JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

APPLICATION OF QuEChERS METHOD FOR THE DETERMINATION OF PHENYLUREA HERBICIDES IN BEETROOT BY HPLC WITH UV-VIS DETECTION

Magdalena Surma, Anna Sadowska-Rociek, Ewa Cieślik*

Address(es): Magdalena Surma, PhD.
University of Agriculture in Krakow, Faculty of Food Technology, Malopolska Centre of Food Monitoring, Balicka St. 122, 30-149 Krakow, Poland, +48 12 6624827.

*Corresponding author: m.surma@ur.krakow.pl

doi: 10.15414/jmbfs.2015.4.special3.145-147

ARTICLE INFO

Received 28. 11. 2014
Revised 3. 12. 2014
Accepted 4. 12. 2014
Published 2. 2. 2015

Short communication

OPEN ACCESS

ABSTRACT

Phenylurea herbicides are an important group of herbicides utilized in weed control. They have been on sale since the 1950s and are still in common use throughout the world from pre- and post-emergence control of many annual and perennial broad-leaved weeds. The aim of this work was to evaluate the utility of the QuEChERS method for the determination of phenylurea pesticides (chlortoluron, isoproturon, linuron, metobromuron, metoxuron, monolinuron) in beetroot by HPLC with UV/Vis detection. Different types of sorbents (PSA, C18, SAX and NH2) and solvents (hexane, ethyl acetate) were applied. The obtained results showed that the best recovery ratios were received for the method with PSA and GCB sorbents and using acetonitrile as an extraction solvent with RSD lower than 15% for most compounds. The linearity of calibration curves was higher than 0.98 for all target analytes. The results show that the QuEChERS method can be successfully applied for the determination of phenylurea herbicides in beetroot.

Keywords: Phenylurea herbicides, HPLC, QuEChERS method, beetroot

INTRODUCTION

Chemical contamination of food are referred to as critical differentiators of quality and food safety. Most of them, detected in food, belongs to a group of contamination difficult or even impossible to avoid due to their widespread occurrence in the environment, sustainability and the ability to accumulate in the food chain. To this group belong compounds ex.: pesticide residues (**Łazowska, 2009**).

Pesticides are toxic agents that have been introduced into general use, to eliminate pests in agriculture and households. They allow to increase food production by destroying weeds and pests attacking crops and agricultural products, reduce losses during transportation and storage of food, also play a large role in reducing the number of human diseases transmitted by insects (**Biziuk, 2001**). Pesticides are characterized by persistence in the environment, toxicity, ability to accumulate and mobility (**Beyer *et al.*, 2008**). A major issue with the use of plant protection products are their side effects such as immunization pathogens buildup of harmful microflora and entofauna, inhibition of growth, development and yield of plants, disorders of subsequent metabolism of plants, as well as the systematic deepening decline in their life.

The aim of the ideal, but unattainable in practice, is selectivity of action, i.e. destructive to undesired forms, and harmless to humans and animals (e.g. beneficial insects) and plants. Toxicity of pesticides, particularly chlorinated in relation to the human body was discovered relatively late. Then it began to gradually withdraw from use and replace ones prone to rapid biodegradation (**Beyer *et al.*, 2008**).

Additionally to carbamates and pyrethroids now often used in plant protection products are also phenylurea herbicides, which include i.e.: linuron, isoproturon, monolinuron, chloroproturon, metobromuron and metoxuron. These pesticides are applied respectively to control dicotyledonous weeds and broom corn plantations cereals (**Wysmułek *et al.*, 2009**) and to reduce the weed in the cultivation of root vegetables (eg. carrots) (**Jaźwa *et al.*, 2008**).

The great diversity of samples due to the composition of the matrix requires the use of multiple methods of sample preparation and many efficient techniques for analytes extraction and their treatment before the final determination stage (**Żwir-Ferenc *et al.*, 2004**). Every step of the sample preparation involves the risk of loss of analytes or contamination of the sample. Particularly high risk of losing the analytes at the trace levels, involves the preparation of a sample using a multi-step purification operations (**Beyer *et al.*, 2007**).

Current trends in the analysis of contaminants in environmental samples focused on the development of the so-called multimethods (multiresidue methods), also known as methods or techniques of simultaneous determination of residues of multiple components (**Gnusowski *et al*, 2010**). These techniques allow to determine in one group of food products contamination belonging to different chemical groups. At the same time modern analytical chemistry aims to develop methods of preparation and chemical analysis, which use small amounts of samples and reagents, simple operations and uncomplicated analytical laboratory equipment and harmless to health and environmental chemicals. One of them is the QuEChERS (ang. Quick, Easy, Cheap, Effective, Rugged and Safe) (**Anastasiades, 2011**). The advantage of this method is the low consumption of sample and reagents, simplicity of implementation, low cost, efficient removal of interfering components of the sample matrix and the high recoveries of the analyzed compounds (**Garcia-Reyes *et al*, 2007**). QuEChERS method was developed initially for the determination of organochlorine pesticide residues in products of plant origin with a low fat content. The effectiveness and popularity of the method attempts this methodology is also being used for the determination of residues of other pesticides (**Romero-Gonzalez *et al*, 2011**).

The aim of this work was to evaluate the utility of the QuEChERS method for the determination of phenylurea pesticides (chlortoluron, isoproturon, linuron, metobromuron, metoxuron, monolinuron) in beetroot by QuEChERS method with the use of different types of sorbents (PSA, C18, SAX and NH2) followed by HPLC with UV/Vis detection.

MATERIAL AND METHODS

Chemicals

Acetonitrile HPLC grade, sodium chloride p.a., and formic acid, 98%, p.a. were purchased from Merck KGaA, Germany. Magnesium sulphate anhydrous p.a. and sodium sulphate anhydrous p.a. were purchased from POCh SA, Poland. Na_3Citrate dihydrate p.a., was obtained from Riedel-de Haen, Germany, Na_2HCitrate sesquihydrate, 99%, p.a., from Sigma-Aldrich Chemie GmbH, Germany. Bondesil PSA, SAX, NH_2, GCB and C_{18} sorbents were purchased from Varian Inc., USA. Phenylurea herbicides were obtained from Dr Ehrenstorfer Standards, UK. Stock, intermediate and working standard solutions of herbicides at concentration 100 μg mL^{-1} and the 5% (v/v) solution of formic acid were prepared in acetonitrile.

Equipment

The HPLC system (ELITE LaChroma, MERCK, Germany) consisted of multi-channel pump, autosampler, column oven, UV-Vis detector and controlled by the EZChrome Elite software was used to perform the LC analyses. All chromatographic determinations were performed at 25 °C with the flow rate of 0.75 mL min-1. Compounds were eluted in gradient system composed of water

(phase A) and acetonitrile (B). Gradient was as follows: 30% B (0 min), 30-50% B (0-13.0 min), 50-70% B (13.0-31.0 min), 70-30% (31.0-32.0 min) and 30% (32.0-35.0 min). Phenylurea herbicides determination were performed using LiChrospher® 100 RP-18 endcapped (10 µm) LichroCART® 250-4 column (Merck, Germany).
AccuTM Thermoblock (Labnet, USA) with nitrogen (Linde Gas, Poland) was used to evaporate the solvent, and concentrate the extracts. MPW 350 R Centrifuge (MPW Med. Instruments, Poland) was used for sample preparation.

QuEChERS sample preparation method for phenylurea herbicides determination

In the study we focused on adaptation of QuEChERS method for phenylurea herbicides determination in beetroot.
A series of experiments were performed for the optimisation the sample preparation techniques, including selection the appropriate, additional sorbent for clean-up the samples (C18 or/and GCB), apart from PSA or SAX or NH_2 sorbents. The methods were marked as follow: 1 (PSA), 2 (PSA+C18), 3 (PSA+GCB), 4 (PSA+C18+GCB), 5 (SAX), 6 (SAX +C18), 7 (SAX+GCB), 8 (SAX+C18+GCB), 9 (NH_2), 10 (NH_2+C18), 11 (NH_2+GCB), 12 (NH_2+C18+GCB). The usefulness of the method was verified on the basis of the recovery ratio of analysed compounds (analysis of spiked samples). Homogenized samples with no pesticides detected previously were used for recovery studies. Recovery study involved samples of beetroot being spiked with the standard solution of herbicides to the fortification level of 0.025 mg kg-1. This level has been adapted to the MRL's limit set in EU (0.01 and 0.05 mg kg^{-1}).
A representative portion of beetroot was cut, macerated and homogenized in a blender. 10 g of each homogenizate was weighted into a 50 mL centrifuge tube, the samples were spiked with mixture of herbicides, mixed and left to stand for 15 min at room temperature prior to extraction. Then 10 mL of acetonitrile was added to each tube and the mixture was shaken vigorously for 1 min. After that 1 g Na_3Citrate dihydrate, 0.5 g Na_2HCitrate sesquihydrate, 1 g NaCl and 4 g $MgSO_4$ were added, and the tube were shaken immediately after addition of the salt. Then each sample was shaken vigorously for 1 min., and centrifuged for 15 min. at 8700 RCF. 6 mL of the supernatant was transferred into a PP 15 mL tube containing 0.150 g PSA or SAX or NH_2, and/or 0.300 g C_{18} and/or 0.050 g GCB) and 0.900 g MgSO4. The tubes were shaken for 30 s and centrifuged for 5 min at 5000 RCF. 4 mL from each extracts was transferred into a screw cup vial, and acidified with 40 µL of 5% formic acid in acetonitrile. In the last step of the procedure samples were evaporated under a stream of N_2 at a temperature of 40 °C to dryness and the residues were dissolved in 1 mL of acetonitrile. Finally, all extracts were analysed by HPLC/UV-Vis. A series of standard solutions in pure solvent were prepared by dilution of the standard mixture solution in acetonitrile at the same ranges from 5 to 100 µg mL^{-1}.

RESULTS AND DISCUSSION

The pesticides involved in this study were identified by comparing the retention time with retention time of the standards. Calibration curves were constructed by plotting the peak area against concentration of the analyte. The recovery values for all twelve methods are presented in Table 1.

Table 1 The recovery values [%] for all tested methods

Sorbent	Chlorotoluron	Isoproturon	Linuron	Metobromuron	Metoxuron	Monolinuron
PSA	103.7	109.0	123.8	102.5	112.9	114.5
PSA + C18	113.1	128.2	250.7	125.5	167.3	131.3
PSA + GCB	69.5	75.0	104.8	94.1	100.0	81.5
PSA + C18 + GCB	12.8	60.0	78.5	89.1	123.2	84.1
SAX	97.7	129.6	105.0	125.4	116.4	101.5
SAX + C18	89.3	107.7	64.3	91.2	100.8	94.1
SAX + GCB	55.2	58.7	125.2	68.8	89.9	64.3
SAX + C18 + GCB	58.3	58.7	132.1	61.3	87.1	61.4
NH2	42.4	46.8	97.8	50.6	50.4	44.7
NH2 + C18	32.3	33.9	74.5	32.0	43.5	34.0
NH2 + GCB	52.1	56.3	123.0	64.4	85.3	60.6
NH2 + C18 + GCB	67.0	69.2	161.2	46.4	73.6	61.6

The obtained results showed that the best recovery ratios (70-120% according to the document SANCO/12495/2011) with RSD lower than 15% for most compounds were received for the method 1 (PSA), 3 (PSA+GCB), 5 (SAX) and 6 (SAX+C18). In the other variants of the QuEChERS method, the recovery values did not fit within the prescribed range. The red colour highlighted results matching to SANCO regulation. Comparison of the results obtained for four most efficient methods are presented in the Figure 1.

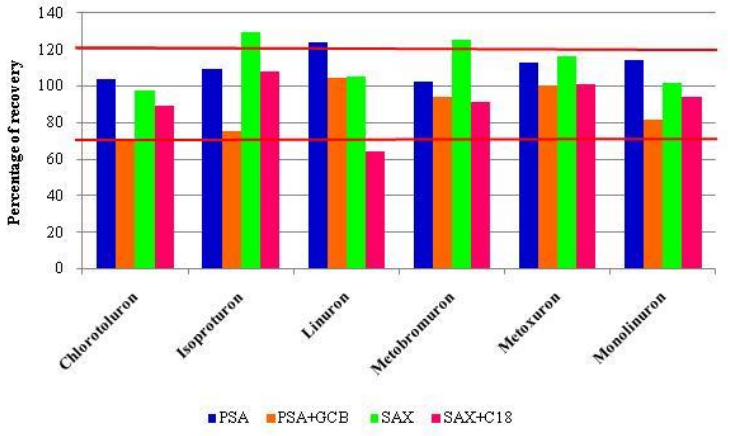

Figure 1 Comparison of the recovery ratios obtained for the most efficient methods.

The method with PSA and GCB as a sorbents can be considered as the most appropriate of all others, because recovery values for all analysed herbicides ranged from 70 to 105% and matching to SANCO regulation. Moreover, the RSD value did not exceed 15%.
Both PSA and GCB sorbents can eliminate pigments, so we can believe that their combination allowed to clean samples from betalaines. However, the combination of SAX and C_{18} sorbents also remove pigments from the samples. SAX is characterized by similar properties as PSA so can be also used to clean up the extracts from the betalaines. It can be assumed that the main role in samples purification play PSA or SAX sorbents, and GCB enhances this effect. Visual assessment confirmed that the method 3 is the most effective in pigment elimination in beetroot sample preparation for phenylurea herbicides determination.
Table 2 summarizes an analytical performance of the method. The values of R^2 were higher than 0.99 for all compounds. Limit of detection (LOD) and limit of quantification (LOQ) were estimated basing on the signal of the background noise measured from the chromatograms of standard at the lowest calibration level. The limit of detection was calculated as three times higher than the level of noise, and the limit of quantification were equal to three times of the detection limit. LOQs for all compounds were lower than 3 µg kg^{-1}, except of metabromuron.

Table 2 Retention times, calibration curves (y=ax) parameters, coefficients of determination (R_2), limits of detection (LOD) and limits of quantification (LOQ)

Compounds	R_t [min]	Calibration slope	Determination coefficient, R^2	LOD [µg kg^{-1}]	LOQ [µg kg^{-1}]	MRL [mg kg^{-1}]
Metoxuron	10.34	329703	0.998	0.98	2.94	-
Chlorotoluron	15.70	229521	0.996	0.85	2.55	0.01
Monolinuron	16.91	346099	0.999	0.92	2.76	0.05
Isoproturon	17.19	210743	0.999	0.99	2.97	0.01
Metabromuron	18.44	423929	0.998	1.02	3.06	-
Linuron	22.48	126771	1	0.79	2.37	0.05

CONCLUSION

In this study, the results show that the QuEChERS method can be successfully applied for the determination of phenylurea herbicides in beetroot. The experiment revealed that the use of PSA and GCB sorbents is the optimal variant of QuEChERS method for the phenylurea herbicides analysis in beetroot.

Acknowledgments:

The research was performed with the financial support of the University of Agriculture in Crakow for young scientist and PhD students (grant for Magdalena Surma, PhD, no. BM/4728/KTGiK/2012), which is hereby gratefully acknowledged.

REFERENCES

ANASTASSIADES M., Maštovská, K., & Lehotay, S. J. 2003. Evaluation of analyte protectants to improve gas chromatographic analysis of pesticides. *Journal of Chromatography A*, 1015, 163–184, http://dx.doi.org/10.1016/s0021-9673(03)01208-1

BEYER A., BIZIUK M. 2008. Polish regulations, comparing with European Union legislation, relating to pesticides content in foodstuffs. *Ecological Chemistry and Engineering S*, 14(1), 30-43.

BIZIUK M. 2001. Pestycydy, występowanie, oznaczanie i unieszkodliwianie. WNT, Warszawa.

GARCÍA-REYES J. F., FERRER C., GÓMEZ-RAMOS J. M., MOLINA-DÍAZ A., FERNÁNDEZ-ALBA A.R. 2007. Determination of pesticide residues in olive oil and olives. *Trends Anal. Chem.*, 26 (8), 828-841, http://dx.doi.org/10.1016/j.trac.2007.01.004

GNUSOWSKI B., NOWACKA A., WALORCZYK S., DROŻDŻOWSKI D. 2010. Pesticide residues in crop samples collected in compliance with a community control programme. *Progress in Plant Protection*, 50(4), 1897-1902.

JAŹWA A., ROGOZIŃSKA K., SADŁO S., KUŹMENKO A. 2008. Studies on disappearance of chlorpyrifos, cypermethrin, linuron and fluorochloridone in soil and in carrot roots. *Progress in Plant Protection*, 48(4), 1999-1203.

ŁAZOWSKA B. 2009. Chemical contaminants in plant food. *Progress in Plant Protection*, 49 (4), 2071-2080.

ROMERO-GONZALEZ R., GARRIDO FRENICH A., MARTINEZ VIDAL J.L., PRESTES O.D., GRIO S.L. 2011. Simultaneous determination of pesticides, biopesticides and mycotoxins in organic products applying a quick, easy, cheap, effective, rugged and safe extraction procedure and ultra-high performance liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*, 1218, 1477-1485, http://dx.doi.org/10.1016/j.chroma.2011.01.034

WYSMUŁEK A., CIESIELSKA A., ŁĘGOWIAK Z., PISKORZ B. 2009. The efficacy of mixtures of diflufenican with isoproturon and diflufenican with chlorotoluron in winter cereals. *Progress in Plant Protection*, 2009, 49(2), 844-848.

ŻWIR-FERENC A., BIZIUK M. 2004, An Analysis of Pesticides and Polychlorinated Biphenyls in Biological Samples and Foods. *Critical Reviews in Analytical Chemistry*, 34, 95-103, http://dx.doi.org/10.1080/10408340490475894.