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## Persistence of Quizalofop Ethyl in Soil and Safety to Ground Nut by Ultrasonic Bath Extraction and HPLC-DAD Detection

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### Abstract

Quizalofop ethyl applied regularly in broad leaved field crops as post emergence to control annual and perennial grass weeds. The experiment was carried out to determine the harvest time residues of quizalofop ethyl in soil and ground nut plant using ultra sonic bath extraction and HPLC-DAD detection in Randomized block design. The quizalofop ethyl (5% EC) was applied at four rates along with control treatment. Standardized methodology supported by recovery studies was adopted to estimate quizalofop ethyl residues on groundnut and soil. The recoveries at different spiking levels ranged from 74.0 to 99.3 % with the quantification limit of 0.01  $\mu\text{g g}^{-1}$ . The quizalofop ethyl residue in soil was ranged from 0.012 to 0.038 mg/kg at harvest. The residues were below the quantification limit (0.01 mg/kg) and maximum residue limit (MRL) in groundnut haulm and kernels. The study established that quizalofop ethyl at 50 g/ha can be used safely to control grass weeds with the pre harvest interval of 110 days.

**Keywords:** Ground nut, HPLC, kernel, quizalofop ethyl, soil, ultrasonic extraction

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## 1. Introduction

The herbicides become the vital part of the crop production and its current use is approximately 47% of the world's pesticide consumption. In India, due to acute labor scarcity and boom in cost of weed management, the herbicide use has been increased 30-33% currently (Janaki et al. 2009; Sondhia, 2014). Owing to rigorous use, the residues are frequently detected in soil environment and aquatic system. Consequently, monitoring the herbicide residues in environment has been regularly achieved all over the world. Herbicides are extremely heterogeneous in nature and traces of some compounds and their degradation products could be the source of health hazards. Among the different type of herbicides, the recently developed acetyl-CoA carboxylase (ACCase) or hydroxy phenyl pyruvate dioxygenase (HPPD) inhibitors are applied by the farmers frequently for weed control due to their high efficiency at low dose.

Among the ACCase inhibitors, quizalofop-ethyl is a 2-[4-(6-chloroquinoxalin-2-yloxy) phenoxy] propionate, belonging to aryloxyphenoxy propionates (FOP's) family is a systemic herbicide, released primarily during 1980's (Gibson, 2004). It is used to control annual and perennial grasses in broad leaved field crops. It is a colorless, crystalline compound, soluble in water (0.3 mg/L at 20°C), in benzene (290 g/L) and ethanol (9 g/L). It is normally stable at pH 7.0 at 50°C (for 90 days) and in the pH range of 3 – 7 at 25°C but unstable in light and has DT<sub>50</sub> of 10-30 days (Robert et al. 1998). The WHO and EPA classify this compound as slightly hazardous (Class III) pesticide moderately toxic to mammals and highly toxic to aquatic organisms. Although it is banned in the European Union, it is registered in USA and India as herbicide (Paranjape et al. 2014). It rapidly degrades in soil to quizalofop acid by hydrolysis with the DT<sub>50</sub> value of 20 hrs to 2 days (Robert et al. 1998; Paranjape et al. 2014). The quizalofop acid has a DT<sub>50</sub> of 60 days and its main metabolite is 4-(6-chloro-2-quinoxalinyloxy) phenol in soil. It is found unchanged in plants at the site of application and it's absorption and translocation is limited to broad leaved plants only (Robert et al. 1998). The tolerant plants metabolize it through conjugation and cleavage. Koeppe et al. (1990) studied the metabolic fate in soybean and cotton plants and noticed a residue of below 0.01 mg/kg in mature soybean and pods while 0.08 and 0.09 mg /kg in cotton fibre and seed respectively. Sahoo et al. (2013) reported the half-life of 0.85 and 0.79 days for quizalofop ethyl on onion and the residue at harvest was below 0.01 mg/ kg at 50 and 100 g/ha application rates. Similar results were reported by Mandal et al. (2014) in black gram seed, foliage and soil.

Current challenge in herbicides residue analysis is developing methods that not only provide option for determining residues from samples of different origin, but also it should detect the compounds below maximum residue limit (MRL) imposed by the regulatory agencies. The determination of residues or target analytes from sample involves a number of steps viz., extraction, clean-up and concentration with least matrix interference. Due to the low levels of herbicide that may be found in soil/crop, an enrichment of the analyte

concentration must be achieved before its instrumental determination. Analytical methods for the quantification of FOP's residues particularly quizalofop ethyl from an environmental matrix was achieved by Gas chromatography with thermo ionic detection, Gas chromatography with electron capture detection (Sahoo et al. 2013; Mandal et al. 2014), liquid chromatography tandem mass spectroscopy (EFSA, 2008) etc., following different extracting solvents, clean-up and pre-concentration procedures. Mostly the compound was extracted by shaking and liquid-liquid partitioning using toluene (Mandal et al. 2014) or derivatized using diazomethane. Though the high-performance liquid chromatography (HPLC) method was reported for the determination of FOP's like cyhalofop ethyl (Sondhia and Kharee, 2014), metamifop (Janaki and Chinnusamy, 2012) from soil and rice crop, report on quizalofop ethyl is very scanty (Sahoo et al. 2013; Mandal et al. 2014). The traditional analyte extraction techniques consumes a large quantity of solvent and time, though offer efficient recoveries. Hence the green extraction methods have been proposed including ultrasonic solvent extraction (USE), microwave assisted extraction (MAE), supercritical fluid extraction (SFE) etc. Among these USE is not explored much for the herbicides extraction from soil and plants. Ultrasonic extraction provides more efficient contact between the solid and solvent than the mechanical shaking, typically resulting in a greater recovery of analytes and also is an efficient method for the extraction of pesticides from several matrices (Babic et al. 1998; Sanchez-Brunete et al. 2003). The ultrasound-assisted extraction has been so far developed for extracting atrazine (Delgado-Moreno et al. 2009; Andreea Dragus et al. 2014), imidazolinones (Assalin et al., 2014) and sulfonyleureas (Ghobadi et al. 2015) from soils. However the USE has not been applied for the extraction of FOP's particularly the quizalofop ethyl from soil and plant matrices. Hence the present research work was focused on the development and evaluation of a sample preparation approach based on ultrasonication coupled with solid phase extraction (SPE) for the extraction of quizalofop ethyl from soil and ground nut plant samples followed by determination with HPLC- DAD. Applicability of the developed method was also tested to extract the quizalofop ethyl from the environmental samples.

## **2. Materials and Methods**

### **2.1. Chemicals, Reagents and Soil**

A certified reference standard of quizalofop ethyl (purity 98%) was purchased from Sigma-Aldrich. The test chemical of quizalofop ethyl 5% EC was supplied by Crystal Crop Protection Pvt. Ltd., (CCPPL), New Delhi, India. All the solvents were of analytical grade and purchased locally. The anhydrous sodium sulfate (AR grade) was used as a drying agent for different samples. For HPLC analysis and the quizalofop ethyl stock solution and working standards preparation, HPLC-grade methanol and 0.2 $\mu$ m filtered Milli-Q water were used.

## 2.2. Field experiment details

Field experiment was conducted to estimate the harvest time residues of quizalofop-ethyl in/on groundnut crop (Variety – TMC Gn 13) and soil during rabi 2012 (December 2012 to March 2013) at ARS, Bhavanisagar Farm, TNAU, Bhavanisagar, Tamil Nadu, India. The experiment was conducted in randomized block design in a plot size of 30 m<sup>2</sup> and each treatment was replicated thrice. The experimental field soil was red sandy loam in texture has 6.8 pH and 0.18 dS m<sup>-1</sup> electrical conductivity. The organic carbon content was 0.55 percent. The maximum temperature during the cropping period ranged from 24°C to 45°C with a mean of 34.7°C. The minimum temperature ranged from 15.5°C to 27°C with a mean of 21°C. The relative humidity ranged from 59 to 92.2 per cent with a mean of 85.7 per cent in the morning (0722 hrs) and 21 to 57 per cent with a mean of 41.7 in the evening (1422 hrs). A total rainfall of 73.1 mm was received in 12 rainy days during the cropping period. A single post emergence application of the test chemical (quizalofop-ethyl 5% EC) was done at different doses (T<sub>1</sub> - 37.5 g ha<sup>-1</sup>, T<sub>2</sub> - 50 g ha<sup>-1</sup>, T<sub>3</sub> - 75 g ha<sup>-1</sup> and T<sub>4</sub> - 100 g ha<sup>-1</sup>) along with untreated control on 15 days after crop sowing. The spraying of herbicide was done using knapsack sprayer fitted with flat fan nozzle and water alone was sprayed in control plot. The samples of groundnut haulm and kernel were collected at random during harvest, air dried and immediately processed (Janaki et al. 2013) for residue estimation. The soil samples at harvest were air-dried, homogenized to fine powder and passed through a 2 mm sieve prior to herbicide residue extraction.

## 2.3. Ultra sonic bath extraction and Clean up

Ultrasonic bath assisted extraction (USBE) was carried out in a bench top ultrasonic bath (Soniclean, Australia) under the constant frequency of 20 Hz pulses at a sweep bandwidth of 45 kHz. The device was equipped with a digital timer and a temperature controller (0-60°C). Spiked soil and plant samples were extracted with 60 mL methanol - water 1:1 (v/v) for 20 min at 40° C. The matrix sediment suspension was prepared in a glass beaker and covered with aluminum paper before placing in an ultrasonic bath. The water in the bath was maintained above the volume of the solvent in the beaker (three fourth of the bath capacity was filled with distilled water) and the condition was maintained constant during the experiments as it affects the ultrasound transmission from the transducer to the sample. After sonication, methanol layer was drawn separately and filtered through anhydrous sodium sulfate and the matrix sediment was washed with additional volume of extractant once. The combined filtrate was concentrated to dryness, then dissolved with 10 mL petroleum ether and subjected to Solid Phase Extraction (SPE). Florisil cartridges were conditioned with 2 mL acetic ether and 4 mL petroleum ether. The cartridges were loaded with 2 ml extract solution, this elute was discarded. The cartridges were washed with 4 mL petroleum ether-acetic ether 98:2 (v/v), and discarded. Analytes were eluted with 5 mL petroleum ether-acetic ether 90:10 (v/v). The elute was concentrated on rotary vacuum

evaporator at 60°C and volume was made to approximately 2 ml using acetonitrile for HPLC-DAD analysis.

#### **2.4. Equipment and conditions of HPLC**

Quizalofop residues were analyzed using Agilent HPLC system (1200 series) having 20 µL injection loop equipped with diode array detector and autosampler under binary elution mode. The mobile phase of acetonitrile: 0.1 % formic acid in water (75:25, v/v) at a flow rate of 1.0 mL min<sup>-1</sup> was used to elute the sample through column. The separation of the compound was performed on Agilent Eclipse C18 (4.6 x 150mm, 5µm) column kept in thermostated oven maintained at 30°C. The instrument was connected to a computer which records the response in terms of peak area and height using the Ezchrom software and the detection was performed at 250 nm.

#### **2.5. Recovery and detection limits**

The stock solution of quizalofop ethyl and its working concentrations were prepared in acetonitrile by diluting the stock solution as described by Janaki et al. (2013) for oxyfluorfen. Then, 20 µL of each working standards were injected into HPLC and the peak area was measured for linearity check study. Validation of the extraction method was performed in terms of recovery studies before analyzing unknown sample (Janaki et al. 2013). The recovery of the active substance of quizalofop-ethyl was determined by fortification of the soil and plant samples with the known concentrations of 0.005, 0.01, 0.05, 0.1, 0.5 and 1.0 mg lit<sup>-1</sup> in three replicates, mixed well and extracted as described for samples. The quantification of residue was accomplished by comparing the peak response for samples with peak area of the standards.

### **3. Results and Discussion**

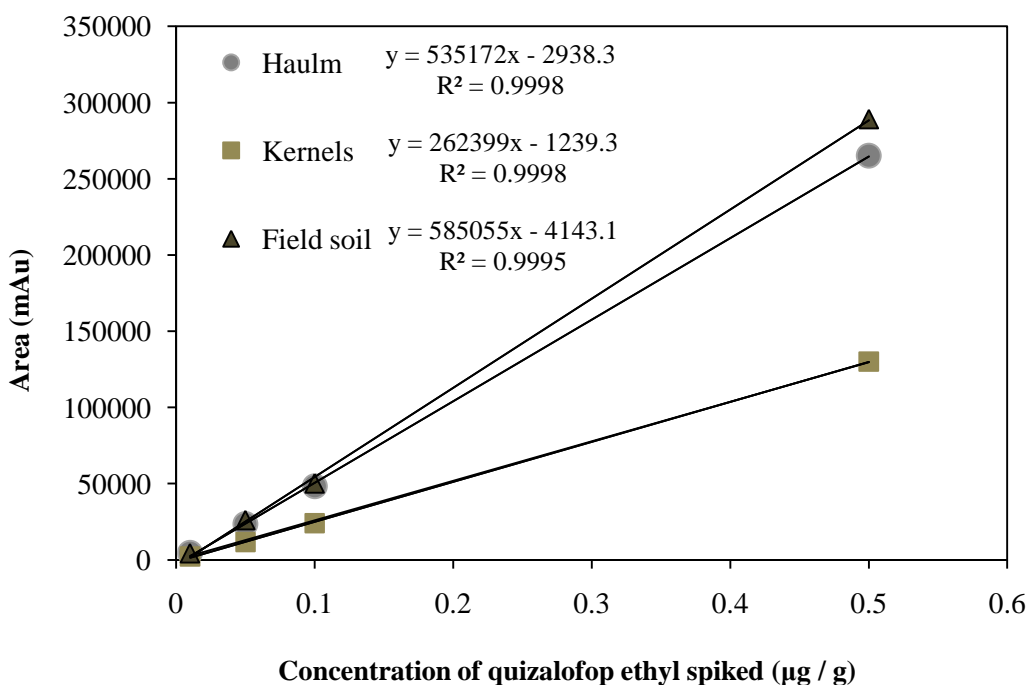
#### **3.1. Validation of extraction Method and Recovery**

To ensure the superiority and consistency of the results for all analytical applications, the validation is a vital requirement. The linearity, accuracy, precision (relative standard deviation (RSD) %), determination coefficient (R<sup>2</sup>), limit of detection (LOD) and limit of quantification (LOQ) are the factors used in the present study for validating the USBE method coupled with HPLC-DAD detection.

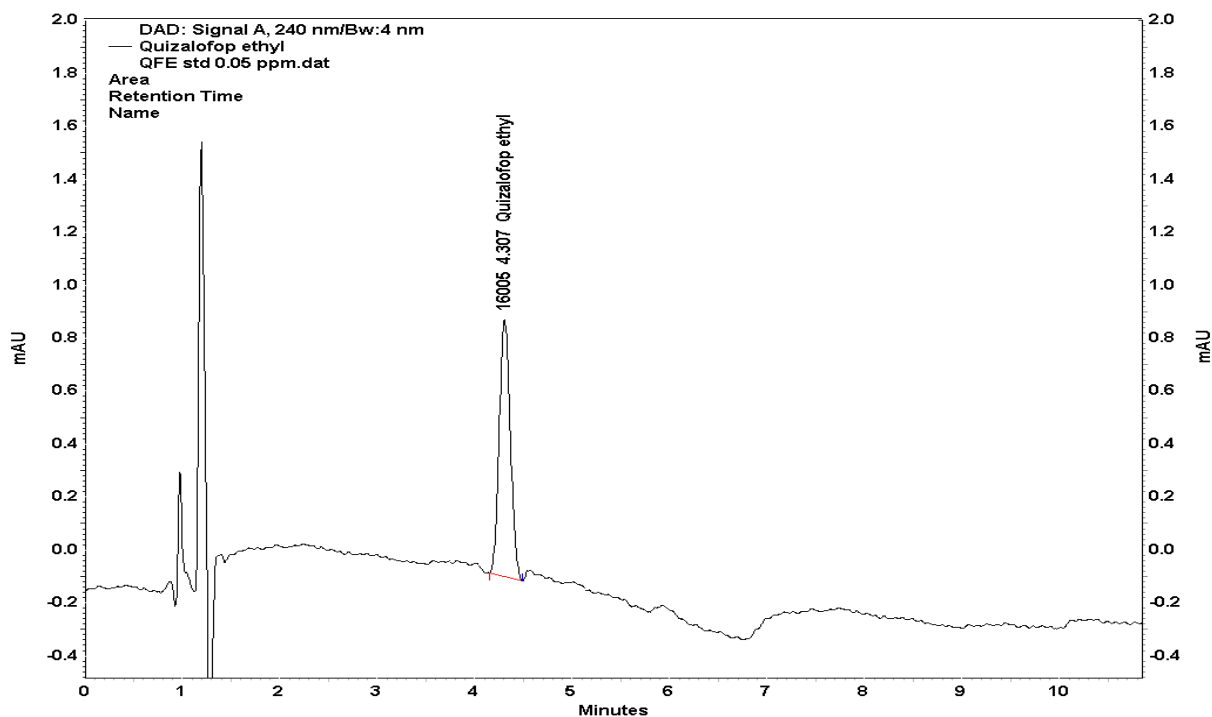
Under the given conditions of HPLC, quizalofop ethyl was resolved at 4.3 min (Figure 2) as a single sharp peak at a peak width of 2.5 Hz with a response time of 0.20 seconds. The analytical calibration graph equation obtained by plotting peak areas in 'y' axis versus concentrations of quizalofop ethyl in 'x' axis was  $y = 283287x - 549.32$  within the range of 0.5 to 0.01 µg mL<sup>-1</sup>. This showed good linearity with the correlation coefficient of 0.975. The LOD for quizalofop ethyl was found to be 0.005µg mL<sup>-1</sup>. The linearity was also determined by fortifying the blank soil and groundnut samples at different concentrations ranging from 0.01 to 0.5 µg g<sup>-1</sup> (Figure 1). The LOQ calculated using the regression

equation was found to be  $0.01 \mu\text{g g}^{-1}$  for all the matrices. Similar quantification limit for quizalofop ethyl in soil, onion and black gram was reported by Sahoo et al. (2013) and Mandal et al. (2014) using GC-ECD.

The mean recovery of quizalofop ethyl through USBE at different fortification levels was found to be 87.2, 83.4 and 82.7 percent for soil, ground nut haulm and kernel, respectively (Table 1). The adopted method was found to be precise, since the RSD (%) was below 10 percent. The described extraction and clean up method was found to be satisfactory since the analytical recovery of the quizalofop ethyl from different substrates was within the range of 74.0 – 99.3 percent. The result of recovery study also confirms the consistent repeatability of the method.



**Figure 1-** Calibration curve of quizalofop-ethyl in spiked matrices determined by HPLC-DAD



**Figure 2-** HPLC chromatograms of standard quizalofop ethyl at 0.05 µg mL<sup>-1</sup>

**Table 1-** Recovery of quizalofop-ethyl in groundnut plant and field soil samples.

Substances	Amount Fortified (in µg/g)	*Recovery (%)	**RSD (%)	Average Recovery (%)
Groundnut haulm	0.01	78.68	2.3	83.4
	0.05	80.88	1.5	
	0.10	82.87	2.1	
	0.50	91.08	3.3	
Groundnut kernels	0.01	77.99	3.7	82.7
	0.05	80.58	2.9	
	0.10	82.77	3.6	
	0.50	89.43	2.8	
Field soil	0.01	74.04	2.9	87.2
	0.05	89.49	2.6	
	0.10	86.11	4.8	
	0.50	99.33	3.9	

\* Average of three replicates; \*\*RSD- Replicate standard deviation percent.

### 3.2. Residue of quizalofop ethyl in soil, ground nut haulm and kernel

The highest concentration of quizalofop ethyl residue was recorded in soil sample from plots (Table 2 and Figure 3) which received 100 g ai ha<sup>-1</sup> followed by the treatments where quizalofop ethyl was applied at 75 and 50 g ai ha<sup>-1</sup>. The decrease in dose of application decreased the quizalofop ethyl residue concentration in soil. The mean residue concentration in soil varied from 0.012 to 0.038 4 µg g<sup>-1</sup> across different treatments. Such a lower residue concentration in soil could have been attributed to the accelerated degradation of quizalofop ethyl in soil by the organic matter content and microbial population in the soil. Kim et al. (1996) reported that more than 60 percent of the applied quizalofop degraded from the soils within 7 days and has the half life of 15-16 days. They also observed that both the incubation temperature and Fusaria population affected the decomposition of quizalofop ethyl in soil. It's residue on 110 days after single application was below the quantification limit at the lower rate of 37.5 g ai ha<sup>-1</sup>; however it was detected at higher rates of 50, 75 and 100 g ai ha<sup>-1</sup>. The low solubility of quizalofop ethyl in water (0.30 mg L<sup>-1</sup> at 20°C) might have retained it in the surface for longer period of time and could augment the photo degradation of this molecule from soil. The detection of quizalofop ethyl residue in soil at higher dose of application could be attributed to the binding of certain amount of herbicide by the humic fraction in soil and has not been availed for the degradation (Janaki and Chinnusamy, 2012) at the early period of application.

**Table 2-** Residue of quizalofop-ethyl (µg g<sup>-1</sup>) in groundnut plant parts and soil at harvest.

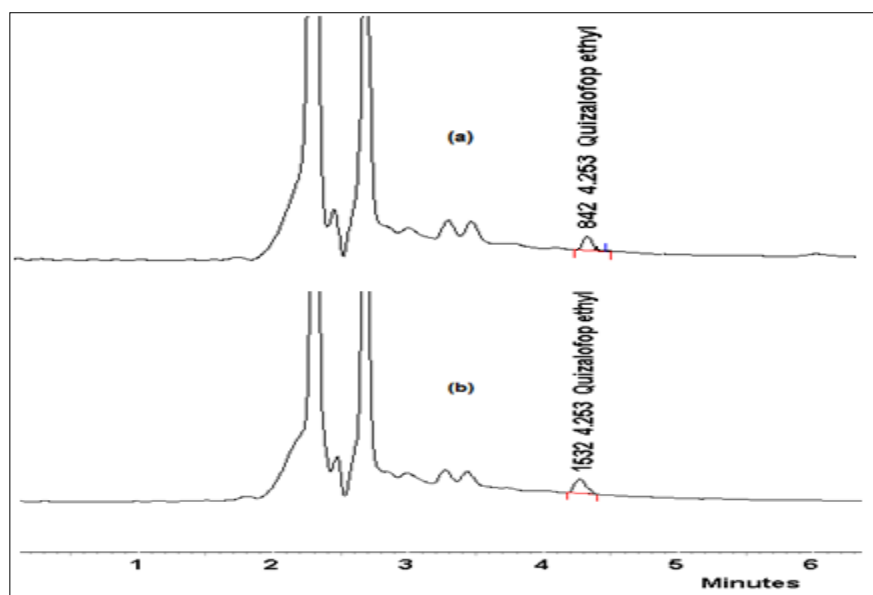
Substrates	Dose of quizalofop-ethyl (g a.i ha <sup>-1</sup> ) applied			
	37.5	50.0	75	100
Haulm	BDL*	BDL	BDL	BDL
Kernel	BDL	BDL	BDL	BDL
Soil	BDL	0.012 ± 0.003	0.023 ± 0.008	0.038 ± 0.011

\*BDL- Below detectable limit (0.005µg g<sup>-1</sup>).

The quizalofop ethyl residue concentration in ground nut haulm and kernel at harvest (110 days after application) was below the quantification limit of 0.01 µg g<sup>-1</sup> (Table 2) irrespective of dose of quizalofop application. It was found that the residues were below the maximum residue limit (MRL) of 0.05 µg g<sup>-1</sup> fixed for the oilseed crops like soybean by FSSAI (2011) and sugar beet by EFSA (2008). Further the residues in the groundnut haulm and kernels are well below the MRL of 0.1 mg kg<sup>-1</sup> proposed for peanut by the Japan (FFCR, 2006) even when it was applied at 100 g ai ha<sup>-1</sup>. Similarly, PSD (1987) reported that the residue of quizalofop ethyl was not detected in the sugar beet, rape seed even when it was applied 2.5 times higher than the recommended rate of 37.5 g ai ha<sup>-1</sup>. The presence of quizalofop ethyl residues below the quantification limit of 0.01 mg/kg in haulm and kernel even at very high dose of application could be ascribed to the slow translocation and metabolism of the parent compound in the plants (USEPA, 1993). The quizalofop ethyl is



mostly metabolized in the plant into its acid and other metabolites like 2-4-hydroxyphenoxy propionic acid and 2-(4-hydroxyphenoxy) propionate through the cleavage of enol-ether linkage as reported by PSD (1987) and USEPA (1993) in potato and soybean plants, respectively.



**Figure 3-** Detection of residue in field soil at the time of ground nut harvest by HPLC-DAD which received quizalofop ethyl 5% EC at 50 (a) and 100 (b) g ai ha<sup>-1</sup>

Based on the harvest time residues of quizalofop ethyl detected in soil, groundnut haulm and kernels, it is established that this molecule can be used safely to control grass weeds (Sahoo et al. 2013; EFSA, 2008) in ground nut at the dose of 50 g ai ha<sup>-1</sup> under tropical conditions of South India with the pre harvest interval of 110 days. Mandal et al. (2014) reported that residue of the quizalofop ethyl substance was below detectable limit in black gram seed, foliae and soil at the harvest and suggested that it is a safe kind of herbicide to be applied for grassy weed control in black gram without any residual effect.

In this study a novel method, USBE was evaluated for the quizalofop ethyl residues extraction from soil and groundnut plant parts. The results established that the accuracy and precision of the proposed method was satisfactory for the extraction of quizalofop ethyl residues from different matrices. The LOQ achieved by the USBE method essentially met the maximum residue limit proposed by the food safety authority of Europe (EFSA), India (FSSAI) and Japan (FFCR) for quizalofop ethyl residue in different crops. The developed method was adopted successfully to analyze the residue of quizalofop ethyl in soil and groundnut parts from the field experiment treated with different levels of quizalofop ethyl. It was found that the residues were below the quantification limit of 0.01 mg/kg and MRL in groundnut haulm and kernels which proven that this molecule can be used safely to control grass weeds with the pre harvest interval of 110 days. However the quizalofop ethyl

residue was detected in the soil and grain at higher doses of application. Hence a detailed study is needed on the persistence, sorption and leaching potential of quizalofop ethyl and its metabolites in soil, field water and its level of biomagnifications in crop produce needs to be studied in future since it is classified as toxic to aquatic organisms.

### **Conflict of interest**

Authors declare no conflict of interest.

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