METHOD DEVELOPMENT AND DETERMINATION OF NEONICOTINOID AND CARBAMATE PESTICIDE RESIDUES IN VEGETABLE AND FRUIT MATRIX BY HPLC-MS

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Abstract

In this work, a chemometric approach to positive electrospray ionization (ESI) optimization for the simultaneous determination of the cyromazine, carbendazim, methomyl, imidacloprid, and thiophanate methyl in vegetable samples by liquid chromatography- mass spectrometry (LC-MS) has been developed. The effects of the operational parameters such as mobile phase modifier concentrations, mobile phase flow rate, column temperature, drying gas flow rate, sampling speed, percentage of formic acid/water at first stage and percentage of formic acid/water at second stage were evaluated by the 2^{8-3} fractional factorial experimental design using Design Expert software 7.0. The best experimental conditions observed were 0.06% formic acid/water and 0.13% formic acid/acetonitrile; 0.13 mL/min of mobile phase; 28°C column temperature; 13.6 L/min drying gas flow rate; 11µL/sec sampling speed; and 77% v/v of 0.06% formic acid/water at first stage, 5% v/v of 0.06% formic acid/water at second stage.

Throughout the last decade, many official multiresidue methods were implemented for pesticide analysis. In this study, the citrate-buffered of Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) was tested. Primary secondary amine and C18 were studied as the extra sorbent for cleanup step. The matrices were spinach, onion, eggplant, paprika, ginger, okras, and mango. Recoveries ranged from 82.0 – 89.0% except for cyromazine that got 35.1% to 37.2% of recovery values. The method detection limit (MDL) ranged from 0.01 – 0.03mg/kg. The values of intra-day and inter-day precision and accuracy were ≤9.8 and ≤10.3, respectively. These values were within the acceptable ranges. Therefore, it was concluded that the method could produce reproducible and accurate results.

Key word: Chemometric, liquid chromatography- mass spectrometry, design expert, multiresidue, QuEChERS.

1. Introduction

Crops are treated with pesticides to against pests and these chemicals may leave residues in products of the plant. During production, processing, storage, and transport of food a variety of residues and contaminants may enter the food chain. Thus, the determination of pesticide residues in food matrices has become a necessity in view of the toxicity and stability of these xenobiotics. The application of multiresidue methods and the multiresidue determination of pesticides in vegetable and fruit matrix are routinely preferred in most of the laboratories due to the simplicity of determining several pesticides after a single extraction, facilitating the demands of more efficient and rapid monitoring. The aim of the present study was to develop a multi-residue method for carbamate and neonicotinoid pesticides with citrate buffer and dispersive clean-up using combined C18/PSA (Primary Secondary Amine).

Over the years, there have been reports proving the ability to apply experimental design methods (DOE) to develop cost-effective and effective LC method. Attempts were, therefore, made to develop a straight, rapid, sensitive, robust, effective and economical HPLC method employing DOE. Experimental design was used for optimization of mobile phase and MS parameters by taking CH_3CN, pH, flow rate and drying gas as variables and their effects.

The proposal method developed on seven matrices was optimized and validated as recommended in SANTE/11945/2015.The confirmation of suitability citrate QuEChERS optimized method was to use for routine testing and assuring the security of farm-produced crops.

2. Experimental

2.1. Chemicals and solvents

Insecticide standards (cyromazine, carbendazim, methomy, imidacloprid, and thiophanate methyl) were purchased from Fluka, Germany. Buffer-salt-mixture for second extraction and partitioning was: 4g±0.2g of magnesium sulfate anhydrous, 1g±0.05g of sodium chloride, 1g±0.05g of trisodium citrate dihydrate and 0.5g±0.03g of disodium hydrogencitrate sesquihydrate. Bondesil-PSA® 40µm and C-18-sorbent (Octadecyl-silyl-modified silica gel), Bulk material 50µm were obtained from Agilent. Acetonitrile (HPLC grade, 99.9%) and formic acid were obtained from Scharlau (Spain) and filtered through the 0.45µm membrane. The high pure water (18.2$M\Omega$.cmresistivity, Milli-Q) was produced with an ElGa water purification system.

2.2. Instrumentation

A Shimadzu LCMS – 2020 system, was used, which consisting of a vacuum degasser, a high-pressure binary solvent delivery system (LC-20AD) and a SIL 20AC autosampler, a column oven, and a single quadrupole MS analyzer with an electrospray (ESI) interface in the following conditions: electrospray ionization (ESI) at +4.5 kV (positive) with nebulizer gas at 1.5 L/min, DL temperature at 250^0C, heat block 200^0C. A C18 column (150 mm x 2.1 mm id, 5µm) was used to perform the separation, with a security guard column C18, 40 x 2.1 mm id. After the optimization study the optimization study, the mobile phase selected was 0.06% formic acid in water (solvent B) and 0.13% formic acid in acetonitrile (solvent A) applied at a flow rate of 0.13ml/min in the following gradient mode (i) 0min (A-B, 23:77, v/v); (ii) 1.8min (A-B, 30:70, v/v); (iii) 7min (A-B, 30:70, v/v); (iv) 18min (A-B, 95:5, v/v); (v) 20min (A-B, 95:5, v/v); (vi) 23 min (A-B, 23:77, v/v) and (vii) 30 min (A-B, 23:77, v/v). The injection volume and column temperature were set at 5µL and 28^0C, respectively. The most abundant ion of each compound was quantified in selected ion monitoring (SIM) mode and a further two ions were used to confirm the presence of each analyte (Table 1)

Table 1. Quantification and confirmation ions selected for each insecticides to perform the ESI-MS detection in positive SIM mode

Compounds	Ion source SEI (+)	
	Quantification	Confirmation
Cyromazine	167 (100%)	168 (11%)
Carbendazim	192 (100%)	193 (65%)
Methomyl	163 (100%)	164 (82%)
Imidacloprid	256 (100%)	258 (76%)
Thiophanate methyl	343 (100%)	344 (37%)

Table 2. Factors and their "low" (−1), "high" (+1) and "zero" (0) values

Name	Units	Low Actual	Mean	High Actual	Low Coded	Mean	High Coded
% HF/H2O	%	0.04	0.12	0.2	-1	0	1
% HF/CH3CN	%	0	0.1	0.2	-1	0	1
Flow rate	ml/min	0.04	0.17	0.3	-1	0	1
Column temp	^{0}C	15	25	35	-1	0	1
Drying gas	L/min	10	14	18	-1	0	1
Sampling Speed	uL/sec	5	10	15	-1	0	1
%HF/H2O_initial	%	70	80	90	-1	0	1
%HF/H2O_second	%	0	10	20	-1	0	1

2.3. Sample preparation

The extraction conditions were determined after the optimization studies described below had been performed. Briefly, 10g ±0.1g of the homogenized sample and 10ml of CH_3CN were transferred to a 50ml centrifuge tubes made of poly-tetrafluoroethylene with screw caps. Close the tube and shake vigorously for 5 min. If the sample's degree of comminution is insufficient or the residues do not readily extract from the matrix, the extraction time may be prolonged. The mixture had been cooled for 5 min in cold water. After that, add the prepared buffer-salt mixture to the suspension. Close the tube and immediately shake vigorously for 5 min and centrifuge for 5 min at 4000rpm. Following this, the aliquot of 8ml of the acetonitrile phase was transferred into a PP-single use centrifuge tube already containing 150mg PSA, 150mg C18 and 900 mg of magnesium sulfate. Close the tube, shake vigorously for 2 min and centrifuge for 5 min at 4000rpm. The extract was passed through a syringe filter, after which a 5µL aliquot was injected into the LC-ESI-MS system.

2.4. Optimization and development of HPLC method

The optimization of mobile phase condition was performed by the 2^{8-3} fractional factorial experimental design with 8 central points using Design Expert software 7.0 by selecting the % formic acid/H2O(X1), % formic acid/CH3CN (X2), flow rate (X3), column temperature (X4), drying gas (X5), sampling speed (X6), % formic/H2O at initial stage (X7) and % formic/H2O at second stage as independent variables, while the peak area, tailing factor and number of theoretical plates as responses. Factors and their "low" (−1), "high" (+1) and "zero" (0) values are presented in Table 2.

3. Results and discussion

3.1. Development and optimization of HPLC method

Analysis of data gained from screening experiments allowed an insight into the basic operation of the ESI source when exposed to determined conditions according to 2^{8-3} fractional factorial design. The repetition of the central experimental point provided a precise estimation of the experimental errors and the measure of the adequacy of the models (lack of fit). Analysis of variance (ANOVA) was performed on the models to determine the statistical significance of the coefficients and interaction between themselves. The ANOVA calculations or the total variation in response calculations, examine the overall significance of each term in the model compared to the residual error. Terms found to have a probability value of less than 0.05 are considered to be

significant. Table 3 resumes the ANOVA results obtained from the design expert 7.0 software. The ANOVA coefficients for all compounds were statistically valid with 95% confidence level.

Table 3. Resume the ANOVA results by the models proposed.

Compounds	P-value		Coefficient of correlation (R^2)
	Regression model	Lack of fit	
Cyromaine	<0.0001	0.4739	0.9976
Carbendazim	<0.0001	0.2724	0.9968
Methomyl	0.0054	0.2069	0.9855
Imidacloprid	0.0044	0.5385	0.9911
Thiophanate methyl	0.0006	0.1005	0.9938

Table 4. The analytical conditions determined after the optimization studies

Parameters	Optimization
% $HCOOH/H_2O$ (Solvent B)	0.06%
% $HCOOH/CH_3CN$ (Solvent A)	0.13%
Flow rate	0.13ml/min
Column temperature	28^0C
Drying gas	13.6 L/min
Sampling speed	11µL/sec
Ratio for % $HCOOH/H_2O$ at initial stage	77%
Ratio for % $HCOOH/H_2O$ at second stage	5%

The lack-of-fit values were also observed, which indicated that the quadratic model was valid for the present study. The coefficient of correlation (R^2>0.9) showed the good fitness of the model. The optimal values of the selected variables were obtained by solving the regression equation. After calculation by the Design Expert software, the optimal conditions were shown in Table 4. The chromatograph was optimally illustrated in Fig. 1.

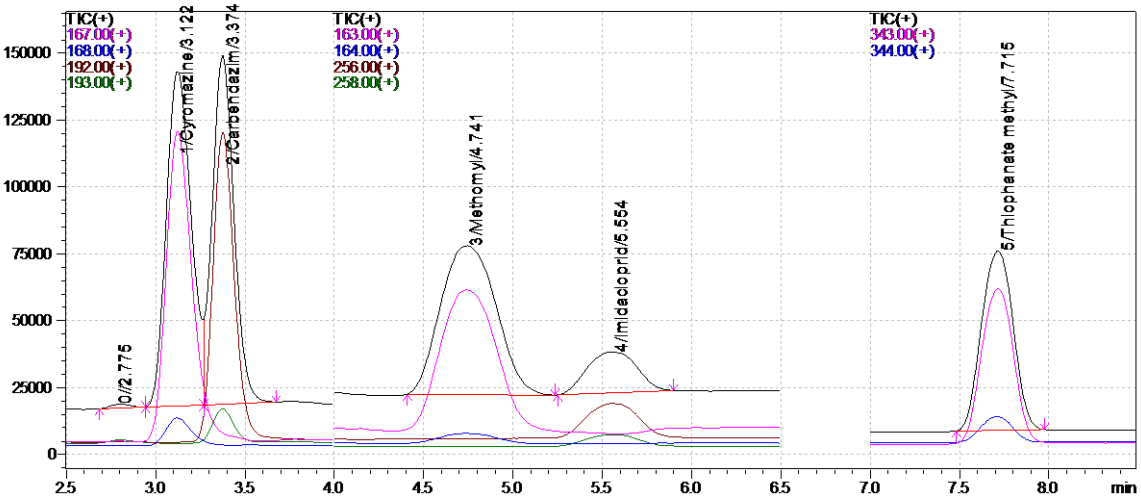

Fig.1. The SIM chromatograph of 5 compounds at optimal conditions

3.2. Validation of the procedure

The method developed was validated for seven matrices studied in this work: spinach, onion, egg plant, paprika, ginger, okras and mango. The validation scheme followed was based on the SANTE guidelines [2]. The specificity of the method was tested by the analysis of blank samples. The absence of any chromatographic peak in

every matrix, at the same retention times as target pesticides, indicated that there were no matrix compounds that might give a false positive signal in these blank samples. Recovery of the pesticides from the fortified samples was calculated relative to that from matrix-matched standard and tested against the 70 – 120% criterion for evaluation of routine analytical quality-control samples [2]. The accuracy of the method was estimated by means of recovery experiments at 0.02mg/kg. For all matrices, the results obtained for most of the compounds were satisfactory, with recoveries between 82.0–89.0% except for cyromazine that got 35.1% to 37.2% of recovery values. The values of intra-day and inter-day precision and accuracy were ≤9.8 and ≤10.3, respectively. The poor recoveries for cyromazine also illustrate the difficulties of development a unique multiresidue method for the determination of a number of pesticides with a wide range of polarities. The method detection limit (MDL) ranged from 0.01 – 0.03mg/kg.

4. Conclusions

The method was successfully developed and optimized through DOE using Design expert version 7.0 software. The significant effect of independent factors was analyzed using ANOVA. The DOE provides efficient tools for the optimization of variable factors for HPLC method development. A multiresidue method has been developed for screening in different matrices by HPLC–MS. The procedure has been validated for representative species from different commodity groups obtaining satisfactory accuracy and precision for most of analyte/matrix combinations. The proposed method allows the simultaneous determination of pesticides of different chemical families and physico-chemical properties in one single determination step, monitoring the most sensitive transition for every compound. Confirmation of pesticides detected in samples is performed by an injection into the HPLC–MS system. The method is stability indicating and it can be used for the routine analysis of sample.

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PHÁT TRIỂN PHƯƠNG PHÁP VÀ XÁC ĐỊNH DƯ LƯỢNG HÓA CHẤT BẢO VỆ THỰC VẬT NHÓM NEONICOTINOID VÀ CARBAMATE TRONG RAU CỦ QUẢ BẰNG PHƯƠNG PHÁP HPLC-MS

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TÓM TẮT

Hóa tin (chemometric) trở thành công cụ hữu ích cho các nhà hóa học trong nhiều năm trở lại đây. Trong công trình này chúng tôi đã ứng dụng hóa tin vào việc tối ưu hóa các thông số hoạt động thiết bị HPLC-MS để xác định đồng thời cyromazine, carbendazim, methomyl, imidacloprid và thiophanate methyl trong các mẫu rau củ quả. Hai loại pha động được sử dụng là acid formic/nước và acid formic/acetonitril và một số thông số khác có ảnh hưởng đến quá trình tách sắc kí trên thiết bị HPLC-MS đã được lựa chọn gồm: tỷ lệ hàm lượng % của acid formic/nước và % của acid formic/acetonitril, lưu tốc dòng pha động, lưu tốc khí làm khô (drying gas), tốc độ hút mẫu, tỷ lệ % của acid formic/nước tại nấc thứ nhất của chương trình gradient pha động và tỷ lệ % của acid formic/nước tại nấc thứ hai của chương trình gradient pha động. Tất cả 8 thông số được tối ưu hóa thông qua ma trận yếu tố riêng phần 2^{8-3} bằng phần mềm Design Expert 7.0. Các điều kiện tối ưu của mỗi thông số đã được đưa ra sau quá trình tối ưu hóa: 0.06% acid formic/nước, 0.13% acid formic/ acetonitril, nhiệt độ cột tách 28°C, lưu tốc khí làm khô 13.6L/phút, tốc độ hút mẫu 11µL/sec, tỷ lệ 0.06% acid formic/nước tại nấc thứ nhất của chương trình gradient pha động: 77% và tỷ lệ 0.06% acid formic/nước tại nấc thứ hai của chương trình gradient pha động: 5%.

Cho đến thời điểm này, có rất nhiều phương pháp phân tích đa hợp phần dư lượng để phân tích dư lượng hóa chất bảo vệ thực vật (DLHCBVTV). Nổi bật hơn hết là phương pháp QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) đã trở thành phương pháp tiêu chuẩn trong hầu hết các phòng thí nghiệm, với việc giảm thiểu lượng dung môi tiêu thụ và tương thích với cả kỹ thuật GC-MS và LC-MS. Trong đề tài này, chúng tôi đã sử dụng phương pháp QuEChERS với đệm citrate làm phương pháp tham khảo để phát triển phương pháp trong đề tài này. Qua đó chúng tôi đã sử dụng hỗn hợp PSA (Primary secondary amin) và C18 để làm sạch dịch trích theo kiểu phân tán pha rắn. Phương pháp đã được phát triển thành công trên các nền mẫu pó xôi, hành lá, ớt chuông, gừng, đậu bắp và xoài, độ thu hồi trung bình đạt được với đa số các hoạt chất khảo sát là từ 82.0 đến 89.0%, ngoại trừ cyromazine chỉ đạt hiệu suất thu hồi 35.1% - 37.2%. Giới hạn phát hiện của phương pháp (MDL) của các hoạt chất nằm trong khoảng nồng độ 0.01 – 0.03 mg/kg. Giá trị độ lặp lại ≤ 9.8% và tái lặp ≤ 10.3% và đều cho thấy nhỏ hơn giá trị cho phép trong phòng thí nghiệm khi tính theo hàm Horwitz. Phương pháp đề xuất trong đề tài có khả năng áp dụng vào thực tế kiểm nghiệm với độ lặp lại và độ chính xác chấp nhận được.

Từ khóa: Hóa tin (chemometric), HPLC-MS, design expert, đa dư lượng, QuEChERS