

## Feasibility for diethylhexyl phthalate(DEHP) analysis in water samples using online and offline SPE-LVI-GC-MS

Suk-Young Hong<sup>1\*</sup>, Jong-Yeon Hwang, Ki-Joon Kim<sup>1</sup>, Jin-Seon Lee<sup>1</sup>, Min-Seob Kim<sup>1</sup>, Suk-Hee Yoon<sup>1</sup>, Oh-Sang Kwon<sup>1</sup>, Won-Seok Lee<sup>1</sup>

<sup>1</sup>National Institute of Environmental Research, 42 Hwangyeong-ro, Seo-Gu, Incheon, Republic of Korea

### Introduction

Primary methodologies for the analysis of semi-volatile organic compounds (SOCs), especially for phthalates, are currently useful for solid phase extraction (SPE) or liquid-liquid extraction (LLE). Even LLE method for phthalates had been established in general<sup>3</sup>, the method of SPE is little available. Phthalates are lately categorized as environmental toxic compounds from various sources such as plastics, glues, carpet, spray, paints, and so on. Because of its low solubility in water and volatility, it exists on the particle surface and transfers into water and air easily<sup>2</sup>. Based on the characteristics of phthalate, SPE using C18 cartridge might have better efficiency of the separation than LLE for extracting from complicated matrix. In addition, advanced online system can be applied with SPE technique and combined with GC-MS for online system. Unfortunately methodologies for online SPE-GC-MS are only few. Therefore this study has purposes to develop methods using automated SPE, large-volume-injector, and GC-MS individually and to fortify the specific method of online SPE-LVI-GC-MS for diethylhexyl phthalate (DEHP) analysis followed by method validations.

### Materials and methods

#### Chemical standards and reagents

An internal standard (ISTD) and phthalate standard were purchased from AccuStandard (New Haven, CT, USA) and Supelco (North Harrison Road Bellefonte, PA, USA). The ISTD of 100 $\mu$ g/mL concentration contained fluoranthene-d10 that was used to monitor the injections. Phthalate standard at 1000 $\mu$ g/mL contained diethylhexyl phthalate (DEHP). Both are first prepared and diluted in methanol for further stock solutions. High purity methanol (MeOH), dichloromethane (DCM), acetone, and ethyl acetate (EtAc) solvents were obtained from Kanto (chuo-ku, Japan). Water was obtained from a Milli-Q Ultra-pure water treatment purification system (Millipore, Bedford, MA, USA)

#### Instrumentations

##### *Automated SPE*

Automated SPE has a key principle for the analysis of phthalate in online and offline SPE-GC-MS system. Symbiosis<sup>TM</sup> Environ SPE system (SPARK Holland, Netherlands) was utilized in this study and its components are described in Fig 1. In detail, Symbiosis<sup>TM</sup> Environ SPE system consist of four modules including Triathlon (used only for offline) which is an autosampler, HPD (High Pressure Dispenser) that is able to control injections and flows of sample, ACE (Advanced Cartridge Exchanger) to perform actual extractions through various cartridges, and Pump extracting analytes by solvents from cartridges and transferring to LVI-GC-MS. Due to a small cartridge volume (2X10mm) packed by 20mg sorbents with 7~10 $\mu$ m particle sizes, Automated SPE only require smaller volumes of sample and solvents than current manifold or semi-automated SPE equipments. Furthermore it is definitely able not only to reduce the pretreatment time and the experimental error but to increase the reproducibility<sup>5</sup>.

##### *Large-volume-injector (LVI) and GC-MS*

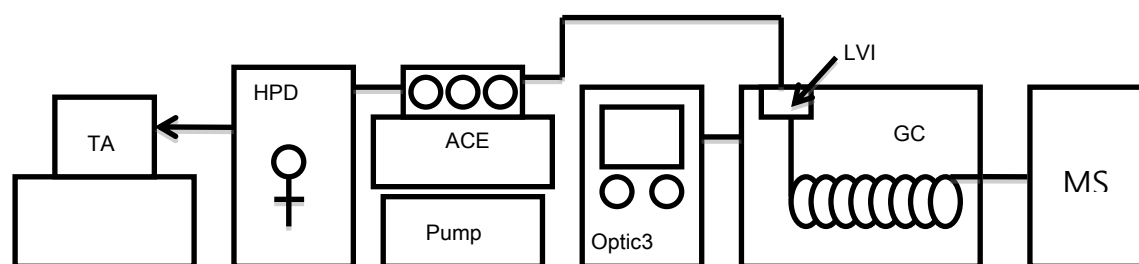
Online SPE-LC-MS system has a little easy to set up the system and to develop the method because extraction solvents in SPE are similar to mobile phases in LC. Unlike this system, online GC-MS require specific equipments to transfer liquid sample into the injector which is specially designed for large volume<sup>7</sup>. LVI (Optic

3 ATAS GL, Netherlands) that is able to accommodate upto 150µl was used in this study. Not only the evaporation of transferred samples but also major conditions including carrier flow, split flow, temperature, etc. can be controlled by unique software for better method development. Perkin Elmer Clarus 600 GC-MS system was involved to analyze phthalate in water samples and validated prior to the method validations.

#### Offline and online methodology

As an approach for setting up specific methods, the method development must be followed by the method validation. However, according to the purpose of this study, the specific method for offline system should be completed first and it could enlarge into online system finishing with validation procedures.

As shown in Fig 1, the system configuration of offline are only automate SPE and GC-MS in respective.



**Fig. 1** Online and offline SPE-LVI-GC-MS configuration for phthalate analysis; TA: Triathlon autosampler (only for offline); HPD: High pressure dispenser; ACE: Advanced cartridge exchanger; LVI: Large-volume-injector

#### Calibration and quantitation

For the quantitation of analytes, commercial standard solutions of ISTD and DEHP were diluted in methanol which is the stock solution. For offline methodology of method development, the stock solution was diluted once more to establish standard calibrators ranged 0.2 to 1.0µg/ml for direct injections. For online system standard calibrators for calibration curve made by spiking different volumes of stock solution into 100ml Milli-Q water. QC and alternative samples were prepared with the same to calibrators. Therefore concentrations of calibrators and QCs in water were ranged from 5 to 55ng/ml and extracted through automated SPE with concentration factor of 100. Since this study has concentrated on methodologies of offline or online SPE-GC-MS, any field samples were not performed.

## Results and discussion

### *Method developments of automated SPE and LVI*

For setting up SPE method in offline automated system, three major steps had been performed such as manual SPE procedure. First the screening process to select the cartridge was performed using HySphere™ cartridge kit for method development including various types of polar and nonpolar. It can be easily selected by acquiring appropriate peak shape after injecting standard solutions. As a result, PLRP cartridge for DEHP had a good separation and this result is same with several articles<sup>4, 5, 6</sup>. Secondly, once the cartridge was chosen, the solutions for cartridge equilibrium and solvation must be determined in order. In this study, solutions of methanol and water had good results for activating and conditioning PLRP cartridge. The last step is to choose the elution solution that is able to extract analytes adsorbed onto cartridge. Pure acetone and mixture with ethyl acetate were dominated as candidates but mixture of acetone and ethyl acetate (90:10, v/v) was even better than pure acetone for the elution solvent<sup>4, 6</sup>. Using this method in offline automated SPE, three spiking levels of extracted samples were tested for recovery and concentration effect. Mean recovery and repeatability (R.S.D.) were 90.9 and 13.1%. S.T. Yang et al<sup>2</sup> presented its recovery for manual SPE and LLE were 77.5 and 88.5%. It indicates that phthalate recovery of automated SPE is better than manual SPE as well as LLE.

For LVI method, several parameters were identified in respect of sample transfer from automated SPE to GC-MS. They are injector temperature, column flow, split flow, separation time, equilibrium and vent time, etc. Due to the characteristics of LVI which is able to accommodate upto 150µl of sample volume, transferred volume of

sample could be controlled around 2.5 $\mu$ l by these parameters that had been sustained to the end of GC-MS running.

#### Method development of GC-MS

According to the analytical method established from Ministry of Environment in Korea, standard solution (0.1 $\mu$ g/ml) was injected in direct and Fig. 2 show analytes information including fragmented ions and retention times. Method development of GC-MS compared the result of ion modes that SIM has better than TIC with lower R.S.D. of repeatability and higher  $r^2$  resulted from five different spiked levels.

#### Method validation of online SPE-LVI-GC-MS

Based on offline methods developed in automated SPE, LVI, and GC-MS respectively, the method validation of online system was performed. Not only six standard calibrators but also several spiked levels were prepared daily for three-day validation. They were all extracted by automated SPE and automatically transferred into GC-MS for quantitations of Fluoranthene-d10 as an internal standard and DEHP. Chromatograms for ISTD and DEHP are shown in Fig. 2 and retention times were 15.8 and 20.1min. In addition, blank did not contain any analytes that is used to be detected even in Milli-Q water and proved that the online system can be totally compatible for phthalate analysis.

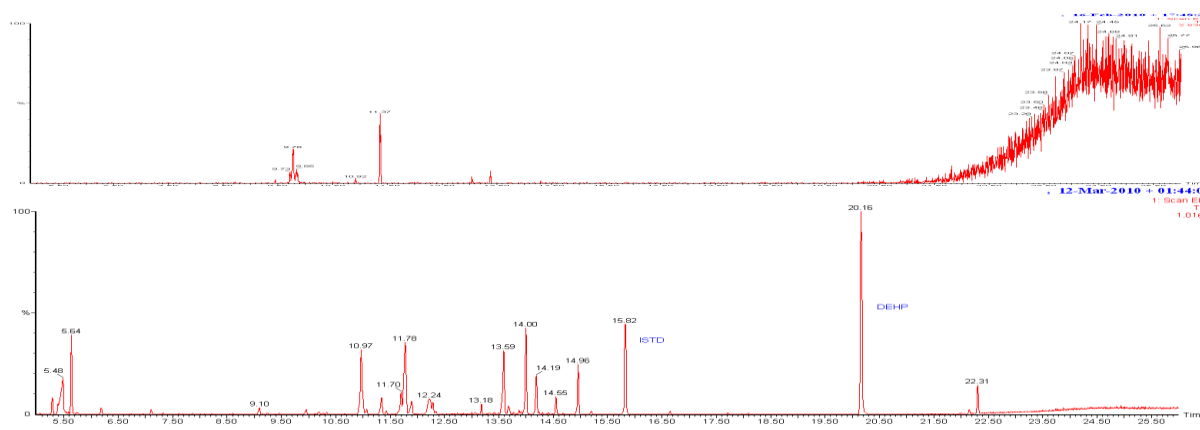
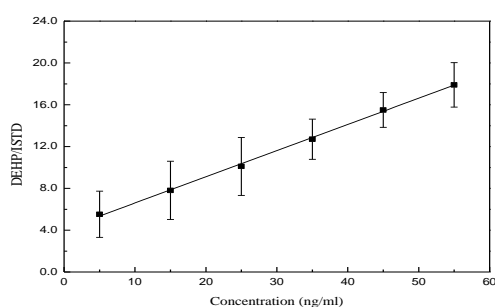


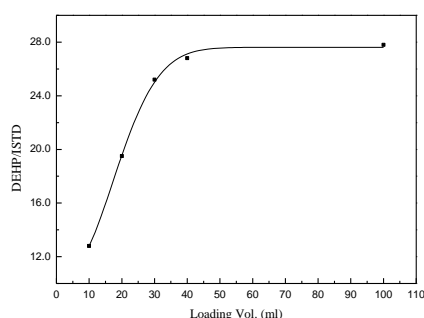
Fig. 2 Chromatograms of blank and spiked sample (55ng/ml)

First, Limit of detection (LOD) and limit of quantitation (LOQ) for online system were completed by diluting lower level of QC samples and calculated as 0.68 and 2.26ng/ml in respective. The calibration range was determined due to automated SPE method and LOD/LOQ. Fig. 3 indicates acceptable linearity ( $r^2=0.999$ ) and error range for each point during three days. The slopes from three calibration curves had variations of 5.45% R.S.D. meaning that quantitations of DEHP could be accepted in appropriate. The average recovery was 93.6% with 13.1% R.S.D. for all extracted samples that is rather better than method development (90.9%) but less accurately with higher R.S.D. on offline system. In respects of system reproducibility for three days, Inter- and Intra-day repeatability were determined using two spiked levels (37.5 and 18.8ng/ml). R.S.D. of Inter- and Intra-day repeatability are 8.46 and 6.48% with deviations of 99.7 and 85.2% compared on target concentrations. Instrumental precision must be considered as one of validation procedures and was completed with one spiked level (36ng/ml). Deviation and R.S.D. of instrumental precision are 103 and 5.28% representing that online system is quiet precise for DEHP analysis in water. The accuracy of online method was calculated from one spiked level (37.5ng/ml) and its deviation is 103% with 6.35% R.S.D. concluded that the method is particularly accurate. The sensitivity test completed to check how the online system could be performed and what the stable range can be available. Therefore five spiked levels in one concentration (35ng/ml) had analyzed for each day prior to other samples running. Its deviation and R.S.D. values are 108 and 10.5% meaning a little high but still acceptable in processing of method validations.

Finally the concentration effect was validated for setting up the actual sample amounts on automated SPE. Five different samples with the same concentration but different total volumes were prepared and analyzed to determine the appropriate sample volume. Fig. 4 indicates no critical change between 40 and 100ml of sample loadings but the range from 10 to 40ml presented a proportional linear. According to the respects of automated SPE and sample pretreatment, 40 or 50ml of sample loading volume could be optimized for this study. It is successful alternatives to increase injection volumes or quantitative sensitivity which are able to damage online system.



**Fig. 3** Calibration curve and error range for online system ( $r^2=0.999$ , slope=0.249)



**Fig. 4** Concentration effect according to sample loading volumes

## Conclusions

Offline SPE-GC-MS and online SPE-LVI-GC-MS have been successfully applied to the method development and validation for DEHP analysis in field water samples. Several characteristics or verifications of this methodology are summarized as followings. First, methods for automated SPE, LVI, and GC-MS have been specifically established and validated. Based on developed methods in respective, method validation of online SPE-LVI-GC-MS concluded that LOD and LOQ are 0.68 and 2.26ng/ml regarding to 100 folds concentration of SPE. Also the recovery resulted in range of 80.5 to 107% even with acceptable R.S.D. (13.1%). Accuracy and precision are 103% both with 5.28 and 6.35% R.S.D. The sensitivity of online system has 108% of deviation and 10.5% of R.S.D. As a result of SPE concentration, sample volume could be considered below 50ml and it compares to manual SPE or LLE required large volume over 200ml. This study verifies specific method for DEHP analysis in field samples using online SPE-LVI-GC-MS.

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