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The Use of Radiotracer Techniques for QA/QC Principles in Pesticide Residue Analysis

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Abstract: In the process of Turkish Republic being an EU member, wholesomeness of agricultural products is very important at the pesticide residue concept. The reliability of analytical data produced in the pesticide residue laboratory, and validity of method must be checked with documented evidence. Quality Assurance (QA) and Quality Control (QC) principles are very useful tools for the proving correctness of analytical data. Most of the analytical steps must be controlled in QA/QC system. The purpose of the article reported here was to demonstrate the use of radiotracer technique (^{14}C-pesticide) for some analytical steps such as, the purity/purification of active ingredient, homogenity of sample processing, extraction efficiency (bound residues) cleanup and recovery in QA/QC system.

Key Words: ^{14}C-pesticide, radiotracer technique, quality control/quality assurance

Pestisit Kalıntı Analizlerinde Kalite Kontrol ve Kalite Güvencesi Prensipleri İçin Radyoizotop İzleme Tekniğinin Kullanımı

Öz: Avrupa Birliği'ne girme sürecinde olduğumuz şu günlerde tarımsal ürünlerin pestisit kalıntıları yönünden güvenilir olması çok önemlidir. Pestisit kalıntı analiz laboratuvarlarında elde edilen verilerin doğruluğunun ve analiz metodunun geçerliliğinin dokumente edilen delillerle ispatlanması gerekir. Kalite kontrol (QC) ve kalite güvencesi (QA) sistemleri analitik verilerin doğruluğunu kontrol etmede önemli bir araçtır. Bu makalenin amacı QC/QA sisteminde, pestisit etkili madde (e.m.) saflığı/saflaştırılması, örnek işlemenin homojenliği, ekstraksiyon verimi (bağlı kalıntılar), temizleme-arıtma (cleanup), geri alım (recovery) gibi analitik işlemlerde radyoizotop izleme tekniğinin (^{14}C-pestisit) kullanılmasını göstermektir.

Anahtar Kelimeler: ^{14}C-pestisit, radyoizotop izleme, kalite kontrol/kalite güvencesi

Introduction

The implementation of quality system in analytical laboratories is now a formal requirement to introduce quality assurance measures to ensure that the data are required quality. Such measures include the use of validated methods of analysis; the use of defined internal quality control procedures; accrediation based on ISO 17025, and establishing traceability of the results of the measurements. While these requirements deeply modified the organization of the laboratories, it has also improved the quality of the results (Anonymous 2000, Feinberg and Laurentie 2005). Appropriate quality control procedures for pesticide residues are essential to demonstate the validity of results, without incurring uncessary cost (Visi 2002).

The reliability of pesticide residue data is very important at the concept of national consumption and international trade. OA/QC parameters are useful tools to control of reliability of the results. These parameters should be certain limits and confirmation must be done statistically. They are also part of Internal Quality Control. Working based on QA/QC is an insurance for analyst. Confirmation of analysis step by step provides time saving and not to consume more solvent. It is also an evidence to show any analytical laboratory performance (Anonymous 2004, Visi 2002).

The use of radiolabeled compound gives a great advantage in residue analysis because it allows one to quickly quantify the analyte directly in the extract,

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without cleanup. Radiotracer technique is also used not only to determine total, extractable, bound and conjugated residues, but also in quality control procedures such as, purity of pesticide active ingredient, uncertainty of sample processing, extraction and clean-up efficiency, and recovery studies (Führ 1987 and 1991, Powley 2004, Khan 1995, Maestroni et al. 2000).

QA/QC in pesticide residue analysis: QA is a prospective quality approving system and means all activities to provide an evidence that QC measures are utilised in a manner to ensure work products are of the highest quality and to provide evidence that results obtained in an investigation are reliable through documentation of practices, conditions and controls in the laboratory. QC is a retrospective quality approving system and confirms that the work was performed with suitable quality. It is the combination of systems, procedures, instructions and activities that are performed to control and maintain work quality. Laboratory operations should meet the requirements of a recognised accreditation scheme, e.g. ISO 17025 or Good Laboratory Practice (GLP) (Anonymous 2004, Visi 2002).

The following steps should be controlled in QA/QC system (Visi 2002);

pesticide standards, purity of standards
sample processing uncertainty
extraction efficiency
control samples
stability during the analysis
contamination and interference (equipment,solvent)
calibration solutions
control in instrumental analysis (SST)
analytical calibration (sample matrix effect)
chromatographic integration
analytical methods and analytical performance
recovery determinations spiking levels
proficiency testing and analysis of reference materials

Some QA/QC analytic steps controlled by using ^{14}C-labeled compounds

Purity and/or purification of pesticide active ingredient: Although active ingredient is in the solvent not causing any degradation, it is necessary to determine inpurity of ^{14}C-pesticide before use. For this aim Thin Layer Chromatography (TLC) is used.

In the previous study, to check the purity of ^{14}C-trifluralin, ^{14}C-trifluralin was spotted to the layer. The TLC layer was developed with hexane:benzene (2:1) developing solvent and visualisation of the spot was performed by using Camag UV-chamber with 254 nm wavelength. Retention factor (R_f) of trifluralin was 0,634. The plate separated in 8 radioactive bands with 2 cm width beginning of 1 cm below from the start. The material in the bands was scrapped off the plate to scintillation vials, then radioassayed by using Liquid Scintillation Analyzer (LSC). The 23789 dpm of the total recovered radioactivity (25139 dpm) was localized at the 5th and 6th band (Figure 1). These 2 bands were the region of trifluralin's R_f. The purity of trifluralin was found as 95 % (Tiryaki 1995).

Uncertainty of Sample Processing and Sampling Constant: It is a requirement under ISO/IEC 17025 that laboratories determine and make available the uncertainty associated with each analytical method and result (Ömeroğlu et al. 2005).

In the past, laboratories concentrated only on the precise performance of the core analytical procedure from the point of extraction to the instrumental determination. The effects of sample processing on the accuracy and uncertainty of the results, and also on the stability of residues attracted very little attention, despite the fact that accuracy and precision of the analytical result can be affected more by the sample processing technique than by subsequent analytical steps (Fussell 2004).

Sample processing is the procedure used to make the analytical sample acceptably homogenous with respect to the analyte distribution. Inhomogeneity of analytical sample will result in underestimates of the residue levels with implications for both MRL compliance monitoring and consumer risk assessment. The sampling costant (K_s) is the weight of single increment that must be withdrawn from a well-mixed material to hold the relative sampling uncertainty to 1 % with 68 % level of confidence (Ambrus et al. 1996).

The use of ^{14}C-labelled compound is preferable because the analyte can be quantified without clean-up. By eliminating the effects of the rest of the analytical procedure, the precision of the final results is significantly improved and the uncertainty of sample processing may be kept at $\leq$2%. For the same purpose, unlabeled pesticides can also be used, but their applications take much longer and the estimated

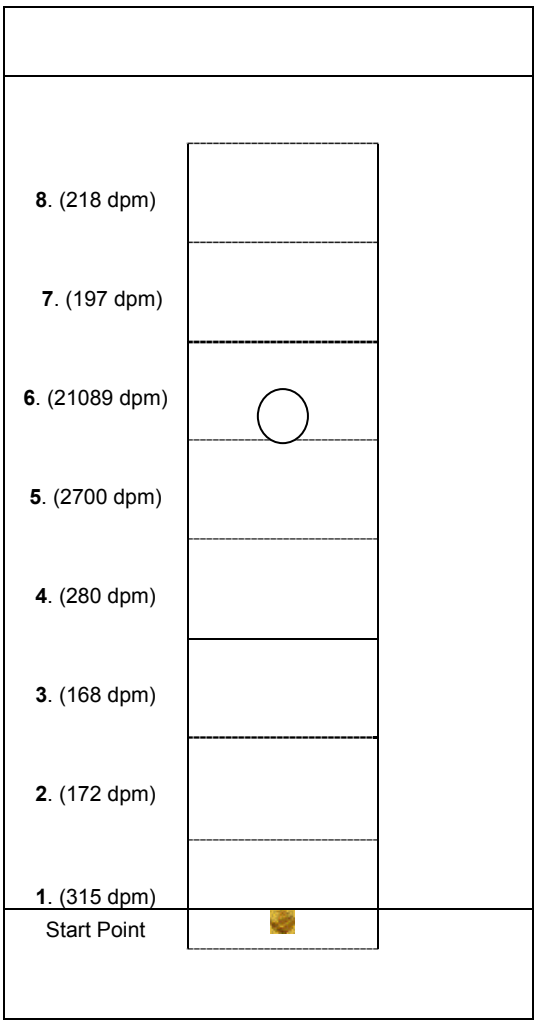

Figure 1. Use of TLC for the purification of ^{14}C-trifluralin (Tiryaki 1995)

uncertainty of sample processing may be less precise (Maestroni et al. 2000).

When the processed sample is statistically homogenous, the efficiency of sample processing is characterized by typical sampling constant (K_s), and the uncertainty of sample processing (CV_{SP}) within the tested analytical portion mass (W) range, is calculated from $K_s=W*CV_{sp}^2$ (Suszter et al. 2006).

Tiryaki and Baysoyu (2006) worked on sample processing uncertainty by using ^{14}C-chlorpyrifos for cucumber commodity. After the serial analysis, sampling constant (K_s) was found as 1.03 kg for Waring Blender sample processor. The K_S value was in agreement with the reported experimental K_s ranges (0.1-1.3 kg) which is indicated by Meastroni (2002).

Extraction efficiency, bound residues, conjugated residues: Pesticide residues absorbed/adsorbed and translocated in soil and plant tissues may be present in three possible forms: freely extractable residues, extractable conjugates bound to natural components of plant, and unextractable or bound residues incorporated into the plant constituents and soil particle (Führ 1987 and 1991, Powley 2004, Khan 1982 and 1995).

Extractability of pesticide is changed by solvent solubilty. The success of conventional chromatographic techniques is depend on extractability of analyt from the plant and/or soil sample. This is called extraction efficiency. Demonstrating the efficiency of extraction is one of the most difficult parts of the method validation. Generally ethyl acetate (EtAc), acetone, and acetonitril are used as an extraction solvent in the multi residue analysis. In many cases, extraction efficiency has not been demonstrated, but if it is very low there would be cause of systematic error or bias. It should be determined for the registration procedure in the USA. Current EU regulations contain no such requirements, but they are under consideration. Fortifications made prior to extraction does not reveal extraction efficiency. Situations where laboratory fortifications may not matched field–aged fortifications include compounds that are hydrogen-bounding, very polar or ionic, systemic or have the tendency to form conjugates (Powley 2004, Ambrus 2004).

Several workers have investigated the localization of binding sites in soil fractions and in plant constituents. Bound pesticide residues have been detected in the three classical organic matter fractions of soil, ie. humic acid (HA), fulvic acid (FA), and humin. It is also detected in the lignin, carbohydrates and protein constituent of plant tissue. Much more information is given on bound residues in the earlier publication (Helling and Krivonak 1978, Khan 1982, Führ 1987).

The most convenient way to determine extraction efficiency is to use radiolabeled material. Registrants typically perform the studies using ^{14}C-compounds applied in the greenhouse or field. Total, extractable and bound residues are determined in the sample. If the extraction efficiency is acceptable, consequently analysis can be continued. When this is done, it is important to chromatograph the extracts and measure

the radioactivity only due to the components in the residue definition. Simply determining total

radioactivity can give misleading results when substantial amounts of non-relevant metabolites or mineralised residues are present (Powley 2004).

Concentration: The volume of the extract obtained after the extraction is very large. To increase active ingredient (a.i.) in the per unit volume, it should be concentrated before the qualitative and quantitative assesment. Generally rotary evaporator and N_2 probe are used for large volume and small volume extract, respectively. During the evaporation, possible losses should be checked regularly in the evaporation waste. ^{14}C-pesticide can be used reliably for this aim.

Clean-up: Whichever technique is used for the extraction, various components with high molecular size such as lipids, pigments and resins are always present in the extract. As they may cause interference in chromatographic system, it is necessary to remove in the extract before chromatographic identification. These dirty material in the extract may damage GC or HPLC equipments (Ahmed 2001).

In order to perform cleaning-up extract, Florisil column, Gel Permeation Chromatography (GPC), and TLC have been used. Recently a new clean-up method known as the quick, easy, cheap, effective, rugged, and safe (QuEchERS) was developed. Unlike traditional methods, the QuEchERS method does not entail a solvent evaporation step to further concentrate the analytes in the final extract prior to analysis (Lehotay et al. 2005).

Calibration of clean-up system and determination clean-up efficiency has high priority in the laboratory. For this aim ^{14}C-pesticide used, with the advantage of having result shorter time and necessity of less solvent.

Fractioning of extract in the GPC system are done based on molecular size. Since big molecular size compounds can not go inside pore of gel, they pass through the column rapidly, whereas small molecular size compounds such as pesticide go inside pore of gel, consequently pass through the column later. It differs from the other clean-up system being the total elution characteristic.

In the previous study the GPC column was calibrated by using ^{14}C-chlorpyrifos at the flow rate of 1 ml/min. Total 30469 dpm ^{14}C-chlorpyrifos was injected in 250 µl EtAc:cyclohexane (1:1) mixture solution to the column. The collected fractions were radioassayed by using 1550 Tri-Carb Liquid Scintillation Analyzer (Tiryaki et al. 2003). Figure 2 shows the elution profiles of ^{14}C chlorpyrifos. 94.35 % of ^{14}C-chlorpyrifos came through the column in the 9-13 ml fractions at the 1 ml/min flow rate of eluent (EtAc:cyclohexane, 1:1).These fractions can only be analysed with the conventional chromatography after evaporation, concentration, solvent change. Another important point is that each laboratory has to use determined laboratory's own elution profile. The other laboratory's elution profiles can not be transferred to the any laboratory.

Recovery: Recovery, the main part of the method validation, is carried out by fortification at the levels of MRL (i.e, 0.5 MRL, 1 MRL, 2 MRL). After completing necessary analysis, chromatographic assesment is done and then recovery is calculated.

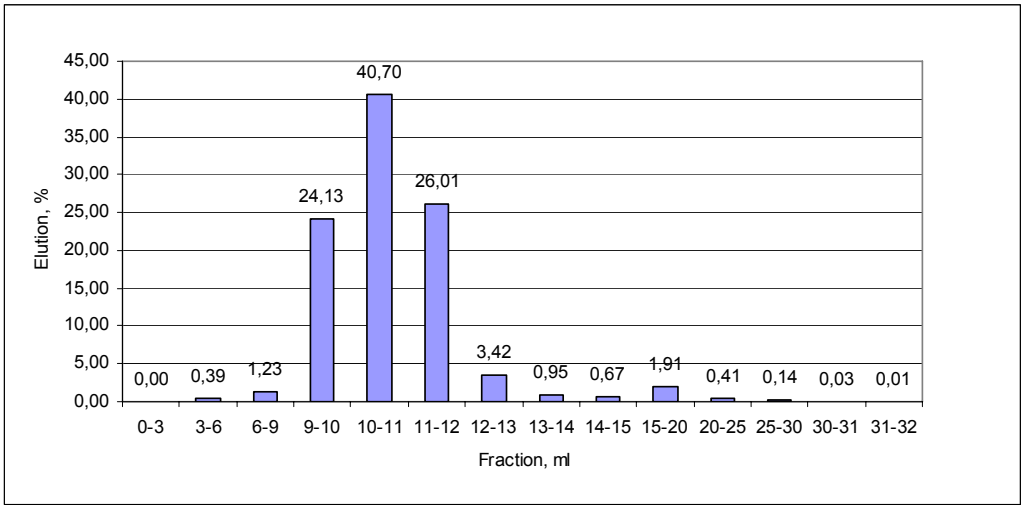

Figure 2. Distribution of ^{14}C-Chlorpyrifos on Bio-Beads SX-3 gel with EtAc:cyclohexane 1/1, (Tiryaki et al., 2003).

Since each analytic step can be controlled and/or recovery and performance of them can be easily determined, the use of ^{14}C-compound is assurance for an analyst as mentioned before.

At the our previous method validation study carried out with chlorpyrifos-ethyl, malathion and dichlorvos by using cucumber commodity, analyses were performed with 4 different fortification level and at least 4 extraction from each level. For the determination of recovery of the analytic steps, ^{14}C-Carbaryl was added to the sample. To determine extraction repeatabilty and recovery, 1ml aliqout was taken from each replication after the extraction (before clean-up), and radioassayed with LSC. Repeatability and recovery of the GPC clean-up were also determined by subjecting samples to LSC counting. After necessary calculations the recovery of extraction and cleanup analytic step were within the required limits based on fortification level (unpublished data).

Results

As mentioned above, the use of ^{14}C-labeled compound gives great advantage in the pesticide residue laboratory. In other words, the radiotracer technique facilities in the laboratory increases not only reliability of analytical data, but also laboratory performance with getting precise results very quickly.

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