Simultaneous Determination of Chlorothalonil and Hexachlorobenzene in Technical and Formulated Materials by Capillary Gas Chromatography: Collaborative Study

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A collaborative study was conducted for the capillary gas chromatographic (GC) method for the simultaneous determination of the fungicide chlorothalonil (CTL) and the accompanying impurity, hexachlorobenzene (HCB), in technical and formulated materials. The method calls for the dissolution of technical and dry formulations of CTL and HCB from the aqueous flowable formulation. The 10 participating laboratories were asked to analyze the samples by adhering to the method as closely as their instrumentation and data systems allowed, and to note any deviations from the method. Collaborators were asked to prepare the standards and samples, set up the capillary GC systems, analyze the samples, and calculate the results. CTL produced reproducibility relative standard deviations (RSDR) of 0.4–2.5 (active ingredient concentrations ranged from approximately 52 to 98% by weight). HCB produced RSDR values of 5.2–22% (HCB concentrations were 0.02–0.04% by weight). The method was adopted First Action by AOAC INTERNATIONAL.

Chlorothalonil (tetrachloroisophthalonitrile, CTL) is a contact fungicide registered for use on a wide variety of fruit crops, vegetables, ornamentals, and turf, and in specialty applications worldwide. It is also being used as a mildewcide in paints and for sapstain control on wood. Hexachlorobenzene (HCB) is a known chemical impurity of CTL, typically present at relatively low levels. HCB has been deemed significant by the U.S. Environmental Protection Agency (EPA) and other international regulatory organizations.

A capillary gas chromatographic method was developed for the simultaneous determination of CTL and HCB in technical and formulated materials. The method and the results of a preliminary interlaboratory study of the method were published previously (1).

Collaborative Study

This study was sponsored by ISK Biosciences Corp., the primary manufacturer of CTL worldwide. The sponsor’s objective of the study was to produce a worldwide standard method of analysis for CTL and HCB, as approved by AOAC INTERNATIONAL.

Eleven laboratories outside of Ricerca, Inc., were initially solicited, and they agreed to participate in this collaborative study. Two collaborators eventually dropped out, one because of the use of hydrogen carrier gas and the other because of inexperience with capillary gas chromatography (GC), instrument problems, and laboratory backlog. The 9 participating laboratories represented academia, industry, and government agencies. Two collaborators were from outside the United States. A laboratory within Ricerca, Inc., also participated in this study. Ten laboratories, including Ricerca, Inc., submitted data packages.

Each collaborator received a copy of the protocol, a practice sample of technical material, duplicates of each of 4 samples (2 blind duplicates and 2 Youden pairs), reference materials, internal standard reagent, and material safety data sheets for all chemicals and samples. All materials were to be stored under ambient conditions. Eight samples were sent to each participating laboratory. The sample types consisted of technical concentrate (TC), water dispersible granular (WG) and wettable powder (WP) formulations, and an aqueous-based suspendable concentrate (SC) flowable. All samples used in this study were produced by ISK Biosciences of Houston, TX, and Mentor, OH. Collaborators were sent worksheets for

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The recommendation was approved by the Methods Committee on Pesticide and Disinfectant Formulations, and was adopted by the Official Methods Board of AOAC INTERNATIONAL. See “Official Methods Board Actions,” (1999) Inside Laboratory Management, July issue.
weights, notes, etc. A subjective method evaluation was also requested on completion of the study.

**Collaborator Overview**

Data were returned in 45–90 days. At least 2 participating laboratories had no previous experience with capillary GC. Eight of the participating collaborators used automatic sampling equipment. One collaborator made all injections manually. Gas chromatographs from 3 different manufacturers were used. One collaborator used helium as the makeup gas rather than nitrogen.

One collaborator did not send a usable data package because of a bad capillary column from which HCB could not be determined. This data package was rejected as incomplete. The other 9 collaborators submitted usable packages. However, some collaborators did not calculate data according to the protocol. For example, a few collaborators took a mean response factor from the entire automated run to calculate results for samples after the fact. Some did not correct for the purity of the CTL before doing the calculations. The study design dictated that all standards and samples be prepared in duplicate and that all preparations be injected in duplicate. One collaborator prepared single standards and samples. All data packages received, worksheets, chromatograms, and calculations were verified, and in some cases the numbers were recalculated to ensure that each package was handled as instructed in the protocol. All data were used in the presentation of results and were annotated appropriately. All data, after being reviewed, were analyzed by the statistical computer program provided by AOAC INTERNATIONAL.

Several collaborators initially questioned the use of hydrogen carrier gas and were not initially equipped to use it in their laboratories. A potential collaborator withdrew immediately because of a bad capillary column from which HCB could not be determined. Hydrogen carrier gas and were not initially equipped to use it in their laboratories. A potential collaborator withdrew immediately because of the use of hydrogen carrier gas. One of the reasons for choosing hydrogen is its use as the common carrier gas outside of the United States. In addition, hydrogen carrier gas allows high column efficiency at high linear gas velocities. All collaborators did use hydrogen as the carrier gas.

**999.04 Determination of Chlorothalonil and Hexachlorobenzene in Technical and Formulated Materials—Capillary Gas Chromatography**

**First Action 1999**

[This method is applicable to analysis of technical material (TC), dry formulations (WP and WG), and aqueous-based (SC) formulations from 50 to 99% chlorothalonil (CTL) and 0.01 to 0.1% hexachlorobenzene (HCB).]

*See Table 999.04A and B for the results of the interlaboratory study supporting acceptance of the method.*

**A. Principle**

Chlorothalonil (tetrachloroisophthalonitrile, CTL) in TC, WG and WP products is dissolved with toluene containing n-butyl phthalate as an internal standard (IS). A minor modification of solvent (1 + 1 by volume, methanol + toluene with IS) is made for SC formulations. The CTL and one chemical impurity, hexachlorobenzene (HCB), are analyzed by capillary FID gas chromatography with an internal standard.

**B. Apparatus**

(a) *Gas chromatograph.*—With a flame ionization detector and split/splitless injector. Temperatures: column, 205°C; injection port, 330°C; detector, 300°C. Gas flows, H carrier at 25 psi (175 kPa) column head pressure; split flow, approximately 140 mL/min. Linear gas velocity (LGV), approximately 74 cm/s. Injection volume, 1 μL. Adjust H and air for flame gases as per manufacturer’s specifications. Makeup gas, N at 30 mL/min. Split liner, single taper with glass wool (taper down; a high temperature septum is recommended). Split flow, approximately 140 to 180 mL/min, adjusted to ensure that the LC peak is not clipped (see Determination section).

(b) *Column.*—30 m, 0.25 mm id, 50% phenylmethylpolysiloxane, 0.5 μm film. Approximate expected retention times are HCB, 3.4 min; CTL, 6.0 min; IS, 7.0 min.

(c) *Integrator.*—Automatic digital or chromatographic data system, or personal computer/software package.

(d) *Disposable Teflon™ syringe filters.*—0.45 μm.

(e) *Mortar and pestle.*

**C. Reagents**

(a) *Toluene.*—Reagent grade or better.

(b) *Methanol.*—Reagent grade or better.

**Table 999.04A Results of interlaboratory study for CTL**

<table>
<thead>
<tr>
<th></th>
<th>TC</th>
<th>WG</th>
<th>WP</th>
<th>SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of labs</td>
<td>9</td>
<td>8</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Mean (wt %)</td>
<td>98.04</td>
<td>90.44</td>
<td>75.62</td>
<td>52.55</td>
</tr>
<tr>
<td>s_r</td>
<td>0.48</td>
<td>0.11</td>
<td>0.51</td>
<td>0.66</td>
</tr>
<tr>
<td>s_R</td>
<td>1.00</td>
<td>0.38</td>
<td>0.51</td>
<td>1.34</td>
</tr>
<tr>
<td>RSD_r, %</td>
<td>0.49</td>
<td>0.12</td>
<td>0.68</td>
<td>1.25</td>
</tr>
<tr>
<td>RSD_R, %</td>
<td>1.02</td>
<td>0.42</td>
<td>0.68</td>
<td>2.55</td>
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</table>

For TC, WP, and SC, no outliers were detected. For WG, one lab was identified as an outlier by Cochran’s test.

TC, technical concentrate; WG, water dispersible granular; WP, wettable powder; SC, suspendable concentrate.

**Table 999.04B Results of interlaboratory study for HCB**

<table>
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<th>TC</th>
<th>WG</th>
<th>WP</th>
<th>SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of labs</td>
<td>8</td>
<td>8</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Mean (wt %)</td>
<td>0.0201</td>
<td>0.0376</td>
<td>0.0210</td>
<td>0.0185</td>
</tr>
<tr>
<td>s_r</td>
<td>0.0007</td>
<td>0.0019</td>
<td>0.0010</td>
<td>0.0006</td>
</tr>
<tr>
<td>s_R</td>
<td>3.5</td>
<td>5.0</td>
<td>4.8</td>
<td>3.4</td>
</tr>
<tr>
<td>RSD_r, %</td>
<td>3.5</td>
<td>5.0</td>
<td>4.8</td>
<td>3.4</td>
</tr>
<tr>
<td>RSD_R, %</td>
<td>6.0</td>
<td>5.5</td>
<td>21.6</td>
<td>5.4</td>
</tr>
</tbody>
</table>

For TC, WG, and SC, one laboratory was identified as an outlier by the single Grubb’s test. For WP, no outlier tests were significant.
weigh of analyte

**Internal standard (IS).—** Prepare IS solution by weighing 2.0 g (±0.01) n-butyl phthalate and quantitatively transferring to 1000 mL volumetric flask. Dissolve in toluene for analysis of TC, WG, and WP formulations and in toluene–methanol (1+1) for SC formulations. The IS for the SC products is amended with 4–6 drops phosphoric acid added for each liter.

**Calibration standards.—** Prepare 2 HCB stock solutions. Weigh and record to the nearest 0.1 mg, 30 mg (±3 mg) directly into 100 mL volumetric flasks. Dilute these stock preparations to volume with IS. Mix well until all HCB dissolves. Dilute stock solutions with IS 2/100 in each of two 100 mL volumetric flasks by pipetting 2.0 mL stock solution into each volumetric flask and adding IS to volume. Label these solutions Diluent 1 and Diluent 2.

**System Suitability**

(a) Weigh and record to 4 decimal places, 1.0 g (±0.01 g) analytical grade CTL of known purity into 2 respective bottles. Correct CTL weight for label purity and identify one of these bottles as STD 1 and the other as STD 2. Pipet 50.0 mL Diluent 1 into STD 1. Pipet 50.0 mL Diluent 2 into STD 2.

(b) Express CTL and HCB concentrations for use in STD 1 (and STD 2) as the weight in mg/50 mL (after correction for purity). Analyze any CTL reference material used for HCB content prior to using. Do not use reference material which contains detectable concentrations of HCB (approximately 0.0025%).

(c) Chromatograph each STD solution to ensure they are properly prepared. Consider each STD properly prepared if relative response factors for each is within 1% of the other for CTL and 10% for HCB.

Relative response factor = \[
\frac{\text{weight of analyte \times area of IS}}{\text{area of analyte}}
\]

(d) If relative response factors meet these criteria, combine STD 1 and STD 2 for use as a calibration solution by pipetting 40 mL of each into a clean 4 oz bottle. Use as weights for this calibration solution the average corrected weights for the 2 compounds in each of the 2 standards. This solution is stable for 7 days.

(e) Determine column efficiency by calculating effective theoretical plates for the CTL peak in the calibration solution by using the following formula:

\[
\text{Effective theoretical plates (N)} = 5.545 \times \left( \frac{t_R (\text{CTL}) - t_R (C_8 H_{10})}{W_h} \right)^2
\]

where \(N\) = number of effective theoretical plates; \(t_R\) = retention time of CTL or butane; \(W_h\) = peak width at half height.

Retention time for butane is determined using a disposable cigarette lighter and a 10 μL syringe. Open valve of lighter (without lighting) and insert syringe needle into lighter jet. Fill and expel gas several times, then draw back syringe plunger to 3 or 4 μL. Inject gas aliquot using the same techniques as with liquids.

(f) System suitability is acceptable when \(N\) (effective theoretical plates) is a minimum of 1560 plates/m for HCB, 700 plates/m for CTL, and 1800 plates/m for the IS. All peaks of interest must be baseline resolved. Resolution for HCB peak must be >1.5. Resolution for CTL and IS peaks must be > 3.

Resolution is calculated as:

\[
R = \frac{1.18(t_{R2} - t_{R1})}{W_{h1} + W_{h2}}
\]

where \(R\) = resolution, \(t_{R1}\) = retention time for peak 1 in resolution pair, \(t_{R2}\) = retention time for peak 2 in resolution pair, \(W_{h1}\) = peak width, at half height, for peak 1 in resolution pair, and \(W_{h2}\) = peak width, at half height for peak 2 in resolution pair.

**Preparation of Test Solution**

(a) **Technical (TC) samples.—** Grind test sample with mortar and pestle prior to weighing. Weigh test portions directly into a vessel. Weigh in duplicate and record to 4 decimal places, 1.0 ± 0.02 g of test sample; then dissolve with 50.0 mL IS solution (20 mg/mL).

Mix preparations until CTL is in solution as described below. An ultrasonic bath may be effectively used by placing bottles in approximately 2.5 cm of water and sonicating for 20 min. Some solids present in TC products may not dissolve in the IS solution. These solids may be carbon black inherent in the technical material and the preparation may take on a dark gray hue in solution. When an ultrasonic bath is used, allow preparations to return to room temperature prior to opening. If an ultrasonic bath is not available, preparations may be placed on a magnetic stirrer for 20 min. Let stand until any carbon black insoluble material settles. If needed, filter suspensions through 0.45 μm disposable filters and a disposable syringe to which the filter is attached. If test solutions are filtered, then the standard preparation should be filtered as well.

(b) **Dry flowables.—** Grind WP and WG formulations with a mortar and pestle prior to dissolution. Adjust test portion weights for each formulation, based on label concentration, to a CTL concentration of 20 mg/mL. Prepare all test solutions in duplicate. Allow preparations to settle prior to analysis, or test solutions and standards may be filtered as instructed above.

(c) **Aqueous based (SC) flowables.—** Shake laboratory samples well prior to subsampling to ensure homogeneity. A 3 min shaking by hand is sufficient. (Note: SC formulations contain approximately 40% water.) The solvent or solvent system used must be miscible with water and effectively solubilize the CTL. The IS solution used for these formulations requires a solvent mixture of 1+1 by volume, methanol–toluene, containing the phosphoric acid as in (c). Prepare in duplicate.

Prepare separate standards for the analyses of these products diluted with toluene–methanol (1+1) solvent mixture/IS. Adjust test portion weights based on label concentration of the formulation to produce CTL concentrations of 20 mg/mL.
Prepared solutions may be sonicated or stirred as above for technical products. (Note: Take care that the test portions are completely extracted. This may require extra time in sonication, or more vigorous shaking than was required for the dry products. When sonicating or shaking, allow enough time to ensure that there are no large clumps of nonextracted formulation present.) Test products and standards may be filtered as above.

**F. Determination**

(a) Adjust the GC conditions and ensure that the CTL peak does not overload the integrator or the detector. A slight column overload may be observed as a leading edge asymmetrical peak, but must be endured to ensure enough sensitivity for the HCB. Adjust the split flow and initially bring the CTL peak on scale.

(b) Examine the apex of the peak to ensure the peak is not “clipped.” Split flow must be optimized by each laboratory and the peak apex examined for round rather than flat apex. A typical chromatogram is shown in Figure 999.04.

(c) Set up an analysis using 1 µL injections. The analysis sequence should be thus: CS S1 S2 S3 CS S4 S5 S6 CS, etc. Duplicate injections should be made from each vial, where CS = calibration standard, S = test solutions 1, 2, 3, etc.

(d) **Criteria.**—Test injections may begin when the calibration standard relative response factors agree within 1 and 10% for CTL and HCB, respectively.

**G. Calculations**

Calculate results based on internal standard methodology by the following formulas: Calculate the mean relative response factor (RRF) for CTL based on the duplicate injections of the standard:

\[
RRF = \frac{\text{weight}_1 \times \text{area}_2}{\text{area}_1}
\]

where weight 1 = CTL in mg/50 mL in CS (purity corrected weight); area 1 = chromatographic peak area of CTL from CS; area 2 = chromatographic peak area of IS from CS.

Calculate weight percentage of CTL as follows:

\[
\text{Wt \% CTL} = \frac{\text{area}_3 \times \text{RRF} \times 100}{\text{weight}_3 \times \text{area}_4}
\]

where area 3 = chromatographic peak area of CTL from test; weight 3 = weight of test portion in mg/50 mL; area 4 = chromatographic peak area of IS from test.

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**Figure 999.04**—Typical chromatogram. (From optimized conditions. The display sensitivity has been maximized to permit visual detection of CTL.)
Average the results of the injections for the final, reportable value.

Calculate the mean RRF for HCB, based on the duplicate injections of the standard, for CTL, where weight 1 of HCB in mg/50 mL in CS; area 1 = chromatographic peak area of HCB from CS; area 2 = chromatographic peak area IS from CS.

Calculate weight percentage of HCB as for CTL, where area 3 = chromatographic peak area of HCB from test; weight 3 = weight of test portion in mg/50 mL; area 4 = chromatographic peak area of IS from test.

Average the results of the injections to produce the final, reportable value.

Report CTL results of individual injections (weight percent) to 4 significant figures. For CTL, the final average result will be 3 significant figures. Individual injections for HCB are initially 4 decimal places (3 significant figures), and final results to 3 decimal places (2 significant figures).


Results and Discussion

Technical CTL is typically 97–98% pure. The WP formulation is nominally formulated to contain 75% CTL, but was somewhat higher in this study. The WG formulation used in this study was formulated to contain 90% CTL. This formulation was then altered, by weight, to produce a second material 3% lower than the original. The SC formulation was formulated to contain 54% CTL. It too was then altered, by weight, to produce a second material 2% lower than the original.

Results of the individual analyses for CTL and HCB are presented in Tables 1 and 2, respectively. The presentation of laboratory data was randomized with regard to the collaborators listed in this paper.

Laboratory 5 was determined to be an outlier by the Cochran’s test with respect to the determination of CTL in the WG formulation. This result was due to the significant variation for that formulation.

Laboratory 4 produced consistently low results for HCB. For TC, WG, and SC samples, that laboratory was classified as an outlier for HCB by the single Grubbs test. However, for the WP formulation, no outlier tests were significant, although the double Grubbs test was within 0.4. When those results from Laboratory 4 were removed because of overall low bias, the single Grubbs test was also significant, and the HCB data from Laboratory 9 were also removed.

Overall, the collaborative study produced a reproducibility relative standard deviation (RSDr) of approximately ≤1% for CTL in technical and dry formulations and an RSDr of 2.5% for CTL in the SC formulations. HCB results produced an RSDr of ≤7.3% after removal of the biased data.

All statistical data are presented in Tables 999.04A and 999.04B.

Collaborators’ Comments

Of the 9 laboratories submitting complete data packages, only 5 made any comments and/or suggestions regarding the method. Of these 5, 4 collaborators said that the method was “useful,” “viable,” “easy to follow as written,” and “...when applied rigorously, will produce precision data.” The fifth collaborator provided specific comments but did not provide an overall reflection on the method.

Three of the 5 participants commenting on the method reported problems with the analysis of the SC sample. One had initially prepared the sample for analysis and had obtained results up to 20% lower than expected. Another had duplicate preparations that gave results that were quite different. These 3 collaborators immediately consulted with the protocol author, and all collaborators then prepared the samples again, taking special care, as directed, to ensure that the prepared sample appeared to be completely dissolved, with no obvious, large clumps of undissolved formulation present and floating in the solvent. When these collaborators repeated the analyses, they reported no further problems. A visible description of the formulation in the solvent would have been helpful to avoid incomplete dissolution. The other 6 collaborators made no comment about problems with this formulation.

One participant reported evidence of a “chased peak” that initially interfered with the analyses, but with the extension of the run time, posed no further problems. No other participants noted this phenomenon.

Two comments challenged the necessity of preparing 2 reference standards. This requirement was included to ensure

Table 1. CTL precision estimates

<table>
<thead>
<tr>
<th>Sample type</th>
<th>No. of labs</th>
<th>Mean, % by weight</th>
<th>s, % by weight</th>
<th>sR, % by weight</th>
<th>RSDr, %</th>
<th>RSDR, %</th>
<th>r, % by weight</th>
<th>R, % by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>9</td>
<td>98.0</td>
<td>0.47</td>
<td>0.99</td>
<td>0.48</td>
<td>1.01</td>
<td>1.31</td>
<td>2.78</td>
</tr>
<tr>
<td>WG</td>
<td>8a</td>
<td>90.4</td>
<td>0.10</td>
<td>0.37</td>
<td>0.41</td>
<td>0.41</td>
<td>0.28</td>
<td>1.03</td>
</tr>
<tr>
<td>WP</td>
<td>9</td>
<td>75.6</td>
<td>0.50</td>
<td>0.50</td>
<td>0.67</td>
<td>0.67</td>
<td>1.41</td>
<td>1.41</td>
</tr>
<tr>
<td>SC</td>
<td>9</td>
<td>52.6</td>
<td>0.67</td>
<td>1.33</td>
<td>1.27</td>
<td>2.54</td>
<td>1.87</td>
<td>3.73</td>
</tr>
</tbody>
</table>

a The within-laboratory variation for Laboratory 5 was identified as an outlier by the Cochran's test, which exhibited statistical significance at the 0.01 level.
that no errors were made in the preparation of a calibration solution. One collaborator argued that they were “professional chemists trained in preparation of chemical standards.” However, we feel that, in some cases, this is not always true. Technicians and field operatives often analyze samples. In addition, anyone can make an error, and this method of preparation will rapidly display errors, because the relative response factors will not agree if one solution is different from the other.

One participant asked why we did not include a range of standards to determine linearity rather than the use of a 1-point calibration standard. Linearity was determined during method development. Both compounds exhibited more than adequate linearity. Because each sample is weighed for analysis on the basis of CTL content, the peak area responses for samples and the calibration standard should be quite comparable.

One collaborator suggested that because the injector temperature is 330°C, a high-temperature septum, good to 350°C, should be used. This is a point well noted.

A residue and trace contaminants laboratory did not care for the grinding of the samples before preparation. The possibility of dust and potential contamination of the work area troubled this laboratory. This procedure was done only to ensure the homogeneity of the samples. Not all formulated samples, particularly WG formulations, are always completely homogeneous. Each laboratory must take care not to contaminate its work area.

There is potential for some CTL reference materials to contain quantifiable HCB. If HCB-free reference material is not available, a procedure for HCB correction is detailed in the Journal of AOAC INTERNATIONAL (1).

### Recommendation

On the basis of the results of this study, it is recommended that the gas chromatographic method for the simultaneous determination of CTL and HCB in technical and formulated materials be adopted First Action by AOAC INTERNATIONAL.

### Acknowledgments

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- B. Ripley and M. Denomme, Ontario Ministry of Agriculture, Guelph, Ontario, Canada
- D. Fox, Zeneca, Inc., Richmond, CA
- J. Byington, U.S. Customs Laboratory, San Francisco, CA
- W. Shultz, Minnesota Valley Testing Laboratories, New Ulm, MN
- D. Kennedy, Zeneca Specialties, Blackley, Manchester, UK
- J. Schetter, Ricerca, Inc., Painesville, OH

### Reference