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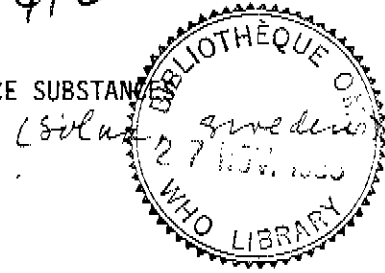
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WHO COLLABORATING CENTRE FOR CHEMICAL REFERENCE SUBSTANCES

Report on the work in 1984

by M. Abrahamsson and B. Öhrner



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Distribution of reference substances in 1984

In 1984 the Centre distributed 2643 packages of International Chemical Reference Substances and 20 sets of Melting Point Reference Substances to drug control laboratories in 47 different countries. In comparison with the distribution figures for the previous year this represents an increase of 12 per cent. The five most frequently requested substances in 1984 were, in order of demand, Ampicillin, Ampicillin Trihydrate, 4-Epitetracycline Ammonium Salt, Prednisolone and Cloxacillin Sodium. Detailed figures for the distribution of the individual substances are given in Appendix 1.

Establishment of reference substances in 1984

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), 13 International Chemical Reference Substances were established in 1984. The substances are listed in Appendix 2 to this report. Of these substances Carbenicillin Monosodium, Hydrocortisone, Ouabain and Prednisolone are replacement batches for previously existing International Chemical Reference Substances, the stocks of which had become depleted. It should be noted that the new Carbenicillin reference material consists of Carbenicillin Monosodium, which is less hygroscopic and more stable than the previously used disodium salt.

A complete list of all the International Chemical Reference Substances available from the Centre in January 1985, with information about package sizes and control numbers for the current batches, is given in Appendix 3 to this report. The list also includes 4 substances mentioned below, which are expected to be formally adopted during the first half of 1985.

Work on new reference substances completed in 1984

The Centre has continued its work to provide new reference substances required to support specifications in the third edition of the International Pharmacopoeia. During 1984 the analytical examinations of the following new reference substances were completed: Flucytosine, Fluoracil and Procarbazine Hydrochloride. The analytical reports for these materials are given in Appendices 7, 8 and 9, respectively. All the three substances were considered suitable for their intended uses and were consequently proposed for adoption as International Chemical Reference Substances.

Since the stocks of the International Chemical Reference Substance for Digoxin with control no 478011 were nearing depletion it was decided to re-examine a batch of Digoxin, which the Centre obtained in 1977, and which has been kept in reserve for possible future use. The original analysis, which was reported in the document WHO/PHARM/78.494, Appendix 8, indicated that the substance was satisfactory for the intended use. Since the re-examination revealed no signs of degradation it is proposed that the material with control number 377011 now be adopted as International Chemical Reference Substance. The analytical results from the re-examination of the batch are given in Appendix 6 to this report.

Stability testing

Each year a number of the International Chemical Reference Substances held in stock at the Centre are being re-examined to control their storage stability. During 1984 the re-examination included most of the steroids and the penicillins in the collection as well as a few other 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 but a guiding principle that is generally applicable 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 re-examination 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.

The Centre has introduced new methods for the stability testing of several substances in 1984, i.a. high performance liquid chromatography and differential thermal analysis for purity estimation and a mercurimetric titration method for the assay of penicillins. The latter also gives an estimate of degradation products in the penicillins examined. In several cases also modifications of earlier used methods were introduced. As could be expected the results obtained with the new methods differed to some extent from those obtained in the initial analysis. In no case, however, was the newly obtained result judged to indicate that the reference substance was no longer suitable for its intended use. In a few cases the newly obtained data have resulted in slight amendments of the analytical certificates for the substances. In a few other cases, where there remains some doubt as to whether the observed differences are entirely caused by the change of analytical method, more frequent re-examination of the substances will be introduced.

The results obtained in the re-examination together with the results from some of the 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

The work to establish the chemical reference substances required for the third edition of the International Pharmacopoeia continues. There are still 11 substances missing for volume 2, but for most of these the analytical work is nearly completed. To support the specifications in volume 3 a further 43 new reference substances are needed. In addition to that at least 4 existing International Chemical Reference Substances should be replaced by new batches in the coming year. Taken together this represents a formidable task. The Centre will do its utmost to carry out the work as quickly as possible, but with the limited resources at present available to the Centre it is realistic to predict that at least three years will be needed to bring the present work programme to an end. As a consequence of this situation it may also be necessary, at least for the time being, to refrain from replacing depleted stocks of existing International Chemical Reference Substances, which are not considered essential for the third edition of the International Pharmacopoeia. The reference substances which the Centre has to establish are listed in Appendix 5 to this report with indication of the substances which are already under work. Any assistance the Centre could get with the supply and/or examination of the remaining substances would be most welcome.

Administrative and financial matters

The Centre has noted with gratitude that the WHO Secretariat has acted in compliance with a resolution of the Thirty-sixth World Health Assembly (WHA 36.15) and contacted a number of international organizations (GATT, UPU and CCC) to seek their support for facilitating the distribution of International Chemical Reference Substances to the WHO member states with a minimum of delay through customs and other administrative procedures. The Centre has been informed about the reply of one of these organizations, the Customs Co-operation Council, which indicates that the matter is rather complicated and will take some time for resolving, a fact that is readily accepted by the Centre.

The financial problems mentioned in the previous annual report of the Centre remain essentially unchanged. Despite an extra contribution received from WHO headquarters the deficit in the budget of the Centre for 1984 amounted to more than 160,000 US\$. The slight improvement in comparison with the figures of last year was brought about by the price increase to 25 US \$ per package for the reference substances delivered to industrial laboratories. However, this represents only 16 per cent of the total distribution. The Centre has continued during the whole of 1984 to deliver the International Chemical Reference Substances free of charge to government drug control laboratories. It is possible that the Centre may not be able to continue this policy in the future.

Acknowledgements

This year the Director of the Centre would first of all like to express his most sincere thanks to Miss Siv Johansson, who is now leaving the Centre to take up a position as head of a group for orphan drugs at our host laboratory, the Central Laboratory of the National Corporation of Swedish Pharmacies. She has directed the laboratory work at the Centre for a large number of years in an extremely competent way and has been such a pleasant colleague to work with that there is no doubt that I have the entire staff as well as many WHO fellowship holders and other temporary workers at the Centre with me when I express our most sincere thanks and wishes her all the best for her future job.

I would then also like to welcome her successor Ms Monika Abrahamsson who came from KabiVitrum, a pharmaceutical manufacturing company in Stockholm, and express my sincere hope that she will find the work for WHO rewarding despite the sometimes seemingly limited resources for execution of the important work.

As usual the Centre also has reason to express the most sincere thanks to Mr C. A. Johnson, Scientific Director and Secretary to the British Pharmacopoeia Commission, and 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. Finally the Centre would like to express its sincere gratitude to all the 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 Hoffman-La Roche and Sandoz in Basle, Switzerland, who supplied the substances for which analytical reports are given here.

APPENDIX 1

DISTRIBUTION OF CHEMICAL REFERENCE SUBSTANCES IN 1984

Aceclidine Salicylate	1 items	Ethinylestradiol	25 items
p-Acetamidobenzalazine	3 "	Ethisterone	14 "
Allopurinol	14 "	Ethosuximide	12 "
3-Aminopyrazole-4-carboxamide		Etocarlide	1 "
Hemisulfate	7 "	Fluphenazine Decanoate	
Amitriptyline Hydrochloride	20 "	Dihydrochloride	18 "
Ampicillin	98 "	Fluphenazine Enantate	
Ampicillin Sodium	61 "	Dihydrochloride	10 "
Ampicillin Trihydrate	92 "	Fluphenazine Hydrochloride	15 "
Anhydrotetracycline Hydrochloride	51 "	Folic Acid	44 "
Atropine Sulfate	26 "	Furosemide	32 "
Azathioprine	7 "	Griseofulvin	39 "
Bendazol Hydrochloride	2 "	Haloperidol	13 "
Benzobarbital	5 "	Hydrochlorothiazide	20 "
Benzylamine Sulfate	4 "	Hydrocortisone	49 "
Benzylpenicillin Potassium	36 "	Hydrocortisone Acetate	24 "
Benzylpenicillin Sodium	56 "	(-)-3-(4-Hydroxy-3-methoxyphenyl)-	
Bephenium Hydroxynaphthoate	6 "	2-methylalanine	9 "
Betamethasone	12 "	Ibuprofen	12 "
Betanidine Sulfate	5 "	Imipramine Hydrochloride	17 "
Bupivacaine Hydrochloride	4 "	Indometacin	20 "
Caffeine	11 "	o-Iodohippuric Acid	3 "
Carbenicillin Monosodium	18 "	Lanatoside C	22 "
Chloramphenicol	59 "	Levodopa	19 "
Chloramphenicol Palmitate	36 "	Lidocaine	17 "
Chloramphenicol Palmitate		Lidocaine Hydrochloride	21 "
(Polymorph A)	14 "	Mefenamic Acid	6 "
5-Chloro-2-methylaminobenzophenone	8 "	Melting Point Reference Substances	20 "
2-(4-Chloro-3-sulfamoylbenzoyl)		(set of 13 substances)	
benzoic Acid	11 "	Metazide	1 "
Chlorphenamine Hydrogen Maleate	21 "	Methaqualone	6 "
Chlorpromazine Hydrochloride	21 "	Methyldopa	18 "
Chlortalidone	7 "	Methyltestosterone	22 "
Cloxacillin Sodium	62 "	Meticillin Sodium	18 "
Cortisone Acetate	33 "	Metronidazole	21 "
Dapsone	8 "	Nafcillin Sodium	6 "
Desoxycortone Acetate	12 "	Nicotinamide	25 "
Dexamethasone	59 "	Nicotinic Acid	20 "
Dexamethasone Acetate	12 "	Ouabain	11 "
Diazepam	25 "	Oxacillin Sodium	44 "
Diazoxide	15 "	Pheneticillin Potassium	8 "
Dicloxacillin Sodium	47 "	Phenoxyethylpenicillin	35 "
Dicolinium Iodide	3 "	Phenoxyethylpenicillin Calcium	9 "
Dicoumarol	6 "	Phenoxyethylpenicillin Potassium	43 "
Diethylcarbamazine Dihydrogen		Phenytoin	23 "
Citrate	13 "	Prednisolone	64 "
Digitoxin	27 "	Prednisolone Acetate	36 "
Digoxin	47 "	Prednisone	23 "
NN'-Di-(2,3-xyl)anthranilamide	3 "	Prednisone Acetate	14 "
4-Epianhydrotetracycline		Procaine Hydrochloride	10 "
Hydrochloride	60 "	Progesterone	34 "
4-Epitetracycline Ammonium Salt	69 "	Propicillin Potassium	15 "
Ergometrine Hydrogen Maleate	43 "	Pyridostigmine Bromide	13 "
Ergotamine Tartrate	35 "	Riboflavin	47 "
Estradiol Benzoate	20 "	Rose Bengal Sodium	-- "
Estrone	18 "	Sulfamethoxazole	26 "
Etacrynic Acid	6 "	Sulfamethoxy-pyridazine	12 "
Ethambutol Hydrochloride	21 "	Sulfanilamide	28 "

Testosterone Propionate	27	items
Tetracycline Hydrochloride	41	"
Thioacetazone	3	"
4,4'-Thiodianiline	5	"
Tolbutamide	10	"
Tolnaftate	9	"
Trimethoprim	26	"
Trimethylguanidine Sulfate	3	"
Tubocurarine Chloride	7	"
Vitamin A Acetate (solution)	46	"
Warfarin	12	"
Total	<u>2 663</u>	items

APPENDIX 2

INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES ESTABLISHED IN 1984

Reference Substance	Control number	Analytical Report	Remarks
Atropine Sulfate	183111	WHO/PHARM/84.513 Appendix 5	--
Bephenium Hydroxy- naphthoate	183112	WHO/PHARM/84.513 Appendix 6	--
Betamethasone	183113	WHO/PHARM/84.513 Appendix 7	--
Carbenicillin Monosodium	383043	WHO/PHARM/84.513 Appendix 8	Replaces No 276043
Chlortalidone	183114	WHO/PHARM/84.513 Appendix 9	--
Dapsone	183115	WHO/PHARM/84.513 Appendix 10	--
Hydrocortisone	283020	WHO/PHARM/84.513 Appendix 11	Replaces No 167020
Ibuprofen	183117	WHO/PHARM/84.513 Appendix 12	--
Metronidazole	183118	WHO/PHARM/84.513 Appendix 13	--
Ouabain	283026	WHO/PHARM/84.513 Appendix 14	Replaces No 167026
Prednisolone	283029	WHO/PHARM/84.513 Appendix 15	Replaces No 167029
Procaine Hydrochloride	183119	WHO/PHARM/84.513 Appendix 16	--
4,4'-Thiodianiline	183116	WHO/PHARM/84.513 Appendix 17	--

LIST OF AVAILABLE INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES

1985

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 re-examination 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
P.O. Box 3045
S-171 03 SOLNA
SWEDEN (Telex: 115 53)

The price for the International Chemical Reference Substances is US \$ 25.- per package which includes the cost for dispatch by air mail. Advance payment is not required.

Pharmacopoeia commissions and national authorities or other organizations carrying out official quality control of drugs on a non-profit basis may receive the International Chemical Reference Substances free of charge. When not known to the Centre such bodies may be requested to provide evidence that makes it clear that they are entitled to receive the substances without charge.

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
Allopurinol	100 mg	172049
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	379004
Chloramphenicol Palmitate	1 g	175072
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
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	377011
NN'-Di-(2,3-xyllyl)anthranilamide	50 mg	173067
4-Epianhydrotetracycline Hydrochloride	25 mg	180097
4-Epitetracycline Ammonium Salt	25 mg	180098
Ergometrine Hydrogen Maleate	50 mg	277012
Ergotamine Tartrate	50 mg	276013
Estradiol Benzoate	100 mg	167014
Estrone	100 mg	279015
Etacrynic Acid	100 mg	281056
Ethambutol Hydrochloride	100 mg	179081
Ethinylestradiol	100 mg	167016
Ethisterone	100 mg	167017
Ethosuximide	100 mg	179088
Etocarlide	100 mg	172057
Flucytosine	100 mg	184121
Fluouracil	100 mg	184122

<u>Reference substance</u>	<u>Package size</u>	<u>Control number for current batch</u>
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
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)	13 x 4 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
Nicotinamide	100 mg	179090
Nicotinic Acid	100 mg	179091
Ouabain	100 mg	283026
Oxacillin Sodium	200 mg	382027
Pheneticillin Potassium	200 mg	167028
Phenoxymethylpenicillin	200 mg	179082
Phenoxymethylpenicillin Calcium	200 mg	179083
Phenoxymethylpenicillin 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
Pyridostigmine Bromide	100 mg	182110
Riboflavin	250 mg	382035
Rose Bengal Sodium	100 mg	173070
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

<u>Reference substance</u>	<u>Package size</u>	<u>Control number for current batch</u>
Tolbutamide	100 mg	179086
Tolnaftate	100 mg	176074
Trimethoprim	100 mg	179093
Trimethylguanidine Sulfate	100 mg	172059
Tubocurarine Chloride	100 mg	170037
Vitamin A Acetate (solution)	5 caps. (*)	581038
Warfarin	100 mg	168041

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

STABILITY TESTING

The storage stability of the International Chemical Reference Substances is monitored by regular re-examination of the substances held in stock at the Centre. The results obtained for the substances re-examined in 1984 are summarized below. For comparison results obtained at earlier occasions are included in the summaries. For the sake of brevity the following abbreviations are used in the tables:

DTA Differential Thermal Analysis
 ETSI Estimated Total Solid Impurities
 HPLC High Performance Liquid Chromatography
 KF Karl Fischer method for determination of water
 PSA Phase Solubility Analysis
 TLC Thin-layer Chromatography

The estimates of total solid impurities obtained by HPLC are expressed as area per cent, by DTA as mole per cent, and by PSA as weight per cent. Assay values are calculated with reference to the dried or anhydrous substance.

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

Ampicillin, Control No 274001

Initial analytical report: WHO/PHARM/75.485, Appendix 4

Examination year:	1974	1978	1981	1984
pH, 0.25% solution	4.9	-	4.9	4.8
Water (KF), %	0.8	-	0.3	0.5
ETSI (HPLC), area %	-	0.5	0.6	0.5
Degradation products, % (mercurimetric)	-	-	-	1.0
Assay (penicillinase), %	99.9	-	98.7	-
Assay (mercurimetric), %	-	-	-	98.9

Ampicillin Sodium, Control No 274002

Initial analytical report: WHO/PHARM/75.485, Appendix 5

Examination year:	1974	1978	1981	1982	1984
pH, 0.25% solution	8.8	-	9.0	9.1	8.9
Water (KF), %	1.2	-	0.9	0.7	1.3
ETSI (HPLC), area %	-	2.2	-	2.3	2.6
Degradation products (mercurimetric)	-	-	-	-	4.5
Assay (penicillinase), %	96.6	-	95.7	95.5	-
Assay (mercurimetric), %	-	-	-	-	94.6

Ampicillin Trihydrate, Control No 274003

Initial analytical report: WHO/PHARM/75.485, Appendix 6

Examination year:	1974	1978	1981	1982	1984
pH, 0.25% solution	5.1	-	5.1	5.1	5.1
Water (KF), %	13.9	-	13.9	13.5	13.3
ETSI (HPLC), area %	-	0.3	0.6	0.3	0.9
Degradation products, % (mercurimetric)	-	-	-	-	0.9
Assay (penicillinase), %	98.5	-	99.0	-	-
Assay (mercurimetric), %	-	-	-	-	98.6
PSA, %	1.0	-	-	-	-

Benzylpenicillin Potassium, Control No 180099

Initial analytical report: WHO/PHARM/81.508, Appendix 6

Examination year:	1980	1984
pH, 2% solution	6.2	6.2
Loss on drying, 105°C, %	0.04	0.11
ETSI (HPLC), area %	<1.0	0.5
Degradation products, % (mercurimetric)	-	0.15
Assay (penicillinase), %	98.3	-
Assay (mercurimetric), %	-	98.8

Benzylpenicillin Sodium, Control No 280047

Initial analytical report: WHO/PHARM/81.508, Appendix 7

Examination year:	1980	1984
pH, 2% solution	6.9	6.8
Loss on drying, 105°C, %	0.0	0.1
ETSI (HPLC), area %	0.7	0.5
ETSI (PSA), %	0.8	-
Degradation products, % (mercurimetric)	-	0.2
Assay (penicillinase), %	98.8	-
Assay (mercurimetric), %	-	98.8

Chloramphenicol, Control No 379004

Initial analytical report: WHO/PHARM/80.504, Appendix 5

Examination year:	1979	1984
UV-absorption 278 nm, E(1 %, 1 cm)	294.5	295.3
ETSI (DTA), mole %	0.14	0.15
ETSI (HPLC), area %	<0.1	<0.1

Chlorpromazine Hydrochloride, Control No 178080

Initial analytical report: WHO/PHARM/79.499, Appendix 4

Examination year:	1970	1984
UV-absorption, E(1 %, 1 cm)		
257 nm	1011	1003
310 nm	125	112
pH, 1% solution	4.1	4.3
ETSI (TLC), %	<0.5	<0.5
ETSI (DTA), mole %	0.37	0.33
Loss on drying, %	0.1%	0.0%

Cloxacillin Sodium, Control No 274005

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

Examination year:	1974	1978	1979	1982	1984
pH, 2% solution	-	-	-	5.9	5.9
pH, 10% solution	6.3	-	-	6.2	-
Water (KF), %	4.2	-	4.0	3.8	4.0
ETSI (HPLC), area %	-	0.5	-	0.9	1.0
Degradation products, % (mercurimetric)	-	-	-	-	0.7
Assay (alcalimetric), %	100.2	-	99.1	100.2	-
Assay (mercurimetric), %	-	-	-	-	98.9

Cortisone Acetate, Control No 167006

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

Examination year:	1966	1975	1984
UV-absorption 238 nm, E(1 %, 1 cm)	402	404	396
Loss on drying, %	0.0	0.2	-
TLC	3 sec.	2 sec.	3 sec.
	spots	spots	spots
ETSI (HPLC), area %	-	-	0,3
ETSI (PSA), %	<0.5	-	-

Desoxycortone Acetate, Control No 167007

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

Examination year:	1965	1975	1980	1984
UV-absorption 242 nm, E(1%, 1cm)	456	452	-	455
Loss on drying, %	0.0	0.1	-	-
ETSI (DTA), mole %	-	-	0.67	0.73
TLC	No sec.	No sec.	-	2 sec.
	spot	spot	-	spots
ETSI (HPLC), area %	-	-	-	0.2
ETSI (PSA) %	<1	-	-	-

Dexamethasone, Control No 279008

Initial analytical report: WHO/PHARM/80.504, Appendix 6

Examination year:	1979	1984
UV-absorption 239 nm, E(1%, 1 cm)	392	396
Loss on drying, %	0.1	-
TLC	3 sec.	4 sec.
	spots	spots
ETSI (TLC) %	1	-
ETSI (HPLC), area %	1.0	1.1
ETSI (PSA), %	0.8	-

Dexamethasone Acetate, Control No 168009

Initial analytical report: WHO/PHARM/69.452, Appendix 2

Examination year:	1968	1975	1984
UV-absorption 239 nm E(1 %, 1 cm)	355	355	355
Loss on drying, %	0.1	0.2	0.1
TLC	2 sec. spots	2 sec. spots	4 sec. spots
ETSI (HPLC), area %	-	-	2.3
ETSI (PSA), %	1.4	-	1.8 ± 0.2

Dicloxacillin Sodium, Control No 174071

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

Examination year:	1974	1982	1984
pH, 1% solution	5.6	5.9	5.8
Water (KF), %	3.8	3.9	3.8
ETSI (HPLC), area %	-	0.3	0.4
Degradation products, % (mercurimetric)	-	-	0.6
Assay (alcalimetric), %	99.5	99.7	-
Assay (mercurimetric), %	-	-	99.5

Estradiol Benzoate, Control No 167014

Initial analytical report: WHO/PHARM/66.431, Appendix 5

Examination year:	1965	1975	1984
UV-absorption 230 nm, E(1 %, 1 cm)	507	508	510
Loss on drying, %	0.1	0.2	-
TLC	No sec. spot	2 sec. spots	3 sec. spots
ETSI (HPLC), area %	-	-	1.2
ETSI (PSA), %	0.5	-	-

Estrone, Control No 279015

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

Examination year:	1978	1984
UV-absorption 281 nm, E(1 %, 1 cm)	784	774
Loss on drying, %	0.0	-
TLC	2 sec. spots	4 sec. spots
ETSI (TLC), %	0.2	-
ETSI (HPLC), area %	<0.2	0.1
ETSI (PSA), %	0.6	-

Ethinylestradiol, Control No 167016

Initial analytical report: WHO/PHARM/67.441, Appendix 2

Examination year:	1966	1975	1980	1984
UV-absorption 281 nm, E(1 %, 1cm)	72	71	-	72
Loss on drying, %	0.1	0.4	-	-
ETSI (DTA), mole %	-	-	0.44	0.53
TLC	1 sec. spot	1 sec. spot	-	1 sec. spot
ETSI (HPLC), area %	-	-	-	0.5
ETSI (PSA), %	0.5	-	-	-

Ethisterone, Control No 167017

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

Examination year:	1965	1975	1984
UV-absorption 242 nm, E(1 %, 1 cm)	525	521	522
Loss on drying, %	0.0	0.3	-
TLC	2 sec. spots	2 sec. spots	3 sec. spots
ETSI (HPLC), area %	-	-	0.5
ETSI (PSA), %	0.5	-	-

Methyldopa, Control No 179084

Initial analytical report: WHO/PHARM/80.504, Appendix 10

Examination year:	1979	1984
UV-absorption 280 nm, E(1 %, 1 cm) (anhydrous)	133.4	132.0
Water (KF), %	11.5	11.5
ETSI (HPLC), area %	0.2	0.2

Methyltestosterone, Control No 167023

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

Examination year:	1964	1975	1980	1984
UV-absorption 242 nm, E(1 %, 1 cm)	541	540	-	537
Loss on drying, %	0.3	1.2	-	0.8
ETSI (DTA), mole %	-	-	0.5	0.6
TLC	1 sec. spot	2 sec. spots	-	-
ETSI (HPLC), area %	-	-	-	0.2
ETSI (PSA), %	0.2	-	-	-

Meticillin Sodium, Control No 274024

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

Examination year:	1974	1978	1984
pH, 10% solution	6.3	-	-
pH, 1.0% solution	-	-	6.0
Water (KF), %	4.7	-	4.6
ETSI (HPLC), area %	-	0.0	0.2
Degradation products, % (mercurimetric)	-	-	0.4
Assay (alcalimetric), %	99.9	-	-
Assay (mercurimetric), %	-	-	99.5

Nafcillin Sodium, Control No 272025

Initial analytical report: WHO/PHARM/72.471, Appendix 6

Examination year:	1972	1978	1979	1984
pH, 3% solution	6.3	-	6.2	6.2
Water (KF), %	4.0	-	4.3	4.2
ETSI (HPLC), area %	-	0.0	-	0.1
ETSI (PSA), %	1.0	-	-	-
Degradation products, % (mercurimetric)	-	-	-	0.3
Assay (alcalimetric), %	99.9	-	99.8	-
Assay (mercurimetric), %	-	-	-	99.1

Oxacillin Sodium, Control No 382027

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

Examination year:	1982	1984
pH, 1% solution	5.7	5.6
Water (KF), %	4.3	4.5
ETSI (HPLC), area %	1.1	1.0
Degradation products, % (mercurimetric)	-	0.3
Assay (alcalimetric), %	100.0	-
Assay (mercurimetric), %	-	99.4
ETSI (PSA), %	0.9	-

Pheneticillin Potassium, Control No 167028

Initial analytical report: WHO/PHARM/68.448, Appendix 7

Examination year:	1967	1974	1977	1978	1982	1984
pH, 10% solution	6.1	6.1	6.2	-	-	-
pH, 1% solution	-	-	-	-	5.9	5.8
Water (KF), %	0.5	0.9	0.6	-	0.3	0.4
ETSI (HPLC), area %	-	-	-	0.4	0.5	0.6
Degradation products, % (mercurimetric)	-	-	-	-	-	0.6
Assay (penicillinase), %	-	99.1	99.3	-	98.4	-
Assay (mercurimetric), %	-	-	-	-	-	99.5

Phenoxymethylpenicillin, Control No 179082

Initial analytical report: WHO/PHARM/79.499, Appendix 9

Examination year:	1977	1984
pH; 0.5% suspension	3.0	2.8
Water (KF), %	0.2	0.2
ETSI (HPLC), area %	0.8	0.6
Degradation products, % (mercurimetric)	-	0.2
Assay (alcalimetric), %	100.1	-
Assay (mercurimetric), %	-	99.6
ETSI (PSA), %	1.0	-

Phenoxymethylpenicillin Calcium, Control No 179083

Initial analytical report: WHO/PHARM/79.499, Appendix 10

Examination year:	1979	1984
pH, 0.5% solution	5.7	5.6
Water (KF), %	4.9	4.9
ETSI (HPLC), area %	2.0	1.3
Degradation products, % (mercurimetric)	-	0.3
Assay (penicillinase), %	100.0	-
Assay (mercurimetric), %	-	99.2

Phenoxymethylpenicillin Potassium, Control No 176075

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

Examination year:	1976	1978	1984
pH, 0.5% solution	6.0	-	5.9
Loss on drying, %	0.1	-	0.1
ETSI (HPLC), area %	-	0.7	0.5
Degradation products, % (mercurimetric)	-	-	0.2
Assay (penicillinase), %	99.6	-	-
Assay (mercurimetric), %	-	-	100.0
ETSI (PSA), %	0.5	-	-

Prednisolone Acetate, Control No 167030

Initial analytical report: WHO/PHARM/66.431, Appendix 7

Examination year:	1966	1975	1984
UV-absorption 242 nm, E(1 %, 1 cm)	382	377	377
Loss on drying, %	0.0	0.2	-
TLC	2 sec. spots	1 sec. spot	3 sec. spots
ETSI (HPLC), area %	-	-	1.8
ETSI (PSA), %	0.5	-	-

Prednisone, Control No 167031

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

Examination year:	1966	1975	1984
UV-absorption 238 nm, E(1 %, 1 cm)	431	419	426
Loss on drying, %	0.0	0.1	-
TLC	No sec. spot	No sec. spot	2 sec. spots
ETSI (HPLC), area %	-	-	0.7
ETSI (PSA), %	0.5	-	-

Prednisone Acetate, Control No 169032

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

Examination year:	1969	1975	1984
UV-absorption 238 nm, E(1 %, 1 cm)	372	371	383
Loss on drying, %	0.1	0.3	-
TLC	4 sec. spots	2 sec. spots	2 sec. spots
ETSI (HPLC), area %	-	-	1.5
ETSI (PSA), %	<0.5	-	-

Progesterone, Control No 167033

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

Examination year:	1966	1975	1980	1984
UV-absorption 242 nm, E(1 %, 1 cm)	545	544	-	538
Loss on drying, %	0.0	0.1	-	-
ETSI (DTA), mole %	-	-	0.1	0.1
TLC	No sec. spot	No sec. spot	-	-
ETSI (HPLC), area %	-	-	-	0.1
ETSI (PSA), %	<0.5	-	-	-

Propicillin Potassium, Control No 274034

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

Examination year:	1974	1978	1982	1984
pH, 10% solution	5.9	-	-	-
pH, 1.0% solution	-	-	5.3	5.3
Water (KF), %	0.3	-	0.3	0.4
ETSI (HPLC), area %	-	0.4	1.0	0.8
Degradation products, % (mercurimetric)	-	-	-	0.8
Assay (penicillinase), %	98.3	-	97.4	-
Assay (mercurimetric), %	-	-	-	98.2

Testosterone Propionate, Control No 167036

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

Examination year:	1964	1975	1980	1984
UV-absorption 241 nm, E(1 %, 1 cm)	506	499	503	487
Loss on drying, %	0.1	0.1	0.0	-
ETSI (DTA), mole %	-	-	0.2	0.2
TLC	No sec. spot	No sec. spot	No sec. spot	-
ETSI (HPLC), area %	-	-	-	0.8
ETSI (PSA), %	0	-	-	-

Warfarin, Control No 168041

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

Examination year:	1967	1980	1984
UV-absorption 307 nm E(1 %, 1 cm) (0.01 N NaOH)	474	-	463
TLC	No sec. spot	No sec. spot	No sec. spot
Loss on drying, %	0.0	0.0	-
Assay, %	100.0	99.4	-
ETSI (HPLC), area %	-	-	<0.1

INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES - PROJECT LIST 1984

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

Volume 2

Acetazolamide (*)	Papaverine Hydrochloride (*)
Chlortetracycline Hydrochloride (*)	Propylthiouracil (*)
Colecalciferol	Propranolol Hydrochloride
Isoniazid (*)	Reserpine (*)
Norethisterone	Trimethadione (*)
Norethisterone Acetate (*)	

Volume 3

2-Amino-5-nitrothiazole (*) (impurity in Niridazole)	Methotrexate
Amodiaquine Hydrochloride	Neomycin B Sulfate
Amphotericin B	Neamine Hydrochloride (impurity in Neomycin B Sulfate)
Bacitracin Zinc	Neostigmine Metilsulfate (*)
Beclometasone Dipropionate	Nifurtimox
Betamethasone Valerate	Niridazole
Calcium Folate	Niridazole-chlorethylcarboxamide (*) (impurity in Niridazole)
Carbamazepine (*)	Noroxymorphone Hydrochloride (impurity in Naloxone)
Cimetidine	Nystatin
Clomifene Citrate, Z-isomer	Oxytetracycline Dihydrate
Dexamethasone Sodium Phosphate	Oxytetracycline Hydrochloride
Dopamine Hydrochloride	Paromycin Sulfate
Doxorubicin Hydrochloride	Praziquantel
Emetine Hydrochloride	Probenecid
Ergocalciferol	Prednisolone Sodium Phosphate
Fludrocortisone Acetate	Pyrantel Embonate
Gentamicin Sulfate	Salazosulfapyridine
Hydrocortisone Sodium Succinate	Spectinomycin Hydrochloride
(-)-3-(4-Hydroxy-3-methoxyphenyl)-2-hydrazino- 2-methylalanine (impurity in Carbidopa)	Sulfacetamide
(-)-3-(4-Hydroxy-3-methoxyphenyl)- 2-methylalanine (impurity in Carbidopa)	Testosterone Enantate
Levonorgestrel	Vincristine Sulfate
Loperamide Hydrochloride	

Replacements

The following existing International Chemical Reference Substances should be replaced by new batches in 1985:

Ergotamine Tartrate (*)	Chloramphenicol Palmitate
Chloramphenicol	Vitamin A Acetate

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

APPENDIX 6

D I G O X I N

Control No 377011
Report on re-examination

The monograph for Digoxin in the 3rd edition of the International Pharmacopoeia requires a reference substance to be used for identity testing by thin-layer chromatography and infrared spectrophotometry and for the colorimetric assay.

Material

A sample of Digoxin, obtained from Sandoz Ltd, Switzerland in 1977, and which has been stored protected from light and moisture at about +5 °C, has been re-examined with the view of assessing whether it remains suitable as reference substance for the purposes mentioned above. The initial analysis was reported in WHO/PHARM/78.494, Appendix 8.

Analytical data

High performance liquid chromatography: The total amount of impurities was estimated by peak area measurement at 0.4 - 0.5 per cent.

Column: Spheri S, 5 ODS 1, 5 µm particles, 25 cm long
Mobile phase: Acetonitrile, Water. Gradient elution with 40 per cent acetonitrile at T= 0 min. and 70 per cent at T= 25 min.
Flow rate: 1 ml per minute
Loading: Approx. 10 µg. 10 µl of a 1,1 mg/ml solution in Acetonitrile, water, 50 + 50.
Detection: UV spectrophotometer at 220 nm connected to an electronic integrator (Varian 4270)

Thin-layer chromatography: The chromatogram was similar to that obtained in 1977.

Plate: Silica gel GF (Merck)
Mobile phase: Methylene Chloride R, Methanol R, 9 + 1. The plate was developed twice.
Loading: 100 µg
Detection: After spraying with phosphoric acid 20 per cent the plate was heated for 15 minutes at 100 °C and then examined in UV-light at 366 nm.

Water: 0.1 per cent (n= 2), determined by the Karl Fischer method.

Assay: 99.6 per cent (n= 3), calculated with reference to the anhydrous substance and determined by the colorimetric method of the International Pharmacopoeia.

Conclusion

Digoxin No 377011 does not seem to have undergone any change during storage and is consequently considered to remain satisfactory for use as International Chemical Reference Substance. It is proposed that when calculating the results of colorimetric assays according to the International Pharmacopoeia, the content of $C_{41}H_{64}O_{14}$ (digoxin) in the reference substance is taken to be 99.6 per cent, calculated with reference to the anhydrous substance (corresponding to 99.5 per cent, calculated on the "as is" basis).

FLUCYTOSINE

Control No 184121
Analytical Report

The monograph for Flucytosine proposed for the 3rd edition of the International Pharmacopoeia requires a reference substance to be used in the infrared spectrophotometric and thin-layer chromatographic tests for identity and for related substances.

MATERIAL

A sample of flucytosine was generously offered by Hoffman-La Roche, Basle. About 150 g of the sample (lot no 202003) were received at the WHO Centre in June 1982. 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 184121). The spectrum is concordant with the spectrum obtained from the USP Ref. stand. Lot 1074 F.

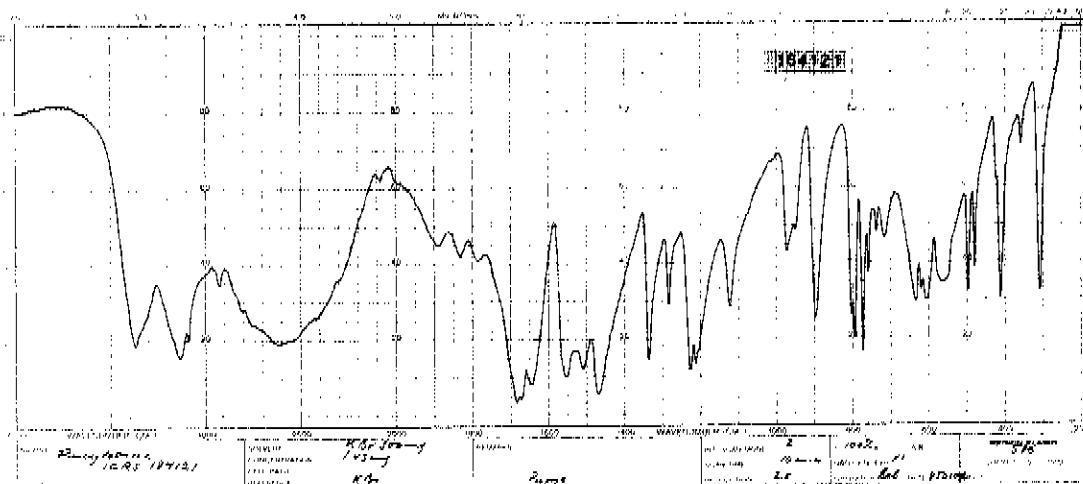


Figure 1. IR-spectrum of 1.43 mg of Flucytosine in 300 mg KBr, recorded against a KBr reference disc. Instrument: Perkin Elmer 580.

Elemental analysis

	C (%)	H (%)	N (%)
Theoretical	37.21	3.12	32.55
Found	37.0	3.0	32.5

The analysis was performed at Mikro Kemi AB, Uppsala.

Nuclear magnetic resonance spectra (NMR)

^1H NMR spectrum was recorded on a JEOL 90 Q NMR Spectrometer. The following structural assignments have been made ^1H NMR (90 MHz, CD_3OD) δ 7.52 (d, 1H, $J_{\text{HF}} = 5,8$ Hz)

UV-spectrum

A UV spectrum in 0.1 M hydrochloric acid is given in Figure 2.
 λ_{max} in 0.1 M HCl = 286 nm. E (1%, 1 cm) = 709 (n= 4)

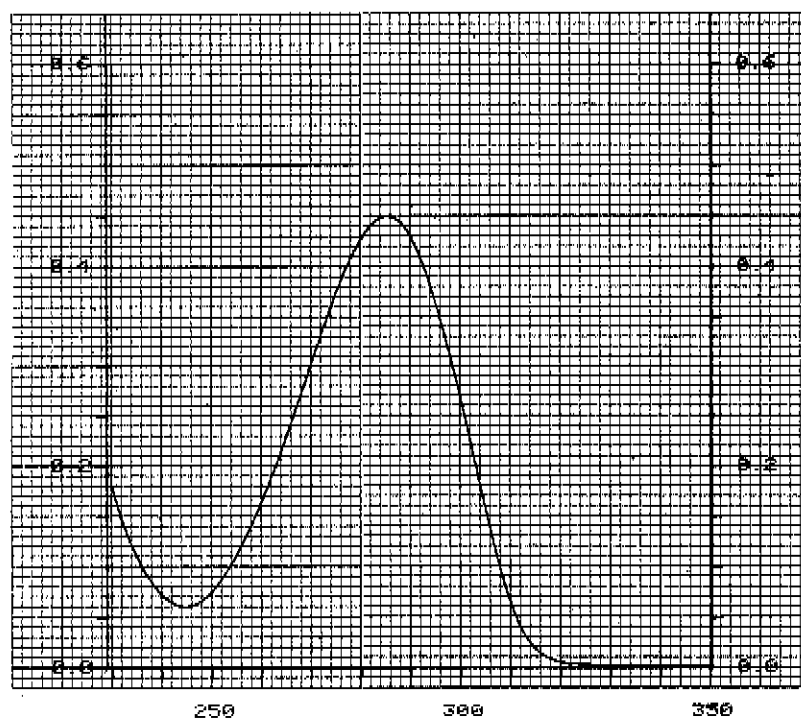


Figure 2. UV-spectrum of Flucytosine 6.3 $\mu\text{g}/\text{ml}$ in 0.1 M hydrochloric acid.

ASSAY

Titration with perchloric acid according to the International Pharmacopoeia slightly modified by dissolving the substance in acetic acid anhydride + glacial acetic acid (1+2).

Result: 99.8% w/w (n= 4)

Calculations are performed with reference to dried substance.

Loss on drying

0.0% (105 $^{\circ}\text{C}$)

PURITY

Thin-layer chromatography

The TLC system described in the International Pharmacopoeia Volume 3 was used.

Adsorbent: Silica gel 60, F-254 (E. Merck)

Mobile phase: Nitromethane: Ethanol 95%: Litium chloride 1% (70 + 20 + 10)

200 μg of flucytosine was applied in each spot. After development the chromatogram was examined in UV-light of 254 nm. The detection limit for flucytosine was less than 0.2 μg .

R_f (flucytosine) = 0.32

0.2 µg of fluorouracil was applied as reference, which corresponds to 0.1% w/w of the flucytosine spot.

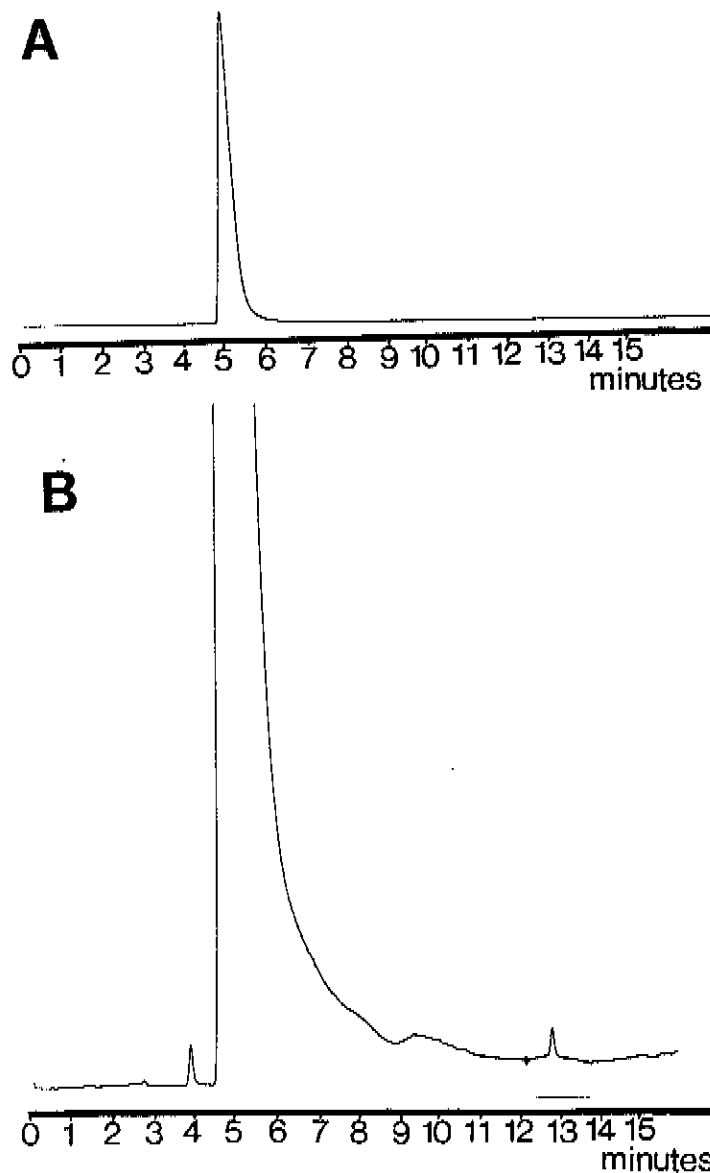
R_f (fluorouracil) = 0.56

Result: No extra spots were detected
USP Ref. stand. Lot 1074 F gave the same result.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.05%. Chromatograms are shown in Figure 3 A+B.

Figure 3.
Chromatogram of Flucytosine
No 184121.



The following conditions were used. Gradient elution:
A= Methanol B= Water

Time (min)	%A	%B
0	5	95
3	5	95
10	60	40
13	60	40
16	5	95

Column: Spheri S 5 ODS 1 (250 mm x 4.5) (Phase Sep)
Detector: Varian UV 200 operated at 285 nm
Pump: Varian 5560 Flow rate: 1.0 ml/min.
Integrator: Varian 4270 Att: 512 (Fig 3A), Att: 4 (Fig 3B)
Sample: 1 mg/ml dissolved in the mobile phase.
10 μ l corresponding to 10 μ g was injected.

STABILITY

Flucytosine was exposed to air of different relative humidity at room temperature (about 20 °C) for a period of five weeks as described in WHO/PHARM/ 82.509. The samples stored at 79% and 93% relative humidity had gained about 14% in weight after 1 week and 2 days respectively. After 4 months storage the samples were investigated by the HPLC method described under purity. No signs of degradation were observed.

DATA GIVEN BY THE MANUFACTURER:

Appearance	white crystalline powder, almost odourless
Heavy metals	< 20 ppm
Residue on ignition	0%
Loss on drying	0.04%
Fluorouracil (Thin-layer chromatography)	< 0.1%
Assay	100.1%
(HClO ₄ titration)	(on dry material)
Polymorphic form	A, which exhibits a band at 900 cm ⁻¹ in the infrared spectrum

CONCLUSION

Flucytosine No 184121 can be considered suitable as International Chemical Reference Substance for the intended purpose.

FLUOROURACIL

Control No 184122
Analytical Report

The monograph for Fluorouracil proposed for the 3rd edition of the International Pharmacopoeia requires a reference substance to be used in the infrared spectrophotometric test and UV-absorption test for identity as well as in the thin-layer chromatographic test for related substances. The reference substance is also used in the test for related substances by thin-layer chromatography in the monograph for Flucytosine.

MATERIAL

A sample of fluorouracil was generously offered by Hoffman-La Roche, Basle. About 150 g of the sample (lot no 202001) were received at the WHO Centre in June 1982. 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 184122). The spectrum is concordant with the spectrum obtained from the USP Ref. stand. Lot G.

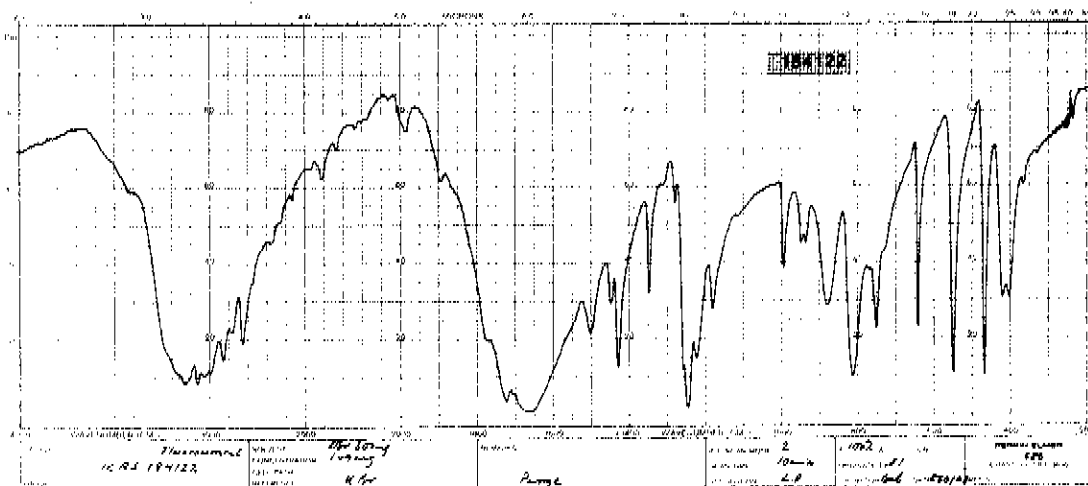


Figure 1. IR-spectrum of 1.49 mg of Fluorouracil in 300 mg KBr recorded against a KBr reference disc. Instrument: Perkin Elmer

Elemental analysis

	C (%)	H (%)	N (%)
Theoretical	36.93	2.32	21.54
Found	36.6	2.2	21.4

The analysis was performed at Mikro Kemi AB, Uppsala.

Nuclear magnetic resonance spectra (NMR)

^1H NMR spectrum was recorded on a JEOL FX 90 Q NMR Spectrometer. The following assignments have been made:

^1H NMR (90 MHz, DMSO-d_6) δ 11.1 (broad, 2H, NH's); 7.76 (d, 1H, $J_{\text{HF}} = 6.1$ Hz)

UV-spectrum

A UV spectrum in acetate buffer pH 4.7 is given in Figure 2.

λ_{max} in acetate buffer pH 4.7 = 266 nm. E (1%, 1 cm) = 550 ($n = 4$)

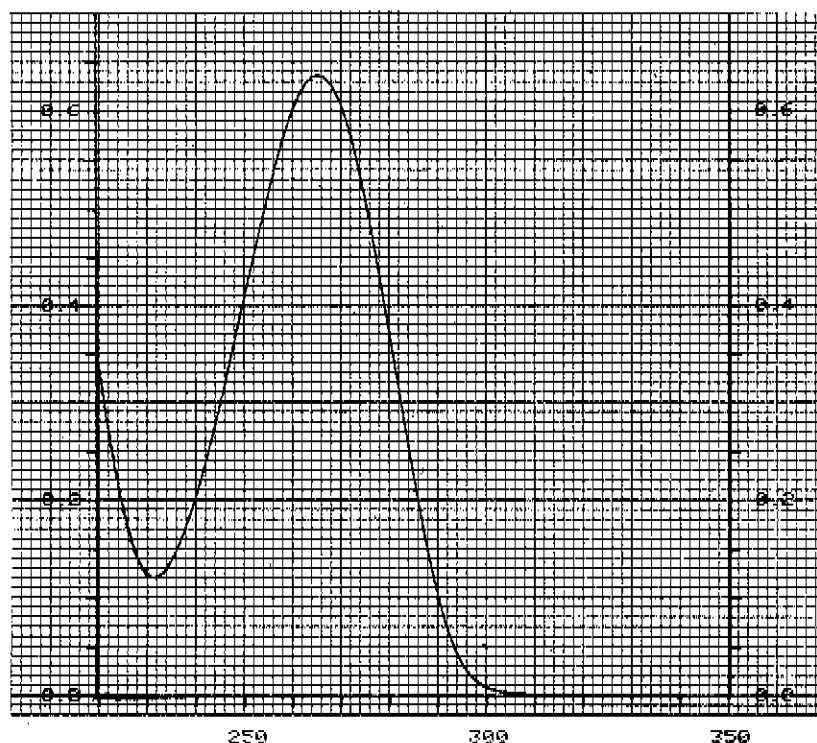


Figure 2. UV-spectrum of Fluorouracil 11.5 $\mu\text{g/ml}$ in acetate buffer pH 4.7.

ASSAY

Titration with 0.1 N tetrabutylammonium hydroxide according to the International Pharmacopoeia.

Result: 100.9% w/w ($n = 3$). Calculations are performed with reference to dried substance.

Loss on drying

0.0% (105°C)

PURITY

Thin-layer chromatography

The TLC system described in the International Pharmacopoeia Volume 3 was used.

Adsorbent: Silica gel 60. F-254 (E. Merck)

Mobile phase: Ethyl acetate: methanol: water (70 + 15 + 15)

100 μg of fluorouracil was applied. After development the chromatogram was examined in UV-light of 254 nm. The detection limit for fluorouracil was less than 0.25 μg .

R_f (fluorouracil) = 0.51

Result: No extra spots were detected. In USP Ref. stand. Lot G one faint extra spot with $R_f = 0.32$ was observed.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.03%. Chromatograms are shown in Figure 3 A + B.

The following conditions were used.

Gradient elution: A= Methanol

B= Water

Time (min)	%A	%B
0	5	95
3	5	95
10	60	40
13	60	40
16	5	95

Column: Spheri S 5 ODS 1 (250 mm x 4.5) (Phase Sep)

Detector: Varian UV 200 operated at 266 nm

Pump: Varian 5560 Flow rate: 1.0 ml/min.

Integrator: Varian 4270 Att: 1024 (Fig 3A), Att: 4 (Fig 3B)

Sample: 1 mg/ml dissolved in the mobile phase. 10 μ l corresponding to 10 μ g was injected.

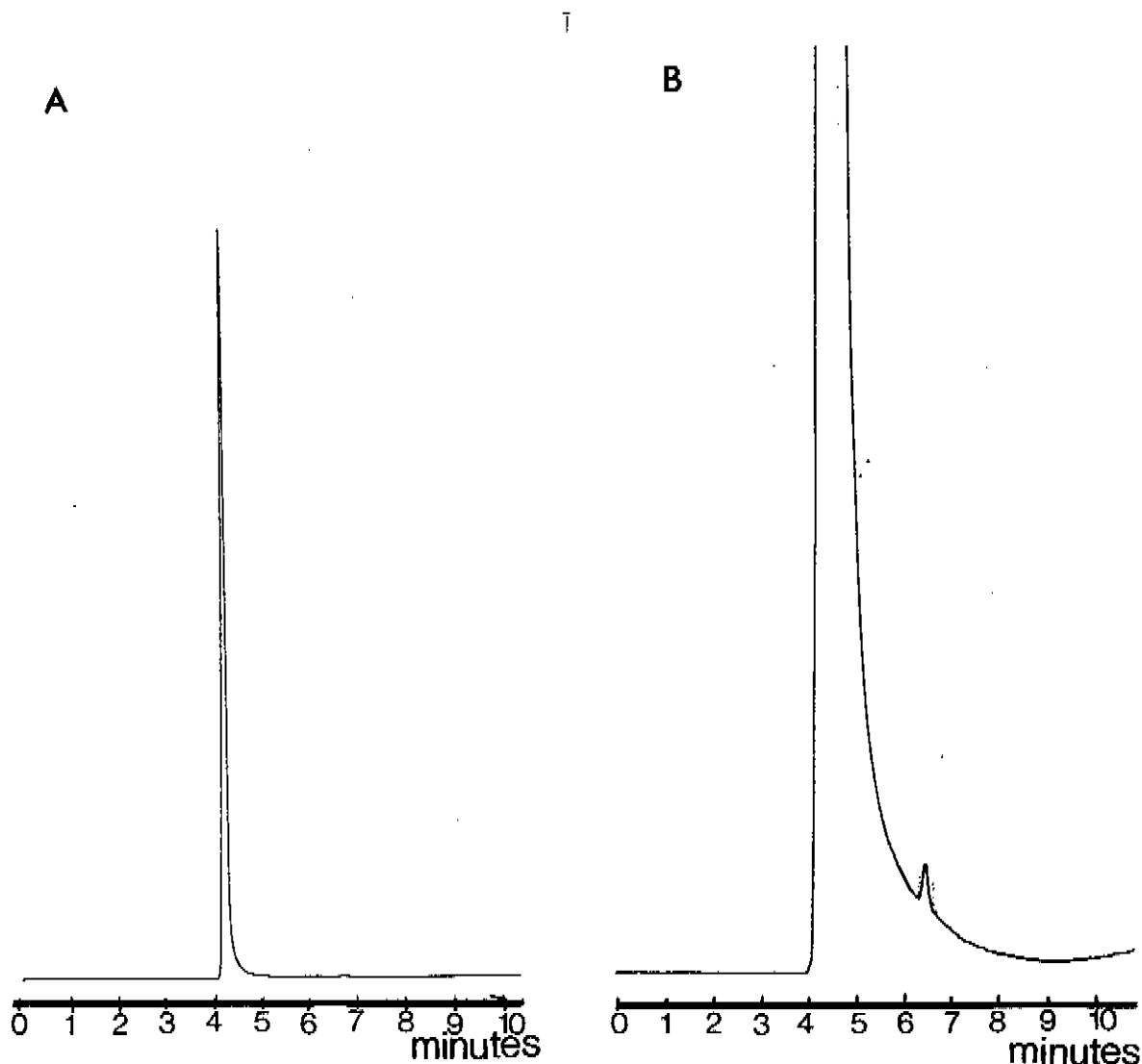


Figure 3. Chromatogram of Fluorouracil Control No 184122.

STABILITY

Fluorouracil was exposed to air of different relative humidity at room temperature (about 20 °C) for a period of five weeks as described in WHO/PHARM/ 82.509. No gain in weight was observed even after 4 months. The sample stored at 93% relative humidity was investigated by the HPLC method described under purity. No signs of degradation were observed.

DATA GIVEN BY THE MANUFACTURER:

Appearance	white crystalline powder, almost odourless
Heavy metals	< 20 ppm
Residue on ignition	0.02%
Loss on drying	0.03%
Related substances	not detected
Assay	100.6%
(Titration with 0.1 N Tetrabutylammonium hydroxide)	(on dry material)

CONCLUSION

Fluorouracil No 185122 can be considered suitable as International Chemical Reference Substance for the intended purpose.

PROCARBAZINE HYDROCHLORIDE

Control No 184120
Analytical Report

The monograph for Procarbazine Hydrochloride proposed for the 3rd edition of the International Pharmacopoeia requires a reference substance to be used in the infrared spectrophotometric test.

MATERIAL

A sample of procarbazine hydrochloride was generously offered by Hoffman-La Roche, Basle. About 150 g of the sample (lot no 087121) were received at the WHO Centre in June 1982. The material has been stored protected from light in tightly closed containers in an atmosphere of inert gas at +5° C.

ANALYTICAL DATA

Description: A yellowish-white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (no 184120). The spectrum is concordant with the spectrum obtained from the USP Ref. stand. Lot 1174-F.

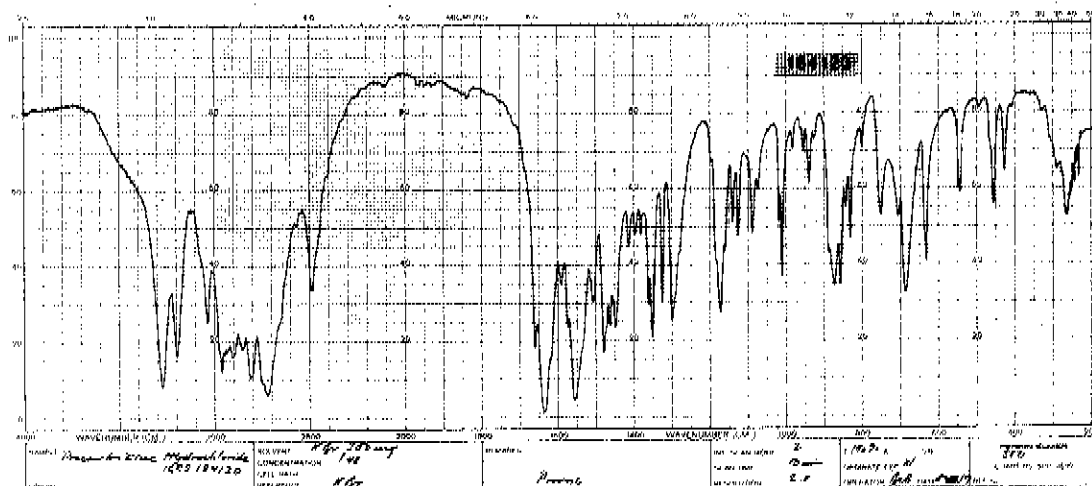


Figure 1. IR-spectrum of 1.48 mg of Procarbazine hydrochloride in 300 mg KBr, recorded against a KBr reference disc. Instrument: Perkin Elmer 580.

Elemental analysis

	C (%)	H (%)	N (%)
Theoretical	55.91	7.82	16.30
Found	56.2	8.1	16.4

The analysis was performed at Mikro Kemi AB, Uppsala.

Nuclear magnetic resonance spectra (NMR)

^1H NMR and ^{13}C NMR spectra were recorded on a JEOL 90 Q NMR Spectrometer. The ^1H NMR spectrum is given in Figure 2.

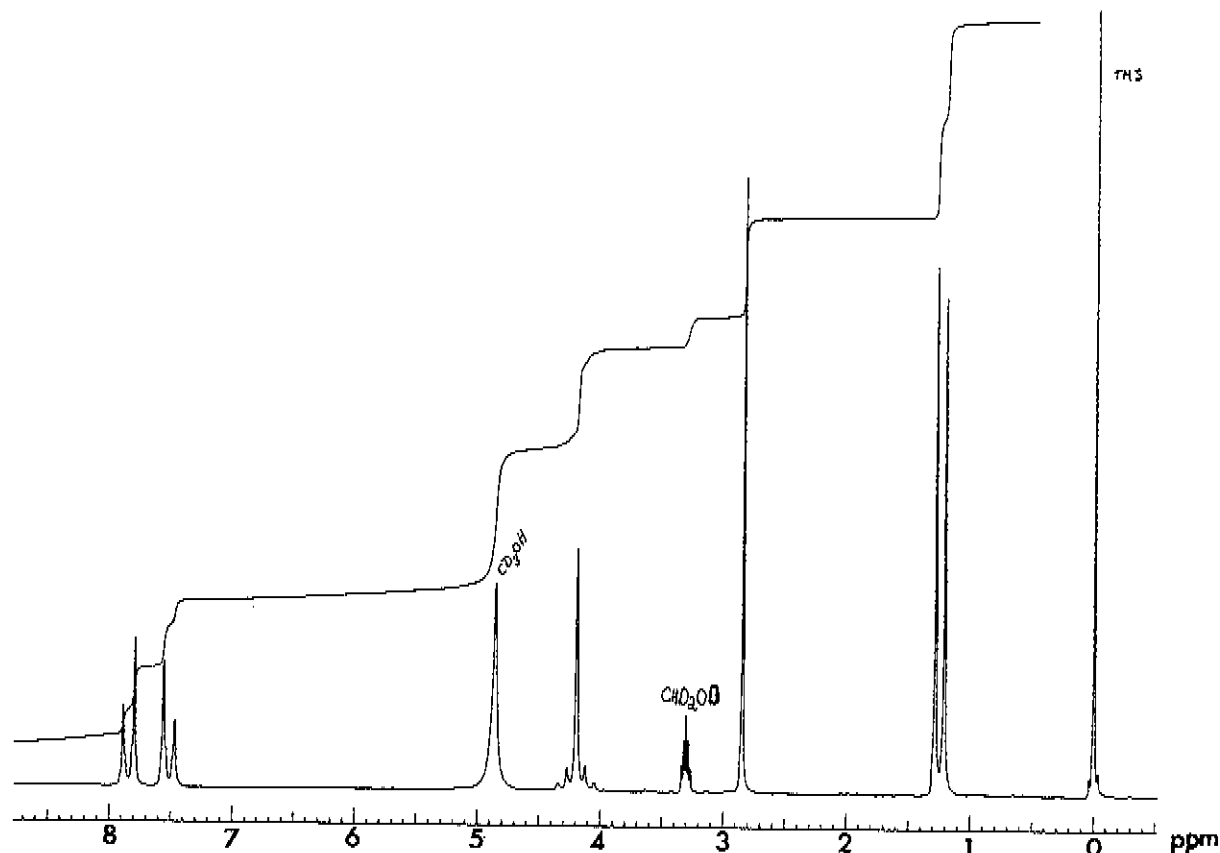


Figure 2. ^1H -NMR spectrum of procarbazine hydrochloride ($\sim 46 \mu\text{g/ml}$ in $\text{CD}_3\text{OD} + \text{TMS}$). The following structural assignments have been made.

^1H NMR (90 MHz, CD_3OD) δ 7.95 - 7.45 (m, 4H, ArH), 4.4 - 4.05 (m, 1H, CH); 4.20 (s, 2H, CH_2); 1.25 (d, $J = 7\text{Hz}$, 6H, CH_3 s)

^{13}C NMR (22.51 MHz, CD_3OD) δ 168.9 (CONH); 139.3, 136.1, 130.1, 128.8 (Ar), 52.9, 43.18, 35.52 (NHCH_3 , CH_2NH , NHCH); 22.5 ($\text{CH}(\text{CH}_3)_2$).

ASSAY

1. Titration with 0.1 N NaOH according to USP XX.
Result: 100.2%, w/w (n= 4)
2. Titration with perchloric acid according to the International Pharmacopoeia slightly modified by dissolving the substance in formic acid.
Result: 99.0% w/w (n= 4)
All calculations are performed with reference to dried substance.

Loss on drying

0.0% (105°C)

PURITY

Due to the low stability of the substance it was impossible to use methods as TLC (degradation on the plate) and thermal analysis (melting under decomposition).

High performance liquid chromatography

A slightly modified version of the method presented by Burce and Boehlert in J. Pharm. Sci., 67 (3), 424-426 (1978), was used. It is stability-indicating and rapid. If the sample is dissolved immediately before injection the risk for degradation is minimized.

The total amount of impurities was estimated by peak area measurement to about 0.7%. The corresponding value for the USP Ref. Std. Lot 1174-F was about 0.4%.

A chromatogram is shown in Figure 3.

Mobile phase: Methanol/Ammonium phosphate buffer 0.1M (50 + 50)
pH in the mixture was 5.4
Column: Spherisorb S 5 ODS 1,
(250 mmx4.5) (Phase Sep)
Detector: Shimadzu SPD-2A operated at
254 nm, 0.64 AUFS
Pump: Waters M-6000
Flow rate: 1.0 ml/min
Integrator: Hewlett Packard 3390 A
Sample: 1 mg/ml dissolved immediately
before injection in the mobile
phase.
20 µl corresponding to 20 µg
were injected.

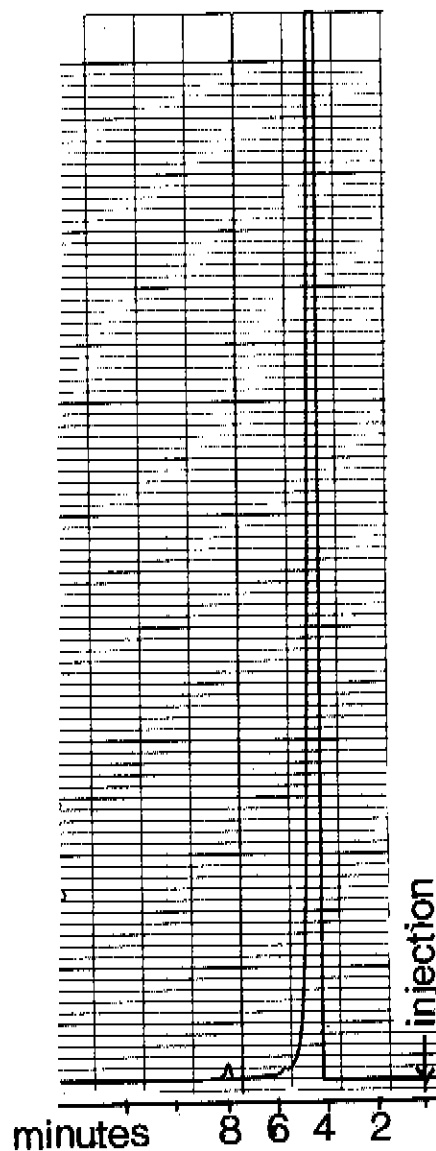


Fig. 3. Chromatogram of Procarbazine hydrochloride No 184120.

STABILITY

Procarbazine hydrochloride was exposed to air of different relative humidity at room temperature for a period of five weeks as described in WHO/PHARM/ 82.509. No gain in weight was observed except for at 93% relative humidity where the substance started to deliquesce after two days and had gained in weight with 44% after five weeks.

At the end of the experiment (i.e. after about 3 months storage) the samples were analyzed by the HPLC method described above. No degradation was observed except for in the sample stored at 93% humidity where only 55% of intact procarbazine hydrochloride was found.

DATA GIVEN BY THE MANUFACTURER:

Appearance	yellowish, crystalline powder, odourless
Heavy metals	< 20 ppm
Residue on ignition	0%
Loss on drying	0.04%
pH-value (5% water-solution)	3.3
Assay (HClO ₄ titration)	99.0% (on dry material)

CONCLUSION

Procarbazine hydrochloride No 184120 can be considered suitable as International Chemical Reference Substance for the intended purpose.

Storage

At low temperature, preferably under inert gas, with protection from light and humidity.

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