International Multidisciplinary Research Journal 2012, 2(9):01-04
ISSN: 2231-6302
Available Online: http://irjs.info/

INTERNATIONAL MULTIDISCIPLINARY RESEARCH JOURNAL

A Simple, reversed-phase Ultra performance liquid chromatographic method for the simultaneous determination of Monocrotophos, Thiram, carbendazim, Carbaryl and imidacloprid pesticides by Quechers method in chilli samples.

Dinesh C Bilehal [1], M. B. Chetti [2], P.T. Goroji [2] and Mahadev C. Khethagoudar[2]

[1]Reva Institute of Technology and Management, Kattigenahalli, Yelahanka, Bengaluru-560064, Karnataka. India
[2]University of Agricultural Sciences, Dharwad, 580 005, Karnataka. India

Abstract
A simple, reversed-phase Ultra-performance liquid chromatographic (RP-UPLC) method for the simultaneous determination of five pesticides namely Monocrotophos, Thiram, Carbendazim, Carbaryl and Imidacloprid has been developed. This method involves sample preparation by QueCherS method and quantification by ultra performance liquid chromatography with tunable dual wavelength detector. The mobile phase composition was varied to improve peak resolution and peak sensitivity. Choosing the match between the stationary phase and mobile phase composition, the developed RP-UPLC method not only can simplify the procedure but also significantly reduce the analysis time. The method presents an average recovery of 87.9% and 96.9%, in repeatability and intermediate precision conditions, respectively, with adequate precision (RSD from 0.8 to 20.7%), for all compounds. The method was applied to determine these pesticide residues in chilli samples.

Keywords: UPLC, pesticide analysis, chilli, and Quechers.

INTRODUCTION

A group of artificially synthesized substances, called pesticides, has been used in agriculture to control pests and to increase crop production[1]. These substances protect the agricultural crops from pests, but overuse and incorrect use can pose risks to human health and the environment[2, 3]. The increase in the amount of pesticides and in variety of products applied to agriculture makes it necessary to monitor residues in the fruits and vegetables. Therefore, the analysis of pesticides has received increasing attention in the last few decades. Due to the low detection levels required by regulatory bodies and the complex nature of the matrices in which the target compounds are present, efficient sample preparation and trace-level detection and identification are important aspects of analytical methods.

Gas chromatography (GC) and high performance liquid chromatography (HPLC) coupled with various detection systems are the most powerful tools for the analysis of pesticides[4-9]. However, many classes of pesticide that have polar characteristics, low volatility or thermal instability, cannot be analyzed directly by GC and require special conditions such as derivatisation procedures. Liquid chromatography (LC) is the preferred approach for these polar and thermally labile pesticides, with a conventional UV detection or diode array detection (DAD). The use of liquid chromatography combined with mass spectrometry (LC-MS-MS) has been proposed for determining some of these pesticides[10-12]. These methods are much more specific and sensitive analytical techniques, but they are not affordable in most research laboratories because of the high cost of the equipment.

This paper reports a simple, relatively fast, and efficient QueCherS and UPLC-TUV method which was developed for the determination of Carbendazim, Carbaryl, Imidacloprid, Thiram and Monocrotophos in chilli samples. To obtain efficient pre-concentration with good precision and recovery, a QueCherS method was applied. The method was validated and the parameters involved in the validation were linearity and range, limit of detection (LOD) and quantification (LOQ), precision (repeatability and intermediate precision), and accuracy (recovery).

UPLC refers to Ultra Performance Liquid Chromatography. It improves in three areas namely chromatographic resolution, speed and sensitivity analysis. The main advantage is a reduction of analysis time, which also meant reduced solvent consumption. It uses fine particles as stationary phase which saves time and reduces solvent consumption[13-17]Analysis time, solvent consumption, and analysis costs are very important in many analytical laboratories. It was found that the sensitivity of UPLC was much higher than that of conventional HPLC.

The most common methods in current use for pesticide residue monitoring from methods developed in the 1960s and 1970s. However, due to the increasing cost of labor, solvents, equipment, and laboratory space, there is an urgent need for pesticide residue chemists to develop and use more cost-effective procedures. Moreover, many advances have been made in residue analysis in recent decades, even in the traditional case of liquid chromatography.

EXPERIMENTAL

Reagents and chemicals

Carbendazim, Carbaryl, Imidacloprid, Thiram and

Received: July 18, 2012; Revised: Aug 20, 2012; Accepted: Sept 25, 2012.

*Corresponding Author
Dinesh C Bilehal
Reva Institute of Technology and Management, Kattigenahalli, Yelahanka, Bengaluru-560064, Karnataka. India

Email: drbilehal@gmail.com

Monocrotophos analytical standard (purity > 99%) were supplied by Sigma Aldrich (India). HPLC grade acetonitrile were supplied by Rankem (New Dehli, India). Water was purified with a Direct-Q UV3® (resistivity 18.2 MΩ cm, Millipore, USA) water purification system (Millipore, Bedford, MA, USA).

Equipment

UPLC analyses were performed on a Waters Acquity Ultra Performance Liquid Chromatographic system with dual wavelength detector, cooling autosampler, and column oven enabling temperature control of the analytical column. Data were collected and processed using Empower chromatographic software. Special analytical columns Acquity UPLC BEH C18 of dimensions 2.1 X100 mm and 1.7 µm particle size were used are connected with UPLC system.

Chromatographic conditions were as follows: For all above-stated stationary phases the same mobile phase consisting of Acetonorile and water with isocratic mode (65:35)was used. Different flow rates, temperature were chosen and optimized for all tested columns so as to obtain the results as fast as possible, taking system back-pressure into consideration. Detection of analytes was accomplished at 210 nm. Injection volume used were 1 µL on all the chromatographic run. All analyses were performed at 30 C.

Preparation of solutions and mobile phases

Individual pesticide stock solutions containing 100 ppm of the target compounds were prepared in acetonitrile : water (50:50) mixture and stored at −4 °C. Intermediate working standard for each pesticide of 1, 5, 10, 20, 40 80, 100 ppm, were prepared and used to prepare the analytical curves. Working standard solutions were prepared monthly, while the dilutions used for the analytical curves were prepared daily. The mobile phase consisted of acetonitrile and milli-Q water. The mobile phases were degassed for 30 min in an ultrasonic bath before use.

QueCheRs extraction procedure

The “quick, easy, cheap, effective, rugged, and safe” (QuEChERS) method was first published by Anastassiades et al. in 2003 for the monitoring of pesticide residues in fruits and vegetables[18]. The method uses acetonitrile (MeCN) for extraction followed by the addition of anhydrous Na_2SO_4 to induce partitioning of the MeCN extract from the water in the sample. The initial extract is then mixed with primary secondary amine (PSA) sorbent and anhydrous $MgSO_4$ and graphitized carbon black (GCB) in a simple approach termed dispersive solid-phase extraction (dispersive-SPE) cleanup. Dispersive-SPE with PSA effectively removes many polar matrix components common in food matrices, such as organic acids and certain polar pigments. The method (with minor modification differences) has become an Official Method of AOAC International and the European Standard Organization (CEN) [19,20]. However, as previously stated, prescribed methods are not required in many cases, and the QuEChERS approach is flexible and easily adaptable.

In the original paper,[18] four different extraction solvent combinations were evaluated, all of which were known to achieve high recoveries for a wide range of pesticides. Both MeCN and ethyl acetate (EtOAc) showed a similar degree of matrix co-extractrives in fruits and vegetables, but MeCN was chosen in the final method because EtOAc presented problems with fatty matrices, was not amenable in reversed- phase LC, and gave lower recoveries for certain pH dependent analytes. Also, EtOAc is a stronger partitioning solvent in dispersive-SPE than MeCN, thus it gave slightly dirtier extracts than MeCN after cleanup. Other recent papers also have compared the use of different solvents, including EtOAc and/or MeCN in pesticide residue analysis, essentially showing advantages and disadvantages in each instance[21,22].

Sample preparation

The entire chilli sample (1kg) was homogenized in two steps. The samples (15 g) were extracted with Acetonitrile (15 mL) plus anhydrous sodium sulphate (10 g) by homogenization followed by centrifugation at 2000 rpm for 3 min. An aliquot of 1mL was drawn from the supernatant and cleaned by dispersive solid phase extraction (DSPE) with PSA (25 mg). The cleaned extract was placed in a 10mL test tube and mixed thoroughly by vortexing. This mixture was subsequently evaporated to near dryness under a gentle stream of nitrogen in a low volume concentrator at 50 ◦C. Typically 50 samples could be evaporated simultaneously in a low-volume concentrator within <20min. This supernatant was filtered through 0.2µm polyvinylidene fluoride (PVDF) membrane filters and then analyzed by UPLC. Fresh chili, which did not receive any treatment with the test pesticides, were used as blanks.

Precision and Accuracy

Precision under the conditions of repeatability (3 different analysts prepared 5 samples each on a single day) and intermediate precision (3 different analysts prepared 5 samples each on 5 different days) were determined separately for a standard concentration of 20ppm of all analytes. Accuracy was evaluated through with 5 replicates at 6 concentration levels of 1, 10, 20, 40, 80 and 100ppm.

Analytical curves and linearity

Linearity corresponds to the capacity of the method to supply results directly proportional to the concentration of the substance under investigation, within one determined application range. Range is the interval between the upper and the lower levels of analyte that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. The linearity of a method can be observed by the equation of the linear regression ($y = ax + b$). The results should not show a significant deviation from linearity, which is taken to mean that the correlation coefficient $r > 0.99$. The analytical curves and linearity of the detector response for the test compounds was evaluated by injecting a total of six calibration working standard solutions in the concentration levels 1, 5, 10, 20, 40 80, 100 ppm with three replicate injections *per* concentration.

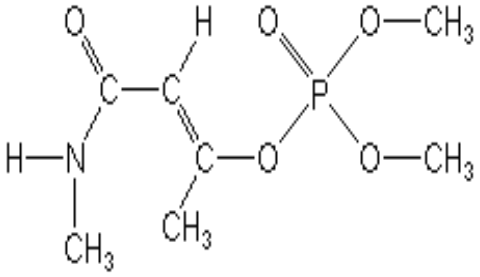
Limit of detection (LOD) and quantification (LOQ)

LOD is the lowest concentration of analyte that can be detected and reliably distinguished from zero (or the noise level of the system), but not necessarily quantified; the concentration at which a measured value is larger than the uncertainty associated with it, and the limit of quantification (LOQ) is the lowest solute

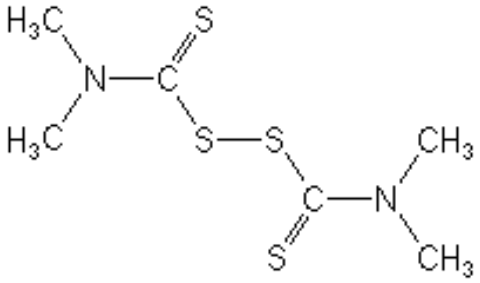
concentration that can be determined with acceptable precision and accuracy, under the stated experimental conditions. It is also expressed in concentration units. In this study, LOD and LOQ were determined considering the LOD as 3 times the baseline noise and the LOQ as the concentration that produced a signal 10 times the baseline noise, in a time close to the retention time of the analyte.

Sampling

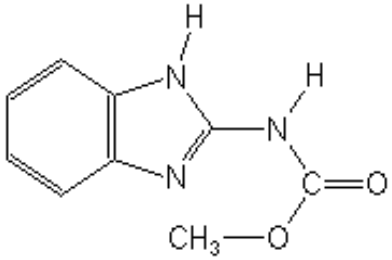
Chilli samples were collected in Byadagi Karnataka. The area is well known for intense chilli production, and consequently the use of pesticide.



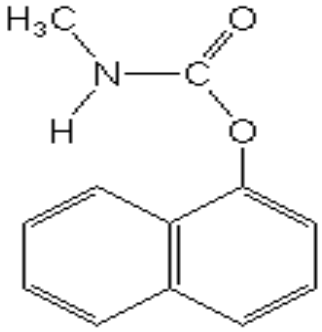
Monocrotophos



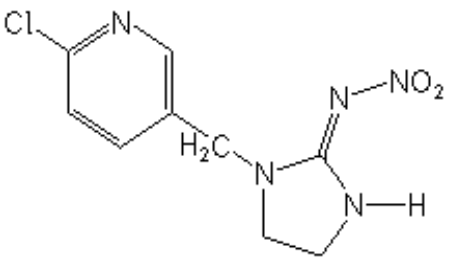
Thiram



Carbendazim



Carbaryl



Imidacloprid

Fig 1. The chemical structures of pesticides.

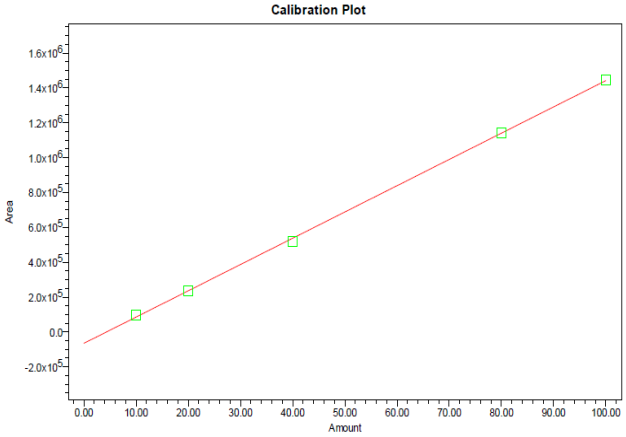


Fig 2. The calibration curve of imidacloprid

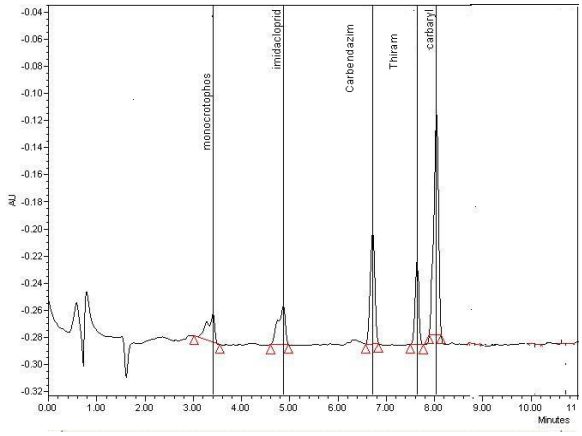


Fig 3. UPLC Chromatographic separation of Monocrotophos, Thiram, carbendazim, Carbaryl and imidacloprid.

SAMPLE ANALYSIS

Applicability of the method

The developed method was applied to determine the pesticides selected in chilli samples. The samples were analysed in triplicate. Results show that some chilli samples show detectable pesticide residues. Thiram was found in all chilli samples ranging from 0.763 to 2.388 (mg/Kg) and also Carbaryl is found in only one ie chilli sample no 4. Monocrotophos is found in almost all the samples except one the concentration is ranging from 0.624 to 14.749 (mg/Kg). Carbendazim is found in many chilli samples ranging from 0.039 to 0.578 (mg/kg). But Imidacloprid is not found in any of the samples.

Table 1. Comparative multiresidue analysis (Monocrotophos, Thiram, carbendazim, Carbaryl and imidacloprid) of pesticides in UPLC by chilli samples

	Monocrotophos (mg/Kg)	Thiram (mg/Kg)	Carbaryl (mg/Kg)	Carbendazim (mg/Kg)	Imidacloprid (mg/Kg)
Chilli sample 1	Not detected	0.763	Not detected	Not detected	Not detected
Chilli sample 2	10.761	2. 350	Not detected	Not detected	Not detected
Chilli sample 3	8.252	1.540	Not detected	Not detected	Not detected
Chilli sample 4	12.592	1.789	0.272	0.517	Not detected
Chilli sample 5	14. 749	1. 562	Not detected	0.156	Not detected
Chilli sample 6	6.212	1. 884	Not detected	Not Quantitable	Not detected
Chilli sample 7	0.624	2.388	Not detected	0.039	Not detected
Chilli sample 8	10.156	2.147	Not detected	Not detected	Not detected
Chilli sample 9	7.444	1.955	Not detected	Not detected	Not detected
Chilli sample 10	2.044	1.163	Not detected	0.578	Not detected
Chilli sample 11	6.058	1.168	Not detected	0.219	Not detected

CONCLUSIONS

The proposed UPLC-TUV method has been evaluated in terms of linearity, precision and accuracy, in a concentration range of 1-100 ppm, with a correlation coefficient higher than 0.999. The method presented an average recovery of 87.9% and 96.9%, in repeatability and intermediate precision conditions for all compounds. It offers good accuracy and precision to determine pesticides in chilli samples. The short analytical run time of 8.0 min leads to an effective cost and fast chromatographic procedure.Thus,the proposed methodology is rapid and selective with a simple sample preparation procedure that could be used for the convenient and effective determination of pesticide residues in chilli samples.

REFERENCES

[1] Acero, J. L.; Benítez, F. J.; Real, F. J.; González, M.; 2008. *J. Hazard. Mater. 153*, 320.

[2] Spadotto, C. A.; Gomes, M. A. F.; Luchini, L. C.; Andréa, M. M.; 2004.*Monitoramento do Risco Ambiental de Agrotóxicos: Príncipios e Recomendações,* Embrapa Meio Ambiente: Jaguariúna, p. 29.

[3] Coutinho, C. F. B.; Tanimoto, S. T.; Galli, A.; Garbellini, G. S.; Takayama, M.; Amaral, R. B.; Mazo, L. H.; Avaca, L. A.; Machado, S. A. S.;2005. *Pesticidas: Revista de Ecotoxicologia e Meio Ambiente 15*, 65.

[4] D'Archivio, A. A.; Fanelli, M.; Mazzeo, P.; Ruggieri, F.; 2007.*Talanta 71*, 25.

[5] Ahmed, F. E.; 2001.*Trends Anal. Chem. 20*, 649.

[6] Palma, G.; Sánchez, A.; Olave, Y.; Encina, F.; Palma, R.; Barra, R.; 2004.*Chemosphere 57*, 763.

[7] Sánchez-Ortega, A.; Sampedro, M. C.; Unceta, N.; Goicolea, M. A.; Barrio, R. J.;2005. *J. Chromatogr., A 1094*, 70.

[8] He, Y.; Lee, H. K.; 2006.*J. Chromatogr., A 1122*, 7.

[9] Tran, A. T. K.; Hyne, R. V.; Doble, P.; 2007.*Chemosphere, 67*, 944.

[10] Sannino, A.; Bolzoni, L.; Bandini, M.; 2004.*J. Chromatogr., A 1036*, 161.

[11] Rodrigues, A. M.; Ferreira, V.; Cardoso, V. V.; Ferreira, E.; Benoliel, M. J.; 2007.*J. Chromatogr., 1150*, 267.

[12] Pirard, C.; Widart, J.; Nguyen, B. K.; Deleuze, C.; Heudt, L.; Haubruge, E.; De Pauw, E.; Focant, J. F.; 2007.*J. Chromatogr., 1152,* 116.

[13] Jerkovich A.D., Mellors J.S., and Jorgenson J.W., 2003. *LCGC,* 21(7), 660–611.

[14] Wu N. , Lippert J.A., and Lee M.L., 2001.*J. Chromotogr.*, 911(1).

[15] Unger K. K., Kumar D. , Grun M., Buchel G., Ludtke S., Adam Th., Scumacher K., and Renker S.,2000. *J. Chromatogr., A,* 892(47) .

[16] Swartz M. E. and Murphy B., 2004.*Lab Plus Int.*, 18(6).

[17] Swartz M. E. and Murphy B., *Pharm.*2004. *Formulation Quality* ,6(5), p. 40.

[18] Anastassiades, M.; Lehotay, S.J.; tajnbaher, D.; Schenck, F.J,2003. J. AOAC mt. *86(2).* 412—431

[19] Lehotay, S.J. 2007.J. AOAC . 90, 485—520.

[20] Anastassiades. M. http://wwwquechers.com/, viewed 21 March 2007 Chemisches und Veterinäruntersuchung Sant Stuttgart. Germany.

[21] Mol, ll.G.J.; van Darn, R.C.J.; Steijger, O.M.2003. *J. Chromatogr. 1015,* 119— 127.

[22] Diez, C.; Traag, W.A.; Zommer. P.; Marinero, P.; Atienza, J. 2006.*J. Chromatogr. 1131,* 1 1-23.