# Comparison of Sample Extraction Methods for the Determination of Bis(*p*-chlorophenyl)dichloroethylene (*p,p'*-DDE) in Rice Flour Using Isotope Dilution Mass Spectrometry

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The determination of pesticide residues in agricultural products and food is great public and regulatory concerns. It is well known that the measurement results of the pesticides show a strong dependence on the extraction method used and following sample clean-up methods employed for the analysis.[1] In this respect, isotope dilution mass spectrometry (IDMS) has been wildly accepted as a reliable analytical method for the accurate determinations of trace organic compounds in complex matrix as the method overcomes difficulty of correcting recovery yield in sample preparation and separation. Therefore, the IDMS method makes the measurement results traceable to the SI units directly without significant empirical correction factors.[2-4]

For usual pesticide analysis, an ideal extraction method should yield quantitative recovery of target analytes without loss or degradation.[5] The same criteria are applied to the sample clean-up processes following the sample extraction. In IDMS method, isotope labeled analogues of target analytes are spiked to sample as internal standards before the sample pretreatment. The basic idea of the IDMS method is that a target analyte and its isotope labeled analogue have same recovery yield in sample preparation and separation. The idea is usually well applied to the sample clean-up processes such as solid-phase extractions, chromatographic separation using a gel permeation chromatograph or a preparative LC column, and concentration by gas purging or vacuum evaporation.[3-4] However, equal recovery of a target analyte and its isotope labeled analogue in sample extraction processes is not simply guaranteed and must be addressed before applying the IDMS method for the specific sample type and the target analyte.[1] The issue of equal recovery for the analyte and its isotope analogue is especially important when the sample is in solid form or biological materials. Appropriate sample extraction and clean-up methods must be employed to make the externally spiked isotope labeled analogue have equal recovery with the native target analyte which is already captured inside solid sample particles or bounded to functional sites of biological materials. The equal recovery for the analyte and its isotope analogue can be achieved if the two compounds results in a complete equilibrium before the isolation from the sample matrix.[4,6]

We are currently preparing rice flour certified reference material (CRM) for the analysis of pesticide residues. The IDMS methods are chosen as a primary certification method. In this letter, we report the intercomparison results of several sample extraction methods with variable extraction conditions for the determination of chlorinated pesticide residues in the rice flour CRM using the IDMS method. The rice flour CRM were prepared three years ago by spraying appropriate amounts of several chlorinated pesticides. The CRM candidate material was then homogenized, bottled in 500 g unit, and sterilized by irradiation of 20 kGy $\gamma$-ray. *p,p'*-DDE was chosen as a target analyte, which is considered to represent chlorinated pesticides.

Rice flour from a single CRM bottle was used for this study. 10 g (5 g for super critical fluid extraction) of sample was taken into an appropriate apparatus that was directly used for the selected sample extraction method. About 0.7 mL (0.35 mL for SCF extraction) of a *p,p'*-DDE-$^{13}C_{12}$ ($^{13}C$-labeled *p,p'*-DDE in two benzene rings) standard solution, 2 $\mu$g/g in 2,2,4-trimethylpentane, was spiked to sample. The amount of the internal standard solution to be spiked was determined to make the isotope ratio for the analyte in the spiked sample to be near 1:1. The sample was then extracted by one of extraction methods list below. The extract was further cleaned up. Water in the extract was removed by adding excess amount of anhydrous sodium sulfate when it was necessary, and the extract was concentrated to 1 mL. The oil matrix was removed by using a gel permeation chromatography (10 mm I.D. column packed upto 150 mm height with Bio-Bead SX-3 with 200-400 mesh from Bio-Rad Laboratories), and by using a solid-phase extraction cartridge (Silica, 500 mg from Waters). The final extract was concentrated to an appropriate volume and analyzed by GC/MS in comparison with a calibration standard mixture containing known amount of *p,p'*-DDE and *p,p'*-DDE-$^{13}C_{12}$ in 1:1 ratio. The mass spectrometer selectively monitored ions at m/z 318 and at m/z 330 for the detection of *p,p'*-DDE and *p,p'*-DDE-$^{13}C_{12}$.

The followings are list of extraction methods and their conditions tested in this study. In our preliminary test, acetonitrile and acetone showed fairly good recovery for *p,p'*-DDE in the rice flour sample compared to other solvents. Thus, acetonitrile was employed for all types of solvent extraction methods in this work.

1) For solvent extraction assisted by sonication using

**Table 1**. Comparison of IDMS measurement results of *p,p'*-DDE in a rice flour CRM using several extraction methods

| Extraction Method[a] | Observed Concentration (ng g)[b] |
|---|---|
| Solvent ($CH_3CN$) extraction assisted by sonication (2 hours) | 130 ± 5 |
| $CO_2$ Supercritical Fluid Extraction (SFE) | 139 ± 4 |
| Soxhlet ($CH_3CN$), t – 20 hours | 171 ± 2 |
| ASE ($CH_3CN$, 2000 psi, 120 °C) static time – 5 minutes | 171 ± 2 |
| static time – 10 minutes | 170 ± 2 |
| Solvent ($CH_3CN$) extraction with refluxing at boiling point, t – 2 hours | 168 ± 3 |
| t – 4 hours | 170 ± 2 |
| t – 10 hours | 171 ± 2 |
| t – 20 hours | 170 ± 2 |

[a]See Text for details on the conditions of each extraction method. [b]The numbers after "±" are the expanded uncertainties of the proceeding IDMS results. The uncertainties are mostly attributed to the standard deviation of 4 replicate IDMS measurement results.

Branson 5200 Sonication Cleaner, sample and 100 mL of acetonitrile were taken into a flask, spiked with the internal standard solution, and sonicated for 2 hours.

2) For solvent extraction with refluxing the solvent, sample was taken into flat-bottomed flask equipped with water-cooled condenser on the top of it, and acetonitrile and the internal standard solution were added into the flask. The solvent was mildly heated up to its boiling point while the contents inside the flask were well stirred by a magnetic bar. The durations of reflux extraction tested were 2, 4, 10, and 20 hours.

3) For accelerated solvent extraction (ASE), acetonitrile was pressurized to 150 bar at 120 °C. The IDMS results were obtained for 5 and 10 minute static extraction times.

4) Soxhlet extraction was done with acetonitrile for 20 hours.

5) For supercritical fluid extraction (SCF), $CO_2$ supercritical fluid (60 °C, 200 bar) was used. The static extraction time was near 20 minutes and the following dynamic extraction was done for 40 minutes at 1.0 mL/min flow rate.

The IDMS measurement results with the selected sample extraction methods were listed in Table 1. Each value is the mean of 4 replicate analytical results. SFE and solvent extraction with sonication show relatively lower observed concentration compared to the other extraction methods. The lower measurement results from the two extraction methods are attributed to the inefficient recovery of the native target analyte from sample compared to the spiked internal standard. The IDMS measurement results with 20 hours of soxhlet extraction, ASE, solvent extraction with reflux for more than 2 hours agree together within the measurement uncertainty. The results from ASE did not change when the static extraction time changed from 5 to 10 minutes and the two results are in good agreement with the results from the soxhlet extraction, indicating that the target analyte and the internal standard reached to equilibrium within 5 minutes and that they showed the same recovery. The results from solvent extraction with reflux for variable durations also show that the two compounds were in equilibrium after at least 4 hours of refluxing.

In conclusion, it is demonstrated that using a proper extraction is very important to get an accurate and bias-free analytical results even with using IDMS methods. For the analysis of rice flour, ASE, 20 hours of soxhlet extraction, and solvent extraction with refluxing for more than 4 hours can give equal recovery for native *p,p'*-DDE and spiked *p,p'*-DDE-$^{13}C_{12}$, which makes the IDMS results traceable to SI unit. Thus, the IDMS methods with using one of those verified extraction methods can be used for the certification of the chlorinated pesticides in rice flour CRMs, whose certified values can be used to test the validations of any analytical methods currently in use or newly developed.

## References

1. Quevauviller, Ph. *Trends Anal. Chem.* **1998**, *17*, 289.
2. Dube, G.; Henrion, A.; Richter, W. *Metrologia* **1997**, *34*, 83.
3. De Leenheer, A. P.; Thienpont, L. M. *Mass Spectrom. Rev.* **1992**, *11*, 249.
4. Lawson, A. M.; Gaskell, S. J.; Hjelm, M. *J. Clin. Chem. Clin. Biochem.* **1985**, *23*, 433.
5. de Castro, M. D. L.; Jiménez-Carmona, M. M. *Trends Anal. Chem.* **2000**, *19*, 223.
6. Welch, M. J.; Cohen, A.; Hertz, H. S.; Ng, K. J.; Schaffer, R.; van der Lijn, P.; White, V. E. *Anal Chem.* **1986**, *58*, 1681.
7. Kim, D. H.; Heo, G. S.; Lee, D. W. *J. Chromatogr. A* **1998**, *824*, 63.