

PESTICIDE RESIDUE ANALYSIS IN FRUIT JUICES BY USING NON USING NON CONVENTIONAL METHOD

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Solid phase extraction method in combination with Gas Chromatography – Electron Capture Detector (GC-ECD) was used for the extraction and quantification of aldrin, butachlor, chlordane, dicofol, and alpha endosulfan pesticides. The results showed higher responses of the pesticides after addition of aliquots of water and an organic solvent to the fruit samples. The extracting solvent consisting of a mixture of methanol/acetonitrile (1:1) when added to the sample in the proportion of 2% volume/weight (v/w) showed the best recovery, resulting in more than 80% recovery of the pesticides for all the investigated fruit samples. Relative standard deviations (RSD) for triplicate analyses of samples were less than 10%. Solid phase extraction techniques has been shown to be a simple extraction technique, which has a number of advantages such as, simplicity and capability with the chromatographic analytical system.

KEYWORDS : Solid phase extraction, pesticide, vegetables, fruits.

INTRODUCTION

During last few decades numerous improved methodology for the analysis of pesticide residues in fruit and vegetables. In addition, it is also intended to further the discussion on how to meet the difficulties in pesticide residue analysis and the future trends in chemical analysis of pesticides. The divergent chemical and physical properties of pesticides and the awareness of their toxicity put great demands on analytical techniques to identify and detect low levels of residue. Furthermore, since the varieties of pesticides are applied on very different commodity classes, from aqueous samples to samples with high fat content, the development of multi residue methods becomes an analytical challenge.

Despite the versatile application to many different types of crops and pesticides, this clean up techniques is a bottleneck in the method. The use of organic solvents and subsequently evaporation and re-dissolving steps will make the sample throughput limited. Therefore, the use of solid phase extraction (SPE) columns has been an alternative procedure of continuing

increased interest. Application to matrices in non polar solvents is a new approach. The columns has, surprisingly, been found to retain different types of pesticides with as good efficiency as under polar solvent conditions. The retention mechanism cannot be fully explained, since II-II interaction of semi-polar compounds, presumably, even OH groups are attached on the surface. Preliminary studies have shown promising results for 5 pesticides. Recovery studies of 5 pesticides in fruit and vegetables gave acceptable result for most pesticides studies.

Because trace amounts of pesticides are usually found in juice samples, pre concentration and purification steps are required. The presence of pesticides in fruit juices generally requires the concentration of these compounds in a clean extract before they can be determined. The development of appropriate methods to monitor pesticide residues in juices is demanding, because it requires simple and fast sample treatment procedures that may be easily implemented in routine laboratories. Classical analytical methodology is based on liquid-liquid extraction (Soler, C., and Pico Y. 2007). Nowadays, sample treatment strategies based on solid – liquid extraction are the more widely used approach because of the simplicity and robustness of these extraction procedures, together with the low requirement of organic solvents. These techniques are mainly based on the extraction of pesticides in a solid phase, which allows for the concentration of analyses in the sorbent and their subsequent elution or desorption, frequently in a selective way. Solid phase extraction SPE (Albero B. *et al* 2004, Tadeo J. L. *et al* 2003, Albero B. *et al* 2003), matrix solid phase dispersion *i.e.* MSPD (Albero B. *et al* 2003, Lambropoulou D. A. 2002), soil- phase microextraction *i.e.* SPME (Young M.S. *et al* 2001), and stir-bar sorptive extraction (SBSE) are the main examples of these extraction techniques applied for multiclass pesticide analysis in juices (Soler, C., Pico Y. 2007). In these cases, a simultaneous extraction and cleanup of extracts occurs, which often allows for the direct analysis. Several methods have been developed for the analysis of pesticide residues in fruits and vegetables (Alder L. *et al.* 2006, Hiemstra M, De kok A. 2007, Albero B. 2005, Sagratini, G. *et al.* 2007, Khrolenko M. *et al.* 2002) but few methods are available in the scientific literature for their determination in juice (Topuz S. *et al.* 2005, Young M.S. *et al* 2001).

In the case of volatile and thermally stable pesticides, so that gas chromatography (GC) analysis is feasible, the most frequently used technique for the determination of pesticides in juices is GC with different selective detector, such as an electron-capture detector (ECD), a nitrogen phosphorus detector (NPD), and flame photometry detector (FPD) (Soler C, Pico Y 2007). The confirmation of residue identity is usually performed by gas chromatography/mass spectrometry (GC-MS) (Will F., Kruger E. 1999). However, the number of compounds that cannot be determined by GC because of their poor volatility and thermal instability has grown dramatically in the past few years.

EXPERIMENTAL PROCEDURES

Chemical and Materials: HPLC-grade acetonitrile and methanol were obtained from Merck (Bombay). Formic acid was obtained from Fluka (Bombay). A Milli-Q-Plus ultrapure water system from Millipore (Milford, MA) was used throughout the study to obtain the HPLC-grade water used during the analysis. Fungicide analytical standards were purchased from M/s Phosphorous Limited Mumbai, Individual fungicide stock solution (200-300 $\mu\text{g MI}^{-1}$) were prepared in methanol and stored at -20°C . Oasis HLB SPE cartridges (200 mg, 6 mL) purchased from Waters (Milford. M.A.) and a Supelco (India). Visiprep SPE vacuum systems were also used.

Sample Treatment : Different brands of juices were purchased in different part of the country. The fungicides were extracted using off-line SPE, before the SPE was performed, a sample aliquot of 50 ml was centrifuged (3000 rpm) for 3 min to avoid blockage of the cartridges. The SPE procedure involves a preconditioning step of the cartridges with 5 mL of MeOH and 5 mL of milli-Q water at a flow rate of 2 mL min⁻¹. After that, aliquots of 25 mL of centrifuged juice sample (without pH adjustment) were loaded at a flow rate of 3 mL min⁻¹. The retained analytes were eluted with 5 mL of MeOH at 1 mL min⁻¹, and this eluate was collected in a 15 mL centrifuge tube, evaporated until near dryness by gentle nitrogen stream, and taken up with 1 mL of MeOH and 2 mL of milli-Q water (pre concentration factor of 10:1). Prior to GC-ECD analysis, this extract was filtered through a 0.45µm PTFE filter (Millex F.G., Millipore, and Milford, M.A.) and transferred into a vial.

For quantization purposes, matrix-matched standards were prepared by spiking the extracts with an appropriate volume of working standard solutions of the studied analytes. For recovery studies, vegetable and fruit juice samples were spiked before the extraction procedure with the mixture of the studied fungicides at two concentration levels of 10 and 20µg L⁻¹.

Gas Chromatography - Electron Capture Detector (GC-ECD)

A Shimadzu GC 17A version gas chromatograph with an electron capture detector ECD was used. A SGE BPX5, 30m x 0.32 mm id capillary column with a 0.25µm film was used in combination with the following oven temperature program: initial temperature 120°C, then heated at 7°C/ min to a final temperature of 250°C, and then held for 4.5 min. The total run time was 23.07 min. The split less mode was used for the injection. The injector temperature was at 240°C and the detector temperature was at 300°C. Nitrogen gas (99.999%) was used as the carrier gas with a gas flow at 24.4 cm/sec linear velocity and the pressure at 94 kPa.

Recovery Studies : For the SPE step, 25 mL of juice sample was selected as the loaded volume. The pre concentration factor was set at 10:1 because of the complexity of the matrix. It should be noted that the proposed method is based on direct SPE procedure without further clean up stages. In this sense, the use of such a pre concentration factor would enable the reduction of the size of the procedure, in terms of, i.e., using smaller SPE cartridges, reduced sample loading volume (5-10 mL), and reduced eluting solvent volumes, thus increasing the throughput of the procedure.

On the other hand, we did not find any significant loss of pesticides using centrifuged juices. Recovery studies were performed by spiking sample before proceeding to centrifuge them. The results were not affected significantly by this step. With regard to the sorbent material used for the SPE step, we also performed experiments with C₁₈ cartridges and found that the best suited for these purposes were oasis HLB cartridges. The recoveries using C₁₈ were poor for most analytes, and the extracts obtained were not particularly clean. For these reasons, the combination of Oasis and methanol was used to isolate and pre concentrate polar pesticides in this matrix, as successfully used in environmental water. Besides, this cartridge is the most suitable one to extend the scope of the method including other classes of pesticides (herbicides, insecticides, etc.) because it offers high recovery rates for a large number of compounds with different physicochemical properties.

To evaluate the effectiveness of the extraction method, different recovery studies were carried out using an orange juice sample of 1L. We chose the orange juice matrix as the more representative juice matrix and negligible variations on the method performance in terms of recovery percentage would have been obtained if different fruit juice matrices were used. In this sense, the effect of the sample matrix of a juice does not play a crucial role. Several

portions of 50 mL were spiked at two different concentrations levels (10 and 20 $\mu\text{g L}^{-1}$) with the working standard solution. Then, the spiked samples were centrifuged and extracted with the SPE method described. The obtained extracts were analyzed with the developed GC-ECD method, obtaining recoveries between 73.6 and 106.1%, as seen in Table 1. These results show the feasibility of the studied extraction method for pesticide residue analysis in vegetable and fruit juices. **Values are the mean of 5 replicate experiments as shown in Table 1. Relative standard deviations for above analysis of samples will have less than 10%.**

Table 1. Recovery Studies on Juice Extracts Fortified with the Pesticide Mixture at the 10 and 20 $\mu\text{g L}^{-1}$ Concentration Levels

Pesticides	Spiking level ($\mu\text{g L}^{-1}$)	Recovery (%)	RSD (%) (n = 5)
Aldrin,	10	76.43	11.3
	20	95.6	8.6
Butachlor	10	98.2	9.8
	20	101.7	7.6
Chlordane	10	89.8	10.2
	20	83.4	8.9
Dicofol,	10	73.6	11.2
	20	80.4	9.6
Endosulfan.	10	94.2	7.9
	20	106.1	7.5

Quality control:

Each congener was identified by matching the retention time in the sample with that in the standard. Procedural blank, consisting of all reagents and glass ware used during the analysis, were periodically determine to check the cross contamination. Since no compound that interfered with is detected the sample values were not corrected for procedural blank. Recovery studies with fortified sample have indicated that overall recovery values exceeded 86%. In any case, it is highly desirable to improve the accuracy and precision for OCPs quantification in vegetable samples. One of the most important way to established a common basis for accuracy measurement and quantification is the existence of a reliable certified reference material (CRM). The objective of the present analysis was to test the hypothesis that commercially available vegetable samples may contains amount of OCPs that can be reliably quantified.

RESULT AND DISCUSSION

The addition of an organic solvent could also promote the release of organic compounds from the vegetable and fruit samples. However, the presence of a high concentration of an organic solvent would lead to a significant decrease in the extraction efficiency of the analytes. Therefore, only a small amount of solvent is recommended for use as the additive. Previous works Based on related to the analysis of pesticides in complex matrice (Todeo J. L., 2004). The organic solvent tested were methanol, acetone, acetonitrile, ethylacetate, methanol/acetone (1:1), methanol/acetonitrile (1:1), methanol/ethylacetate (1:1) and acetone/acetonitrile (1:1). In this study, 2% (volume/weight) of organic solvent was added to the fruit samples.

The influence of water (dilution factor of 5) and the addition of organic solvent in grape samples are shown in figure 1. The average percentage shown is the average percentage recovery (%) of all the investigated pesticides. From the results, an average percentage recovery (%) obtained using a mixture of methanol/acetone (1:1) was much higher compared to that using the other organic solvents. The average recovery of all the investigated pesticides of grape sample under these conditions was 91.9%. Besides the extraction efficiency, a mixture of methanol/acetone (1:1) was selected because it is relatively non-toxic, easy to volatilize and readily obtainable in the laboratory.

For 10 mg fruit samples, the limit of detection was 0.20 $\mu\text{g}/\text{kg}$ for aldrin, butachlor, chlordane and endosulphan. The highest level of limit of detection was found in dicofol *i.e.* 0.10 $\mu\text{g}/\text{kg}$. However, limit of quantification was 0.8 $\mu\text{g}/\text{kg}$ for aldrin, butachlor and endosulphan, whereas limit of quantification of for chlordane and dicofol was found 0.1 and 0.3 $\mu\text{g}/\text{kg}$.

The study showed a good agreement of the results using both the size exclusion and the solid phase extraction technique. Use of a more powerful solvent would be advantageous. The essential advantage of this clean up step is the use of established extraction solvent. After slight modification of the solvent composition, the sample can be loaded on the cartridge and eluted with ethyl acetate. Furthermore, no drying or evaporation steps are needed, which makes it preferable also for highly volatile compounds (Figure 1).

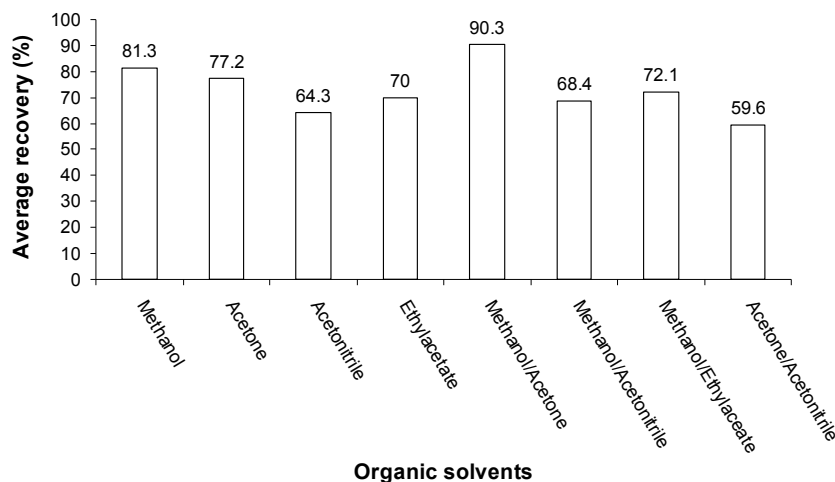


Fig. 1. Effect of organic solvents addition on extraction efficiency in Grape samples with dilution factor of 5.

From an environmental point of view the development of the early part of the analytical chain, e.g. the sample extraction has an increasingly important role in the future. Environmental concern deals not only with pesticides; it also deals with the use of harmful organic solvents. The use of organic solvents like benzene, toluene, chloroform and dichloromethane has therefore been banned or restricted in order to limit the risk on safety and health of users or to avoid hazardous environmental consequences. These restrictions, will, of course, effect the method development of pesticide residue analysis. The development of solvent free extraction techniques, like solid phase micro extraction (SPME), is one way to solve this issue that has gained interest as an alternative in the analysis of aqueous samples, Enzyme linked immune sorbent assay (ELISA) procedure, ultrasonic solvent extraction and

microwave assisted extraction (MAE) are other examples that have been developed to replace the liquid-liquid extraction.

The extraction of pesticide residues from vegetables and fruits using solid phase extraction (SPE), have shown to fulfill the requirements on alternative techniques. The traditional liquid-liquid techniques are thus replaced by efficient techniques that, at the same time, contribute to the beneficial influence on the environment and finally to reduced analytical costs.

CONCLUSION

The complexity of the vegetable and fruit matrix makes it difficult to obtain a quantitative extraction of pesticides, but the decrease in concentration of the interfering components by a simple dilution and the addition of a small amount of organic solvent to the sample makes it possible to quantify pesticide residues. Optimized SPE procedure can be proposed as a fast and accurate method for analyzing pesticides in vegetable and fruit samples and can be used instead of the conventional extraction technique, which involves large volumes of solvents, clean up procedures and time consuming steps.

Although many aspects of the application of SPE for analyzing pesticide residues in complex samples have to be resolved, this extraction technique may be considered as a viable alternative in multi residue techniques. The convenience, simplicity and reliability of the SPE techniques should undoubtedly make it a valuable tool for the environmental screening of pesticides in the future.

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