JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 8911–8917 8911
DOI:10.1021/jf1008346

Analytical Method Validation for the Determination of Meptyldinocap As 2,4-Dinitrooctylphenol Metabolite in Mango and Soil Using LC−MS/MS and Dissipation Study of the Fungicide in Indian Mango Field Ecosystem

Sudeb Mandal,[†] Bappaditya Kanrar,[‡] Saktipada Das,[†] and Anjan Bhattacharyya*,[‡]

[†]Department of Chemistry, Kalyani University, Kalyani-741235, WB, India, and [‡]Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur-741252, WB, India

An analytical method for the quantitative determination of meptyldinocap (2,4-DNOPC) as 2,4-dinitrooctylphenyl (2,4-DNOP) in mango and soil was developed as well as validated using liquid chromatography tandem mass spectrometry (LC−MS/MS). The method comprised an extraction with an acetone:methanol:4 N HCl (100:10:5, v/v/v) mixture followed by hydrolytic conversion of parent 2,4-DNOPC to the corresponding phenol metabolite (2,4-DNOP), and cleanup was done by liquid:liquid partition using ethyl acetate. Final quantitation was performed by LC−MS/MS of 2,4-DNOP with negative electrospray ionization using gradient elution. The method was validated at concentrations ranging from 0.025 to 2 μg/g, and the limit of quantification (LOQ) of meptyldinocap in mango and soil samples was 0.025 μg/g. The recovery of meptyldinocap from mango and soil sample was found to be 93−98% spiked at different levels with analyte, and the relative standard deviation for repeatability (RSD_r) and reproducibility (RSD_R) were acceptable (2−6%). The method was rugged as evident from a low measurement uncertainty at 0.05 μg/g. In order to evaluate its safety use in India a multilocational field dissipation study on meptyldinocap in mango was conducted by following the proposed analytical method.

KEYWORDS: Meptyldinocap; method validation; dissipation; LC−MS/MS; mango; soil

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important tropical fruits worldwide in terms of production and consumer acceptance (*1*). India is the largest producer of mango (10.8 million tonnes) in the world, accounting for about 41% of world output in 2004 (*2*). Powdery mildew is an important disease in most of the mango growing areas of the world causing a considerable crop loss. Powdery mildew caused by *Oidium mangiferae* Berthet is widely distributed and can be a major foliar disease of field-grown mango trees (*3*), on which it can infect leaves, bloom clusters and young fruits.

Dinocap is a mixture of six dinitrooctylphenyl crotonate (2,4-DNOPC) isomers (both ortho and para methylheptyl, ethylhexyl, and propylpentyl crotonate isomers (*4*) (**Figure 1**). The new meptyldinocap, an enhanced offering of the single 2,4-DNOPC methylheptyl isomer, is going to be introduced in the Indian market by Dow AgroSciences to replace dinocap. Meptyldinocap has an improved toxicological profile relative to the older dinocap. It is a novel powdery mildew (*Erysiphe necator*) fungicide which shows protectant and postinfective activities. It was primarily to be used for the control of powdery mildew in grapevine, but is also being developed for use on cucurbits and strawberries (*5*). The efficacy of meptyldinocap against grape powdery mildew is the same as that of dinocap (*6*). The maximum residue limit (MRL) of meptyldinocap (sum of 2,4-DNOPC and 2,4-DNOP expressed as meptyldinocap) was 0.05 μg/g in mangoes set by the European Union (EC-No 839/2008) (*7*).

Several analytical methods are available for determining dinocap in foods, and the majority are classical methods based on gas chromatography (GC) techniques with electron-capture detector (GC/ECD) that had been used for the determination of trace amounts of dinocap in wine (*8*). Identification and qualified estimation of dinocap was done using GC/IR and GC/MS (*9*) as well as using HPLC (*10*, *11*). A procedure for high-performance liquid chromatographic (HPLC) determination of four active ingredients of dinocap in crops was done by Duxiang et al. (*12*). Two improved methods for the determination of dinocap in apples, strawberries and cucumbers using GC and spectrophotomety and qualitative confirmation of the presence of dinocap are performed by thin-layer chromatography (TLC) (*13*). The LC−UV methods often have low sensitivity and lack of sufficient selectivity toward endogenous compounds, which absorb at the same wavelength. Christer Jansson et al. (*14*) presented ethyl acetate extraction and determination by means of LC−MS/MS in a multiresidue method. But there is no reliable validated LC−MS/MS method for measuring trace levels of meptyldinocap where the fungicide is determined as a derivative

*Corresponding author. Tel: +91-33- 25827139 (R), +91-9433007139 (M). Fax: +91-33-25828460. E-mail: anjan_84@rediffmail.com.

Published on Web 07/22/2010

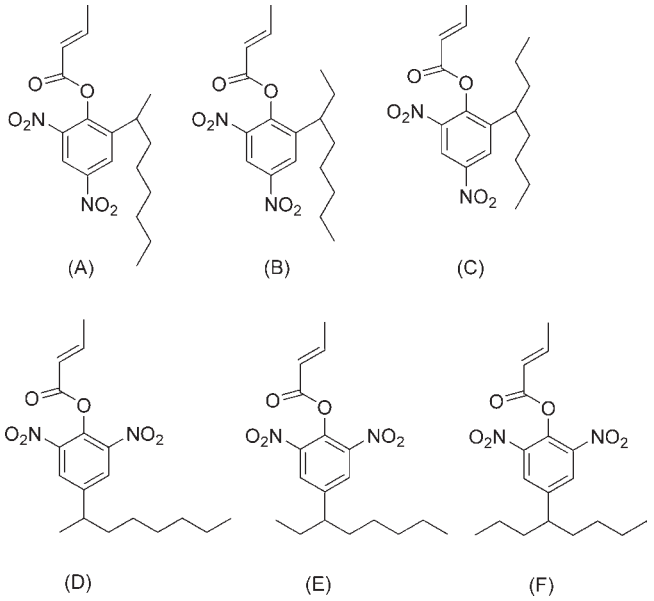

Figure 1. Chemical structure of six isomers of dinocap: **A** = 2,4-dinitro-6-(1-methylheptyl)phenyl crotonate or meptyldinocap or 2,4-DNOPC; **B** = 2,4-dinitro-6-(1-ethylhexyl)phenyl crotonate; **C** = 2,4-dinitro-6-(1-propylpentyl)phenyl crotonate; **D** = 2,6-dinitro-4-(1-methylheptyl)phenyl crotonate; **E** = 2,6-dinitro-4-(1-ethylhexyl)phenyl crotonate; **F** = 2,6-dinitro-4-(1-propylpentyl)phenyl crotonate).

(2,4-DNOP) with accuracy, precision, and repeatability in food and soil samples. The proposed analytical method in this paper is comparable to other methods described in Federal Register/ Vol. 74, No. 69/Monday, April 13, 2009 (*15*), but improvements have been made regarding sample preparation and other crucial points.

Although the consumption of mango is widespread, there is no report dealing with the methodological approach for the analysis of meptyldinocap in mango. In particular, there is a lack of data regarding the disappearance in-field of meptyldinocap on mango for determination of the most realistic PHI (preharvest interval) to ensure the safe use of this fungicide.

The primary aim of this work was to develop an analytical method for the determination of meptyldinocap (2,4-DNOPC) as 2,4-DNOP by optimizing the preparation of sample and optimizing LC−MS/MS conditions as well as validate the method in mango and soil samples. Moreover, the developed and validated method was used to study its dissipation and residue (at harvest) of meptyldinocap in the mango field ecosystem under the Indian tropical climate with a view to ensure human and environmental safety.

MATERIALS AND METHODS

Apparatus. An Alliance 2695 separations module (Waters, Milford, MA) was coupled to a Micromass Quattro Micro triple-quadrupole mass spectrometer (Micromass, Manchester, U.K.). MassLynx $V_{4.1}$ software was used for instrument control and QuanLynx for data analysis. A homogenizer (Polytron, PT-3100, Switzerland), pH meter (CL 46 type; Toshniwal Instruments, Pvt, Ltd., India) and rotary-vacuum-evaporator of Eyela (SB-1000, Japan) were used in this study. The shaker (ZHWY-200D; Zhicheng, China) and the centrifuge were from Beckman Coulter (Avanti J-30I, USA) and were used for sample preparation.

Chemicals. An analytical standard of meptyldinocap (2,4-DNOPC, 99.23% pure) was procured from M/S Dow AgroSciences India Pvt. Ltd., Mumbai. An analytical standard of 2,4-DNOP (99.00% pure) was prepared in our laboratory. Pesticide residue analysis grade acetone, acetonitrile, *n*-hexane, methanol, and ethyl acetate were purchased from Rankem (India). Sodium sulfate (anhydrous) and sodium hydroxide were purchased from Qualigen (India). Hydrochloric acid, acetic acid and ammonium acetate were purchased from Emerck (India). Purified water was prepared by using a Milli-Q (Millipore, Bedford, MA) water purification system.

Table 1. Monthly Meteorological Data during the Experimental Period

		temp (°C)		rel humidity (%)		
trial location	month and year	max	min	max	min	rainfall (mm)
I	Jan 2008	25.5	12.3	94.7	50.2	60.5
	Feb 2008	26.8	13.9	94.7	46.3	27.8
	March 2008	33.7	21.9	91.9	46.9	16.2
	April 2008	37.5	21.3	93.4	49.4	40.8
II	Jan 2008	32.8	16.9	90.2	58.4	0.0
	Feb 2008	30.6	18.6	85.6	62.6	0.0
	March 2008	32.9	22.1	89.7	65.1	51.4
	April 2008	33.6	25.4	84.3	68.3	0.0
III	March 2008	31.5	10.2	85.8	25.8	1.8
	April 2008	33.4	13.6	66.7	28.0	46.6
	May 2008	35.5	18.8	70.0	36.5	63.2
	June 2008	31.4	23.8	92.3	72.2	301.5
IV	Dec 2007	27.8	9.2	82.7	75.8	0.0
	Jan 2008	29.4	9.6	54.8	48.6	0.0
	Feb 2008	30.4	16.2	67.5	55.9	0.0
	March 2008	32.1	19.0	75.7	68.5	0.0

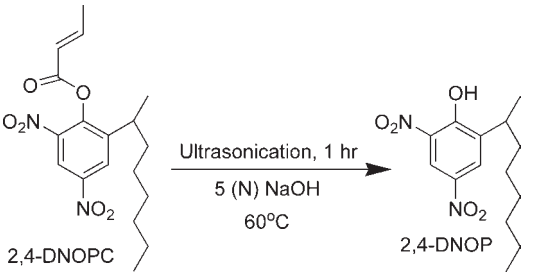

Figure 2. Conversion of 2,4-DNOPC to 2,4-DNOP.

Preparation of 2,4-DNOP Metabolite. An amount of 10 mg of 2,4-DNOPC was dissolved in 25 mL of methanol in a 100 mL round-bottom flask, and 4 mL of 4 (N) NaOH was added to it. The flask was heated at 60 °C temperature in an ultrasonic bath for 1 h. After cooling of the round-bottom flask, the pH of the reaction mixture was neutralized by addition of concentrated HCl dropwise and the methanol solvent was evaporated in a rotary vacuum evaporator at 40 °C. Then the reaction mixture was partioned with (50 + 50) mL of ethyl acetate which was concentrated by rotary-vacuum-evaporator and purified by column chromatography. The purity (99.00%) was confirmed by IR, LC−MS/MS and NMR spectroscopy.

Standard Solutions. Stock solutions of 2,4-DNOPC and 2,4-DNOP standards were prepared by weighing 10 ± 0.02 mg in volumetric flasks (certified "A" class) and dissolving each in 100 mL of methanol. Five levels of calibration concentration containing 0.01, 0.025, 0.05, 0.1, and 0.5 μg/mL of 2,4-DNOP were obtained by serial dilution of 2,4-DNOP stock solution with methanol. Three levels of fortification concentration containing 0.025, 0.05, and 0.1 μg/mL were prepared by serial dilution of 2,4-DNOPC stock solution with methanol required for recovery study.

Field Experiment. A multilocation field study was conducted in mango fields in four different locations with different variety in India, viz., (I) Bidhan Chandra Krishi Viswavidyalaya, Mohanpur (BCKV), West Bengal (Local), (II) Konkan Krishi Vidyapeeth (KKV), Dapoli, Maharashtra (Alphonso), (III) G. B. Pant University of Agricultura & Technology (GBPUAT), Pantnagar, Uttarakhand (Dessheri), and (IV) Acharya N. G. Ranga Agricultural University (ANGRAU), Hyderabad (M. Vikarabad) on mango during December, 2007, to June, 2008. A plot size of 1 Plant/Trt was selected for the control and each treatment of the fungicide under study, and the average ages of the mango trees were 23−30 years. Meptyldinocap (35% EC) was applied to mango at the rate of 270 gai/ha (T_1) and 540 gai/ha (T_2) along with untreated control (T_3). Each treatment including control was replicated thrice in a randomized block

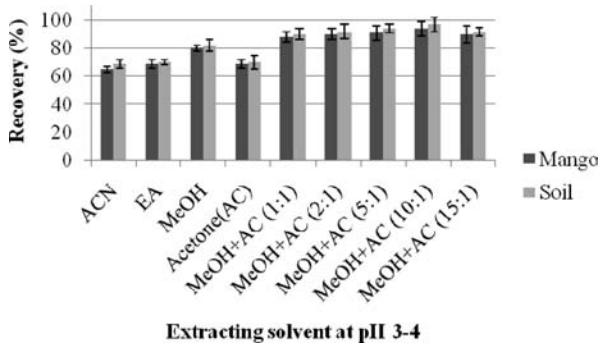

Figure 3. Comparison of extraction capabilities of different solvent systems (50 mL) in the final method from mango and soil (10 g) spiked at 0.1 μg/g . Error bars signify standard deviation (n = 6).

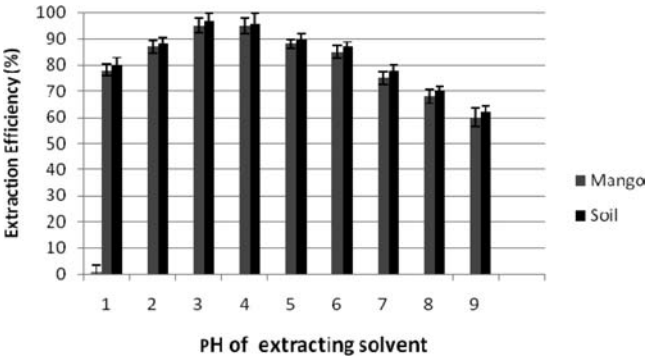

Figure 4. Effect of pH of the extracting solvent (acetone:methanol:4 N HCl;100:10:5; v/v/v) for meptyldinocap (2,4-DNOPC) extraction from mango and soil samples.

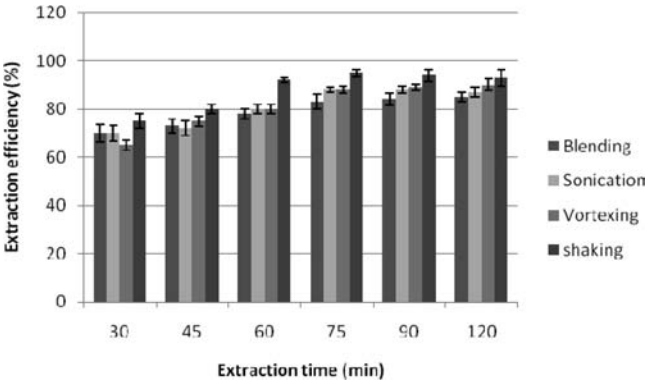

Figure 5. Extraction capabilities of blending over shaking, sonicating and vortexing of meptyldinocap after 2 h of spiking at 0.1 μg/g in soil sample using 50 mL of acetone:methanol:4 N HCl (100:10:5; v/v/v) as extracting solvent. Error bars signify standard deviation (n = 6).

design (RBD). Two sprays were given at a 15 day interval. Mango samples were randomly collected at 0 (3 h after application), 1, 3, 5, 7, and 15 (at harvest) days after second application (DAA). Soil samples were randomly collected at 15 days (at harvest) after the second application. The samples were transported to the laboratory in dry ice bags and kept at −20 °C until analysis in order to avoid any degradation of residues between sampling and analysis. Meteorological data including rainfall, humidity, and temperature were recorded using a field weather station during the period of field experiments and are given in **Table 1**.

Sample Extraction. Chopped mango sample (10 g) was macerated with 50 mL of acetone:methanol:4 N HCl (100:10:5; v/v/v) using a Polytron homogenizer, and the homogenate was centrifuged. The supernatant was transferred to a clean bottle via a glass wool. An aliquot (30 mL) of acetone:methanol:4 N HCl (100:10:5; v/v/v) was added to the residuum and macerated as previously. The sample was centrifuged, and the supernatants were combined. The total volume was adjusted to 100 mL using the extraction solvent. An aliquot (20 mL) of extract was transferred to a round-bottom flask and reduced to a low volume (< 1 mL) by rotary evaporation at 35 °C.

Soil samples (10 g) were taken in a 250 mL conical flask, and then 50 mL of acetone:methanol:4 N HCl (100:10:5; v/v/v) was added to the flask. The samples were then kept for two hours and shaken in a shaker (ZHWY-200D) for one hour. Then the extracts were filtered in a Buchner funnel using 50 mL of the same extracting solvent (2 × 25 mL) for washing. The total volume was adjusted using the extraction solvent to 100 mL. An aliquot (4 mL) of the extract was transferred to a flask and reduced to a low volume (< 1 mL) by rotary evaporation at 35 °C.

The extracted soil and mango samples were then transferred to a graduated tube with 20 mL of ultrapure water, 1 N HCl (2 mL) and 10 mL of hexane:ethyl acetate (50:50, v/v). Then it was shaken vigorously, and the phases were allowed to settle. The upper organic phase was transferred into a round-bottom flask, and the extraction was repeated with a further two aliquots (10 mL) of the hexane:ethyl acetate mixture. The combined organic phase was collected in the same round-bottom flask. The solvent was removed by rotary vacuum evaporator at 35 °C.

Conversion of crotonates to phenol (**Figure 2**) was done by taking aliquots of methanol (10 mL), 5 (N) sodium hydroxide (5 mL) in the concentrated round-bottom flask. The flask was then ultrasonicated and the sample transferred to a graduated tube. The sample was placed in an ultrasonic water bath set at 60 °C for 60 min.

Cleanup and Analysis. The sample was removed from the water bath, the remaining methanol was evaporated in a rotary vacuum evaporator, and the sample was allowed to cool at room temperature. Aliquots of water (8 mL), concentrated hydrochloric acid (3 mL), and ethyl acetate (10 mL) were then added in a separatory funnel. The sample was shaken and the upper ethyl acetate phase was transferred through a glass funnel containing 75 g of sodium sulfate into a round-bottom flask. The partition was repeated with (10 + 10) mL of ethyl acetate, and the combined organic phase was evaporated to dryness in a rotary vacuum evaporator at 35 °C. The sample was reconstituted in 10 mL of methanol:water (70:30; v/v) containing 0.1% glacial acetic acid for final quantification by LC−MS/MS analysis.

According to the SANCO/10684/2009 (*16*) guideline a calibration curve was constructed by plotting 0.01−0.1 ppm of 2,4-DNOP standards injected versus peak area. From the calibration curve the concentration of 2,4-DNOP in samples was determined. Consequently the concentration of meptyldinocap (2,4-DNOPC) was calculated by the following equation:

$$C_{2,4\text{-DNOPC}} = \frac{\text{MW}_{2,4\text{-DNOPC}}}{\text{MW}_{2,4\text{-DNOP}}} \times C_{2,4\text{-DNOP}} = 1.23 C_{2,4\text{-DNOP}}$$

The PHI, i.e. the time period (in days) required for dissipation of the initial residue deposits to below the maximum residue limit (MRL) for first-order kinetics, was determined by the equation

$$\text{PHI} = [\log(\text{intercept}) - \log(\text{MRL})]/(\text{slope of first-order equation})$$

LC−MS/MS analysis. *HPLC Conditions*. Optimization of chromatographic separation was performed on a Waters 2695 liquid chromatograph (Milford, MA) equipped with a quaternary solvent delivery system, an autosampler and a Waters 2996 DAD detector. Different columns were assayed for 2,4-DNOP analysis. A reverse phase column Symmetry C18, 5 μm, 2.1 × 100 mm, showed an excellent retention for 2,4-DNOP (retention times0.99 ± 0.06 min). The first mobile phase assayed was composed of 50:50 acetonitrile:water with 10 mM ammonium acetate. The second mobile phase assayed was 50:50 acetonitrile:water with 10 mM ammonium acetate and added with 0.1% of acetic acid. The third mobile phase assayed was 50:50 acetonitrile:water with 10 mM ammonium acetate and 0.1% formic acid. The mobile phase containing acetic acid significantly improves the response of 2,4-DNOP (m/z 295.14) about 1.35-fold compared to formic acid, and 2.1 times higher than the mobile phase with only ammonium acetate. Acetic acid was selected as acidic modifier. The mobile phase was composed of (A) a mixture of 0.01 M ammonium acetate with 0.1% acetic acid in water:acetonitrile (80:20 v:v) and (B) 0.1%

Table 2. Linearity Range, Limit of Quantification (LOQ), Recovery (Rec), Relative Standard Daviation for Repeatability (RSD_r), Relative Standard Daviation for Reproducibility (RSD_R) and Matrix Effect (ME) of Meptyldinocap from Mango and Soil

substrate	linear range (μg/g)	spiked level (μg/g)	R^2	RSD_r (%)	RSD_R (%)	Rec (%)	ME[a] (%)	LOQ (μg/g)
mango	0.025−2	0.025	0.9851	5.5	5.9	93.8	−19	0.025
		0.05	0.9751	4.8	4.3	95.1	−17	
		0.1	0.9826	4.1	3.9	94.6	−12	
soil	0.025−3	0.025	0.9912	5.2	4.2	97.6	−17	0.025
		0.05	0.9862	3.4	3.8	96.8	−14	
		0.1	0.9754	2.2	2.6	98.2	−10	

[a] Minus sign designates induced signal suppression.

acetic acid in acetonitrile (v/v). 75% solvent B was used initially; then, its concentration was changed from 75% to 100% in 3 min, keeping these conditions for 1 min. Finally, the program is switched to 75% B in 6 min and kept for at least 5 min before starting a new analysis at a flow rate of 0.3 mL/min.

MS/MS Conditions. A Micromass (Manchester, U.K.) Quattro Micro triple-quadrupole mass spectrometer equipped with an electrospray source was used for detection and quantification. The optimized MS instrument parameters obtained by the tuning were as follows: capillary voltage, 1.20 kV; cone voltage, 46 V; source temperature, 120 °C; desolvation temperature, 350 °C; desolvation gas flow, 650 L h^{-1} nitrogen; cone gas flow, 25 L h^{-1}; argon collision gas (argon) pressure to 3.5 e^{-3} psi for MS/MS. The collision energy for each monitored transition was 13 eV in MRM mode. In the MRM transitions the dwell and interscan times were 0.3 and 0.1 s, respectively. The LC−MS/MS run time was 6 min per sample.

MS/MS Transitions. The collision energy for each monitored transition was optimized in MRM mode. The transitions monitored for 2,4-DNOP were m/z 295.14 > 193.42 at 29 V, 295.14 > 134.50 at 40 V, and 295.14 > 208.9 at 46 V. The MRM mode of the degradation patterns m/z 295.14 > 193.42 was used for quantification, and 295.14 > 134.50 and 295.14 > 163.1 were used for confirmation, respectively. MS conditions were optimized by observing responses for loop injections of 20 μL of 2,4-DNOP standard (10 μg/mL) in sample matrices. A dwell time of 100 ms per transition was used. 2, 4-DNOP was detected using electrospray ionization in the negative ion mode.

Method Validation. The analytical method was validated as per the single laboratory validation approach (*17*). The performance of the method was evaluated considering different validation parameters that include the following items.

Calibration Range. The calibration curves of 2,4-DNOP in pure solvent and matrix were obtained by plotting the peak area against the concentration of the corresponding calibration standards at five calibration levels ranging between 0.01 and 0.5 μg/mL.

Sensitivity. The limit of detection (LOD) was determined by considering a signal-to-noise ratio (S/N) of 3 with reference to the background noise obtained from blank sample, whereas the limits of quantification (LOQ) were determined by considering a S/N of 10 using matrix matched standards.

Precision. Method precision was checked in terms of intraday repeatability and interday reproducibility at three concentration levels (0.025, 0.05, and 0.1 μg/g). The intraday precision study was carried out by the injection of the same standard solution five consecutive times ($n = 5$) in the same day under the same conditions. The interday precision was carried out for three successive days using the same solution. The tested products were mango and soil.

Accuracy-Recovery Experiments. Accuracy was evaluated through recovery study by spiking untreated mango and soil samples (10 g) in triplicate with meptyldinocap separately at three concentration levels (25, 50, and 100 μg/g of 2,4-DNOPC). The mixture was extracted, derivatized, cleaned up and analyzed using the method mentioned above.

Matrix Effect. The matrix effect (ME) was assessed by employing matrix-matched standards. The slope of the calibration graph based on the matrix matched standards of mango and soil sample was compared with the slope of the pure solvent based calibration graph. A higher slope of the matrix calibration indicates matrix induced signal enhancement, whereas, a lower slope represents signal suppression. The matrix effect (ME %) was evaluated by the following equation:

$$\text{ME, \%} = \frac{(\text{peak area of matrix standard} - \text{peak area of solvent standard}) \times 100}{\text{peak area of solvent standard}}$$

Measurement Uncertainty. Sources and quantification of the global uncertainty for the applied method were evaluted for meptyldinocap at the level of 0.05 μg/g as per the statistical procedure of the EURACHEM/CITAC Guide CG 4 (*18*) in the same way as reported by Banerjee et al. (*19*). Five individual sources of uncertainty were taken into account, viz., uncertainty associated with the calibration graph (U_1), day wise uncertainty associated with precision (U_2), analyst wise uncertainty associated with precision (U_3), daywise uncertainty associated with accuracy/bias (U_4), and analystwise uncertainty associated with accuracy/bias (U_5). The global uncertainty (U) was calculated as

$$U = (U_1^2 + U_2^2 + U_3^2 + U_4^2 + U_5^2)^{1/2}$$

and was reported as expanded uncertainty, which is twice the value of the global uncertainty.

RESULTS AND DISCUSSION

Optimization of Sample Preparation. The pH of the extracting solvent plays an important role in controlling the degree of extraction of the target molecule from mango and soil sample. Optimization of the solvent pH was done from pH 2 to 9. **Figure 3** shows that at pH values between 3 and 4 an optimal efficiency had been reached, and a pH value of 3−4 was chosen for the experiments.

For the selection of extraction solvent nine organic solvents and solvent mixtures, viz., acetonitrile, ethyl acetate, methanol, acetone, methanol + acetone (1:1; v/v), acetone + methanol (1:1; v/v), acetone + methanol (2:1; v/v), acetone + methanol (5:1; v/v), acetone + methanol (10:1; v/v) and acetone + methanol (15:1; v/v) at pH 3−4 were evaluated for their extraction efficiency. From this study combining the pH effect it is clearly revealed that a mixture of acetone:methanol:4 N HCl (10:2:1; v/v/v) gave higher recovery percentage than other solvent or solvent mixtures used for extraction (**Figure 4**).

Different approaches have been described for soil sample extraction, such as shaking, vortexing, blending and ultrasonication. To check for effectiveness of the extraction, the same sample was incubated at different extraction times. From our results it is revealed that shaking gave better recovery for the fungicide compared to vortexing, blending and ultrasonication based methods (**Figure 5**). It was observed that 1 h gave higher fungicide extraction and that prolonged time did not exert additional advantages.

Extraction of free meptyldinocap (2,4-DNOPC) is not possible because it is likely to be converted to 2,4-DNOP. Hence the parent 2,4-DNOPC must be derivatized to its metabolite 2,4-DNOP (**Figure 2**). The hydrolyzed sample must be methanol free to get optimum recovery. The analyte might be lost in the methanol−water phase in the liquid−liquid partition step with

ethyl acetate. The method is based on the rapid hydrolysis of dinocap by means of ethanolamine, but it is a toxic, costly viscous liquid. So we have used sodium hydroxide for hydrolysis purpose instead of ethanolamine. After hydrolysis of analyte to 2,4-DNOP from 2,4-DNOPC using NaOH solution, it was neutralized prior to liquid−liquid partition to get maximum recovery. For the development of a sensitive method to determine the presence of pesticides with a 2,4-dinitrophenolic structure, it is crucial to know whether the compounds are present in the free phenolic or in the dissociated phenolate form, which depends on the appropriate selection of pH (*20*). The results show that the recovery of the compounds after the hydrolysis step increased when it was neutralized at pH around 7.5.

Validation of the Proposed Method. The linearity in the response was studied using matrix-matched calibration solutions prepared by spiking 2,4-DNOP at five concentration levels, ranging from 0.005 to 5 μg/mL in the mango and soil samples. The calibration curves were obtained by plotting the peak area against the concentration of the corresponding calibration standards in all matrices. Good linearity was observed in the studied range with R^2 values higher than 0.97. The mean recovery percentages and the associated standard deviation are given in **Table 2**. It can be seen that the mean recoveries ranged from 93.8 to 95.1% for mango and from 97.6 to 98.2% for soil and the relative standard deviation (RSD) values obtained from run-to-run (RSD_r) and day-to-day (RSD_R) precision are summarized (2−6%) in **Table 3**. From the results obtained, the developed method was found to be precise (*21*) for quantitative purposes.

Table 3. Individual and Global Uncertainties[a] for Triasulfuron in Mango and Soil Expressed as % Relative Standard Deviation

matrices	U_1	U_2	U_3	U_4	U_5	U	$2U$
mango	2.2	0.24	0.26	2.2	2.2	3.83	7.65
soil	1.8	0.28	0.25	2.6	1.7	3.61	7.22

[a] Calculated at 50 ng/g.

An estimated value of LOD was 0.01 μg/mL, whereas LOQ values were in the range from 0.02 to 0.25 μg/mL. The results are summarized in **Table 2**. However, calculated LODs and LOQs were at least 2 orders of magnitude lower than the established MRLs for mango, indicating that the proposed method is suitable for quantification of meptyldinocap in mango.

No interfering endogenous compound peaks were observed at the retention time of 2,4-DNOP in chromatograms obtained from control blank food (mango and soil) samples (**Figure 2**). It was therefore expected that assay of food samples by use of this method would not be prevented by interfering peaks. Representative chromatograms obtained from extracted control and 2,4-DNOP spiked food samples are shown in **Figure 2**, from which it is apparent that the retention time of 2,4-DNOP was approximately 0.99 min (**Figure 6**).

The uncertainty values for meptyldinocap are reported as % relative standard deviation in **Table 3**. Calculated U values ranged below 4% for mango and soil matrices. The global uncertainty of meptyldinocap in different matrices varied below 10% ($U < 4$) and had lower uncertainties associated with precision ($< 0.3\%$ for both U_2 and U_3) as well as the accuracy/bias ($< 3\%$ for both U_4 and U_5). This suggests that the method gave repeatable and reliable results for this fungicide, without any major loss of residues during sample preparation.

Result of Field Sample Analysis and Safety Evaluation. The residue of meptyldinocap in/on mango after its second application at 15 day interval at the rate of 270 gai/ha (T_1) and 540 gai/ha (T_2) in four locations in India is presented in **Tables 4** and **5**. The results of this experiment showed that meptyldinocap dissipated with gradual and continuous deterioration after application. The dissipation trend of meptyldinocap in mango followed first order kinetics. The possible routes of meptyldinocap dissipation and transformation in the environment include plant species, climatic conditions and biotransformation via soil microorganisms on soil and photo conversion to simpler products on plant surfaces. The initial deposit of meptyldinocap in mango of four locations was

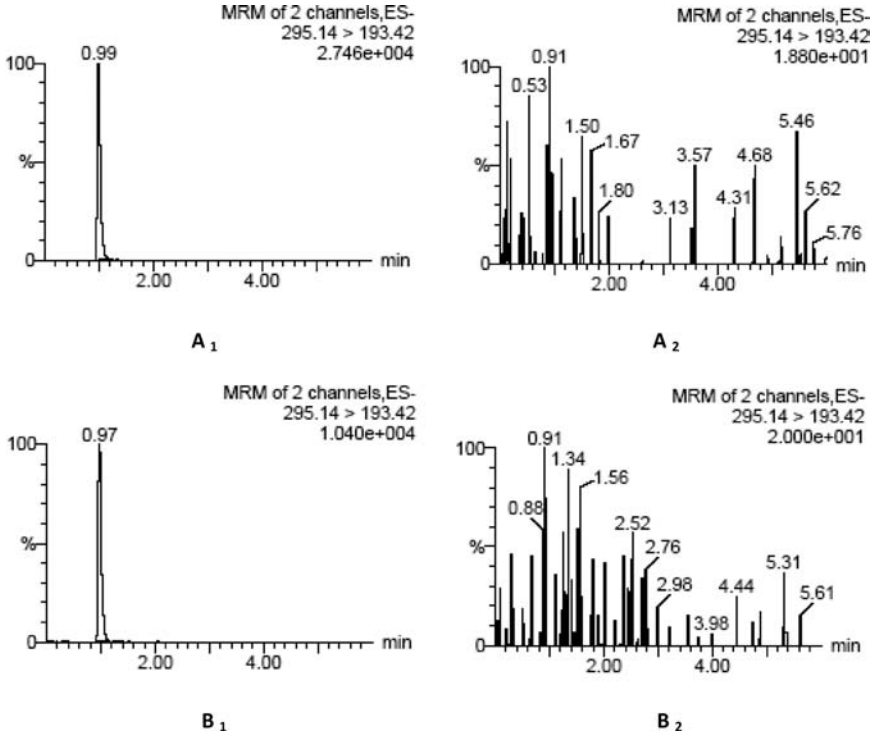

Figure 6. Mass chromatographic profiles of product ion [m/z 295.14 → 193.42 (b)] for 2,4-DNOP from mango ($\mathbf{A_1}$) and soil ($\mathbf{B_1}$) matrices and chromatograms obtained from control mango ($\mathbf{A_2}$) and soil ($\mathbf{B_2}$) sample monitored at m/z 295.14 → 193.42 of 2,4-DNOP.

Table 4. Dissipation of Meptyldinocap Residues in Mango in Four Locations[a]

		amt of meptyldinocap recovered ± SD (μg g^{-1}) (% dissipation)	
trial location	DAA	T_1 (270 gai ha^{-1})	T_2 (540 gai ha^{-1})
BCKV	0	1.32 ± 0.06 (—)	1.72 ± 0.01 (—)
	1	0.81 ± 0.02 (38.38)	1.41 ± 0.02 (17.83)
	3	0.55 ± 0.02 (58.08)	0.83 ± 0.06 (51.74)
	5	0.32 ± 0.03 (75.76)	0.60 ± 0.14 (64.92)
	7	BDL	BDL
	regression equation	$Y = 3.0793 - 0.1153X$	$Y = 3.2349 - 0.0944X$
	half life ($t_{1/2}$)	2.61 days	3.19 days
	waiting period (PHI)	15.52	19.18
KKV	0	1.31 ± 0.04 (—)	1.86 ± 0.07 (—)
	1	0.87 ± 0.04 (53.55)	1.56 ± 0.03 (53.55)
	3	0.57 ± 0.02 (69.50)	1.12 ± 0.05 (69.50)
	5	0.34 ± 0.03 (81.91)	0.61 ± 0.06 (81.91)
	7	BDL	BDL
	regression equation	$Y = 3.0919 - 0.1131X$	$Y = 3.2863 - 0.0939X$
	half life ($t_{1/2}$)	2.66 days	3.21 days
	waiting period (PHI)	15.84	19.36
GBPUAT	0	1.45 ± 0.02 (—)	2.02 ± 0.02 (—)
	1	0.90 ± 0.07 (52.30)	1.61 ± 0.05 (49.37)
	3	0.57 ± 0.06 (69.50)	1.24 ± 0.02 (61.01)
	5	0.28 ± 0.05 (85.28)	0.71 ± 0.07 (77.57)
	7	BDL	BDL
	regression equation	$Y = 3.138 - 0.1358X$	$Y = 3.3141 - 0.0885X$
	half life ($t_{1/2}$)	2.22 days	3.40 days
	waiting period (PHI)	13.24	20.58
AGRAU	0	1.27 ± 0.10 (—)	1.69 ± 0.01 (—)
	1	0.84 ± 0.05 (55.14)	1.41 ± 0.03 (55.77)
	3	0.59 ± 0.01 (68.79)	0.93 ± 0.05 (70.65)
	5	0.32 ± 0.03 (83.16)	0.43 ± 0.01 (86.58)
	7	BDL	BDL
	regression equation	$Y = 3.0847 - 0.1132X$	$Y = 3.2561 - 0.1127X$
	half life ($t_{1/2}$)	2.66 days	2.67 days
	waiting period (PHI)	15.81	16.09

[a] SD, standard deviation; average of three replicates. DAA, days after application. BDL: below detectable level. BCKV, Bidhan Chandra Krishi Viswavidyalaya. KKV, Konkan Krishi Vidyapeeth. GBPUAT, G. B. Pant University of Agricultura & Technology. ANGRAU, Acharya N. G. Ranga Agricultural University.

Table 5. Harvest Residue of Meptyldinocap in Mango and Mango Cropped Soil in Four Locations[a]

			amt of meptyldinocap recovered ± SD (μg g^{-1}) (% dissipation)	
trial location	DAA	substrate	T_1 (270 gai ha^{-1})	T_2 (540 gai ha^{-1})
I	15	mango	BDL	BDL
	15	soil	BDL	BDL
II	15	mango	BDL	BDL
	15	soil	BDL	BDL
III	15	mango	BDL	BDL
	15	soil	BDL	BDL
IV	15	mango	BDL	BDL
	15	soil	BDL	BDL

[a] SD, standard deviation; average of three replicates. DAA, days after application. BDL, below detectable levels.

found in the range of 1.31−1.45 μg/g for recommended dose (T_1) and 1.69−2.02 μg/g for double the recommended dose (T_2) respectively. The residues declined with time, and 64−86% dissipation appeared on the fifth DAA.

The dissipation of meptyldinocap was slightly faster at recommended dose (270 gai/ha) ($T_{1/2}$ = 2.22−2.66 day) than the double the recommended dose (540 gai/ha) ($T_{1/2}$ = 2.67−3.40 day). Among the locations, dissipation of meptyldinocap in mango was faster at ANGRAU [$T_{1/2}$ = 2.66 day (T_1); 2.67 day (T_2)] followed by BCKV [$T_{1/2}$ = 2.61 day (T_1); 3.19 day (T_2)], KKV [$T_{1/2}$=2.66 day (T_1); 3.21 day (T_2)] and GBPUAT ($T_{1/2}$=2.22 day (T_1); 3.40 day (T_2)]. The difference could be attributed to weather conditions and the growth characteristics of different varieties of mango grown at these locations. Though meptyldinocap was applied on mango, part of the spray material falls on the field soil.

The residues of meptyldinocap in mango were found below its LOQ of 0.025 μg/g, after 7 days irrespective of dose and location. At harvest (15 DAA), the residues of meptyldinocap in mango and soil samples were found to be lower than the European Union's maximum residue limit (MRL) of 0.05 μg/g in mango (**Table 5**). So it is suggested that a waiting period (PHI) of 15 days should be done at the recommended dose before consumption of mangoes, as it will be safe for the consumer's health.

ACKNOWLEDGMENT

We gratefully acknowledge M/S Dow AgroSciences, Mumbai, India, for providing analytical standard. We are also thankful to Export testing Laboratory (ETL), Bidhan Chandra Krishi Viswavidyalaya, Mohanpur (BCKV), West Bengal, India, for providing instruments.

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Received for review March 1, 2010. Revised manuscript received July 8, 2010. Accepted July 12, 2010. We gratefully acknowledge M/S Dow AgroSciences, Mumbai, India, for financial support.