LGC

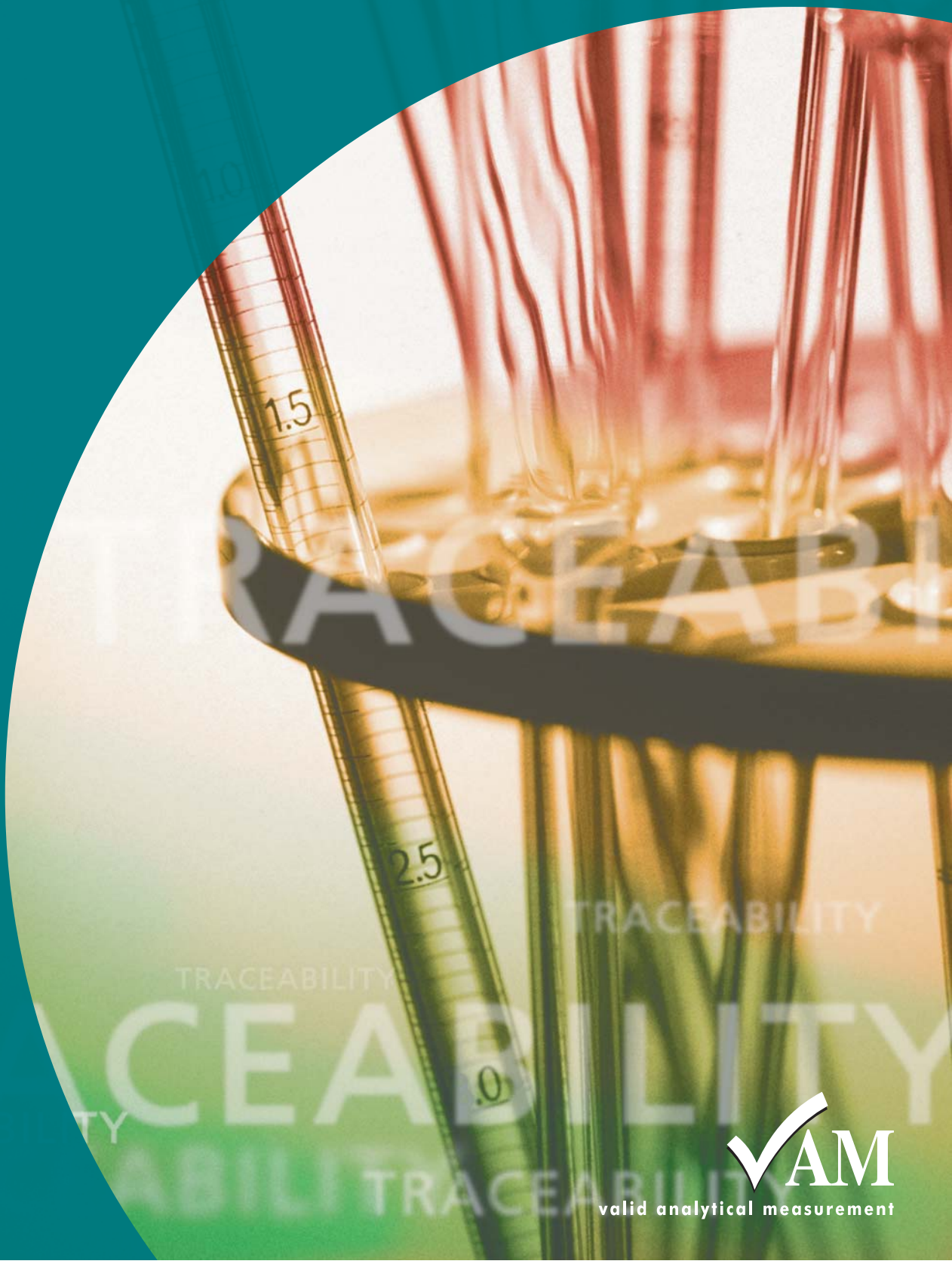
*Setting standards in analytical science*

# Meeting the Traceability requirements of ISO17025

An Analyst's Guide

Second Edition

November 2003




VAM

valid analytical measurement

The work described in this guide was supported under contract with the Department of Trade and Industry as part of the National Measurement System's Valid Analytical Measurement (VAM) Programme

ISBN 0-948926-20-1



Published by LGC Limited

# Meeting the Traceability Requirements of ISO17025

# An Analyst's Guide

**Second Edition**

**November 2003**

Editors

Richard Lawn and
Steve Wood

LGC
Queens Road
Teddington
Middlesex
TW11 0LY

Email: vam@lgc.co.uk

# Preface

Many analysts are aware of the traceability requirements of ISO17025. These place the long-standing practice of obtaining traceable calibrations for equipment such as balances or volumetric glassware on a more formal basis. More importantly, they extend this requirement to the chemical standards and reference materials used to calibrate or validate analytical methods. Recent investigations carried out by LGC within the VAM programme have demonstrated the practical benefit of establishing the traceability of routine test results to chemical measurement standards of known quality. Hence all laboratories, regardless of whether their methods are accredited to ISO 17025, can benefit from implementing the principles needed to obtain traceable measurement results. Unfortunately, many laboratory managers have difficulty in envisaging how this can be done in a straightforward and cost-effective manner.

This guide provides essential practical advice to analysts and laboratory managers on how to establish the traceability of their results to reliable and appropriate measurement standards. Such traceability is the key to obtaining results that are fit-for-purpose, particularly in terms of accuracy, between-laboratory comparability, and consistency of data over periods of time. Following the guidance given here should ensure compliance with the traceability requirements of ISO17025.

The approach adopted in this guide is based on the recently published Eurachem/CITAC document *Traceability in Chemical Measurement*. The main aim of the present guide is to provide an interpretation for analysts of the practical requirements associated with the Eurachem/CITAC document. Hence we have focussed on those essential practical steps involved in a typical analytical procedure for which traceability must be established and explained how the analyst can judge what is required *in their particular circumstances*. In order to simplify this process we have adopted a colour coding system which, we hope, will help analysts to classify the traceability requirements of their methods in accordance with the impact on the final analytical result of each individual traceable calibration.

The guidance is illustrated using extensive examples of several analytical methods and standard operating procedures taken from the food and environmental measurement sectors. The examples are based on real analytical procedures, simplified where necessary to aid clarity. We hope they will facilitate an understanding of the principles of traceability and prove useful for both private study and training courses.

The examples and the procedures described in this guide were presented, applied and reviewed at two workshops organised by LGC and involving analysts, managers, experts on traceability, and representatives of accreditation bodies. The final version of the guide and examples incorporates many suggestions from the workshop participants (see Appendix 3) and we are grateful for their help. We are also grateful to the Chairman of the Eurachem/CITAC Working Group for his advice and for early access to their draft document. This work was supported by the Department of Trade and Industry Valid Analytical Measurement Programme (VAM).

Mike Sargent

LGC

September 2003

# CONTENTS

# 1. Introduction

## 1.1 Why is Traceability Important?

All chemical measurement results depend upon and are ultimately traceable to the values of measurement standards of various types, such as those for mass, volume and the amount of a particular chemical species. If results obtained by different laboratories are to be comparable, it is essential that all results are based on reliable measurement standards whose values are linked to a stated reference. If there are differences in the quality of the measurement standards used in different laboratories, discrepancies will inevitably arise when different laboratories analyse the same sample.

Recent investigations carried out within the VAM programme have shown the practical benefit of establishing the traceability of routine test results to measurement standards of known quality. In an interlaboratory exercise to determine iron in river water at the level of 280 μg/L, each laboratory sourced its own measurement standard for iron and the between-laboratory coefficient of variation of the results was 41%. When the traceability of each laboratory's result to a common, high quality iron standard was established, the coefficient of variation was reduced to 11%.

Whilst it is generally not practicable to ensure the use of common standards for all of the measurements involved in a chemical analysis, steps should be taken by analysts to ensure that the measurement standards they are using are of an appropriate quality. This effectively requires the analyst to check that the stated values of the standards have been established by valid procedures and are accompanied by an uncertainty estimate that is appropriate to the particular analyses being carried out.

This guide provides practical advice and guidance to analysts on how to establish the traceability of their measurements to reliable measurement standards. It is based upon the principles described in the Eurachem/CITAC document entitled Traceability in Chemical Measurement (March 2003). The latter document may be viewed on the Eurachem website *(www.eurachem.ul.pt)*

## 1.2 Traceability in Chemical Measurements

A typical chemical analysis, usually involves a number of individual operations, such as the following:

1. Measurement of the amount (e.g. mass) of sample taken for analysis
2. Preparation (e.g. dissolution, digestion, extraction or cleanup) of the sample, according to fixed and defined experimental conditions, such as time, temperature, acid concentration, solvent composition, etc.
3. Measurement of the amount (e.g. volume) of the prepared sample extract
4. Calibration of an instrument with a standard solution of known concentration
5. Measurement of the instrument response obtained for the sample extract
6. Calculation of the concentration of analyte in the original sample

Examination of the above shows that a typical analytical procedure requires measurements to be made (e.g. sample mass, extract volume, etc) and fixed experimental conditions to be realised (e.g. time, temperature, reagent concentration for sample extraction, etc.).

The essential task of the analyst is to ensure that all of these experimentally measured or realised values are traceable to reliable measurement standards. Ideally, the measurement standards selected for the purpose of establishing traceability should be internationally recognised as being fit for that purpose, as emphasised in the VIM[1] definition of traceability:

*Property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties.*

It will be noted that the VIM definition refers to 'national or international standards'. However, whilst national and international standards exist for physical measurements such as mass (i.e. the international standard kilogram), there are currently no such standards for chemical measurements. For example, if we were to analyse a sample of drinking water for lead content, we would soon find that there is no national or international measurement standard for lead.

Given this situation, the task of the analyst is to chose, for use as measurement standards, stated references that are appropriate for the particular analysis that is to be carried out. To identify appropriate stated references for traceability purposes, a systematic approach should be adopted. Section 2 of this guide suggests an approach that was the subject of two user workshops and which was found to be useful and workable by the participants.

Having completed an evaluation of the traceability for each critical parameter, the analyst should prepare an appropriate traceability statement which may be written into the SOP itself or the validation report. When reporting the results of an analysis carried out using the SOP it will usually be sufficient for the laboratory to state that all critical parameters used in the method are traceable to recognised national or international standards.

1. International Vocabulary of Basic and General Terms in Metrology. ISO, Geneva, 1993, 2nd edition. ISBN 92-67-01075-1

# 2. Practical Attainment of Traceability

## 2.1 Overview

Based on the approach described in the Eurachem/CITAC Guide, the analyst must undertake the following tasks as a pre-requisite to obtaining traceable measurement results when carrying out a particular analytical method or standard operating procedure (SOP).

1. Write down and understand the equation used to calculate the analytical result
2. Identify any reagents or equipment with specified values
3. Identify the fixed experimental conditions used in the SOP
4. Obtain appropriate stated references (measurement standards) for use in the practical measurement or realisation of the experimental values identified in 1,2 and 3.

Additionally, it is important to note that the SOP concerned must have been properly validated and must be applied within its stated scope. If these conditions are not met, an erroneous result may still be produced, even if all of the measurements and values referred to in the SOP are carried out or realised in a traceable manner. Guidance on method validation is beyond the scope of this document, but further information may be found in:

*The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics. (www.eurachem.ul.pt)*

Before commencing the analysis therefore, the analyst must first review the SOP and carry out steps 1, 2 and 3. This will enable those values, which appear in the equation or are specified in the reagents, equipment or fixed conditions to be identified.

The SOP must be carried out in a manner that establishes the traceability of these values to appropriate stated references. For this purpose, the analyst must then carry out step 4 and obtain the appropriate stated references. The issues involved in this approach are discussed below.

## 2.2 Appropriate Stated References

### 2.2.1 What are Stated References?

Put simply, a stated reference is any ‘reference point’ that an analyst uses to measure, obtain or realise a particular experimental value in practice. Some examples of stated references and their potential applications are tabulated below:

**Typical Examples of Stated References**

| Stated reference | Could be used* to provide traceability for practical realisations of the following values: |
|---|---|
| Balance | mass |
| Standard weight | mass |
| Pipette, burette, graduated flask (or other volumetric glassware) | volume |
| Automatic pipette | volume |
| Measuring cylinder | volume |
| Graduated syringe | volume |
| Hg in glass thermometer | temperature |
| Platinum resistance thermometer | temperature |
| Clock or stopwatch | time |
| UV/optical/IR filter | absorbance; wavelength |
| Buffer solution | pH |
| Sieve | particle size |
| Filter paper; membrane filter; sintered glass filter | particle size |
| Published tables and compilations of physical/chemical data | atomic and molecular weights; density; etc. |
| Pure chemical or solution prepared from a pure chemical | instrument calibration response factors; molarity of volumetric reagents |
| Certified reference material comprising a pure chemical or a solution of a pure chemical | instrument calibration response factors; molarity of volumetric reagents |
| Commercial chemical with a producer's stated specification | composition of reagents used for sample digestion/extraction |

* At the analyst's discretion (see Section 2.2.2)

The chosen stated reference may be a formally certified artefact, item of equipment or chemical material, issued by a calibration laboratory or a reference material producer and accompanied by a certificate. However, this is not an automatic and mandatory requirement for a stated reference. For example, for certain applications volumetric glassware of stated tolerance, but without a certificate of calibration, may be appropriate for volume measurements. Likewise, a reagent grade chemical of stated, but not formally certified, purity may be appropriate for the preparation of an instrument calibration standard.

It is the analyst's responsibility to decide what stated references are appropriate for a particular analytical determination, such that the final results obtained and reported on the test samples are fit for their intended purpose.

### 2.2.2 What is Appropriate?

The stated reference that is appropriate for a given application in a given SOP depends on the 'degree of control' that the analyst needs to apply in practice when measuring or realising a particular experimental value (e.g. mass, temperature, concentration of a calibration standard, etc.). 'Degree of control' refers, in general terms, to the uncertainty that is acceptable in the measured experimental value. The two examples below illustrate the issues involved.

**(a) Degree of Control and Instrument Calibration**

When carrying out an SOP for the determination of the pesticide p,p'-DDE in animal fat or a soil sample by some instrumental technique (e.g. GC/MS), the analyst will need to choose an appropriate sample of p,p'-DDE for use in the preparation of an instrument calibration solution. Two choices might be available:

- A commercial grade chemical, stated purity >95%
- A formally certified reference material, certified purity 99.6 ± 0.4%

The former material would contribute about 5% to the calibration uncertainty whereas the latter will contribute only 0.4%. There will be similar uncertainty contributions associated with the result ultimately obtained on the test sample. Depending on the purpose of the analysis, the analyst makes a choice as to the appropriate calibration material. If the analysis is being carried out for the purpose of screening a large number of test samples to evaluate the proportion of samples contaminated with p,p'-DDE, an uncertainty contribution of about 5% due to instrument calibration might well be acceptable. If however, the analysis was being carried out to check whether a specific test sample complied with a legislative limit and it was suspected that the sample value was close to the limit, the smaller uncertainty provided by the certified calibration material would be required. Thus the degree of control that is exercised in the calibration of the instrument depends on the ultimate purpose of the analysis – there is no one single degree of control that is applicable to all instrument calibration procedures.

**(b) Degree of Control and Sample Preparation**

The following is a typical set of operations that might be involved in the preparation of a sample extract prior to measurement by some classical or instrumental technique:

*…..make the sample extract to 50mL, take a 10mL aliquot and pass it through a column containing approx 2g granular anhydrous sodium sulphate. Collect the eluate in a rotary evaporator flask and rinse the column with about 5mL of iso-octane, collecting the rinsings in the flask. Evaporate the extract to dryness at a temperature not exceeding 50°C…….*

The analyst must review this aspect of the SOP in terms of the degree of control that should be applied when each of the values and quantities specified are measured or realised in practice.

When making the sample extract to 50mL and subsequently taking a 10mL aliquot, a significant degree of control must be exercised in the measurement of these volumes, since the actual volumes concerned will have a direct effect on the accuracy of the final analytical result. An appropriate degree of control would be provided by using volumetric glassware (volumetric flasks, pipettes, etc). These volume measuring devices typically have measurement tolerances of <0.5%. This degree of control will be more than adequate for many chemical analyses.

The anhydrous sodium sulphate is used to dry the extract and therefore the exact mass is not critical to the accuracy of the final result. A sufficient degree of control in realising the 2g quantity would be obtained by use of a top-pan balance, or even by simply filling the column to an appropriate depth.

The rinsing of the column specifies using about 5mL of iso-octane. This volume could be realised with sufficient accuracy by use of a 10mL measuring cylinder.

The temperature of the water bath of the rotary evaporator is specified not to exceed 50°C. If the water bath is actually operated at a temperature significantly lower than this, say 40°C, a sufficient degree of control of the bath temperature would be provided by an ordinary laboratory mercury in glass thermometer, since any uncertainty in the temperature reading of the thermometer would be expected to be much less than 10°C .

However, if it was decided to operate the water bath at 49°C, although a mercury in glass thermometer would still be suitable, the accuracy of the graduations would require checking against a properly calibrated thermometer. Thus a greater degree of control of the water bath temperature is required when it is close to the specified upper permissible limit.

## 2.3 Choosing the Appropriate Degree of Control

When deciding what degree of control is appropriate when a particular value or experimental parameter has to be measured or realised in practice, the analyst may find the following sources of guidance helpful.

### 2.3.1 Fitness for Purpose Criteria

Whenever an analysis is carried out, the analyst should be aware of the ultimate end-use to which the results will be put. This will determine the uncertainty in the final result that is acceptable and fit for purpose. For example, if a contaminated land site is being surveyed to assess the distribution pattern of hot-spots, measurements with an uncertainty of ± 50% might be acceptable. Conversely, if a sample of blood is to be analysed to determine whether the ethanol content exceeds that permitted in drink/driving legislation, a much smaller uncertainty, perhaps ±5% or less, might be required. The degree of analytical control that must be applied in these two situations therefore differs markedly.

Clearly, the individual steps in the analytical procedure that make a significant contribution to the overall uncertainty must be controlled to a smaller degree of uncertainty than that required in the final analytical result. Ideally, experimental values

having a significant effect on the final result should be measured or realised with an uncertainty that is one-fifth (or less) of the overall target uncertainty for the final result. When this condition is met the individual step concerned will make a negligible contribution to the overall uncertainty.

### 2.3.2 Method Validation Data

It has already been emphasised (Section 2.1) that the use of a properly validated method, operated within its scope, is essential if reliable results are to be obtained. Information from the method validation studies will often assist in identifying the degree of control that is required for particular steps in the SOP.

For example, information may be available on the effect that variations in extraction conditions (e.g. time, temperature, extractant composition) have on the final analytical result. If changing the extraction temperature by ±5°C has no significant effect on the final analytical result, then it will be adequate to control the extraction temperature to ±5°C. This could be achieved using an ordinary laboratory mercury in glass thermometer.

If changing the extractant composition from, say, a specified value of 10% v/v nitric acid to 15% v/v has a significant effect on the final result, then the acid concentration would have to be controlled to better than 10 ± 5%. How much closer the control would have to be depends upon the magnitude of the variation of the final result with variations in acid concentration. In the immediate absence of such knowledge, the analyst with responsibility for applying the SOP should control the acid concentration to 10 ± 0.5%. This recommendation is made because the acid concentration in the SOP is specified as 10% v/v, that is to the nearest whole number, which carries the implication that it should lie between 9.5 and 10.5%.

### 2.3.3 Uncertainty Data

It will be appreciated from preceding discussions that the issue of traceability is closely linked to measurement uncertainty. Information on the uncertainty budget for an SOP and the individual sources of uncertainty will therefore be of value when considering the traceability requirements for the SOP.

Comprehensive guidance on measurement uncertainty has been published by Eurachem and further information may be found in the following document:

*Quantifying Uncertainty in Analytical Measurement, 2nd edition, (www.eurachem.ul.pt)*

### 2.3.4 Analysts' Experience

In addition to the above more formal sources of information, the analysts' general experience will often provide useful guidance as to those parts of an analytical procedure where a greater degree of control is required and those parts where lesser control is acceptable.

## 2.4 Obtaining the Appropriate Degree of Control

Once the appropriate degree of control has been identified for a particular step in an SOP, consideration must be given as to how this degree of control will be obtained in practice. This effectively means identifying appropriate stated references that may be used to realise the experimental values concerned with an appropriate uncertainty. Because there are many different degrees of control and various ways to obtain them, it is helpful to allocate the degree of control required for a particular experimental value to one of three categories. This will help the analyst to identify traceability requirements for an SOP in a focused and systematic manner. The three categories, 'colour coded' green, amber and red, are discussed in detail below.

### 2.4.1 Green Category

This represents a minimal or basic degree of control in which normal, routine laboratory equipment, reagents, etc. are able to provide appropriate stated references. This degree of control would be readily obtainable even in a basically equipped laboratory and would not require the analyst to make any special arrangements. It is applicable to those steps in an analytical procedure that do not have a significant effect on the uncertainty of the final analytical result. By way of example, it would be applicable where the following situations are encountered in an SOP:

- approximate volume measurements, where it is reasonable to conclude that a graduated beaker or measuring cylinder would be adequate (e.g. 'dissolve the residue in about 10mL of hexane')
- measurements of time, where it is reasonable to rely on a clock or stopwatch (e.g. 'shake the mixture for 60 minutes')
- measurements of length, where it is reasonable to use a ruler (e.g. 'fused silica crucibles, 57mm diameter')
- reagents with specified approximate concentrations (e.g. approx. 6M HCl)
- equipment with approximate specifications (e.g. 'medium grade porosity filter paper')
- temperatures with approximate specifications (e.g. 'room temperature; red heat')

Once a particular experimental value has been allocated to the green category the analyst may, for all practical purposes, regard the realisation of those values as easily achievable using basic knowledge, skills and procedures.

### 2.4.2 Amber Category

This represents a significant degree of control, such as that provided by properly maintained and calibrated equipment for common measurements such as mass, volume, temperature, instrument response, etc. All values appearing in the equation used to calculate the final analytical result would either be in the amber category or the red category (see next Section).

The quality assurance (QA) system of a properly equipped and appointed laboratory will normally provide the appropriate stated references, via a defined policy for ensuring the quality of common measurements. For example, the regular maintenance and calibration of balances by a service engineer should ensure that measurements of mass are traceable to the national/international standard kilogram. The purchase of volumetric glassware with a stated specification from a reputable supplier, combined with procedures for regular maintenance and checking of the glassware, should provide appropriate stated references for volume measurements. The availability of a calibrated thermometer for checking the accuracy of working thermometers will provide traceability for temperature measurements.

A laboratory's QA system should also include a policy for the purchase of common chemical reagents (e.g. conc. nitric acid, SG 1.42; phenol, 80%w/w; potassium iodate, >99.5%; acetonitrile, HPLC grade) from recognised producers and suppliers. Where such producers/suppliers are certified to ISO9001, the purchasing laboratory has the added assurance that the quality systems used in the production and supply of common laboratory chemicals have been the subject of a third party audit. The purchase of laboratory chemicals from a reputable supplier, combined with a policy for their storage and setting a shelf-life once they are received in the laboratory, should ensure that reagents of the specified grade may be realised by the analyst without the need for further special arrangements. The central provision of laboratory services, such as demineralised water and piped gases to a particular specification should also be covered by the QA system.

It is important for the analyst to be aware of exactly what is covered by the laboratory's QA system and that the specifications involved meet the analyst's requirements for a particular SOP. If they do not, the analyst will have to make special arrangements over and above that provided by the QA system and the amber category. Such arrangements will fall into the red category.

### 2.4.3 Red Category

This also represents a significant degree of control, but one which requires the analyst to select the 'special' stated references needed to carry out a particular SOP. It is important to note that these 'special' stated references are not necessarily difficult to obtain, nor do they necessarily provide a greater degree of control than those in the amber category. However, their selection does require the analyst to give some special consideration as to what will be appropriate, since it cannot be automatically assumed that the laboratory's QA system will cover the requirements of the SOP.

Examples of stated references that would be allocated to the red category include the following:

- Materials with specified values (e.g. purity, concentration, physical properties) that are used for instrument calibration purposes.
- Materials with specified values (e.g. purity, concentration) that are used either as standard titrants, or for standardising titrants, in volumetric procedures.
- Matrix reference materials, where the SOP specifies that a reference material must be included with each batch of test samples analysed.

- Physical properties (e.g. molecular weights, density values) that appear in the equation used to calculate the final analytical result.
- Individually calibrated items of volumetric glassware, where the tolerance of a class A item is too large to be fit-for-purpose
- Sample extractants where the composition has a significant effect on the final analytical result, e.g. 0.07M hydrochloric acid used to simulate stomach acid in testing paint on toys for available toxic elements.

# 3. Identifying the Traceability Requirements for a Standard Operating Procedure – An Example

The following discussion describes the application of the above approach to a typical SOP – the determination of potassium iodide in vitamin tablets. The SOP is given in Appendix 1.

## 3.1 Key Steps in the Attainment of Traceability

Summarising the discussion of the previous Section, the analyst must carry out the following steps in order to obtain valid and traceable results.

1. Select a properly validated method, with a scope that is applicable to the test sample, both in terms of matrix composition and analyte concentration.

2. Identify the acceptable uncertainty in the final result, i.e. the uncertainty that is consistent with the result being fit-for-purpose.

3. Write down and understand the equation that is used in the SOP to calculate the final analytical result.

4. Identify any reagents or equipment in the SOP with specified values.

5. Identify the fixed experimental conditions used in the SOP.

6. Allocate the values identified in steps 3, 4 and 5 to either the green, amber or red category, depending on the degree of control that needs to be applied when that value is measured or realised in practice.

7. Obtain any 'special' stated references (measurement standards), i.e. those in the red category.

## 3.2 Application of the Key Steps to the SOP

### 3.2.1 Steps 1 and 2: Method Selection and Acceptable Uncertainty

For the purposes of this example it is presumed that the SOP has been validated and is applied within its stated scope (step 1). Also the acceptable uncertainty in the final result is taken to be ±5% (step 2). Therefore the degree of control ideally required in any experimentally measured or realised values having a significant effect on the final result (those in the amber and red category) is ±1% or better, i.e. one-fifth of the acceptable overall uncertainty (see Section 2.3.1).

### 3.2.2 Step 3: Equation

Section 7 of the SOP gives the equation used to calculate the final analytical result. For convenience the equation is also set out below. The method uses a volumetric determination and is based on the equivalence $1KI \equiv 6Na_2S_2O_3$.

$$\text{Iodide Content as KI } (\mu\text{g/tablet}) = \frac{(T - B) \times M \times MW_{KI} \times 10^6 \times A}{6 \times 1000 \times W} \quad \text{.....eq. 1}$$

Where,

T = Titre (mL)

B = Blank titre (mL)

M = Molarity of sodium thiosulphate (mol/litre)

A = mean weight of one tablet (g)

W = weight of sample used [equivalent to 20 tablets](g)

$MW_{KI}$ = molecular weight of KI

All experimental values in the equation will fall into either the amber or red categories, as they all obviously have a direct and significant effect on the final result. Therefore, all of the values, except the unit conversion factors (1000; $10^6$) and the volumetric equivalence factor (6), must be traceable to appropriate stated references. The degree of control that the chosen stated reference must provide is ±1% or better (i.e. one-fifth of the uncertainty that is acceptable in the final result – see Section 2.3.1).

***The titre volumes (T and B)*** are measured using a burette. A laboratory's QA system would normally be expected to provide volumetric glassware that conforms to a recognised specification (e.g. BS846, ISO385) and is obtained from a reputable supplier. Therefore, provided this is the case, the appropriate stated reference (i.e. a burette) for realising the titre volumes with an appropriate degree of control would fall into the amber category.

Examination of manufacturers'specifications given in laboratory supply catalogues shows that various options are available when selecting a burette for a particular application. The table gives three examples:

| Type of Burette | Capacity | Graduations | Cost |
|---|---|---|---|
| Class A, borosilicate glass, BS846 | 10mL | 0.02mL | £58 |
| Class A, borosilicate glass, BS846 | 10mL | 0.05mL | £32 |
| Class B, Schellbach glass, BS846 | 10mL | 0.1mL | £19 |

Any of these burettes will provide the necessary degree of control as they will all enable an expected 10mL titre to be measured to about ±1% or better, although the Schellbach glass burette is exactly at this limit. The only task for the analyst is to check what

specification of volumetric glassware is provided by the laboratory's QA system. If the specification provided by the QA system does not give the necessary degree of control required for a particular SOP, the analyst will have to make special arrangements to obtain the necessary stated reference, i.e. it will fall in the red category and not the amber category.

***The stated reference for the molarity of the sodium thiosulphate*** solution could, in principle, be provided by a commercially produced volumetric standard solution with a stated molarity value. For this purpose the analyst will need to identify a suitable source of the volumetric reagent. For example, 1litre of a 0.1M sodium thiosulphate solution, with a tolerance factor of ±0.001M (i.e. ±1%), may be purchased from recognised and reputable laboratory reagent suppliers for about £7.

Alternatively, and as actually specified in the SOP, the molarity value of the sodium thiosulphate solution could be established experimentally by standardisation against potassium iodate. For this purpose the analyst will need to understand the principle underlying the standardisation and the way the molarity value is calculated. The standardisation is based on the following equivalence: $1KIO_3 \equiv 6Na_2S_2O_3$. The calculation of the molarity is based on the equation below :

$$\text{M (mol/litre)} = \frac{\text{mass of } KIO_3 \times \text{Purity of } KIO_3 \times 1000 \times 6}{MW_{KIO_3} \times \text{volume of } Na_2S_2O_3} \quad \ldots\ldots\ldots\ldots \text{eq. 2}$$

In terms of establishing traceability, the principal task of the analyst is to identify and obtain an appropriate source of potassium iodate, as this is now the stated reference on which the molarity of the sodium thiosulphate is based and to which it is traceable. The important property of the potassium iodate is its purity and the uncertainty of the purity value. Examination of catalogues from various suppliers shows that a number of options are available:

| Type of Potassium Iodate | Purity | Cost |
|---|---|---|
| General purpose grade (GPR) | >99.5% | £15/100g |
| Analytical grade (AR) | >99.9% | £18/100g |
| Certified reference material (CRM) | 99.96 ± 0.03% | £30/50g |

The uncertainty (degree of control) provided by the different potassium iodate materials improves progressively from the general purpose grade chemical through to the certified reference material with a formally certified purity value.

For the present example, any of these materials could be used to standardise the sodium thiosulphate, since even the GPR material would contribute <±1% to the uncertainty of the experimentally determined molarity of the sodium thiosulphate solution. However, in view of the small cost difference, selection of the AR grade chemical might be considered the preferred choice.

The CRM, issued by the National Institute of Technology and Evaluation, Japan, has been certified in accordance with international guidelines e.g. ISO Guide 35: 'Certification of reference materials – general and statistical principles'. Consequently this material would provide traceability to a value established by internationally recognised procedures, which additionally has full documentation and a stated uncertainty. In certain critical applications (e.g. where an analysis may be part of a legal dispute), the use of a formally certified material might be preferable, since there is less scope for criticism of a result on the grounds that an inappropriate standard has been used.

Finally, if the cost of using the CRM on a frequent basis is considered prohibitive, an option may be to use the CRM occasionally to verify the purity of a large batch of the AR grade material. The latter may then be used on a daily basis for the routine analysis purposes.

The above considerations show that the analyst must give some special thought to identifying an appropriate stated reference to establish the traceability of the sodium thiosulphate molarity value (M) in equation 1. It is considered unlikely that a stated reference would be provided by a laboratory's QA system. This value is therefore allocated to the red category.

***Values for molecular weights (MW)*** appear in both equations 1 and 2. The analyst will need to adopt appropriate values for these. This is a straightforward matter, simply requiring up-to-date tables and an accurate addition of the component atomic weights. Calculation to three decimal places will provide molecular weight values with an uncertainty of $< \pm 0.1\%$, which will be fit-for-purpose for use in virtually all SOPs.

Because the analyst is required to do the calculations (it is considered unlikely that the laboratory's QA system would provide molecular weight values), they are allocated to the red category.

***Measurements of mass*** also appear in both equations 1 and 2. A tablet weight (A) of the order of 1g should be measurable to about ± 0.0004g on a 4-figure analytical balance. This corresponds to a degree of control in the measurement of such mass values of about ±0.04%, which is more than adequate for the purposes of this example. In contrast, a 2-figure top-pan balance would be expected to provide an uncertainty of about ± 0.04g, equivalent to a degree of control of ±4%, which is not fit for purpose.

The combined mass (W) of 20 tablets (about 20g) taken for the actual analysis could be measured to within ±0.2% on a 2-figure top-pan balance, which is fit-for-purpose. A 4-figure analytical balance would also, of course, be fit-for-purpose.

Therefore balances properly calibrated and maintained as part of a laboratory's QA system and properly selected by the analyst will provide the necessary degree of control for the mass measurements. They are therefore allocated to the amber category.

Similar considerations show that if the $KIO_3$ stock solution used in the standardisation of the sodium thiosulphate (equation 2) is prepared using a 4-figure analytical balance, an adequate degree of control will be obtained. A known aliquot volume of this solution is then taken using volumetric glassware (a pipette). The term 'mass of $KIO_3$' in equation 2 is actually the product of the concentration of the stock solution and the aliquot volume taken. As discussed previously in relation to the use of burettes, appropriate volumetric glassware for realising volumes will normally be provided by the laboratory's QA system.

***Special Note***

Where an SOP gives a specific instruction as to the degree of control required in a particular step, such as:

- weigh on a 4-figure balance;
- use calibrated volumetric glassware with an individual certificate;
- calibrate the instrument with NIST SRM 3108 [Cd solution in $HNO_3$ :9.12±0.03 mg/g]

this degree of control must be applied, even if the considerations discussed above indicate that a less stringent degree of control would be fit-for-purpose.

***Summary***

The outcome of the above discussion is summarised in the table below, in which each of the values referred to in equations 1 and 2 are assigned to the colour category which identifies how the appropriate degree of control may be obtained.

| **Value in Equation** | **Colour Category** | **Minimum Action Required by Analyst to Obtain the Appropriate Stated References** |
|---|---|---|
| T = Titre (mL) | Amber | Use volumetric glassware |
| B = Blank titre (mL) | Amber | Use volumetric glassware |
| A = mean weight of one tablet (g) | Amber | Use analytical balance (4-fig) |
| W = weight of sample used (g) | Amber | Use top-pan balance (2-fig) |
| $MW_{KIO3}$ = molecular weight of $KIO_3$ | Red | Calculate to 3 d.p. using up-to-date tables |
| $MW_{KI}$ = molecular weight of KI | Red | Calculate to 3 d.p. using up-to-date tables |
| M = Molarity of $Na_2S_2O_3$ | Red | Standardise using $KIO_3$ |
| Mass of $KIO_3$ (g) | Amber | Use analytical balance (4-fig) |
| Purity of $KIO_3$ | Red | Choose reagent with required purity and uncertainty |
| Volume of $Na_2S_2O_3$ | Amber | Use volumetric glassware |

The table above reinforces the earlier comments that values appearing in the equation will always fall in either the amber or red categories since they all have a direct and significant effect on the final analytical result.

It is important to note that the equation must always be written out in full and explicitly. Occasionally, SOPs will be found with equations in a shortened form. For example, dilution factors, unit conversion factors and certain physical constants may be combined into a single numerical value. Equation 3 below is an example of this, in which the unit

conversion factors ($10^6$ and $10^3$), the volumetric equivalence factor (6) and the molecular weight value for KI (166.002) of equation 1 have been combined to give a single numerical factor, 27667.

$$\text{Iodide Content as KI } (\mu g/\text{tablet}) = \frac{(T - B) \times M \times A \times 27667}{W} \quad \text{.....eq 3}$$

When an equation of this type is encountered the analyst must identify all of individual component parts making up the numerical factor, in this case:

$$27667 = \frac{MW_{KI} \times 10^6}{6 \times 10^3} \quad \text{.....eq 4}$$

The traceability and corresponding degree of control requirements for each component may then be properly considered.

SOPs may be encountered that do not present an equation in any form. Instead they may simply include a statement to the effect that the calculations are carried out using software and a data processing system. In such instances the analyst must establish the exact form of the equation that has been programmed into the data system and use this to assess the traceability requirements.

Finally, if the data processing software performs additional data manipulations to those involving the equation, the analyst must take due account of this. For example, if the software also carries out automatic corrections for interferences or non-linear calibration plots, the validity of these procedures must be established as part of the requirement to select a properly validated method, as per step 1, Section 3.1.

### 3.2.3 Step 4: Identify Reagents and Equipment in the SOP with Specified Values

**(a) Equipment with Specified Values**

Section 3 of the SOP (Appendix 1) lists the equipment requirements and examination of these shows that certain values are specified, e.g.:

- Fused silica crucibles, 50mL capacity, 57mm diameter;
- Whatman filter paper 541, 18.5cm diameter

However, an experienced analyst will readily appreciate that these values are generally provided for indicative information purposes only. They clearly will have no significant effect on the final analytical result. Only minimal control is required in the 'realisation' of these values. They are therefore allocated to the green category.

**(b) Reagents with Specified Values**

Section 4 of the SOP lists the reagent requirements and many of these specify values or other information regarding the reagent, e.g.:

- Purified water
- Phenol 80% w/w, reagent grade
- Phenol solution 5% v/v
- Bromine, reagent grade
- Potassium carbonate, reagent grade
- Orthophosphoric acid, 88% reagent grade
- Potassium iodate, reagent grade
- Sodium thiosulphate, 0.1M analytical volumetric solution

Certain of the specified values clearly refer to chemical reagents that are produced and sold by commercial manufacturers, such as phenol 80% w/w, reagent grade and sodium thiosulphate, 0.1M analytical volumetric solution. It is reasonable for the analyst to presume that such values may be realised in practice with an appropriate degree of control by using chemicals of the prescribed specification that have been obtained from reputable manufacturers. Additionally, such chemicals should be properly stored once received in the laboratory and assigned an 'expiry date', after which they should not be used. For certain reagents, it may also be appropriate for the laboratory policy to stipulate, say, that the last 10% or 5% of reagent remaining in a bottle should not be used, but discarded. The foregoing approach to sourcing and using common chemical reagents with a 'commercial' specification would normally be documented in a laboratory's QA system. Such values are therefore allocated to the amber category.

Some of the reagents in the above list do not have a stated value attached to them, for example bromine is just specified as 'reagent grade'. In such cases the analyst will need to judge whether a grade suitable for general laboratory work or analytical work is required. In either case, the sourcing and use of such chemicals should be covered by the QA system and therefore the corresponding traceability requirements are allocated to the amber category.

The reagent list also specifies 'purified' water, but gives no further details of the required purity level. In such circumstances the analyst's experience might suggest that water of a purity typically provided by a properly functioning de-ionisation system would be appropriate. The supply of demineralised water to a specification appropriate for typical analytical work would be expected to be covered by the laboratory's QA system. It would therefore be allocated to the amber category. If a particular analysis posed special water purity requirements over and above that appropriate for more routine analytical work the analyst would need to address these requirements. The traceability requirements for the purity specification would then be allocated to the red category.

Examination of the SOP, as discussed in step 3 (Section 3.2.2), shows that potassium iodate is used to standardise the sodium thiosulphate solution, the latter being used to determine the KI content of the tablet samples. Therefore, sodium thiosulphate solution with the specified value (0.1M) controlled to a sufficient degree may be obtained from a reputable supplier of such reagents. It therefore falls into the amber category.

The potassium iodate is simply specified as 'reagent grade'. However, in view of its function as the essential measurement standard in the SOP, to which the final analytical result is ultimately traceable, the analyst must consider its traceability and degree of control requirements. It is therefore allocated to the red category. See Section 3.2.2 for more discussion regarding the choice of the particular grade of potassium iodate that could be used to provide traceability for results obtained using this SOP.

### 3.2.4 Step 5: Identify the Fixed Experimental Conditions used in the SOP

Section 5 of the SOP refers to 'fixed experimental conditions' at various stages of the sample preparation procedure, for example:

- Add 7g of potassium carbonate…..ignite the mixture for 25 minutes in a muffle furnace at 675°C to 700°C
- Cool, add 20mL of water

The analyst must obtain or realise values such as these experimentally, with an appropriate degree of control.

The sample preparation requires 7g of potassium carbonate to be mixed with the ground tablet sample. Reviewing this step in the context of the entire method shows that the value of 7g does not contribute significantly to the final analytical result. As the SOP does not specify a tolerance for the specified 7g, a reasonable assumption on the part of the analyst is that it could be controlled to an appropriate degree by weighing on a top-pan balance to 1 decimal place. The calibration of the balance used is expected to be covered by the laboratory's QA system, therefore the required degree of control for the 7g measurement is allocated to the amber category.

The practical realisation of a time of 25 minutes is a very simple task. Almost any clock or watch will provide the necessary degree of control, time intervals easily being measured to within ± 1 minute. The required degree of control is therefore allocated to the green category.

The degree of control required when obtaining the specified furnace temperature depends on the effect departures from the specified value will have on the final analytical result. As discussed in Section 2.3.2, information obtained from method validation work may be helpful in addressing such issues. In general terms, if the ignition temperature is too low, there may be incomplete release of the KI from the tablet matrix. Conversely, if it is too high, losses by volatilisation may occur.

The fact that the SOP specifies a temperature range of 675°C to 700°C suggests that an actual temperature of 687°C ± 12°C should be appropriate. A degree of control of ±12°C should be readily obtainable using the temperature read-out device attached to the muffle furnace. It would be sensible to verify the accuracy of this read-out, say on a yearly basis, by making a cross-check with a calibrated device, such as a platinum resistance thermometer. The provision and use of formally calibrated reference thermometers for checking the performance of working thermometers is an activity that would normally be covered by a laboratory's QA system. Stated references for temperature measurement are therefore allocated to the amber category.

If a laboratory's QA system does not provide calibrated reference thermometers, the analyst will have to make special arrangements when realising experimentally specified temperatures. It would then be allocated to the red category.

The addition of 20mL of water to the residue from the ignition stage, when reviewed in the context of the entire method, is seen to require only a basic degree of control. The 20mL could be reasonably dispensed using a measuring cylinder, or even a graduated beaker. The degree of control required of this experimental value is therefore allocated to the green category.

### 3.2.5 Step 6: Traceability Statement

The outcome of the above evaluation of the traceability requirements for the SOP for determining potassium iodide in vitamin tablets is summarised by the colour coding of the relevant text, as given in Appendix 1. Having completed the traceability evaluation, the analyst must then include a traceability statement for those stated references assigned to the 'red category', which may be written into the SOP or the Validation Report. This statement should indicate the principles and procedures on which the property values are based and identify where traceability can be related to stated references e.g. calibrated volumetric glassware, calibrated weights and balances and where traceability is achieved through the use of certified reference materials. In the latter case, details of the material must be given, which itself must contain a traceability statement. Documentary evidence of the traceability chain should be kept and made available to customers on request.

A suitable traceability statement for those values identified as requiring the highest degree of control (i.e. assigned to the red category) in determining potassium iodide in vitamin tablets would be:

*"An evaluation of the traceability requirements for the method for determining potassium iodide in vitamin tablets identified four property values requiring a high degree of control. Traceability of these values has been achieved as follows:*

- *potassium iodate*
  - *supplied by XXXXX, product code YYYYY with a purity of ZZ.Z%*
- *molarity of standardised sodium thiosulphate*
  - *standardised using potassium iodate and calibrated volumetric glassware*
- *vitamin tablet sample weight*
  - *all weighings carried out on calibrated balances traceable to national standards*
- *molecular weights of potassium iodate and potassium iodide*
  - *to 3 decimal places from IUPAC tables (www.chem.qmul.ac.uk/iupac/AtWt/index.html)"*

When reporting the results of the analysis carried out using the SOP, the laboratory should state that all critical parameters used in the method are traceable to recognised national or international standards.

# 4. Other Examples

Appendix 2 gives other examples of analytical procedures that have been evaluated for their traceability and degree of control requirements according to the approaches described in this Guide.

The analytical procedures concerned are:

1. **Determination of Nutritional Elements in Food and Biological Materials by Dry Ashing**

   This procedure is based on two SOPs:

   SOP: FDS/3. Sample Preparation by Dry Ashing

   SOP: INS/1. Quantitative Analysis of Aqueous Extracts by Inductively Coupled Plasma-Mass Spectrometry

2. **SOP: FDS/2. Determination of Dimetridazole in Animal Feedingstuffs by High Performance Liquid Chromatography**

3. **Determination of Extractable Metals in Soil**

   This procedure is based on two SOPs

   SOP: ENV/1. Extraction of Metals from Soil by Aqua-Regia

   SOP: INS/1. Quantitative Analysis of Aqueous Extracts by Inductively Coupled Plasma-Mass Spectrometry

4. **SOP: ENV/3. Determination of Water-Soluble Sulphate in Soil**

5. **SOP: ENV/2. Determination of Common Anions in Waters by Ion Chromatography**

# 5. Bibliography

*Traceability in Chemical Measurement. A guide to achieving comparable results in chemical measurements. (www.eurachem.ul.pt)*

*The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics. (www.eurachem.ul.pt)*

*Quantifying Uncertainty in Analytical Measurement, 2nd edition, (www.eurachem.ul.pt)*

# Appendix 1

**SOP: FDS/1. Determination of Potassium Iodide in Vitamin Tablets**

This SOP is discussed in detail in section 3 of the guide.

NOTE

This example of an SOP has been compiled from various sources for the specific purpose of illustrating the principles of measurement traceability. In the form it is written it is not intended for use on the bench.

The degree of control requirements for the various experimental values, as indicated by the colour coding, are based on group discussions at LGC and the two workshops organised by LGC and held in July 2003. They are provided for discussion purposes, not as definitive and final answers. Readers may well have their own views.

**SOP:FDS/1: DETERMINATION OF POTASSIUM IODIDE IN VITAMIN TABLETS**

## 1. SCOPE

The method is for the analysis of potassium iodide in multivitamin tablets, where the potassium iodide is in the range 117-143 µg/tablet.

## 2. PRINCIPLE

The tablets are ashed to remove any organic impurities and to free the potassium iodide. The ashed sample is then extracted with boiling water and the potassium iodide is converted to potassium iodate by reaction with bromine water. Phosphoric acid is added to liberate excess bromine, which is then removed by boiling. Iodide is added to react with the iodate to yield iodine. The free iodine is then determined by titrating against a standardised solution of sodium thiosulphate.

## 3. APPARATUS

In addition to normal laboratory equipment, the following is required:

3.1 Fused silica crucibles with lids, (50ml capacity, 57mm diameter).

3.2 Filter paper, Whatman, No.541, 18.5cm diameter

3.3 Muffle furnace

## 4. REAGENTS

4.1 Purified water

4.2 Phenol, 80% w/w, reagent grade

4.2.1 Phenol solution, 5% v/v

Prepare by diluting 5ml of phenol (4.2) with water (4.1) to 88ml in a measuring cylinder. Transfer to a suitable container and mix well before use.

4.3 Bromine reagent grade

4.3.1 Saturated Bromine Water

Prepare in a fume cupboard

Pipette 1ml of bromine (4.3) into a 50ml volumetric flask. Make up to volume with water (4.1), stopper and mix well.

4.4 Potassium carbonate reagent grade

4.5 Potassium iodide, reagent grade

4.5.1 Potassium iodide solution, 16% w/v

Weigh approximately 16g of potassium iodide, into a 50ml beaker. Transfer to a 100ml graduated flask with water (4.1). Make up to volume with water (4.1), stopper flask and mix well.

4.6 Orthophosphoric acid 88%, reagent grade

4.6.1 Orthophosphoric acid, 50% v/v

Prepare in a fume cupboard

Prepare by diluting 50ml of orthophosphoric acid (4.6) to 88mls of water in a measuring cylinder. Transfer to a suitable container and mix well before use.

4.7 Thyodene, indicator

4.8 Potassium iodate, reagent grade

4.9 Sodium thiosulphate 0.1M, analytical volumetric solution

4.9.1 Sodium thiosulphate, 0.01M

Pipette 50ml of sodium thiosulphate (4.9) into a 500ml graduated flask. Make up to volume with water, stopper flask and mix well. Standardise the sodium thiosulphate against a solution of potassium iodate. The method used is that detailed in *Quantitative Inorganic Analysis, A.I. Vogel, fourth edition, page 375*.

## 5. SAMPLE PREPARATION

5.1 Weigh 20 tablets to four decimal places using an analytical balance and record the weight. Calculate the mean tablet weight.

5.2 Grind the above 20 tablets plus another 20 tablets as finely as possible using a pestle and mortar.

## 6. METHOD

The analysis is carried out in duplicate, for each batch of tablets. A blank determination, omitting the sample, is also carried out.

6.1 Using long tongs place crucible and lid in a muffle set at 675ºC, for 25 minutes. Remove immediately from muffle, place on a heatproof mat and cool to room temperature.

6.2 Weigh to four decimal places a sample weight equivalent to twenty tablets into a dry crucible.

6.3 Add 7 grams of anhydrous potassium carbonate (4.4) mix carefully, and gently tap the crucible several times to compact the mixture. Overlay with an additional 10 grams of potassium carbonate, and again compact the mixture thoroughly by tapping. Ignite the mixture for 25 minutes at 675ºC to 700ºC in a muffle furnace preheated to that temperature.

6.4 Cool, add 20ml of water, or more if necessary, heat gently to boiling, and decant through a filter (3.2) into a conical flask of suitable size (for example 500ml).

6.5 Repeat the extraction by boiling with 20ml of water, then wash the crucible and the char on the filter with hot water until the filtrate measures approximately 200ml.

6.6 Add 7ml of freshly prepared bromine water (4.3.1), then slowly add 40ml of dilute phosphoric acid (4.6.1), and boil until starch iodide paper is no longer coloured amber by the vapours. During the boiling add water from time to time, as necessary, to maintain a volume of at least 200ml.

6.7 Wash down the walls of the flask with water and continue the boiling for 5 minutes.

6.8 Cool, add 5mls of phenol solution (4.2.1), again rinse the walls of the flask and allow to stand for 5 minutes.

6.9 Add 2mls of dilute phosphoric acid (4.6.1) and 5mls of potassium iodide (4.5.1) and titrate immediately with 0.01M sodium thiosulphate (4.9.1) adding thyodene (4.7) as the end point is neared.

## 7. CALCULATION OF RESULTS

Essentially the above procedure is based on the following reaction:

$$1KI \equiv 6Na_2S_2O_3$$

$$\text{Iodide Content as KI } (\mu g/\text{tablet}) = \frac{(T - B) \times M \times MW_{KI} \times 10^6 \times A}{6 \times 1000 \times W}$$

standardisation of sodium thiosulphate: $1KIO_3 \equiv 6Na_2S_2O_3$

$$M \text{ (mol/litre)} = \frac{\text{mass of } KIO_3 \times \text{Purity of } KIO_3 \times 1000 \times 6}{MW_{KIO_3} \times \text{volume of } Na_2S_2O_3}$$

Where:

T = Titre (ml)

B = Blank titre (ml)

M = Molarity of sodium thiosulphate after standardisation (mol/litre)

A = mean weight of one tablet (g) (mean of 20 tablets)

W = weight of sample used (g)

$MW_{KIO3}$ = molecular weight of $KIO_3$

$MW_{KI}$ = molecular weight of KI

# Appendix 2

## Examples of Other SOPs

NOTE

These examples of SOPs have been compiled from various sources for the specific purpose of illustrating the principles of measurement traceability. In the form they are written they are not intended for use on the bench.

The degree of control requirements for the various experimental values, as indicated by the colour coding, are based on group discussions at LGC and the two workshops held in July 2003. They are provided for discussion purposes, not as definitive and final answers. Readers may well have their own views.

DETERMINATION OF NUTRITIONAL ELEMENTS IN FOOD AND BIOLOGICAL MATERIALS

The determination of nutritional elements in food and biological materials involves two processes. First the sample is prepared by the dry ashing method (SOP FDS/3), which is followed by the quantitative analysis of aqueous extracts by inductively coupled plasma-mass spectrometry (ICP-MS) (SOP INS/1).

SOP:FDS/3:. SAMPLE PREPARATION BY DRY ASHING

1. SCOPE AND PRINCIPLE

The method applies to the quantitative analysis of trace elements of nutritional importance, in food and biological materials. (It is not suitable for oils and fats, and special care is required for foods with high fat or sugar content). The method is applicable to applicable for the following elements - Na, K, Ca, Mg, P, Cu, Fe, Mn and Zn.

The method involves the removal of organic matter by controlled combustion in a muffle furnace, the inorganic residue being dissolved in hydrochloric acid solution, ready for analysis by inductively coupled plasma mass spectrometry (SOP:INS/1).

2. APPARATUS

In addition to normal laboratory apparatus, the following is required:

2.1 Muffle furnace, 0-1000°C model.

2.2 Silica dishes, 55mm x 30mm deep, each is uniquely labelled before use by burning on a code number written with a wax pencil. Only dishes which are not chipped, scratched or otherwise damaged, may be used.

2.3 Tilt measures, 5ml and 10ml.

All glassware and plastic vessels should be cleaned before use by rinsing with 5% nitric acid (3.5), followed by thorough rinsing with purified water (3.4).

3. REAGENTS

3.1 Nitric acid, concentrated, s.g 1.42 `Aristar` grade (BDH)

3.2 Hydrochloric acid, concentrated, s.g. 1.18 `Aristar` grade (BDH)

3.3 Hydrochloric acid solution 50% v/v (aq) prepared by diluting (3.2), with (3.4) and stored in a plastic vessel.

3.4 Purified water from an Elgastat.

3.5 Nitric acid, 5% v/v (aq) prepared by diluting (3.1) with (3.4) and stored in a plastic vessel.

3.6 Nitric acid 10% v/v (aq) prepared by diluting (3.1) with (3.4).

4. QUALITY CONTROL MATERIALS

A variety of matrix reference materials are available. Normally the material most closely resembling the material under test will be analysed.

## 5. INSTRUMENT OPERATING CONDITIONS

The temperature control of the muffle furnace is set via the Eurotherm controller on the instrument. Full operating instructions may be found in the manual, but the following parameters must be set:

| | | | | |
|---|---|---|---|---|
| Ramp Rate 1 | (rl) | 1.6°C/minute | } | |
| Level 1 | (L1) | 400°C | } | CHARRING |
| Dwell 1 | (d1) | 30 minutes | } | |
| | | | | |
| Ramp Rate 2 | (r2) | 5°C/minute | } | |
| Level 2 | (L2) | 500°C | } | ASHING |
| Dwell 2 | (d2) | 960 minutes | } | |

Total duration of programme is approximately 21 hours.

## 6. SAMPLE PREPARATION

The sample must be comminuted as finely as possible (mixer, chopper, mincer etc.,) and then homogenised in a food processor or liquidiser. Care must be taken not to contaminate the sample, only stainless steel or plastic implements should be used where possible.

## 7. METHODS OF ANALYSIS

### 7.1 Dish cleaning procedure

Silica dishes (2.3) must be cleaned before each analysis as follows:

7.1.1 Soak the silica dishes (2.3) at least overnight in 10% nitric acid (3.6) in a plastic container. This container must be stored in the fume cupboard.

7.1.2 Rinse the soaked silica dishes copiously with purified water. If the dish contains any particle residues, clean it with a plastic brush and rinse with purified water.

7.1.3 Place the dish on the hot plate heated to 150°C to dry, then cool in a desiccator until required for use.

### 7.2 Ashing Procedure

7.2.1 Weigh a cleaned labelled silica ashing dish, to 0.1mg.

7.2.2 Transfer approximately 2g of freeze dried sample or 5g fresh sample into the dish, spread the sample into a thin layer, and reweigh to 0.1mg.

7.2.3 If the sample is mostly liquid, it must be dried down gradually on a hot plate before transferring to the muffle furnace. Samples containing lots of sugar or fat are liable to spit, and must be charred over a Bunsen flame or under an infra-red lamp, before placing in the muffle furnace.

7.2.4 Place into a muffle furnace, whilst still cold, the sample dishes, at least one empty dish to act as a sample blank, and the appropriate quality control material(s) to act as quality control standard(s). Reset and ensure the programme is as specified in section 5. Press the RUN button to start the programme.

7.2.5 After 16 hours at 500°C+/-10°C open the furnace door and leave to cool to 200°C according to the digital muffle readout. Heating will stop automatically as soon as the door is opened.

7.2.6 If the ash contains black carbonaceous material, allow the dishes to cool to room temperature. Add sufficient purified water to dampen the ash, dry down on a hot plate, and place back in the muffle furnace for a further 3 hours at 500+/-10°C.

7.3 Preparation of solutions

7.3.1 Allow the dishes to cool to less than 100°C according to the digital muffle and remove using long tongs (2.9).

7.3.2 Add 10ml of 50% hydrochloric acid (3.3) using a tilt measure (2.7), and carefully transfer the dishes to a hot plate in a fume cupboard. Evaporate to dryness, but do not bake the sample.

7.3.3 Add a further 10ml of 50% hydrochloric acid (3.3), warm the dish for approximately 30 seconds, and then transfer the solution, with the aid of a funnel to a plastic volumetric flask using distilled water for washing. Care should be taken not to allow the solution to run down the outside of the dish.

7.3.4 Add a further 10ml of 50% hydrochloric acid, warm the dish for approximately 20 seconds then transfer the solution to the same flask. Rinse the dish with purified water into the flask. Allow the solutions to cool to room temperature and then make up to volume (V), mixing well. On standing if a solution contains particles, filter through an ashless filter paper and treat blank(s) and reference material(s) similarly. Store the solution in a plastic bottle previously rinsed with 5% nitric acid (3.5) and distilled water.

7.3.5 Measure the trace element concentration by inductively coupled plasma emission spectrometry (SOP:INS/1).

7.4 Calculation of Results

7.4.1 In-put the results measured by ICP-MS into the following equation (applicable for the following elements - Na, K, Ca, Mg, P, Cu, Fe, Mn and Zn):

$$\text{Concentration of element (mg/100g)} = \frac{(C_S - C_B) \times V}{(10 \times W)}$$

Where:

$C_S$ = Concentration of element in sample solution , (µg/mL), as determined by ICP-MS

$C_B$ = Concentration of element in sample blank solution (µg/mL), as determined by ICP-MS

V = Final volume that the ashed sample is made up to (mL)

W = weight of sample taken for digestion (g)

7.5 Acceptability Criteria for QC material results

7.5.1 The results obtained for the QC material must lie within the acceptable in-house limits.

7.5.2 If the results falls outside the action limits, fail the associated batch and repeat the samples. Inform the responsible analyst and record the failure in the trace element QC failure action book (trace element laboratory) together with the corrective action taken to remedy the situation.

SOP INS/1: QUANTITATIVE ANALYSIS OF AQUEOUS EXTRACTS BY INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY

1. SCOPE

The method is applicable to the quantitative determination of elements in the mass range 7 to 260 amu in diluted and undiluted aqueous extracts at concentrations in the range 0.01ng/mL to 0.5g/mL. The method is not applicable to the determination of organometallic compounds such as alkyl lead or mercury compounds.

2. PRINCIPLE

The sample extract is aspirated into the plasma of the ICP-MS instrument and the positive elemental ions that are produced are passed into a quadrupole mass spectrometer where they are separated according to their mass/charge ratio. The ions are detected by an electron multiplier tube and are quantified by comparison to a previously prepared calibration curve for the isotopes/elements of interest. Major suppression or enhancement effects are compensated for by use of an internal standard (rhodium).

3. APPARATUS

3.1 Perkin Elmer Elan 5000A inductively coupled mass spectrometer operating under the following typical conditions:

Power: 1000 – 1100W

Gas flows:-

Coolant: 15 L/min

Auxiliary: 0.80 L/min

Nebuliser: 0.85 to 0.95 L/min

Sample uptake: 0.5 to 1.5 mL/min

Note: the sample uptake rate affects the sampling position in the plasma, ion intensities and the formation of oxides and doubly charged ions. Once set, all standards and samples shall be analysed using the same sample uptake rate.

4. REAGENTS

4.1 Nitric, sulphuric and hydrochloric acids (Baker Ultrex II acids, or equivalent)

4.2 De-ionised water (from Elga maxima purification unit), >17.8MΩ resistivity

5. CALIBRATION STANDARDS

5.1 Certified plasma emission standards (nominal 10000 µg/mL) covering the elements of interest (e.g. from BDH, Alfa Aesar, NIST, etc)

5.2 Rhodium internal standard solution (nominal 10000 µg/mL) (supplier as above)

6. SAMPLE PREPARATION

If necessary, sample extracts must be diluted, immediately prior to analysis, using 1% (v/v) nitric acid (or another acid, as appropriate) to bring the elemental concentration into the calibration range of the instrument (<1 μg/mL). To achieve this an accurately measured aliquot is diluted to a known volume. A suitable internal standard (e.g. rhodium) is also added at a concentration of 5 ng/mL, which is appropriate for most situations.

## 7. VALIDATION OF INSTRUMENT PERFORMANCE

The instrument must be set up and its performance validated as described in the manufacturer's user manual.

## 8. INSTRUMENT CALIBRATION

### 8.1 Multi-Element Stock Solutions of the Elements to be Determined

A multi-element stock solution containing each of the elements of interest at 10 μg/mL is prepared in 1% (v/v) nitric acid, using individual concentrated solutions purchased from a commercial supplier (5.1).

### 8.2 Internal Standard (Rhodium) Stock Solution

A stock solution of rhodium at 10μg/mL in 1% (v/v) nitric acid is prepared using the concentrated solution purchased from a commercial supplier (5.2)

### 8.3 Calibration Standards

Standard solutions for calibrating the instrument are prepared from the multi-element stock solution (8.1), in a matrix which matches as closely as possible that of the samples to be analysed. At least two calibration solutions are prepared covering the expected concentrations in the samples, plus a blank (e.g. 0, 50 and 100 ng/mL). The multi-element calibration standard solutions must also contain the internal standard (8.2) at a concentration of 5 ng/mL.

### 8.4 Calibration Procedure

Calibration is performed according to the procedures detailed in the instrument user's manual and before any test samples are analysed. Where possible, at least two isotopes of each element are to be measured. The calibration solutions are aspirated into the instrument and between 3 and 5 replicate measurements are obtained per calibration solution. A parameter file is created for the elements of interest for the purposes of data acquisition. Acquisition is performed in the 'peak-hop' mode using one point per peak.

The blank solution is also measured and blank readings are subtracted from all subsequent measurements of standards. A calibration line is constructed that is 'linear thru zero'.

## 9. SAMPLE ANALYSIS

The test samples are analysed in the same manner as the calibration solutions, using the same parameter file for the acquisition of data. A wash step using 1 to 5% (v/v) nitric acid is included between each sample and standard of at least 1 min at 1mL/min uptake rate. Some elements are prone to memory effects in the sample introduction system and may require the wash time to be doubled and a subsequent blank analysis to check such effects have been eliminated.

One calibration standard is analysed every sixth sample to check for instrument drift. If significant drift is observed the instrument is re-calibrated and all samples since the last check standard are compensated for the observed drift, or re-analysed. Drift is deemed significant at twice the quoted precision of the measurements.

## 10. CALCULATION AND REPORTING RESULTS

The instrument software automatically calculates the concentration of the elements present in the aqueous sample extract ($C_{sample}$), by comparison to the relevant calibration data.

The software uses an equation of the following type:

$$C_{sample} = C_{calib} \times \frac{R_{sample}}{R_{calib}} \times \frac{R_{IS\ in\ calib}}{R_{IS\ in\ sample}} \times F$$

$C_{sample}$ = concentration of element in the aqueous extract (ng/mL)

$C_{calib}$ = concentration of element in the prepared calibration standard (ng/mL)

$R_{sample}$ = instrument response for element in sample

$R_{calib}$ = instrument response for element in calibration standard

$R_{IS\ in\ calib}$ = instrument response for internal standard (Rh) in calibration standard

$R_{IS\ in\ sample}$ = instrument response for internal standard (Rh) in sample

F = dilution factor, if appropriate (final volume, mL/aliquot volume, mL)

The software also automatically corrects for isobaric interferences. However, all concentrations reported on each isotope should be checked for polyatomic interferences or residual isobaric interferences. Care should always be exercised when interpreting results.

## 11. BIAS AND PRECISION

Short-term precision is typically 2% relative.

Accuracy is checked using either certified reference materials or the method of standard additions, as described in the users manual.

A certified plasma emission standard (5.1), from a source different to that used to prepare the calibration standards (8.3) may be suitable as CRMs for the purposes of checking bias.

SOP:FDS/2: THE DETERMINATION OF DIMETRIDAZOLE, IN ANIMAL FEEDING STUFFS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

1. SCOPE

This method specifies a procedure for the determination of dimetridazole in animal feeds. The lower limit of determination is 1mg/kg.

2. PRINICIPLE

The analyte is extracted from the sample with dichloromethane. The extract is cleaned up on a silica cartridge and the analyte quantified using reverse phase high performance liquid chromatography, using ultra-violet (UV) absorbance detection (320nm).

3. REAGENTS

NB. Unless otherwise specified all reagents must be of analytical reagent quality or better. The water must be glass distilled or of at least equivalent purity.

3.1 Acetonitrile, HPLC grade

3.2 Dichloromethane

3.3 Methanol

3.4 Ammonium acetate

3.5 Acetic acid, glacial

3.6 Potassium dihydrogen orthophosphate

3.7 Di-potassium hydrogen orthophosphate, trihydrate

3.8 Dimetridazole, >99% purity available from Sigma Chemicals

3.9 Ammonium acetate buffer

Dissolve 3.2g of ammonium acetate (3.4) in 900ml of water. Adjust the pH to 4.4 to 4.5 with glacial acetic acid (3.5) and make up to 1 litre with water.

3.10 Di-potassium hydrogen orthophosphate, trihydrate, 0.2M aqueous solution.

Dissolve 11.41g of di-potassium hydrogen orthophosphate (3.7) in water and make up to 250ml in a graduated flask.

3.11 Potassium dihydrogen orthophosphate, 0.009M aqueous solution.

Dissolve 1.2248g of potassium dihydrogen orthophosphate (3.6) in water and make up to 1 litre in a graduated flask.

3.12 Phosphate Buffer

Mix equal quantities of 0.2M di-potassium hydrogen orthophosphate solution (3.10) with 0.009M potassium dihydrogen orthophosphate (3.11). This will give a buffer solution with a pH of between 8.0 and 8.5 – this should be confirmed using a pH meter.

3.13 Mobile Phase

800ml of ammonium acetate buffer (3.9)

200ml of acetonitrile (3.1)

3.14 Dimetridazole stock standard solution (1mg/ml)

Dissolve 100.0mg of dimetridazole (3.8) in methanol (3.3) in a 100ml graduated flask. Make up to the mark with methanol and mix thoroughly. This solution can be kept for up to 1 month if stored in an amber container and refrigerated (at $\leq$ 4ºC).

3.15 Intermediate Standard (50µg/ml)

Pipette 2.5ml of the stock standard solution (3.14) into a 50ml graduated flask. Make up to the mark with mobile phase (3.13) and mix thoroughly. This solution can be kept for up to 1 week if stored in an amber container and refrigerated (at ≤ 4ºC).

3.16 Calibration Standards

Prepare standards at 0.25µg/ml, 0.5µg/ml, 1µg/ml, and 2µg/ml by diluting the intermediate standard solution (3.15) with mobile phase (3.13). These must be prepared fresh daily.

3.17 Spiking Standard Solution (200µg/ml of the standard)

Pipette 5ml of the stock standard (3.14) into a 25ml graduated flask, make up to the mark with methanol and mix thoroughly. This solution can be kept for up to 1 week if stored in an amber container and refrigerated (at ≤ 4ºC).

4. QUALITY CONTROL MATERIAL

A previously tested blank feed (10g) is spiked with 200µl of the spiking standard (3.17), to give a QC material with a dimetridazole concentration of 4mg/kg.

5. APPARATUS

In addition to normal laboratory apparatus, the following is required:

5.1 Sep-Pak silica cartridges, Whatman Part No. WAT051900

5.2 HPLC system consisting of the following items:

Pump capable of pumping at 1ml/min

Injection system capable of injecting 100µl

UV detector capable of operating at 320nm

Data acquisition system

5.3 HPLC Column

Phenomenex phenyl hexyl column, particle size 5µm, 250mm x 4.6mm or equivalent should be used.

5.4 pH Meter

The pH meter should be calibrated with appropriate buffer solutions and used according to the manufacturers instructions.

6. PROCEDURE

NB During extraction and clean up stages care must be taken to ensure that the dichloromethane is not lost by evaporation.

6.1 Preparation of QC Materials

Weigh two 10g ± 0.1g portions of a previously tested blank feed into two separate conical flasks. Record the weight to 0.001g. Add 200µl of the spiking standard (3.17) to one of these test portions. The second portion of blank feed is analysed as a blank. Analyse these 2 samples along with the other samples comprising the batch from point 6.2.2.

6.2 Extraction and Clean up

6.2.1 Sample preparation: all samples must be ground to pass a 0.5mm sieve and mixed thoroughly prior to analysis.

6.2.2 Weigh between 9.9g and 10.1g of the sample into a conical flask and record the weight to 0.001g. Add 15ml of phosphate buffer (3.12) using a measuring cylinder and allow to soak for about 10 minutes.

6.2.3 Using a pipette add 50ml of dichloromethane (3.2) to the flask. Stopper tightly and shake the contents of the flask vigorously by hand to ensure none of the sample is stuck to the bottom of the flask and that there are no large lumps present. Follow this by shaking on a shaker for about 15 minutes. If there is no evidence of an aqueous phase, filter the extract through a filter funnel containing a little glass wool and collect approximately 30mls of the extract.

6.2.4 If the sample has separated into 2 layers, filter the extract through a filter funnel containing a little glass wool and collect the extract in a separating funnel. Allow the layers to separate and collect the lower dichloromethane layer.

6.2.5 Condition a silica cartridge (5.1) by passing 5ml of dichloromethane (3.2) through it. Then using a pipette add 10ml of the dichloromethane sample extract to the cartridge. When the meniscus has reached the surface of the packing – **do not let the cartridge go dry** – wash the cartridge with a further 2ml of dichloromethane (3.2) and then dry the cartridge with a gentle stream of air.

6.2.6 When the cartridge is completely free of dichloromethane elute the compounds from the cartridge with 8ml of mobile phase (3.13). Collect the eluate in a 10ml graduated flask and make up to the mark with mobile phase (3.13). This solution is now ready for examination by HPLC as described in section 7.

## 7. HPLC DETERMINATION

### 7.1 System suitability for the screening test

Allow the HPLC system to equilibrate by running the mobile phase for at least 30 minutes before any injections are made. Inject 100µl of the 2µg/ml calibration standard. Ensure that the retention time for dimetridazole is not less than 10 minutes. Repeat the injections a further two times. The peak heights obtained from the data acquisition system should agree to within ± 5% of the mean value.

### 7.2 Calibration

Calibrate the HPLC system using the calibration standards prepared in 3.16. Construct a calibration curve by plotting the mean peak height values of all calibration standards versus the corresponding concentration value.

Using a suitable spreadsheet package construct a linear regression curve and determine both the slope (m) and intercept (c) of the curve.

### 7.3 Sample Analysis

When satisfactory repeatability has been obtained from repeated injections of the calibration standard, injections of the QC solutions from 6.1 and sample solutions from 6.2 can be made. All sample solutions are injected in duplicate. An injection of a standard is made after every fourth sample such that the full range of standards (3.16) are incorporated into the sample extract sequence. When necessary a measured aliquot of the sample extract solution should be diluted to a measured volume using the mobile phase (3.13), to ensure that the response does not exceed the response of the top calibration standard solution. After injection of all the sample solutions, two injections of each of the calibration standards should be made.

## 8. CALCULATION OF RESULTS

The concentration (x) of the drug in the extract solution can be calculated from the equation:using the expression:

$$X\ (\mu g/ml) = \frac{(y - c)}{m}$$

where:

y = observed mean sample peak height

m = slope of calibration linear regression curve

c = intercept of calibration linear regression curve

The concentration of dimetridazole can then be calculated from:

$$G = \frac{X \times V \times F}{M}$$

where:

G = dimetridazole content in sample (mg/kg)

X = concentration of dimetridazole in injected sample solution (µg/ml)

V = total volume of the dichloromethane extract (50 ml)

F = dilution factor, if applicable

M = mass of sample taken for analysis (g)

DETERMINATION OF EXTRACTABLE METALS IN SOIL

The determination of extractable metals in soil samples is based on two SOPs:

SOP ENV/1: Extraction of Metals from Soil using Aqua-Regia

This is followed by:

SOP INS/1: Quantitative Analysis of Aqueous Extracts by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

SOP ENV/1: EXTRACTION OF METALS FROM SOIL BY AQUA-REGIA

1. SCOPE

The method is applicable to soils containing not more than 33% (m/m) of organic matter

2. PRINCIPLE

The sample is extracted using a hydrochloric acid/nitric acid mixture under specified conditions (BS7755. ISO11466:1995). The extraction procedure does not necessarily extract the total metal content.

3. REAGENTS

All reagents should be of at least analytical grade. They are obtainable from major chemical suppliers, unless otherwise stated.

3.1 Water (demineralised)

3.2 Nitric acid (69% v/v SG 1.42)

3.3 Hydrochloric acid (37% v/v SG 1.18)

3.4 0.5mol/litre nitric acid:

Using a measuring cylinder add 32 ± 0.5mL of nitric acid (3.2) to a 1 litre volumetric flask containing about 900 mL of water (3.1) and swirl to mix. Allow to cool, make to the mark with water (3.1), stopper and mix. Transfer to a polythene bottle and store at room temperature.

4. QUALITY CONTROL MATERIALS

A previously analysed test sample may be used to verify that within-laboratory repeatability is acceptable.

A certified reference material may be used from time-to-time to check for bias, e.g

BCR143R Sewage sludge-amended soil

LGC6135 Brick-works soil

NIST SRM2710 Montana soil

5. APPARATUS

In addition to normal laboratory glassware and equipment, the following is required

5.1 Reflux condenser, assembled length 340mm, with ground glass joints

5.2 150µm sieve

6. SAMPLE PREPARATION

The soil must be air-dried (according to SOP ENV/1A) and ground to pass a 150μm sieve (5.2) and then mixed.

7. SAMPLE EXTRACTION PROCEDURE

Note: With each batch of 10 soil samples, a reagent blank and a soil QC sample (4) must be run.

7.1 Weigh 3.00 ± 0.01g of the prepared sample into a 250mL reaction vessel.

7.2 Moisten the soil with 2.0 ± 0.1mL of water (3.1)

7.3 Using a measuring cylinder add 21.0 ± 0.5mL of hydrochloric acid (3.3), followed by 7.0 ± 0.5mL of nitric acid (3.2), dropwise if necessary to reduce foaming.

7.4 Allow to stand overnight at room temperature

7.5 Add 15.0 ± 0.5mL of 0.5mol/litre nitric acid (3.4) to the absorption vessel and connect the absorption vessel to the reaction vessel, via the reflux condenser (5.1).

7.6 Heat the soil/acid mixture under reflux for 2 hours ± 5 minutes, ensuring that the condensation zone is lower than 1/3 of the height of the condenser.

7.7 Add the contents of the absorption vessel to the reaction vessel, via the condenser, by rinsing the absorption vessel with 2 x 10.0 ± 0.5mL portions of 0.5 mol/litre nitric acid (3.4).

7.8 Filter the contents of the reaction vessel into a Buchner flask.

7.9 Rinse the reaction vessel with 20.0 ± 0.5mL 0.5 mol/litre nitric acid (3.4) and filter the rinsing through the same filter paper into the conical flask.

7.10 Quantitatively transfer the combined filtrate and rinsings to a 100mL volumetric flask

7.11 Rinse the Buchner flask with 10.0 ± 0.5mL of 0.5 mol/litre nitric acid (3.4) and add the rinsing to the volumetric flask.

7.12 Make up to the mark with 0.5 mol/litre nitric acid (3.4). Stopper the flask and mix.

8. ANALYSIS OF THE EXTRACT

Determine the metal concentration in the prepared extract (7.12) using inductively coupled plasma - mass spectrometry (SOP INS/1)

9. CALCULATION OF RESULT

The concentration (C, in mg/kg) of the element in the soil is calculated using the following equation:

$$C\,(mg/kg) = \frac{C_{ext}}{W} \times \frac{V}{1000}$$

$C_{ext}$ = element concentration (μg/L) in the extract, determined by ICP-MS

V = volume of extract (mL)

W = mass of sample (g) taken for extraction

The result obtained for the QC sample is plotted on the QC chart. The result should lie within the control limits. If it does not the matter should be investigatedand, if necessary, the analyses on the test samples repeated

SOP ENV/3: DETERMINATION OF WATER-SOLUBLE SULPHATE IN SOIL

1. SCOPE

The method is applicable to the determination of the concentration of water-soluble sulphate in soils and soil-like matrices, at concentrations in the range 50 to 25000 mg/kg.

## 2. PRINCIPLE

1 part by mass of the soil sample is shaken with 5 parts by volume of water for 16 hours. The sulphate content of the water extract after filtration is determined gravimetrically, following precipitation as barium sulphate.

## 3. APPARATUS

In addition to normal laboratory equipment the following is required:

Mechanical shaker: capable of keeping 10g of soil sample in continuous suspension in 50mL of water

3.1 Filter papers – ashless, medium grade porosity, 8µm
3.2 Filter papers – fine grade porosity, 3µm
3.3 Sieve, 2mm

## 4. REAGENTS

The following reagents are required:

4.1 Demineralised water

4.2 Hydrochloric acid (35.5 – 37.5% v/v; SG 1.18), analytical grade

4.3 Barium chloride dihydrate, analytical grade

4.4 Sodium hydroxide pellets, analytical grade

4.5 Silver nitrate, analytical grade

4.6 Methyl orange

4.7 Hydrochloric acid, approx. 6 mol/litre: Carefully mix 500mL ± 10mL of conc. hydrochloric acid (4.2) with water (4.1) and dilute to 1 litre in a measuring cylinder. Transfer the solution to a polythene bottle.

4.8 Barium chloride solution, approx 100g/litre: Dissolve 100g ± 1g of barium chloride hydrate (4.3) in about 800ml of water (4.1). Warm the solution on a hot plate to aid dissolution. Cool to room temperature, dilute to 1 litre in a measuring cylinder and transfer to a glass or polythene bottle.

4.9 Sodium hydroxide solution, approx. 5 mol/litre: Dissolve 20g of sodium hydroxide pellets (4.4) in 100mL of water (4.1), with stirring to aid dissolution. Transfer to a glass or polythene bottle.

4.10 Methyl orange indicator solution, approx. 1g/litre: Dissolve 100mg of methyl orange (4.5) in about 50mL of water (4.1). Warm the solution on a hot plate to aid dissolution. Cool to room temperature and dilute to 100mL in a measuring cylinder. Transfer to a glass or polythene bottle.

4.11 Silver nitrate solution, approx. 0.1 mol/litre: Dissolve 17g ± 1g of silver nitrate (4.5) in about 800mL of water (4.1) and dilute to 1 litre in a measuring cylinder with water (4.1). Transfer the solution to an amber glass bottle and store in the dark.

## 5. QUALITY CONTROL MATERIALS

Either of the following may be used as a quality control material:

5.1 A previously analysed soil sample

5.2 A CRM may be used (e.g. LGC6144, Contaminated Soil).

6. PROCEDURE

6.1 Sample Preparation

6.1.1 The soil sample must be air-dried (according to SOP ENV/1A), ground to pass a 2mm sieve and mixed.

6.1.2 A quality control material (5) must be included with each batch of samples.

6.2 Extraction of Samples

6.2.1 Extractions should be carried out at a temperature in the range 20°C to 25°C.

6.2.2 A reagent blank must be included with each batch of samples.

6.2.3 Transfer 10g ± 0.1g of the prepared sample to an extraction bottle.

6.2.4 Add 50mL ± 0.5mL of water (4.1) to the extraction bottle and stopper tightly.

6.2.5 Place the extraction bottle on the mechanical shaker (3.1) and agitate for 16 hours.

6.2.6 Centrifuge the soil suspension and filter the supernate under vacuum through a suitable filter paper (3.2) into a Buchner flask.

6.2.7 Measure the volume of the filtrate ($V_E$) and retain the filtrate for determination of the sulphate content.

6.3 Determination of Sulphate in the Extract

6.3.1 Accurately transfer a measured volume ($V_A$) of the extract, using a pipette, to a 250mL beaker

6.3.2 The volume ($V_A$) of the extract taken for analysis shall be between 10mL and 50mL and shall not contain more than 50mg of sulphate ions. A preliminary analysis may be required to establish the appropriate volume required.

6.3.3 Add 2 drops of methyl orange indicator (4.10) to the solution and neutralise (pink↔orange-yellow) the test portion with dilute hydrochloric acid (4.7) or sodium hydroxide (4.9), according to the initial pH.

6.3.4 Add 2mL ± 0.2mL dilute hydrochloric acid (4.7) and, if necessary, add water to bring the total volume to 200mL ± 20mL.

6.3.5 Boil the solution on a hot plate for at least 5 minutes.

6.3.6 If the solution is clear after boiling proceed to step 6.3.8

6.3.7 If insoluble matter is present, filter the hot mixture through a fine porosity filter paper (3.3) and wash the paper with a small quantity of hot water (4.1), combining the washings with the filtrate.

6.3.8 Transfer the solution quantitatively to a 500mL beaker and boil the solution on a hot plate; slowly add, using a pipette, 10mL ± 5mL of hot (about 80°C) barium chloride solution (4.8)

6.3.9 Heat the solution for at least 1 hour and then allow to cool and stand overnight.

6.3.10 Filter the mixture through an ashless filter paper (3.2), ensuring that the precipitate is transferred quantitatively to the filter paper.

6.3.11 Wash the precipitate several times with hot water (4.1) until the washings are free from chloride, as indicated by the absence of turbidity when a drop of is tested with the solution of silver nitrate (4.11).

6.3.12 Transfer the filter paper and precipitate to a previously ignited and weighed ($m_1$) porcelain or silica crucible.

6.3.13 Place the crucible in an electric muffle furnace at room temperature and then raise the temperature gradually to red heat (800°C).

6.3.14 Hold the crucible at red heat for 15 minutes.

6.3.15 Transfer the crucible and contents to a desiccator and allow to cool to room temperature. Weigh the crucible and contents ($m_2$)

## 7. CALCULATION OF RESULTS

The sulphate concentration, C, in the original test sample is calculated using the equation:

$$C\,(mg/kg) = \frac{(m_2 - m_1) - m_0}{m_s} \times \frac{V_E}{V_A} \times \frac{MW_{SO4}}{MW_{BaSO4}} \times 10^6$$

$m_2$ = mass of crucible + precipitate, g

$m_1$ = mass of crucible, g

$m_0$ = mass of residue in blank crucible, g

ms = mass of sample taken for extraction, g

$V_A$ = volume of extract taken for gravimetric analysis, mL

$V_E$ = total volume of extract, mL

$MW_{SO4}$ = molecular weight of sulphate (SO4)

$MW_{BaSO4}$ = molecular weight of barium sulphate (BaSO4)

Check that the results obtained on the QC material are within the set limits.

SOP ENV/2: DETERMINATION OF COMMON ANIONS IN WATERS BY ION CHROMATOGRAPHY

1. SCOPE

The method is applicable to the determination of fluoride, chloride, phosphate, nitrate and sulphate in potable water, swimming pool water and effluents.

2. PRINCIPLE

The sample is injected onto an ion-exchange chromatography column and eluted with an aqueous carbonate/bicarbonate mobile phase. The anions are detected and quantified using a conductivity detector.

3. APPARATUS

In addition to normal laboratory glassware and equipment, the following is required

3.1 Dionex DX-500 Ion Chromatograph

4. REAGENTS

4.1 Ultra-pure water (Elgastat UHP water), with a conductivity <0.1μS/cm

4.2 Sodium carbonate

4.3 Sodium bicarbonate

4.4 Mobile phase: 1.8mM sodium carbonate-1.7mM sodium bicarbonate

Weigh 0.960 ± 0.005g sodium carbonate (4.2) and 0.710 ± 0.005g sodium bicarbonate (4.3) into a 5 litre volumetric flask. Add water (4.1) to dissolve the salts, make to the mark with water (4.1) and mix.

5. CALIBRATION STANDARDS

5.1 Pure Substances used to Prepare Calibration Standards

5.1.1 Sodium fluoride

5.1.2 Sodium chloride

5.1.3 Sodium nitrate

5.1.4 Potassium dihydrogen phosphate

5.1.5 Potassium sulphate

5.2 Stock Standard Solutions

The following stock standard solutions are prepared by dissolving the stated quantity of each particular compound in water (4.1). The solution is transferred to a 1 litre volumetric flask, which is then made to the mark with water (4.1) and inverted several times to mix the contents.

| Anion | Concentration of anion in stock solution (mg/litre) | Compound to be used to prepare stock solution | Quantity (g) of compound to be dissolved in 1 litre of water |
|---|---|---|---|
| Fluoride | 1000 | NaF | 2.210 ± 0.005 |
| Chloride | 3000 | NaCl | 4.945 ± 0.005 |
| Nitrate | 2000 | $NaNO_3$ | 2.742 ± 0.005 |
| Phosphate | 1000 | $KH_2PO_4$ | 1.433 ± 0.005 |
| Sulphate | 3000 | $K_2SO_4$ | 5.442 ± 0.005 |

5.3 Mixed Stock Solution

Using glass pipettes, transfer the following aliquots of each of the single stock solutions into a 1 litre volumetric flask, make up to the mark with water (4.1) and mix.

| Stock Solution | Aliquot (mL) | Concentration in the mixed stock solution (mg/litre) |
|---|---|---|
| Fluoride | 4 | 4 |
| Chloride | 20 | 60 |
| Nitrate | 25 | 50 |
| Phosphate | 5 | 5 |
| Sulphate | 25 | 75 |

5.4 Calibration Solutions

Using glass pipettes, dilute 60mL, 20mL and 5 mL aliquots of the mixed stock solution (5.3) to 100mL, 100mL and 200mL respectively, in volumetric flasks.

The three diluted mixed solutions and the undiluted mixed solution (5.3) provide four calibration solutions, as tabulated below:

| | Calib Std 4<br>mg/litre | Calib Std 3<br>mg/litre | Calib Std 2<br>mg/litre | Calib Std 1<br>mg/litre |
|---|---|---|---|---|
| Fluoride | 4 | 2.4 | 0.8 | 0.1 |
| Chloride | 60 | 36 | 12 | 1.5 |
| Nitrate | 50 | 30 | 10 | 1.25 |
| Phosphate | 5 | 3 | 1 | 0.125 |
| Sulphate | 75 | 45 | 15 | 1.875 |

## 6. QUALITY CONTROL MATERIALS

Two types of QC material may be used.

6.1 A solution is prepared in-house, by diluting 25 mL of an independently prepared mixed stock solution (5.2) to 100mL.

6.2 A certified reference material, e.g. BCR-616 Ground water

## 7. ION CHROMATOGRAPHY

Set up the equipment according to the manufacturer's instructions. Ensure there is sufficient eluent in the reservoir and set the pump at the appropriate flow-rate. Once the system has stabilised (after about 10 minutes), check that the background conductivity of the eluent is <20μS/cm. If it is not, replace the eluent.

Load portions (5mL) of the samples, calibration standards and QC material into polyvials, up to the mark. Place the polyvials in the autosampler. Calibration standards are placed at the start of a run and at about every 20 sample vials. In each run there should be at least one QC material and one replicate test sample.

Set up a file for the acquisition of data from the chromatography run.

To verify the system is operating correctly, firstly run a mixed calibration standard. The peak areas and retention times should be comparable to those obtained in previous runs. The retention time of the sulphate peak should be within ± 1 minute of that observed in previous runs.

Provided the system is working satisfactorily, run the complete set of polyvials.

If the area of a sample peak exceeds that of the top calibration standard, dilute a measured aliquot to a known volume to bring the sample peak area within the calibration range and re-inject the diluted sample.

## 8. CALCULATION OF RESULTS

Using the data station, process the raw peak data to obtain the calculated anion concentrations in the samples and the QC material.
The data station processes the data using an equation of the following type:

$$C_{sample} = C_{calibstd} \times \frac{A_{sample}}{A_{calibstd}} \times F$$

where:

$C_{sample}$ = concentration of anion in sample, mg/L

$C_{calibstd}$ = concentration of anion in calibration standard, mg/L

$A_{sample}$ = peak area of anion in sample

$A_{calibstd}$ = peak area of anion in calibration standard

F = dilution factor, if appropriate (final volume/aliquot volume)

The result for the QC material should lie within the set limits for the particular QC material concerned.

# Appendix 3

List of workshop participants:

| | |
|---|---|
| Vicki Barwick | LGC |
| Malcolm Burn | LGC |
| Mary Butts | Public & Agricultural Analyst, Somerset |
| Charlotte Byrne | Harwell Scientifics |
| Gaelle Chapelais | Chemex Environmental International Ltd |
| Yuk Cheung | UKAS |
| Rasa Cooke | Chemex Environmental International Ltd |
| Nan Fernandez | Harwell Scientifics |
| Jonathan Fisher | Harwell Scientifics |
| Simon Freeman | Nestle |
| Rosie Gutreich | Alcontrol Technichem |
| Jonathan Hamlet | Cleanaway |
| Paul Hancock | Public & Agricultural Analyst, Somerset |
| Henry Hau | Rhodia Consumer Speciality Division |
| Christopher House | NRM Ltd |
| Stephen Humphry | NRM Ltd |
| Warren Jackson | RHM |
| Richard Lawn | LGC |
| Darren Lewis | City Analytical |
| David Lowe | UKAS |
| Sheila Merson | LGC |
| Jenny Newfor | Harwell Scientifics |
| Yvonne Rainey | City Analytical |
| Anne Rothin | Worcestershire Scientific Services |
| Jeff Ruddle | LGC |
| Mike Sargent | LGC |
| Andrew Scott | Direct Labs |
| Dr Rachel Siertsema | Dextra Labs |
| Margaret Walker | Direct Labs |
| Alec Williams | Chairman - Eurachem/CITAC Traceability Working Group |
| Steve Wood | LGC |