

**CIPAC/4105/R**

**GUIDELINES FOR THE DESIGN OF CHROMATOGRAPHIC  
ANALYTICAL METHODS INTENDED FOR CIPAC  
COLLABORATIVE STUDY**

**Prepared for CIPAC by Dr M J Tandy\*, P M Clarke and B White (UK)**

The rapid expansion in use of chromatographic methods for pesticide product analysis has led to a wide choice of chromatography columns and variation in experimental parameters, along with increased demands on instrumentation. Whilst this approach may suit agrochemical manufacturers and suppliers of a limited range of specific products, its inherent complexity can cause problems when implementing on a global basis in laboratories where many different pesticide products have to be analysed and there is less familiarity with specific product analysis. Authors of analytical methods, intended for global use via CIPAC collaborative study, should be encouraged to streamline their procedures preferably during in-house development but certainly prior to collaborative study.

This paper presents guidelines on how to simplify the design of capillary gas chromatography (GC) and high performance liquid chromatography (HPLC) methods. Emphasis is placed on those important experimental parameters which can be changed to optimise method performance or kept constant in order to minimise method variation. Other parameters are identified as less critical. The key choice of chromatography column is simplified significantly to a recommended short list. Guidance is also given on the need and use of solvents, gases and internal standards. Each parameter is listed within a method format, to provide clarity along with individual explanation of its importance.

These guidelines concentrate on chromatographic parameters. They are not intended to provide guidance on sample preparation or indeed acceptable quality of results. These are covered in other CIPAC guidelines such as 'Guidelines for CIPAC Collaborative Study Procedures for Assessment of Performance of Analytical Methods' and 'Guidelines on method validation to be performed in support of analytical methods for agrochemical formulations'. However, one benefit of using such a method format is to encourage the standardised layout of chromatographic parameters within a CIPAC method.

**THE DETERMINATION OF ACTIVE INGREDIENT IN FORMULATED MATERIALS  
BY CAPILLARY GAS CHROMATOGRAPHY**

Prepared for CIPAC by P M Clarke, M J Tandy and B White (UK)

## 1 SCOPE

This capillary gas chromatography (GC) method provides for the determination of active ingredient in formulated material.

## 2 SUMMARY OF METHOD

The active ingredient content is determined by capillary gas chromatography, using an **internal standard** procedure.

Significant parameters of the method include :-

- \* **split injection**
- \* **medium bore fused silica capillary column**
- \* **flame ionisation detection.**

## 3 CHEMICALS

*[State grade and recommended supplier]*

### **Safety Information**

All chemicals should be handled according to normal laboratory safety procedures, in a fume cupboard, wearing a laboratory coat, eye protection and suitable gloves.

If in any doubt about the nature and hazards of the chemicals used in this method consult an appropriate safety manual such as:

Hazards in the Chemical Laboratory, Edited by Luxon, Royal Society of Chemistry, Fifth Edition, 1992, London, ISBN 0-85186-229-2.

The Sigma-Aldrich Library of Chemical Safety Data, Edited by Lenga, Second Edition, 1988, Milwaukee, WI, ISBN 0-941633-16-0.

**Organic Solvent, analytical grade (typically acetone, butyl/ethyl acetate or haloalkanes)**  
*Ideally this solvent should also be able to be used as extraction solvent, in which case it may be necessary to check for reaction of active ingredient during method development. Solvent chosen should not be of toxicological concern.*

**Internal Standard, high purity (98% +)**  
*The volatility and structural functionality of the internal standard are critical to the precision obtained. Ensure internal standard is **readily** available from a global supplier. A high purity is desirable to minimise possible interferences.*

**Active Ingredient**, Analytical Standard of certified purity. Store refrigerated (where appropriate).

#### 4 APPARATUS AND OPERATING CONDITIONS

The apparatus listed below is 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.

|                          |   |
|--------------------------|---|
| <b>Instrument</b>        | GC system, equipped with split/splitless injection and flame ionisation detection, operated in <b>split mode</b> .  |
| <b>Injection Mode</b>    | <i>Use of an autoinjector is recommended to enable reproducible volume and speed of injection.</i>  |
| <b>Injection Liner</b>   | <i>The choice of liner, amount and type of packing, can have a critical effect on precision.</i><br><br><i>Liners need to be checked or replaced on a regular basis. Otherwise a build up of less volatile material can result in adsorption, or reaction.</i><br><br><i>Used split injection liners should be decontaminated as appropriate prior to silanisation.</i> |
| <b>Injection load</b>    | 0.5 - 2 $\mu$ l, usually 1 $\mu$ l (syringe size typically 10 $\mu$ l).   |
| <b>Column Dimensions</b> | Fused silica; length : 10 - 25 m<br>internal diameter : 0.2 - 0.25 mm (0.32 mm may need to be considered)<br><i>These dimensions offer a good compromise between column efficiency, analysis time and capacity.</i>   |
| <b>Film Thickness</b>    | 0.1 - 0.25 $\mu$ m ( <i>sufficient phase to minimise capacity factors whilst still maintaining column efficiency and minimise elution temperature</i> ).  |
| <b>Stationary Phase</b>  | Crosslinked dimethyl polysiloxane.<br><br><i>This is the preferred phase as it is robust with a wide range of applications, and should be used where there are no specific benefits in using another phase.</i>   |

*Other recommended stationary phases:-*

| <b>Phase</b>   | <b>Comments</b>   |
|--|---|
| <i>Crosslinked 5% phenyl polysiloxane 95% dimethyl polysiloxane.</i> | <i>Improved selectivity for aromatics over dimethyl polysiloxane phase.</i> |
| <i>Crosslinked 14% cyanopropyl-phenyl 86% dimethyl polysiloxane.</i> | <i>Medium polarity phase, unique selectivity.</i>                           |
| <i>Crosslinked 50% phenyl 50% dimethyl polysiloxane.</i>             | <i>Applicable to analysis of medium polarity compounds.</i>                 |
| <i>Crosslinked 50% trifluoropropyl 50% dimethyl polysiloxane</i>     | <i>Highly selective phase for resolution of halogenated compounds.</i>      |

|   |   |
|---|---|
| <b>Injection Port Temp</b>  | <i>This will need to be evaluated to minimise breakdown and optimise precision.</i>   |
| <b>Oven Temperature</b>   | <p>Isothermal temperature :</p> <p><i>The temperature at which the active ingredient elutes between 5 - 10 minutes (H<sub>2</sub> carrier gas) or 10 - 15 minutes (He carrier gas). A shorter elution time will generally reduce peak efficiency and resolution. Ensure temperature chosen is within the limits set by the column manufacturer.</i></p> <p>Initial time :</p> <p><i>Sufficient to elute active ingredient and internal standard.</i></p>  |
| <b>Temperature programme to remove formulation adjuvants if necessary</b> | <p>Programme rate 1 : <i>Maximum ramp rate of GC system</i></p> <p>Final temperature 1 : <i>Within the temperature limit of the column</i></p> <p>Final time 1 : <i>Sufficiently long to elute all components</i></p>   |
| <b>Detector Temperature</b>   | <i>325 °C or 25 °C above final temperature 1.</i>   |
| <b>Gas Filtration</b>   | All gases should be <b>purified through molecular sieves</b> . The carrier gas should be further purified through an oxygen trap.   |
| <b>Detector Gas Flow Rates</b>  | According to manufacturers recommendations.   |
| <b>Carrier gas</b>  | <p><i>Hydrogen or helium, preferably hydrogen. The benefits of using hydrogen over helium are less critical instrument setup and quicker analysis times. However hydrogen may be reactive with reducible compounds, eg nitro compounds.</i></p> <p>Average Linear Gas Velocity : <i>45 - 55 cm s<sup>-1</sup> (hydrogen carrier gas)<br/>25 - 35 cm s<sup>-1</sup> (helium carrier gas)<br/>The above values cover optimum performance for the recommended column internal diameter range.</i></p> <p><i>A higher linear velocity may be desirable to shorten the analysis if there is sufficient resolution.</i></p> |
| <b>Split flow</b>   | <p><i>60 - 250 ml min<sup>-1</sup> (hydrogen carrier gas)<br/>30 - 200 ml min<sup>-1</sup> (helium carrier gas)</i></p> <p><i>This parameter is not generally critical when used within the above ranges. The term "split flow" is recommended over "split ratio" for ease of method setup.</i></p>   |
| <b>Data handling</b>  | <i>Correct setting of integration parameters is important for accurate definition of peak start and end points.</i>   |

## 5 SUITABILITY

Stored or new columns may require conditioning prior to use.

Perform replicate injections of calibration solution until acceptable, repeatable chromatography is obtained.

Compare the chromatogram obtained with that shown in Figure 1.

*Provide an example chromatogram, Figure 1, with the peak of interest on scale clearly showing peak shape and retention.*

Measure the retention time of the active ingredient (*give an acceptable time window in minutes e.g.  $4.5 \pm 0.5$  minutes*).

If the peak retention time is not within the quoted window the oven temperature or the column head pressure may be adjusted but only within stipulated values (*e.g.  $\pm 10^{\circ}\text{C}$  or  $\pm 1$  psi, respectively*)

*Further adjustments could indicate unacceptable experimental procedure.*

## 6 DETERMINATION

Perform an injection of a sample solution. *Provide an example chromatogram, Figure 2. This enables comparison with Figure 1 to illustrate the separation of other constituents eg formulation adjuvants.*

Perform replicate injections of the calibration and sample solutions following the recommended sequence of injection.

*If column performance deteriorates substantially during use, check the condition of the split injection liner and replace if necessary. If column performance remains unacceptable, then remove approximately 10 cm length from the column at the injection end.*

## 7 METHOD VALIDATION SUMMARY

**Precision** (*The method should state the maximum relative standard deviation allowable relevant to the appropriate active ingredient content e.g.  $\text{RSD} < 2.1$  for 5% active ingredient*).

**Accuracy** (*The method should state the allowable recovery range of the active ingredient from the sample, relevant to the appropriate active ingredient content e.g. 97.0 - 103.0% for 1-10% active ingredient*).

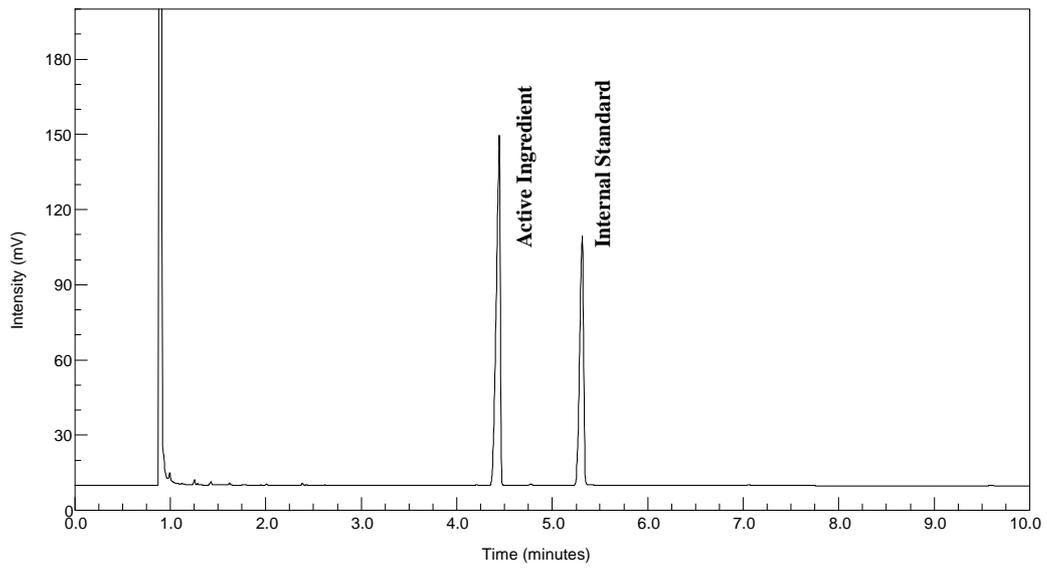
**Linearity** (*The method should state the range over which response is linear e.g.  $\pm 20\%$  of the nominal active ingredient concentration*).

## 8 REFERENCES

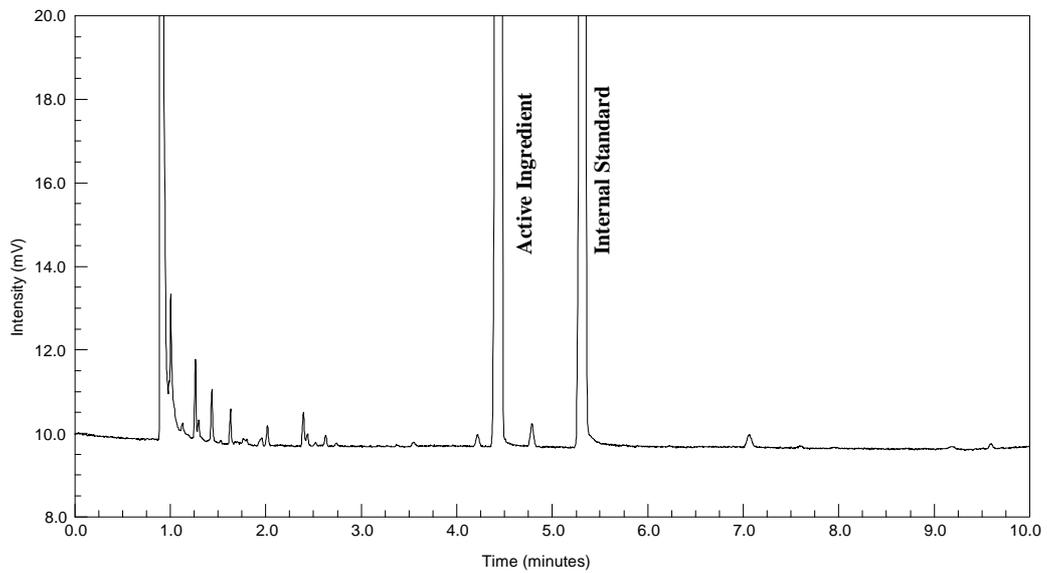
CIPAC/3807R Guidelines on method validation to be performed in support of analytical methods for agrochemical formulations.

Guidelines for CIPAC collaborative study procedures for assessment of performance of analytical methods.

**Figure 1: Chromatogram Of Calibration Solution**



**Figure 2: Chromatogram Of Sample Solution**



**THE DETERMINATION OF ACTIVE INGREDIENT IN FORMULATED MATERIALS BY  
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY**

Prepared for CIPAC by P M Clarke, M J Tandy and B White (UK)

## 1 SCOPE

This high performance liquid chromatography (HPLC) method provides for the determination of active ingredient in formulated material.

## 2 SUMMARY OF METHOD

The active ingredient content is determined by high performance liquid chromatography using an **external standard** procedure where possible. *Internal standards are generally not essential with automated or overfilled loop injectors but may be required to ensure accurate preparation of sample solution.*

Significant parameters of the method include:

- \* **reversed phase chromatography** with a base deactivated endcapped octadecylsilyl bonded silica column (where possible)
- \* **column temperature control**
- \* **ultra-violet (UV) detection** (where possible)
- \* **samples prepared in initial mobile phase** (when achievable)

## 3 CHEMICALS

*[State grade and recommended supplier]*

### Safety Information

All chemicals should be handled according to normal laboratory safety procedures, in a fume hood wearing a laboratory coat, eye protection and suitable gloves.

If in any doubt about the nature and hazards of the chemicals used in this method, consult an appropriate safety manual such as:

Hazards in the Chemical Laboratory, Edited by Luxon, Royal Society of Chemistry, Fifth Edition, 1992, London, ISBN 0-85186-220-2.

The Sigma-Aldrich Library of Chemical Safety Data, Edited by Lenga, Second Edition, 1988, Milwaukee, WI, ISBN 0-941622-16-0.

**Organic solvent**, HPLC grade (*typically methanol or acetonitrile*).

**Water**, purified conforming to ASTM type 2 or HPLC quality.

**Active Ingredient**, Analytical Standard of **certified** purity. Store refrigerated (where appropriate).

#### 4 APPARATUS AND OPERATING CONDITIONS

The apparatus listed below is 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.

|                          |  |
|--------------------------|--|
| <b>Instrument</b>        | HPLC system equipped with pump, auto-injector, column oven and UV variable wavelength detector.  |
| <b>Injection Mode</b>    | <i>Use of an automated injector system or manually overfilled loop is recommended for consistency of volume of injection.</i>  |
| <b>Injection Load</b>    | 5 - 20 $\mu$ l.<br><i>Injection volume is not generally critical.<br/>Ensure that the response of the component of interest is within the linear range of the detector.</i>  |
| <b>Column Dimensions</b> | Stainless steel; length : 100 - 250 mm<br>internal diameter : 3 - 5 mm<br><i>A length of less than 100 mm may give a less robust method.<br/>A diameter of less than 3 mm requires specialist equipment.<br/>A short guard column, packed with appropriate stationary phase, is recommended.</i> |
| <b>Particle Size</b>     | Nominal particle size 3 - 5 $\mu$ m. <i>Deviation from this size may give rise to operational or performance problems.</i>   |
| <b>Stationary Phase</b>  | Base deactivated endcapped octadecylsilyl bonded silica. <i>This is the preferred phase as it is robust with a wide range of applications.</i>   |

##### Alternative Stationary Phases

| <b>Column</b>                           | <b>Comments</b>                            |
|---|--|
| Strong Anion Exchange (SAX)             | For ion exchange chromatography of anions. |
| Strong Cation Exchange (SCX)            | For ion exchange chromatography of cations |
| Bonded Phase Silica (eg Cyano or Amino) | For specialist applications.               |
| Silica                                  | For normal phase chromatography.           |

*Other stationary phases may be used where these give a significant benefit.*

|                             |  |
|-----------------------------|--|
| <b>Column Temperature</b>   | <p>Temperature control eg at 40 °C ensures consistent retention times.</p> <p>Other values may be used where temperature is significant in improving separation.</p> <p>Avoid using the description “ambient” as a column temperature.</p>   |
| <b>Mobile Phase</b>         | <p>Express each component as a percentage by volume before mixing where possible.</p> <p>Measure organic and aqueous portions separately.</p> <p>pH may be critical for ionisable compounds.</p> <p>Avoid gradients unless necessary for removal of strongly retained formulation adjuvants.</p> <p>Include example recipe.</p> <p>Degassing of mobile phase is recommended.</p> |
| <b>Flow Rate</b>            | <p>0.5 - 2.0 ml min<sup>-1</sup>. Choose appropriate flow rate for column diameter eg 1.0 ml min<sup>-1</sup> for 4.6mm id, 0.5 ml min<sup>-1</sup> for 3.2mm id.</p>  |
| <b>Detector Wavelength</b>  | <p>Wavelength selected should be a wavelength in the UV spectrum of active ingredient where the change in slope is not pronounced, eg maximum or minimum. Always use a wavelength more than 10nm away from the mobile phase UV cut-off.</p>  |
| <b>Data Handling System</b> | <p>Correct setting of integration parameters is important for accurate definition of peak start and end points.</p>  |

## 5 SUITABILITY

Stored or new columns may require conditioning with mobile phase(s) prior to use. *State mobile phase(s), time and flow rate.*

Perform replicate injections of calibration solution until acceptable, repeatable chromatography is obtained.

Compare the chromatogram obtained with that shown in Figure 1.  
*Provide an example chromatogram, Figure 1, with the peak of interest on scale clearly showing peak shape and retention.*

Measure the retention time of the active ingredient (*give an acceptable time window in minutes eg 6.3 ± 0.5 minutes*).

If the peak retention time is not within the quoted window, the mobile phase may be adjusted but only within stipulated values *eg for mobile phase containing 50 % methanol, the methanol concentration may be adjusted by ± 5%*).

*Further adjustments could indicate unacceptable experimental procedure.*

## 6 DETERMINATION

Perform an injection of a sample solution. *Provide an example chromatogram, Figure 2. This enables comparison with Figure 1 to illustrate the separation of other constituents eg formulation adjuvants.*

Perform replicate injections of the calibration and sample solutions following the recommended sequence of injection.

*If column performance deteriorates substantially during use, replace the guard column.*

*Columns may require washing prior to storage. State mobile phase(s), time and flow rate.*

## 7 METHOD VALIDATION SUMMARY

**Precision** *(The method should state the maximum relative standard deviation allowable relevant to the appropriate active ingredient content eg  $RSD_r < 2.1$  for 5% active ingredient).*

**Accuracy** *(The method should state the allowable recovery range of the active ingredient from the sample, relevant to the appropriate active ingredient content eg 97.0 - 103.0% for 1-10% active ingredient).*

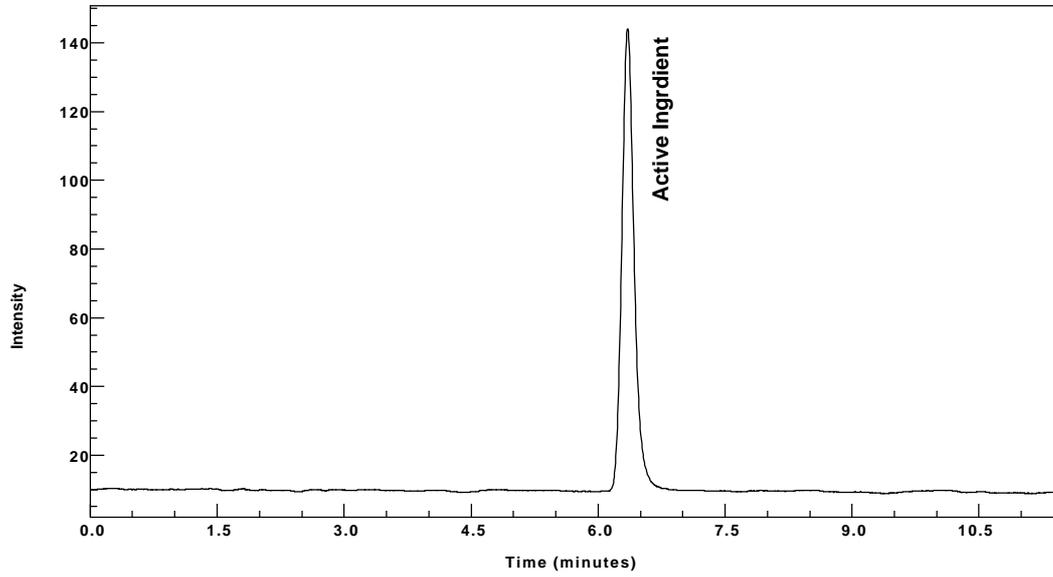
**Linearity** *(The method should state the range over which response is linear eg  $\pm 20\%$  of the nominal active ingredient concentration).*

## 8 REFERENCES

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Guidelines for CIPAC collaborative study procedures for assessment of performance of analytical methods.

**Figure 1: Chromatogram Of Calibration Solution**



**Figure 2: Chromatogram Of Sample Solution**

