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 1.25×10^{-3} and 1.5×10^{-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 5×10^{-3}, 3.75×10^{-3}, 2.5×10^{-3}, 1.25×10^{-3} and 0.25×10^{-3} mg/ml of ethylene dibromide in iso-hexane. See summary table.

Calibration Solution	Nominal Amount EDB present in final calibration solution (mg)	Nominal Concentration (mg/ml)	Sample Equivalent Concentration (mg/kg)
C1	0.0125	1.25×10^{-3}	5
C2	0.05	5.0×10^{-3}	20
C3	0.0025	2.5×10^{-4}	1
C4	0.025	2.5×10^{-3}	10
C5	0.0375	3.75×10^{-3}	15

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 1.25×10^{-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 5.0×10^{-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**.

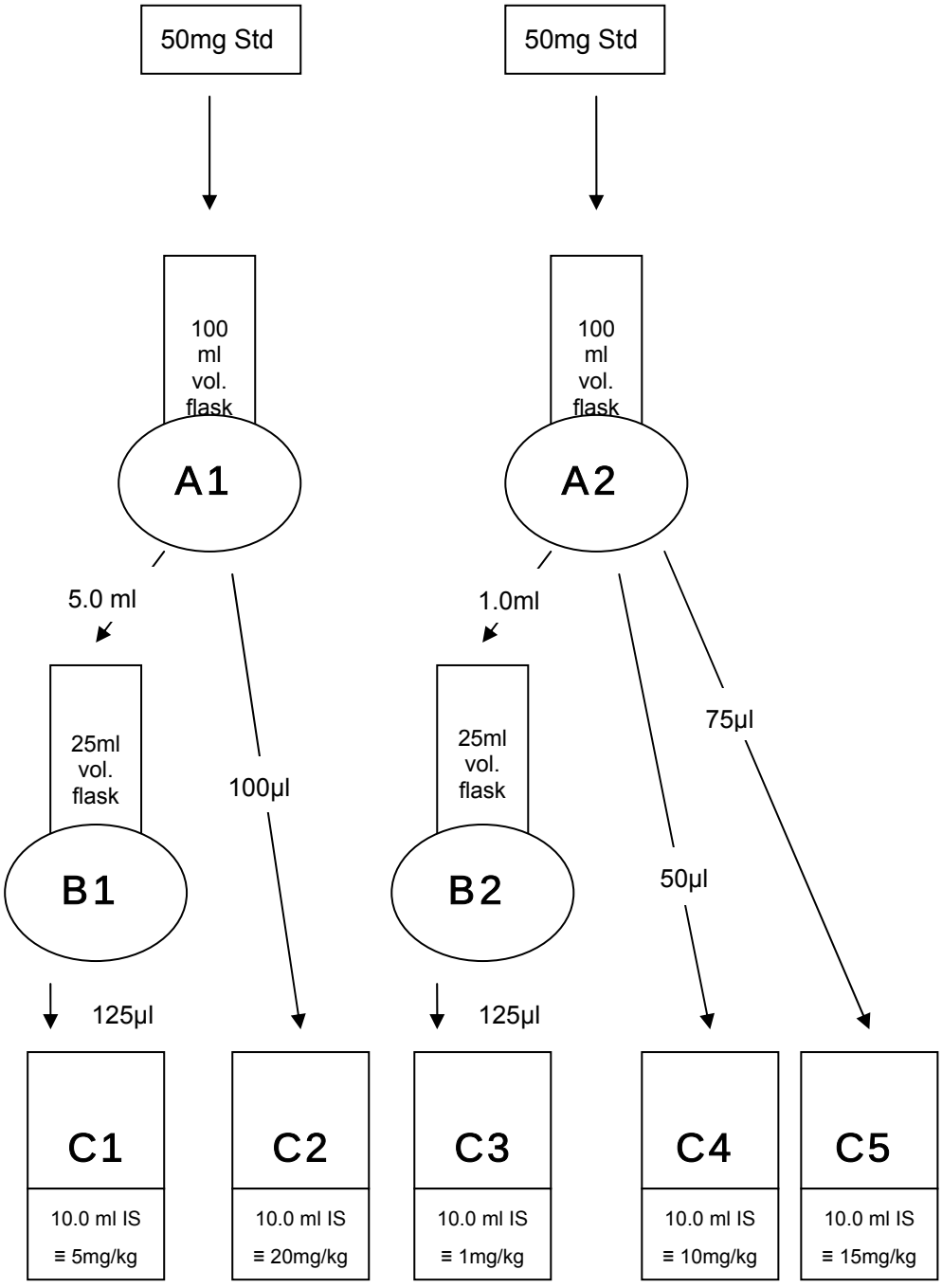
To prepare C3 (1mg/kg ethylene dibromide equivalent), nominal concentration 2.5×10^{-4}mg/ml: To a 14ml trident vial (with a tight fitting lid) add 10.0 ml of IS solution. Transfer 125µl of solution B2 to the vial using a suitable syringe or pipettor and mix well. Call this solution **C3**.

To prepare C4 (10mg/kg ethylene dibromide equivalent), nominal concentration 2.5×10^{-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 x 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



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 Programme	Initial temperature:	60	ºC
	Initial time:	0	min
	Programme rate 1:	20	ºC/min
	Temperature 1:	160	ºC
	Time 1:	0	min
	Programme rate 2:	35	ºC/min
	Final Temperature:	300	ºC
	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	320ºC
MS Quad Temperature	150ºC

MS Source Temperature 230ºC

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

Group	Components	Retention Window (mins)	Resolution	Mass	Dwell Time (ms)
1	Ethylene Dibromide	1.60-3.0	Low	107.0	100
				109.0	100
2	IS	3.0-9.0*	Low	57.0	100
				85.0	100
				170.0	100

* 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 2.5×10^{-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, C5

or

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

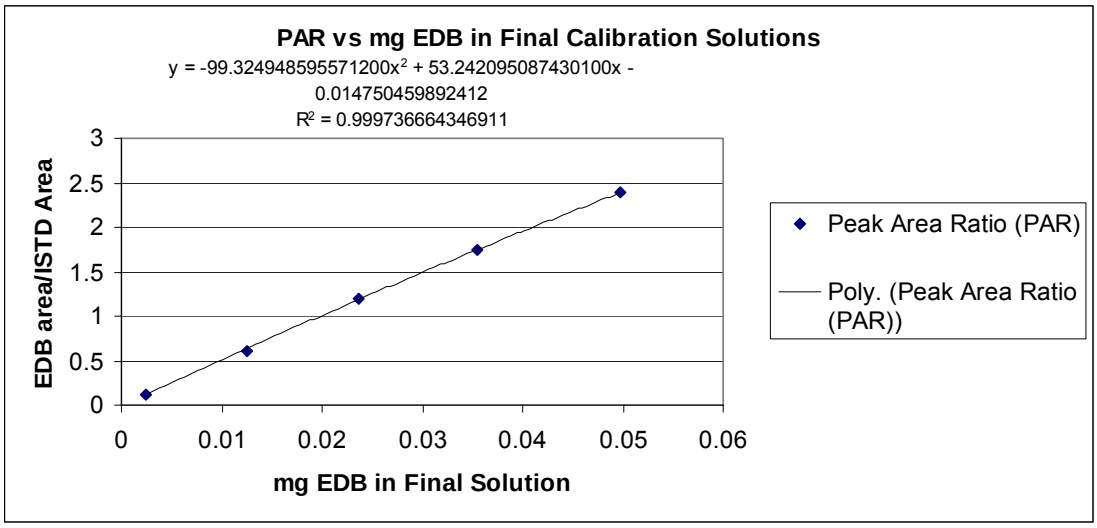
Where S1 is the solution resulting from the first weighing of a sample and S2 is from the second weighing.

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, **$S1_1$**, **$S1_1$**, C2, **$S1_1$**, **$S2_1$**, C3, **$S2_1$**, C4, **$S2_1$**, C5, C1, **$S1_2$**, **$S1_2$**, C2, **$S1_2$**, **$S2_2$**, C3, **$S2_2$** C4, **$S2_2$**, C5, C1, **$S1_3$**, **$S1_3$**, C2, **$S1_3$**, **$S2_3$**, C3, **$S2_3$**, C4, **$S2_3$**, 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



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:

$$PAR_{Standard} = \frac{A_{Standard}}{I_{Standard}}$$

Where

$A_{Standard}$ = Peak area of ethylene dibromide in the calibration solution.

$I_{Standard}$ = 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:

$$AM_{B1} = \frac{(W_{A1} \times P) \times 5}{(100 \times 100)}$$

$$AM_{B2} = \frac{(W_{A2} \times P) \times 1}{(100 \times 100)}$$

$$AM_{C1} = \frac{AM_{B1} \times 0.125}{25}$$

$$AM_{C2} = \frac{(W_{A1} \times P) \times 0.100}{(100 \times 100)}$$

$$AM_{C3} = \frac{AM_{B2} \times 0.125}{25}$$

$$AM_{C4} = \frac{(W_{A2} \times P) \times 0.050}{(100 \times 100)}$$

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

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 2nd 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:

$$AM' = \frac{-b+\sqrt{(b^2 - 4a(c-PAR_S))}}{2a}$$

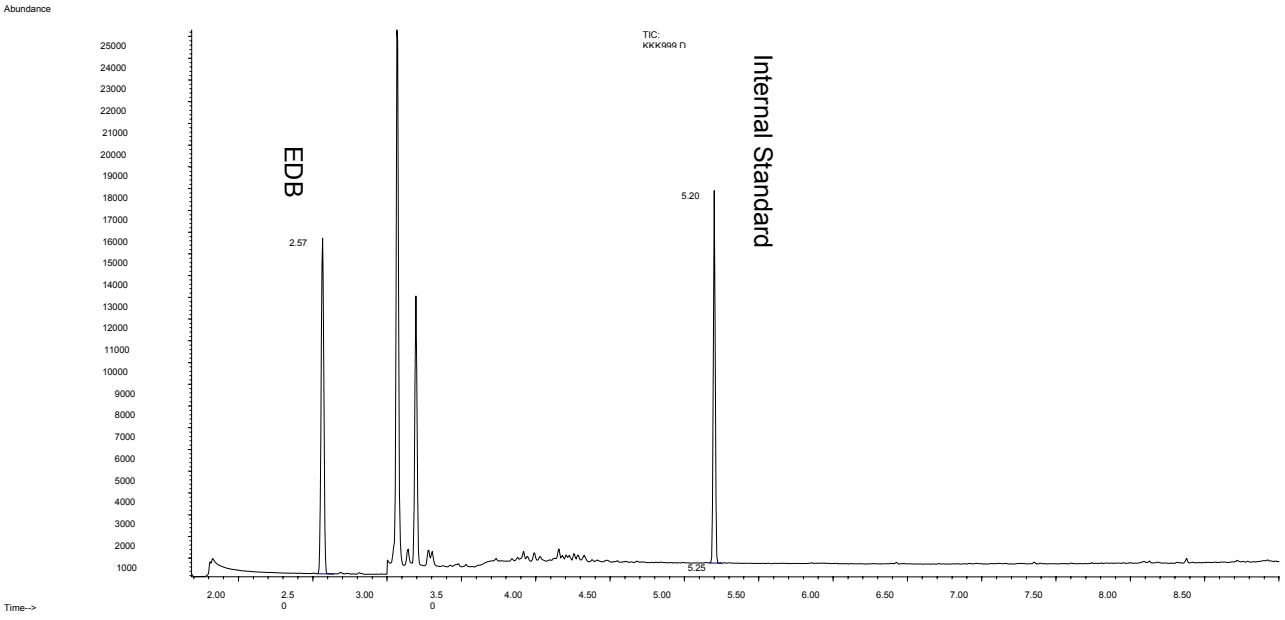
Where a, b and c are the numerical coefficients from the equation of the line.

For sample Solution S the mg/kg ethylene dibromide content is calculated as follows:

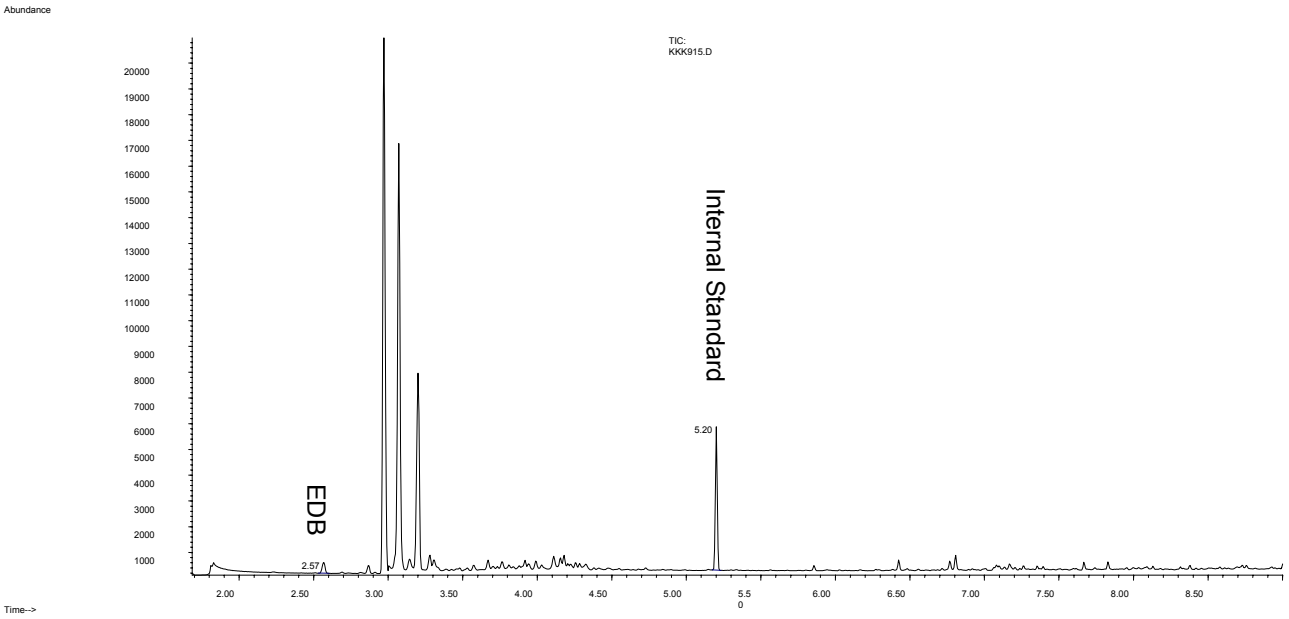
$$mg\ kg^{-1} = \frac{AM' \times 10^6 \times IS_S}{W_S \times IS_C}$$

Where

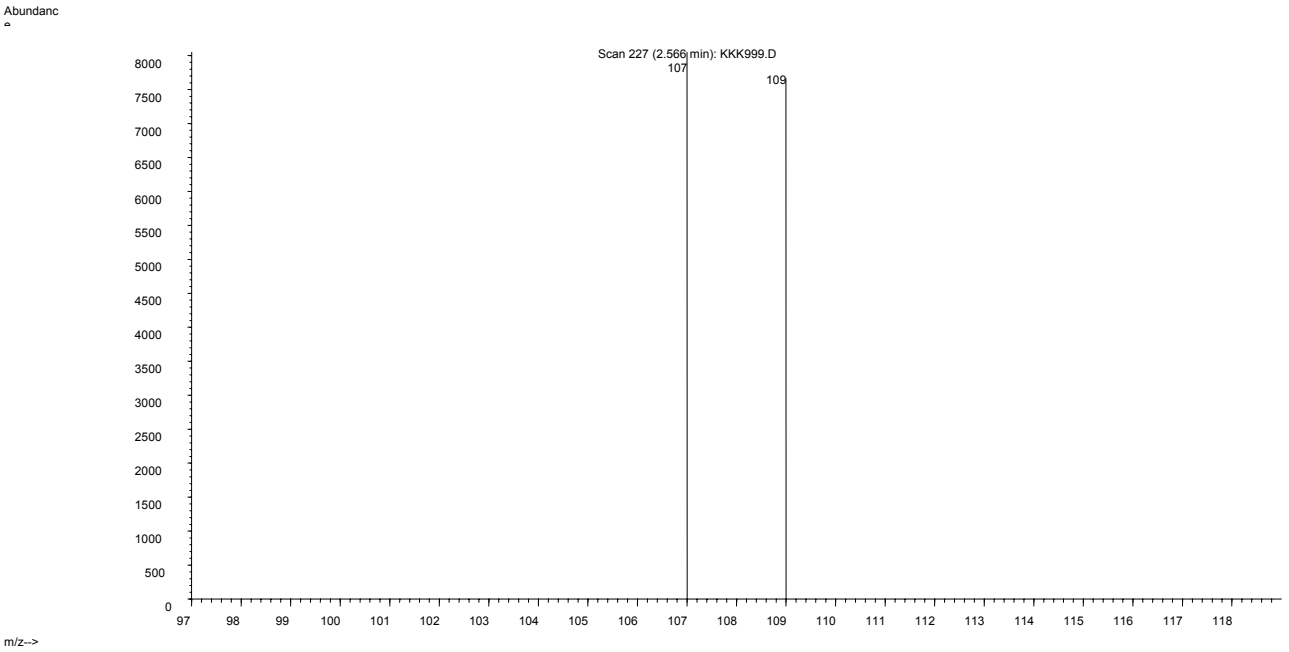
W_S	=	Weight of sample (mg)
IS_S	=	Volume of IS solution added to samples (ml)
IS_C	=	Volume of IS solution added to calibration solutions (ml)



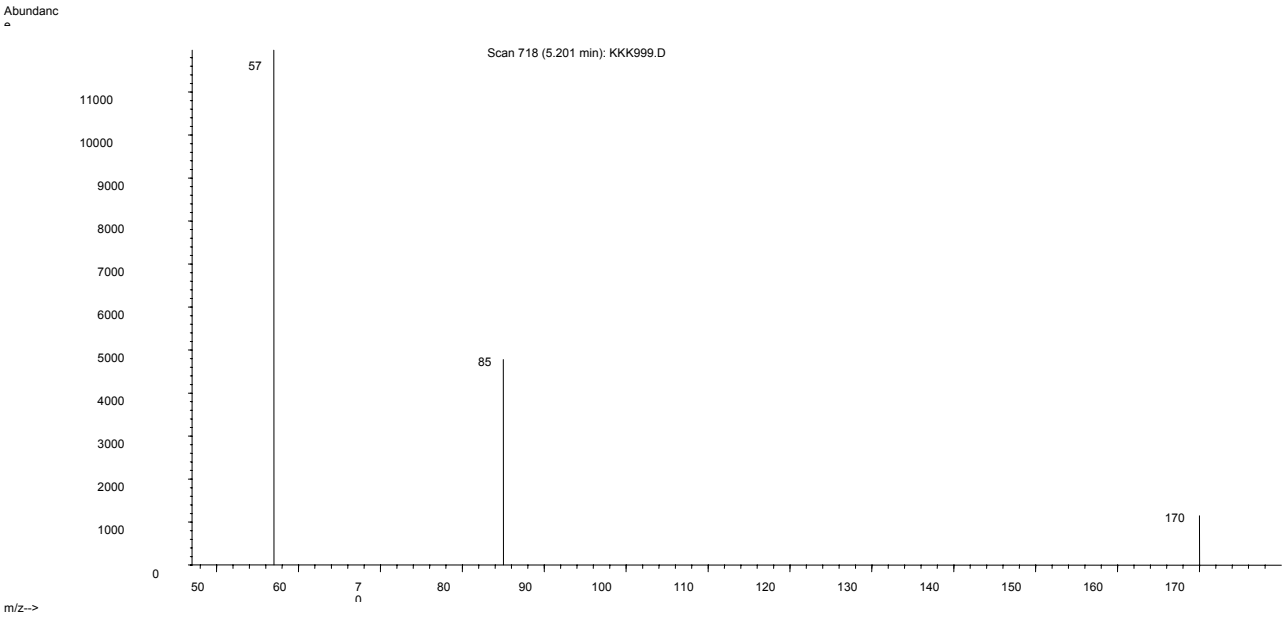
Typical Selected Ion Total Ion Chromatogram of EDB Calibration Solution



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

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

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

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.

$$\% \text{ recovery} = \frac{[\text{fortified sample result (mg/kg)} - \text{sample result (unfortified)}] \times 100}{\text{Level of fortification (mg/kg)}}$$