Liquid Chromatography

DETERMINATION OF KEPONE AND ITS METABOLITE IN WATER AND SOIL BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

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Kepone (chlordecone) is a persistent organic pollutant and may degrade to kepone alcohol. A fast and sensitive high-performance liquid chromatography–electrospray ionization–mass spectrometry (HPLC–ESI–MS) method using multiple reaction monitoring is reported for the accurate determination of kepone and kepone alcohol. Water and soil were enriched by liquid–liquid extraction and liquid–solid extraction. Good linearity was obtained between 1 and 100 ng/mL with \( r^2 = 0.997 \) for kepone and 30 and 200 ng/mL with \( r^2 = 0.996 \) for kepone alcohol. The recovery was in the range of 70% to 90%. This approach provided higher sensitivity compared to gas chromatography methods and avoided the formation of an artifact methanolic hemiketal.

**Keywords**: High-performance liquid chromatography (HPLC-MS); Kepone; Kepone alcohol; Mass spectrometry; Water and soil

INTRODUCTION

Kepone (chlordecone) is a chlorinated pesticide \( (C_{10}Cl_{10}O) \) that has been used in banana and sweet-potato cultivation in Europe, South America, Africa, and Asia (Amalric, Henry, and Berrehouc 2006). Kepone accumulates in the food chain, with potential chronic and acute toxicity, and is a carcinogen (Dolfing et al. 2012). Although its use is prohibited, kepone persists in the environment (Cabidoche et al. 2009; Brunet et al. 2009; Coat et al. 2011; Bocquené and Franco 2005). Kepone may undergo long-range transport and was declared a persistent organic pollutant by the 2009 Stockholm Convention (POPRC May 2009). Methods for kepone determination are primarily based on gas chromatography (GC) (Amalric et al. 2006;
Caplan, Thompson, and Hebb 1979; Carver, Borsetti, and Kamps 1978; Dabrowski and Waliszewski 1979; Deleon et al. 1980; Meus and Ernst 1979; Moseman et al. 1977; Stafford et al. 1978). George, King, and Claxton (1986) reported that kepone may be determined by high-performace liquid chromatography (HPLC), which tolerates higher sample loading and may avoid sample degradation (Harless et al. 1978; Cairns, Siegmund, and Doose 1982). In addition, HPLC–mass spectrometry (MS) typically exhibited higher sensitivity than GC–MS (Moriwaki and Hasegawa 2004), although the analysis may be affected by formation of a methanolic hemiketal.

Moriwaki and Hasegawa (2004) developed an HPLC–ESI–MS method for kepone ([M + H₂O–H]−), which was reported to be more sensitive than GC–MS, but was not employed for environmental samples (Chung and Chen 2011; Bristeau, Amalric, and Mouvet 2014). Recently, the method was reevaluated by our group for real sample analysis. Unexpectedly, in addition to the ion at m/z 507 [M + H₂O–H]− (Moriwaki and Hasegawa 2004; Fariss et al. 1982), an ion at m/z 521 [M + MeOH–H]− (Figure S1, Supplemental Content) was also observed by HPLC–MS when kepone was eluted in methanol/water. The methanolic adduct was also reported by Harless et al. (1978). Therefore, the reported HPLC–ESI–MS method needed improvement.

Kepone alcohol, which has been mistakenly recognized as an artifact in kepone analysis (Harless et al. 1978), has been considered to be a kepone metabolite (Fariss et al. 1982; Houston et al. 1981; Molowa eet al. 1986; Dilling and Dilling 1967). However, to our knowledge, no method is available for the determination of kepone and kepone alcohol in environmental samples. In this work, an improved HPLC–ESI–MS method is reported for these two compounds. The method was simple, rapid, and sensitive, and was applied for the analysis of water and soil.

**EXPERIMENTAL**

**Chemicals and Standards**

Kepone (98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Kepone alcohol was prepared by a method reported by Fariss et al. (1982). HPLC grade acetonitrile was obtained from Merck (Darmstadt, Germany), and deionized water was prepared by a Milli-Q purification system (Millipore, Bedford, MA, USA). Other chemicals and solvents were of analytical reagent grade. The water samples were collected in a creek at different days (samples W1, W2, and W3). The soil samples were collected in a farmland near the creek (samples S1, S2, and S3).

**Standard and Sample Preparation**

A stock standard solution of kepone or kepone alcohol was prepared by the addition of 1 mg of kepone or kepone alcohol into 10 mL of acetonitrile. The stock solutions were stored at 4°C, and diluted with acetonitrile to concentrations of 1 to 100 ng/mL for kepone and 30 to 200 ng/mL for kepone alcohol for working standards.

Creek water (100 mL) was extracted twice with 100 mL of chloroform, and the combined organic layers were dried over Na₂SO₄, filtered, and the solvent was
1 mL of acetonitrile was added to the residue which was centrifuged for 15 min at 12,000 rpm. The supernatant was analyzed by HPLC–MS. Soil (100 g) was ground, added to an open column (3.5 cm × 50 cm), and the analytes were eluted with 200 mL of acetonitrile and then processed in an identical fashion as the water. In order to evaluate the recovery of the sample preparation, 100-mL water or 100-g soil were fortified with kepone and kepone alcohol at 1.5, 2, and 2.5 times the concentration of its original samples.

**HPLC–MS Kepone and Kepone Alcohol**

The study was performed by an Acquity HPLC (Waters Co., Milford, Massachusetts, USA) coupled to a triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source. The desolvation and nebulizer gases were nitrogen. The desolvation gas flow was 600 L/h; the cone gas flow was 1 L/h. The nitrogen desolvation temperature was 600°C. ESI-MS was performed in negative ion mode with multiple reaction monitoring (MRM) with a cone voltage of 44 V and a capillary voltage of 1.5 kV. Transitions of m/z 507 to 427 (collision energy = 22 eV) and m/z 491 to 491 (collision energy = 2 eV) were employed for kepone and kepone alcohol determination, respectively. HPLC was performed on an Acquity HPLC BEH C18 column (2.1 mm × 50 mm i.d., 1.7 μm, Waters Associates). The mobile phase consisted of water/acetonitrile, 95:5, (v/v) (A) and acetonitrile (B) at a flow rate of 0.5 mL/min and the column was maintained at 40°C. A volume of 1 μL was employed for analysis. Solvent A was present at 100% from 0 to 0.5 min, changed linearly to 20:80 (A/B) over 3.5 min, and was varied linearly to 100% B (100%) over 3 min. After each analysis, the mobile phase was switched back to solvent A (100%) 0.5 min before the next injection.

**RESULTS AND DISCUSSION**

**High-Performance Liquid Chromatography–Mass Spectrometry**

Kepone is reported to form its hydrate in water, and hence the hydrate is determined by HPLC (Harless et al. 1978) (Figure S4, Supplemental Content) and electrospray ionization (Moriwaki and Hasegawa 2004). Kepone and kepone alcohol were separated (Figure 1) with a total analysis time of 7 min. Detailed examination of the total ion chromatogram (TIC) produced in methanol revealed several noteworthy features. Although Moriwaki and Hasegawa (2004) developed a HPLC–MS method with a limit of detection (LOD) of 0.007 ng/mL, the formation of the hemiketal was not considered (Figure S2, Supplemental Content). Therefore, acetoni- trile was employed for the analysis (Harless et al. 1978).

In the ESI–MS positive ion mode, no kepone peaks were present. However, ions corresponding to m/z 507 [M + H₂O–H]⁻ of kepone hydrate were observed in the ESI–MS negative ion mode (Figure 1). The [M + H₂O–H]⁻ ions of kepone hydrate were formed by the addition of water to neutral kepone and subsequently loss of H⁺(Figure S4, Supplemental Content). Therefore, kepone (hydrate) were determined in acetonitrile-water to eliminate the formation of the hemiketal in methanol/water. Kepone hydrate was separated on a reversed-phased C₁₈ column.
at a retention time of 3.78 min. Kepone alcohol was not detected in the positive ESI mode, but an ion corresponding to \( m/z \) 491 [M–H]– of kepone alcohol was observed in the negative ion mode (Figure 1). However, the parent ion at \( m/z \) 491 [M–H]– was not fragmented even using a high collision energy. Therefore, the transition at \( m/z \) 491 using a collision energy of 2 eV was employed for the detection of kepone alcohol.

**Validation**

The calibration curve for kepone was linear between 1 ng/mL to 100 ng/mL (Table 1). The calibration curve for kepone alcohol at concentrations from 30 ng/mL to 200 ng/mL was also linear (Table 1). The limits of detection (LOD) and quantification (LOQ) were 2 ng/mL and 30 ng/mL (Table 1), which were determined at signal-to-noise ratios of 3 and 10, respectively.
Table 1. Analytical figures of merit for kepone and kepone alcohol in water and soil by HPLC–MS

| Compound       | Calibration relationship | $R^2$ | Limit of detection (ng/mL) | Limit of quantification (mg/mL) | Recovery (%) | Relative standard deviation (%) | Linear dynamic range (ng/mL) | Concentration (ng/mL) | Sample |
|----------------|--------------------------|-------|----------------------------|---------------------------------|--------------|-------------------------------|-----------------------------|-----------------------|--------|
| kepone         | $y = 18.63x - 53.26$     | 0.997 | 0.5                        | 2                               | 70           | 7.4                           | 1–100                      | 0.57                  | water  |
| kepone         | $y = 18.63x - 53.26$     | 0.997 | 0.5                        | 2                               | 81           | 6.9                           | 1–100                      | 0.76                  | water  |
| kepone alcohol | $y = 19.93x + 98.19$     | 0.996 | 20                         | 30                              | 74           | 9.3                           | 30–200                     | 1.20                  | water  |
| kepone alcohol | $y = 19.93x + 98.19$     | 0.996 | 20                         | 30                              | 90           | 6.7                           | 30–200                     | 1.60                  | water  |
| kepone alcohol | $y = 19.93x + 98.19$     | 0.996 | 20                         | 30                              | 86           | 8.1                           | 30–200                     | 2.00                  | water  |
| kepone         | $y = 18.63x - 53.26$     | 0.997 | 0.5                        | 2                               | 79           | 9.6                           | 1–100                      | 0.54                  | soil    |
| kepone         | $y = 18.63x - 53.26$     | 0.997 | 0.5                        | 2                               | 73           | 7.2                           | 1–100                      | 0.72                  | soil    |
| kepone alcohol | $y = 19.93x + 98.19$     | 0.996 | 20                         | 30                              | 84           | 7.2                           | 1–100                      | 0.90                  | soil    |
| kepone alcohol | $y = 19.93x + 98.19$     | 0.996 | 20                         | 30                              | 77           | 9.4                           | 30–200                     | 0.90                  | soil    |
| kepone alcohol | $y = 19.93x + 98.19$     | 0.996 | 20                         | 30                              | 82           | 8.0                           | 30–200                     | 1.20                  | soil    |
| kepone alcohol | $y = 19.93x + 98.19$     | 0.996 | 20                         | 30                              | 88           | 8.3                           | 30–200                     | 1.50                  | soil    |
The recoveries for kepone and kepone alcohol in water and soil were investigated to assess the efficiency of the method. The intraday precision of the overall method was evaluated and expressed as relative standard deviation (RSD). The developed method had good accuracy and repeatability, with the recoveries between 70% and 90% with RSD values <9.6% (Table 1). Replicate determination \((n = 6)\) of kepone was performed at the concentrations between 57 and 95 ng/mL, while kepone alcohol \((n = 6)\) was determined between 90 and 200 ng/mL (Table 1).

The specificity was investigated to characterize matrix effects. Blank water and soil were analyzed by HPLC–MS. No positive chromatographic or mass spectrometric responses were observed at the retention times of the analytes.

**Figure 2.** Total ion chromatogram of kepone hydrate and kepone alcohol by multiple reaction monitoring (MRM) in water.

**Figure 3.** Total ion chromatogram of kepone hydrate and kepone alcohol by multiple reaction monitoring (MRM) in soil.
Water and Soil Analysis

In order to determine kepone and kepone alcohol, water, and soil were enriched one hundred-fold prior to HPLC–MS analysis. The water and soil contained kepone and kepone alcohol (Figure 2 and Figure 3, and Figures S5 and S6, Supplemental Content). In the water, kepone was detected at 0.44 to 0.46 ng/mL, and kepone alcohol was detected at 0.9 to 1 ng/mL. In the soil, kepone was present between 0.36 and 0.41 ng/g, and kepone alcohol was present between 0.6 to 0.8 ng/g. Harless and co-authors (1978) reported that kepone was present at 0.15 to 3.38 ppm (µg/mL) in water and 1.31 to 13250 ppm (µg/mL) in soil by GC–MS. Amalric et al. (2006) reported a limit of quantification of 1 mg/kg for kepone in soil. The results indicate the developed method was more sensitive than previously reported procedures.

CONCLUSIONS

A high sensitive HPLC–MS–MS method was developed for the determination of kepone and kepone alcohol in water and soil using multiple reaction monitoring. Kepone alcohol was present at approximately twice the concentration of kepone in these environmental samples, suggesting the former is a metabolite of the latter (Fariss et al. 1982; Houston et al. 1981; Molowa et al. 1986; Dilling and Dilling 1967).

FUNDING

This work was financially supported by grants from the 2010 Technological Talents Program of the Chinese Academy of Sciences and the National Natural Science Foundation (No. 21302180) of China.

SUPPLEMENTAL MATERIAL

Supplemental data for this article can be accessed on the publisher’s website at http://dx.doi.org/10.1080/00032719.2014.930867.

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