DIQUAT DIBROMIDE

1,1’-ethylene-2,2’-bipyridyldiylium dibromide

2008

1 Diquat is the ISO common name for the 1,1’-ethylene-2,2’-bipyridyldiylium dication.
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## PART ONE

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FAO specifications are developed with the basic objective of promoting, as far as practicable, the manufacture, distribution and use of pesticides that meet basic quality requirements.

Compliance with the specifications does not constitute an endorsement or warranty of the fitness of a particular pesticide for a particular purpose, including its suitability for the control of any given pest, or its suitability for use in a particular area. Owing to the complexity of the problems involved, the suitability of pesticides for a particular purpose and the content of the labelling instructions must be decided at the national or provincial level.

Furthermore, pesticides which are manufactured to comply with these specifications are not exempted from any safety regulation or other legal or administrative provision applicable to their manufacture, sale, transportation, storage, handling, preparation and/or use.

FAO disclaims any and all liability for any injury, death, loss, damage or other prejudice of any kind that may arise as a result of, or in connection with, the manufacture, sale, transportation, storage, handling, preparation and/or use of pesticides which are found, or are claimed, to have been manufactured to comply with these specifications.

Additionally, FAO wishes to alert users to the fact that improper storage, handling, preparation and/or use of pesticides can result in either a lowering or complete loss of safety and/or efficacy.

FAO is not responsible, and does not accept any liability, for the testing of pesticides for compliance with the specifications, nor for any methods recommended and/or used for testing compliance. As a result, FAO does not in any way warrant or represent that any pesticide claimed to comply with a FAO specification actually does so.

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1 This disclaimer applies to all specifications published by FAO.
INTRODUCTION

FAO establishes and publishes specifications* for technical material and related formulations of agricultural pesticides, with the objective that these specifications may be used to provide an international point of reference against which products can be judged either for regulatory purposes or in commercial dealings.

From 1999, the development of FAO specifications has followed the New Procedure, subsequently described in the 1st edition of “Manual for Development and Use of FAO and WHO Specifications for Pesticides” (2002) and amended with the supplement of this manual (2006), which is available only on the internet through the FAO and WHO web sites. This New Procedure follows a formal and transparent evaluation process. It describes the minimum data package, the procedure and evaluation applied by FAO and the Experts of the FAO/WHO Joint Meeting on Pesticide Specifications (JMPS). [Note: prior to 2002, the Experts were of the FAO Panel of Experts on Pesticide Specifications, Registration Requirements, Application Standards and Prior Informed Consent, which now forms part of the JMPS, rather than the JMPS.]

FAO Specifications now only apply to products for which the technical materials have been evaluated. Consequently from the year 2000 onwards the publication of FAO specifications under the New Procedure has changed. Every specification consists now of two parts, namely the specifications and the evaluation report(s):

Part One: The Specification of the technical material and the related formulations of the pesticide in accordance with chapters 4 to 9 of the “Manual on development and use of FAO and WHO specifications for pesticides”.

Part Two: The Evaluation Report(s) of the pesticide, reflecting the evaluation of the data package carried out by FAO and the JMPS. The data are provided by the manufacturer(s) according to the requirements of chapter 3 of the “FAO/WHO Manual on Pesticide Specifications” and supported by other information sources. The Evaluation Report includes the name(s) of the manufacturer(s) whose technical material has been evaluated. Evaluation reports on specifications developed subsequently to the original set of specifications are added in a chronological order to this report.

FAO specifications developed under the New Procedure do not necessarily apply to nominally similar products of other manufacturer(s), nor to those where the active ingredient is produced by other routes of manufacture. FAO has the possibility to extend the scope of the specifications to similar products but only when the JMPS has been satisfied that the additional products are equivalent to that which formed the basis of the reference specification.

Specifications bear the date (month and year) of publication of the current version. Dates of publication of the earlier versions, if any, are identified in a footnote. Evaluations bear the date (year) of the meeting at which the recommendations were made by the JMPS.

PART ONE

SPECIFICATIONS

DIQUAT DIBROMIDE

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<tr>
<td>DIQUAT DIBROMIDE SOLUBLE CONCENTRATE (FEBRUARY 2008)</td>
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</tr>
</tbody>
</table>
Common name
Diquat (E-ISO, (m) F-ISO, BSI, ANSI, WSSA, JMAF). Refers to the dication, not the salt.

Synonyms
None

Chemical name
IUPAC 1,1'-ethylene-2,2'-bipyridyldiylium dibromide
CA 6,7-dihydrodipyrido[1,2-a:2',1'-c]pyrazinediium dibromide

Structural formula

\[
\begin{array}{c}
\text{N}^+ \text{N}^+ \\
\text{Br}_2
\end{array}
\]

Empirical formula
\[\text{C}_{12}\text{H}_{12}\text{Br}_2\text{N}_2\text{ (dibromide)}\]
\[\text{C}_{12}\text{H}_{12}\text{N}_2\text{ (dication)}\]

Relative molecular mass
344.5 (dibromide)
362.5 (dibromide monohydrate)
184.2 (dication)

CAS Registry number
85-00-7 (dibromide)
6385-62-2 (dibromide monohydrate)
2764-72-9 (dication)

CIPAC number
55.303 (dibromide)
55 (dication)

Identity tests

Chemical. A green colour, following addition of alkaline sodium dithionite to a dilute aqueous solution indicates the presence of diquat.

UV spectroscopy. The UV spectrum over the range 200 to 350nm using water as a reference. The absorption maximum in the sample solution should be similar to that for the standard solution.
HPLC. Using method 55 + 56/SL/M/2.3, but omitting paraquat from the calibration solution, the relative retention time for diquat should not deviate by more than 1% from that of the calibration solution.

**DIQUAT DIBROMIDE TECHNICAL CONCENTRATE** (Note 1)

FAO specification 55.303/TK (February 2008*)

This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose name is listed in the evaluation report (55.303/2005). It should be applicable to TK produced by this manufacturer but it is not an endorsement of those products, nor a guarantee that they comply with the specifications. The specification may not be appropriate for TK produced by other manufacturers. The evaluation report (55.303/2005), as PART TWO, forms an integral part of this publication.

1 **Description**

The material shall consist of diquat dibromide, together with related manufacturing impurities, in the form of an aqueous solution (Note 1) and shall be free from visible extraneous matter and added modifying agents.

2 **Active ingredient**

2.1 **Identity tests** (55/TC/M/2, CIPAC Handbook G, p.47, 1995)

The active ingredient (diquat and bromide components, Note 2) shall comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.

2.2 **Diquat dibromide content** (55/TC/M/3, CIPAC Handbook E, p.74, 1993)

The diquat dibromide content (Note 3) shall be declared (not less than 377 g/kg or 467 g/l at 20 ± 2ºC, Note 4) and, when determined, the average measured content shall not differ from that declared by more than ± 5%.

3 **Relevant impurities**

3.1 **Free 2,2'-bipyridyl** (55/13/M/7.4, CIPAC Handbook 1A, p.1245, 1980)

Maximum: 0.75 g/kg (750 ppm).

3.2 **Total terpyridines** (Note 5)

Maximum: 0.001 g/kg (1.0 ppm).

3.3 **Ethylene dibromide** (Note 6)

Maximum: 0.01 g/kg (10 ppm).

4 **Physical properties**

4.1 **pH range** (MT 75.3, CIPAC Handbook J, p. 131, 2000)

pH range: 3.5 to 7.5.

Note 1  The product must not be allowed to come into direct contact with metal. Containers may be manufactured from suitable polymeric materials or metal and must comply with pertinent national and international transport and safety regulations. If metal is used, containers must be lined with suitable polymeric material, or the internal surfaces treated to prevent corrosion of the container and/or deterioration of the contents.

Note 2  The method for identification of bromide in technical and formulated diquat dibromide (including mixtures with paraquat dichloride) can be downloaded here.

Note 3  To calculate the diquat dibromide content, multiply the diquat ion content (as determined by CIPAC method 55/SL/M/3) by 1.87.

Note 4  If the buyer requires specification of both g/l at 20°C and g/kg, then in cases of dispute the analytical results shall be calculated as g/kg.

Note 5  The method for determination of total terpyridines in technical and formulated diquat dibromide is available from CIPAC at http://www.cipac.org/lnpub.htm.

Note 6  The method for determination of ethylene dibromide in technical and formulated diquat dibromide can be downloaded here.
FAO SPECIFICATIONS AND EVALUATIONS
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DIQUAT DIBROMIDE SOLUBLE CONCENTRATE (Notes 1 and 2)

FAO specification 55.303/SL (February 2008*)

This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose names is listed in the evaluation report (55.303/2005). It should be applicable to relevant products of this manufacturer, and those of any other formulators who use only TK from the evaluated source. The specification is not an endorsement of those products, nor a guarantee that they comply with the specification. The specification may not be appropriate for the products of other manufacturers who use TK from other sources. The evaluation report (55.303/2005), as PART TWO, forms an integral part of this publication.

1 Description
The material shall consist of technical diquat dibromide, complying with the requirements of FAO specification 55.303/TK (February 2008), in the form of an aqueous solution (Notes 1 and 2), together with any other necessary formulants. It shall contain not more than a trace of suspended matter, immiscible solvents and sediment.

2 Active ingredient
2.1 Identity tests (55/SL/M/2, CIPAC G, p.47, 1995)
The active ingredient (diquat and bromide components, Note 3) shall comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.

2.2 Diquat dibromide content (55/SL/M/3, CIPAC E, p.74, 1993, Note 4)
The diquat dibromide content (Note 5) shall be declared (g/kg and/or g/l at 20 ± 2ºC, Note 6) and, when determined, the average content measured shall not differ from that declared by more than the following tolerances.

<table>
<thead>
<tr>
<th>Declared content, g/kg or g/l at 20 ± 2ºC</th>
<th>Permitted tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 up to 100</td>
<td>± 10% of the declared content</td>
</tr>
<tr>
<td>Above 100 up to 250</td>
<td>± 6% of the declared content</td>
</tr>
<tr>
<td>Above 250 up to 500</td>
<td>± 5% of the declared content</td>
</tr>
</tbody>
</table>

Note: the upper limit is included in each range.

3 Relevant impurities
3.1 Free 2,2'-bipyridyl (55/13/M/7.4, CIPAC Handbook 1A, p.1245, 1980)
Maximum: 0.75 g/kg (750 ppm).

3.2 Total terpyridines (Note 7)

* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at: http://www.fao.org/pest-and-pesticide-management/expert-bodies-conventions/faowho-joint-meeting-on-pesticide-specifications-jmps/pesticide-specifications/en/
Maximum: 0.001 g/kg (1.0 ppm).

3.3 Ethylene dibromide (Note 8)
Maximum: 0.01 g/kg (10 ppm).

4 Physical properties

4.1 pH range (MT 75.3, CIPAC Handbook J, p. 131, 2000)
PH range 4.0 to 8.0.

4.2 Solution stability (MT 41, CIPAC Handbook F, p. 131, 1995)
The formulation, after the stability test at 54°C (see 5.2) and following dilution (Note 9) with CIPAC standard water D and standing at 30 ± 2°C for 18 h, shall give a clear or opalescent solution, free from more than a trace of sediment and visible solid particles. Any visible sediment or particles produced shall pass through a 45 µm test sieve (Note 10).

4.3 Persistent foam (MT 47.2, CIPAC Handbook F, p. 152, 1995) (Note 11)
Maximum: 60 ml after one minute.

5 Storage stability

After storage at 0 ± 2°C for 7 days, the volume of solid and/or liquid which separates shall not be more than 0.3 ml.

After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than 97%, relative to the determined average content found before storage (Note 12), and the product shall continue to comply with the clause for:
- pH range (4.1).

Note 1 FAO specifications 55/SL and 56/SL are applied to mixed SL formulations, containing both diquat and paraquat. An emetic is added to all formulations containing paraquat and the extra precautions required for handling solutions of paraquat must be observed when handling the mixed formulation. The method for determination of the emetic in technical and formulated paraquat was peer-validated in 2003 can be downloaded here.

Note 2 The product must not be allowed to come into direct contact with metal. Containers may be manufactured from suitable polymeric materials or metal and must comply with pertinent national and international transport and safety regulations. If metal is used, containers must be lined with suitable polymeric material, or the internal surfaces treated to prevent corrosion of the container and/or deterioration of the contents.

Note 3 The method for identification of bromide in technical and formulated diquat dibromide (including mixtures with paraquat dichloride) can be downloaded here.

Note 4 If the SL contains both diquat and paraquat, CIPAC method 55+56/SL/M/3 (CIPAC Handbook E, p.75, 1993) should be used for determination of active ingredient content.
Note 5  To obtain the diquat dibromide content, multiply the diquat ion content (as determined by CIPAC method 56/SL/M/3) by 1.87.

Note 6  If the buyer requires specification of both g/l at 20°C and g/kg, then in case of dispute the analytical results shall be calculated as g/kg.

Note 7  The method for determination of total terpyridines in technical and formulated diquat dibromide is available from CIPAC at http://www.cipac.org/lnpub.htm.

Note 8  The method for determination of ethylene dibromide in technical and formulated diquat dibromide can be downloaded here.

Note 9  The concentration for the test should not be higher than the highest concentration recommended for use.

Note 10  Some formulations containing additional wetter may show signs of layering and produce a trace of oily precipitate under the test conditions defined in MT 41. This is acceptable and does not affect biological efficacy or spray characteristics at normal spray dilution.

Note 11  The mass of sample used in the test should correspond to the highest concentration recommended for use.

Note 12  Samples of the product taken before and after the storage stability test should be analyzed concurrently after the test to reduce the analytical error.
## PART TWO

### EVALUATION REPORTS

**DIQUAT**

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<td></td>
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<tr>
<td></td>
<td>Supporting information</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Annex 2: references</td>
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</table>
Recommendations

The Meeting recommended the following.

(i) All existing FAO specifications (1994 and 1973) for diquat and diquat + paraquat should be withdrawn.

(ii) The specifications for diquat dibromide TK and SL, proposed by Syngenta Crop Protection AG, as amended, should be adopted by FAO, subject to acceptable validation of the analytical method for determination of terpyridines.

(iii) The manufacturer should endeavour to develop and validate analytical methods which will enable the limits for relevant impurities to be expressed on the basis of active ingredient content, instead of the whole product. As and when such improved methods become available, the specified limits for relevant impurities in diquat should be reviewed accordingly.

Appraisal

The Meeting considered data on diquat, submitted by Syngenta Crop Protection AG, for review of existing FAO specifications. Existing specifications for diquat dibromide were developed under the old FAO procedure in 1994 (TK, SL and mixed SL including paraquat dichloride, AGP:CP/341, 1996). In addition, 1973 FAO specifications for diquat apparently remained in force, including a specification for SG. Revised FAO specifications for the TK, SL and mixed diquat + paraquat SL were proposed. The data submitted were in accordance with FAO/WHO Manual (2002, 1st edition).

Diquat dibromide is no longer under patent.

Diquat dibromide is a non-volatile ionic, non-selective contact herbicide, highly soluble in water and stable at pH 5-7, with <10% loss of diquat observed over 30 days at pH 9. It is subject to slow photolysis. Diquat dication rapidly binds to soils, sediment and plant materials.

The proposer provided the Meeting with commercially confidential information on the manufacturing process for diquat dibromide and on manufacturing limits for impurities. Five batch analysis data were provided for the TK. Mass balances were high, 998-1007g per kg, with a declared minimum for diquat dibromide of 467 g/l (377 g/kg). These data were confirmed as similar to those submitted for registration in the UK.

No relevant impurities at ≥1 g/kg were identified in the TK but three impurities at <1 g/kg were consistently present and were proposed as relevant, due to their exceptional hazards. Their manufacturing limits in the TK were: 2,2'-bipyridyl,

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1 An LC-MS/MS method for determination of terpyridines was peer-validated and accepted by CIPAC in 2007 and is available at [http://www.cipac.org/lnpub.htm](http://www.cipac.org/lnpub.htm).
0.75 g/kg (0.075% or 750 ppm); ethylene dibromide, 0.01 g/kg (0.001% or 10 ppm); and total terpyridines, 0.001 g/kg (0.0001% or 1 ppm). The isomeric 4,4'-bipyridyl and total terpyridines were identified in FAO paraquat specifications (2003) as relevant, on the basis of their high relative toxicity. Paraquat is about 100 times more toxic than diquat and therefore the Meeting agreed that 2,2'-bipyridyl and total terpyridines should also be designated as relevant impurities in diquat. Ethylene dibromide is a carcinogen and the Meeting agreed that it should also be designated as a relevant impurity.

Exceptionally, the proposed limits for these relevant impurities in the TK (containing a minimum of 377 g/kg diquat dibromide) and SL (including the SL plus paraquat) were based on the whole product, not the content of active ingredient. The manufacturer explained that this was unavoidable, due to limitations in the analytical methods, which are currently unable to determine lower levels of impurities in the formulations with acceptable accuracy, although they may be present at levels up to about half the proposed limits. The Meeting acknowledged that analysis and specifications for relevant impurities in paraquat formulations are subject to the same limitations. The manufacturer stated that an LC-DAD method for determination of terpyridines in both diquat and paraquat formulations had been investigated but that efforts had subsequently focused upon LC-MS/MS. Although the work was still in progress, the sensitivity of the LC-MS/MS technique offered the potential for limits in the formulations to be based on diquat dibromide content. This was welcomed by the Meeting but it was agreed that, based on currently achievable analytical performance, the limits in both TK and SL specifications should be on a whole product basis. WHO/PCS advised that the proposed limits were acceptable and the Meeting agreed.

The analytical method for determination of diquat dibromide, in which only the dication is detected, is a full CIPAC method (CIPAC Handbook G, 1995).

The semi-quantitative method for identification of bromide, in the absence (diquat TK, SL) or presence (diquat + paraquat SL) of chloride is based on capillary electrophoresis.

The method for determination of 2,2'-bipyridyl is based on CG/FID (CIPAC 55/S/L/M/7.4, CIPAC A, p1245). The Meeting noted that the CIPAC method for bipyridyl employs packed-column GC, an old technology for which serviceable equipment no longer exists in most laboratories. This problem is often associated with older methods but, although it is expected that capillary GC would form an appropriate and preferable alternative in this case, a method employing this technique has not been peer-validated.

The method for determination of total terpyridines was successfully peer-validated for the analysis of paraquat TK. The manufacturer reported that extension of this GC-MS method to diquat (in the absence and presence of paraquat) had proven problematic and work was still in progress to develop and validate a robust method.

Ethylene dibromide is determined by capillary GC-FID and the method remained unchanged from that supplied to FAO in 1994, in support of diquat specifications

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1 An LC-MS/MS method for determination of terpyridines was peer-validated and accepted by CIPAC in 2007 and is available at [http://www.cipac.org/lnpub.htm](http://www.cipac.org/lnpub.htm).
under the old FAO procedure. Peer validation of the method, for analysis of TK and SL (with and without paraquat) was completed in 2006.

Because all paraquat formulations must contain an effective emetic, an analytical method for the emetic PP796 in diquat + paraquat SL was peer-validated\(^1\).

The physico-chemical properties, the methods for testing them and the limits proposed for the SL formulations complied with the requirements of the FAO/WHO manual (2002). The proposed specifications were in accordance with the requirements of the manual, with the following exceptions.

The proposed description clause in the SL specification differed from guideline given in the manual. The standard phrase: “...free from visible suspended matter and sediment...” was replaced by “...shall contain not more than a trace of suspended matter, immiscible solvents and sediments...”. The proposer explained that certain batches of wetters used in current formulations result in the presence of fine oily droplets but that these do not affect the sprayability or other characteristics of the product and have not resulted in complaints from customers. The Meeting questioned whether the relevant impurities (which are non-ionic) might become concentrated within these droplets and thus create unexpected risks for the user.

The proposer explained that the droplets derive from the surfactants used (a mixture of ionic and non-ionic types) and that droplet formation depends on a combination of surfactant batch and the water used. The droplets were said to be comprised mainly of the calcium salts of the ionic surfactants and the manufacturer stated that lipophilic compounds are not expected to partition into them. The Meeting accepted the modified wording of the clause.

A separate specification for diquat + paraquat SL was proposed. In general, FAO/WHO specifications are expected to apply to all products of a particular formulation type (e.g. SL), irrespective of the presence of other active ingredients. Exceptions are normally made only in cases where the ratio of active ingredients is critical for acceptable performance of the product. The exact ratio of active ingredients was not critical in this case. The proposer stated that, for the diquat + paraquat mixture, a simple diquat SL specification would not address the toxicity of paraquat and the consequent need for an emetic. The Meeting noted that anyone handling the product would only be aware that the proposed specification for a mixed formulation should be applied if the product label indicates the presence of paraquat. The existing paraquat specification incorporates appropriate handling precautions for that active ingredient. The Meeting therefore agreed that, although a separate specification for the mixed SL was not necessary, the diquat SL specification should incorporate a note, drawing attention to requirements which apply when paraquat is also present.

The Meeting noted that diquat dibromide solutions will react with many metals over a wide pH range, especially at low pH, and it was agreed that the specifications should incorporate a note to indicate that containers must be specially designed to avoid corrosion problems.

\(^1\) The method for determination of the emetic in technical and formulated paraquat was peer-validated in 2003 and is available from the Pesticide Management Group of the FAO Plant Protection Service or can be [downloaded here](#).
SUPPORTING INFORMATION
FOR
EVALUATION REPORT 55/2005
Uses

Diquat dibromide is a non-selective contact herbicide, which is absorbed by foliage with some translocation in the xylem. It is active against a broad spectrum of weed species in a wide range of agricultural applications.

Identity of the active ingredient

**Common name**
Diquat (E-ISO, (m) F-ISO, BSI, ANSI, WSSA, JMAF). Refers to the dication, not the salt.

**Synonyms**
None

**Chemical name**

*IUPAC* 1,1'-ethylene-2,2'-bipyridyldiylium dibromide

*CA* 6,7-dihydrodipyrido[1,2-a:2',1'-c]pyrazinediium dibromide

**Structural formula**

\[
\begin{array}{c}
\text{N}^+ \\
\text{N}^+ \\
\text{Br}^2 \\
\end{array}
\]

**Empirical formula**
C\(_{12}\)H\(_{12}\)Br\(_2\)N\(_2\) (dibromide)
C\(_{12}\)H\(_{12}\)N\(_2\) (dication)

**Relative molecular mass**
344.5 (dibromide)
362.5 (dibromide monohydrate)
184.2 (dication)

**CAS Registry number**
85-00-7 (dibromide)
6385-62-2 (dibromide monohydrate)
2764-72-9 (dication)

**CIPAC number**
55.303 (dibromide)
55 (dication)

**Identity tests**

Chemical. A green colour, following addition of alkaline sodium dithionite to a dilute aqueous solution indicates the presence of diquat.

UV spectroscopy. The UV spectrum over the range 200 to 350nm using water as a reference. The absorption maximum in the sample solution should be similar to that for the standard solution.
HPLC. Using method 55 + 56/SL/M/2.3, but omitting paraquat from the calibration solution, the relative retention time for diquat should not deviate by more than 1% from that of the calibration solution.


**Physical and chemical properties**

**Table 1. Physicochemical properties of pure diquat dibromide**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
<th>Purity, %</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vapour pressure</td>
<td>&lt;&lt;1 x 10^-8 kPa at 25°C.</td>
<td>100%</td>
<td>OECD 104</td>
<td>PP901/0024</td>
</tr>
<tr>
<td>Melting point</td>
<td>Decomposes before melting.</td>
<td>100%</td>
<td>OECD 102</td>
<td>PP901/0024</td>
</tr>
<tr>
<td>Boiling point</td>
<td>not applicable.</td>
<td></td>
<td>PP901/0024</td>
<td></td>
</tr>
<tr>
<td>Decomposition temperature</td>
<td>325°C.</td>
<td></td>
<td>PP901/0024</td>
<td></td>
</tr>
<tr>
<td>Solubility in water</td>
<td>718 g/l at 0°C at pH 7.2.</td>
<td>100%</td>
<td>OECD 105</td>
<td>PP901/0024</td>
</tr>
<tr>
<td>Octanol:water partition coefficient</td>
<td>log P K_{OW} = -4.6 at 20°C.</td>
<td>100%</td>
<td>OECD 107</td>
<td>PP901/0024</td>
</tr>
<tr>
<td>Hydrolysis</td>
<td>Stable under acidic, neutral and alkaline conditions. No significant decrease in concentration at pH 5 and 7, &lt;10% decrease at pH 9 after 30 days at 25°C.</td>
<td>Radio-chemical purity &gt;98% (specific activity 0.398 Gbq/mmol)</td>
<td>Analysis of sterile aqueous buffer solutions</td>
<td>PP901/0525</td>
</tr>
<tr>
<td>Photolysis</td>
<td>Environmental half-life of diquat dibromide in water under mid-European conditions calculated to be 2-210 days, depending on seasonal sunlight and depth of water</td>
<td>99.7%</td>
<td>Measurement of UV absorption and quantum yield, half-life estimated by Frank and Klöpffer model</td>
<td>PP901/0926</td>
</tr>
<tr>
<td>Dissociation characteristics</td>
<td>Diquat dibromide exists as the ionized salt. The diquat dication is not deprotonated at pH ≤14</td>
<td>-</td>
<td>Structural assessment</td>
<td>PP901/0024</td>
</tr>
</tbody>
</table>
### Table 2. Chemical composition and properties of diquat dibromide technical material (TK)

| Manufacturing process, maximum limits for impurities ≥ 1 g/kg, 5 batch analysis data | Confidential information supplied and held on file by FAO. Mass balances were 99.8–100.7% and unknowns were insignificant (total <1 g/kg). |
| Declared minimum diquat dibromide content in the TK | 467g/l (377g/kg) as diquat dibromide |
| Relevant impurities ≥1 g/kg and maximum limits for them | None |
| Relevant impurities <1 g/kg and maximum limits for them | 2,2’-bipyridyl, 0.75 g/kg (750 ppm), whole product basis; ethylene dibromide, 0.01 g/kg (10 ppm), whole product basis; total terpyridines: 0.001 g/kg (1 ppm), whole product basis |
| Stabilizers or other additives and maximum limits for them | None |

### Background information on toxicology/ecotoxicology

Diquat was reviewed by WHO and UNEP (WHO 1984), and by IPCS (IPCS 1991). The 1991 IPCS review concluded that residue levels of diquat in food and drinking-water, resulting from its normal use, are unlikely to pose a health hazard for the general population.


US EPA reregistered diquat in 1995 (USEPA 1995a, 1995b) and re-assessed its tolerances in 2002 (USEPA 2002). US EPA categorized diquat toxicity as follows. On the basis of slight to severe eye irritation: Toxicity Category II (2nd highest of 4 categories) for this effect. On the basis of slight acute toxicity by oral and inhalation routes: Toxicity Category II for these effects. On the basis of slight dermal irritation: Toxicity Category IV for this effect. It is not a skin sensitizer. Diquat was classified as a Group E carcinogen, that is, a chemical for which there is evidence of non-carcinogenicity for humans. US EPA determined that dietary food risks are not of concern.

Diquat was evaluated by the European Commission and included in Annex I of Directive 91/414/EEC (EU 2001).

The WHO hazard classification of diquat is: moderately hazardous, Class II (WHO 2002).

### Formulations

Diquat dibromide is registered and marketed in many countries throughout the world. The main formulation type is SL and diquat may be co-formulated with paraquat.

### Methods of analysis and testing

The analytical methods for diquat (including identity tests) in TC and SL are full CIPAC methods (CIPAC Handbook G, p.47, 1995). Diquat is determined by HPLC,
using UV detection at 290 nm using an internal standard. The identity test is a
colorimetric procedure, based on the green diquat free radical ion.

The relevant impurities are determined by GC/FID (2,2'-bipyridyl, CIPAC 55/SL/M/-),
GC/MS or LC-DAD (terpyridines), and capillary GC-FID or capillary GC-MS (ethylene
dibromide). The method for total terpyridines in diquat is undergoing further
development, after peer-validation studies revealed problems. The method for
ethylene dibromide was successfully peer-validated in 2006. The method for
determination of the emetic (PP796), added to paraquat products and present in the
mixed paraquat/diquat SL, is based on reversed-phase HPLC and internal
standardization with 4-nitroacetanilide. This method was also successfully peer-
validated in 2006.

Analytical method(s) for determination of other impurities were based on GC-FID and
CE.

Test methods for determination of physico-chemical properties of the technical active
ingredient were essentially OECD methods, with CIPAC procedures being used for
formulations.

Containers and packaging

It is important to prevent diquat dibromide products from coming into contact with
metals. Requirements for containers are noted in the specifications.

Expression of active ingredient

The active ingredient is expressed as diquat dibromide, in g/kg or g/l at 20 ± 2°C,
which is calculated by multiplying the determined diquat dication content by 1.87.
ANNEX 1

HAZARD SUMMARY PROVIDED BY THE PROPOSER

Note: The proposer provided written confirmation that the toxicological and ecotoxicological data included in the following summary were derived from diquat dibromide having impurity profiles similar to those referred to in Table 2, above.
Table A. Toxicology profile of technical diquat dibromide, based on acute toxicity, irritation and sensitization (all values expressed as diquat ion)

<table>
<thead>
<tr>
<th>Species</th>
<th>Test Description</th>
<th>Duration and conditions</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat, Alpk:ApfSD, m,f</td>
<td>oral</td>
<td>OECD 401, 14 d observation, purity 21.2% w/w</td>
<td>MLD = 1009 mg/kg (m), 1047 mg/kg (f), equivalent to diquat ion at 214 mg/kg (m), 222 mg/kg (f)</td>
<td>PP901/0079</td>
</tr>
<tr>
<td>Rat, Alpk:ApfSD, m,f</td>
<td>dermal</td>
<td>OECD 402, 24 h occluded, 14 d observation, purity 21.2% w/w</td>
<td>MLD &gt;2000 mg/kg (m,f), equivalent to diquat ion at 424 mg/kg</td>
<td>PP901/0080</td>
</tr>
<tr>
<td>Rat, Alpk:Ap, m,f</td>
<td>inhalation</td>
<td>OECD 403, 4 h whole body, 19.5% w/w formulation, 14 d observation, purity 19.5% w/w</td>
<td>LC₅₀ = 0.8 mg/l (m), 1.09 mg/l (f), equivalent to diquat ion at 0.121 mg/l (m), 0.132 mg/l (f)*</td>
<td>PP901/0161</td>
</tr>
<tr>
<td>Rabbit, New Zealand White, f</td>
<td>skin irritation</td>
<td>OECD 404, 4 h occluded, 240g/l SL formulation, 17 d observation, purity 19.9% w/w</td>
<td>Slight skin irritation, all signs of irritation resolved within 17 d**</td>
<td>PP901/1521</td>
</tr>
<tr>
<td>Rabbit, New Zealand White, f</td>
<td>eye irritation</td>
<td>OECD 405, 240g/l SL formulation, 10 d observation, purity 19.9% w/w</td>
<td>Mild eye irritation, all signs of irritation resolved within 10 d***</td>
<td>PP901/1519</td>
</tr>
<tr>
<td>Guinea pigs, Dunkin Hartley, f</td>
<td>skin sensitization</td>
<td>OECD 406, undiluted TK, 31% diquat dibromide, Magnusson &amp; Kligman maximization test, 24 h occluded, 48 h observation, purity 26.7% w/v</td>
<td>Skin sensitizer****</td>
<td>PP901/0078 &amp; PP901/0755</td>
</tr>
</tbody>
</table>

* Diquat dibromide is non-volatile and its formulations are not applied with equipment which generates a significant proportion (>1% w/w) of spray droplets <50 µm. Diquat dibromide is therefore unlikely to be inhaled and the results are not relevant to human risk assessment.

** According to European Commission Directive 2001/59/EC, classification is not required. Based on scores at 72 h, would be assigned to US EPA Category IV.

*** According to European Commission Directive 2001/59/EC, classification is not required. Positive effects cleared within 7 d, placing the material in US EPA Category III.

**** A 1 in 10 dilution would not trigger classification for sensitization.
Table B. Toxicology profile of technical diquat dibromide, based on repeated administration (sub-acute to chronic) (all values expressed as diquat ion)

<table>
<thead>
<tr>
<th>Species</th>
<th>Test</th>
<th>Duration and conditions</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat, Sprague Dawley m,f</td>
<td>Short-term toxicity</td>
<td>13 week dietary, purity 20.5% w/w</td>
<td>NOEL = 60 ppm, equivalent to approximately 4.7 and 5 mg diquat ion/kg bw/day, m &amp; f, respectively  LOEL = 300 ppm, equivalent to approximately 23.2 and 25.3 mg diquat ion/kg bw/day, m &amp; f, respectively</td>
<td>PP901/1387</td>
</tr>
<tr>
<td>Dog, Beagle, m,f</td>
<td>Short-term toxicity</td>
<td>1 year dietary, purity 26.7% w/v</td>
<td>NOAEL = 0.5 mg diquat ion/kg, m &amp; f  LOEL = 2.5 mg diquat ion/kg, m &amp; f</td>
<td>PP901/0116</td>
</tr>
<tr>
<td>Mouse, Alpk Swiss-derived, m,f</td>
<td>Carcinogenicity</td>
<td>2 year dietary, purity 26.7% w/v</td>
<td>Not tumorigenic or carcinogenic  NOAEL = 30ppm, equivalent to 4.2 mg diquat ion/kg bw/day, m &amp; f  LOEL = 100 ppm, equivalent to approximately 14 mg diquat ion/kg bw/day, m &amp; f</td>
<td>PP901/1435</td>
</tr>
<tr>
<td>Rat, Sprague Dawley, m,f</td>
<td>Chronic toxicity &amp; carcinogenicity</td>
<td>2 year dietary, purity 18.4% w/w</td>
<td>Not tumorigenic or carcinogenic  NOAEL = 5 ppm, equivalent to approximately 0.2 mg diquat ion/kg bw/day, m &amp; f  LOEL = 15 ppm, equivalent to approximately 0.65 mg diquat ion/kg bw/day, m &amp; f</td>
<td>PP901/0110-3</td>
</tr>
<tr>
<td>Rat, Alpk:APfSD m,f</td>
<td>Reproductive toxicity</td>
<td>2 generation, dietary, purity 26.7% w/v</td>
<td>No significant effect on reproductive parameters  NOAEL (parental) = 16 ppm, equivalent to approximately 1.4 mg diquat ion/kg bw/day  NOAEL (reproductive effects) = 240/400 ppm, equivalent to approximately 22-32 mg diquat ion/kg bw /day</td>
<td>PP901/0121</td>
</tr>
<tr>
<td>Rat, Alpk:APfSD, m,f</td>
<td>Developmental toxicity</td>
<td>Gavage, purity 26.2% w/v</td>
<td>Not teratogenic  NOAEL (teratogenicity) = 40 mg diquat ion/kg  NOEL (maternal and developmental toxicity) = 4 mg diquat ion/kg bw/day</td>
<td>PP901/0136</td>
</tr>
<tr>
<td>Rabbit, New Zealand White, m,f</td>
<td>Developmental toxicity</td>
<td>Gavage, purity 26.2% w/v</td>
<td>Not teratogenic  NOAEL (teratogenicity) = 40 mg diquat ion/kg  NOEL (maternal toxicity) = 1 mg diquat ion/kg bw/day  NOAEL (developmental toxicity) = 3 mg diquat ion/kg bw/day</td>
<td>PP901/0130</td>
</tr>
</tbody>
</table>
### Table C. Mutagenicity profile of technical diquat dibromide, based on *in vitro* and *in vivo* tests (all values expressed as diquat ion)

<table>
<thead>
<tr>
<th>Species</th>
<th>Test</th>
<th>Conditions</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. typhimurium</em> and <em>E.coli</em></td>
<td>Bacterial gene mutation, OECD 471, <em>in vitro</em></td>
<td>Doses not stated, purity 25.8% w/w</td>
<td>Negative ± S9, cytotoxicity at ≥100 µg/plate</td>
<td>PP901/0140</td>
</tr>
<tr>
<td>Mouse lymphocytes (L5178Y)</td>
<td>OECD 476, mouse lymphoma assay, <em>in vitro</em></td>
<td>Doses not stated, purity 25.8% w/w</td>
<td>Equivocal ± S9, cytotoxicity at highest concentrations</td>
<td>PP901/0143</td>
</tr>
<tr>
<td>Human lymphocytes</td>
<td>OECD 473, cytogenetic study, <em>in vitro</em></td>
<td>Doses not stated, purity 53.5% w/w</td>
<td>Positive ± S9 but only at cytotoxic doses</td>
<td>PP901/0139</td>
</tr>
<tr>
<td>Mouse somatic cells</td>
<td>OECD 474, micronucleus test, <em>in vivo</em></td>
<td>Oral doses up to 100 mg/kg, purity 25.8% w/w</td>
<td>Negative</td>
<td>PP901/0141</td>
</tr>
<tr>
<td>Rat somatic cells</td>
<td>Liver UDS assay, <em>in vivo</em></td>
<td>Oral doses up to 900 mg/kg bw, purity 25.8% w/w</td>
<td>Negative. Evidence of toxicity to hepatocytes</td>
<td>PP901/0145</td>
</tr>
<tr>
<td>Mouse germ cells</td>
<td>Dominant lethal, <em>in vivo</em></td>
<td>Doses up to 10 mg/kg bw/day, purity 28.6% w/v</td>
<td>Negative.</td>
<td>PP901/0137</td>
</tr>
</tbody>
</table>

### Table D. Ecotoxicology profile of technical diquat dibromide (all values expressed as diquat ion)

<table>
<thead>
<tr>
<th>Species</th>
<th>Test</th>
<th>Duration and conditions</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Daphnia magna</em>, water flea</td>
<td>Acute toxicity</td>
<td>EPA-660/3-75-009, static system, 17 ± 1.5°C, 48 h duration, purity 46.6% w/w</td>
<td>EC₅₀ (24 h) = 2.2 mg/l EC₅₀ (48 h) = 1.2 mg/l</td>
<td>PP901/0563</td>
</tr>
<tr>
<td><em>Daphnia magna</em>, water flea</td>
<td>Chronic toxicity</td>
<td>21 d exposure, based on OECD guideline 202, modified by individually separating the <em>Daphnia</em>, static system, growth and reproduction monitored, purity 27.4% w/v</td>
<td>NOEC = 0.125 mg/l</td>
<td>PP901/0566</td>
</tr>
<tr>
<td><em>Oncorhynchus mykiss</em>, rainbow trout</td>
<td>Acute toxicity</td>
<td>EEC Method C1, Static system at 16°C, purity 26.8% w/v</td>
<td>24, 48, 72 and 96 h LC₅₀ = 69, 27, 23 and 21 mg/l, respectively 96-h NOEC = 6.7 mg/l</td>
<td>PP901/0970</td>
</tr>
<tr>
<td><em>Cyprinus carpio</em>, mirror carp</td>
<td>Acute toxicity</td>
<td>OECD 203, static system at 23°C, purity 26.8% w/v</td>
<td>24, 48, 72 and 96 h LC₅₀ = 285, 143, 91 and 67 mg/l, respectively 96 hour NOEC =14 mg/l</td>
<td>PP901/0972</td>
</tr>
</tbody>
</table>
### Table D. Ecotoxicology profile of technical diquat dibromide (all values expressed as diquat ion)

<table>
<thead>
<tr>
<th>Species</th>
<th>Test</th>
<th>Duration and conditions</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oncorhynchus mykiss</em>, rainbow trout</td>
<td>Chronic toxicity</td>
<td>21 d, fish juvenile growth test, based on OECD Method 204, with the exposure period extended to 21 d, broadly in agreement with draft OECD guideline 'Fish, juvenile growth test - 28 days', except exposure was for 21 d; flow-through system at 15ºC, purity 26.2% w/v</td>
<td>NOEC = 1.4 mg/l</td>
<td>PP901/0559</td>
</tr>
<tr>
<td><em>Selenastrum capricornutum</em>, green alga</td>
<td>Effect on growth</td>
<td>Based on OECD guideline 201 but with extension of exposure period to 96 h, static system at 24ºC, biomass and growth rate observed, purity 26.8% w/v</td>
<td><strong>E₅₀C₅₀</strong> = 0.011 mg/l, <strong>E₉₀C₅₀</strong> = 0.019 mg/l, NOEC = 0.0068 mg/l</td>
<td>PP901/0572</td>
</tr>
<tr>
<td><em>Eisenia fetida</em>, earthworm</td>
<td>Acute toxicity</td>
<td>Lab study in artificial soil, 200 g/l SL, based on OECD guideline 207 and EEC guideline C(L1)4, purity 20.3% w/v</td>
<td>14-d <strong>LC₅₀</strong> = 130 mg/kg dry soil</td>
<td>PP901/0556</td>
</tr>
<tr>
<td><em>Apis mellifera</em> (honey bee)</td>
<td>Acute oral toxicity</td>
<td>Based on UK Data Requirements for Approval, COPR Working Document D3 (revised 1979), consistent with EPPO guideline 170, controlled environment at 24-26ºC, purity 20.1% w/v</td>
<td>24, 48, 72, 96 and 120 h <strong>LD₅₀</strong> = 139, 67, 23, 14 and 12 µg/bee, respectively</td>
<td>PP901/0542</td>
</tr>
<tr>
<td><em>Apis mellifera</em> (honey bee)</td>
<td>Acute contact toxicity</td>
<td>Based on UK Data Requirements for Approval, COPR Working Document D3 (revised 1979), consistent with EPPO guideline 170, controlled environment at 24-26ºC, purity 20.1% w/v</td>
<td>24, 48, 72, 96 and 120 h <strong>LD₅₀</strong> = 120, 77, 46, 27 and 14 µg/bee, respectively</td>
<td>PP901/0542</td>
</tr>
<tr>
<td><em>Perdix perdix</em>, partridge</td>
<td>Acute toxicity</td>
<td>Oral intubation in distilled water, 14 d observation, USEPA guideline G 163.71-1, purity 53.7% w/w</td>
<td><strong>LD₅₀</strong> = 158 mg/kg bw</td>
<td>PP901/0534</td>
</tr>
<tr>
<td><em>Anas platyrhynchos</em>, mallard duck</td>
<td>Acute toxicity</td>
<td>Oral intubation in propylene glycol, 14 d observation, USEPA guideline G 163.71-1, purity 45.6% w/w</td>
<td><strong>LD₅₀</strong> = 83 mg/kg bw</td>
<td>PP901/0536</td>
</tr>
<tr>
<td><em>Colinus virginianus</em>, bobwhite quail</td>
<td>Short-term toxicity</td>
<td>5 d treatment, 3 d observation, similar to USEPA guideline 71-2, purity not specified</td>
<td><strong>LC₅₀</strong> = 1570 mg/kg diet</td>
<td>PP901/1891</td>
</tr>
<tr>
<td><em>Anas platyrhynchos</em>, mallard duck</td>
<td>Short-term toxicity</td>
<td>5 d treatment, 3 d observation, similar to USEPA guideline 71-2, purity not specified</td>
<td><strong>LC₅₀</strong> &gt;2677 mg/kg diet</td>
<td>PP901/1891</td>
</tr>
</tbody>
</table>
### Table D. Ecotoxicology profile of technical diquat dibromide (all values expressed as diquat ion)

<table>
<thead>
<tr>
<th>Species</th>
<th>Test</th>
<th>Duration and conditions</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coturnix japonica, Japanese quail</td>
<td>Short-term toxicity</td>
<td>5 d treatment, 3 d observation, similar to USEPA guideline 71-2, purity not specified</td>
<td>LC50 = 721 mg/kg diet</td>
<td>PP901/1891</td>
</tr>
<tr>
<td>Phasianus colchicus, ring-necked pheasant</td>
<td>Short-term toxicity</td>
<td>5 d treatment, 3 d observation, similar to USEPA guideline 71-2, purity not specified</td>
<td>LC50 = 2004 mg/kg diet</td>
<td>PP901/1891</td>
</tr>
<tr>
<td>Colinus virginianus, bobwhite quail</td>
<td>Reproductive toxicity</td>
<td>18 week dietary, egg-laying and collection started after 10 weeks on treated diet and lasted 8 weeks; based on USEPA guideline 71-4 and OECD 206, purity 19.6% w/w</td>
<td>NOEC (toxicity and reproduction) = 100 mg/kg diet</td>
<td>PP901/0538</td>
</tr>
<tr>
<td>Anas platyrhynchos, mallard duck</td>
<td>Reproductive toxicity</td>
<td>3 weeks pre egg-laying plus 6 weeks post egg-laying exposure, dietary treatment, egg-collection lasted for 9 weeks; based on USEPA guideline 71-4, and OECD 206, purity 22.7% w/w</td>
<td>NOEC (reproduction) = 20 mg/kg diet</td>
<td>PP901/1436</td>
</tr>
</tbody>
</table>
## Annex 2. References

<table>
<thead>
<tr>
<th>Syngenta document No. or other reference</th>
<th>Year and title of report or publication details</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP901/0078 &amp; PP901/0755</td>
<td>1990. Diquat: Skin Sensitisation To The Guinea Pig.</td>
</tr>
<tr>
<td>PP901/0079</td>
<td>1990. Diquat Dibromide: Acute Oral Toxicity To The Rat.</td>
</tr>
<tr>
<td>PP901/0080</td>
<td>1995. Diquat Dibromide: Acute Dermal Toxicity To The Rat.</td>
</tr>
<tr>
<td>PP901/0110-3 &amp; PP901/0775</td>
<td>1985. Diquat Dibromide: Evaluation Of Potential Carcinogenicity And Chronic Toxicity By Prolonged Dietary Administration To Rats.</td>
</tr>
<tr>
<td>PP901/0116</td>
<td>1990. Diquat: 1 Year Feeding Study In Dogs.</td>
</tr>
<tr>
<td>PP901/0121</td>
<td>1990. Diquat: Multigeneration Study In The Rat.</td>
</tr>
<tr>
<td>PP901/0556</td>
<td>1993. Diquat: Toxicity to the Earthworm Eisenia foetida of a 200 g Litre⁻¹ Soluble Concentrate</td>
</tr>
<tr>
<td>PP901/0563</td>
<td>1978. 48 Hour Acute Static Toxicity of Diquat Dibromide (SX958) to 1st stage Nymph Water Fleas (Daphnia magna Straus)</td>
</tr>
<tr>
<td>PP901/1436</td>
<td>2004. Diquat: An egg production study with the mallard.</td>
</tr>
<tr>
<td>Syngenta document No. or other reference</td>
<td>Year and title of report or publication details</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>PP901/1891</td>
<td>1975. Lethal Dietary Toxicities of Environmental Pollutants to Birds.</td>
</tr>
</tbody>
</table>
PARAQUAT DICHLORIDE AND DIQUAT DIBROMIDE

Semi-Quantitative Determination and Identity Test for Chloride and Bromide Anions in Paraquat Dichloride Technical and Formulation, Diquat Dibromide Technical and Formulation, and Paraquat Dichloride and Diquat Dibromide Formulation Mixtures

OUTLINE OF METHOD
The weight percent content of chloride anion and bromide anion can be estimated in bipyridinium samples by capillary electrophoresis (CE), using an external standard procedure. The key parameters of this method include the use of an Agilent polyvinyl alcohol (PVA) coated capillary column with indirect ultra-violet (UV) detection and calculation of the chloride anion and bromide anion contents with reference to standards.

The identity of chloride anion and bromide anion can be established by using the CE instrument to fortify samples with calibration solutions.

REAGENTS
0.1N Orthophosphoric acid, aqueous diluted from HPLC grade Orthophosphoric acid (ex Fluka)
Sodium chromate, 98% (ex Aldrich)
Water, purified conforming to ASTM type II
Sodium chloride, standard of known purity
Potassium Bromide, standard of known purity

Electrolyte preparation
Prepare a solution containing approximately 20mM sodium chromate at pH 8.0. For example, to prepare 1 litre: Weigh approximately 3.2g of sodium chromate into a 1 litre plastic bottle. Add 1 litre of ASTM Type II water. Sonicate until completely dissolved and mix thoroughly. Adjust the pH of the electrolyte by gradually adding 0.1N Orthophosphoric acid until pH 8.0 is obtained. Filter the solution using a 0.45μm membrane filter. Degas the electrolyte before use.

Calibration solutions – preparation of solutions for injection
For chloride anion determination or identification: Weigh approximately 40 mg Sodium Chloride standard (WCALCl mg) into a glass bottle (60 ml). Add 50 ml ASTM Type II and shake the bottle until all the sodium chloride has dissolved. Call this solution CCl. Transfer approximately 200μl (using e.g. Gilson Microman pipette) of solution CCl to a CE autosampler vial.

For bromide anion determination or identification: Weigh approximately 50 mg Potassium Bromide standard (WCALBr mg) into a glass bottle (60 ml). Add 50 ml ASTM Type II and shake the bottle until all the potassium bromide has dissolved. Call this solution CBr. Transfer approximately 200μl (using e.g. Gilson Microman pipette) of solution CBr to a CE autosampler vial.

These are the solutions to be injected as calibration solutions, if a semi-quantitative analysis is required. In addition, they can be used as fortifying solutions if an identity test is required.
APPARATUS

The apparatus listed below was that used to establish the method. Consideration must be given to confirmation of the method on other makes of equipment, providing equivalent performance, to ensure that they are suitable.

Capillary Electrophoresis Instrument equipped with a diode array detector (DAD)
Capillary Column 64.5 cm x 50µm (effective length 56 cm) fused silica capillary coated with PVA
Data system

PROCEDURE

(a) Chromatographic conditions (typical)

Capillary 64.5 cm x 50µm (effective length 56 cm) fused silica capillary coated with PVA ex Agilent part no.: G1600-61219. Green interface required for Agilent 3D CE

Capillary Temperature 20°C

Electrolyte Replenishment For quantitative, reproducible results, replenishment of electrolyte vials is recommended prior to each injection. This can be done by filling vials manually or by using the Replenishment capability, if the instrument can do this. For example:

<table>
<thead>
<tr>
<th>Function</th>
<th>cm</th>
<th>Vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>REPLENISH</td>
<td>1.8</td>
<td>InHomeVial</td>
</tr>
<tr>
<td>REPLENISH</td>
<td>1.8</td>
<td>OutHomeVial</td>
</tr>
</tbody>
</table>

Capillary Preconditioning For quantitative, reproducible results, capillary preconditioning is required. An example of the typical entries in the capillary preconditioning table is shown below:

<table>
<thead>
<tr>
<th>Function</th>
<th>Time</th>
<th>Inlet</th>
<th>Outlet</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLUSH</td>
<td>2.00 min</td>
<td>I : InHomeVial</td>
<td>O : 48: Waste</td>
</tr>
</tbody>
</table>
Injection Parameters

For semi-quantitative analyses use the following:

<table>
<thead>
<tr>
<th>Vial</th>
<th>Pressure (mbar)</th>
<th>Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration/Sample</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>I : InHomeVial</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

Where identification of an anion is required use the following:

<table>
<thead>
<tr>
<th>Vial</th>
<th>Pressure (mbar)</th>
<th>Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Calibration (chloride or bromide anion)</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

Electrical Parameters

Negative Polarity

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Voltage (kV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.2</td>
<td>25</td>
</tr>
</tbody>
</table>

This voltage will generate a current of approximately minus 30-36 µA

Run Time

5 minutes

Detector Settings

Wavelength  500 nm, Bandwidth 60 nm
Reference  374 nm, Bandwidth 30 nm

Retention times

Bromide anion approximately 3.4 min
Chloride anion approximately 3.5 min

(b) Equilibration

Inject portions of the CA or CB solution and check that two consecutive electropherograms are similar to the typical electropherograms shown at the end of the method.
(c) Preparation of sample
WARNING: Bipyridinium salts and their solutions are highly poisonous. Bipyridinium salt solutions are corrosive if splashed in the eyes and harmful if allowed to contact the skin or open cuts. Wear eye protection and protective gloves when handling bipyridinium salts, aqueous concentrates and formulated materials.

Weigh approximately 200-250 mg (200µl) of sample (WS mg) into a bottle (60 ml). Add 50 ml ASTM Type II water and mix well. Call this sample solution S. Transfer approximately 200µl (using e.g. Gilson Microman pipette) of each sample solution S to separate CE autosampler vials.

(d) Determination
Make at least duplicate injections of each calibration and sample in the following sequence:

CCl, S, CCl, S …for chloride anion analyses and
CBr, S, CBr, S …for bromide anion analyses

Measure time corrected peak areas in calibration and sample solution injections. The limit of determination for this method is approximately 0.1% w/w for chloride anion and 0.2% w/w for bromide anion in bipyridinium samples.

Confirmation of identity can be established by fortification using the CE instrument. Fortification is achieved by loading a portion of sample solution S onto the capillary followed by a portion of CCl or CBr calibration solution. This can be done by using the appropriate CE instrument injection parameters described earlier in this method. On comparison of an electropherogram resulting from an injection made without fortification with an electropherogram resulting from an injection with fortification, the identity of the anion will be confirmed if the anion peak in question is greater in size for the fortified injection electropherogram and there is an absence of an additional peak.

(e) Calculation
When not calculated by a data system, time corrected peak areas can be calculated as follows:

\[
\text{Time corrected peak area} = \frac{\text{Peak area}}{\text{Migration time (s)}}
\]

The following calculation is appropriate for the semi-quantitative determination of chloride anion content using the weights and dilutions of standard and samples specified in this method:

For each sample injection solution, the percentage chloride anion content (w/w) is:

\[
\% \text{ w/w} = \frac{A' \times P \times 0.607 \times WCALCl \times 100}{A \times WS \times 100}
\]

where:

A' = Time corrected peak area of chloride anion in the sample solution injection
P = Purity of the Sodium Chloride Standard (% w/w)
0.607 = Factor for the proportion of chloride anion in the standard
WCALCl = Weight taken of Sodium Chloride Standard (mg)
A = Time corrected peak area of chloride anion in the calibration solution injection
WS = Weight taken of sample (mg)

The following calculation is appropriate for the semi-quantitative determination of bromide anion content using the weights and dilutions of standard and samples specified in this method:

For each sample injection solution, the percentage bromide anion content (w/w) is:

\[
\% \text{ w/w} = \frac{A' \times P \times 0.671 \times WCALBr \times 100}{A \times WS \times 100}
\]

where:

A' = Time corrected peak area of bromide anion in the sample solution injection
P = Purity of the Potassium Bromide Standard (% w/w)
0.671 = Factor for the proportion of bromide anion in the standard
WCALBr = Weight taken of Potassium Bromide Standard (mg)
A = Time corrected peak area of bromide anion in the calibration solution injection
WS = Weight taken of sample (mg)

Typical Electropherogram Of Sodium Chloride Calibration Solution
Typical Electropherogram Of Potassium Bromide Calibration Solution

Typical Electropherogram Of Paraquat Dichloride and Diquat Dibromide Formulation Mixture Solution
The Determination of Ethylene Dibromide in Diquat Dibromide and Diquat Dibromide / Paraquat Dichloride SL Formulations

OUTLINE OF METHOD
This gas chromatography/mass spectrometry (GC/MS) method provides for the mg/kg determination of ethylene dibromide using an internal standard procedure. A portion of sample is extracted with iso-hexane and subsequently examined by capillary gas chromatography/mass spectrometry. The key parameters of the method include split injection on a HP-5 MS fused silica capillary column, a temperature programme with selected ion monitoring mass spectrometric detection and calculation of the ethylene dibromide with reference to standards. Where possible, to avoid volatilisation of ethylene dibromide, open sample bottles or containers for the first time when carrying out the sample preparation.

REAGENTS
Iso-hexane, HPLC grade (ex Rathburn Chemicals Ltd)
Dodecane, >99% (ex Sigma-Aldrich)
Ethylene dibromide, high purity material used as an impurity reference standard >99% (ex Sigma-Aldrich)

Preparation of Internal Standard (IS) Solution
Prepare a solution containing approximately between $1.25 \times 10^{-3}$ and $1.5 \times 10^{-3}$ mg/ml dodecane internal standard in iso-hexane. For example:

Weigh 25 to 30mg (38µl) of dodecane into a 100ml volumetric flask. Add 40ml iso-hexane and swirl to dissolve, make up to the mark with iso-hexane and shake well. Call this solution IS stock solution.

Transfer 0.5ml of IS stock solution to an appropriate glass bottle using a glass bulb pipette and add 100ml iso-hexane and mix well. Call this IS solution.

Calibration solutions – preparation of solutions for injection
WARNING: ETHYLENE DIBROMIDE and its solutions are highly toxic by inhalation, ingestion and if absorbed through the skin. It is also carcinogenic and teratogenic. Wear eye protection and protective gloves when handling the liquid and its solutions. The pure material and its solutions should only be handled in a fume cupboard.

Prepare a series of calibration solutions containing IS solution, which are equivalent to 20, 15, 10, 5 and 1 mg/kg ethylene dibromide in the sample. This can be achieved by preparing a series of calibration solutions with nominal concentrations $5 \times 10^{-3}$, $3.75 \times 10^{-3}$, $2.5 \times 10^{-3}$, $1.25 \times 10^{-3}$ and $0.25 \times 10^{-3}$ mg/ml of ethylene dibromide in iso-hexane. See summary table.
### Calibration Solution

<table>
<thead>
<tr>
<th>Calibration Solution</th>
<th>Nominal Amount EDB present in final calibration solution (mg)</th>
<th>Nominal Concentration (mg/ml)</th>
<th>Sample Equivalent Concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>0.0125</td>
<td>$1.25 \times 10^{-3}$</td>
<td>5</td>
</tr>
<tr>
<td>C2</td>
<td>0.05</td>
<td>$5.0 \times 10^{-3}$</td>
<td>20</td>
</tr>
<tr>
<td>C3</td>
<td>0.0025</td>
<td>$2.5 \times 10^{-4}$</td>
<td>1</td>
</tr>
<tr>
<td>C4</td>
<td>0.025</td>
<td>$2.5 \times 10^{-3}$</td>
<td>10</td>
</tr>
<tr>
<td>C5</td>
<td>0.0375</td>
<td>$3.75 \times 10^{-3}$</td>
<td>15</td>
</tr>
</tbody>
</table>

A suggested calibration preparation schematic is shown in Figure 1. For example:

Weigh accurately, in duplicate, approximately 50mg (25µl) of ethylene dibromide impurity reference standard into a 100ml volumetric flask. Add 40ml of iso-hexane, stopper tightly and swirl to dissolve. Make up to the mark with iso-hexane and mix well. Call these solutions A1 & A2.

To prepare B1: To a 25ml volumetric flask containing approximately 10ml iso-hexane, transfer 5.0ml of solution A1. Make up to the mark with iso-hexane and mix well. Call this solution B1.

To prepare B2: To a 25ml volumetric flask containing approximately 10ml iso-hexane, transfer 1.0ml of solution A2. Make up to the mark with iso-hexane and mix well. Call this solution B2.

To prepare C1 (5mg/kg ethylene dibromide equivalent), nominal concentration $1.25 \times 10^{-3}$ mg/ml: To a 14ml trident vial (with a tight fitting lid) add 10.0 ml of IS solution. Transfer 125µl of solution B1 to the vial using a suitable syringe or pipettor and mix well. Call this solution C1.

To prepare C2 (20mg/kg ethylene dibromide equivalent), nominal concentration $5.0 \times 10^{-3}$ mg/ml: To a 14ml trident vial (with a tight fitting lid) add 10.0 ml of IS solution. Transfer 100µl of solution A1 to the vial using a suitable syringe or pipettor and mix well. Call this solution C2.

To prepare C3 (1mg/kg ethylene dibromide equivalent), nominal concentration $2.5 \times 10^{-4}$ mg/ml: To a 14ml trident vial (with a tight fitting lid) add 10.0 ml of IS solution. Transfer 125µl of solution B2 to the vial using a suitable syringe or pipettor and mix well. Call this solution C3.

To prepare C4 (10mg/kg ethylene dibromide equivalent), nominal concentration $2.5 \times 10^{-3}$ mg/ml: To a 14ml trident vial (with a tight fitting lid) add 10.0 ml of IS solution. Transfer 50µl of solution A2 to the vial using a suitable syringe or pipettor and mix well. Call this solution C4.
To prepare C5 (15mg/kg ethylene dibromide equivalent), nominal concentration $3.75 \times 10^{-3}$ mg/ml:
To a 14ml trident vial (with a tight fitting lid) add 10.0 ml of IS solution. Transfer 75µl of solution A2 to the vial using a suitable syringe or pipettor and mix well. Call this solution C5.
Solutions to inject are C1, C2, C3, C4 & C5.

Figure 1: Suggested Calibration Preparation Schematic

50mg Std

A1

5.0 ml

25ml vol. flask

B1

100 µl

125µl

C1

10.0 ml IS ≡ 5mg/kg

50mg Std

A2

1.0ml

25ml vol. flask

B2

75µl

50µl

C2

10.0 ml IS ≡ 20mg/kg

C3

10.0 ml IS ≡ 1mg/kg

C4

10.0 ml IS ≡ 10mg/kg

C5

10.0 ml IS ≡ 15mg/kg
APPARATUS

*The apparatus listed below was that used to establish the method. Consideration must be given to confirmation of the method on other makes of equipment, providing equivalent performance, to ensure that they are suitable.*

**Gas Chromatography / Mass Spectrometry Instrument**

*Column* 30 m x 0.25 mm ID fused silica crosslinked 5% (phenyl)-methyl polysiloxane (0.25 µm film thickness) HP-5 MS column

**Data system**

**PROCEDURE**

(a) Chromatographic conditions (typical)

**Instrument**  
Hewlett Packard HP6890, equipped with split/splitless injection system and a 5973 mass spectrometric detection, operated in split mode, with automatic injector.

**Injection Liner**  
Straight silica liner (4mm ID) packed with silanised fused silica wool plug (ex. Restek cat No. 20913). Contaminated split injection liners should be treated as follows:-

Soak in Decon Neutracon (10% solution) for approximately 1 hour, then wash in purified water and dry in an oven at 120 °C. Take the liner while still warm and soak for 5 minutes in a 5% solution of dimethylchlorosilane (DMCS) in hexane. Remove the liner and soak in fresh dry methanol for 1 hour. Wash the liner with acetone and dry thoroughly. The liner is ready for packing with silanised fused silica wool.

**Column**  
30 m x 0.25 mm ID fused silica crosslinked 5% (phenyl)-methyl polysiloxane (0.25 µm film thickness) HP-5 MS column (ex. J & W, cat no. 19091S-433). Maximum operating temperature 350ºC.

It is recommended that a column is dedicated to the analysis of diquat formulations.

**Injection Syringe**  
10µl gas tight syringe with Teflon-tip plunger (ex Agilent Technologies p/n 5183-4730)

**Injection Load**  
3µl

**Pre Injection Washes**  
x 5 Solvent A (iso-hexane)

**Post Injection Washes**  
x 5 Solvent B (iso-hexane)
**Oven Temperature**

Initial temperature: 60 °C

Initial time: 0 min

Programme rate 1: 20 °C/min

Temperature 1: 160 °C

Time 1: 0 min

Programme rate 2: 35 °C/min

Final Temperature: 300 °C

Final Time: 0 min

**Run Time**

9 minutes

**Injection Port Temperature**

250 °C

**Carrier Gas**

Helium

All gases should be purified through molecular sieves. The carrier gas should be further purified through an oxygen trap.

**Constant Flow Mode**

Average linear velocity: 34 cm/sec (at 60 °C)

Flow: 0.9 ml/min

Initial pressure: 6.5 psi

Split ratio: 50:1

Split flow: 42.8 ml/min

**Data Acquisition**

Chemstation Software

(b) **Mass spectroscopy parameters** (typical)

**Ionisation mode**

EI+

**Mode Type**

Selected Ion Monitoring (SIM)

**Tune File**

ATUNE

**EM absolute**

False

**EM offset**

0

**Resulting EM Voltage**

1518

**MS Transfer Line Temperature**

320°C

**MS Quad Temperature**

150°C
MS Source Temperature  230°C

Solvent Delay  1.60 min

*resulting EM voltage during method development.

All other parameters appropriate to the instrument’s operational and tuning requirements.

SIM Parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>Components</th>
<th>Retention Window (mins)</th>
<th>Resolution</th>
<th>Mass</th>
<th>Dwell Time (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethylene Dibromide</td>
<td>1.60-3.0</td>
<td>Low</td>
<td>107.0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>109.0</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>IS</td>
<td>3.0-9.0*</td>
<td>Low</td>
<td>57.0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>85.0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>170.0</td>
<td>100</td>
</tr>
</tbody>
</table>

* The end of the retention time window for the IS should correlate to the IS retention time plus approximately 1 minute. No acquisition is required after this time. For example, if the IS elutes at 4.8 minutes, add 1 minute and stop acquiring at 5.8 minutes.

(c) Equilibration

It is important to note that iso-hexane should be used in the wash vials on the GC instrument.

The analytical system should be demonstrated to be sufficiently sensitive to detect ethylene dibromide in a sample equivalent to 1 mg/kg. This can be achieved for example, using a calibration solution with a nominal concentration of $2.5 \times 10^{-4}$ mg/ml ethylene dibromide, which may contain internal standard. For example, calibration solution C3, prepared as described earlier in this method can be used.

Perform replicate injections of calibration solution C3 to equilibrate the system. Measure the retention times for ethylene dibromide (nominally between 1.6 and 3.0 minutes) and internal standard (nominally between 3.0 and 9.0 minutes). If the EDB peak retention time is not within the quoted time window, then consider checking or even replacing the column. If the column and the instrument are not at fault then consider adjusting the flow rate to obtain an EDB peak retention time that it is within the quoted time window.
(c) Preparation of sample

**WARNING:** BIPYRIDILIUM SALTS and their solutions are toxic, particularly by inhalation of dusts. Bipyridilium salt solutions are an irritant if splashed in the eyes and harmful if allowed to contact the skin or open cuts. Wear eye protection and protective gloves when handling bipyridilium salts, aqueous concentrates and formulated materials.

**IMPORTANT:** The extraction vial caps and GC vial cap septa used for this work must be coated with PTFE or a similar inert material. Contaminants leach from soft plastic such as polyethylene and polypropylene causing interference and non reproducible results. Plastic vials/inserts must not be used.

Wherever possible, only open sample bottles just prior to analysis to avoid volatilisation of ethylene dibromide.

Weigh accurately, in duplicate, 2ml Diquat sample into a 14ml glass screw top (trident) vial and add 4.0ml of IS solution. Cap tightly and shake vigorously for ~10-20 seconds. Allow the sample to stand until two layers form. Using a Pasteur pipette transfer as much of the top iso-hexane layer as possible to another glass screw top vial and cap tightly taking care not to transfer any of the lower aqueous layer. The aqueous layer may vary in colour depending on the formulation, for example brown, blue etc.

Repeat this extraction with 4ml of iso-hexane transferring as much of the top iso-hexane layer as possible to the vial containing the first extract and mix well. Call this solution $S$.

(d) Determination

Ensure the mass spectrometer has been tuned and mass calibrated according to relevant SOPs or procedures prior to analysis.

Perform replicate injections of calibration solutions C1, C2, C3, C4 and C5 and sample solutions, S, following the SIM conditions detailed earlier in this method. A minimum of three injections per sample solution and two injections for each standard solution should be made in any one sequence.

Calibration solution injections should encompass the sample solution injections, with a maximum of two sample solutions between calibration injections, and if possible, calibration and sample solutions should be injected alternately.

Example of an injection sequence for one sample in the sequence:

C1, C2, C3, S1, C4, S1, C5, S1, C1, S2, C2, S2, C3, S2, C4, S2, C5

or

C1, C2, C3, S1, S1, C4, C5, S1, S2, C1, C2, S2, S2, C3, C4, C5.

Where $S_1$ is the solution resulting from the first weighing of a sample and $S_2$ is from the second weighing.
For a sequence containing two or more samples, use the following as an example (in this case 3 samples indicated by subscripts on the S1 & S2):

C1, S11, S11, C2, S11, S21, C3, S21, C4, S21, C5, C1, S11, S12, C2, S12, S22, C3, S22, C4, S22, C5, C1, S13, S13, C2, S13, S23, C3, S23, C4, S23, C5

Solutions C1, C2, C3, C4 and C5 are used to prepare a five level calibration curve to which a quadratic equation is assigned. For a long sequence, it is advisable that each sample (total of 6 injections of S1 + S2) is quantified using its corresponding calibration curve i.e. one calibration curve is generated for each sample. In the case of only analysing one sample in a sequence, the average of the duplicate calibration injections (see above sequence) should be used for quantification. A graph showing a typical ethylene dibromide calibration curve is shown below.

**Graph Showing Typical Calibration Solution Response**

![Graph showing typical calibration solution response](image-url)

Measure the peak areas for ethylene dibromide and the IS from the total ion chromatograms and calculate the mg/kg of ethylene dibromide in the samples.

Typical total ion chromatograms and mass spectra obtained for calibration and sample solutions are shown towards the end of this method.

If the column performance deteriorates substantially during use, check the condition of the split injection liner and replace if necessary. If column performance is not improved by changes to the liner, consider replacing the column.

**Note:** When analysing new formulation types, or when troubleshooting, the accuracy of the method may be checked following the procedure documented in appendix 1.
(e) Calculation

These calculations were used during method development. Other forms of these calculations may be used in data systems and should give comparable results.

It is recommended that results be calculated using data handling software. Alternatively, if results need to be calculated without this, an example follows on how to do this using Microsoft Excel 2003. Any spreadsheets constructed for calculation of results should be thoroughly checked (verified) prior to use.

Calculate the Peak Area Ratio, PAR, for the ethylene dibromide peak in each of the calibration solutions C1, C2, C3, C4 and C5 as follows:

\[
\text{PAR}_{\text{Standard}} = \frac{A_{\text{Standard}}}{I_{\text{Standard}}}
\]

Where

\[
A_{\text{Standard}} = \text{Peak area of ethylene dibromide in the calibration solution.}
\]

\[
I_{\text{Standard}} = \text{Peak area of internal standard in the calibration solution.}
\]

Calculate the amount, AM (mg), of ethylene dibromide in calibration solutions C1, C2, C3, C4 and C5 as follows:

\[
\text{AM}_{B1} = \frac{(W_A1 \times P) \times 5}{(100 \times 100)}
\]

\[
\text{AM}_{B2} = \frac{(W_A2 \times P) \times 1}{(100 \times 100)}
\]

\[
\text{AM}_{C1} = \frac{\text{AM}_{B1} \times 0.125}{25}
\]

\[
\text{AM}_{C2} = \frac{(W_A1 \times P) \times 0.100}{(100 \times 100)}
\]

\[
\text{AM}_{C3} = \frac{\text{AM}_{B2} \times 0.125}{25}
\]
AM_{C4} = \frac{(W_{A2} \times P) \times 0.050}{(100 \times 100)}

AM_{C5} = \frac{(W_{A2} \times P) \times 0.075}{(100 \times 100)}

Where

W_{A1} = Weight of Standard taken to make solution A1
W_{A2} = Weight of Standard taken to make solution A2
P = Purity of the ethylene dibromide standard (% w/w)

Calculate the peak area ratio, PAR_S, for ethylene dibromide in the sample solution S as follows:

PAR_{S} = \frac{A'}{I'}

Where

A' = Peak area of ethylene dibromide in the sample
I' = Peak area of Internal Standard in the sample

Calculate the amount, AM' (mg), of ethylene dibromide in the sample solution S using a data system, Microsoft Excel 2003 or similar package. For example:

1. Produce an Excel workbook containing the raw peak area data for the ethylene dibromide and internal standard peaks for the calibrants and samples.
2. Calculate the peak area ratios for the calibration standards C1, C2, C3, C4 and C5. Plot the calibration amounts (AM_{C1}, AM_{C2}, AM_{C3}, AM_{C4} and AM_{C5}) against these values as a graph; ensuring that the equation of the line is shown after the 2^{nd} order polynomial trend line has been selected.
3. The equation of the line will be of the form y = ax^2 + bx + c and the coefficients a, b and c will be the equation numerical values. These will need to be formatted to give 15 decimal places.
4. Calculate the amount AM' of ethylene dibromide in each injection as follows:

\[ AM' = \frac{-b + \sqrt{b^2 - 4a(c - PAR_{S})}}{2a} \]

Where a, b and c are the numerical coefficients from the equation of the line.
For sample Solution S the mg/kg ethylene dibromide content is calculated as follows:

\[
\text{mg kg}^{-1} = \frac{\text{AM'} \times 10^6 \times \text{IS}_S}{\text{W}_S \times \text{IS}_C}
\]

Where

\[
\begin{align*}
\text{W}_S &= \text{Weight of sample (mg)} \\
\text{IS}_S &= \text{Volume of IS solution added to samples (ml)} \\
\text{IS}_C &= \text{Volume of IS solution added to calibration solutions (ml)}
\end{align*}
\]

Typical Selected Ion Total Ion Chromatogram of EDB Calibration Solution
Typical Total Ion Chromatogram of a Diquat Dibromide / Paraquat Dichloride SL Formulation Solution

Typical Selected Ion EI Peak Apex Spectrum of Ethylene Dibromide in Calibration Solution
Typical Selected Ion EI Peak Apex Spectrum of Internal Standard in Calibration Solution
APPENDIX 1

Preparation of fortified sample solution (only to be carried out when analysing new formulation types or when troubleshooting)

Weigh accurately, in duplicate, 100mg of ethylene dibromide analytical standard to a 100ml volumetric flask. Add 40ml of methanol and swirl to dissolve, make up to the mark with methanol and mix well. Call these solutions E1 and E2.

Weigh accurately, in duplicate, 2g of Diquat sample into a 14ml glass screwtop (trident) vial. Call these samples F1 and F2.

Using a 25µl syringe, transfer 10µl of E1 to sample F1. Call this sample G1.
Using a 25µl syringe, transfer 20µl of E2 to sample F2. Call this sample G2.

To samples G1 and G2, add 4.0ml of IS solution. Cap tightly and shake vigorously for ~10-20 seconds. Allow the sample to stand until two layers form. Using a Pasteur pipette transfer as much of the top iso-hexane layer as possible to another glass screw top vial and cap tightly taking care not to transfer any of the lower brown aqueous layer.

Repeat this extraction with 4ml of iso-hexane transferring as much of the top iso-hexane layer as possible to the same vial as the first extract and mix well. Call these solutions H1 and H2.

To calculate the percentage recovery in the fortified sample solutions

Level of fortification (mg/kg) in sample G1 = \( \frac{10 \times 10^{-3} \times WE1 \times 10^6}{100 \times WF1} \)

Level of fortification (mg/kg) in sample G2 = \( \frac{20 \times 10^{-3} \times WE2 \times 10^6}{100 \times WF2} \)

Where

WE1 = Weight (g) of Ethylene Dibromide in solution E1.
WE2 = Weight (g) of Ethylene Dibromide in solution E2.
WF1 = Weight (g) of diquat sample in solution F1.
WF2 = Weight (g) of diquat sample in solution F2.
% recovery = \frac{\text{fortified sample result (mg/kg)} - \text{sample result (unfortified)}}{\text{Level of fortification (mg/kg)}} \times 100
Determination of PP796 (emetic) in paraquat dichloride technical concentrates (TK)

Information

IUPAC name: 2-amino-4,5-dihydro-6-methyl-4-propyl-s-triazole-[1,5-a]pyrimidin-5-one
CA name: 2-amino-6-methyl-4-propyl-(1,2,4)triazolo[1,5-a]pyrimidine-5-(4H)-one (9CI)
CAS Registry No: [27277-00-5]
Molecular structure:

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{N} \quad \text{N} \\
\text{N} & \quad \text{N} \quad \text{NH}_2 \\
\text{H}_3\text{C} & \quad \text{O}
\end{align*}
\]

Molecular formula: C_{9}H_{13}N_{5}O
Relative molecular mass: 207.2

Scope of method

This capillary gas chromatography (GC) method is for the determination of PP796 emetic, as % w/w, in paraquat dichloride technical concentrates.

Summary of method

A portion of the TK (aqueous solution) is made basic with NaOH and partitioned into dichloromethane, containing octadecane as an internal standard. The extract is analyzed by capillary GC-FID, measuring peak areas.

Safety information

Paraquat dichloride salts are toxic, particularly by inhalation of particulates or ingestion, and, because there is no antidote or treatment for the progressive symptoms which can develop, exposure must be avoided. Paraquat dichloride solutions are irritant if splashed in the eyes and harmful if allowed to contact the skin or open cuts. Wear eye protection and protective gloves when handling paraquat dichloride analytical standards, the TK or formulated materials. Solid paraquat materials must only be handled in a fume cupboard.

If in any doubt about the nature and hazards of the chemicals used in this method, consult the Material Safety Data Sheet (MSDS) or an appropriate safety manual such as:


Chemicals

Dichloromethane, HPLC grade.

Octadecane, laboratory reagent grade. Weigh approximately 50 mg into a 100 ml volumetric flask and add about 80 ml dichloromethane. Shake to dissolve, make to the mark with dichloromethane and mix well, to produce an internal standard solution of approximately 0.5 mg/ml.

Sodium hydroxide solution, 1M.

PP796 emetic, analytical standard grade (obtainable from Syngenta). Weigh accurately about 10 mg into two separate 25 ml volumetric flasks and add 5.0 ml of internal standard solution. Shake to dissolve, make to the mark with dichloromethane and mix well, to produce two solutions (Solutions A₁ and A₂) containing PP796 at 0.4 mg/ml.

Laboratory detergent, non-ionic, e.g. Decon Neutracon.

Dimethyldichlorosilane (DMCS), laboratory reagent grade.

Hexane, laboratory reagent grade.

Methanol, water-free.
**Acetone, laboratory reagent grade**

**Apparatus**

Gas chromatograph, equipped with split/splitless injection system and flame ionisation detection, operated in split mode, with automatic injector and electronic data capture and handling system. All gases should be purified through molecular sieves. The carrier gas should be further purified through an oxygen trap.

**Injection Liner,** straight silica liner (4 mm ID) packed with silanised fused silica wool plug (e.g. Restek cat No. 20790). Contaminated split injection liners should be treated as follows.

Immerse in detergent (10% solution) for about 1 hour, then wash in purified water and dry in an oven at 120°C. Take the liner, while still warm, and immerse in a 5% solution of dimethylchlorosilane in hexane for 5 min. Remove the liner and immerse in fresh dry methanol for 1 hour. Wash the liner with acetone and dry thoroughly. The fused silica liner is ready for packing with silanised fused silica wool.

**Column,** 25 m x 0.25 mm ID fused silica capillary column with 0.25 µm film of BPX-5 (ex SGE) or Chrompack CP-Sil 8CB, or equivalent. Maximum programmed operating temperature 350°C.

**Typical operating conditions**

**Oven temperature programme:** initial temperature 50°C for 2 min.

- programme 1, rate 20°C min⁻¹ to 100°C, held for 2 min.
- programme 2, rate 20°C min⁻¹ to 280°C, held for 10 min.

**total run time,** 25.5 min.

**Injector temperature:** 300°C

**Detector temperature:** 325°C

**Gas flow rates:** hydrogen carrier gas, 50 cm/sec (e.g. 10 psi head pressure)

- nitrogen make-up gas, 30 ml/min.
- hydrogen flame gas, 30 ml/min.
- air, 450 ml/min.

**Split flow ,** 50 ml/min.

**Injection volume:** 1 µl (by autosampler)

**Typical retention times:** octadecane, 12-14 min; PP796, 13-15 min.

**Sample extraction**

Weigh accurately, in duplicate, about 2 g of paraquat dichloride technical concentrate into two 100 ml separating funnels. In each case, add 0.5 ml 1M sodium hydroxide solution and swirl the separating funnel taking care not to foul the stopper. Add 2.0 ml octadecane internal standard solution and swirl the separating funnel, taking care not to foul the stopper. Carefully release the gas pressure, shake well and again carefully release the gas pressure. Leave standing until two clear layers are obtained.

Collect the lower (dichloromethane) layer into a 14 ml glass screw-capped (trident) vial and retain the aqueous layer in the separating funnel. Add 2 ml dichloromethane to the separating funnel and shake well. Leave standing until two clear layers are obtained. Combine the lower (dichloromethane) layer with the initial extract in the glass vial and retain the aqueous layer in the separating funnel. Repeat the extraction with a further 2 ml dichloromethane, combining all three extracts in the glass vial and add 5 ml dichloromethane to the glass vial. Identify the duplicate extracts as Solutions B₁ and B₂.

**Determination**

Make replicate injections of Solution A₁ and/or A₂ at about 2 min. intervals, to equilibrate the GC system. Wait 19 min. then inject Solution A₁ or A₂ again and check that the retention times of the octadecane (12-14 min.) and PP796 (13-15 min.) are within the expected time windows. If not, the column head pressure may be adjusted ± 1 psi, or column temperature programme 1 final temperature may be adjusted ± 10°C. If column performance deteriorates substantially during use, check the condition of the split injection liner and replace if necessary.

Perform replicate injections of calibration and sample solutions in an appropriate sequence, such as: A₁, B₁, A₁, B₁, A₁, B₁, A₂, B₂, A₂, B₂, A₂, B₂. Measure peak areas.
Confirm the identity of PP796 in Solution B by GC-MS or, alternatively, by spiking an aliquot of Solution B with an aliquot of Solution A and check for exact co-elution.

**Calculations**

Calculate the relative response factor, RF, for each injection of the standard Solution A as follows.

\[
RF = \frac{A \times VI \times 100}{P \times I \times WR}
\]

Where:
- \(WR\) = weight of PP796 standard (mg);
- \(VI\) = volume of internal standard added in ml;
- \(A\) = peak area of PP796 peak;
- \(I\) = peak area of octadecane peak;
- \(P\) = % w/w purity of PP796 standard.

Calculate the percentage PP796 content (w/w) of each sample Solution B as follows.

\[
\% \text{ w/w} = \frac{A' \times VI \times 100}{I' \times WS \times RF}
\]

Where:
- \(WS\) = weight of sample (mg);
- \(VI\) = volume of internal standard added in ml;
- \(A'\) = peak area of PP796 peak;
- \(I'\) = peak area of octadecane peak;
- \(RF\) = relative response factor for PP796 obtained from the preceding standard Solution A.

Calculate the average PP796 content from Solutions B₁ and B₂.