CHAPTER V

CONCLUSION

Various sample preparation methods for the analysis of trace phthalate esters (PEs) in milk were studies. The newest medium of solid phase extraction (solid phase extraction membrane,SPEM) was introduced during the study. Two concentration levels of PEs in milk were used: 0.0500 mg/kg (low concentration) and 5.0000 mg/kg (high concentration) against the blank milk.

SPEM had several advantages over SPE cartridge, including high cross-sectional area. The coupling of a glass microfiber prefilter with SPEM decreased the chance of plugging of the matrix. There were four easy and low time consumpting sample preparation methods were prepared.

The first method was direct sample application through SPEM which had been coupled with a glass microfiber prefilter. Ethyl acetate was the solvent used to elute the PEs from C_{18} SPEM. The results shown that the time requirement for 25 mL milk sample was approximate 48 min including time for clean up also. The percent recoveries and precisions of each PEs were 57.37 ± 29.86 to 114.66 ± 8.87 and 66.36 ± 20.30 to118.95 ± 5.86 μg/kg when detected by GC-FID and GC-ECD respectively, and the high concentration spiked levels of standard mixture PEs were 11.03 ± 17.56 to 76.00 ± 1.95 μg/kg by GC-FID detection. The method detection limit of PEs were also presented: 41.44, 33.48, 26.80,21.88, 15.75 and 25.50 μg/kg and 41.44, 33.48, 26.80, 16.41, 10.50 and 20.40 μg/kg for GC-FID and GC-ECD as a sequence of DMP, DEP, DBP, BBP, DEHP and DOP respectively.

The matrix could be diluted with solvents that promote the interaction chosen for isolate retention. In this case, the isolate to be retained by non polar interactions, the matrix should be made as polar as possible. Milk could be

diluted with water. For this reason, the second method was a diluted milk sample with four times dilution and then applied through SPEM as in the first method. This method took longer time than the first method, approximately one hour.The percent recoveries and precision were 86.62 ± 4.97 to 136.12± 18.34 and 93.58 ± 6.73 to 170.80 ± 0.20 when were detected by GC-FID and GC-ECD respectively and 31.37 ± 4.14 to 74.34 ± 4.34 for high concentration spiked levels. The method detection limit of PEs were better results than in the first method, these were 35.52, 27.90, 21.44, 16.41, 10.50 and 20.40 μg/kg and 35.52, 27.90, 21.44, 10.94, 5.25 and 15.30 μg/kg as a consequence of DMP, DEP, DBP, BBP, DEHP and DOP which were detected by GC-FID and GC-ECD respectively.

As in the first method and the second method, the third method and the fourth method were prepared, but differed in the use of a glass bead filter aid. This was coupled with SPEM and the microfiber prefilter in order to help with sample containing solid particles. The third method was 25 mL milk directly passed through these three layers. This method took about 50 min, and the percent recoveries with precision of PEs were 56.49 ± 3.13 to 128.68 ± 7.55 and_71.97 ± 6.77 to 176.83 ± 7.38 when were detected by GC-FID and GC-ECD respectively. For high concentration spiked levels, the percent recovery and precision were 12.69 ± 4.58 to 79.75 ± 0.91 which was detected by GC-FID. The method detection limit of this method for DMP, DEP, DBP, BPP, DEHP and DOP were 41.44, 33.48, 46.80, 21.88, 15.75 and 25.50 by GC-FID and 41.44, 33.48, 26.80, 16.41, 18.50 and 20.40 by GC-ECD respectively.

The fourth method used the diluted milk similar to the second method, but the filter aid was also applied. The results were about 64 min time consumption, and the percent recoveries and precision were 88.99 ±2.95 to 103.10 ± 8.16 and 74.09 ± 1.30 to 126.21 ± 4.72 for low concentration spiked level of PEs when were detected by GC-FID and GC-ECD respectively. For the high concentration spiked level, it was 16.36 ± 1.32 to 87.66 ± 2.36 percent

recoveries and precisions. The method detection limit of DMP, DEP, DBP, BBP, DEHP and DOP were 35.52, 27.90, 21.44, 16.41, 10.50 and 20.40 by GC-FID, and 35.52, 27.90, 21.44, 10.94, 5.25 and 15.30 by GC-ECD.

All of the simple methods (1-4) provided satisfactory results of percent recovery and precision, and the diluted sample gives better results than non diluted sample. The use of Filter aid gave better precision, but a slightlyt longer time consumption.

Not only four simple methods but also sample pretreatment methods were studied. Following the classical sample preparation, many techniques were included with SPEM to prepare milk for determination of PEs.

The fifth method used liquid-liquid extraction to dissolve the PEs in the solvent phase followed by dilution and elution through SPEM. In this method, SPEM was classified as a matrix removal and concentration device. It gave rather bad results because it not undergo a clean up step. The high background was also present when C_{18} SPEM was used to be a matrix removal device for 25 mL milk as a sample. It could be inferred that lipids could be adsorbed with C_{18} similar to PES.

In the sixth, seventh and eighth methods the liquid phase and milk solid were separated before being applied through SPEM. Forthermore, higher sample volume could be used in these methods.

The sixth method, 50 mL of milk was diluted to 100 mL (dilution factor equal two) and then adjusted to pH 2.10 with hydrochloric acid and sodium hydroxide. After that, it was centrifuged. The lipid phase was passed through the SPEM, and the solid phase was sonicated with ethanol and ethyl acetate. The solution was separated from the solid phase and passed through SPEM The time consumption for this method was approximate 63 min and the percent recoveries and precision were 78.74 ± 3.06 to 129.65 ± 4.24 and 63.96 ± 2.95 to 117.95 ± 1.73 μg/kg for low concentration level by GC-FID and GC-ECD, and 27.43 ± 2.34 to 85.59 ± 1.55 for high concentration level by GC-

FID. The method detection limit for DMP, DEP, DBP, BBP, DEHP and DOP were 17.76, 16.74, 10.72, 10.94, 5.25 and 10.20 (detected by GC-FID) and 17.76, 16.74, 10.72, 2.75, 1.31 and 5.10 μg/kg (detected by GC-ECD).

This study used two methods to separate the liquid phase and solid phase of milk. These were mechanical and solid precipitation by chemicals.

The apparance of milk solid which was seperated by centrifugation was fine, but the appearence of solid which was seperated by chemical precipitation as clotting precipitate.

The seventh method used the centrifugation to separate the milk solid. Higher volume of sample could be applied such as 100 mL of milk, and longer time is required. The results of percent recoveries and precisions were 32.78 ± 11.29 to 99.99 ± 15.35 and 30.85 ± 80.86 to 67.07 ± 19.41 μg/kg for low concentration level and 6.44 ± 31.04 to 75.96 ± 1.24 for high concentration level of PEs and the method detection limit of DMP, DEP, DBP, BBP, DEHP and DOP were 23.84, 16.74, 10.72, 16.41, 10.50, and 15.30 μg/kg by GC-FID and 23.84, 16.74, 10.72, 10.94, 5.25 and 10.20 μg/kg by GC-ECD detection respectively.

The eighth method was prepared by chemical precipitation to separate the milk solid and liquid phase. The time requirement of this method was approximate 84 min and the percent recoveries and precisions were 45.62 ± 12.65 to 88.45 ± 22.86 and 6.56 ± 15.52 to 122.30 ± 8.99 for low concentration levels of PEs which were detected by GC-FID and GC-ECD respectively. At high concentration levels this method shown 7.91 ± 40.87 to 64.41 ± 30.81 of percent recoveries and precision of PEs.

The results of high concentration spiked level shown that every method gave less satisfactory than the low concentration spiked levels. This was due to the two factors: the first was the low solubility of PEs in water but high solubility in fat so that could be lost with lipids, the second was the

capacity of SPEM. The lower molecular weight PEs could loss because it had weaker interactions with C_{18} than higher molecular weight PEs.

The sixth method gave the most satisfactory results and precision because of the adjustment of the ionic strength of PEs and matrix.The use of sonication helped to dissolve PEs into the solvent. By these reasons, this method was used to study real samples and could be applied to other dairy products.

Six samples: two milk samples and four yogurt samples, were collected from various markets and the analyzed by sample preparation method six. DEHP were found in all samples in range of 10 - 30 μg/kg which were detected by both GC-FID and GC-ECD. Some PEs, such as BBP and DBP were only found when GC-ECD was used. All of the results were confirmed by GC-MSD. We could suggested that the plasticized plastic packaging has a chance of plasticizer leaching.

This study presented the choice of sample preparation methods. Both pretreatment and non pretreatment were prepared with SPEM. The diluted milk sample were found to be more satisfactory results. The easy methods could be used with SPEM and gave quite satisfactory results. For the other dairy products, the sixth method could used with them, and this method also gave quite satisfactory results .

In expansion, the sample preparation methods which were prepared in this study can be modified to determinations of the other contaminants in dairy products. Especially, in the case of intermediate polar and polar substances in dairy products can be easily determined due to it is not adsorbed on lipids. In this case, the optimum sorbents, eluting solvent will be considered.

Recommendation

According to the study of phthalate esters in milk samples, we may suggest better solution procedures for determination PEs in matrix samples.

1. We can lower the MDL by decreasing sample volume and final volume extraction to gain concentrated and low background.

2. The alternative clean up method such as size exclusion mechanism can be used replace Florisil clean up.

3. In order to measure sample loss, internal standard should be spiked with sample in detection procedure. The percent recovery and precision will be improved as a consequence