

ENVIRONMENTAL CHEMISTRY METHOD FOR THE DETERMINATION OF DPX-KN128/IN-KN127 RESIDUES IN WATER USING GC/ECD

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1.0 SUMMARY

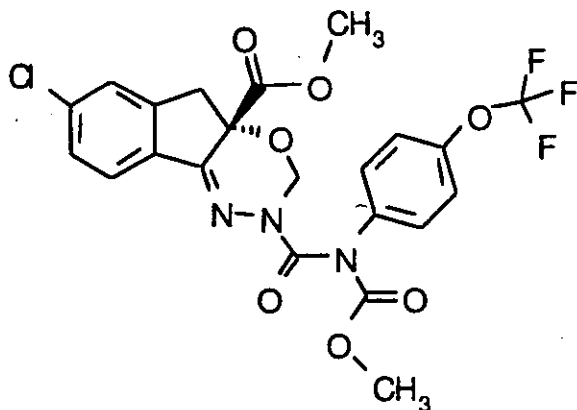
An analytical method is described for determining the residues of DPX-KN128 combined with IN-KN127 in water. DPX-JW062 or DPX-MP062 Experimental Insecticides are formulated containing a mixture of DPX-KN128 and IN-KN127. DPX-JW062 Experimental Insecticide is a formulation containing a racemic (50:50) mixture of DPX-KN128 (insecticidally active enantiomer) and DPX-KN127 (insecticidally inactive enantiomer). DPX-MP062 Experimental Insecticide is a formulation containing a 75% of DPX-KN128 and 25% of DPX-KN127. Residues will be reported as the sum of DPX-KN128 and DPX-KN127 which elute in a single chromatographic peak. Henceforth in this study, DPX-KN128 combined with DPX-KN127 will be referred to as "KN128/KN127".

2.0 INTRODUCTION

DPX-MP062 Experimental Insecticide is a postemergence insecticide developed for insect control in cotton, sweet corn, pome fruit, vegetables, grapes, and root crops production. DPX-MP062 Experimental Insecticide is formulated as a suspension concentration or a water-dispersible granule. DPX-MP062 Experimental Insecticide is effective for control of various insects, such as *Heliothis/Helicoverpa* spp., *Spodoptera exigua*, *S. littoralis*, *Pectinophera gossypiella* (pink bollworm), *Earias* spp. (spiny bollworm), *Plutella xylostella* (diamondback moth), *Tricoplusia ni* (cabbage looper), *Spodoptera exigua* (beet armyworm), *Pieris rapae* (imported cabbageworm), *Mamestra brassicae* (cabbage moth), and other insects.

The method involved the analysis of toluene extracts of water samples using gas chromatography (GC) with an electron capture detector (ECD). The method limit of LOQ is 0.05 ppb with an estimate MDL of 0.02 ppb. GC/MSD can be used as a confirmatory method using the GC column and conditions specified in this report.

The structure and chemical name of the active ingredient DPX-KN128 are shown below.



CAS Registry Number: 173584-44-6
 CAS Name (S)-methyl 7-chloro-2,5-dihydro-2-
 [[[(methoxycarbonyl)[4-(trifluoro-
 methoxy)phenyl]amino]carbonyl]=
 indeno[1,2-*e*][1,3,4]oxadiazine-4a(3*H*)-carboxylate

3.0 MATERIALS

3.1 *Equipment (Equivalent equipment and materials may be substituted unless otherwise specified; note any specifications in the following descriptions before making substitutions. Substitutions should only be made if equivalency/suitability has been verified with acceptable control and fortification recovery data.)*

Balance - AE163 balance, 0.0001 to 160.000g (Mettler, Hightstown, NJ).

Sample Bottles - 120-mL precleaned amber glass bottles and caps, #B7316-120 (Baxter, McGaw Hill Park, IL).

Sample Shaker - wrist-action shaker (Burrell, Pittsburgh, PA).

Micropipets - P.C.R. positive displacement pipets, 25 and 250 μ L (Tri-Continent Scientific, Grass Valley, CA).

Gas Chromatograph - Hewlett-Packard 5890 Series II Gas Chromatograph equipped with a 7673A autosampler, a pressure-programmable split/splitless inlet, and an ECD (Hewlett-Packard, Kennett Square, PA).

GC Inlet Liners - deactivated, packed, tapered liner, #5062-3587 (Hewlett-Packard, Kennett Square, PA).

Column - J&W Scientific 30 m x 0.25 mm DB-5 (1- μ film) column, #C4587-89 (Baxter, McGaw Hill Park, IL).

3.2 Reagents & Standards

Toluene - Mallinckrodt nanograde toluene, #8092-4*NY (Baxter, McGaw Hill Park, IL).

Acetonitrile - Burdick and Jackson UV grade acetonitrile, #015-4*DK (Baxter, McGaw Hill Park, IL).

DPX-JW062 - DPX-JW062 analytical standard, Lot 33, purity 98.6%, Stock #1340 (DuPont Agricultural Products, Global Technology Division, E. I. du Pont de Nemours and Company, Wilmington, DE).

3.3 *Safety & Health*

No unusually hazardous materials are used in this method. Each analyst must be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. All appropriate material safety data sheets should be read and followed, and proper personal protective equipment should be used.

4.0 METHODS

4.1 *Principle of the Analytical Method*

The method involved the analysis of toluene extracts of water samples using GC with an ECD. 100 ± 5 mL of water are collected and are extracted with 10.0 mL of toluene. The sample is shaken for 10 minutes and the phases are allowed to separate. Exactly 1 mL of the toluene extract is transferred to a GC vial and analyzed by GC/ECD.

4.2 *Analytical Procedures*

4.2.1 *Glassware & Equipment Cleaning Procedure*

The effectiveness of any cleaning procedure used should be demonstrated by preparation and analysis of reagent blanks. In general, all glass- and plasticware should be washed in hot tap water with laboratory grade, non-phosphate detergent, rinsed several times with tap water, rinsed several times with deionized water, rinsed once with acetone, and allowed to fully dry before use. Care should be taken to avoid working with high levels of the analyte being monitored in the same laboratory where samples are being extracted and analyzed.

4.2.2 *Preparation & Stability of Reagents Solutions*

No preparation of any reagents was performed for this study. Reagents were used from a commercially available source. Manufacture recommend stability for these reagents was followed.

4.2.3 Standard Preparation and Stability

Stock Standard Solution

A stock standard solution is prepared by accurately weighing 10.0 mg of KN128/KN127 and dissolving in 50.0-mL acetonitrile to obtain a 200- $\mu\text{g}/\text{mL}$ solution. This stock standard solution is stable for several months when stored at 4°C.

Intermediate Standard Solutions

The stock standard solution is diluted serially (1.0 mL in 10.0-mL total) with acetonitrile to obtain 20.0 $\mu\text{g}/\text{mL}$, 2.0 $\mu\text{g}/\text{mL}$, and 0.20- $\mu\text{g}/\text{mL}$ intermediate standard solutions. This standard solution is stable for several months when stored at 4°C.

GC Calibration Standards

Standards for GC calibration are prepared using the intermediate standard solutions as described in Table 1. Aliquots of the intermediate standard solutions are dispensed using the micropipets. Standards for this study were stored in amber glassware in a freezer and are stable for several months, but standards can be stored in clear glassware in a freezer if needed.

KN128/KN127 concentration in intermediate standard solution ($\mu\text{g}/\text{mL}$)	Volume of intermediate standard solution (μL) diluted to 10.0 mL with toluene	KN128/KN127 concentration in GC standard (ng/mL)
0.2	25.0	0.50
2.0	12.5	2.5
2.0	50.0	10.0
20.0	25.0	50.0
20.0	100.0	200.0

4.2.4 Source of Samples

Water was sampled from four different sources and analyzed using this method (Bradenton, FL, Donna, TX, Elkhart, MD, and Madera, CA). There were no interferences in these water matrices.

4.2.5 Storage & Preparation of Samples

Sample Collection

100 mL of water samples are to be collected in 120-mL amber or clear glass bottles marked with a 100-mL graduation. The accuracy on collection should be approximately 100 ± 5 mL. Each sample bottle becomes an individual sample and cannot be split. A sufficient number of sample bottles must be prepared to account for replicate, retain, and fortification samples required by the protocol.

Storage

Water samples must be frozen from the time of sampling until extraction and analysis.

4.2.6 Sample Fortification Procedure

For each set of samples, spike two samples (controls, if available) with intermediate standard solutions to obtain concentrations approximately corresponding to the MDL and five times the MDL. Extract the spiked samples as specified above (Item 2).

For example, if the MDL is 0.020 ppb, two samples would be spiked, one at 0.020 and one at 0.10 ppb, with KN128/KN127. The 0.020-ppb sample is prepared by adding 10 μ L of the 0.20- μ g/mL intermediate standard solution (to obtain 0.002 μ g in 100 mL which is equivalent to 0.020 ppb), and the 0.10-ppb sample is prepared by adding 50 μ L of the 0.20- μ g/mL intermediate standard solution.

4.2.7 Analyte Extraction Procedure

The exterior of each sample bottle should be wiped clean and dry, and the total weight determined (including the bottle and cap). After recording the weight, add 10.0 mL of toluene to each sample, allow the samples to thaw, and shake for 10 minutes on a wrist-action shaker (samples oriented horizontally). After shaking, allow the phases to separate sufficiently to sample exactly 1 mL of the toluene extract. Transfer the aliquot of toluene to a GC vial. Empty the sample bottles, rinse with water and dry. Weigh the bottle and cap after drying. Calculate and record the sample weight - i.e., weight of bottle containing the sample - weight of the dry bottle and cap.

4.2.8 Analyte Purification Procedure

No further purification of the water sample was needed.

4.3 Instrumentation

4.3.1 GC Setup and Calibration

Set up the GC as specified in Table 2. Refer to manufacturer recommendations for setting detector gas flows (N_2 make-up gas). If pressure programming is not available, use a constant pressure of 30 psi for He carrier gas. Analyze standards to

determine the retention time of KN128/KN127 and to confirm the linearity of the GC method. See Table 1 for information on standard preparation.

Table 2. GC Conditions for KN128/KN127 Analysis	
injection volume = 5 μ L, splitless (inlet purge time = 2 min)	
injector temperature = 250°C	
detector temperature = 340°C	
signal range = 0	
Temperature program:	
initial temperature = 150°C	initial time = 1 min
heating rate = 15°C/min	
final temperature A = 330°C	initial time A = 9 min
Pressure program: (He carrier gas)	
initial pressure = 70 psi	initial time = 0.3 min
pressure program rate = 99 psi/min	
final pressure = 30 psi	final time = 40 min

4.3.2 Sample Analysis

The following sequence should be used when analyzing samples.

MDL standard, sample fortified at the MDL, sample 1, sample 2, sample 3, sample fortified at 5 x MDL, 5 x MDL standard, MDL standard, sample 4, etc.

MDL standard is a solution of KN128/KN127 in toluene with a concentration corresponding to the MDL. For example, if the MDL is 0.020 ppb, the MDL standard would be 0.2-ng/mL KN128/KN127.

The concentration of the MDL standard (ng/mL) is determined as follows:

$$C_{\text{MDLSTD}} = \text{MDL}(\text{ppb}) * 10$$

where 10 is the concentration factor for the extraction step.

Analyze the GC data to ensure proper identification and integration of the KN128/KN127 peak. Calculate the KN128/KN127 concentrations using the procedure described in the next section. Water samples with KN128/KN127 concentrations which are higher than the highest calibration standard should be diluted appropriately (i.e., to obtain a concentration lower than the highest standard) and reanalyzed.

4.4 *Calculations*

4.4.1 *Response Factor (RF)*

Quantitation of KN128/KN127 in the water samples is done using the average of the response factors of a standard analyzed before and after each set of three samples.

The response factor for KN128/KN127 is defined as,

$$\text{Response factor} = A_{\text{KN128/KN127}}/C_{\text{KN128/KN127}}$$

- $A_{\text{KN128/KN127}}$ = GC peak area for KN128/KN127.
- $C_{\text{KN128/KN127}}$ = concentration (ng/mL) of KN128/KN127 in the standard.

4.4.2 *Quantitation of KN128/KN127 in Water*

The following equation is used to calculate the concentration of KN128/KN127 in water samples,

$$\text{KN128/KN127 (ppb)} = (A_w/\text{Average RF})/10$$

- A_w = GC peak area for KN128/KN127 in the sample extract.
- Average RF [area/(ng/mL)] = average of the response factors of the standards analyzed before and after the sample (i.e., the MDL standard and 5X MDL standard).
- The divisor of the 10 accounts for the 10-fold concentration in the extraction procedure.

4.4.3 *Recovery of KN128/KN127 from Fortified Samples*

The following equation is used to calculate the % of KN128/KN127 recovered from fortified samples.

$$\% \text{ recovered} = [(A_F - A_S)/A_{\text{STD}}] \times 100$$

- A_F = KN128/KN127 peak area for fortified sample.
- A_S = KN128/KN127 peak area for unfortified sample.
- A_{STD} = KN128/KN127 peak area for the standard concentration corresponding to the spike level.

5.3 *Modifications or Special Precautions*

The half-life for KN128/KN127 in water samples at room temperature is typically less than one week. It is important to extract samples within several hours of thawing.

The use of plasticware for the storage of toluene or for sample extractions is discouraged due to the extraction of plasticizers and additives which may interfere with KN128/KN127 in GC analyses.

5.4.2 *Specificity/Potential Interferences*

Water was sampled from four different sources and analyzed using this method. Chromatograms for the four water samples are shown in Figures 2-5. There were no interferences in these water matrices. Chromatograms for a 0.5-ng/mL and a 10.0-ng/mL KN128/KN127-standard are shown in Figures 6 and 7. Chromatograms for Bradenton, FL, water samples fortified at 0.050 and 2.0 ppb with KN128/KN127 are shown in Figures 8 and 9.

Standards of bromacil and diuron, two potential interferences, were also analyzed. Both compounds eluted more than 9 minutes before KN128/KN127.