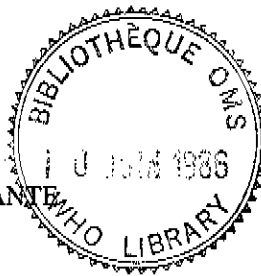




WLOSCC
 WORLD HEALTH ORGANIZATION
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4159
 Drugs - standard
 Reference standard
 WHO/PHARM/86.527
 ORIGINAL: ENGLISH

1355

WHO COLLABORATING CENTRE FOR CHEMICAL REFERENCE SUBSTANCES

(Solna, Sweden)

Report on the work in 1985

by M. Abrahamsson

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Distribution of reference substances in 1985

In 1985 the Centre distributed 2210 packages of International Chemical Reference Substances and 22 sets of Melting Point Reference Substances to drug control laboratories in 46 different countries. In comparison with the distribution figures for the previous year this represents a decrease of 16 per cent. The five most frequently requested substances in 1985 were, in order of demand, Folic Acid, Ampicillin, Digoxin, Benzylpenicillin Sodium and Ergotamine Tartrate. Detailed figures for the distribution of the individual substances are given in Appendix 1.

Establishment of reference substances in 1985

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), 4 International Chemical Reference Substances were established in 1985. The substances are listed in Appendix 2 to this report. Of these substances Digoxin is a replacement batch for a previously existing International Chemical Reference Substance, the stocks of which had become depleted.

A complete list of all the International Chemical Reference Substances available from the Centre in January 1986, with information about package sizes and control numbers for the current batches, is given in Appendix 3 to this report. The list also includes 6 substances mentioned below, which are expected to be formally adopted during the first half of 1986. Rose Bengal Sodium is no longer available and has therefore been deleted from the list. In accordance with a recommendation of the Expert Committee at its 30th meeting this and other substances not required for the third edition of the International Pharmacopoeia will, at least for the present time, not be replaced.

Work on new reference substances completed in 1985

The Centre has continued its work to provide new reference substances required to support specifications in the third edition of the International Pharmacopoeia. During 1985 the analytical examinations of the following new reference substances were completed: Isoniazid, Norethisterone Acetate, Papaverine Hydrochloride, Propylthiouracil and Trimethadione. The analytical reports for these materials are given in Appendices 7, 8, 9, 10 and 11, respectively. All the five substances were considered suitable for their intended uses and were consequently proposed for adoption as International Chemical Reference Substances.

Since the stock of the International Chemical Reference Substance for Ergotamine Tartrate with control number 276013 is depleted a new batch has been investigated and found suitable as replacement batch. It is proposed that the material with control number 385013 be adopted as International Chemical Reference Substance. The analytical results 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 1985 the re-examination was performed on twenty substances including the tetracyclines.

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 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.

The substances investigated have in general turned out to be stable when stored at +5 °C. The only exception is 4-epianhydrotetracycline hydrochloride with control no. 180097, where the content of anhydrotetracycline has increased from 1.7 to 7 per cent during five years. However, since this substance is only used as an impurity reference material in thin-layer chromatography this amount of degradation product may be considered acceptable. In a close future a new batch will anyhow be prepared as the stock will be depleted soon.

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 6 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 46 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. With a realistic appraisal of the work involved and considering the resources available to the Centre it seems likely that bringing the present work programme to an end might take as much as five years. As a consequence of this unsatisfactory 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. The Centre is also very interested in getting indications about which substances in Appendix 5 should be given priority. The Centre has noted with satisfaction that the project to establish a WHO collection of infrared reference spectra for pharmaceutical substances is making progress. This project, when fully implemented, will considerably reduce the number of International Chemical Reference Substances that have to be established and thus relieve the Centre from some of its present heavy work-load. In anticipation of a successful implementation of the project the Centre has already deleted from its work-list (Appendix 5) those reference substances, which are only required for identification by infrared spectrophotometry.

Administrative and financial matters

The financial situation of the Centre remains unsatisfactory. The total cost for running the Centre in 1985 was estimated at 198.000 US\$. The income from sales of reference substances to industrial laboratories was about 13.000 US\$ and the contribution received from WHO headquarters was 19.000 US\$ which leaves a deficit of 166.000 US\$. After consideration of the matter the management board of the National Corporation of Swedish Pharmacies agreed to support the continued operation of the Centre at an unchanged level, but urged that every possibility be explored to reduce the deficit. The options at present available to the Centre to improve the financial situation are essentially the following:

- 1) Increase the sales of reference substances to industrial laboratories by active marketing efforts.
- 2) Increase the price of the substances.
- 3) Introduce a charge for the substances also when delivered to governmental control laboratories.

In line with the first of the above measures the collection of International Chemical Reference Substances was presented at an exhibition arranged in connection with an analytical chemistry conference in Lund, Sweden, in June 1985 and attracted considerable interest among the analytical chemists present.

The fee for the substances has during 1985 been kept unchanged at US\$ 25 per package, but an increase in the coming year seems possible and justified, particularly if the exchange rate of the dollar continues to fall.

The proposal to charge also governmental control laboratories for the substances has been reiterated and is now seriously being considered for implementation. As mentioned in an earlier report from the Centre (WHO/PHARM/84.513) this might make it difficult for a number of governmental laboratories to obtain necessary reference materials due to their restricted budgets and/or lack of allotments in convertible currencies. The Centre has

informed the WHO Regional Offices about the proposal and will discuss the matter further with them with the objective to obtain WHO financial support to those laboratories that would otherwise be deprived of the reference materials necessary for their drug control activities.

The construction of a new building for the central laboratory of Apoteksbolaget and for the Centre is progressing according to plan. The moving into the new premises, which are located some 12 km southwest of central Stockholm, is scheduled to take place end of May 1986. After a necessary running-in period of 3-4 months the Centre can then again offer training facilities for WHO fellowship holders interested in the establishment of reference substances. The new postal address of the Centre is stated in Appendix 3.

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 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 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 Bayer AG in Leverkusen, Schering AG in Berlin and Diamalt AG in Raubling, all in Federal Republic of Germany, Abbott in North Chicago, USA, Recordati in Milano, Italy, and Sandoz in Basle, Switzerland.

Finally it remains to express our heartiest thanks to Miss Eivor Andersson who at the end of 1985 retired after 30 years of extremely creditable service as senior analyst at the Centre. Miss Andersson has an unusual command of all the analytical techniques required for the evaluation of chemical reference substances and she will be long remembered and missed not only by us at the Centre but also by the many WHO fellowship holders who got their training in phase solubility analysis and other intricate methods under her gentle but most instructive guidance. We would now also like to welcome her successor, Miss Lise-Lotte Larsson who joined the Centre in November 1985.

APPENDIX 1

DISTRIBUTION OF CHEMICAL REFERENCE SUBSTANCES IN 1985

Aceclidine Salicylate	4 items	Ethambutol Hydrochloride	19 items
p-Acetamidobenzalazine	7 "	Ethinylestradiol	17 "
Allopurinol	21 "	Ethisterone	15 "
3-Aminopyrazole-4-carboxamide Hemisulfate	15 "	Ethosuximide	11 "
Amitriptyline Hydrochloride	26 "	Etocarlide	3 "
Ampicillin	69 "	Fluphenazine Decanoate Dihydrochloride	12 "
Ampicillin Sodium	30 "	Fluphenazine Enantate Dihydrochloride	9 "
Ampicillin Trihydrate	44 "	Fluphenazine Hydrochloride	10 "
Anhydrotetracycline Hydrochloride	39 "	Folic Acid	81 "
Atropine Sulfate	20 "	Furosemide	18 "
Azathioprine	7 "	Griseofulvin	21 "
Bendazol Hydrochloride	3 "	Haloperidol	23 "
Benzobarbital	15 "	Hydrochlorothiazide	16 "
Benzylamine Sulfate	5 "	Hydrocortisone	25 "
Benzylpenicillin Potassium	18 "	Hydrocortisone Acetate	42 "
Benzylpenicillin Sodium	52 "	(-)-3-(4-Hydroxy-3-methoxyphenyl)- 2-methylalanine	7 "
Bephenium Hydroxynaphthoate	11 "	Ibuprofen	16 "
Betamethasone	22 "	Imipramine Hydrochloride	12 "
Betanidine Sulfate	4 "	Indometacin	23 "
Bupivacaine Hydrochloride	11 "	o-Iodohippuric Acid	3 "
Caffeine	21 "	Lanatoside C	27 "
Carbenicillin Monosodium	24 "	Levodopa	9 "
Chloramphenicol	12 "	Lidocaine	26 "
Chloramphenicol Palmitate	19 "	Lidocaine Hydrochloride	19 "
Chloramphenicol Palmitate (Polymorph A)	17 "	Mefenamic Acid	4 "
5-Chloro-2-methylaminobenzophenone 2-(4-Chloro-3-sulfamoylbenzoyl) benzoic Acid	20 "	Melting Point Reference Substances (set of 13 substances)	22 "
Chlorphenamine Hydrogen Maleate	4 "	Metazide	3 "
Chlorpromazine Hydrochloride	21 "	Methaqualone	5 "
Chlortalidone	26 "	Methylodopa	17 "
Cloxacillin Sodium	13 "	Methyltestosterone	13 "
Cortisone Acetate	15 "	Meticillin Sodium	13 "
Dapsone	26 "	Metronidazole	19 "
Desoxycortone Acetate	11 "	Nafcillin Sodium	6 "
Dexamethasone	16 "	Nicotinamide	27 "
Dexamethasone Acetate	37 "	Nicotinic Acid	16 "
Diazepam	15 "	Ouabain	17 "
Diazoxide	24 "	Oxacillin Sodium	33 "
Dicloxacillin Sodium	8 "	Pheneticillin Potassium	9 "
Dicolinium Iodide	20 "	Phenoxymethylpenicillin	11 "
Dicoumarol	5 "	Phenoxymethylpenicillin Calcium	7 "
Diethylcarbamazine Dihydrogen Citrate	10 "	Phenoxymethylpenicillin Potassium	20 "
Digitoxin	12 "	Phenytoin	13 "
Digoxin	30 "	Prednisolone	31 "
NN'-Di-(2,3-xyllyl)anthranilamide	59 "	Prednisolone Acetate	23 "
4-Epianhydrotetracycline Hydrochloride	4 "	Prednisone	30 "
4-Epitetracycline Ammonium Salt	46 "	Prednisone Acetate	12 "
Ergometrine Hydrogen Maleate	35 "	Procaine Hydrochloride	14 "
Ergotamine Tartrate	17 "	Progesterone	18 "
Estradiol Benzoate	48 "	Propicillin Potassium	3 "
Estrone	44 "	Pyridostigmine Bromide	10 "
Etacrynic Acid	18 "	Riboflavin	25 "
	12 "	Rose Bengal Sodium	1 "
	13 "	Sulfamethoxazole	30 "

Sulfamethoxypyridazine	18	items
Sulfanilamide	17	"
Testosterone Propionate	18	"
Tetracycline Hydrochloride	33	"
Thioacetazone	7	"
4,4'-Thiodianiline	5	"
Tolbutamide	21	"
Tolnaftate	13	"
Trimethoprim	30	"
Trimethylguanidine Sulfate	5	"
Tubocurarine Chloride	12	"
Vitamin A Acetate (solution)	42	"
Warfarin	14	"
Total	<u>2</u>	<u>232</u> items

APPENDIX 2

INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES ESTABLISHED IN 1985

Reference Substance	Control number	Analytical Report	Remarks
Digoxin	377011	WHO/PHARM/85.517 Appendix 6	Replaces No 478011
Flucytosine	184121	WHO/PHARM/85.517 Appendix 7	--
Fluorouracil	184122	WHO/PHARM/85.517 Appendix 8	--
Procarbazine Hydrochloride	184120	WHO/PHARM/85.517 Appendix 9	--

LIST OF AVAILABLE INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES

1986

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
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
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

<u>Reference substance</u>	<u>Package size</u>	<u>Control number for current batch</u>
Azathioprine	100 mg	172060
Bendazol Hydrochloride	100 mg	173066
Benzobarbital	100 mg	172051
Benzylamine Sulfate	100 mg	172052
Benzympenicillin Potassium	200 mg	180099
Benzympenicillin 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 ¹ -Di-(2,3-xylol)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	385013
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
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

<u>Reference substance</u>	<u>Package size</u>	<u>Control number for current batch</u>
(-)-3-(4-Hydroxy-3-methoxyphenyl)- 2-methylalanine	25 mg	179085
Ibuprofen	100 mg	183117
Imipramine Hydrochloride	100 mg	172064
Indometacin	100 mg	178078
Isoniazid	100 mg	185124
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 temperatures 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
Nicotinamide	100 mg	179090
Nicotinic Acid	100 mg	179091
Norethisterone acetate	100 mg	185123
Ouabain	100 mg	283026
Oxacillin Sodium	200 mg	382027
Papaverine hydrochloride	100 mg	185127
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
Propylthiouracil	100 mg	185126
Pyridostigmine Bromide	100 mg	182110
Riboflavin	250 mg	382035
Sulfamethoxazole	100 mg	179092
Sulfamethoxypridazine	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)	5 caps. (*)	581038
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 re-examination of the substances held in stock at the Centre. The results obtained for the substances re-examined in 1985 are summarized below. For comparison results obtained at earlier occasions are included in the summaries. The substances have been stored at +5° C. The following abbreviations are used in the tables:

DTA Differential Thermal Analysis
 HPLC High Performance Liquid Chromatography
 TLC Thin-layer Chromatography
 PSA Phase Solubility Analysis
 KF Karl Fischer titration
 IR Infrared Spectrophotometry

The estimates of total solid impurities by HPLC and TLC are expressed as area per cent if otherwise not stated, 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.

Allopurinol, Control No 172049

Initial analytical report: WHO/PHARM/72.471, Appendix 9

Examination year:	1972	1974	1979	1985
Light absorption, 250 nm	0.57	0.57	0.57	-
TLC	no sec. spots	no sec. spots	no sec. spots	no sec. spots
Loss on drying, %	0.1	0.0	0.04	-
Assay	99.6	99.95	99.98	-

3-Aminopyrazole-4-carboxamide hemisulfate, Control No 172050

Initial analytical report: WHO/PHARM/72.471, Appendix 10

Examination year:	1972	1975	1981	1985
IR	conforms	-	-	conforms
Melting temperature	225° C	-	-	-
TLC	no sec. spots	-	-	2 faint sec. spots (250 µg)
Water (KF), %	13.7	11.2	10.8	10.4* (5.2**)
Assay (potentiometric),	99.8	99.6	99.5	(100.3)

* old dispensed vials

** bulk, dispensed 1985

Amitriptyline hydrochloride, Control No 181101

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

Examination year:	1982	1985
IR	conforms	conforms
TLC, %	<0.1	<0.1
HPLC, %	<0.1	<0.1
Loss on drying, %	0.1	0.1
DTA, %	0.3	0.4
Water (KF), %	-	0.1
Assay (potentiometric)	100.3	100.3
PSA, %	0.3	-

Anhydrotetracycline hydrochloride, Control No 180096

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

Examination year:	1980	1985
Loss on drying, %	1.2	-
Water (KF), %	-	2.4
HPLC, weight % of 4-epianhydrotetracycline	1.4	1.5

5-Chloro-2-methylaminobenzophenone, Control No 172061

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

Examination year:	1972	1985
Melting range	94.9 - 95.2	94.7 - 95.3
Light absorption, 236 nm	0.54	-
TLC	no sec. spots	no sec. spots
DTA, %	0.4	0.5

Diazepam, Control No 172062

Initial analytical report: WHO/PHARM/73.475, Appendix 5

Examination year:	1973	1977	1982	1985
Melting range	132-133	131-132.5	-	-
Light absorption, 241 nm	0.82	0.80	0.80	-
TLC	one sec. spot	one sec. spot	one sec. spot	one sec. spot
DTA, %	0.05	-	0.04	0.02
Loss on drying, %	0.0	0.0	-	-
Assay	100.0	99.8	100.0	-

Digoxin, Control No 377011

Initial analytical report: WHO/PHARM/78.494, Appendix 8

Examination year:	1977	1985
TLC	5 faint sec. spots	5 faint sec. spots
HPLC, %	0.4	0.4-0.5
Loss on drying, %	0.2	-
Water (KF), %	-	0.1
Assay (colourimetric)	99.2	99.6
PSA, %	about 1	-

4-Epianhydrotetracycline hydrochloride, Control No 180097

Initial analytical report: WHO/PHARM/81.508, Appendix 8

Examination year:	1980	1985
HPLC, weight % of anhydrotetracycline	1.7	about 7
Loss on drying, %	3.1	-
Water (KF), %	-	3.9

4-Epitetracycline Ammonium Salt, Control No 180098

Initial analytical report: WHO/PHARM/81.508, Appendix 9

Examination year:	1980	1985
HPLC, weight % of tetracycline	0.4	0.3
4-epianhydrotetracycline	0.1	0.1
4-anhydrotetracycline	traces	-
Loss on drying, %	0	-
Water (KF), %	-	3.9

Ergotamine tartrate, Control No 276013

Initial analytical report: WHO/PHARM/77.491, Appendix 4

Examination year:	1976	1982	1985
Appearance	-	-	slightly discoloured
Light absorption 318 nm	0.59	0.58	
TLC, %	2 sec. spots	2 sec. spots	2 sec. spots and 4 traces (0.4-0.5)
HPLC, % total ergotaminin, weight %	-	0.4	0.5
Loss on drying, %	0.1	-	0.4
Water (KF), %	4.4	-	-
Assay (potentiometric)	-	-	3.0
	99.6	-	100.1

Ethambutol hydrochloride, Control No 179081

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

Examination year:	1979	1985
TLC	no sec. spots	no sec. spots
Loss on drying, %	0.1	0.3
DTA, %	0.3	0.4
Assay (titration)	100.1	-

Folic acid, Control No 277019

Initial analytical report: WHO/PHARM/78.494, Appendix 6

Examination year:	1977	1985
HPLC, %	0.3	0.4
Water (KF), %	8	8
TLC	3 faint sec.spots	-

Furosemide, Control No 171044

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

Examination year:	1971	1976	1985
Melting point, °C	209	-	-
Light absorption			
228 nm	110	108	-
271 nm	60	59	-
334 nm	13,5	13	-
TLC	no sec. spots	trace of 1 sec. spot	no sec. spots
Loss on drying, %	0.1	0.06	0.2
Assay	99.4	100.1	-
PSA, %	<0.5	-	-

Indometacin, Control No 178078

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

Examination year:	1978	1985
TLC	6 trace sec. spots	6 trace sec. spots
Loss on drying, %	0.2	0
DTA, %	0.2	0.3
Assay (potentiometric)	99.9	-
PSA, %	0	-

Phenytoin, Control No 179089

Initial analytical report: WHO/PHARM/80.504, Appendix 13

Examination year:	1979	1985
Loss on drying, %	0	0.2
DTA, %	0.2	0.3
HPLC, weight % of benzophenone	0.02	-
Assay	100.1	-

Sulfamethoxazole, Control No 179092

Initial analytical report: WHO/PHARM/80.504, Appendix 14

Examination year:	1980	1985
TLC	four traces of sec. spots	two sec. spots
DTA, %	0.15	0.16
Loss on drying, %	0.0	0.0
HPLC, %	0.1	-
Assay, (potentiometric)	100.1	-

Sulfanilamide, Control No 179094

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

Examination year:	1980	1985
TLC	no sec. spots	no sec. spots
DTA, %	0.05	0.06
Loss on drying, %	0.0	0.0
Assay (potentiometric)	100.0	-

Tetracycline hydrochloride, Control No 180095

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

Examination year:	1980	1985
HPLC, weight %		
of 4-epitetracycline	1.3	0.7
anhydrotetracycline	0.1	0.1
4-epianhydrotetracycline	<0.05	0.05
unidentified, area %	~0.05	0.1
Loss on drying, %	0.25	-
Water (KF), %	0.4	0.3
TLC	One sec.	-
	spot	
Assay (potentiometric)	99.6	-

Trimethoprim, Control No 179093

Initial analytical report: WHO/PHARM/80.504, Appendix 17

Examination year:	1980	1985
DTA, %	0.03	0.0
TLC, %	6 weak	6 weak
	sec. spots	sec. spots
	<0.1	<0.1
Loss on drying, %	0.0	-
Water (KF), %	-	<0.05

Vitamin A acetate, Control No 581038

Initial analytical report: WHO/PHARM/82.509, Appendix 15

Examination year:	1981	1985
Assay (spectrophotometric)		
Vitamin A acetate, mg/g	38.8	32.7

APPENDIX 5

INTERNATIONAL CHEMICAL REFERENCE SUBSTANCES - PROJECT LIST 1986

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

Volume 2

Acetazolamide (*)
Chlortetracycline Hydrochloride (*)
Colecalciferol
Norethisterone (*)
Propranolol Hydrochloride
Reserpine (*)

Volume 3

2-Amino-5-nitrothiazole (*)
(impurity in Niridazole)
Amodiaquine Hydrochloride
Amphotericin B
Bacitracin Zinc
Beclometasone Dipropionate
Betamethasone Valerate
Calcium Folate
Carbamazepine (*)
Cimetidine
Clomifene Citrate, Z-isomer
(impurity in Clomifene Citrate)
Dexamethasone Sodium Phosphate
Dopamine Hydrochloride
Doxorubicin Hydrochloride
Emetine Hydrochloride (*)
Ergocalciferol
Fludrocortisone Acetate
3-Formylrifamycin SV
(impurity in Rifampicin)
Framycetin (Neomycin B Sulfate)
(impurity in Neomycin Sulfate)
Gentamicin Sulfate
Hydrocortisone Sodium Succinate
(-)-3-(4-Hydroxy-3-methoxyphenyl)-
2-hydrazino-2-methylalanine
(impurity in Carbidopa)
Levonorgestrel

Levothyroxine Sodium
Liothyronine
(impurity in Levothyroxine Sodium)
Loperamide Hydrochloride
Methotrexate
Neamine (impurity in Neomycin Sulfate)
Neostigmine Methylsulfate (*)
Nifurtimex
Niridazole
Niridazole-chlorethyl carboxamide (*)
(impurity in Niridazole)
Noroxymorphone Hydrochloride
(impurity in Naloxone Hydrochloride)
Nystatin
Oxytetracycline Dihydrate
Oxytetracycline Hydrochloride
Paromomycin Sulfate
Praziquantel
Prednisolone Sodium Phosphate
Probenecid
Pyrantel Embonate
Rifampicin quinone
(impurity in Rifampicin)
Salazosulfapyridine
Sodium Cromoglicate
Spectinomycin Hydrochloride
Testosterone Enantate
Vincristine Sulfate

Replacements

The following existing International Chemical Reference Substances should be replaced by new batches in 1986

Allopurinol
Chloramphenicol (*)

Chloramphenicol Palmitate (*)
Vitamin A Acetate

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

APPENDIX 6

ERGOTAMINE TARTRATE

Control No 385013

Analytical Report

The stock of the current batch of the International Chemical Reference Substance for ergotamine tartrate, Control No 276013 is depleted and has to be replaced. The monograph for ergotamine tartrate in the International Pharmacopoeia Ed. III, Vol 2 requires a reference substance to be used in the test for identity by thin-layer chromatography.

MATERIAL

A sample of ergotamine tartrate was obtained from Sandoz Ltd, Basle. About 50 g of the sample (lot no 81001) were received at the WHO Centre in June 1985. The material is being stored protected from light in tightly closed containers at +5 °C.

ANALYTICAL DATA

Description: Almost white, crystalline powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (no 385013). The spectrum is concordant with the spectra obtained from the ICRS 276013 and EPCRS (Batch no 2).

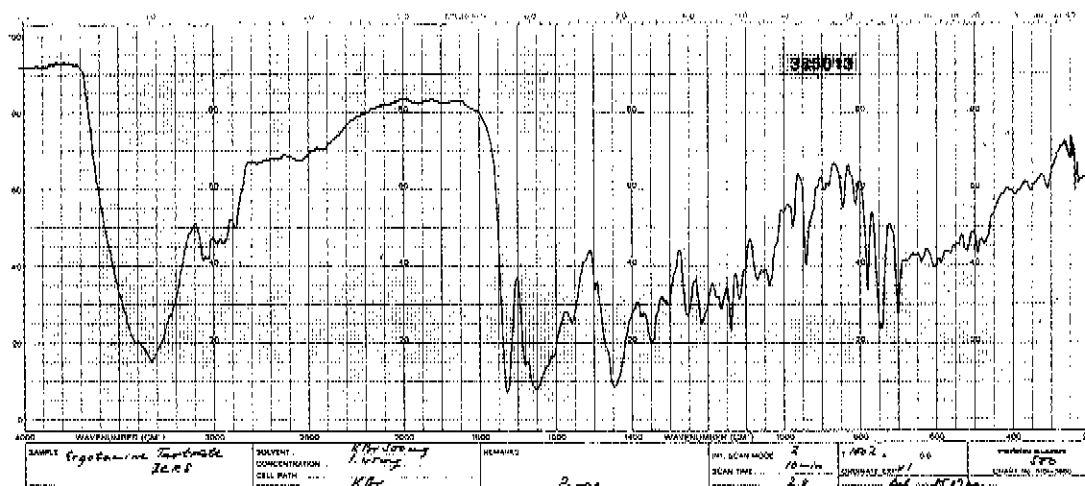


Figure 1. IR-spectrum of ergotamine tartrate No 385013. 1.45 mg in 300 mg KBr, recorded against a KBr reference disc. Instrument: Perkin Elmer 580

Ultra-violet absorption: The ultra-violet absorption spectrum in the range 240 nm to 350 nm of a 0.005% solution in a 1% tartaric acid solution, exhibits a maximum at 318 nm and a minimum at 271 nm; the absorbance of a 1-cm layer at the maximum wavelength is 0.59.

Specific optical rotation of ergotamine base

20°C
[α]_D = -156.3⁰ (n= 3) in ethanol-free chloroform.

The determination was performed according to Ph. Int. Ed. III, Vol 2, but modified according to the monograph in Ph. Eur. 2nd Ed. The determination of ergotamine base was performed by a non-aqueous titration instead of the gravimetric determination prescribed in Ph. Int.

ASSAY

99.2% (n= 7) determined by titration with acetic perchloric acid and potentiometric determination of the end-point. The calculations were performed with reference to the loss on drying reported below.

Loss on drying

24 mg/g (2.4%) when dried for 6 hours to constant weight in vacuo at 95⁰ C.
Desiccant: Silica gel.

Water

1.6% determined by Karl Fischer titration.

Methanol

0.87% determined by gas chromatography.

Column: 1.5 meter long packed with Tenax GC, 60/80, 156 maintained at 80⁰ C.

Oven temperature: 80 - 100 - 200 - 200⁰ C

time: 0 - 4 - 6 - 15 minutes

Detector: Flame ionization detector

PURITY

Clarity and colour of solution: According to Ph. Int. Ed. III, Vol 2. Complies.

Thin-layer chromatography

The following TLC systems were used.

I. Thin-layer: Silica gel 60 (Merck)
Eluent: Chloroform/Methanol 90 + 10
Sample: 50 µg of ergotamine tartrate was applied.
Visualization: UV-light at 366 nm.

R_f (ergotamine tartrate) = 0.3

The detection limit for ergotamine tartrate was less than 0.1 µg (0.2%).

Result: Two secondary spots were observed with R_f = 0.55 (ergotamine) and R_f = 0.37 (unknown). They were estimated to 0.3% when compared to low loadings of ergotamine tartrate. The chromatograms were evaluated using a Zeiss KM3 Chromatogram Spectrophotometer operated in the reflectance mode at 318 nm.

II. Thin-layer: Silica gel 60 (Merck)
Eluent: Chloroform/Methanol 90 + 10
Sample: 50 µg of ergotamine tartrate applied in each spot. After application the spots were exposed to ammonia vapour for 20 sec and cold air for 20 sec. before developing. It is not advisable to put ammonia in the chromatographic chamber as prescribed in Ph. Int. Ed. III, Vol 2. This resulted in further degradation on the plate (0.74% impurities).

Visualization: Spraying with 2%, 4-dimethylaminobenzaldehyde in ethanol/ hydrochloric acid (50/50) and examination in daylight.

The detection limit for ergotamine tartrate was estimated to less than 0.25 µg (0.5%).

Result: One faint secondary spot corresponding to ergotamine was observed, it was estimated to less than 0.25 µg (0.5%).

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurements to about 0.18% at 210 nm. A chromatogram is shown in Figure 2.

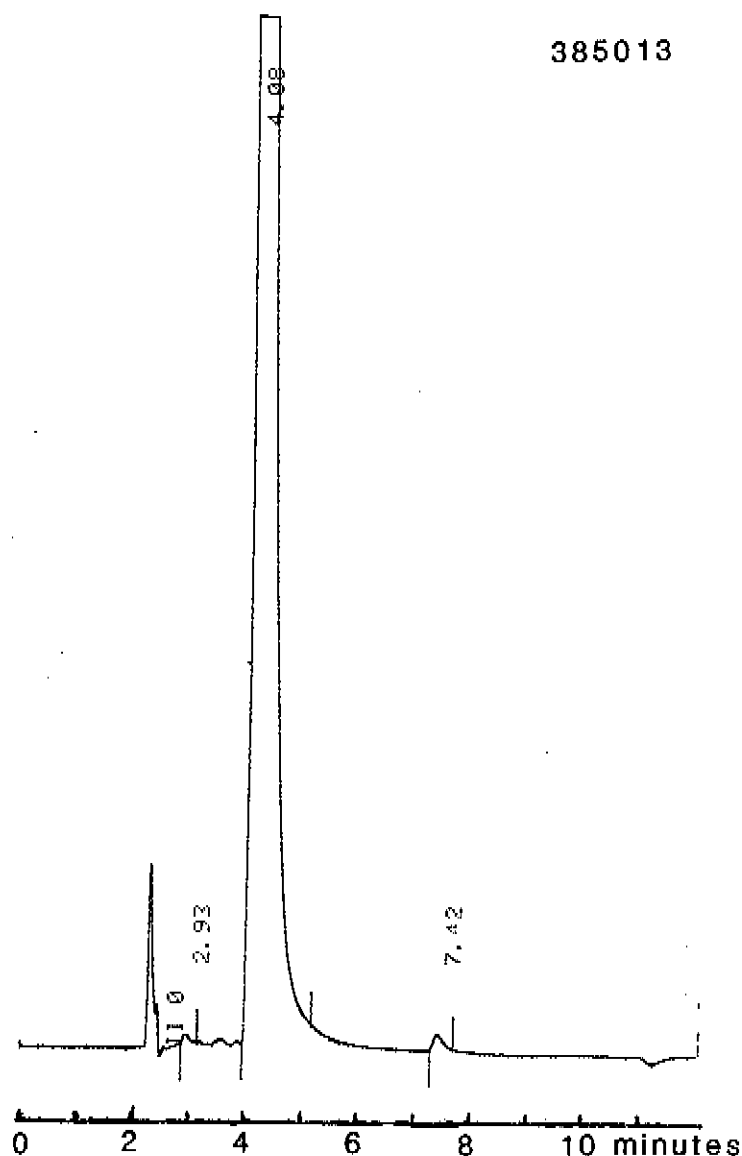


Figure 2. A chromatogram of ergotamine tartrate No 385013.

Two impurities were observed; ergotamine (7.42 min) estimated to 0.12% when compared to a reference of ergotamine, and one unknown (2.93 min). 318 nm was also used as detection wavelength but found to be less sensitive than 210 nm.

It is advisable to prepare fresh solutions as epimerization of ergotamine tartrate takes place. After 30 min about 0.3% of ergotaminine was found (solvent: acetonitrile/water 50/50).

The system is also capable of separating ergocryptine and ergocristine. See Figure 3.

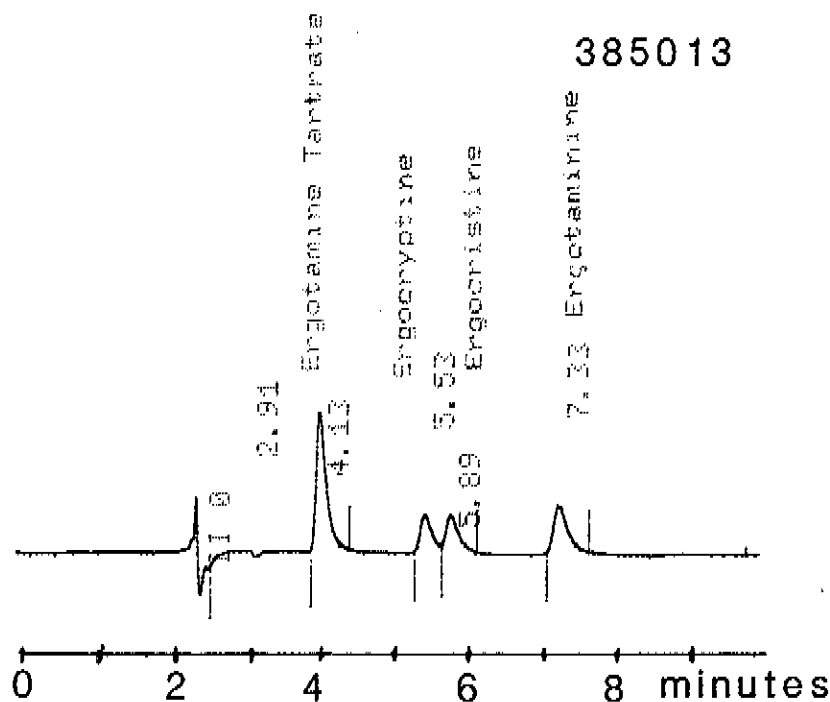


Figure 3. Chromatogram of ergotamine tartrate, ergocryptine, ergocristine and ergotaminine.

The following conditions were used:

Eluent: Acetonitrile/Ammoniumcarbonate (0.02 M in water) (50:50)
 Column: Vydac 218 TP54 (250 mm x 4.5)
 Detector: Varian UV 200 operated at 210 nm and 318 nm.
 Pump: Varian 5560 operated at a flowrate of 1 ml/min
 Integrator: Varian 4270 Attenuation: 1
 Sample: 1 mg/ml dissolved in acetonitrile/water (50/50).
 10 µl corresponding to 10 µg was injected.

DATA GIVEN BY THE MANUFACTURER

Appearance	complies
Odour	complies
Identity	positive
Impurities	complies
Reaction	pH value 4.6
Loss on drying	3.1%
Assay	99.0%

CONCLUSION

Ergotamine tartrate Control No 385013 can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 7

ISONIAZID

Control No 185124

Analytical Report

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

MATERIAL

A sample of isoniazid was generously offered by Bayer AG, Leverkusen. About 100 g of the sample (lot no 915618) were received at the WHO Centre in December 1984. 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 185124). The spectrum is concordant with the spectrum obtained from the USP Ref. stand. Lot F.

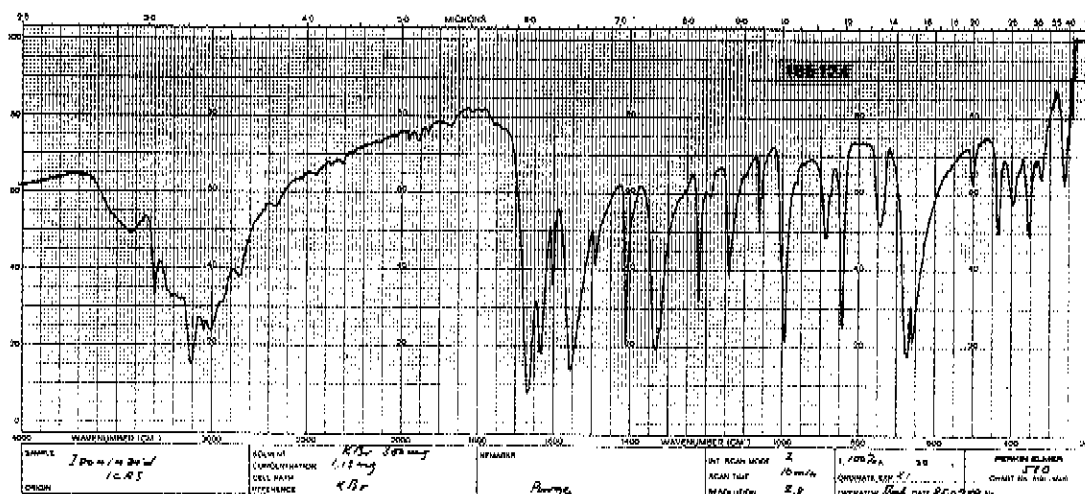


Figure 1. IR-spectrum of 1.1 mg of isoniazid in 300 mg KBr recorded against a KBr reference disc. Instrument: Perkin Elmer 580.

Elemental analysis

	C (%)	H (%)	N (%)
Theoretical	52.5	5.1	30.6
Found	52.7	5.2	30.6

The analysis was performed at Mikro Kemi AB, Uppsala.

UV-spectrum

λ_{max} in 0.01 N HCl = 267 nm and 214 nm
E (1%, 1cm) = 416 (n= 3) at 267 nm
= 434 (n= 3) at 214 nm

For USP Ref. Stand Lot F E(1%, 1 cm) was found to be 415 (267 nm) and 431 (214 nm).

A UV-spectrum in 0.01 N HCl is given in Figure 2.

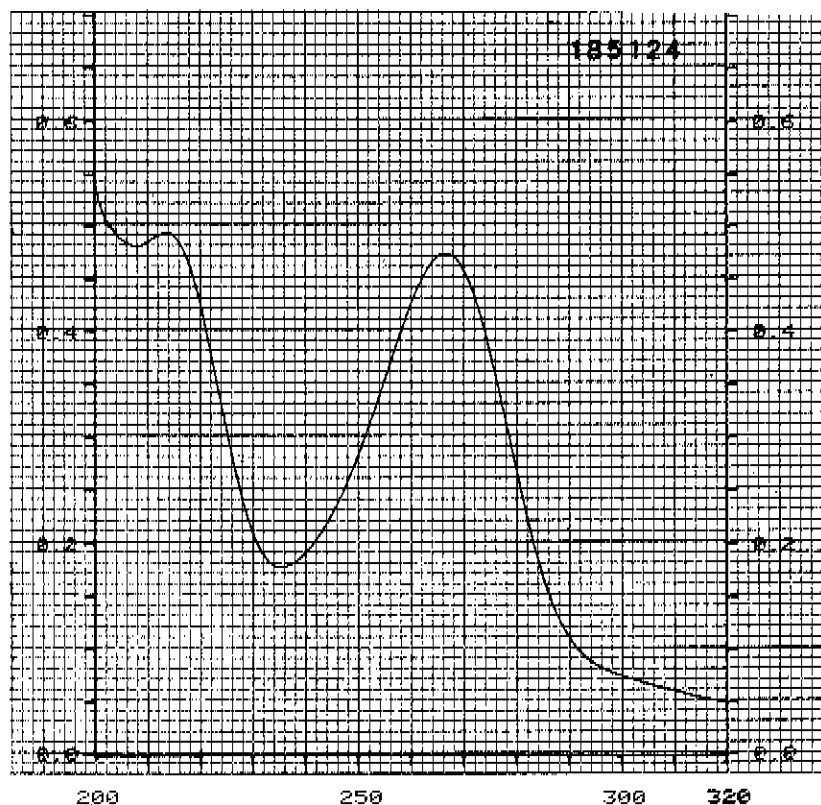


Figure 2. UV-spectrum of isoniazid 11.5 $\mu\text{g/ml}$ in 0.01 N HCl.

ASSAY

Potentiometric titration with 0.1 N perchloric acid. 80 mg was dissolved in 20 ml glacial acetic acid + 10 ml acetic acid anhydride.

Result: 100.0% w/w (n= 4)

Loss on drying

0.0% (105⁰ C)

PURITY

pH value

pH= 7.3 of a 0.05 g/ml solution

Melting temperature: 172.4⁰ C, determined by the capillary method of Ph. Int. III.

Total solid impurities

1) Differential thermal analysis (DTA): The substance shows a tendency to melt under decomposition so it is not possible to estimate the purity by this method. Melting temperature = 170.2° C.

Thin-layer chromatography

Two thin-layer chromatographic systems were used.

System 1 (according to Ph. Int. Ed. III, Vol 2.)

Thin-layer: Silica Gel G (Merck)
Eluent: Acetone/Water (98 + 2)
Sample: 1000 µg of isoniazid was applied. As reference 0.1 µg and 0.2 µg of hydrazine hydrate was applied.
Visualization: Spraying with 4-dimethylamino-benzaldehyde and examination in daylight.
Result: One extremely weak spot corresponding to hydrazine was estimated to less than 0.005%.

System 2

Thin-layer: Silica Gel 60, F-254 (Merck)
Eluent: Ethylacetate / Acetone / Methanol / Water (50 + 20 + 20)
Sample: 1000 µg of isoniazid was applied. As reference 0.1 µg of hydrazine hydrate and 0.2 µg of isonicotinic acid were applied.
Visualization: UV-light of 254 nm

<u>Substance</u>	<u>R_f</u>
Isoniazid	0.4
Hydrazine hydrate	0.6
Isonicotinic acid	0.3

Result: Less than 0.01% impurities was observed in UV-light of 254 nm. They were not identical to any of the references applied.

High performance liquid chromatography

No impurities were found. A chromatogram is shown in Figure 3.

The system was evaluated at 210, 254 and 267 nm but no impurities were found at any of these wavelengths. The same result was obtained with USP Lot F. Isonicotinic acid elutes after 6.9 minutes.

The following conditions were used:

Eluent: Acetonitrile / Acetate buffer pH 3.5 (93 + 7)
Column: LiChrosorb D10L (250 mm x 4) (Merck)
Detector: Varian UV 200 operated at 210, 254 and 267 nm.
Pump: Varian 5560 operated at a flow rate of 1.0 ml/min.
Integrator: Varian 4270 Attenuation: 0.5
Sample: 1 mg/ml dissolved in the eluent. 10 µl corresponding to 10 µg was injected.

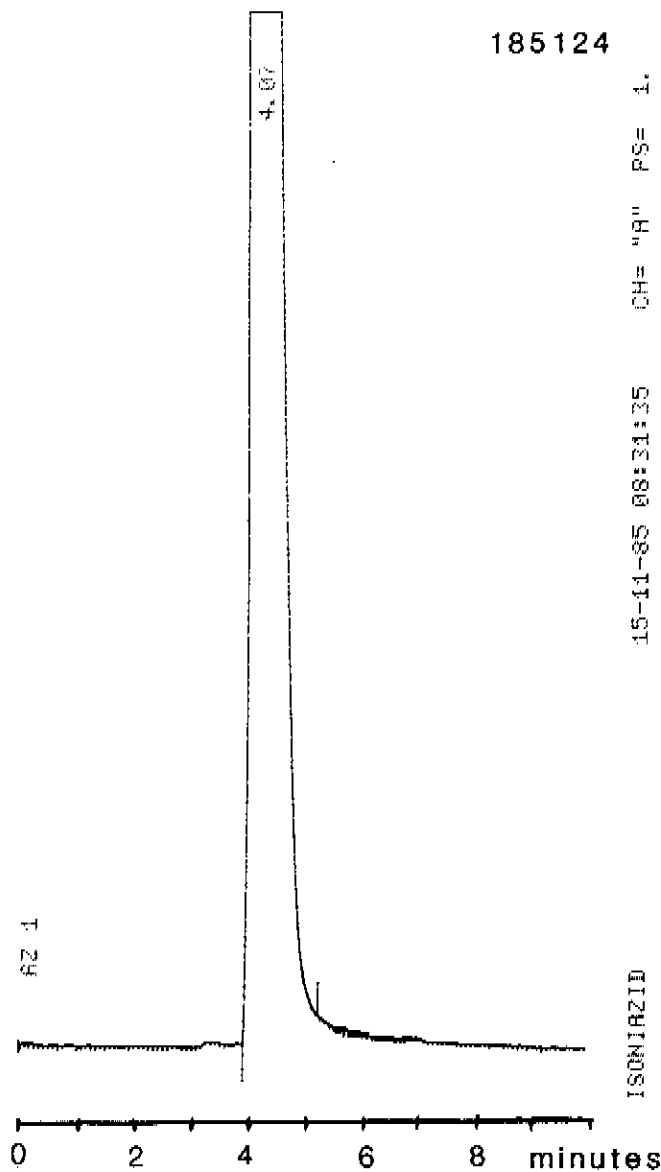


Figure 3. A chromatogram of isoniazid No 185124

STABILITY

Isoniazid was exposed to air of different relative humidity at room temperature (about 20° C) for a period of eleven weeks as described in WHO/PHARM/ 82.509. All samples were unchanged at visual inspection and no weight changes were noted. No signs of degradation were observed when the samples were analyzed by the thin-layer chromatographic system according to Ph. Int.

DATA GIVEN BY THE MANUFACTURER

Melting point	172° C	Sulfate ash	<0.1%
pH in solution	7.4	Loss on drying	0.12%
Sulfate	<0.01%	Hydrazine	complies
Chloride	<0.002%	Assay	99.8%
Heavy metals	<0.001%		

CONCLUSION

Isoniazid No 185124 can be considered suitable as International Chemical Reference Substance for the intended purpose.

APPENDIX 8

N O R E T H I S T E R O N E A C E T A T E

Control No 185123

Analytical Report

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

MATERIAL

A sample of norethisterone acetate was generously offered by Schering AG, Berlin. About 100 g of the sample (lot no 24035009) were received at the WHO Centre in November 1984. 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 185123). The spectrum is concordant with the spectrum obtained from the USP Ref. stand. Lot F1.

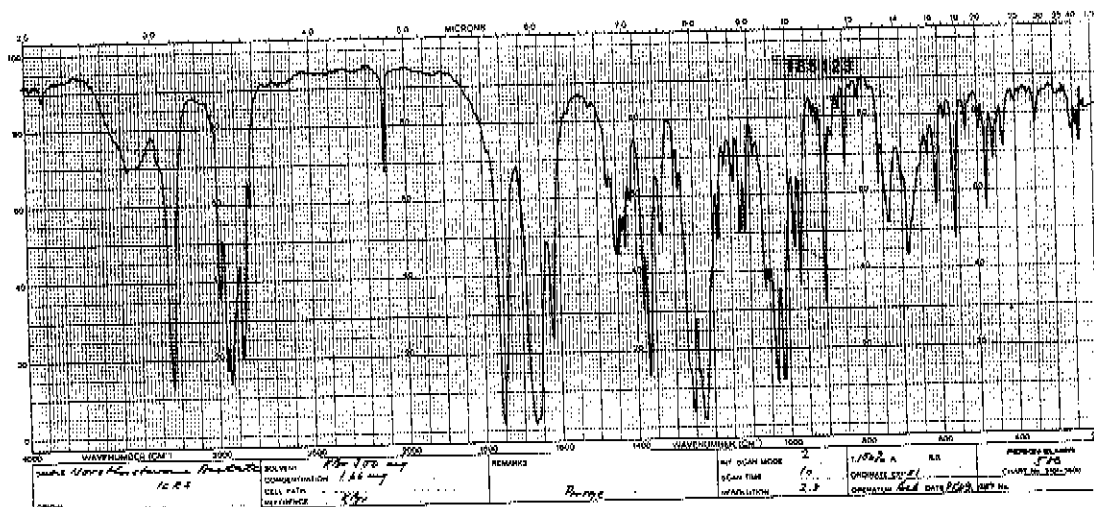


Figure 1. IR-spectrum of 1.7 mg of norethisterone acetate in 300 mg KBr recorded against a KBr reference disc. Instrument: Perkin Elmer 580.

Elemental analysis

	C (%)	H (%)	N (%)
Theoretical	77.6	8.2	-
Found	77.6	8.0	-

The analysis was performed at Mikro Kemi AB, Uppsala.

Nuclear magnetic resonance spectra (NMR)

¹³C NMR spectrum was recorded.

Result: Conforms

The amount of impurities were roughly estimated to less than 1 per cent.

UV-spectrum

A UV-spectrum in ethanol is given in Figure 2.

λ_{max} in ethanol = 240.7 nm
E (1%, 1cm) = 516 (n= 4)

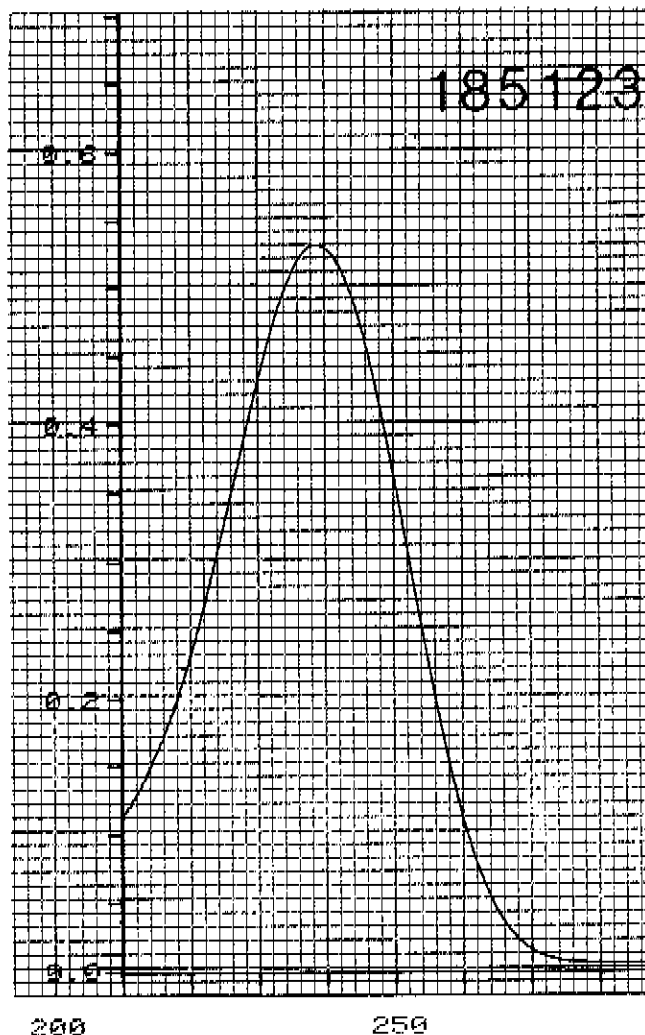


Figure 2.
UV-spectrum of norethisterone
acetate 10 µg/ml in ethanol

Test for acetate

According to Ph. Int. Ed. III, Vol 2, identity test C.

Result: Conforms

ASSAY

The spectrophotometric assay described in the Ph. Int. Ed. III, Vol 2 was used. USP Reference Standard Lot F-1 was used as standard and regarded as 100 per cent.

Result: 100% (n= 4)

Loss on drying

0.05% (105° C)

PURITY

Total solid impurities

1) Differential thermal analysis (DTA): The substance shows a tendency to melt under decomposition and the purity value decreases with decreasing heating rate.
Melting temperature: 163.4^o C (Mettler TA 2000)

Thin-layer chromatography

The following TLC systems were used.

Thin-layer: Silica gel 60, F-254 (Merck) and HPTLC, silica gel 60, F-254 (Merck)
Eluent: Toluene: Ethylacetate (1 + 1)
Sample: 100-200 µg of norethisterone acetate was applied.
Visualization: UV-light of 254 nm and spraying with sulfuric acid/ethanol followed by heating to 105^o C and examination in daylight. R_f (norethisterone acetate) = 0.47
The detection limit for norethisterone acetate was less than 0.1 µg (0.1%)

Result: The total amount of impurities was estimated to about 0.4%. Two impurities were observed with R_f = 0.38 (UV 254) and a weak spot at R_f = 0.60 which appears after spraying with sulfuric acid. The chromatogram was evaluated using a Zeiss KM3 Chromatogram Spectrophotometer operated in the reflectance mode at 240 nm. The dominating peak at R_f = 0.38 was estimated to 0.2% (n = 3).

None of the two spots were identical to ethisterone or norethisterone. USP Reference Standard Lot F-1 had a different purity profile with 6 weak spots all with R_f < 0.47.

The TLC system described above was also applied to a HPTLC plate and the plate was scanned at 240 nm (CAMAG TLC scanner). Two impurities were observed with R_f = 0.56 and 0.82 respectively. Their total amount was estimated to about 0.4%. The spots were more distinct on the HPTLC plate where both could be detected in UV-light.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.3%. A chromatogram is shown in Figure 3. Norethisterone elutes after 4.2 minutes and was not detected in the chromatogram.

The following conditions were used.

Eluent: Acetonitrile/Water (70:30)
Column: Spheri S5 ODS 1 (250 mm x 4.5) (Phase Sep)
Detector: Varian UV 200 operated at 240 nm
Pump: Varian 5560 operated at a flow rate of 1.0 ml/min
Integrator: Varian 4270 Attenuation: 16
Sample: 1 mg/ml dissolved in the eluent.
10 µl corresponding to 10 µg was injected.

The chromatogram was also evaluated with a diode array detector (HP 1040 A). The UV maximum was found at 241 nm for the main impurity (5.8 min) as well as for the main peak (6.4 min).

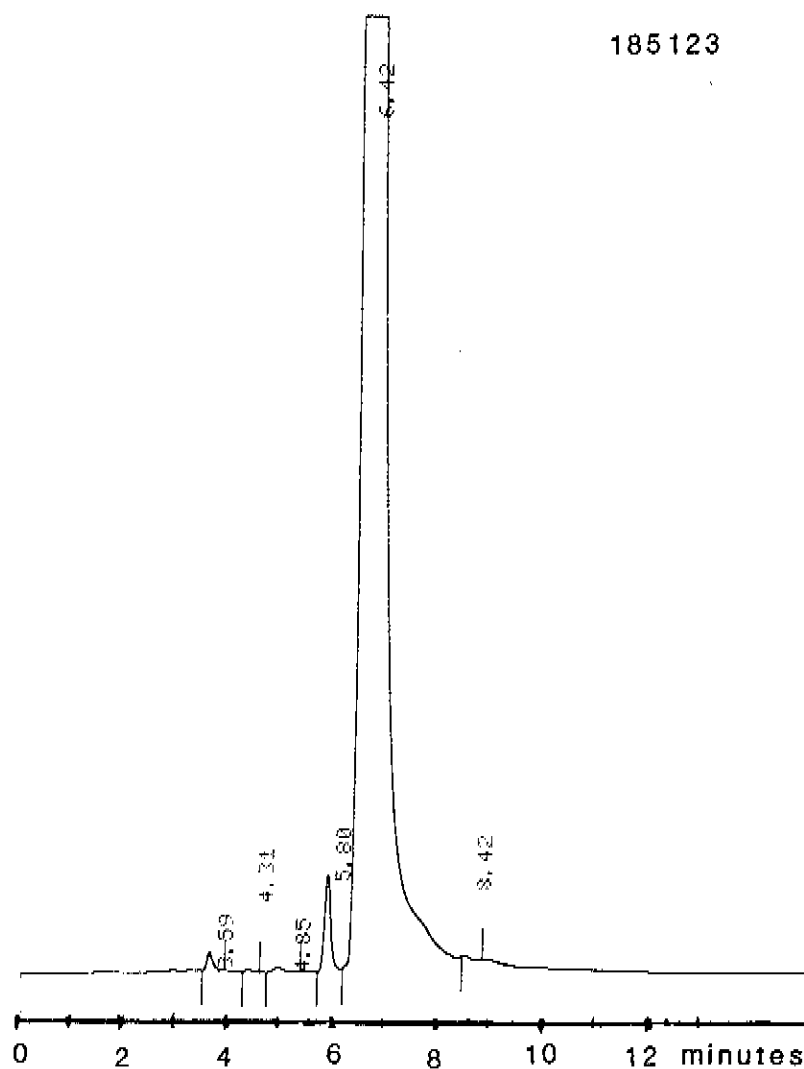


Figure 3. Chromatogram of norethisterone acetate No 185123.

STABILITY

Norethisterone acetate was exposed to air of different relative humidity at room temperature (about 20° C) for a period of 5 weeks as described in WHO/PHARM/82.509. All samples were 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.

DATA GIVEN BY THE MANUFACTURER

Description: White to creamy white, odorless, crystalline powder.
Identity: IR-, UV-spectrum and ethyl acetate; conforms
Melting temp: 162.5^o - 163.5^o C
Spec. rotation: -34.3^o
Spec. extinction: 513
Content: 99.8%
Solution: Clear
Loss on drying: 0.0%
Sulfated ash: 0.02%
TLC test
 byspots 3
 single spot max 1%, conforms
Estrogens: threshold value 0.3 - 1.0 mg

Calculated on the dried basis.

CONCLUSION

Norethisterone acetate No 185123 can be considered suitable as International Chemical Reference Substance for the intended purpose. On the basis of the results obtained the content of norethisterone acetate when used in the spectrophotometric assay is taken to be 100 per cent calculated with reference to the anhydrous substance.

PAP A V E R I N E H Y D R O C H L O R I D E

Control No 185127

Analytical Report

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

MATERIAL

A sample of papaverine hydrochloride was generously offered by Recordati, Milano. About 100 g of the sample (lot no 8414/1011) were received at the WHO Centre in December 1984. 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 STRUCTUREInfrared spectrum

An infrared spectrum is given in Figure 1 (no 185127). The spectrum is concordant with the spectrum obtained from the USP Ref. stand. Lot F1.

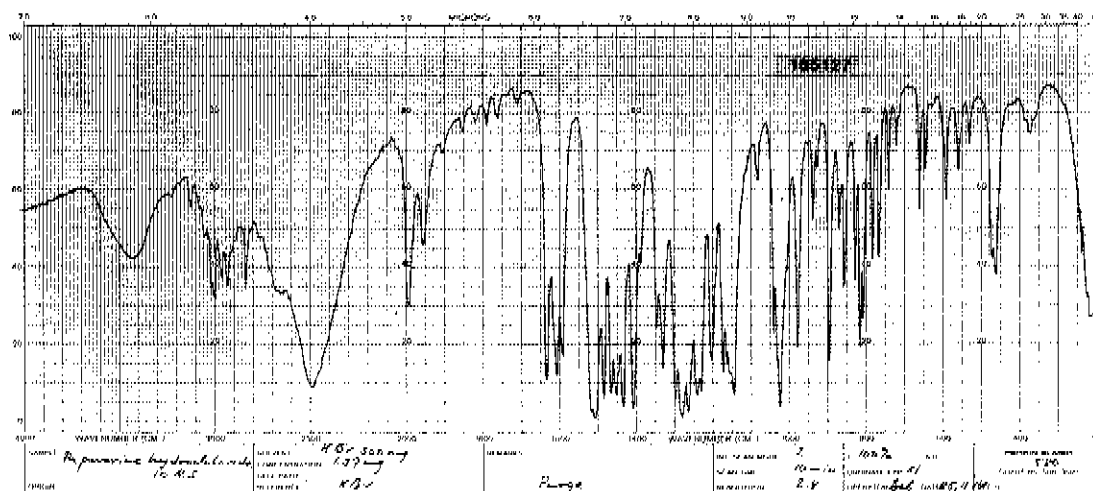


Figure 1. IR-spectrum of 1.4 mg of papaverine hydrochloride in 300 mg of KBr recorded against a KBr reference disc. Instrument: Perkin Elmer 580.

Elemental analysis

	C (%)	H (%)	N (%)
Theoretical	63.9	5.90	3.73
Found	64.0	6.0	3.8

The analysis was performed at Mikro Kemi AB, Uppsala.

Identity for chloride: According to Ph. Int. Ed. III, Vol 2. Conforms.

UV-spectrum

A UV-spectrum in 0.01 N HCl is given in Figure 2.

λ_{max} in 0.01N HCl = 250.4 nm
E (1%, 1cm) = 1660 (n= 3)

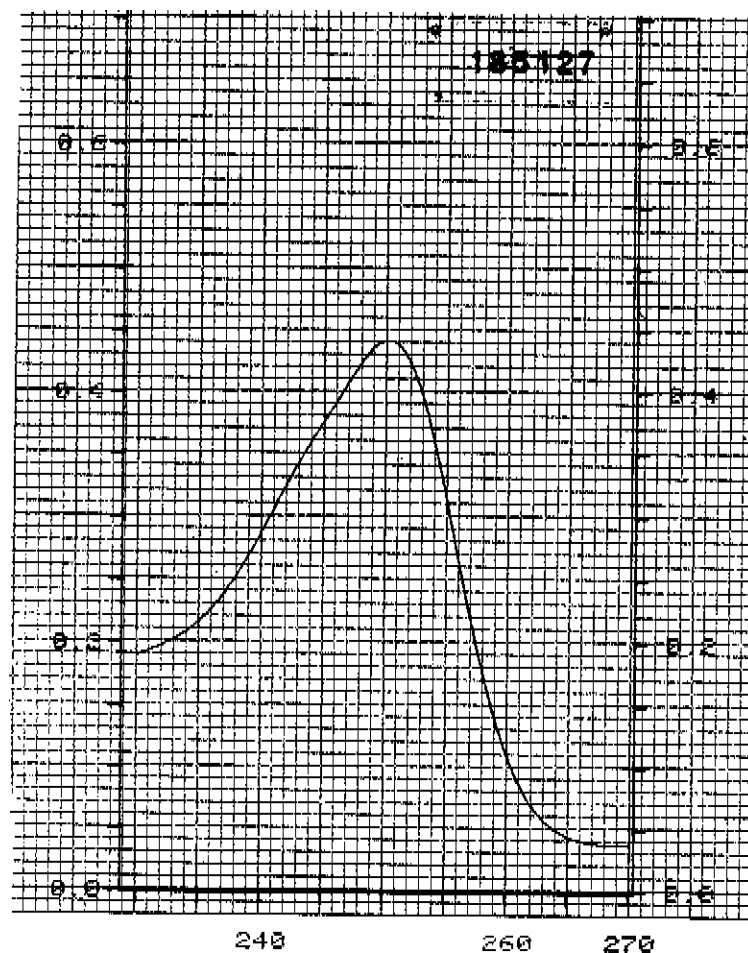


Figure 2. UV-spectrum of papaverine hydrochloride 2.7 $\mu\text{g/ml}$ in 0.01 N HCl.

ASSAY

100.0% (n= 5). Determined by potentiometric titration with 0.1N perchloric acid according to Ph. Int. Ed. III, Vol 2.

Loss on drying

0% (105° 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 under decomposition.

pH value: 3.25 in a 2% aqueous solution.

Clarity and colour of solution: According to Ph. Int. Ed. III, Vol 2. Conforms.

Thin-layer chromatography

The system described under related substances in Ph. Int. was used.

Thin-layer: Silica gel 60. F-254 (Merck)
Eluent: Toluene: ethyl acetate: diethylamine (70 + 20 + 10)
Sample: 500 µg of papaverine hydrochloride was applied
Visualization: UV-light of 254 nm.
 R_f (papaverine hydrochloride) = 0.4, R_f (codeine) = 0.2

Result: The total amount of impurities was estimated to less than 0.02% (det. limit). One very weak spot with R_f = 0.5 was observed. The chromatogram was evaluated using a Zeiss KM3 Chromatogram Spectrophotometer operated in the reflectance mode at 250 nm.

High performance liquid chromatography

No impurities were found. A chromatogram is shown in Figure 3.

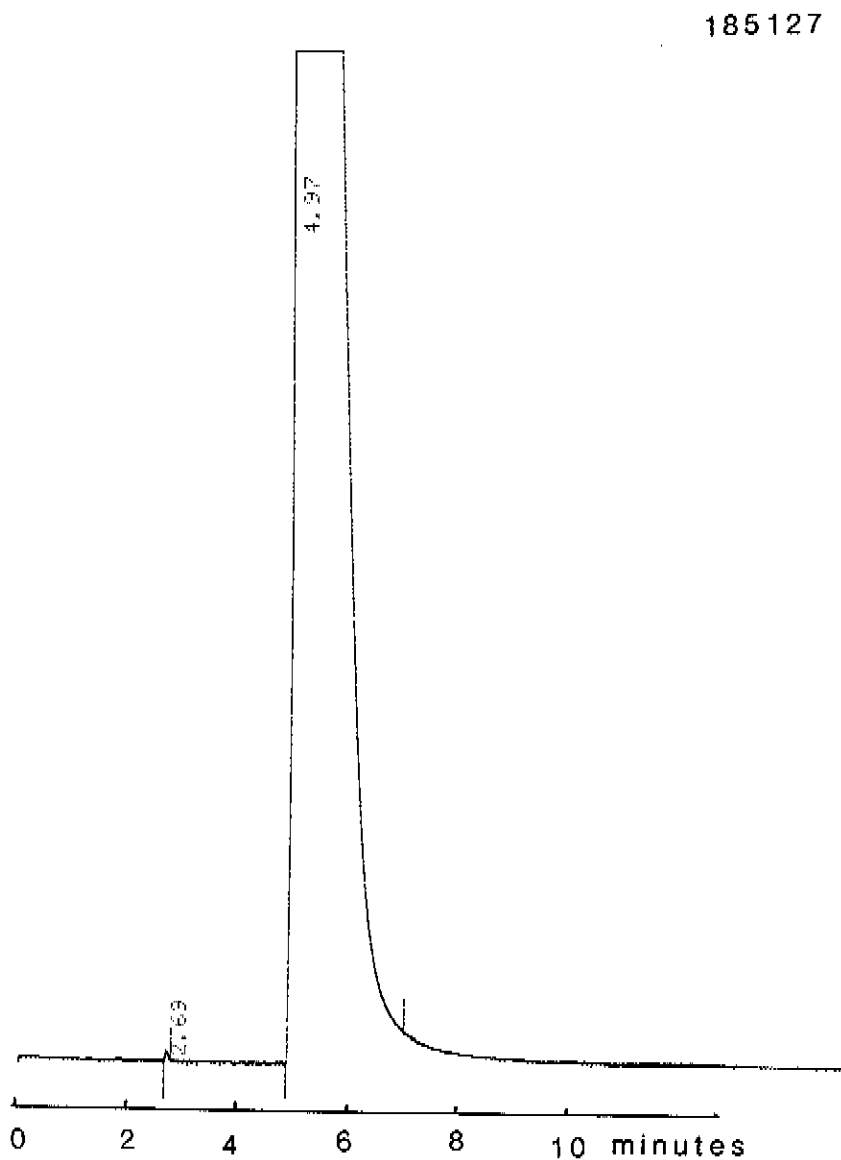


Figure 3. A chromatogram of papaverine hydrochloride No 185127.

The following conditions were used:

Eluent: Phosphate buffer pH 4.5/Acetonitrile (30/70)
Column: RP-18, Spheri-5 (Brownlee)
Detector: Varian UV 200 operated at 238 nm
Pump: Varian 5560 operated at a flow rate of 1 ml/min
Integrator: Varian 4270 Attenuation:1
Sample: 1 mg/ml dissolved in acetonitrile/water (50/50). 10 μ l corresponding to 10 μ g was injected

The same result was obtained for USP standard Lot F-1.

STABILITY

Papaverine hydrochloride was exposed to air of different relative humidity at room temperature (about 20^o C) for a period of 5 weeks as described in WHO/ PHARM/82.509. All samples were unchanged at visual inspection and no weight changes were noted. No signs of degradation were observed when selected samples were analyzed by the liquid chromatographic method described above.

DATA GIVEN BY THE MANUFACTURER

Description: White, crystalline powder, odourless
Identification: IR spectrum, UV-spectrum, test for chlorides
Assay: 100.11%
pH: 3.24
Loss on drying: 0.053%
Residue on
Ignition: 0.02%

Cryptopine, thebaine or other organic impurities: Passes test
Completeness of solution: Passes test

CONCLUSION

Papaverine hydrochloride No 185127 can be considered suitable as International Chemical Reference Substance for the intended purpose.

PROPYLTHIOURACIL

Control No 185126

Analytical Report

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

MATERIAL

A sample of propylthiouracil was generously offered by Diamalt AG, Raubling. About 100 g of the sample (lot no 1194) were received at the WHO Centre in October 1984. The material is being stored protected from light in tightly closed containers at +5 °C.

ANALYTICAL DATA

Description: Colourless crystalline, powder.

EVIDENCE OF CHEMICAL STRUCTURE

Infrared spectrum

An infrared spectrum is given in Figure 1 (no 185126). The spectrum is concordant with the spectrum obtained from the USP Ref. stand. Lot 1074-F.

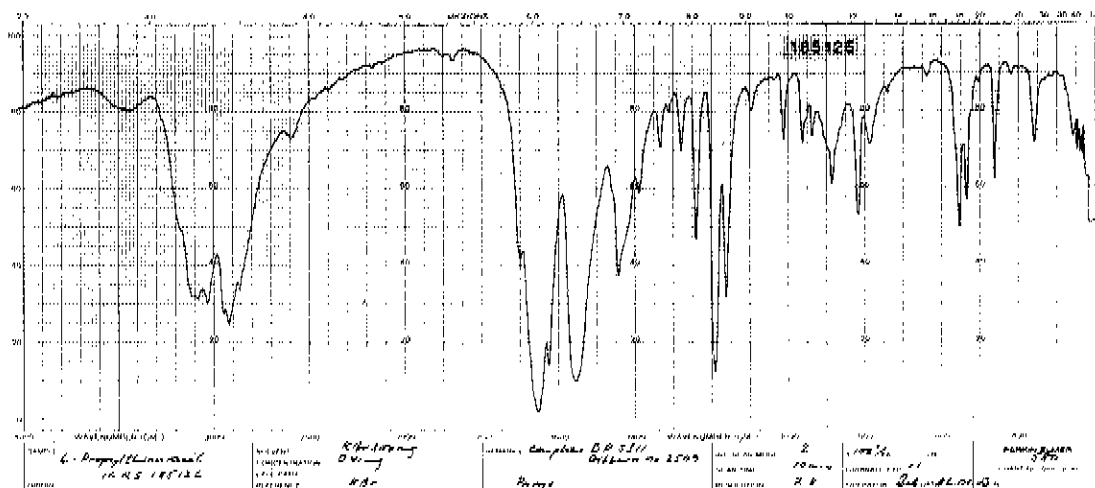


Figure 1. IR-spectrum of 0.41 mg propylthiouracil in 300 mg KBr recorded against a KBr reference disc. Instrument: Perkin Elmer 580.

Elemental analysis

	C (%)	H (%)	N (%)
Theoretical	49.4	5.9	16.5
Found	49.3	6.0	16.3

The analysis was performed at Mikro Kemi AB, Uppsala.

Identity test B According to Ph. Int. Ed. III, Vol 2. Conforms. The method was modified by dissolving the substance in ethanol instead of boiling water.

Melting temperature: 219.5° C, determined by the capillary method of Ph. Int. Ed. III.

UV-spectrum

A UV-spectrum in methanol is given in Figure 2.

λ_{max} in methanol = 274.7 nm and 213.8 nm
E (1%, 1cm) 274.7 nm = 950 (n= 8) Relative standard deviation= 0.3%

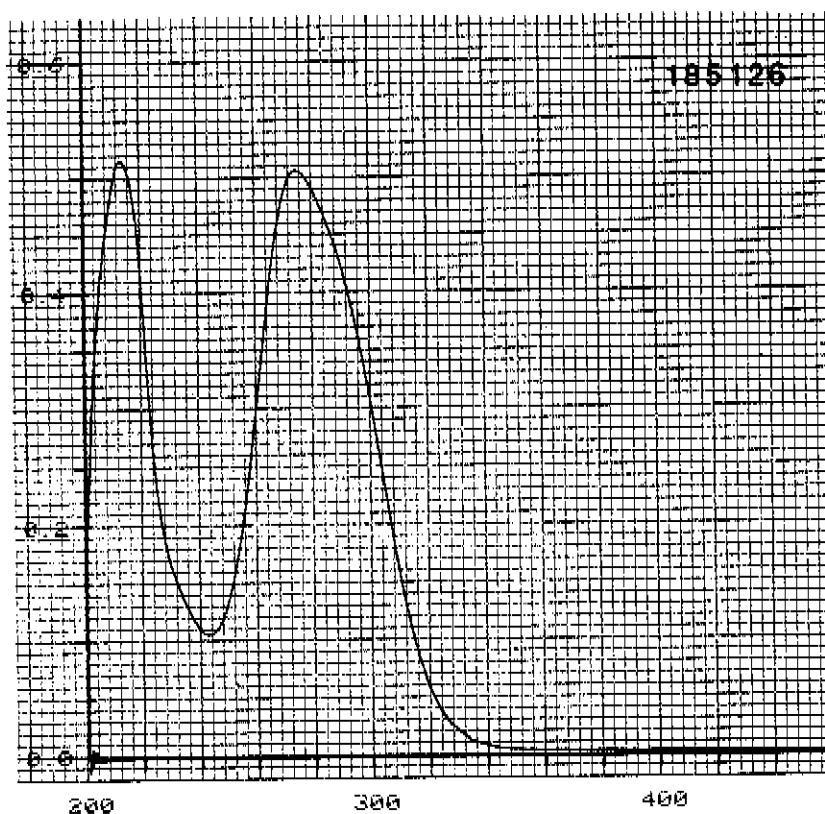


Figure 2. UV-spectrum of propylthiouracil 5.3 µg/ml in methanol

ASSAY

100.0 (n= 5) determined by titration with 0.1 N NaOH according to Ph. Int. Ed. III, Vol 2. 99.9% (n= 5) Determined by titration as above but with potentiometric determination of end-point (according to USP XXI).

Loss on drying

0% (105° C)

PURITY

Total solid impurities

1) Differential thermal analysis (DTA): About 0.2 mole per cent (n= 7). The determination was carried out on 2 mg using a heating rate of 2° C per minute.

Melting temperature: 218.3° C

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 following thin-layer chromatographic system was used.

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

Eluent: Chloroform/Methanol (1 + 1)

Sample: 200 µg of propylthiouracil was applied

Visualization: UV-light of 254 nm and spraying with sodium nitroprusside reagent.

R_f (propylthiouracil) = 0.75

The detection limit for propylthiouracil was less than 0.2 µg (0.1%).

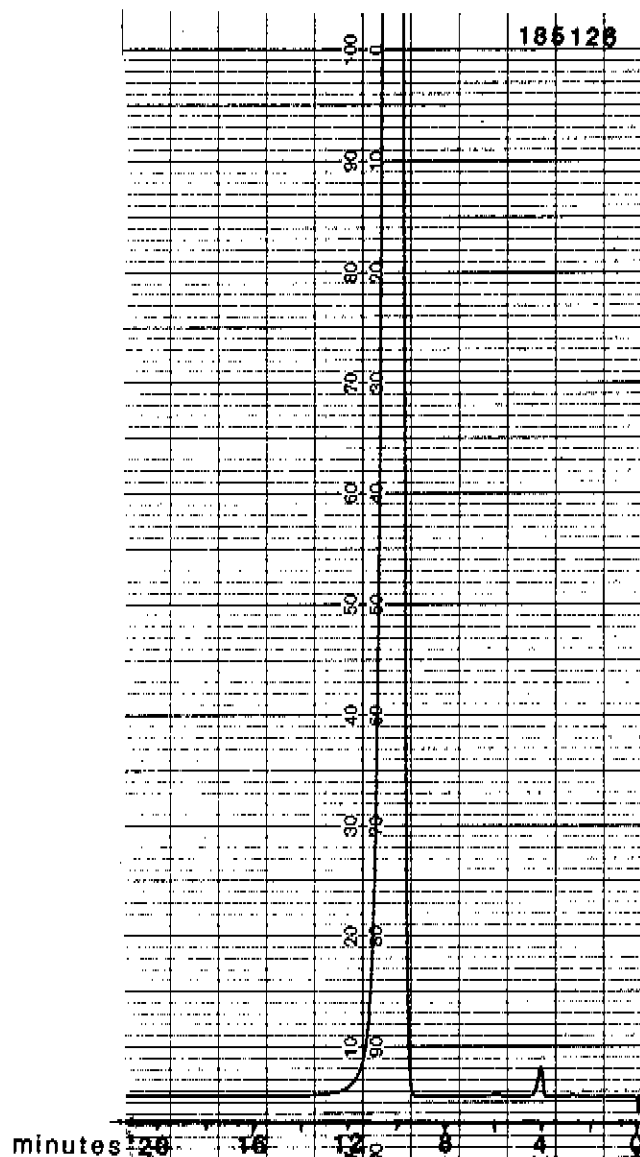
Thiourea was applied as reference (R_f = 0.6)

Result: No extra spots were detected.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.35%. A chromatogram is shown in Figure 3.

Figure 3. Chromatogram of propylthiouracil No 185126.



Two minor impurities were observed eluting after 4 minutes and 5.8 minutes respectively. None of them is identical to thiourea which elutes after 2.6 minutes. Thiourea was not detected, the detection limit is less than 0.05%. In USP reference standard lot 1074-F none of these impurities were found.

The following conditions were used:

Eluent: Phosphate buffer pH 3.5 / Acetonitrile (95 + 5)
Column: μ Bondapak C₁₈ from Waters (250 mm x 4.5)
Detector: Shimadzu SPD-2A operated at 215 nm. Sensitivity: 0.08 AUFS
Pump: Waters, M-6000 operated at a flow rate of 1.5 ml/min.
Integrator: Hewlett Packard 3390 A Attenuation: 2 + 3
Sample: 1 mg/ml dissolved in the eluent. 20 μ l corresponding to 20 μ g was injected.

STABILITY

Propylthiouracil was exposed to air of different relative humidity at room temperature (about 20^o C) for a period of 8 weeks as described in WHO/PHARM/ 82.509. All samples were unchanged at visual inspection and no weight changes were noted. No signs of degradation were observed when selected samples were analyzed by the liquid chromatographic method described above.

DATA GIVEN BY THE MANUFACTURER

Identification: IR spectrum corresponds to reference standard
Description: Colourless crystalline powder
Melting point: 220.5 - 221.5^o C
Water: 0.01% (Karl Fischer)
Ash: 0.1%
Content: 99.4%
Heavy metals: < 20 ppm

CONCLUSION

Propylthiouracil No 185126 can be considered suitable as International Chemical Reference Substance for the intended purpose.

TRIMETHADIONE

Control No 185125

Analytical Report

The monograph for trimethadione in the International Pharmacopoeia Ed. III, Vol. 2. requires a reference substance to be used in the infrared spectrophotometric test for identity as well as in a gas chromatographic assay.

MATERIAL

A sample of trimethadione was generously offered by Abbott, North Chicago, USA. About 200 g of the sample (lot no 22-593-AC) were received at the WHO Centre in October 1984. The material is being stored protected from light in tightly closed containers at +5 °C.

ANALYTICAL DATA

Description: Colourless crystals with slightly camphoraceous odour.

EVIDENCE OF CHEMICAL STRUCTUREInfrared spectrum

An infrared spectrum is given in Figure 1 (no 185125). The spectrum is concordant with the spectrum obtained from the USP Ref. stand. Lot G.

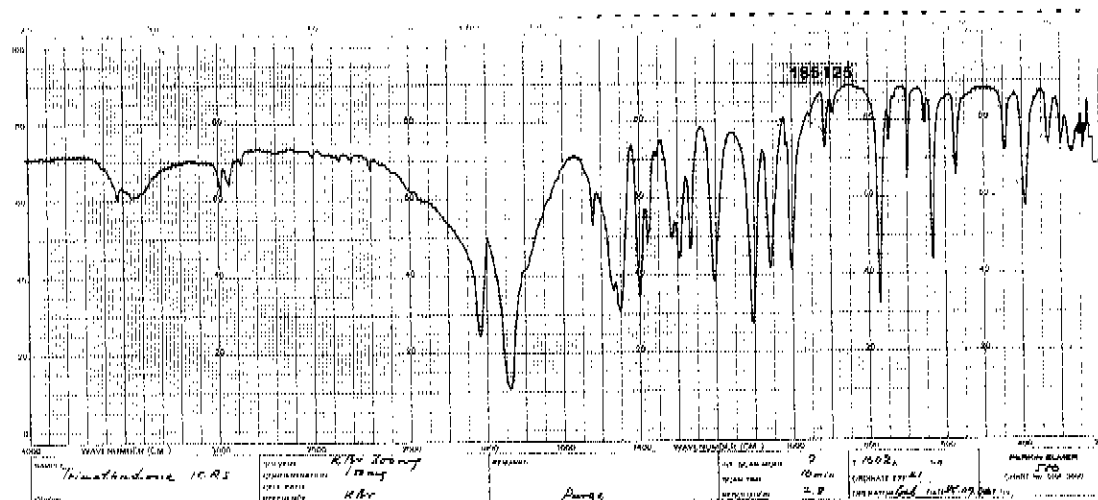


Figure 1. IR-spectrum of 1.50 mg of trimethadione in 300 mg KBr recorded against a KBr reference disc. Instrument: Perkin Elmer 580.

Elemental analysis

	C (%)	H (%)	N (%)
Theoretical	50.3	6.3	9.8
Found	50.6	6.4	9.6

The analysis was performed at Mikro Kemi AB, Uppsala.

Nuclear magnetic resonance spectra (NMR)

^1H -NMR and ^{13}C -NMR spectra were recorded.

Result: Conforms. The amount of impurities were roughly estimated to less than 1%.

UV-spectrum

A UV-spectrum in water is given in Figure 2.

λ_{max} in water = 223 nm
E (1%, 1cm) = 11 (n= 3)

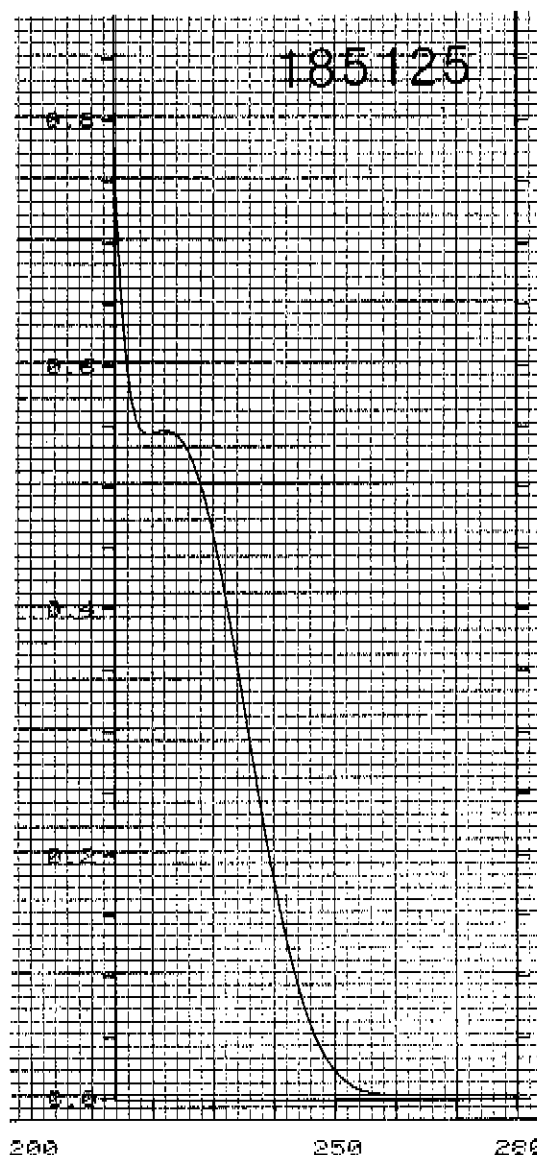


Figure 2. UV-spectrum of trimethadione
499 $\mu\text{g/ml}$ in water.

Melting range: 45.5 - 46.7 $^{\circ}$ C, determined with the Mettler FP 800 Thermosystem (capillary method).

ASSAY

99.8% (n= 8)

The gas chromatographic method described in the International Pharmacopoeia Ed. III, Vol. 2 was used. USP Ref. stand. (Lot F-1) was used as reference standard and regarded as 100%. A chromatogram is shown in Figure 3.

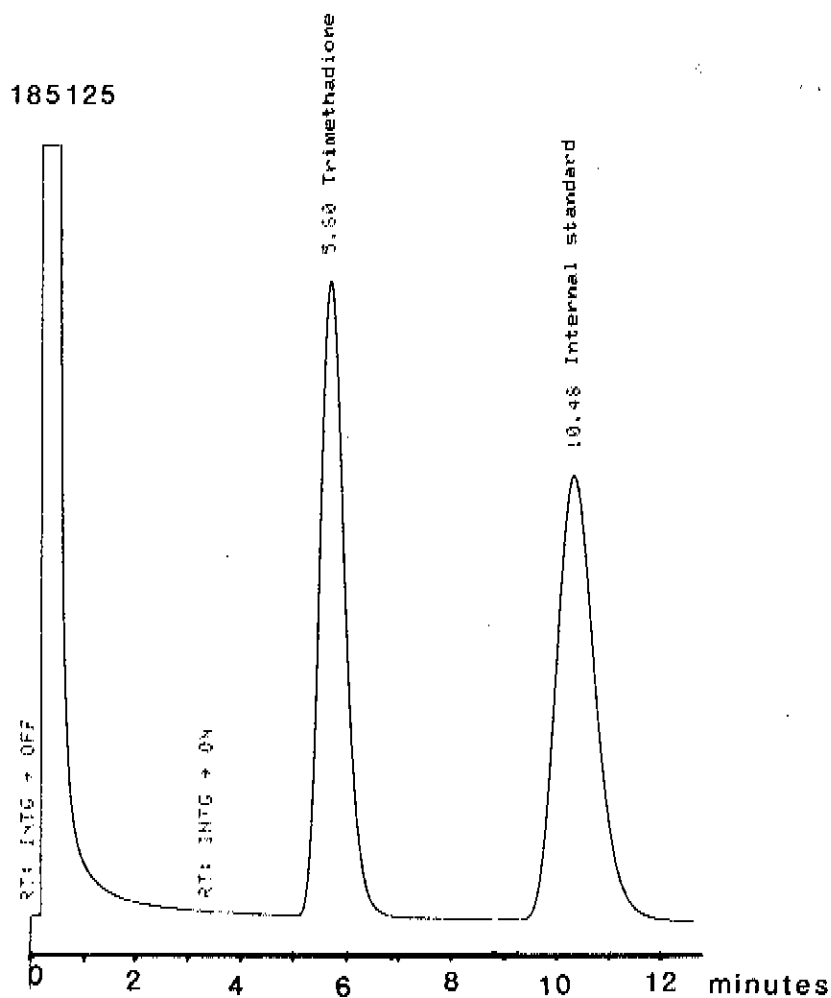


Figure 3. Chromatogram of trimethadione No 185125 and internal standard.

Sample solution: 4 mg/ml
Column: Homepacked Chromosorb W coated with diethylene glycol succinate.
Carrier gas: Nitrogen
Oven temperature: 115^o C
Injection port: 150^o C
Detector: 150^o C
Injection volume: 1 µl
Attenuation: 2 + 7
Gas chromatograph: Hewlett Packard 5880

No contaminants were noted when chromatograms with only trimethadione and internal standard respectively were run.

Loss on drying

As the substance is volatile loss on drying is not a suitable method for this substance. The loss of weight with time is shown in Table 1.

Table 1

<u>Time (hours)</u>	<u>Loss in weight (%)</u>
1.5-2	0.3
3.5	0.42
5.0	0.49
6.5	0.66
8.0	0.84
10.0	1.03
12.0	1.23

The experiment was performed at ambient temperature over silica gel.

Water

0.6 mg/g (0.06%) determined by Karl Fischer titration.

PURITY

Total solid impurities

1) Differential thermal analysis (DTA):
About 0.1 mole per cent ($n=5$)
Melting temperature: 45.5⁰ C

Instrument: Mettler TA 2000 system, operated on-line with a Hewlett Packard calculator (9815A)
Heating rate: 2⁰ C per minute
Sample: About 2 mg
Calculation: By the Mettler standard computer program for purity analysis.

The same purity was obtained for USP Reference standard Lot F-1.

2) Phase solubility analysis (PSA):
It was not possible to perform PSA as the substance is volatile, a fact that disturbs the gravimetric measurements.

High performance liquid chromatography

The total amount of impurities was estimated by peak area measurement to about 0.1%. Chromatograms are shown in Figure 4 a and b.

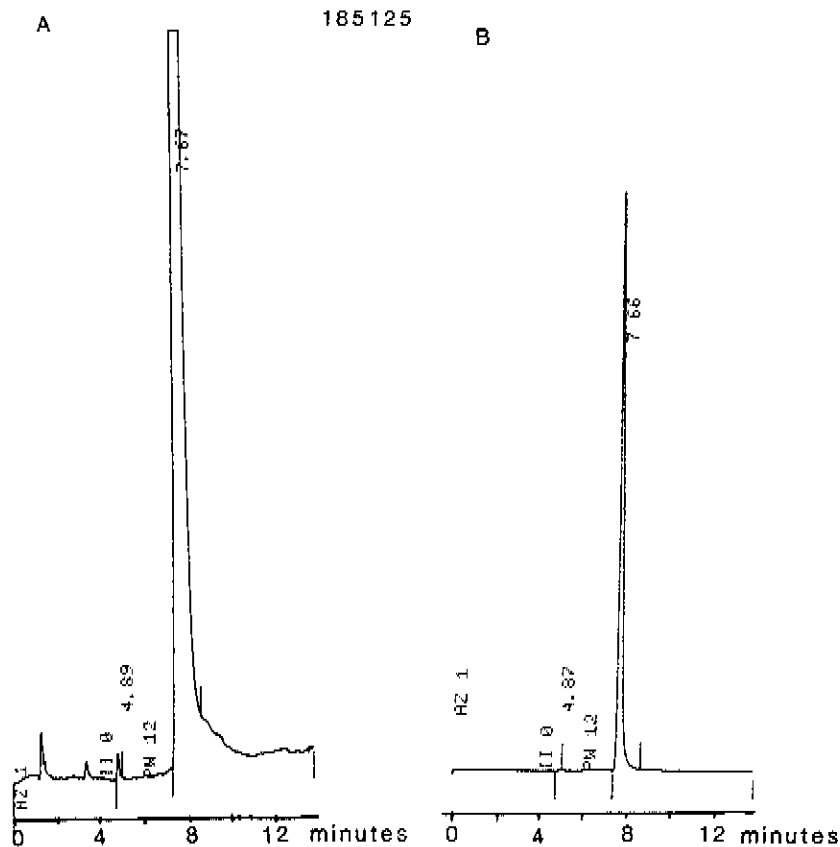


Figure 4. Chromatograms of trimethadione 185125 A) Att: 128, B) Att: 4

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

Time (min)	%A	%B
0	15	85
8	15	85
20	90	10

Column: Spheri S 5 ODS 1 (250 mm x 4.5)
Detector: Varian UV 200 operated at 210 nm
Pump: Varian 5560 Flow rate: 1.0 ml/min
Integrator: Varian 4270 Att: 128 and 4
Sample: 1 mg/ml dissolved in water
10 μ l corresponding to 10 μ g was injected

STABILITY

Trimethadione was exposed to air of different relative humidity at room temperature (about 20^o C) for a period of 5 weeks as described in WHO/PHARM/ 82.509. Throughout a loss in weight was observed, after five weeks as high as 60 - 80%. The samples were analyzed with the HPLC method described above but no signs of degradation were observed. The substance is volatile.

CONCLUSION

Trimethadione No 185125 can be considered suitable as International Chemical Reference Substance for the intended purpose. On the basis of the results obtained the content of trimethadione when used in the gas-chromatographic method is taken to be 99.9 per cent, calculated with reference to the anhydrous substance.

Storage

In a tightly closed container at a cool place. Due to the low melting point it should not be transported at temperatures exceeding 35⁰ C.

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