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Comparison between UV-spectrophotometry and HPLC Methods to Determine Napropamide Concentration in Soil Sorption Experiment

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ABSTRACT

In this study we compared the High Performance Liquid Chromatography (HPLC-UV) and the Ultraviolet (UV) spectrophotometry methods for measuring the herbicide napropamide concentrations in soil solution during batch equilibrium sorption study. Experiments were conducted to compare the calibration curves, recovery in background solution and spiked soil samples, intra and inter day precision and mass balance of the two methods. Mass balance of napropamide in the sorption study was determined from the difference between the initial napropamide content in the supernatant and the equilibrium concentration. Although DOC interfered with UV wave absorption by napropamide in the UV-spectrophotometry method, it was easily corrected by using a blank solution containing the same amount and type of DOC as used in the sample. The intra and inter day precision experiments indicated the measured napropamide concentrations using both methods were equally reproducible and consistent. The recovery of napropamide from spiked soil samples decreased with increasing amount of clay and organic matter but recoveries for both methods exceeded 88% and the recovery of the UV-spectrophotometry method was comparable to the HPLC. The measured concentrations of napropamide in soil solution supernatant used for the sorption study showed no significant differences between the two methods. We found that the UV-spectrophotometry method was reliable to determine the concentration of napropamide in the supernatant of the soil solution used in the sorption study. Therefore, the UV-spectrophotometry method can be used as an alternative method to HPLC for determination of napropamide concentration in soil batch equilibrium sorption study.

Key words: Napropamide, HPLC, UV-spectrophotometry, dissolved organic matter, supernatant

INTRODUCTION

Pesticide fates in the environment can be affected by its sorption onto the soil colloids. The batch equilibrium method is frequently used to determine the soil sorption capacity of pesticide. Determination of soil sorption capacity of a pesticide is vital in evaluating its fate in the environment. Napropamide [N, N-Diethyl-2-(1-naphthalenyloxy) propionamide] is a quite polar herbicide and fairly soluble in water, used to control several grasses and broadleaf weeds in many agricultural cultivation. The analytical methods for determination of napropamide concentration

are numerous and the most frequently used method are the Gas Chromatography (GC-NPD) (Antonious and Patterson, 2005; Antonious *et al.*, 2005; Kim, 2004) and the High Performance Liquid Chromatography (HPLC) (Biswas *et al.*, 2007; Lu *et al.*, 2002). However, not all researchers have access to these two instruments especially in the less developed countries. Furthermore, these methods require extraction and cleaning procedures to remove the unwanted constituents from the soil extract that may interface with subsequent analysis (Eagle *et al.*, 1991).

A cheaper, less complicated instrument that can be used as an alternative to the GC and HPLC for the determination of pesticide in soil is an UV-spectrophotometer (Jakczyk, 1977). The UV-spectroscopy method is routinely used in the quantitative determination of transition metal ions and highly conjugated organic compounds in solution. The UV-spectrophotometry method has been successfully used to measure the concentration of triazine herbicides in soils (Jakczyk, 1977). Rodriguez-Rubio *et al.* (2006) measured the 2, 4-D on natural and organic amended soil using the UV-spectrophotometry method. However, in literature there is no study on the use of UV-spectrophotometry method to measure napropamide in solution. The objective of this study was to compare the HPLC with the UV-spectrophotometry in measuring the concentration of napropamide in soil solution supernatant used for batch equilibrium sorption study.

MATERIALS AND METHODS

Chemicals: An analytical grade napropamide (99% purity) was supplied by Sigma-Aldrich (Seelze, Germany). Napropamide is a polar nonionic herbicide and has an aqueous solubility of 74 mg L^{-1} ; vapor pressure of $1.7 \times 10^{-7} \text{ mm Hg}$; K_{OC} of 700 L kg^{-1} and degradation half-life of 70 days (Wauchop *et al.*, 1992). The chemical structure of napropamide is shown in Fig. 1. Due to the low solubility of napropamide in water, stock solution of napropamide (5000 mg L^{-1}) was prepared in methanol. Then the stock solution was added to a distilled water containing 0.01 M CaCl_2 and $200 \text{ mg L}^{-1} \text{ HgCl}_2$ and this solution was later used for our sorption study. Total methanol concentration in the solution did not exceed 0.1% (v/v) to avoid any changes in the solution properties.

Napropamide determination using UV-UV-spectrophotometry method: The UV-visible dual beam spectrophotometer (Shimadzu, UV-1650 PC) was used for determination of napropamide. The solution containing napropamide was scanned to find the absorption wavelength of napropamide. The wavelength for maximum absorbance of napropamide was determined to be 288 nm and this wavelength was used throughout the study. A blank solution contained 0.01 M CaCl_2 , $200 \text{ mg L}^{-1} \text{ HgCl}_2$ and methanol in distilled water but without any napropamide. The detection and quantification limits for both HPLC and UV methods were defined as the concentration of napropamide that gave signals 3 and 10 times than the noise, respectively. The detection limit for

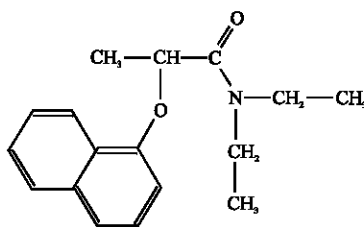


Fig. 1: Chemical structure of napropamide

UV-spectrophotometer method was 0.05 mg L^{-1} while and the quantification limit was 0.15 mg L^{-1} . Figure 2 illustrate the UV-spectrophotometer chromatograms for the background solution with and without the addition of 20 mg L^{-1} napropamide.

Napropamide determination using HPLC: The concentration of napropamide was determined using a HPLC equipped with a UV detector (Model 1050, Hewlett Packard, USA). The stationary phase was a ZORBAX 300SB- C_{18} ($4.6 \mu\text{m} \times 250$) column. The analyses were done under the following conditions: flow rate of 0.6 mL min^{-1} ; mobile phase was water/acetonitrile (45/55 v/v); detection wavelength was 288 nm and injection volume of $20 \mu\text{L}$. Limits of detection and quantification were 0.005 and 0.016 mg L^{-1} , respectively. The retention time was 10.2 min. Figure 3 shows the HPLC-UV chromatograms for the background solution with and without 20 mg L^{-1} napropamide.

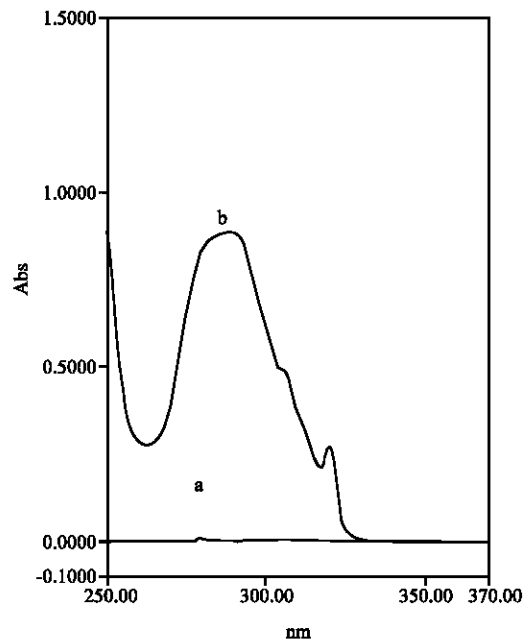


Fig. 2: The UV spectra of (a) background solution and (b) background solution added with 20 mg L^{-1} napropamide

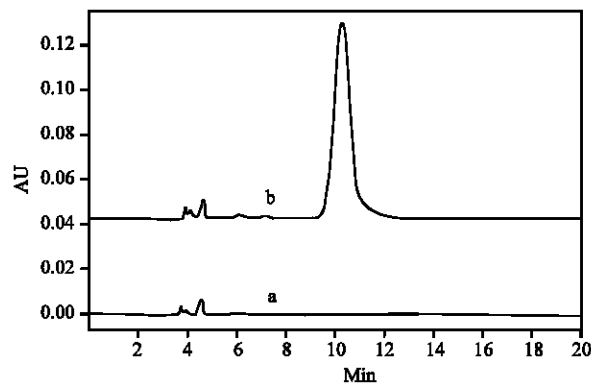


Fig. 3: The HPLC chromatograms of (a) background solution and (b) background solution added with 20 mg L^{-1} napropamide

Linearity of absorbance against concentration of napropamide: The linearity of absorbance with different napropamide concentrations was determined using 0.5, 1, 5, 10, 15, 20, 30 and 40 mg L⁻¹ of napropamide. Calibration curves for both UV-spectrometry and HPLC methods were plotted and linear regressions were used for both plots.

Recovery, inter and intra-day precision: The recovery study was conducted by using known concentrations of napropamide (5, 10, 15, 20, 25, 30, 35 and 40 mg L⁻¹) in solution containing 0.01 M CaCl₂ and 200 mg L⁻¹ HgCl₂. The solution was shaken for 24 h, centrifuged at 7000 rpm for 10 min and the supernatant was analyzed using both the HPLC-UV and UV-spectrophotometer.

The concentration of napropamide in the supernatant was calculated from the calibration curve. The percent recovery was calculated based on the initial concentration and the concentration recovered after the shaking and centrifugation processes.

The intra-day precision was assessed using 0.5, 1, 5, 10, 15, 20, 30 and 40 mg L⁻¹ of napropamide and the solution were analyzed seven times on the same day but at different times. For the inter-day precision solution samples of 0.5, 1, 5, 10, 15, 20, 30 and 40 mg L⁻¹ of napropamide were analyzed at different times.

Recovery in spiked soil samples: Five gram of sample was taken from each of four different soil types and placed into 50 mL centrifuge tubes separately and fortified with 0.5 mL of 50 µg mL⁻¹ analytical grade napropamide to give a final concentration of 5 µg napropamide g⁻¹ of soil. The soil samples were left at room temperature for 24 h and then each of them were mixed thoroughly and left for another 1 h after which a 15 mL mixture of methanol and water (3:1 v/v) was added, followed by shaking on a reciprocal shaker at 250 rpm for 2 h. The samples were then centrifuged at 7000 rpm for 10 min. The supernatant was transferred to a round bottomed flask and evaporated on a rotary evaporator to evaporate the methanol. The concentration of napropamide in the remaining water solution was measured by the HPLC-UV and UV-spectrophotometer.

The effects of Dissolved Organic Matter (DOC) on the napropamide absorbance using UV-spectrophotometer: The DOC was extracted from sandy soils receiving 0, 10, 20, 30, 40, 50, 60, 70 and 80 Mg ha⁻¹ Chicken Dung (CD). The extracting solution was 0.01 M CaCl₂ and the soil: CaCl₂ ratio was 1:10 (w/v). The mixture of soil and solution was shaken for 24 h then it was centrifuged at 7,000 rpm for 10 min. The supernatant was filtered using 0.45 µm membrane filter and the filtrate was labeled as solution of DOC. The DOC in the filtrate was measured using a total organic carbon analyzer (ANA TOC Series II). The Dissolved Organic Carbon (DOC) concentration from soils receiving 0, 10, 20, 30, 40, 50, 60, 70 and 80 Mg ha⁻¹ CD were 3, 7, 11, 15, 19, 26, 30, 35 and 39 mg L⁻¹, respectively. The absorbance of this solution was measured at 288 nm using the UV-spectrophotometer. Then, 12.6 mg L⁻¹ (the concentration which usually added to soil for controlling the weeds) of napropamide was added to the filtrate and the absorbance was again measured using the UV-spectrophotometer. Since DOC interfered with napropamide absorption at 288 nm, addition of DOC in a solution of napropamide will increase the UV sorption by the napropamide. Therefore, in this study we assumed that the UV wave absorption in solution containing DOC only was the same as the napropamide sorption. The concentration of napropamide in solution was calculated using the napropamide standard curve of absorbances against its concentrations. The concentrations of napropamide in the solutions containing different amount of DOC and the same solution of DOC but with the addition of 12.6 mg L⁻¹ napropamide were plotted against the amount of chicken dung added to the soil Fig. 4. Finally, the concentration of napropamide in DOC solutions containing 12.6 mg L⁻¹ napropamide was calculated by the

differences between the concentrations of napropamide in DOC solutions containing 12.6 mg L⁻¹ napropamide and the DOC solutions without napropamide.

Determination of soil characteristics and napropamide sorption study: Four different soils were used for soil sorption study. Soils were labeled as soil A, B, C and D (Table 1). The soil Total Organic Carbon (TOC) was determined using the Walkley and Black method (Nelson and Sommers, 1982), particle size distribution was determined by the pipette method (Gee and Bauder, 1986) Cation Exchange Capacity (CEC) was determined using 1 N ammonium acetate and soil pH and Electrical Conductivity (EC) were determined using a soil to double-distilled water ratio of at a 1:2.5.

The batch equilibrium method was used to determine napropamide sorption in soils. Two grams air-dried soil from each soil was added to 20 mL of background solution containing 0, 5, 10, 15.0, 20, 25, 30, 35 and 40, mg L⁻¹ napropamide. The soil suspensions were shaken in 50 mL centrifuge tubes for 24 h in a rotating shaker and which the samples were centrifuged for 10 min at 7000 rpm. Ten milliliter of supernatant was removed to determine the napropamide concentration by the HPLC-UV and UV-spectrophotometer. We have previously determined that

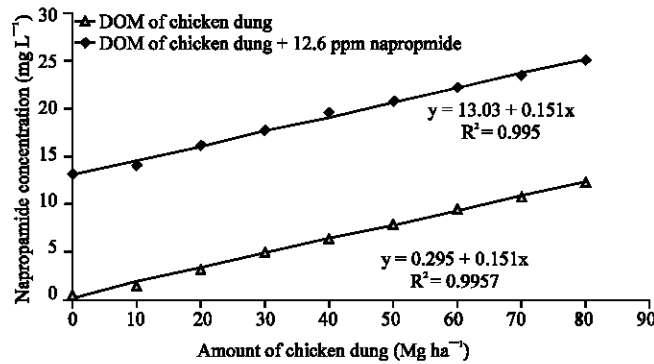


Fig. 4: Plots of napropamide concentrations in solution containing DOM extracted from soil receiving different rates of chicken dung and the same solution added with 12.6 mg L⁻¹ napropamide

Table 1: Percentage of recovery by HPLC-UV and UV-spectrophotometer

Napropamide concentrations (mg L ⁻¹)	Recovery (100%)	
	UV- spectrophotometer	HPLC-UV
5	98.1±0.7†	98.4±0.4
10	98.4±0.8	98.1±0.2
15	98.2±0.3	98.3±0.5
20	98.3±0.7	98.5±0.6
25	98.3±0.7	98.3±0.3
30	98.1±0.8	98.6±0.5
35	98.6±0.5	98.3±0.9
40	98.2±0.3	98.2±0.2

† Values are Mean±standard error

equilibrium time for all soils was reached within 24 h and no biodegradation occurred because of HgCl_2 efficiently inhibited the microbial growth.

Mass balance determination: The soil samples used for the sorption study were retained to determine the mass balance of napropamide in the sorption studies. The napropamide that remained in the soil sample after decantation of supernatant in the sorption study was extracted with 6 mL solution of methanol and water (3:1; v/v). The sample was shaken for 2 h and then centrifuged at 7000 rpm for 10 min. The methanol in the solution was evaporated then concentration of napropamide in the water was measured by the HPLC-UV and UV-spectrophotometer.

RESULTS AND DISCUSSION

The results showed that the linear regressions for absorbance/peak area for both methods were high, exceeding 99.99% (graph not shown). This indicated that both methods were highly reliable at concentration range between 0 to 40 mg L⁻¹ of napropamide which are the range usually used for napropamide sorption study (Auger *et al.*, 2000). The UV spectra of background solution with and without 20 mg L⁻¹ napropamide are shown in Fig. 2 while the HPLC chromatograms are shown in Fig. 3. The retention time of napropamide by the HPLC was 10.2 min and running time for each sample took 15 min whereas determination by UV-spectrophotometer took only a few seconds. Therefore, UV-spectrophotometry method is much faster than HPLC-UV method. Determining of napropamide using HPLC-UV needs organic solvent (HPLC grad) as flushing (e.g., methanol) and mobile phase (e.g., acetonitrile). Moreover, HPLC is more expensive than the UV-spectrophotometer.

Table 1 shows the percent recovery of napropamide at different concentrations from the background solution using both the UV-spectrophotometer and HPLC. The results indicated that the recovery of napropamide at all concentrations were satisfactory for both UV-spectrophotometer and HPLC. However, the measurement of napropamide recovery was from background solution without any interference. When separation of target compound from impurities in sample matrix is needed, HPLC offer better separation and therefore better sensitivities (Baskaran and Bolan, 1998). In order to examine the matrix effect on the UV absorption by napropamide using the UV-spectrophotometer, we carried out an experiment to determine the effect of DOC in the background solution on napropamide absorption. The UV absorption by the DOC at 288 nm increased linearly with the increasing amount of DOC Fig. 4. The UV absorption of background solution containing both the DOC and napropamide (12.6 mg L⁻¹) also increased linearly with the increasing amount of DOC. The UV wave absorption by the solution without napropamide was lower than the solution with napropamide, at the same DOC concentration. We converted the UV wave absorbance by the solution containing DOC only to concentration of napropamide in order to calculate the concentration of napropamide in solution of DOC plus napropamide. When we calculated the concentration of napropamide in the solution of DOC plus napropamide by subtracting the value obtained from the top plot in Fig. 4 with the value obtained from the bottom plot, the mean concentration of napropamide that we got was very close to 12.6 mg L⁻¹. The results showed that the matrix effect can be easily corrected by subtracting the absorbance of a sample with the absorbance of a blank solution obtained by extracting similar soil but without napropamide. We did not encounter any other UV wave absorption interferences at 288 nm other than the DOC absorption.

The intra and inter day precisions by both methods are shown in (Table 2). The average Standard Deviation (SD) for intra day precision was 0.25 and 0.2 for UV-spectrophotometer and HPLC-UV, respectively which indicated the measurements using the UV-spectrophotometer method were as reproducible as the HPLC method. The average of SD for inter assay precision was 0.7 and 0.45 for UV-spectrophotometer and HPLC-UV, respectively. Although the SD for inter day precision of the UV-spectrophotometry method was slightly higher than the HPLC method, it was way below the acceptable value. The results indicated that the napropamide measurement using the UV-spectrophotometry method was consistent.

The percent recoveries of napropamide from spiked soils are shown in (Table 3) and the properties of the soils are shown in (Table 5). The percent recoveries for both methods were quite high, the lowest was 88.2% and the highest was 95.4%. The percent recoveries for both methods decreased with the increasing amount of clay and organic content of soil. Stronger sorption of napropamide in soils with high amounts of organic matter and clay may be the reason for lower recoveries in those soils.

The napropamide in supernatant solutions measured at equilibrium for the sorption study determined using HPLC-UV and UV-spectrophotometer are shown in Fig. 5(a-d). There was no difference in napropamide concentrations at all concentrations of napropamide added to the soil solution mixture between the two methods at $p < 0.05$ for all soils that we studied. At any given napropamide concentration in the soil solution, the concentration of napropamide in the supernatants decreased with soil types according to the following trend; soil A > soil B > soil C > soil D. Again, the reason for the decreasing amount of napropamide can be attributed to the differences in clay and organic matter contents of the soils as well as the CEC. The results showed that UV-spectrophotometer was a reliable method to determine napropamide concentration in the batch equilibrium method for different soils which have different amount of OM and clay content. The result is in agreement with the result of Rodriguez-Rubio *et al.* (2006) which showed that the 2, 4-D concentration in soil solution can be measured by UV-spectrophotometry method. However, Baskaran and Bolan (1998) reported that the presence of water soluble organic matter interfered with the measurement of the same pesticide using UV-spectrophotometry method. We have shown that this can be corrected easily using a dual beam UV spectrophotometer which was not done by Baskaran and Bolan (1998).

The napropamide mass balance calculations which involved the initial amount of napropamide added to the soil solution mixture, its concentration in the supernatant and the amount adsorbed by the soil are shown in Table 4. The percent recoveries of napropamide in the four soils are calculated from the mass balance and presented on the same (Table 4). In all four soils that we studied, the percent recoveries of napropamide for the sorption study using the UV-spectrophotometry method were as good as the HPLC-UV method. Similar to the sorption trend, percent recoveries of napropamide from soils with higher clay and organic matter contents were lower probably because napropamide was strongly adsorbed in those soils and were not completely released by extraction with the methanol/water mixture.

Table 2: The inter and intra day precision of HPLC-UV and UV-spectrophotometer

Napropamide concentration (mg L ⁻¹)	0.5	5	10	20	30	40
UV-spectrophotometer intra day precision	99.2±0.4†	99.4±0.3	99.4±0.3	99.4±0.1	99.5±0.1	99.4±0.3
HPLC intra day precision	99.3±0.1	99.5±0.2	99.6±0.2	99.4±0.3	99.6±0.3	99.4±0.1
UV-spectrophotometer inter day precision	98.8±1.1	99.5±0.5	98.9±0.7	99.6±0.4	99.0±0.9	98.5±0.6
HPLC inter day precision	99.1±0.5	98.8±0.3	99.3±0.6	98.8±0.4	98.7±0.6	99.3±0.3

†Values are Mean±standard deviation

Table 3: The recovery of napropamide in spiked soils determined by HPLC-UV and UV- spectrophotometer

Soil	Percentage of recovery	
	HPLC	UV- Spectrophotometer
A	95.2±0.4†	95.4±0.7
B	94.3±0.6	93.7±0.9
C	92.5±0.9	91.3±1.1
D	89.8±1.2	88.2±1.5

† Values are Mean±standard error

Table 4: Percent recovery of napropamide in the sorption study for soil A-D

Results	Concentration (mg L ⁻¹)				
	5	10	20	30	40
Soil A					
Total amount of napropamide (µg) in the supernatant measured with HPLC	89	184	342	516	694
Total amount of napropamide (µg) in the supernatant measured with UV-spectrophotometer	88	180	346	520	706
Total extracted amount of napropamide (µg) measured with HPLC	5	6	37	55	78
Total extracted amount of napropamide (µg) measured with UV-spectrophotometer	5	8	35	56	70
Percentage of napropamide recovery in the HPLC	94	95	94.7	95.1	96.5
Percentage of napropamide recovery in the UV-spectrophotometer	93	94	95.2	96	97
Soil B					
Total amount of napropamide (µg) in the supernatant measured with HPLC	82	152	334	486	666
Total amount of napropamide (µg) in the supernatant measured with UV-spectrophotometer	83	156	330	492	670
Total extracted amount of napropamide (µg) measured with HPLC	11	37	44	80	100
Total extracted amount of napropamide (µg) measured with UV-spectrophotometer	10	32	47	78	95
Percentage of napropamide recovery in the HPLC	93	94.5	94.5	94.1	95.7
Percentage of napropamide recovery in the UV-spectrophotometer	93	94	94.2	94	95.6
Soil C					
Total amount of napropamide (µg) in the supernatant measured with HPLC	64	126	268	410	587
Total amount of napropamide (µg) in the supernatant measured with UV-spectrophotometer	66	124	278	416	590
Total extracted amount of napropamide (µg) measured with HPLC	25	56	97	142	153
Total extracted amount of napropamide (µg) measured with UV-spectrophotometer	22	58	90	143	151
Percentage of napropamide recovery with the HPLC	89	91	91.2	92	92.5
Percentage of napropamide recovery with the UV-spectrophotometer	88	90	92	92.1	92.6
Soil D					
Total amount of napropamide (µg) in the supernatant measured with HPLC	12	54	150	264	406
Total amount of napropamide (µg) in the supernatant measured with UV-spectrophotometer	12	58	146	266	410
Total extracted amount of napropamide (µg) measured with HPLC	74	110	204	270	301
Total extracted amount of napropamide (µg) measured with UV-spectrophotometer	73	116	209	263	307
Percentage of napropamide recovery with the HPLC	86	87	88.5	89	89.3
Percentage of napropamide recovery with the UV-spectrophotometer	85	87	88.7	88.1	88.8

Table 5: The physical and chemical characteristics of the soils used in the sorption study

Soils	OM (g kg ⁻¹)	Clay (g kg ⁻¹)	CEC (cmole kg ⁻¹)	EC (dS m ⁻¹)	pH
A	3	0	0.6	0.1	5.2
B	7	0	1.6	0.5	5.7
C	10	160	9.2	0.3	5.9
D	39	280	17.8	0.4	6.1

EC: electrical conductivity; OM: organic matter; CEC: cation exchange capacity

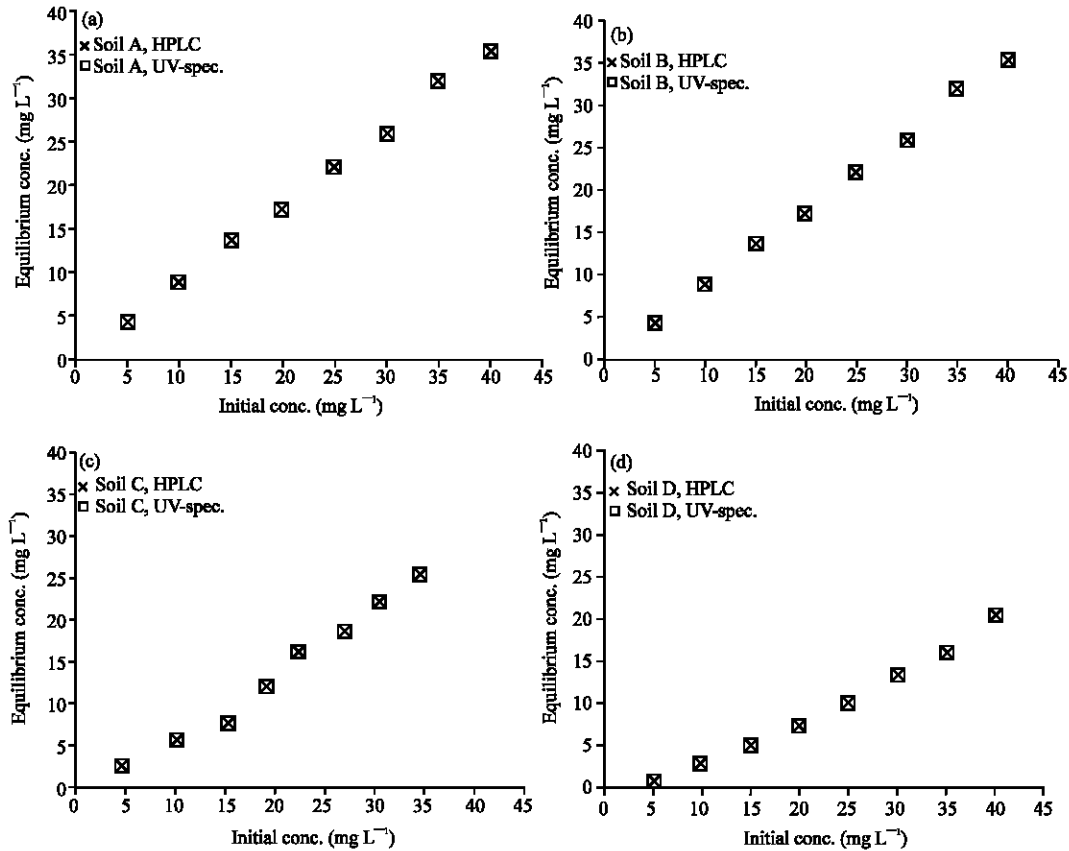


Fig. 5: The napropamide concentration in soil solution supernatant used in the sorption study for different soils using HPLC-UV and UV-spectrophotometer plotted against the initial

CONCLUSIONS

The results showed that the percent recoveries of napropamide in background solution, spiked soil sample and in the sorption study were quite high for both methods. The intra and inter day precision indicated that napropamide measurements using both methods were highly reproducible and stable at different times of the day as well as at different days. There were no differences between napropamide concentration in the supernatant measured by UV-spectrophotometry and HPLC method. Although DOC interfered with napropamide UV wave absorption, it can be easily corrected on the dual beam UV-spectrophotometer. As the UV-spectrophotometry method is fast and a reliable method to determine napropamide concentration in the supernatant of batch equilibrium sorption study, it can be used as an alternative to the HPLC method.

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