Validation protocol for whole-body dosimetry in an agricultural exposure study

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Abstract Agricultural workers exposed to pesticides can experience adverse health impacts depending on toxicity and exposure amount. Whole-body dosimetry (WBD) is the most reliable, practical, and realistic method for measuring exposure. Since validation of analytical and experimental methodologies is critical for quantitative determination of exposure, we conducted a validation procedure to design an essential protocol for WBD exposure studies. Using the fungicide kresoxim-methyl, matrix-matched standards were prepared with various matrices including outer cloth, inner cloth, washing solution for gloves and hands, gauze, and glass fiber filter (IOM sampler) to determine the instrumental limit of quantitation for high-performance liquid chromatography (HPLC) (2 ng) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) (10 pg). Method limits of quantitation (MLOQ) were also set for HPLC (0.1 mg/L) and LC–MS/MS (0.005 mg/L). We observed good analysis repeatability (coefficient of variation < 6%), and the linearity of the calibration curves was reasonable ($r^2 > 0.998$) in the range of 0.001–10 mg/L in various matrices. Recovery tests were carried out at three levels of concentration (MLOQ, 10 MLOQ, and 100 MLOQ) and resulted in good recoveries (72.7–105.6%). We did not observe breakthrough of the compound in tests of holding capacity for glass fiber pesticide filters. The procedures established in the present study are applicable as an essential, comprehensive protocol for exposure assessment studies using WBD.

Keywords Exposure · IOM sampler · Kresoxim-methyl · Method validation · Pesticide · Whole-body dosimetry

Introduction

Agricultural pesticides can be deposited on workers’ bodies or inhaled during the mixing/loading and application of spray solution, as well as harvesting of crops. Since direct exposure to pesticides can lead to negative health impacts depending on pesticide toxicity and exposure amount, concern regarding risks associated with human exposure to pesticide has become a major agricultural issue. One of the main exposure routes is dermal [1–3], which is measured by the patch method [1, 3–10] or whole-body dosimetry (WBD) [2, 11–16]. The conventional patch method using small patches attached to outer clothing is simple and economical. However, WBD evaluates an individual’s outer and inner clothing to measure dermal exposure in a more realistic and practical manner. Therefore, WBD exposure assessments require the outer clothing, inner clothing, gloves, and gauze for dermal exposure measurements, as well as glass fiber filters or resin tubes for inhalation measurements [11, 13, 14].

Whether the patch or WBD method is used, the validation of analytical methodologies is critical to obtaining reliable results for risk assessment on exposure of pesticides to applicator. Several studies of the patch method have included method validations [1, 3–5, 7–10, 17, 18], but only one study of WBD has done so and lacked a breakthrough test [13].
The purpose of the present study was to establish a critical method validation protocol for measuring dermal and inhalation exposure using WBD. These evaluations will be carried out in apple orchards when a fungicide, kresoxim-methyl, is applied to control crop disease. We calculated instrumental and method limits of quantitation, derived a matrix-matched calibration curve, and assessed linearity, repeatability, matrix effect, and recovery of kresoxim-methyl from various exposure matrices for method validation. We also performed a breakthrough test using glass fiber filter (IOM sampler) for assessments of inhalation exposure to ensure that our method obtained sensitive, quantitative, and reliable results.

Materials and methods

Chemicals and materials

Kresoxim-methyl standard (96.1%) and formic acid (> 99.7%) were purchased from Sigma-Aldrich (St. Louis, MO, the USA). All HPLC-grade solvents were obtained from Fisher Scientific (Seoul, Republic of Korea). Outer (35% polyester/65% cotton) and inner clothing (100% cotton) were purchased from Uniseven (Seoul, Republic of Korea) and TRY (Seoul, Republic of Korea), respectively. Nitrile gloves (Sol-vex 237-676, Ansell, Malaysia) and aerosol OT-75 detergent (Jakyu Chemical, Republic of Korea) were purchased from a local supplier. Gauze (10 × 10 cm) was obtained from Kukjae Co. (Republic of Korea). Glass fiber filter (25 mm), IOM sampler cassettes (225.70A), and XAD-2 resin tubes (400/200 mg) were purchased from SKC (Eighty Four, PA, the USA). A personal air monitoring pump was obtained from Gillian (Gilarir-3, Sensidyne, the USA).

Instruments and analytical conditions

Kresoxim-methyl residue on outer clothing was detected by HPLC (Agilent 1100 series, Agilent Technologies Inc., Santa Clara, CA, the USA) with a Luna C18 column (5 µm particle size, 4.6 × 250 mm, Phenomenex, Torrance, CA, the USA) using a mixture of acetonitrile and water as the mobile phase. The mobile phase was programmed in a gradient system as follows: 20% acetonitrile at initial time, increased to 50% acetonitrile for 10 min, held for 20 min, decreased to 20% acetonitrile for 1 min, and held for 4 min. The flow rate was 1.0 mL/min, and injection volume was 20 µL. A diode array detector (Agilent Technologies Inc., Santa Clara, CA, the USA) was used for detection at 254 nm. Kresoxim-methyl residues on inner clothing and in solution used to wash gloves/hands were analyzed with UHPLC–MS/MS (Nexera LC-30AD and Shimadzu LCMS 8040 triple-quadrupole system) with a Kinetex C18 column (2.6 µm particle size, 2.1 × 100 mm, Phenomenex, Torrance, CA, the USA). The mobile phase was acetonitrile and 0.1% formic acid aqueous solution. The gradient system of the mobile phase was programmed as follows: 5% acetonitrile at initial time, held for 1 min, increased to 95% acetonitrile for 3.5 min, held for 3.5 min, decreased to 5% acetonitrile for 7.5 min, and held for 5 min. The flow rate was 0.2 mL/min, and the injection volume was 2 µL. The electrospray ionization (ESI) mode was used for MS/MS analysis, and two transitions including a quantifier ion (314 > 222, m/z) and a qualifier ion (314 > 116, m/z) were selected and optimized for selected reaction monitoring of kresoxim-methyl.

Preparation of solvent standard and matrix-matched standard solutions

Kresoxim-methyl standard was precisely weighed and dissolved in acetonitrile to prepare a stock solution at a concentration of 1000 mg/L. Solvent standard solutions were prepared at concentrations of 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01, 0.05, 0.02, and 0.001 mg/L with acetonitrile. Outer and inner clothing was cut into 11 parts (left and right upper arms, forearms, thighs and shins; front, back, pelvis/hip parts) and the cut clothes, gauze, and glass fiber filter were extracted with various volumes of acetonitrile (Table 1) by shaking at 300 rpm for 1 h in a shaker (SR-2w, Taitech, Japan). An aliquot (1 mL) of each extract was filtered through a 0.2-µm PTFE syringe filter (4 mm, Sartorius, Germany) to obtain a matrix extract. Matrix extracts for gloves and hands were prepared by washing with 1000 mL of 0.01% aerosol OT-75 solution and filtration. Finally, matrix-matched standard solutions (0.0025–0.01 mg/L) were prepared by mixing solvent standard solution (500 µL) with matrix extract (500 µL).

Instrumental limit of quantitation (ILOQ) and method limit of quantitation (MLOQ)

Aliquots of standard solutions (0.001–0.1 mg/L) were analyzed with HPLC or LC–MS/MS to determine the instrumental limit of quantitation (ILOQ). The method limit of quantitation (MLOQ) was calculated using ILOQ and injection volume.

Repeatability

Two levels (MLOQ and 10 MLOQ) of matrix-matched standard solution were consecutively injected seven times to determine the coefficient of variation (CV) values of peak area and retention time.
Linearity of calibration curves

To establish the calibration curve for HPLC, solvent standard solutions (0.5–10 mg/L) were analyzed, while for LC–MS/MS, solvent standard solutions (0.001–0.1 mg/L) and matrix-matched standard solutions (0.001–0.1 mg/L) were analyzed.

Matrix effects (MEs)

MEs (%) were calculated by comparing the slope of the matrix-matched standard calibration curve with that of the solvent calibration curve, using the following equation:

\[
\text{ME} (\%) = \left( \frac{\text{Slope of matrix matched calibration curve}}{\text{Slope of solvent only calibration curve}} - 1 \right) \times 100.
\]

Recovery test

For recovery tests, three levels of kresoxim-methyl standard solutions (MLOQ, 10 MLOQ, and 100 MLOQ) were fortified for each control sample of exposure matrix, and pesticide was extracted and analyzed as described in “Preparation of solvent standard and matrix-matched standard solutions” section (Table 1). All tests and analyses were conducted in triplicate.

Breakthrough test

To perform breakthrough tests, glass fiber filter and the XAD-2 resin tube were connected and air was passed through them at 2 L/min for 4 h with an air pump after 100 MLOQ of pesticide standard solution was spiked on the glass fiber filter (Fig. 1). After 4 h, the glass fiber filter and XAD-2 resin were extracted and analyzed.

Results and discussion

ILOQ and MLOQ

The ILOQ is the minimum amount of pesticide that can be quantified on an analytical instrument and is generally defined as a peak of signal-to-noise ratio > 9–10. The MLOQ is the minimum concentration of pesticide that can be reliably quantified considering the ILOQ and all sample preparation and analytical procedures [19, 20]. Pesticides on outer clothing were analyzed with HPLC because higher levels of exposure were predicted compared to inner clothing or other exposure routes such as gloves, hands, gauze, and glass fiber filter. The ILOQ for HPLC analysis was determined to be 2 ng, while that for LC–MS/MS of the inner clothing and other routes expected to comprise less exposure was set at 10 pg. Using the appropriate equation (ILOQ/injection volume), the MLOQ of outer clothing was determined to be 100 mg/L, while that of inner clothing, gloves, hands, gauze, and glass fiber filter was 0.005 mg/L.

Repeatability

Repeatability tests are usually performed to verify the stability of an analytical instrument [19, 20]. We repeated the analysis at two levels (MLOQ and 10 MLOQ) seven times using matrix-matched standard solutions and found

| Matrix                        | Extraction Bottle size (mL) | Volume (mL) | Solvent          |
|-------------------------------|-----------------------------|-------------|------------------|
| Outer clothing                | 1000                        | 500         | Acetonitrile     |
| Inner clothing                | 1000                        | 200         | Acetonitrile     |
| Glove washing solution        | 1000                        | 1000        | 0.01% of aerosol OT-75 |
| Hand-washing solution         | 1000                        | 1000        | 0.01% of aerosol OT-75 |
| Gauze                         | 125                         | 100         | Acetonitrile     |
| Glass fiber filter            | 50                          | 10          | Acetonitrile     |
that the CVs of peak area and retention time were lower than 6%, confirming the stability of the instrument.

**Linearity of calibration curves**

A calibration curve should have good linearity ($r^2$, correlation coefficients) in a certain concentration range in order to allow precise quantitative analysis [19, 20]. Calibration curves of solvent standards and matrix-matched standards showed good linearities ($r^2 > 0.998$), indicating that reliable quantification of kresoxim-methyl is possible (Table 2).

### Table 2  Linearity of calibration curves

| Instrument | Standard          | Part                      | Calibration curve | Linearity ($r^2$) |
|------------|-------------------|---------------------------|-------------------|------------------|
| HPLC       | Solvent standard  | –                         | $y = 69.3x + 0.34$ | 0.999            |
| LC–MS/MS   | Solvent standard  | –                         | $y = 6628.0x + 14,235$ | 0.999          |
|            | Matrix-matched    | Inner clothing            |                   |                  |
|            | standard          | Upper arm                 | $y = 6625.2x + 12,687$ | 0.999           |
|            |                   | Fore arm                  | $y = 6154.4x + 13,470$ | 0.999           |
|            |                   | Body (chest and stomach/back) | $y = 4392.2x + 15,553$ | 0.999          |
|            |                   | Pelvis/hip                | $y = 7962.6x + 6833.5$ | 0.999           |
|            |                   | Thigh                     | $y = 7.004x + 16,823$ | 0.999           |
|            |                   | Shin                      | $y = 4.254x + 3257.5$ | 0.999           |
|            |                   | Gloves                    | $y = 8546.3x + 14,571$ | 0.999           |
|            |                   | Hands                     | $y = 7310.3x + 6313.4$ | 0.999           |
|            |                   | Gauze                     | $y = 8635.4x + 2509.8$ | 0.999           |
|            |                   | Glass fiber filter        | $y = 7984.7x + 7504.3$ | 0.998           |

**Matrix effects (MEs)**

In ESI mode of LC–MS/MS analysis, unlike HPLC, the presence of matrix extract in analytical sample extracts either enhances or suppresses the peak height and area, thereby affecting quantitative results. The MEs depend on the chemical characteristics of the pesticide, the types of analytical instrument and matrix, and the methods of extraction and purification [19–21]. The ME (Fig. 2) showed a range of $-36$ to $31\%$, suggesting that matrix-matched standards must be used in LC–MS/MS analysis.
Recovery test

Recovery tests demonstrate the accuracy and precision of the method by confirming the recovery rate of the analyte in the sample according to established analysis methods [19–21]. A recovery test was carried out to confirm that the pesticide was extracted successfully from the exposure matrix by the extraction solvent, because reasonable recovery is necessary to obtain reliable quantitative analytical results. Recoveries from various matrices ranged from 72.7 to 105.6% (CV = 1.0–10.1%), indicating that the extraction efficiencies of acetonitrile were reliable (Table 3).

Breakthrough test

Since some pesticide can be escaped from glass fiber filter by air flow after trapping, breakthrough tests are used to measure the holding capacity of glass fiber filter for pesticide. In experimental design, an XAD-2 resin tube was connected to the glass fiber filter as a second adsorbent to trap any compound escaping from the glass fiber filter (Fig. 1). If the amount of pesticide adsorbed on XAD-2 resin exceeds 20% of the pesticide amount on glass fiber filter, pesticide measurements using glass fiber filter are considered unreliable [13, 17, 22]. There were no pesticides trapped in the XAD-2 resin in our samples, and the average recovery of kresoxim-methyl from glass fiber filter was 96.3% (CV = 7.8), indicating that the holding efficiency of glass fiber filter for kresoxim-methyl was suitable for use in field exposure experiments.

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Table 3 Recoveries of various matrices

| Matrix           | Spiked levels | 1   | 2   | 3   | Average (%) | CV (%) |
|------------------|---------------|-----|-----|-----|-------------|--------|
| Outer clothing   | MLOQ          | 82.9| 91.6| 86.9| 87.1        | 5.0    |
|                  | 10 MLOQ       | 90.4| 96.7| 94.7| 93.9        | 3.4    |
|                  | 100 MLOQ      | 99.4| 98.7| 93.1| 97.0        | 3.5    |
| Inner clothing   | MLOQ          | 96.4| 87.6| 84.1| 89.4        | 7.1    |
|                  | 10 MLOQ       | 95.4| 105.1| 92.7| 97.7        | 6.7    |
|                  | 100 MLOQ      | 102.6| 105.3| 100.0| 102.7        | 2.6    |
| Gloves           | MLOQ          | 70.0| 75.8| 72.4| 72.7        | 4.0    |
|                  | 10 MLOQ       | 88.9| 86.4| 85.6| 87.0        | 2.0    |
|                  | 100 MLOQ      | 82.0| 76.6| 70.7| 76.4        | 7.4    |
| Hands            | MLOQ          | 73.2| 76.9| 77.4| 75.9        | 3.0    |
|                  | 10 MLOQ       | 95.5| 93.9| 95.4| 95.0        | 1.0    |
|                  | 100 MLOQ      | 94.1| 92.6| 91.3| 92.7        | 1.5    |
| Gauze            | MLOQ          | 80.3| 78.8| 89.5| 82.9        | 7.0    |
|                  | 10 MLOQ       | 85.4| 92.9| 94.0| 90.8        | 5.1    |
|                  | 100 MLOQ      | 74.5| 81.0| 78.2| 77.5        | 4.2    |
| Glass fiber filter| MLOQ         | 72.8| 84.1| 80.5| 79.1        | 8.0    |
|                  | 10 MLOQ       | 102.8| 108.6| 105.3| 105.6        | 2.7    |
|                  | 100 MLOQ      | 78.5| 92.1| 95.3| 88.6        | 10.1   |
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