International Chemical Reference Substances available from the Centre in January 1988, with information about package sizes and control numbers for the current batches, is given in Appendix 3 to this report. The list also includes 8 substances mentioned below, which are expected to be formally adopted during the first half of 1988.

Work on new reference substances completed in 1987

Work is being continued on new reference substances required to support specifications in the third edition of the International Pharmacopoeia. During 1987 the following new reference substances for volume 3 were examined: Chlortetracycline hydrochloride, Clomifene citrate, Clomifene citrate Z-isomer, Emetine hydrochloride, Neostigmine metilsulfate and Propranolol hydrochloride. The analytical reports for these materials are given in Appendices 7, 8, 9, 11, 12 and 13. All these substances were considered suitable for their intended uses and were proposed for adoption as International Chemical Reference Substances.

The following two stocks of International Chemical Reference Substances were depleted and have been replaced by new batches during 1987. Allopurinol No 172049 was replaced by No 287049 and Digoxin No 377011 was replaced by No 587011. The analytical reports are given in Appendices 6 and 10 to this report.

Stability testing

Each year a number of the International Chemical Reference Substances held in stock at the Centre are being reexamined to control their storage stability. During 1987-1988 the re-examination was performed on twelve substances.

The selection of analytical methods to be used for the stability monitoring requires careful reflection. The choice of method, is of course, much depending on the nature of the substance concerned. However, a generally applicable guiding principle is to use methods of high reproducibility and to adhere as closely as possible to the same methods and the same experimental conditions for the reexamination of a reference material as were used in the initial analysis. This will reduce the influence of analytical errors and facilitate early detection of onset of degradation of the material. It is, however, also prudent to consider from time to time the progress of analytical chemistry and to introduce new methods if they are considered to be more informative and/or more convenient.

During 1987 thermogravimetric analysis (TGA) has been introduced as a replacement/complement to loss on drying.

The results obtained in the reexamination together with the results from earlier studies are summarized in Appendix 4 to this report. Details about the methods used can be obtained from the Centre.

Work in progress and future work

Work on the establishment of new chemical reference substances is being continued. There is still one substance left to support a monograph in volume 2 of the International Pharmacopoeia. To support the monographs in volume 3 there is today a need for 42 new reference substances. Six of these are already in work at the Centre. Older batches have also to be replaced because the stocks are depleted. At present 8 substances have to be replaced during 1988-1989 but this figure may increase depending on the distribution. A great deal of the work load originates from the growing demand for regular reexamination of already existing reference substances. Some substances are very old and the extended total amount of reference substances results in still more work. The reference substances the Centre has to establish are listed in Appendix 5 to this report. The substances that are already in work are indicated with an asterisk.

During 1987 computerization of the activities concerning the work on reference substances has continued. The system consists of an IBM XT Personal Computer. Today information about bulk ordering, analytical schemes, dispensing worksheets and a plan for regular reexamination are available in the computer. Plans for computerized orders and an inventory of the stock of existing reference substances are in progress. Collaboration with other laboratories to decrease the workload on the Centre in Stockholm has also started. During 1987 the Centre has in particular received valuable help from the National Biological Standards Laboratory in Australia. Hopefully this will facilitate the preparation of the new reference substances for volume 3.

Administrative and financial matters

The financial situation of the Centre remains unsatisfactory. The total cost for running the Centre in 1987 was estimated at 307.300 US\$. The income from sales of reference substances to industrial laboratories was about 31.000 US\$ and the contribution received from the WHO Headquarters was 16.000 US\$, which leaves a deficit of 260.300 US\$. The management board of the National Corporation of Swedish Pharmacies has agreed to support the continued operation of the Centre at an unchanged level, provided all possibilities to reduce the deficit would be investigated.

From February 1987 the fee was increased to US\$ 40 per package and a freight and handling charge of US\$ 10 was also added to each order.

In order to alleviate the financial deficit of the Centre, National Research Centres have been requested to offer analytical support and WHO Regional Offices have been approached for financial assistance.

Acknowledgements

As usual the Centre has reason to express the most sincere thanks to Dr C. A. Johnson, Scientific Director and Secretary to the British Pharmacopoeia Commission, and a member of the WHO Expert Advisory Panel on the International Pharmacopoeia and Pharmaceutical Preparations, for his never-failing interest in our work and extremely valuable help as counsellor to the Centre in all matters concerning the establishment of reference substances. The Centre would also like to express its sincere gratitude to all pharmaceutical industries who have assisted the Centre by provision of candidate reference materials as well as by participation in the analytical testing. This year we want particularly to thank Andard-Mount Company in Wembley, England, Boehringer Ingelheim GmbH in West Germany, Farmitalia Carlo Erba, Milan, Italy, Hoffman-La Roche, Basle, Switzerland, Imperial Chemical Industries (ICI) in Macclesfield, England, Merrel Dow Pharmaceuticals Inc. in Cincinnati, Ohio, USA and The Wellcome Foundation in Dartford, England.

APPENDIX 1

DISTRIBUTION OF CHEMICAL REFERENCE SUBSTANCES IN 1987

Aceclidine salicylate	1 items	Estrone	11 items
p-Acetamidobenzalazine	2 "	Etacrynic acid	5 "
Acetazolamide	- "	Ethambutol hydrochloride	12 "
Allopurinol	- "	Ethinylestradiol	23 "
2-Amino-5-nitrothiazole	- "	Ethisterone	13 "
3-Aminopyrazole-4-carboxamide		Ethosuximide	3 "
hemisulfate	12 "	Etocarlide	4 "
Amitriptyline hydrochloride	11 "	Flucytosine	3 "
Ampicillin	101 "	Fluorouracil	9 "
Ampicillin sodium	36 "	Fluphenazine decanoate	
Ampicillin trihydrate	48 "	dihydrochloride	19 "
Anhydrotetracycline hydrochloride	19 "	Fluphenazine enantate	
Atropine sulfate	16 "	dihydrochloride	7 "
Azathioprine	3 "	Fluphenazine hydrochloride	23 "
Bendazol hydrochloride	5 "	Folic acid	84 "
Benzobarbital	5 "	Furosemide	21 "
Benzylamine sulfate	2 "	Griseofulvin	23 "
Benzylpenicillin potassium	19 "	Haloperidol	9 "
Benzylpenicillin sodium	28 "	Hydrochlorothiazide	16 "
Bephenium hydroxynaphthoate	1 "	Hydrocortisone	22 "
Betamethasone	14 "	Hydrocortisone acetate	14 "
Betanidine sulfate	1 "	(-)-3-(4-hydroxy-3-methoxyphenyl)-	
Bupivacaine hydrochloride	14 "	2-methylalanine	1 "
Caffeine	12 "	Ibuprofen	11 "
Carbenicillin monosodium	14 "	Imipramine hydrochloride	15 "
Chloramphenicol	45 "	Indometacin	10 "
Chloramphenicol palmitate	4 "	o-Iodohippuric acid	5 "
Chloramphenicol palmitate		Isoniazid	8 "
(Polymorph A)	7 "	Lanatoside C	14 "
5-Chloro-2-methylaminobenzophenone	7 "	Levodopa	12 "
2-(4-Chloro-3-sulfamoylbenzoyl)		Lidocaine	14 "
benzoic acid	1 "	Lidocaine hydrochloride	20 "
Chlorphenamine hydrogen maleate	8 "	Mefenamic acid	15 "
Chlorpromazine hydrochloride	14 "	Metazide	4 "
Chlortalidone	7 "	Methaqualone	9 "
Cloxacillin sodium	53 "	Methyldopa	6 "
Cortisone acetate	17 "	Methyltestosterone	19 "
Dapsone	11 "	Meticillin sodium	19 "
Desoxycortone acetate	9 "	Metronidazole	21 "
Dexamethasone	20 "	Nafcillin sodium	9 "
Dexamethasone acetate	8 "	Nicotinamide	20 "
Diazepam	32 "	Nicotinic acid	15 "
Diazoxide	2 "	Niridazole	- "
Dicloxacillin sodium	46 "	Niridazole-chlorethylcarboxamide	- "
Dicolinium iodide	1 "	Norethisterone	2 "
Dicoumarol	7 "	Norethisterone acetate	4 "
Diethylcarbamazine dihydrogen		Ouabain	22 "
citrate	7 "	Oxacillin sodium	46 "
Digitoxin	26 "	Papaverine hydrochloride	11 "
Digoxin	- "	Phenethicillin potassium	9 "
NN'-Di-(2,3-xylyl)anthranilamide	4 "	Phenoxymethylpenicillin	26 "
4-Epianhydrotetracycline		Phenoxymethylpenicillin calcium	3 "
hydrochloride	40 "	Phenoxymethylpenicillin potassium	36 "
4-Epitetracycline ammonium salt	21 "	Phenytoin	12 "
Ergometrine maleate	12 "	Prednisolone	35 "
Ergotamine tartrate	32 "	Prednisolone acetate	15 "
Estradiol benzoate	9 "	Prednisone	18 "

Prednisone acetate	11	items
Procaine hydrochloride	20	"
Procarbazine hydrochloride	1	"
Progesterone	18	"
Propicillin potassium	49	"
Propylthiouracil	6	"
Pyridostigmine bromide	4	"
Reserpine	2	"
Riboflavin	38	"
Sulfamethoxazole	19	"
Sulfamethoxypyridazine	8	"
Sulfanilamide	12	"
Testosterone propionate	24	"
Tetracycline hydrochloride	34	"
Thioacetazone	6	"
4,4'-Thiodianiline	1	"
Tolbutamide	4	"
Tolnaftate	10	"
Trimethadione	7	"
Trimethoprim	28	"
Trimethylguanidine sulfate	4	"
Tubocurarine chloride	10	"
Vitamin A acetate (solution) (\bar{a} 25000 IU)	27	"
Warfarin	11	"
Total	2 013	items

Melting Point Reference Substances 28 x 13 substances (approx. 4 g of each).

APPENDIX 2

INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES ESTABLISHED IN 1987

Reference Substance	Control number	Analytical Report	Remarks
Acetazolamide	186128	WHO/PHARM/87.532 Appendix 6	
2-Amino-5-nitrothiazole	186131	WHO/PHARM/87.532 Appendix 7	
Chloramphenicol	486004	WHO/PHARM/87.532 Appendix 8	Replaces No 379004
Chloramphenicol palmitate	286072	WHO/PHARM/87.532 Appendix 9	Replaces No 175072
Niridazole	186129	WHO/PHARM/87.532 Appendix 10	
Niridazole - chlorethyl- carboxamide	186130	WHO/PHARM/87.532 Appendix 11	
Norethisterone	186132	WHO/PHARM/87.532 Appendix 12	
Reserpine	186133	WHO/PHARM/87.532 Appendix 13	
Vitamin A acetate	686038	WHO/PHARM/87.532 Appendix 14	Replaces No 581038

APPENDIX 3

LIST OF AVAILABLE INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES

1988

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.

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 these substances to be used.

Directions for use and analytical data as required for the use intended in the relevant specifications of The International Pharmacopoeia are given in the certificates enclosed with the substances when distributed. More detailed analytical reports on the substances may be obtained on request from the WHO Collaborating Centre for Chemical Reference Substances.

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 accompanying leaflet.

The stability of the International Chemical Reference Substances kept at the Collaborating Centre is monitored by regular reexamination and deteriorated materials are replaced by new batches when necessary. Lists giving control numbers for the current batches are issued in the annual reports from the Centre and may be obtained on request.

Ordering Information

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

WHO Collaborating Centre for Chemical Reference Substances
APOTEKSBOLAGET AB
Centrallaboratoriet
S-105 14 STOCKHOLM
SWEDEN

(Telex: 115 53 APOBOL S)

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

<u>Reference substance</u>	<u>Package size</u>	<u>Control number for current batch</u>
Aceclidine salicylate	100 mg	172048
p-Acetamidobenzalazine	100 mg	171042
Acetazolamide	100 mg	186128
Allopurinol	100 mg	287049
2-Amino-5-nitrothiazole	25 mg	186131
3-Aminopyrazole-4-carboxamide hemisulfate	100 mg	172050
Amitriptyline hydrochloride	100 mg	181101
Ampicillin	200 mg	274001
Ampicillin sodium	200 mg	274002
Ampicillin trihydrate	200 mg	274003
Anhydrotetracycline hydrochloride	25 mg	180096
Atropine sulfate	100 mg	183111
Azathioprine	100 mg	172060
Bendazol hydrochloride	100 mg	173066
Benzobarbital	100 mg	172051
Benzylamine sulfate	100 mg	172052
Benzylpenicillin potassium	200 mg	180099
Benzylpenicillin sodium	200 mg	280047
Bephenium hydroxynaphthoate	100 mg	183112
Betamethasone	100 mg	183113
Betanidine sulfate	100 mg	172053
Bupivacaine hydrochloride	100 mg	172054
Caffeine	100 mg	181102
Carbenicillin monosodium	200 mg	383043
Chloramphenicol	200 mg	486004
Chloramphenicol palmitate	1 g	286072
Chloramphenicol palmitate (Polymorph A)	200 mg	175073
5-Chloro-2-methylaminobenzophenone	100 mg	172061
2-(4-Chloro-3-sulfamoylbenzoyl)benzoic acid	50 mg	181106
Chlorphenamine hydrogen maleate	100 mg	182109
Chlorpromazine hydrochloride	100 mg	178080
Chlortalidone	100 mg	183114
Chlortetracycline hydrochloride	200 mg	187138
Clomifene citrate	100 mg	187136
Clomifene citrate Z-isomer (Zuclomifene)	50 mg	187137
Cloxacillin sodium	200 mg	274005
Cortisone acetate	100 mg	167006
Dapsone	100 mg	183115
Desoxycortone acetate	100 mg	167007
Dexamethasone	100 mg	279008
Dexamethasone acetate	100 mg	168009
Diazepam	100 mg	172062
Diazoxide	100 mg	181103
Dicloxacillin sodium	200 mg	174071
Dicolinium Iodide	100 mg	172055
Dicoumarol	100 mg	178077
Diethylcarbamazine dihydrogen citrate	100 mg	181100
Digitoxin	100 mg	277010
Digoxin	100 mg	587011
NN'-Di-(2,3-xylyl)anthranilamide	50 mg	173067
Emetine hydrochloride	100 mg	187134
4-Epianhydrotetracycline hydrochloride	25 mg	180097
4-Epitetracycline ammonium salt	25 mg	180098
Ergometrine hydrogen maleate	50 mg	277012
Ergotamine tartrate	50 mg	385013
Estradiol benzoate	100 mg	167014
Estrone	100 mg	279015
Etacrynic acid	100 mg	281056
Ethambutol hydrochloride	100 mg	179081
Ethinylestradiol	100 mg	167016

<u>Reference substance</u>	<u>Package size</u>	<u>Control number for current batch</u>
Ethisterone	100 mg	167017
Ethosuximide	100 mg	179088
Etocarlide	100 mg	172057
Flucytosine	100 mg	184121
Fluorouracil	100 mg	184122
Fluphenazine decanoate dihydrochloride	100 mg	182107
Fluphenazine enantate dihydrochloride	100 mg	182108
Fluphenazine hydrochloride	100 mg	176076
Folic acid	100 mg	277019
Furosemide	100 mg	171044
Griseofulvin	200 mg	280040
Haloperidol	100 mg	172063
Hydrochlorothiazide	100 mg	179087
Hydrocortisone	100 mg	283020
Hydrocortisone acetate	100 mg	280021
(-)-3-(4-Hydroxy-3-methoxyphenyl)- 2-methylalanine	25 mg	179085
Ibuprofen	100 mg	183117
Imipramine hydrochloride	100 mg	172064
Indometacin	100 mg	178078
o-Iodohippuric acid	100 mg	171045
Isoniazid	100 mg	185124
Lanatoside C	100 mg	281022
Levodopa	100 mg	172065
Lidocaine	100 mg	181104
Lidocaine hydrochloride	100 mg	181105
Mefenamic acid	100 mg	173068
Melting Point Reference Substances (set of 13 substances with melting temp- eratures ranging from +69 °C to +263 °C)	13x4 g	
Metazide	100 mg	172058
Methaqualone	100 mg	173069
Methyldopa	100 mg	179084
Methyltestosterone	100 mg	167023
Meticillin sodium	200 mg	274024
Metronidazole	100 mg	183118
Nafcillin sodium	200 mg	272025
Neostigmine metilsulfate	100 mg	187135
Nicotinamide	100 mg	179090
Nicotinic acid	100 mg	179091
Niridazole	200 mg	186129
Niridazole-chlorethylcarboxamide	25 mg	186130
Norethisterone	100 mg	186132
Norethisterone acetate	100 mg	185123
Ouabain	100 mg	283026
Oxacillin sodium	200 mg	382027
Papaverine hydrochloride	100 mg	185127
Phenethicillin potassium	200 mg	167028
Phenoxyethylpenicillin	200 mg	179082
Phenoxyethylpenicillin calcium	200 mg	179083
Phenoxyethylpenicillin potassium	200 mg	176075
Phenytoin	100 mg	179089
Prednisolone	100 mg	283029
Prednisolone acetate	100 mg	167030
Prednisone	100 mg	167031
Prednisone acetate	100 mg	169032
Procaine hydrochloride	100 mg	183119
Procarbazine hydrochloride	100 mg	184120
Progesterone	100 mg	167033
Propicillin potassium	200 mg	274034

<u>Reference substance</u>	<u>Package size</u>	<u>Control number for current batch</u>
Propranolol hydrochloride	100 mg	187139
Propylthiouracil	100 mg	185126
Pyridostigmine bromide	100 mg	182110
Reserpine	100 mg	186133
Riboflavin	250 mg	382035
Sulfamethoxazole	100 mg	179092
Sulfamethoxypyridazine	100 mg	178079
Sulfanilamide	100 mg	179094
Testosterone propionate	100 mg	167036
Tetracycline hydrochloride	200 mg	180095
Thioacetazone	100 mg	171046
4,4'-Thiodianiline	50 mg	183116
Tolbutamide	100 mg	179086
Tolnaftate	100 mg	176074
Trimethadione	200 mg	185125
Trimethoprim	100 mg	179093
Trimethylguanidine sulfate	100 mg	172059
Tubocurarine chloride	100 mg	170037
Vitamin A acetate (solution) (Retinol)	5 caps. (*)	686038
Warfarin	100 mg	168041

(*) About 9 mg in 250 mg oil per capsule

APPENDIX 4

STABILITY TESTING

The storage stability of the International Chemical Reference Substances is monitored by regular reexamination of the substances held in stock at the Centre. The results obtained for the substances reexamined in 1987-1988 are summarized below. For comparison results obtained at earlier occasions are included in the summaries. The substances have been stored at +5⁰ C. The following abbreviations are used in the tables:

DSC Differential Scanning Calorimetry
 DTA Differential Thermal Analysis
 HPLC High Performance Liquid Chromatography
 IR Infrared Spectrophotometry
 KF Karl Fischer titration
 LOD Loss on drying
 TLC Thin-layer Chromatography
 PSA Phase Solubility Analysis
 TGA Thermogravimetric analysis

The estimates of total solid impurities by HPLC and by TLC are expressed as area per cent (area %), if not otherwise stated, by DSC and by DTA as mole per cent (mole %), and by PSA as weight per cent (w/w %). LOD and TGA (loss in weight) are expressed as weight per cent (w/w %). Assay values are calculated with reference to the dried or the anhydrous substance.

More details about the analytical methods used can be obtained from the Centre.

Dapsone, Control No 183115

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

Examination year:	1983	1988
HPLC, %	1.2	1.9 (1.1) ¹⁾
IR	conforms	-
LOD, %	0.1	-
TGA, %		< 0.1
TLC	2-4 sec spots	3-4 sec spots ²⁾
Assay, % (titrimetric)	99.9	100.0

- 1) The initial substance was recrystallized and the HPLC value 1.1 was obtained after re-recrystallization
- 2) System A (according to Ph Int III, Vol. 2) about 1.2%
 System B (according to initial report) about 1.1%

Fluphenazine hydrochloride, Control No 176076

Initial analytical report: WHO/PHARM/77.491, Annex 5

Examination year:	1976	1988
HPLC, %	0.5	0.4
IR	conforms	conforms
LOD, %	0	-
TGA, %	-	0
TLC	2 sec spots	A: 3 sec spots (one very weak) ¹⁾ B: 4 sec spots (one very weak) ²⁾
UV-absorption; 260 nm	0.65	0.65

1) System A according to the initial analytical report

2) System B according to the monograph in Ph Int, Ed III, Vol 2 (Related substances)

Griseofulvin, Control No 280040

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

Examination year:	1980	1988
HPLC, %	0.6 (236 nm) 0.9 (291 nm)	0.6 (236 nm) 0.9 (291nm)
IR	conforms	conforms
LOD, %	0.1	-
TGA, %	-	0
TLC	1 sec spot	1 sec spot
UV-absorption, 291 nm	0.68	0.68
Assay, % (spectrophotometric)	100.0	99.9

Haloperidol, Control No 172063

Initial analytical report: WHO/PHARM/73.475, Appendix 6

Examination year:	1972	1977	1979	1988
DSC, mole %	0.36 [*])	-	-	0.1
DTA, mole %				0.1
IR	conforms	-	-	conforms
LOD, %	0.3	0	0	-
TGA, %				0
TLC, System A	3 sec spots	1 sec spot	3 sec spots	2-3 very ¹⁾ weak sec spots
System B				no sec ²⁾ spots
UV-absorption, 245 nm	0.53	0.54	0.52	0.53
Assay, % (potentiometric)	99.6	99.9	99.8	-

*) manual evaluation, 1) System A: according to the initial analytical report.

2) System B: according to the monograph in Ph Int, Ed III, Vol 2 (only one secondary spot when applying solution aged 24 hours)

Imipramine hydrochloride, Control No 172064

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

Examination year:	1972	1977	1983	1988
DSC, mole %	0.22 ^{*)}	-	-	0.19
DTA, mole %			0.56	0.53
IR	conforms	conforms	-	conforms
LOD, %	0	0.05	0	0.08
TLC	4 sec spots	3 sec spots	3 sec spots	3 sec spots
Assay, % (potentiometric)	99.9	99.1	99.1	99.2

^{*)} manual evaluation

Lidocaine, Control No 181104

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

Examination year:	1981	1988
DTA, mole %	0.3	0.4
HPLC, %	< 0.1	-
IR	conforms	conforms
LOD, %	0	0.1
TLC	no sec spots	no sec spots
Assay, % (potentiometric)	99.6	99.8

Lidocaine hydrochloride, Control No 181105

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

Examination year:	1981	1988
DSC, mole %	-	0.3
DTA, mole %	0.8	0.8
IR	conforms	conforms
KF, %	6.4	6.2
TLC	no sec spots	no sec spots
Assay, % (potentiometric)	100.2	100.0

Methyltestosterone, Control No 167023

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

Examination year:	1964	1975	1980	1984	1988
DTA, mole %	-	-	0.5	0.6	0.6
HPLC, %	-	-	-	0.2	0.2
IR	conforms		-	-	conforms
LOD, %	0.3	1.2	0.3	0.8	1.2
UV-absorption, 241 nm	0.54	0.54	0.54	0.54	0.54
Assay, % (spectrophotometric)	-	-	-	100	100

Oxacillin sodium, Control No 382027

Initial analytical report: WHO/PHARM/83.510, Appendix 8

Examination year:	1982	1984	1987
pH, 1% solution	5.7	5.6	-
KF (water), %	4.3	4.5	4.1
HPLC, %	1.1	1.0	1.3
Degradation Products, % (mercurimetric)	-	0.3	0.3
Assay, % (alcalimetric)	100.0	-	-
Assay, % (mercurimetric)	-	99.4	99.4
PSA, %	0.9	-	-

Phenoxymethylpenicillin potassium, Control No 176075

Initial analytical report: WHO/PHARM/77.491, Appendix 6

Examination year:	1976	1978	1984	1987
pH, 0.5% solution	6.0	-	5.9	-
LOD, %	0.1	-	0.1	-
TGA, %	-	-	-	0.3
HPLC, %	-	0.7	0.5	1.4
Degradation products, % (mercurimetric)	-	-	0.2	0.2
Assay, % (penicillinase)	99.6	-	-	-
Assay, % (mercurimetric)	-	-	100.0	100.0
PSA, %	0.5	-	-	-

Propicillin potassium, Control No 274034

Initial analytical report: WHO/PHARM/75.485, Appendix 8

Examination year:	1974	1978	1982	1984	1987
pH, 10% solution	5.9	-	-	-	-
pH, 1.0% solution	-	-	5.3	5.3	-
KF (water), %	0.3	-	0.3	0.4	-
TGA, %	-	-	-	-	0.35
HPLC, %	-	0.4	1.0	0.8	0.5
Degradation products, % (mercurimetric)	-	-	-	0.8	0.8
Assay, % (penicillinase)	98.3	-	97.4	-	-
Assay, % (mercurimetric)	-	-	-	98.2	98.3

Testosterone propionate, Control No 167036

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

Examination year:	1964	1975	1980	1984	1988
DTA, mole %	-	-	0.2	0.2	0.3
HPLC, %	-	-	-	0.8	0.9
IR	conforms	-	-	conforms	conforms
LOD, %	0.1	0.1	0	-	-
TGA, %	-	-	-	-	0.1
TLC	no sec spots	no sec spots	no sec spots	-	no sec spots
UV-absorption, 240 nm	0.506	0.499	0.503	0.487	0.488
Assay, % (spectrophotometric)	-	-	-	100	100

APPENDIX 5

INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES - PROJECT LIST 1988

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

Volume 2

Colecalciferol

Volume 3

Amodiaquine hydrochloride	Methotrexate
Amphotericin B (*)	Neamine (*)
Bacitracin zinc (*)	(impurity in Neomycin sulfate)
Beclomethasone dipropionate	Neomycin B sulfate
Betamethasone valerate	(impurity in Neomycin sulfate)
Calcium folinate	Nifurtimox
Carbamazepine (*)	Noroxymorphone hydrochloride
Cimetidine (*)	(impurity in Naloxone hydrochloride)
Dexamethasone sodium phosphate	Nystatin
Dopamine hydrochloride	Oxytetracycline dihydrate (*)
Doxorubicin hydrochloride	Oxytetracycline hydrochloride (*)
Ergocalciferol	Paromomycin sulfate
Fludrocortisone acetate	Praziquantel
3-Formylrifamycin SV (*)	Prednisolone sodium phosphate
(impurity in Rifampicin)	Probenecid (*)
Gentamicin sulfate	Pyrantel embonate (*)
Hydrocortisone sodium succinate	Rifampicin quinone (*)
(-)-3-(4-Hydroxy-3-methoxyphenyl)-2-hydrazino- 2-methylalanine (impurity in Carbidopa)	(impurity in Rifampicin)
Levonorgestrel	Sodium cromoglicate
Levothyroxine sodium (*)	Spectinomycin hydrochloride
Liothyronine (*)	Sulfacetamide
(impurity in Levothyroxine sodium)	Sulfasalazine
Loperamide hydrochloride	Testosterone enantate
	Vincristine sulfate

Replacements

The following existing International Chemical Reference Substances should be replaced by new batches in 1988-1989

p-Acetamidobenzalazine (*)
Anhydrotetracycline hydrochloride (*)
Dexamethasone (*)
Dexamethasone acetate (*)
4-Epianhydrotetracycline hydrochloride (*)
Folic acid (*)
Prednisolone (*)
Prednisolone acetate (*)

(*) Denotes that work on the substance is in progress at the Centre.

APPENDIX 6

ALLOPURINOL

Control No 287049

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

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

MATERIAL

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

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

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

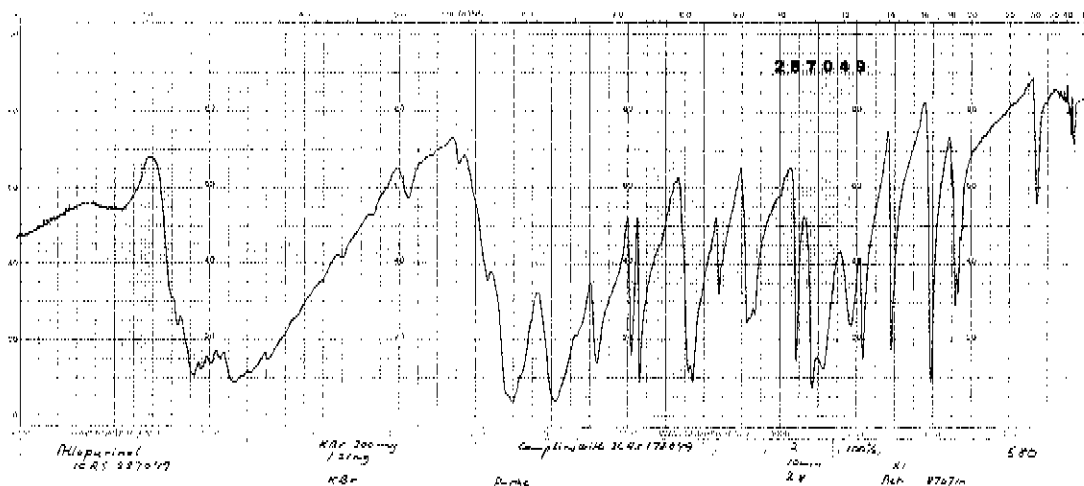


Figure 1. IR-spectrum of 1.2 mg of allopurinol in 300 mg KBr recorded against a KBr disc. Instrument: Perkin Elmer 580.

UV-spectrum

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

λ max in 0.1 M hydrochloric acid = 250 nm.

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

The absorbance of a 10 μ g/ml solution was 0.56.

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

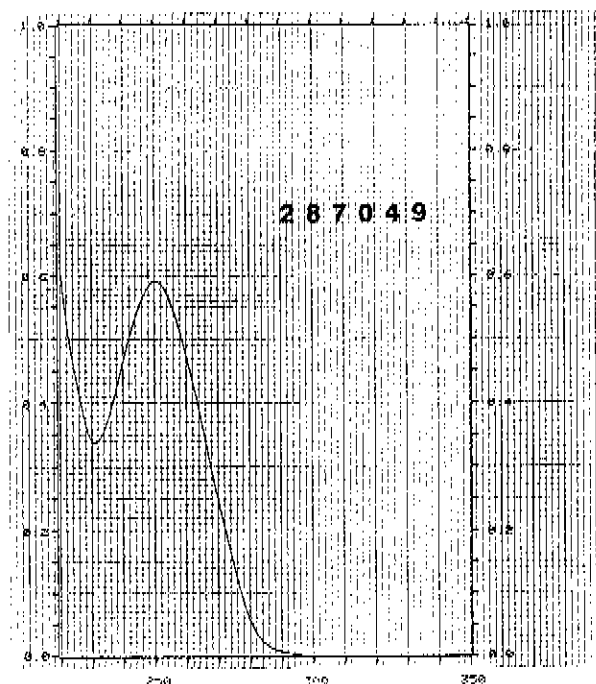


Figure 2. UV-spectrum of allopurinol 10.0 µg/ml in 0.1 M hydrochloric acid.

ASSAY

Thermogravimetric analysis

0% loss in weight.

Titrimetric assay

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

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

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

PURITY

Total solid impurities

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

Thin-layer chromatography

The following thin-layer chromatographic system was used:

Thin-layer: Cellulosa F-254 (Merck)

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

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

Visualization: UV-light of 254 nm.

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

High performance liquid chromatography

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

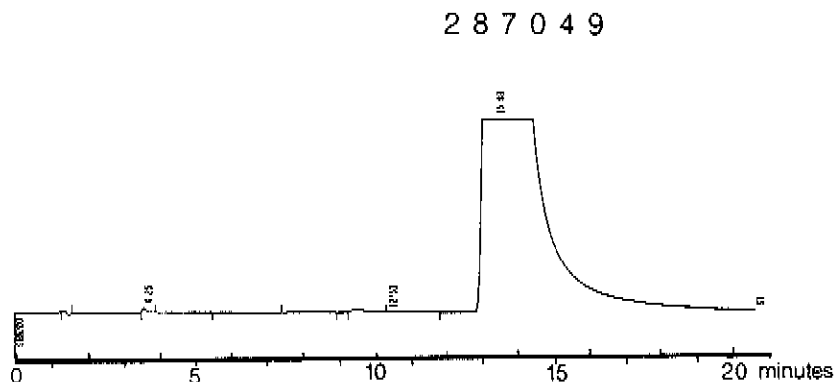


Figure 3. A chromatogram of allopurinol Control No. 287049.

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

The following conditions were used:

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

DIODE-ARRAY DETECTION

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

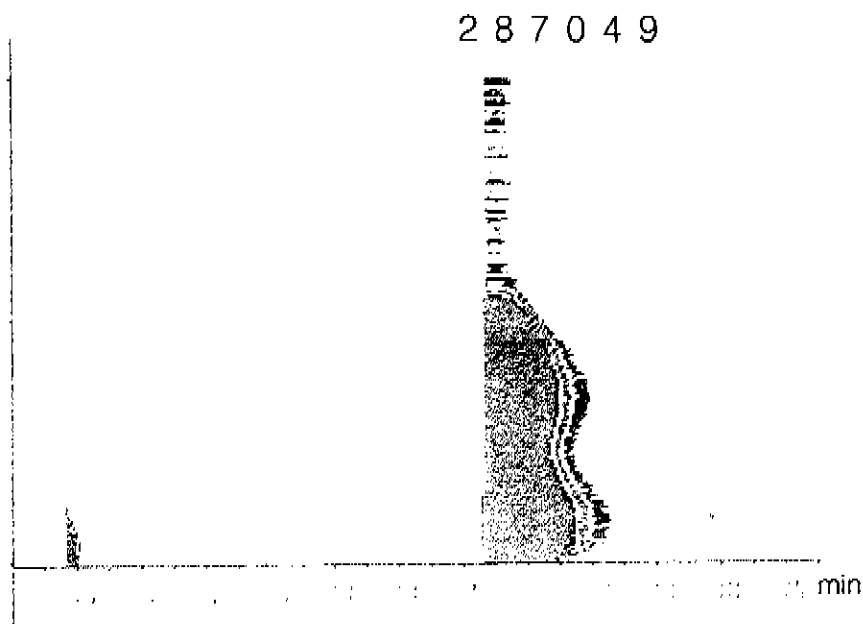


Figure 4. Isogram of allopurinol, Control No 287049. Sensitivity: 0.002

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

STABILITY

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

DATA GIVEN BY THE MANUFACTURER

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

CONCLUSION

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

APPENDIX 7

CHLORTETRACYCLINE HYDROCHLORIDE

Control No 187138

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

MATERIAL

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

ANALYTICAL DATA

Description: A yellow, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

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

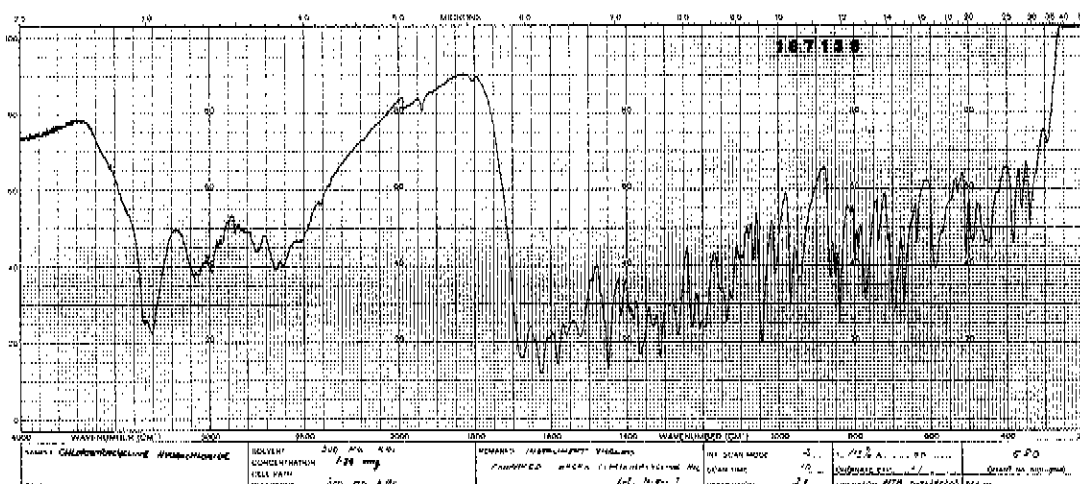


Figure 1. IR-spectrum of 1.4 mg of chlortetracycline hydrochloride in 300 mg KBr recorded against a KBr disc.
Instrument: Perkin Elmer 580.

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

UV-spectrum

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

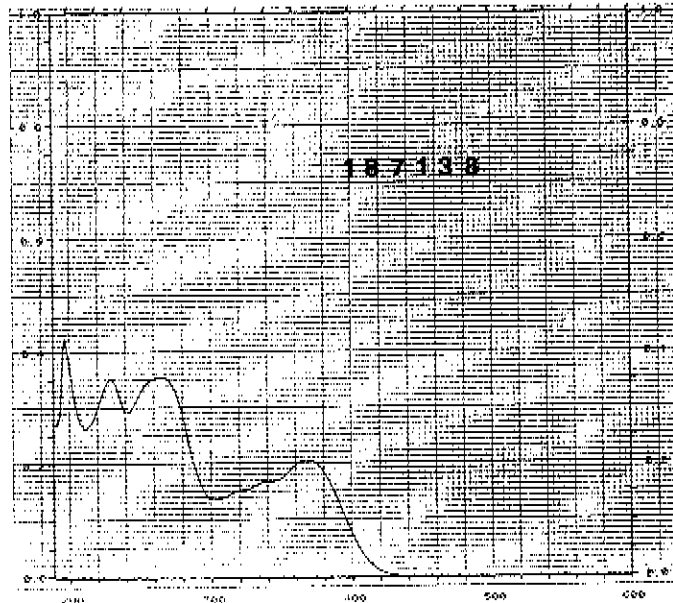


Figure 2. UV-spectrum of chlortetracycline hydrochloride 10 µg/ml in 0.01 M HCl.

Absorption in the ultraviolet region according to Ph.Int:

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

ASSAY

Thermogravimetric analysis: 0.15% loss in weight.

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

PURITY

Thin-layer chromatography

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

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

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

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

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

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

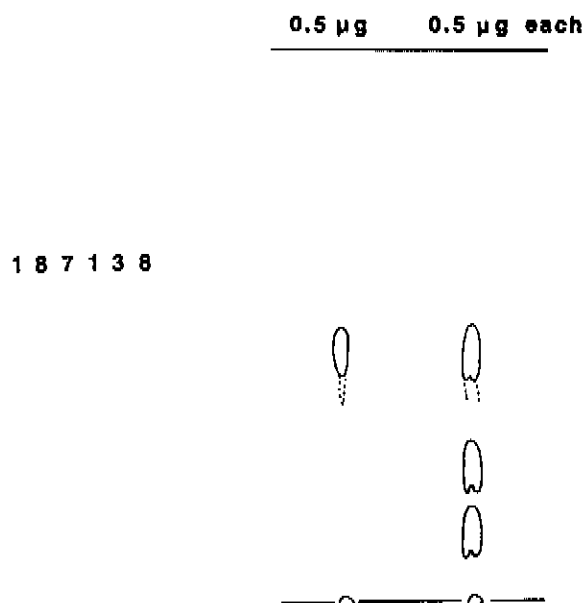


Figure 3. Thin-layer chromatogram of chlortetracycline hydrochloride Control No 187138, tetracycline hydrochloride and oxytetracycline hydrochloride.

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

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

All batches of chlortetracycline hydrochloride showed a Rf of 0.49.

High performance liquid chromatography

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

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

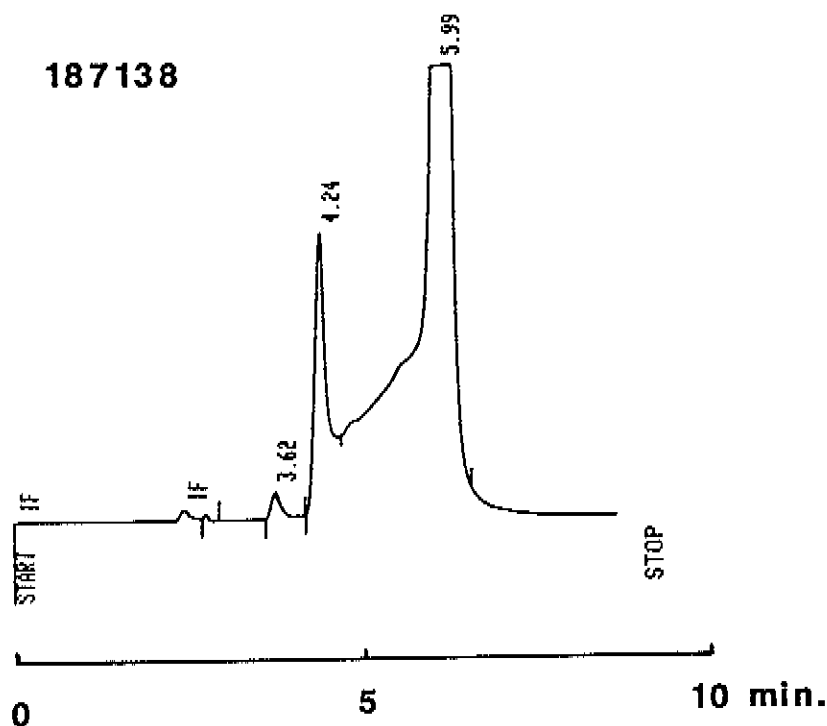


Figure 4. Chromatogram of Chlortetracycline hydrochloride Control No 187138 eluted with 20% acetonitrile: 80% phosphate buffer (0.1 M) pH 2.1.

The following conditions were used:

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

STABILITY

Chlortetracycline hydrochloride was exposed to air of different relative humidity at room temperature (about 20 °C) for a period of 8 weeks as described in WHO/PHARM/82.509. All samples were unchanged at visual inspection. The samples stored at a relative humidity of 55% and 75% picked up moisture and increased 0.3% in weight. The sample stored at 95% relative humidity increased 4.2% in weight. No signs of degradation were observed when the samples were analyzed by the liquid chromatographic method described above.

CONCLUSION

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

APPENDIX 8

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

Control No 187136

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

MATERIAL

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

ANALYTICAL DATA

Description: A white powder; odourless.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

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

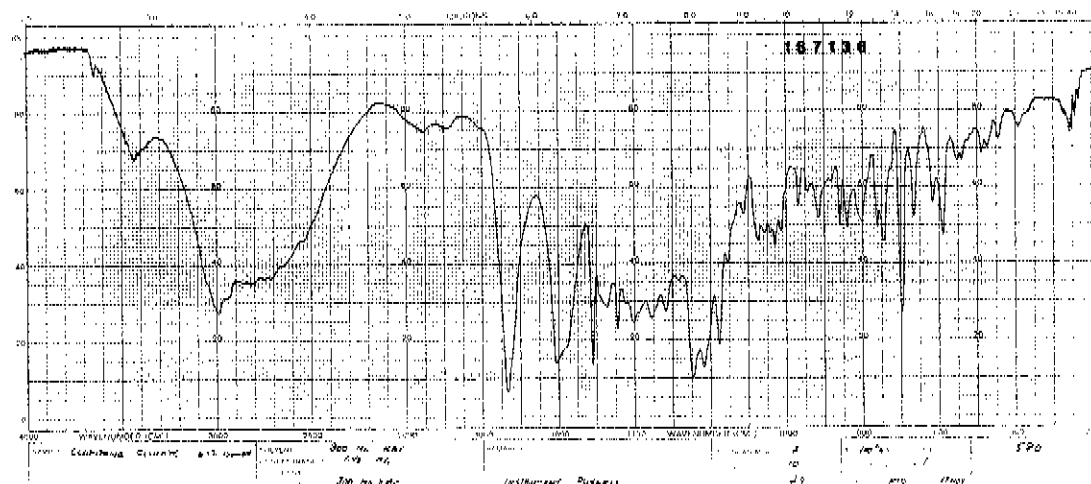


Figure 1. IR-spectrum of 2.1 mg of clomifene citrate in 300 mg KBr recorded against a KBr disc.
Instrument: Perkin Elmer 580.

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

UV-spectrum

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

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

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

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

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

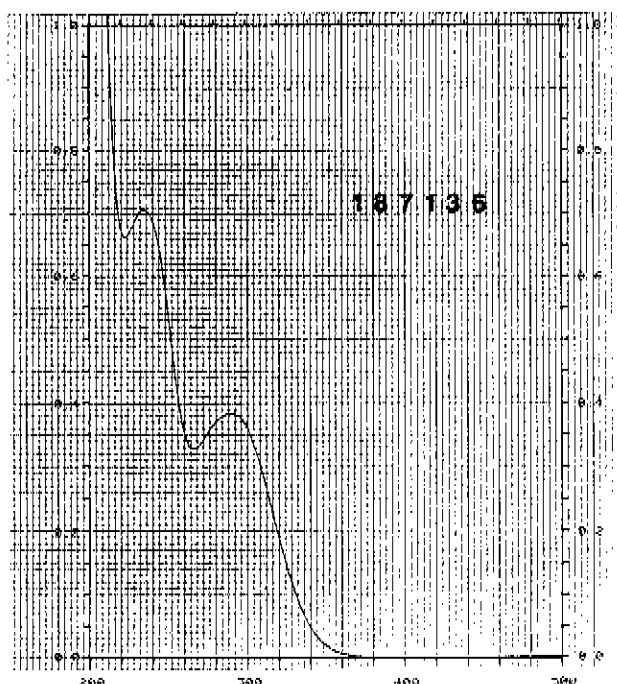


Figure 2. UV-spectrum of clomifene citrate 22 μ g/ml in 0.1 M HCl.

ASSAY

Thermogravimetric analysis

0.3% loss in weight.

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

PURITY

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

Total solid impurities

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

Thin-layer chromatography

The following thin-layer chromatographic systems were used:

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

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

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

System II:

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

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

High performance liquid chromatography

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

System 1. Straight phase

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

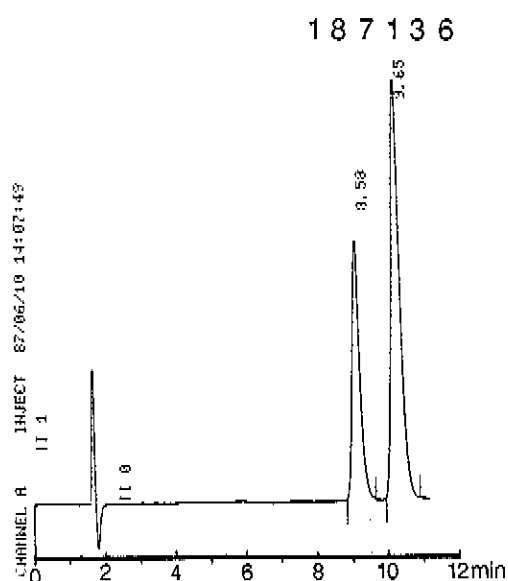


Figure 3. Chromatogram of clomifene citrate (E + Z), Control No 187136.

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

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

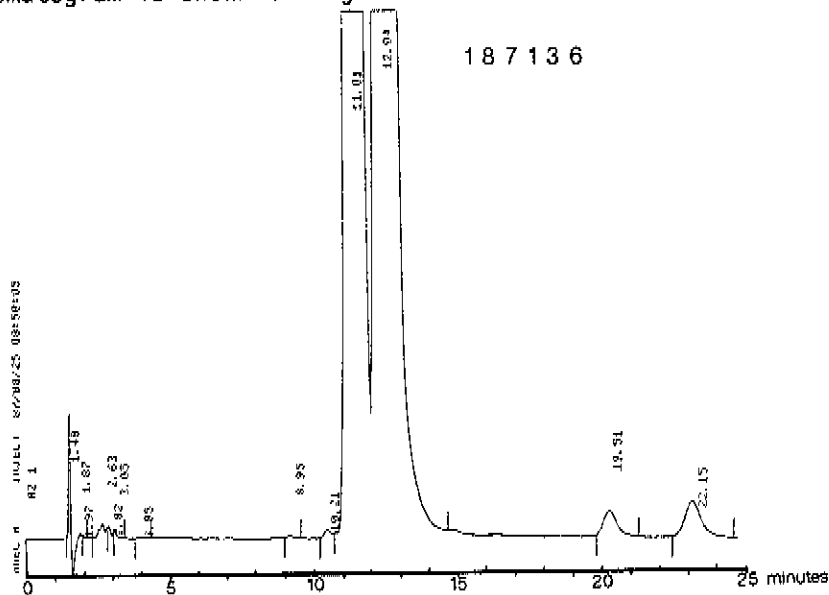


Figure 4. Chromatogram of clomifene citrate (E + Z), Control No 187136 and impurities.

The following conditions were used:

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

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

System 2. Reversed phase

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

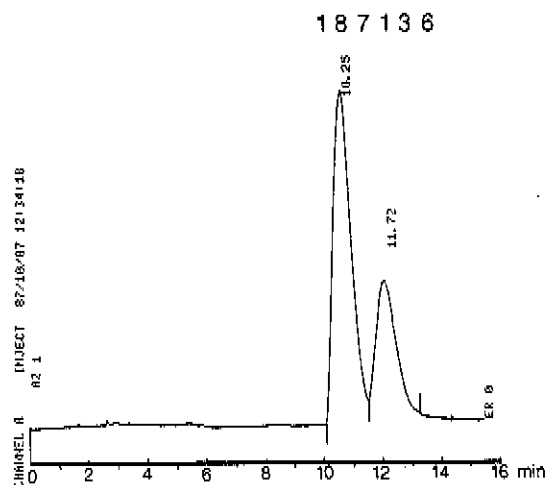


Figure 5. Chromatogram of clomifene citrate (E + Z), Control No 187136.

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

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

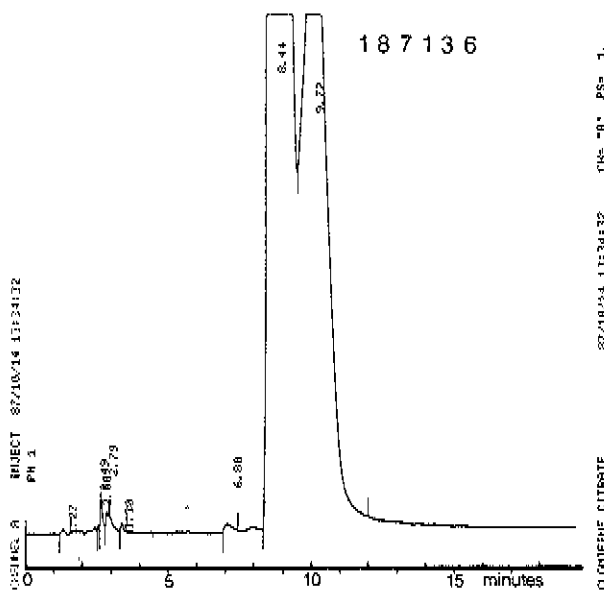


Figure 6. Chromatogram of clomifene citrate (E + Z), Control No 187136 and impurities.

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

DIODE-ARRAY DETECTION

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

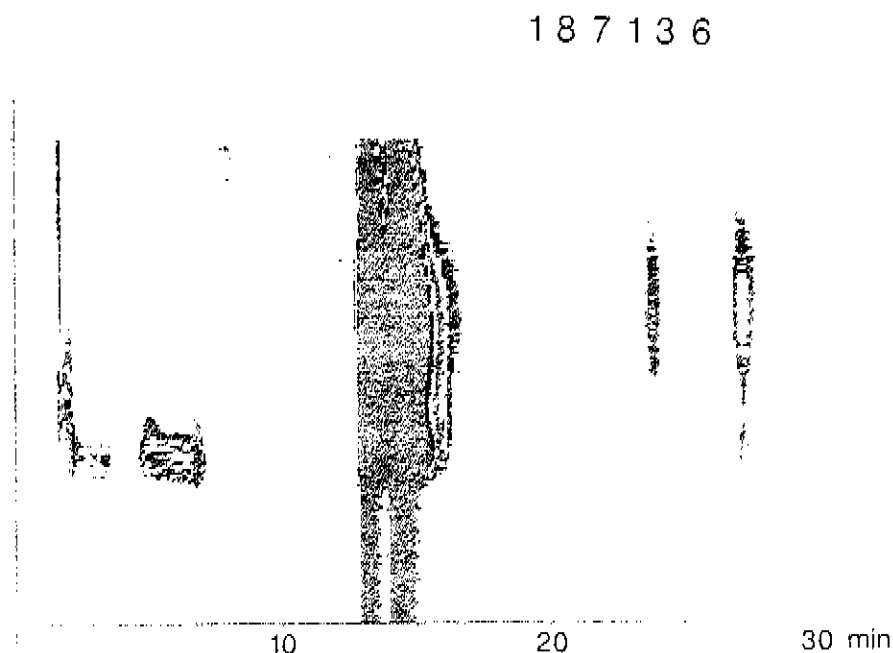


Figure 7. Isogram of clomifene citrate (E + Z), Control No 187136 when chromatographed according to system 1. Sensitivity: 0.002.

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

STABILITY

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

CONCLUSION

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

APPENDIX 9

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

Control No 187137

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

MATERIAL

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

ANALYTICAL DATA

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

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

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

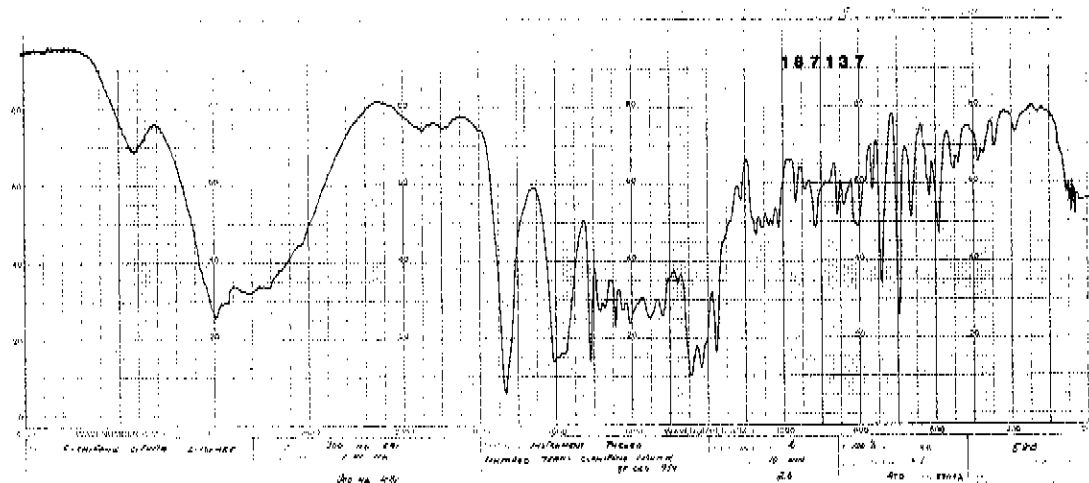


Figure 1. IR-spectrum of 2.1 mg of clomifene citrate Z-isomer in 300 mg KBr recorded against a KBr disc. Instrument: Perkin Elmer 580.

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

UV-spectrum

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

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

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

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

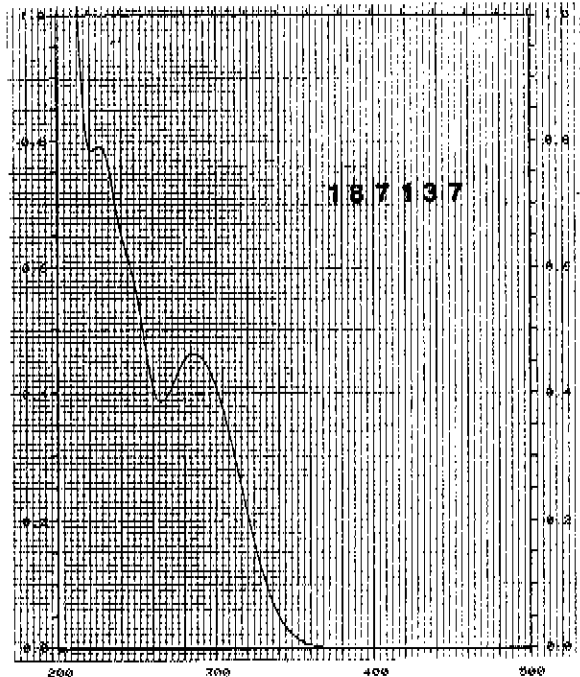


Figure 2. UV-spectrum of clomifene citrate Z-isomer 24.4 μ g/ml in 0.1 M HCl.

ASSAY

Thermogravimetric analysis

0.2% loss in weight.

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

PURITY

Total solid impurities

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

Thin-layer chromatography

The following thin-layer chromatographic systems were used:

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

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

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

System II:

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

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

High performance liquid chromatography

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

System 1. Straight phase

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

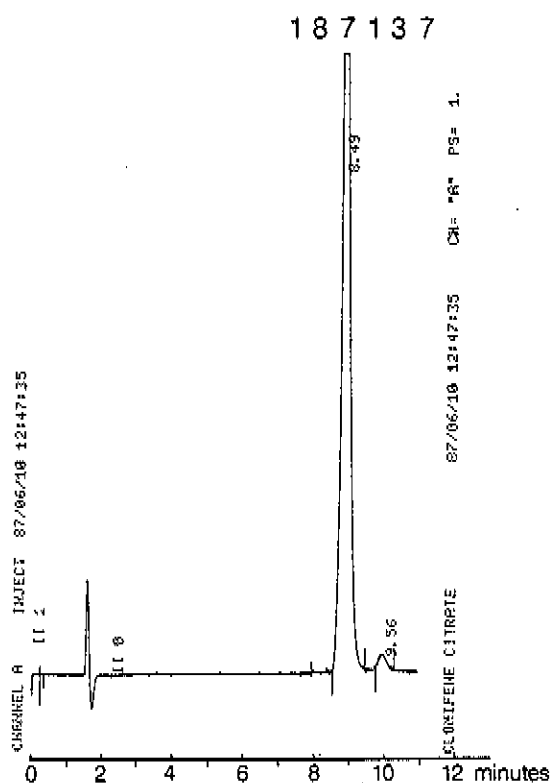


Figure 3. Chromatogram of clomifene citrate Z-isomer, Control No 187137.

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

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

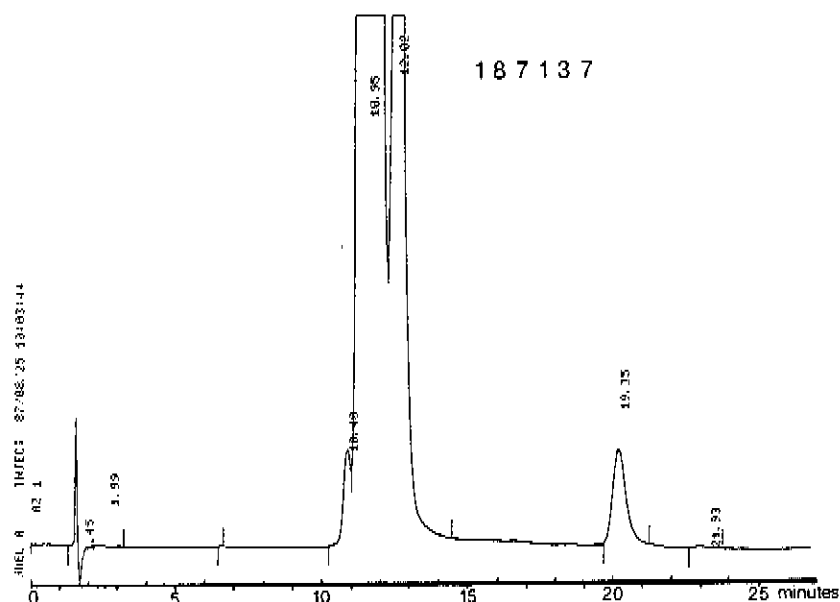


Figure 4. Chromatogram of clomifene citrate Z-isomer, Control No 187137 and impurities.

The following conditions were used:

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

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

System 2. Reversed phase

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

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

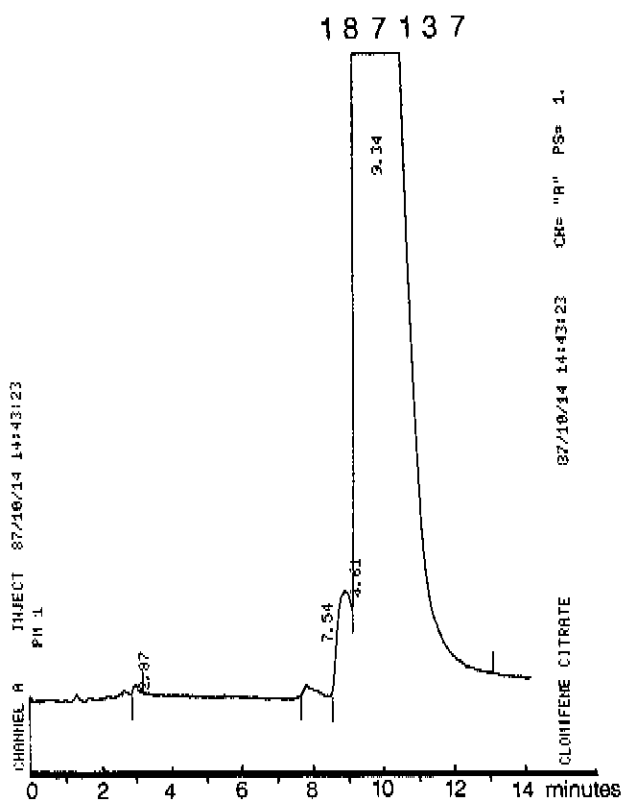


Figure 5. Chromatogram of clomifene citrate Z-isomer, Control No 187137 and impurities.

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

DIODE-ARRAY DETECTION

The chromatogram was also evaluated with a LK8 2140 Rapid Diode Array Detector. The same chromatographic system as described above under system 1 was used, except for the injection volume that was increased to 100 μ l. An isogram is given in Figure 6.

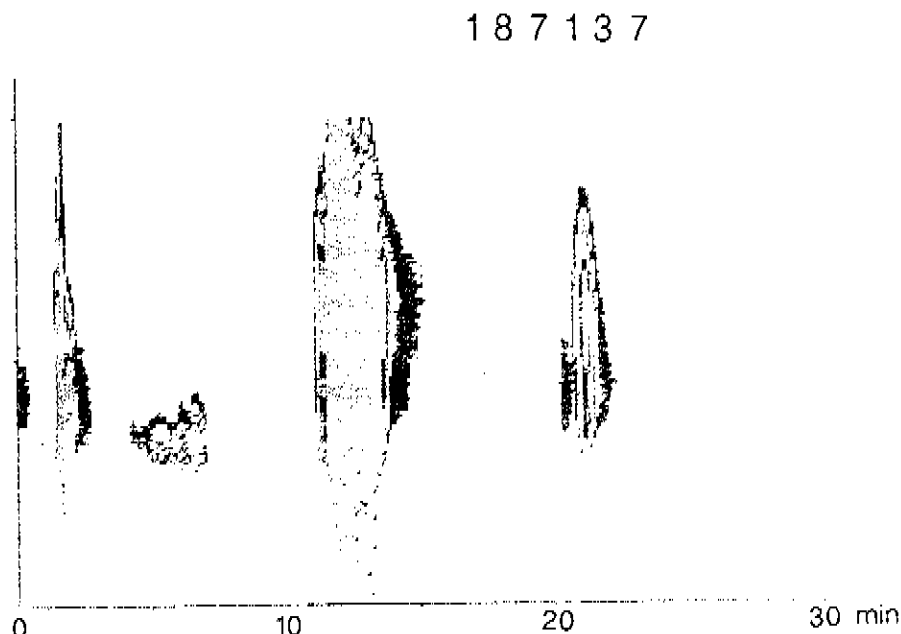


Figure 6. Isogram of clomifene citrate Z-isomer, Control No 187137 when chromatographed according to system 1. Sensitivity: 0.002.

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

STABILITY

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

CONCLUSION

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

D I G O X I N

Control No 587011

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

MATERIAL

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

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

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

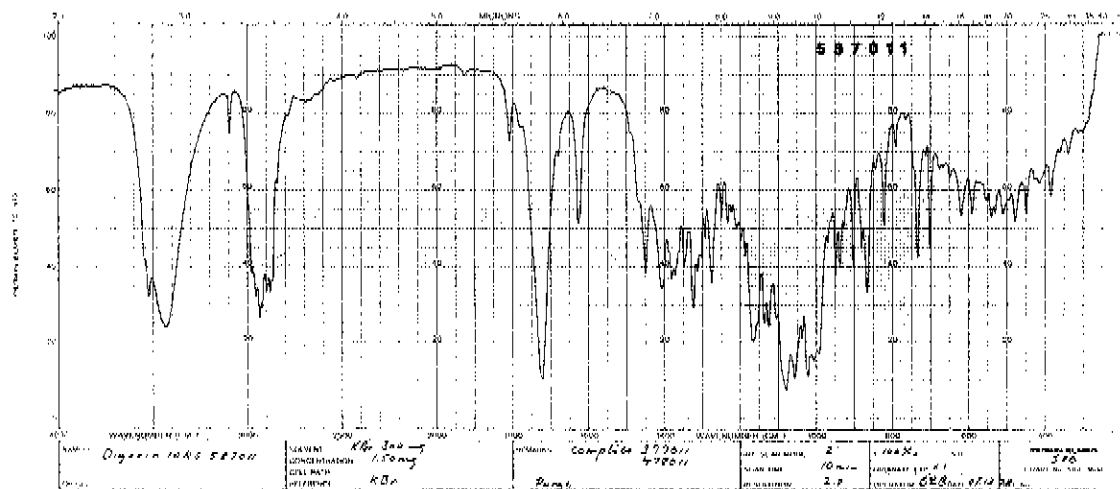


Figure 1. IR-spectrum of 1.5 mg of digoxin in 300 mg KBr recorded against a KBr disc. Instrument: Perkin-Elmer 580.

UV spectrum

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

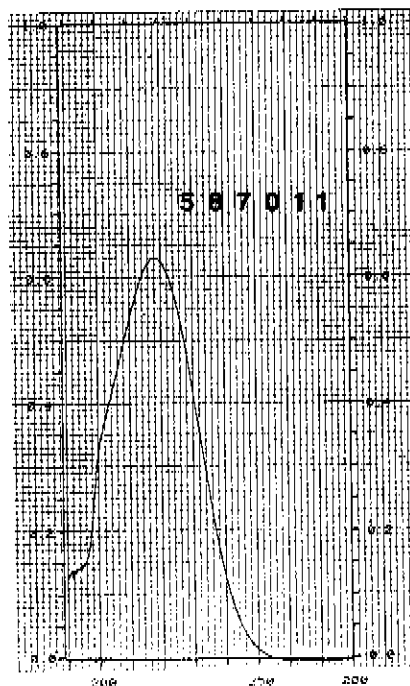


Figure 2. UV-spectrum of digoxin 30 µg/ml in ethanol.

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

ASSAY

Thermogravimetric analysis

0.15% loss in weight.

Water

0.16% determined by Karl Fischer titration.

Colorimetric assay:

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

PURITY

Thin layer chromatography

The following thin-layer chromatographic systems were used:

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

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

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

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

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

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

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

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

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

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

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

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

High performance liquid chromatography

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

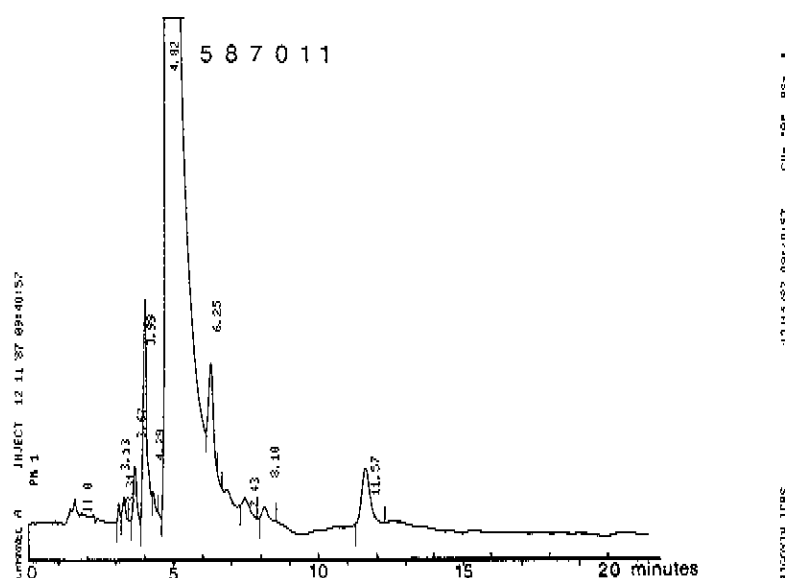


Figure 3. A chromatogram of digoxin, Control No. 587011.

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

The following conditions were used:

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

DIODE-ARRAY DETECTION

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

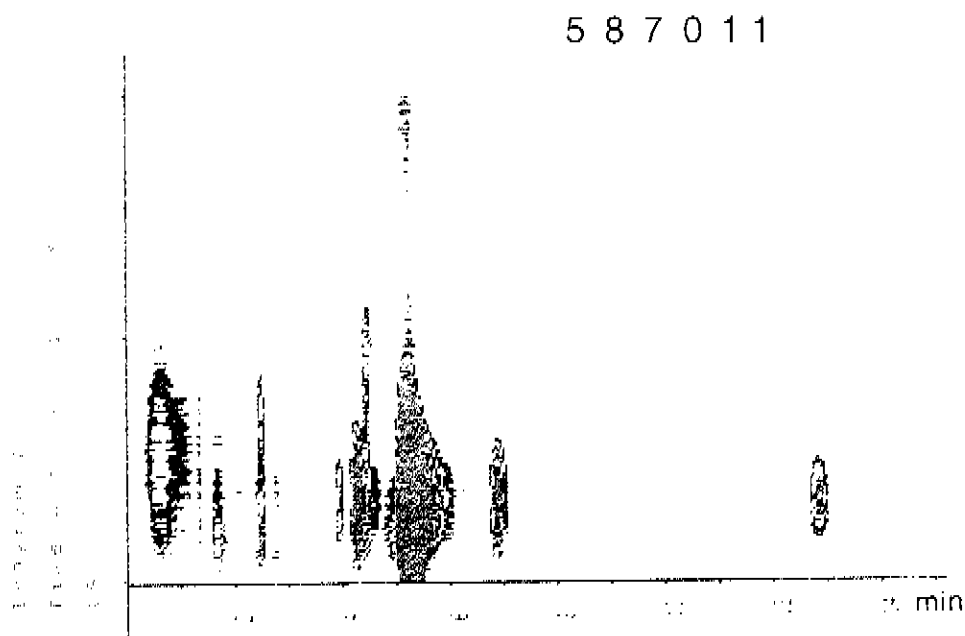


Figure 4. Isogram of digoxin, Control No 587011. Sensitivity: 0.002.

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

STABILITY

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

DATA GIVEN BY THE MANUFACTURER

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

CONCLUSION

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

EMETINE HYDROCHLORIDE

Control No 187134

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

MATERIAL

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

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

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

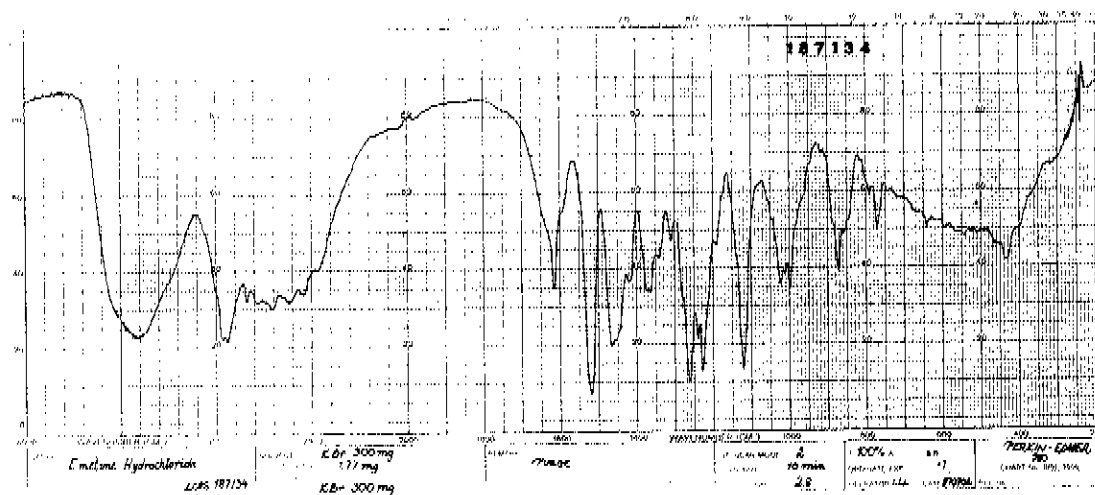


Figure 1. IR-spectrum of 1.77 mg of emetine hydrochloride in 300 mg KBr recorded against a KBr disc.

Instrument: Perkin Elmer 580.

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

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

The calculations were performed on the dried substance.

UV-spectrum

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

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

The determinations were performed with reference to the dried substance.

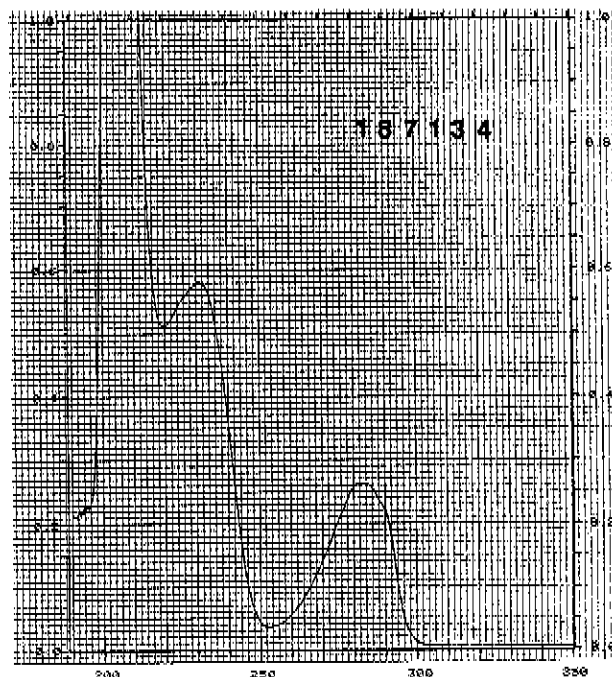


Figure 2. UV-spectrum of emetine hydrochloride 24 μ g/ml in ethanol.

ASSAY

Thermogravimetric analysis

16.8% loss in weight.

Titrimetric assay

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

PURITY

Total solid impurities

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

Thin-layer chromatography

The following thin-layer chromatographic system was used:

Thin-layer: Silica gel 60 (Merck)
Eluent: Chloroform:ethylene glycol monomethyl ether:methanol:water:diethylamine
(100:20:5:2: 0.5).
Sample: 5 and 100 μ g of emetine hydrochloride were applied. The samples were dissolved in methanol:ammonia conc. (99:1).
Visualization: Spraying with iodine/chloroform TS followed by heating at 60 °C for 15 minutes and examination in UV-light of 365 nm.
Rf (emetine hydrochloride) = 0.7

Rf (cephaeline hydrochloride) = 0.2

Rf (isometine hydrobromide) = 0.5.

The detection limit for cephaeline hydrochloride was 0.15 μg (0.15%).

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

High performance liquid chromatography

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

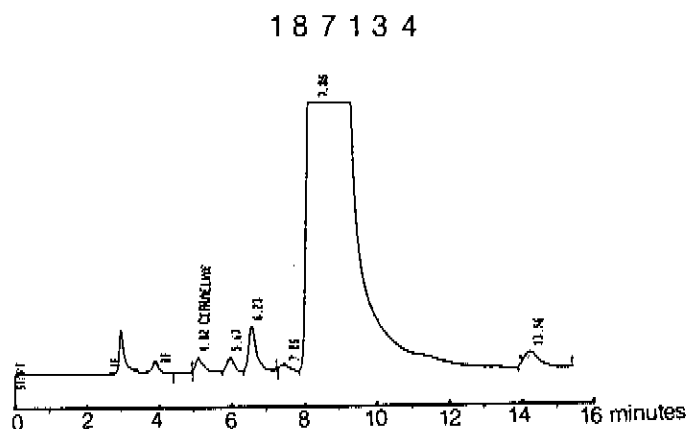


Figure 3. Chromatogram of emetine hydrochloride, Control No 187134.

The following conditions were used:

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

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

DIODE-ARRAY DETECTION

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

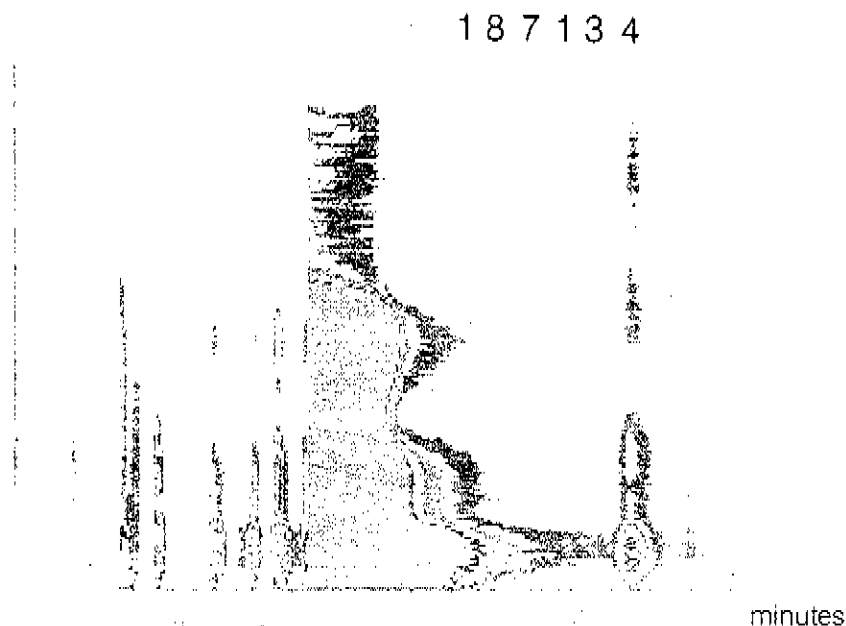


Figure 4. Isogram of emetine hydrochloride, Control No 187134. Sensitivity: 0.002.

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

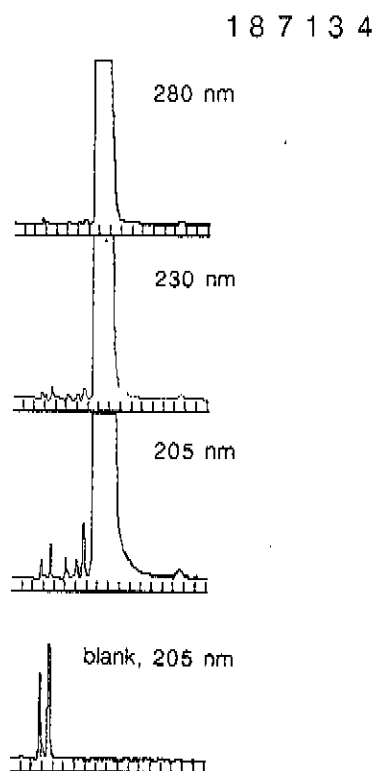


Figure 5. Chromatograms of emetine hydrochloride, Control No 187134 at 280, 230 and 205 nm.

STABILITY

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

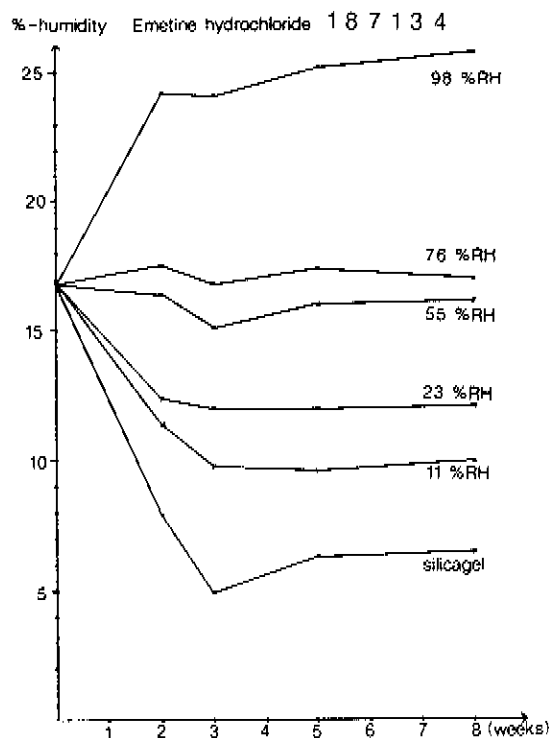


Figure 6. Stability of emetine hydrochloride, Control No 187134 stored at different relative humidity at 20 °C.

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

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

ANALYSIS PERFORMED BY THE MANUFACTURER

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

CONCLUSION

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

APPENDIX 12

NEOSTIGMINE METILSULFATE

Control No 187135

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

MATERIAL

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

ANALYTICAL DATA

Description: A white, crystalline powder; odourless.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

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

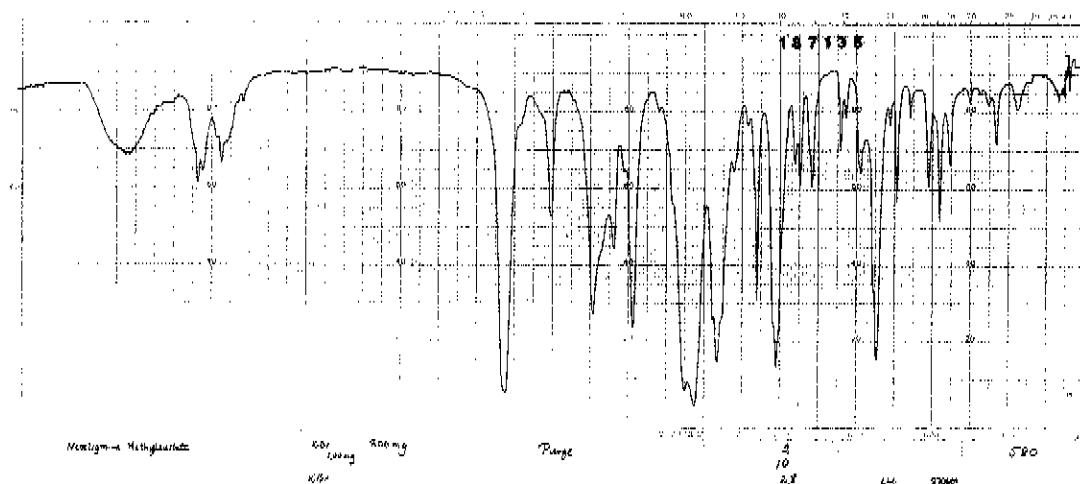


Figure 1. IR-spectrum of 1.0 mg of neostigmine metilsulfate in 300 mg KBr recorded against a KBr disc.
Instrument: Perkin Elmer 580.

UV-spectrum

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

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

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

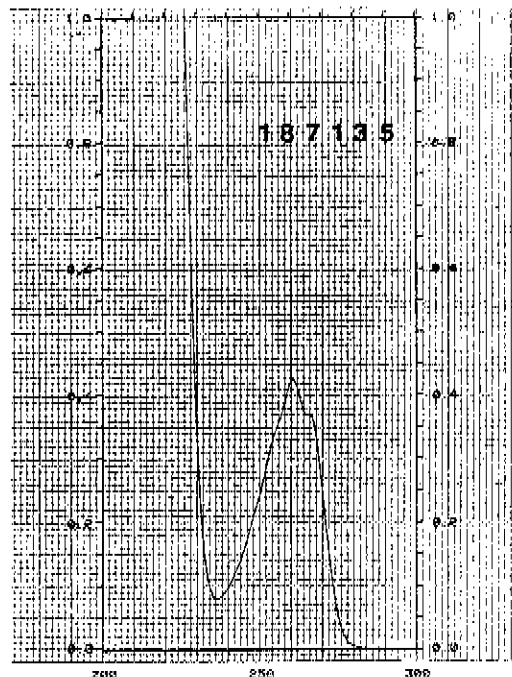


Figure 2. UV-spectrum of neostigmine metilsulfate 255 µg/ml in ethanol.

ASSAY

Loss on drying

0.05% (105 °C) (n= 2)

Thermogravimetric analysis: 0.15% loss in weight.

Titrimetric assay

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

PURITY

Total solid impurities

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

Thin-layer chromatography

The following thin-layer chromatographic systems were used:

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

Eluent: Methanol: 3% sodium chloride in water (4 + 1)

Sample: 1, 2 and 200 µg of neostigmine were applied

Visualization: UV-light of 254 nm and at 260 nm by scanning.

After spraying with a modified solution of ninhydrin (0.3 g ninhydrin, 2 ml glacial acetic acid, 2 g sodium acetate, 5 ml water and the volume adjusted with ethanol to 100 ml) heating in an oven at 130 °C for 5-10 min. After cooling, the plate was sprayed with iodoplatinate TS and observed in day-light. R_f (neostigmine metilsulfate) = 0.36. The detection limit for neostigmine metilsulfate was 1 µg (0.5%), when scanned at 260 nm. By spraying the detection limit was 20 µg (10%).

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

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

High performance liquid chromatography

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

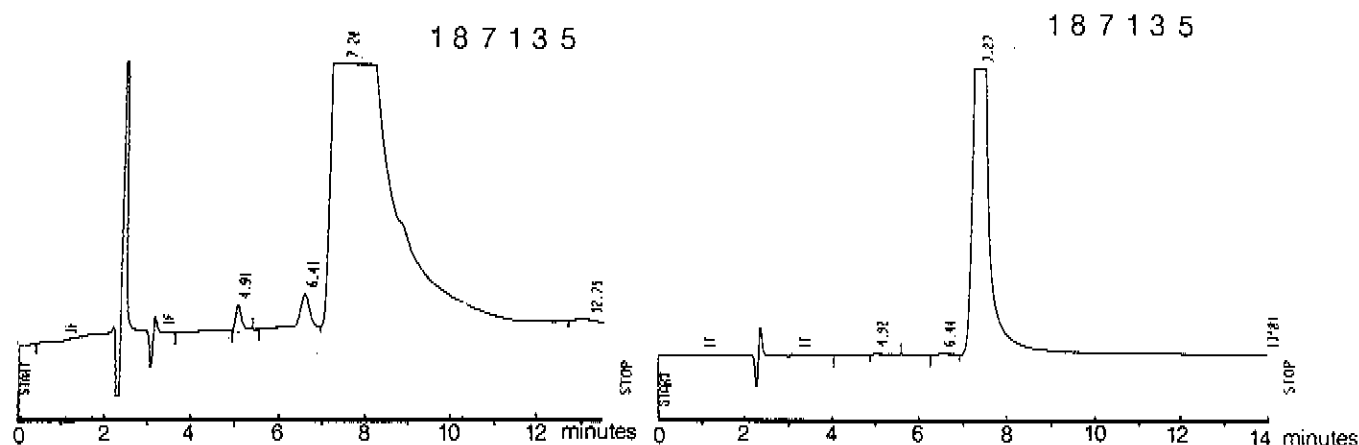


Figure 3 A + B. Chromatograms of neostigmine metilsulfate, Control No 187135. Sensitivity: 4 (A) and 8 (B).

The following conditions were used:

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

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

DIODE-ARRAY DETECTION

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

187135

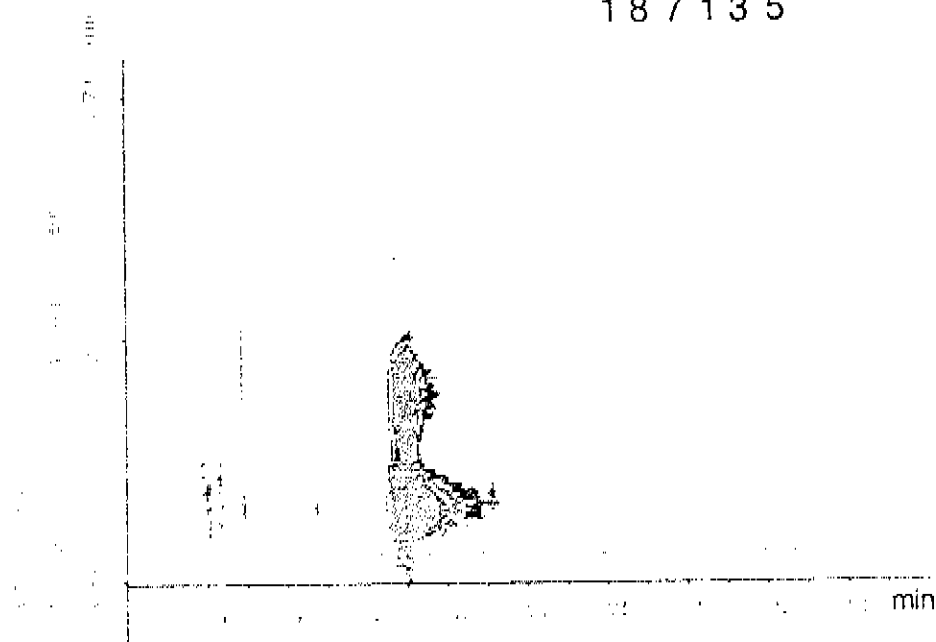


Figure 4. Isogram of neostigmine metilsulfate 187135. Sensitivity: 0.002

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

STABILITY

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

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

Additional data performed by the British Pharmacopoeia Commission Laboratory

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

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

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

PURITY

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

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

Loss on drying

(105° C): 0.23%

ASSAY

99.16% (titration)

99.6% (according to BP assay of injection)

CONCLUSION

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

PROPRANOLOL HYDROCHLORIDE

Control No 187139

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

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

MATERIAL

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

ANALYTICAL DATA

Description: A white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

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

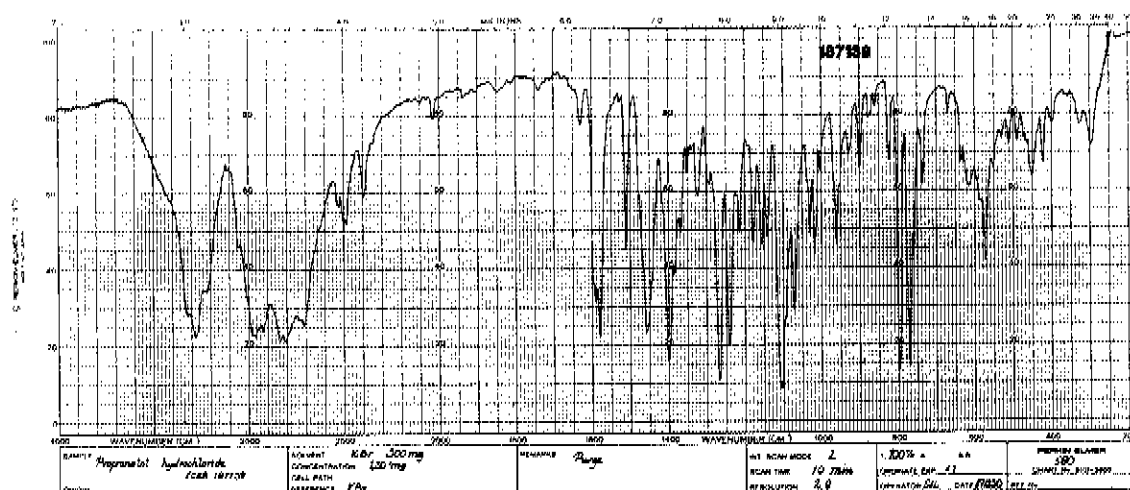


Figure 1. IR-spectrum of 1.30 mg of propranolol hydrochloride in 300 mg KBr recorded against a KBr disc.
Instrument: Perkin Elmer 580.

(*) Infrared spectrum

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

(*) UV spectrum

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

The measurements were made on a Varian DMS 100 spectrophotometer.

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

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

(*) Melting point

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

Result: 164-165 °C.

(*) GC/MS

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

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

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

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

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

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

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

(*) ASSAY

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

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

(*) 2) UV analysis

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

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

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

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

The sample content was determined to be:

289 nm: 100.5% (0.1% RSD)

319 nm: 100.2%

(*) 3) HPLC-analysis:

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

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

Column: Altex 5 µ C₁₈, 3.9 mm x 30 cm

Sample solvent: Methanol

Detection

wave length: 290 nm

Sample R_T: 13.5 minutes

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

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

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

(*) Loss on drying

0.27 - 0.35 g of material were taken and dried to constant weight at 105⁰ C.

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

PURITY

(*) Acidity

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

Total solid impurities

Differential thermal analysis (DTA): About 0.2 mol % (n= 2). The determination was carried out on 2.3 mg using a heating rate of 2⁰ C per minute.

Melting temperature: 163.1⁰C.

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

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

(*) Thin-layer chromatography

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

Coating substance: Silica gel G

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

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

Visualization: After drying in air and spraying with a mixture of 0.5 ml of anisaldehyde, 10 ml of glacial acetic acid, 85 ml of methanol and 5 ml of sulfuric acid, heat at 105⁰ C for fifteen minutes.

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

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

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

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

High performance liquid chromatography

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

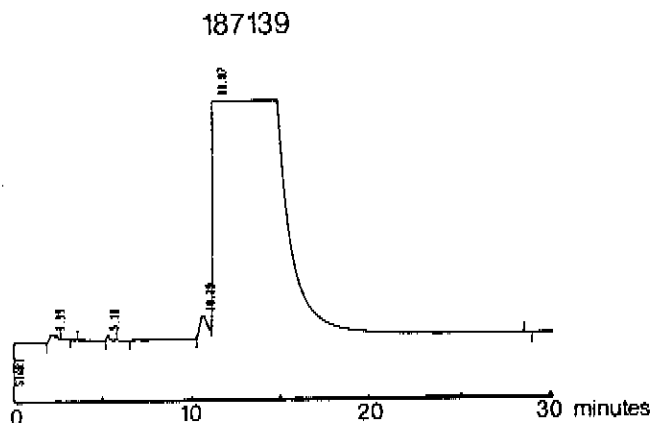


Figure 2. Chromatogram of propranolol hydrochloride, Control No 187139.

The following conditions were used:

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

(*) High Performance Liquid Chromatography

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

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

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

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

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

DIODE-ARRAY DETECTION

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

187139

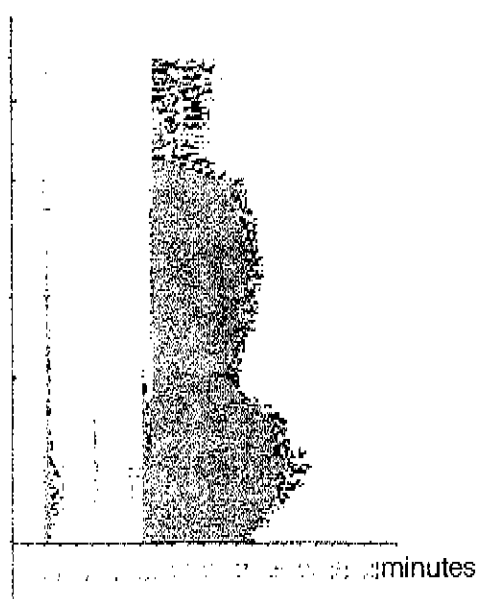


Figure 3. Isogram of propranolol hydrochloride, Control No 187139. Sensitivity: 0.002

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

(*) Purity - Diode array analysis

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

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

(*) Purity - gradient HPLC screen

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

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

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

Detection: UV at 290 nm
Sample: 200 µg/ml methanol

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

STABILITY

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

ANALYSIS PERFORMED BY THE MANUFACTURER

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

CONCLUSION

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

* * *