
Supplementary information

Annotated references

1. Procedure for the selection of recommended international nonproprietary names for pharmaceutical substances and General principles for guidance in devising international nonproprietary names for pharmaceutical substances. In: *International nonproprietary names (INN) for pharmaceutical substances. Cumulative list No. 10*. Geneva, World Health Organization, 2002, pp. ix–xii, available on CD-ROM.

This information is also accessible on the Internet at <http://www.who.int/medicines/>

These two texts are based on World Health Assembly resolution WHA3.11. The procedure for the selection of recommended international nonproprietary names for pharmaceutical substances, and the general principles for selecting international nonproprietary names for pharmaceutical substances, have been updated regularly since the INN programme began in 1950.

2. Guidelines for the graphic representation of chemical formulae. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-fourth report*. Geneva, World Health Organization, 1996, Annex 1 (WHO Technical Report Series, No. 863, pp. 16–49).

These guidelines are also available in *The graphic representation of chemical formulae in the publications of international nonproprietary names (INN) for pharmaceutical substances*. Geneva, World Health Organization, 1995 (document WHO/PHARM/95.579).

These unique guidelines are intended to help scientists to portray chemical names and structures correctly and unambiguously in pharmacopoeias and other compendia. For details of chemical nomenclature conventions, readers are referred to the recommendations of the International Union of Pure and Applied Chemistry.¹

The guidelines should be followed as closely as possible, although it should be noted that unwavering adherence to these principles is not always practicable. Therefore, they may be adapted, with certain exceptions, where necessary to produce accurately drawn structural formulae. Details of the formulae, such as bond lengths, the position of subscripts and superscripts,

¹ International Union of Pure and Applied Chemistry, Organic Chemistry Division, Commission on the Nomenclature of Organic Chemistry. *Nomenclature of organic chemistry, sections, A, B, C, D, E, F, and H*, 4th ed. Oxford, Pergamon, 1979.

Leigh GJ, ed. *Nomenclature of inorganic chemistry: recommendations 1990*. Oxford, Blackwell Scientific, 1990.

and the closeness of apposition of the individual atomic symbols, will depend on the drawing method used, whether computer-based or manual.

The guidelines cover acyclic, cyclic, and ionic structures, isotopically modified and coordination compounds, stereochemistry, carbohydrates, steroids, terpenoids, prostanoids, alkaloids, antibiotics, polypeptides, and polymers.

List of available International Chemical Reference Substances¹

International Chemical Reference Substances (ICRS) are established on the advice of the WHO Expert Committee on Specifications for Pharmaceutical Preparations. They are supplied primarily for use in physical and chemical tests and assays described in the specifications for quality control of drugs published in *The International Pharmacopoeia* or proposed in draft monographs. The ICRS are mainly intended to be used as primary standards to calibrate secondary standards.

Directions for use, and analytical data required for the use described 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 from the WHO Collaborating Centre for Chemical Reference Substances.

ICRS may also be used in tests and assays not described in *The International Pharmacopoeia*. However, the responsibility for assessing the suitability of the substances then rests with the user or with the pharmacopoeia commission or other authority that has prescribed this use.

It is generally recommended that the substances should be stored protected from light and moisture and preferably at a temperature of about 5 °C. When special storage conditions are required, this is stated on the label or in the accompanying leaflet. It is recommended that the user purchase only an amount sufficient for immediate use.

The stability of the ICRS kept at the Collaborating Centre is monitored by regular re-examination, and any material that has deteriorated is replaced by new batches as necessary. Lists giving control numbers for the current batches are issued in the annual reports from the Centre and new yearly lists may be obtained on request.

Orders for the ICRS should be sent to:

WHO Collaborating Centre for Chemical Reference Substances
Apoteket AB
Produktion & Laboratorier Centrallaboratoriet, ACL
Prismavägen 2
S-141 75 Kungens Kurva
Sweden
(Fax: +46 8 740 6040; email: who.apl@apoteket.se)

¹ As updated at the thirty-seventh meeting of the WHO Expert Committee on Specifications for Pharmaceutical Preparations, 22–26 October 2001.

The International Pharmacopoeia

The ICRS are supplied only in the standard packages indicated in the following list:

<i>Reference substance</i>	<i>Package size</i>	<i>Control number</i>
<i>p</i> -acetamidobenzalazine	25 mg	290042
acetazolamide	100 mg	186128
allopurinol	100 mg	287049
amidotrizoic acid	100 mg	196205
2-amino-5-nitrothiazole	25 mg	186131
3-aminopyrazole-4-carboxamide hemisulfate	100 mg	172050
3-amino-2,4,6-triiodobenzoic acid	100 mg	196206
amitriptyline hydrochloride	100 mg	181101
amodiaquine hydrochloride	200 mg	192160
amphotericin B	400 mg	191153
ampicillin (anhydrous)	200 mg	390001
ampicillin sodium	200 mg	388002
ampicillin trihydrate	200 mg	274003
anhydrotetracycline hydrochloride	25 mg	180096
atropine sulfate	100 mg	183111
azathioprine	100 mg	172060
bacitracin zinc	200 mg	192174
beclometasone dipropionate	200 mg	192175
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
betamethasone sodium phosphate	100 mg	196203
betamethasone valerate	100 mg	190145
betanidine sulfate	100 mg	172053
bupivacaine hydrochloride	100 mg	289054
caffeine	100 mg	181102
calcium folinate (leucovorin calcium)	100 mg	194188
captopril	100 mg	197214
captopril disulfide	25 mg	198216
carbamazepine	100 mg	189143
carbenicillin monosodium	200 mg	383043
chloramphenicol	200 mg	486004
chloramphenicol palmitate	1 g	286072
chloramphenicol palmitate (polymorph A)	200 mg	175073
5-chloro-2-methylaminobenzophenone	100 mg	172061

Supplementary information

<i>Reference substance</i>	<i>Package size</i>	<i>Control number</i>
chloroquine sulfate	200 mg	195201
2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid	50 mg	181106
chlorphenamine hydrogen maleate	100 mg	182109
chlorpromazine hydrochloride	100 mg	178080
chlortalidone	100 mg	183114
chlortetracycline hydrochloride	200 mg	187138
cimetidine	100 mg	190150
ciprofloxacin hydrochloride	400 mg	197210
ciprofloxacin by-compound A	20 mg	198220
ciprofloxacin desfluoro-compound	20 mg	198219
ciprofloxacin ethylenediamine-compound	20 mg	198218
cisplatin	100 mg	197207
clomifene citrate	100 mg	187136
clomifene citrate <i>Z</i> -isomer <i>see</i> zuclomifene		
cloxacillin sodium	200 mg	274005
colecalfiferol (vitamin D ₃)	500 mg	190146
cortisone acetate	100 mg	167006
dapsone	100 mg	183115
desoxycortone acetate	100 mg	167007
dexamethasone	100 mg	388008
dexamethasone acetate	100 mg	288009
dexamethasone phosphoric acid	100 mg	192161
dexamethasone sodium phosphate	100 mg	192158
diazepam	100 mg	172062
diazoxide	100 mg	181103
dicloxacillin sodium	200 mg	174071
dicolinium iodide	100 mg	172055
dicoumarol	100 mg	178077
diethylcarbamazine dihydrogen citrate	100 mg	181100
digitoxin	100 mg	277010
digoxin	100 mg	587011
dopamine hydrochloride	100 mg	192159
doxorubicin hydrochloride	100 mg	196202
emetine hydrochloride	100 mg	187134
4-epianhydrotetracycline hydrochloride	25 mg	288097
4-epitetracycline hydrochloride	25 mg	293098
ergocalciferol (vitamin D ₂)	500 mg	190147
ergometrine hydrogen maleate	50 mg	277012
ergotamine tartrate	50 mg	385013
erythromycin	250 mg	191154
erythromycin B	150 mg	194186

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<i>Reference substance</i>	<i>Package size</i>	<i>Control number</i>
erythromycin C	25 mg	194187
estradiol benzoate	100 mg	167014
estrone	100 mg	279015
etacrynic acid	100 mg	281056
ethambutol hydrochloride	100 mg	179081
ethinylestradiol	100 mg	301016
ethisterone	100 mg	167017
ethosuximide	100 mg	179088
etocarlide	100 mg	172057
flucloxacillin sodium	200 mg	195194
flucytosine	100 mg	184121
fludrocortisone acetate	200 mg	195199
fluoroqui-nolonic acid	20 mg	198217
fluorouracil	100 mg	184122
fluphenazine decanoate dihydrochloride	100 mg	182107
fluphenazine enantate dihydrochloride	100 mg	182108
fluphenazine hydrochloride	100 mg	176076
folic acid	100 mg	388019
3-formylrifamycin	200 mg	190149
framycetin sulfate (neomycin B sulfate)	200 mg	193178
furosemide	100 mg	171044
gentamicin sulfate	100 mg	194183
griseofulvin	200 mg	280040
haloperidol	100 mg	172063
hydrochlorothiazide	100 mg	179087
hydrocortisone	100 mg	283020
hydrocortisone acetate	100 mg	280021
hydrocortisone sodium succinate	200 mg	194184
(-)-3-(4-hydroxy-3-methoxyphenyl)-2-hydrazino-2-methylalanine (3-O-methylcarbidopa)	25 mg	193180
(-)-3-(4-hydroxy-3-methoxyphenyl)-2-methylalanine (3-O-methylmethyldopa)	25 mg	179085
ibuprofen	100 mg	183117
imipramine hydrochloride	100 mg	172064
indometacin	100 mg	178078
<i>o</i> -iodohippuric acid	100 mg	171045
isoniazid	100 mg	185124
kanamycin monosulfate	12 mg	197211

Supplementary information

<i>Reference substance</i>	<i>Package size</i>	<i>Control number</i>
lanatoside C	100 mg	281022
levodopa	100 mg	295065
levonorgestrel	200 mg	194182
levothyroxine sodium	100 mg	189144
lidocaine	100 mg	181104
lidocaine hydrochloride	100 mg	181105
liothyronine sodium	50 mg	193179
loperamide hydrochloride	100 mg	194185
mebendazole	200 mg	195195
melting point reference substances		
azobenzene (69 °C)	1 g	192168
vanillin (83 °C)	1 g	299169
benzil (96 °C)	4 g	294170
acetanilide (116 °C)	1 g	297171
phenacetin (136 °C)	1 g	297172
benzanilide (165 °C)	4 g	192173
sulfanilamide (166 °C)	1 g	192162
sulfapyridine (193 °C)	4 g	192163
dicyanodiamide (210 °C)	1 g	192164
saccharin (229 °C)	1 g	192165
caffeine (237 °C)	1 g	299166
phenolphthalein (263 °C)	1 g	299167
metazide	100 mg	172058
methaqualone	100 mg	173069
methotrexate	100 mg	194193
methyl dopa	100 mg	179084
methyltestosterone	100 mg	167023
meticillin sodium	200 mg	274024
metronidazole	100 mg	183118
nafcillin sodium	200 mg	272025
neamine hydrochloride (neomycin A hydrochloride)	0.5 mg	193177
neomycin B sulfate <i>see</i> framycetin sulfate		
neostigmine metilsulfate	100 mg	187135
nicotinamide	100 mg	200090
nicotinic acid	100 mg	179091
nifurtimox	100 mg	194189
niridazole	200 mg	186129
niridazole-chlorethylcarboxamide	25 mg	186130
norethisterone	100 mg	186132
norethisterone acetate	100 mg	185123
nystatin	200 mg	300152

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<i>Reference substance</i>	<i>Package size</i>	<i>Control number</i>
oubain	100 mg	283026
oxacillin sodium	200 mg	382027
oxytetracycline dihydrate	200 mg	189142
oxytetracycline hydrochloride	200 mg	189141
papaverine hydrochloride	100 mg	185127
paracetamol	100 mg	195198
paromomycin sulfate	75 mg	195197
pheneticillin potassium	200 mg	167028
phenoxymethylpenicillin	200 mg	179082
phenoxymethylpenicillin calcium	200 mg	179083
phenoxymethylpenicillin potassium	200 mg	176075
phenytoin	100 mg	179089
piperazine adipate	100 mg	197212
piperazine citrate	100 mg	197213
praziquantel	100 mg	194191
prednisolone	100 mg	389029
prednisolone acetate	100 mg	289030
prednisolone hemisuccinate	200 mg	195196
prednisolone sodium phosphate	200 mg	194190
prednisone	100 mg	167031
prednisone acetate	100 mg	169032
probenecid	100 mg	192156
procaine hydrochloride	100 mg	183119
procarbazine hydrochloride	100 mg	184120
progesterone	100 mg	167033
propicillin potassium	200 mg	274034
propranolol hydrochloride	100 mg	187139
propylthiouracil	100 mg	185126
pyrantel embonate (pyrantel pamoate)	500 mg	192157
pyridostigmine bromide	100 mg	182110
reserpine	100 mg	186133
retinol acetate (solution)	5 capsules ¹	898038
riboflavin	250 mg	382035
rifampicin	200 mg	191151
rifampicin quinone	200 mg	190148
sodium amidotrizoate	100 mg	198221
sodium cromoglicate	100 mg	188140
spectinomycin hydrochloride	200 mg	193176
streptomycin sulfate	100 mg	197215

¹ Each containing about 8 mg in 230 mg of oil.

Supplementary information

<i>Reference substance</i>	<i>Package size</i>	<i>Control number</i>
sulfacetamide	100 mg	196200
sulfamethoxazole	100 mg	179092
sulfamethoxypyridazine	100 mg	178079
sulfanilamide	100 mg	179094
sulfasalazine	100 mg	191155
tamoxifen citrate	100 mg	196208
tamoxifen citrate <i>E</i> -isomer	10 mg	196209
testosterone enantate	200 mg	194192
testosterone propionate	100 mg	167036
tetracycline hydrochloride	200 mg	180095
thioacetazone	100 mg	171046
4,4'-thiodianiline	50 mg	183116
thyroxine sodium <i>see</i> levothyroxine sodium		
tolbutamide	100 mg	179086
tolnaftate	100 mg	176074
toluene-2-sulfonamide	100 mg	196204
trimethadione	200 mg	185125
trimethoprim	100 mg	179093
trimethylguanidine sulfate	100 mg	172059
vincristine sulfate	9.7 mg/vial	193181
vitamin A acetate (solution) <i>see</i> retinol acetate (solution)		
warfarin	100 mg	168041
zuclomifene	50 mg	187137

List of available International Infrared Reference Spectra¹

International Infrared Reference Spectra are established on the advice of the WHO Expert Committee on Specifications for Pharmaceutical Preparations. Full-scale reproductions of spectra produced from authenticated material on a suitable instrument are supplied for use in identification tests described in the specifications for quality control of drugs, published in *The International Pharmacopoeia* or proposed in draft monographs.

Precise instructions for the preparation of spectra are given on the label of each reference spectrum. All International Infrared Reference Spectra are distributed together with a document giving further details on the use of such spectra, entitled "General recommendations for the preparation and use of infrared spectra in pharmaceutical analysis".²

Orders for International Infrared Reference Spectra should be sent to:

WHO Collaborating Centre for Chemical Reference Substances
Apoteket AB
Produktion & Laboratorier Centrallaboratoriet, ACL
Prismavägen 2
S-141 75 Kungens Kurva
Sweden
(Fax: +46 8 740 6040; email who.apl@apoteket.se)

The following International Infrared Reference Spectra are currently available from the Centre:

aceclidine salicylate	caffeine (anhydrous)
acetazolamide	calcium folinate
allopurinol	carbidopa
amiloride hydrochloride	chlorphenamine hydrogen maleate
amitriptyline hydrochloride	clofazimine
ampicillin trihydrate	cloxacillin sodium
	colchicine
beclometasone dipropionate	cytarabine
benzylpenicillin potassium	
biperiden	dexamethasone
biperiden hydrochloride	dexamethasone acetate, mono-
bupivacaine hydrochloride	hydrate

¹ As updated at the thirty-seventh meeting of the WHO Expert Committee on Specifications for Pharmaceutical Preparations, 22–26 October 2001.

² *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-fourth report.* Geneva, World Health Organization, 1996, Annex 4 (WHO Technical Report Series, No. 863).

dextromethorphan hydrobromide

diazepam

dicolinium iodide

dicoumarol

diethylcarbamazine dihydrogen

citrate

diphenoxylate hydrochloride

erythromycin ethylsuccinate

erythromycin stearate

etacrynic acid

ethionamide

ethosuximide

furosemide

gallamine triethiodide

glibenclamide

haloperidol

hydrochlorothiazide

ibuprofen

imipramine hydrochloride

indometacin

isoniazid

lidocaine

lidocaine hydrochloride

lindane

metronidazole

miconazole nitrate

niclosamide

nicotinamide

noscapine

oxamniquine

papaverine hydrochloride

phenobarbital

phenoxymethylpenicillin calcium

phenytoin

primaquine phosphate

propylthiouracil

protionamide

pyrimethamine

salbutamol

salbutamol sulfate

sulfadimidine

sulfadoxine

sulfamethoxazole

sulfamethoxy pyridazine

tiabendazole

trihexyphenidyl hydrochloride

trimethoprim

valproic acid

verapamil hydrochloride

General guidelines for the establishment, maintenance, and distribution of chemical reference substances

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Introduction

In 1975, the WHO Expert Committee on Specifications for Pharmaceutical Preparations recommended “General guidelines for the establishment, maintenance and distribution of chemical reference substances” (1).¹ At that time these general guidelines were aimed at fostering greater collaboration and harmonization among various national and regional authorities responsible for collections of chemical reference substances. This aim is still relevant. The guidelines were initially drawn up for particular use by the WHO Collaborating Centre for Chemical Reference Substances in Sweden, which provides International Chemical Reference Substances (ICRS). These substances are primarily intended for use with pharmacopoeial monographs included in *The International Pharmacopoeia* (2).

It became evident that in order to meet particular national or regional pharmacopoeial requirements, it was necessary to establish chemical reference substances external to the WHO Collaborating Centre for Chemical Reference Substances. Another difficulty was to ensure prompt dispatch of the substances. Since the meticulous work of the WHO Collaborating Centre establishing the international collection would have to be duplicated in local or regional laboratories, guidelines were necessary to ensure the integrity of national or regional collections. In order to clarify the need for national and regional collections, the 1975 guidelines were reviewed and modified in 1982 (3). In view of refinements in pharmaceutical and analytical methods since then, the present revision was considered essential.

The purpose of having chemical reference substances is to achieve accuracy and reproducibility of the analytical results required by pharmacopoeial testing and pharmaceutical control in general. These substances are normally prepared and issued by the regional/national pharmacopoeial commission or the regional/national quality control laboratory on behalf of the drug regulatory authority. In the context of these guidelines, the general use of a chemical reference substance should be considered an integral part of a compliance-oriented monograph or test procedure used to demonstrate the identity, purity and content of pharmaceutical substances and preparations.

The establishment of chemical reference substances should be based on reports in which the results of analytical testing have been evaluated. These reports should subsequently be approved and adopted by a certifying body, normally the relevant pharmacopoeial committee or the drug regulatory authority. Such establishment can be on an international, national or regional basis. Each substance is generally established for a specific analytical purpose, defined by the issuing body. Its use for any other purpose becomes the responsibility of the user and a suitable caution is included in the information sheet accompanying a reference substance. The present guidelines are concerned

¹ The term *chemical reference substances*, as used in this text, refers to an authenticated uniform material that is intended for use in specified chemical and physical tests, in which its properties are compared with the properties of a product under examination, and which possesses a degree of purity adequate for its intended use.

with both primary and secondary chemical reference substances as defined below.

The preparation of a chemical reference substance should comply with the requirements for quality assurance systems, including principles of good manufacturing practices (GMP) and good control laboratory practices (4–6).

Adequate training programmes are also required. Both the WHO Collaborating Centre and other laboratories concerned with the evaluation and establishment of chemical reference substances give assistance in training, subject to the availability of resources.

Primary chemical reference substance

A designated primary chemical reference substance is one that is widely acknowledged to have the appropriate qualities within a specified context, and whose value is accepted without requiring comparison to another chemical substance.

Secondary chemical reference substance

A secondary chemical reference substance is a substance whose characteristics are assigned and/or calibrated by comparison with a primary chemical reference substance. The extent of characterization and testing of a secondary chemical reference substance may be less than for a primary chemical reference substance. This definition may apply *inter alia* to some substances termed “working standards”.

Part A. Primary chemical reference substances

1. Assessment of need for the establishment of chemical reference substances

The production, validation, maintenance and distribution of chemical reference substances is a costly and time-consuming undertaking. It is therefore of great importance to determine critically whether a need for a given substance exists. Requests for new chemical reference substances usually arise when a particular approach to developing a specification for a new substance or product has been adopted. Methods may have been proposed in a specification that require the establishment of a chemical reference substance for use as a comparative standard. Therefore, the first matter that should be assessed is whether an alternative, equally satisfactory, procedure could be adopted that does not require a comparative standard.

Analytical procedures currently used in specifications for pharmaceutical substances and products that may require a chemical reference substance are:

- (a) infrared (IR) spectrophotometry, whether for identification or quantitative purposes;
- (b) quantitative methods based on ultraviolet (UV) absorption spectrophotometry;

- (c) quantitative methods based on the development of a colour and the measurement of its intensity, whether by instrumental or visual comparison;
- (d) methods based on chromatographic separation for identification or quantitative purposes;
- (e) quantitative methods (including automated methods) based on other separation techniques that depend on partition of the substance to be determined between solvent phases, where the precise efficiency of the extraction procedure might depend upon ambient conditions that vary from time to time and from laboratory to laboratory;
- (f) quantitative methods, often titrimetric but sometimes gravimetric, that are based on non-stoichiometric relationships;
- (g) assay methods based on measurement of optical rotation; and
- (h) methods that might require a chemical reference substance consisting of a fixed ratio of known components (for example, *cis/trans* isomers, spiked samples).

2. Obtaining source material

Source material of satisfactory quality can be selected from a batch (lot) of the substance originating from the normal production process, if the purity is acceptable. Further purification techniques may be needed to render the material acceptable for use as a chemical reference substance.

The purity requirements for a chemical reference substance depend upon its intended use. A chemical reference substance proposed for an identification test does not require meticulous purification, since the presence of a small percentage of impurities in the substance often has no noticeable effect on the test.

On the other hand, chemical reference substances that are to be used in assays should possess a high degree of purity. As a guiding principle, a purity of 99.5% or higher is desirable, calculated on the basis of the material in its anhydrous form or free of volatile substances. However, where the selectivity of the analytical procedure for which the chemical reference substance is required is low, such a degree of purity may not be necessary. In making a decision about the suitability of a chemical reference substance, the most important consideration is the influence of the impurity on the attribute measured in the assay when used in a non-specific assay procedure. Impurities with physicochemical characteristics similar to those of the main component will not impair the usefulness of a chemical reference substance, whereas even traces of impurities with significantly different properties may render a substance unsuitable as a chemical reference substance.

When source material to be used as a chemical reference substance is obtained from a supplier, the following should be supplied with the material:

- Certificate of analysis with complete information as to test methods employed, values found and number of replicates used, where applicable, and relevant spectra and/or chromatograms.

- Information on optimal storage conditions required for stability (temperature and humidity considerations).
- Results of any hygroscopicity study and/or statement of the hygroscopicity of the source material.
- Results of any accelerated stability studies.
- Identification of detected impurities (by preference), and/or specific information on the relative response factor as determined in compendial methods concerning the principal component, and/or the percentage mass of the impurity.
- Updated Material Safety Data Sheet outlining any health hazards associated with the material.

For new drug substances, manufacturers should be aware that elaboration of pharmacopoeial monographs will be needed and a batch of the new substance should be set aside to be used if necessary as the chemical reference substance. It is desirable for bodies that issue chemical reference substances to provide each other with a sample of the same batch of material, even if the substance will be employed for different test methods. This will require the exchange of information concerning the establishment process, supplier(s), availability and conditions of supply.

3. Evaluation of chemical reference substances

The suitability of a substance proposed for use as a chemical reference requires careful evaluation by the issuing body. It is necessary to consider all data obtained from testing the material by a wide variety of analytical methods. When taken as a whole, this will ensure that the substance is suitable for its intended use. The extent of the analyses required depends on the purpose(s) for which the chemical reference substance is to be employed, and may involve a number of independent laboratories.

3.1 Use in identification tests

For use in identification tests (infrared spectrophotometry and/or chromatographic methods), a batch of good quality material selected from the normal production process is satisfactory if it is of acceptable purity. Additional purification by the supplier may be necessary. The most important check is the application of the test(s) for which the substance is intended. It is usual for at least one laboratory to apply all the tests described in the relevant monograph.

3.2 Use in purity tests

The characterization of a chemical reference substance used in the determination of a specific impurity is more extensive, especially when used in a limit

test. If the technique employed is thin-layer chromatography (TLC) an acceptable minimum purity is recommended (normally at least 90%), but purer material may be required for liquid chromatography (LC) or gas chromatography (GC). If the proposed reference substance is being prepared or isolated for the first time, appropriate chemical and physicochemical tests, such as nuclear magnetic resonance (NMR), mass spectrometry (MS) and elemental analysis, must be applied to characterize it.

3.3 Use in assays

If the chemical reference substance is to be used in an assay (colorimetry, LC, GC or UV spectrophotometry), the extent of testing is very much greater. Several (a minimum of three) laboratories should collaborate in testing the proposed substance, using a variety of established and validated techniques, including the method used in the pharmacopoeial specification. The relative reactivity or relative absorbance of the impurities present must be checked when a non-specific assay method is employed, e.g. by colorimetry or UV spectrophotometry. When a selective assay method is employed, it is particularly important to determine the quantity of impurities. In such a case, it is best to examine the proposed reference substance by as many methods as practicable including, where possible, absolute methods. For substances that are acidic or basic a titration with alkali or acid is simple, but other reactions which are known to be stoichiometric may be used. Phase solubility analysis and differential scanning calorimetry may also be employed in certain cases.

The total of the determinations of water content, organic solvents, mineral impurities and organic components should amount to 100%. For most chemical reference substances intended for assays, the content may be expressed "as is". When establishing the chemical reference substance it is therefore essential to determine the content of water and residual solvents for a non-specific assay, and also to determine the content of impurities for a selective assay.

3.4 Use in the calibration of an instrument

Where the chemical reference substance is to be employed as calibration material, the extent of testing is similar to that for a chemical reference substance used in assays. Several laboratories should collaborate in testing the proposed substance using a variety of techniques to check that its purity is adequate. An appropriate number of collaborating laboratories should also participate, after the reference substance has been deemed suitable, to establish a value for the essential property of the substance using an appropriate instrument.

4. Chemical and physical methods used in evaluating chemical reference substances

It is important to establish by individual testing that a substance proposed for use as a chemical reference is suitable.

The methods used to establish the suitability of such a substance fall into two broad groups: those intended primarily to identify the substance and those used to establish its purity. With most methods, the percentage purity of a chemical reference substance cannot be expressed as an absolute value if the impurities have not been identified. The quoted purity is then an estimate based upon the data obtained by the various analytical methods.

4.1 Methods used to verify the identity of chemical reference substances

Where a proposed substance consists of a compound whose structure has been satisfactorily defined, its identity may be confirmed by matching the IR spectra of the substance to that of an authentic compound. Particular care should be taken when polymorphism exists (7). Other highly specific techniques, such as NMR spectroscopy, MS, or X-ray diffraction crystallography, may also be used for such comparisons. The identity of a substance that is intended to replace an established chemical reference substance of the same molecular constitution must be verified, to determine that the characteristic properties of the two specimens are identical. For this purpose it is often sufficient to compare their IR absorption spectra.

However, where no authentic specimen of the proposed substance is available for comparison, and definitive data about its properties are lacking, it may be necessary to verify its identity by applying several analytical techniques currently used to characterize new compounds. Such analytical methods may include elemental analyses, crystallographic studies, MS, NMR spectroscopy, functional group analyses, and IR or UV spectrophotometry, as well as other supplementary tests as required to establish that the proposed substance is fully characterized.

4.2 Methods used to determine the purity of chemical reference substances

The analytical methods to be employed in examining a substance should be considered in relation to its intended use. These analytical methods may be divided into three broad categories: those that require comparison with an external chemical reference substance (e.g. chromatographic or spectrophotometric methods), those that depend solely on an intrinsic dynamic property (e.g. phase solubility analysis and differential scanning calorimetry) and other methods.

4.2.1 Separation techniques

The methods used for the determination of purity should be established and validated with system suitability requirements as appropriate.

Chromatographic methods. Methods of analysis based on chromatographic separation are especially useful for detecting and determining impurities in

chemical reference substances. High-performance liquid chromatography (HPLC) is the most widely used chromatographic method, but TLC and GC are also used. The individual components separated by chromatographic methods may sometimes be recovered for characterization.

The selectivity of HPLC and of GC usually exceeds that of TLC. Both the first two methods also have the advantage of being readily applicable on a quantitative basis, but they require more complex equipment. HPLC, employing a spectrophotometric method of detection, is of particular value in the examination of chemical reference substances intended for use in UV spectrophotometric assays. The UV wavelength of detection employed for determining the impurity content of the chemical reference substance should be chosen so that the detection responses of the substance and its known impurities are similar. When the response factors are significantly different at the optimal wavelength of detection, appropriate corrections must be made to estimate the content of impurities. LC with diode-array detection is very useful for recording the UV spectra of both the main peak and the impurities. LC with MS detection is used for identification of separated impurities as well as for the main component, and is particularly important for chemical reference substances where no other reference standards or IR reference spectra are available.

In a GC method used for an assay, as with LC, the detection responses of the known impurities are determined. Generally, GC monograph methods are of particular value in detecting and determining volatile impurities, including solvent residues, in chemical reference substances.

TLC uses apparatus that is simple and cheap; the technique is easy to carry out and is readily applicable even in the microgram range. It can separate closely related compounds, such as geometric isomers and the members of a homologous series. All the constituents of a substance submitted to chromatography appear somewhere on the chromatogram. However, some constituents may remain on the starting line, some may move with the solvent front, some may migrate at the same rate as the main component, and some may remain undetected. For this reason, the usefulness of the method may be greatly enhanced by means of two-dimensional chromatography and by using a number of different solvent systems and a variety of detection methods. In some cases the method may be used quantitatively with acceptable accuracy by using a densitometer.

Capillary electrophoresis. Capillary electrophoresis is an increasingly common method. It may be considered as complementary to LC for detecting impurities.

4.2.2 *Methods based on intrinsic thermodynamic properties*

Methods in this group measure total impurity levels in absolute terms.

Differential scanning calorimetry. This technique is used to check the presence of different polymorphic forms and to determine the total amount of solid impurities. Purity estimation is based on determination of the heat of fusion

of the sample and of the change in its melting point caused by the presence of impurities. This analytical method can be performed rapidly and with high precision. However, it is not applicable if the substance decomposes on melting. This limits its value as a general procedure for purity estimation of chemical reference substances. It is also inapplicable if solid solutions are formed.

Phase solubility analysis. The method has occasionally been used, but its value is limited and the procedure is time consuming. It may be employed to detect contaminating substances, including isomeric species, and to estimate their concentration. Some factors that may make the method inapplicable are degradation of the substance during the course of analysis, formation of a solid solution, and polymorphism in the main component.

4.2.3 Other methods

Spectrophotometric methods. UV spectrophotometry is occasionally used to determine purity. Since it depends upon the presence of a characteristic chromophore, it can detect impurities that contribute excessively to the absorbance value and may indicate the presence of impurities that have a negligible or distinctive absorbance.

However, the utility of the method is limited by the small number of absorption maxima in the UV range, the large numbers of compounds containing similar characteristic chromophores, and the need for an external chemical reference substance.

IR spectrophotometry may be used to identify and determine the proportions of geometric isomers. NMR spectroscopy, a powerful spectroscopic identification tool, is also occasionally useful in the determination of purity.

Titrimetric methods. Titrimetric methods provide a valuable means of confirming the identity and purity of a proposed chemical reference substance and are useful in confirming purity values obtained by other methods.

Optical rotation methods. Many chemical reference substances are optically active and the relative proportion of optical isomers can sometimes be determined by an optical rotation method, but generally such methods lack sensitivity. However, the quantitative use of these techniques is well established and can yield results of high precision, depending on the solvent and the wavelength chosen for measurement. Chiral chromatography and NMR are becoming increasingly important.

Determination of water and organic volatiles. It is essential that an accurate assessment of the moisture content and the content of volatile contaminants be made. These total values may often be obtained by drying under defined conditions that are appropriate to the proposed substance. Sometimes this may not be possible or may yield misleading results. In such cases, thermogravimetric analysis may be used to determine the water and volatile content. Alternatively,

the water content may be determined by Karl Fischer titration and the content of volatile solvents by GC. Without an accurate assessment of these values at the time that other determinations are being made, judgements of the acceptability of the proposed chemical reference substance will be invalid.

5. Assignment of content

If a content is to be assigned to a chemical reference substance, it should be borne in mind that the value is based on the results of a collaborative interlaboratory programme using different analytical methods. This experimentally obtained value represents the best estimate of the true value. In general, the assignment of content for a chemical reference substance is 100% minus the content of water and volatiles, and when a substance is intended for use as an assay standard based on a separation technique the impurity content, as determined by that method, must also be subtracted. Sometimes the chemical reference substances must be dried before use, in which case the content is expressed on the basis of the dried material.

6. Handling and distribution of chemical reference substances

The handling, distribution and use of established chemical reference substances must ensure that their integrity is safeguarded and maintained throughout their period of use.

6.1 Packaging operations

Current GMP requirements (5) should be observed. The various stages in packaging chemical reference substances should be clearly defined and controlled, to avoid contamination of the sample, mislabelling of containers, or any other event which might result in mishandling or mismanagement.

Containers for chemical reference substances should protect their contents from moisture, light and oxygen and must be tested for moisture permeability.

Additional measures may be necessary to ensure long-term integrity and stability. The best containers for chemical reference substances from the point of view of stability are sealed glass ampoules, but these have certain disadvantages. There is the risk of contaminating the substance with glass particles when the ampoules are opened, and reclosure is difficult. Sealable glass ampoules are therefore principally used for substances that must be kept in an oxygen-free atmosphere. Certain other substances may require even more elaborate protection. Most chemical reference substances, however, are conveniently supplied in reclosable containers which should be uniform in type and size to facilitate distribution. The lack of permeability to moisture is an important factor in determining the suitability of container closure systems.

Before undertaking any packaging operations, the health hazards of the item to be packaged should be assessed through information sources, e.g. the Material Safety Data Sheet. Appropriate precautions should be taken to protect the person handling the chemical reference substance.

The packaging of a batch of a chemical reference substance into containers is a small-scale operation for which suitable equipment is now always available to the manufacturer of the material. Therefore, the packaging of chemical reference substances is usually undertaken by the responsible issuing body. Screw-type feeders have been constructed, but generally the packaging of chemical reference substances is carried out manually. Substances which are expensive or only available in very small quantities may have to be divided between containers in solution and then lyophilized, or evaporated to dryness.

Some chemical reference substances must be packaged under an inert gas or in conditions of controlled humidity. Therefore, the use of a glove-box or an air-tight cabinet is necessary.

6.2 Storage

Information about suitable storage conditions can often be obtained from the manufacturer of the source material and should be requested routinely when a new chemical reference substance is established. Theoretically, the stability of the substances should be enhanced by keeping them at low temperatures but, for substances that contain water, storage below 0°C may impair the stability. It should also be remembered that the relative humidity in normal refrigerators or cold-rooms may be high and, unless ampoules or other tightly closed containers are used, the improvement in stability may be more than offset by degradation due to the absorption of moisture. Storage at about +5°C, with precautions to prevent such absorption, has proved satisfactory for most chemical reference substances.

6.3 Stability

A chemical reference substance is an integral part of the drug specification. Thus, if the reference substance deteriorates, this will change the specification of the drug. It is therefore of the utmost importance that the stability of chemical reference substances should be monitored by regular re-examination and that they should be replaced as soon as a significant change in a property is noted.

The definition of what is a "significant change" differs according to the intended use of the chemical reference substance. Several per cent of degradation products found in a substance may not impair the usefulness of the material in identification tests. For chemical reference substances that are used in chromatographic assays, however, even small amounts of impurities may be unacceptable. When establishing a chemical reference substance, consideration must be given to its intended use and to the performance characteristics of the analytical methods in which it will be used. The tolerable degree of degradation will be different from case to case.

Laboratories in charge of collections of chemical reference substances should have a system for regular re-examination of the materials in stock. The frequency of re-testing may be modified according to the need. It must be borne

in mind that the stability of a specially prepared chemical reference substance may not always be the same as that of commercial samples of the same material.

The selection of suitable analytical methods for monitoring the stability of chemical reference substances depends on the nature and intended use of the substance. A substance used solely for identification purposes will normally only require demonstration that it is still suitable for this use, e.g. that the IR spectrum is identical to that obtained during establishment. If substances are employed for other purposes, the testing must be more extensive but should use methods which are rapid and sensitive so as not to consume too much of the existing stock. It is important to check that there has been no significant uptake of moisture, which could result in degradation by hydrolysis and/or a decrease in the assigned content of the substance. Chromatography is employed extensively, as well as absolute methods such as differential scanning calorimetry where applicable. Changes in the impurity profile or purity determination usually mean that the batch must be replaced. Changes which compromise the integrity of the batch indicate it should immediately be withdrawn from use. Sometimes a batch of a chemical reference substance will discolour or otherwise change in appearance. Steps should be taken to replace this substance whether or not the results of subsequent analyses indicate significant degradation. Such changes in physical appearance reduce the confidence of the user in the suitability of the chemical reference substance. Appropriate testing of active bulk substance should be carried out before further dispensing into vials or ampoules.

6.4 Information to be supplied with chemical reference substances

Labels on chemical reference substances should give the following information:

- the appropriate name of the substance: the international nonproprietary name (INN) should be used wherever possible;
- name and address of the issuing body;
- approximate quantity of material in the container; and
- batch or control number.

Where associated documents are provided they should incorporate relevant items from the list above. The following information should be given, as necessary, on the labels and/or in associated documents:

- recommended storage conditions (if special conditions apply);
- intended use of the chemical reference substance;
- directions for use (e.g. storage and handling);
- information about assigned analytical value of the chemical reference substance (needed for calculation of the results of tests in which the substance will be used);

- a disclaimer of responsibility when chemical reference substances are misused, or stored under inappropriate conditions, or used for other purposes than those intended by the issuing body; and
- health hazard information or warning in conformity with national and regional regulations or international agreements.

If analytical data are to be supplied with the chemical reference substances, it is recommended that the data provided be limited to what is necessary for the proper use of the substances in the tests and assays.

6.5 Distribution and supply

Distribution of chemical reference substances within the same country usually does not present problems. However, when samples are to be sent to other countries, both the sender and the receiver of the goods may encounter difficulties because of the vagaries of postal and customs regulations, e.g. the application of special procedural requirements applicable to substances under international control. Distributors of chemical reference substances waste considerable resources in seeking information on different international import regulations, and in completing the required forms. A way of reducing such difficulties and barriers to effective distribution of chemical reference substances should be sought. There should be the minimum delay in providing the chemical reference substances to the users, and the most speedy means of transport should be chosen.

6.6 Period of use

Chemical reference substances do not carry an “expiry date” in the conventional sense. To avoid the unnecessary discarding of satisfactory substances, a mechanism for general control of the batch of a chemical reference substance may be used by the issuing body. If the issuing body applies stability considerations and a monitoring procedure based on its experience to its collection, this should guarantee the user of the acceptability of the chemical reference substance for its intended use.

If it is considered necessary to specify a beyond-use date, it should be stated on the label and/or on a document accompanying the chemical reference substances. Adequate shipping records should exist to enable contact with the purchaser of a batch for recall or other notification.

The storage and maintenance of unopened containers of the chemical reference substance in accordance with information provided are integral to its suitability of use. To avoid potential doubts concerning the integrity of opened containers, it is suggested that potential users obtain only the quantities of substances necessary for short-term need and obtain fresh stocks (held under controlled and known conditions) when needed. Long-term storage of substances in opened containers is to be avoided. Similarly, efforts should be made to avoid

possible degradation, contamination and/or introduction of moisture during the repeated use of a substance.

Part B. Secondary chemical reference substances

The establishment of secondary chemical reference substances calibrated against a primary chemical reference substance may be desirable for various practical reasons, e.g. the latter may not be available in adequate quantities to supply all local needs. Moreover, the availability of such secondary chemical reference substances (for example, on a regional basis) would reduce the delay in receiving the reference material.

The body which establishes a secondary chemical reference substance for national/regional use should be clearly defined by the competent drug regulatory authority. Clear documentation must exist to establish the relationship between the secondary and the primary chemical reference substance.

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