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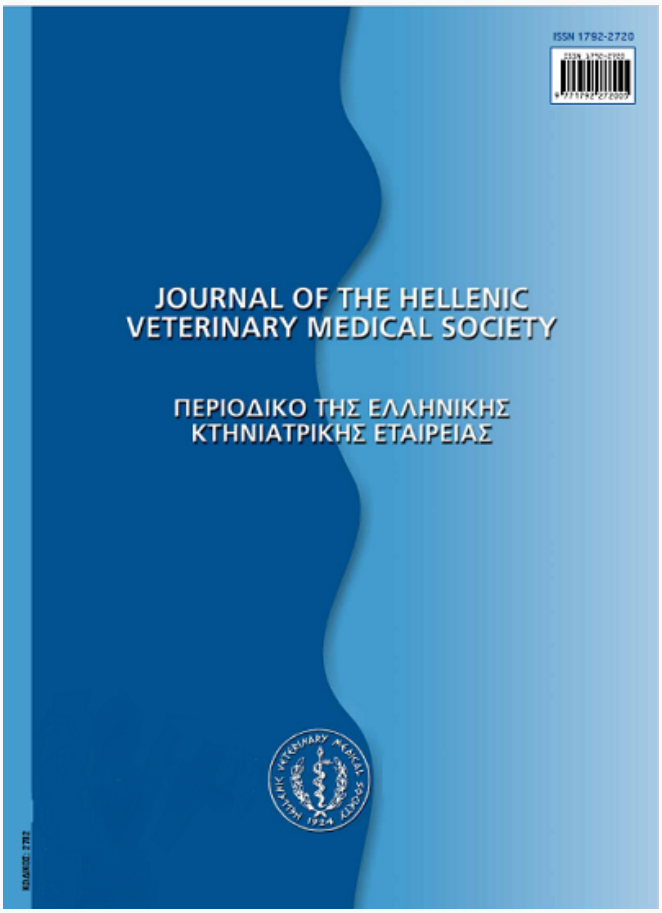

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Research article
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Carry over effect in pesticide residues analysis by LC - MS/ MS

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Προβλήματα carry over στον προσδιορισμό υπολειμμάτων φυτοφαρμάκων με LC-MS/MS

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ABSTRACT. The Carry over effect, i.e. the appearance of a peak in the chromatogram of a blank analysis due to sample remaining from the previous analysis, was studied. The study was conducted for pesticide residues analysis by LC-MS/MS. In total 128 pesticides that belong to 13 different chemical classes were analyzed in order to investigate the cases that the effect is significant; i.e. a peak higher than 1% of the peak of the previous chromatogram appears. Carry over was found for 32 of the 128 studied pesticides (25%), at concentration levels between the LOD and 0.167 μg/mL. For 28 out of the 32 substances, more than two injections of a blank sample were required, as to reduce the effect significantly. Compounds presenting Carry over effect were mainly non-polar with logkow values between 4 and 7, char-

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acterized with very low water solubility, between 0.001 mg/L and 2 mg/L. On the contrary, the vapor pressure did not seem to be obviously related to the effect, as the substances presenting Carry over had various vapor pressure values, from 10-12 to 0.2 mP.

Keywords: *Pesticides residues, Carry over, LC-MS/MS*

ΠΕΡΙΛΗΨΗ. Μελετήθηκε το φαινόμενο carry over που εμφανίζεται στη χρωματογραφία, της «μεταφοράς» δηλαδή μιας ουσίας από ένα δείγμα στο επόμενο. Η μελέτη έγινε κατά τον προσδιορισμό υπολειμμάτων φυτοφαρμάκων με LC-MS/MS. Εξετάστηκαν 128 φυτοφάρμακα που ανήκουν σε 13 χημικές κατηγορίες προκειμένου να διαπιστωθεί σε ποιές περιπτώσεις το φαινόμενο είναι σημαντικό, οι τιμές δηλαδή της μεταφερόμενης ουσίας ξεπερνούν το 1% της αρχικής συγκέντρωσης. Συνολικά, 32 από τις 128 προσδιοριζόμενες ενώσεις (ποσοστό 25%) εμφάνισαν carry over, σε επίπεδα συγκεντρώσεων από το όριο ανίχνευσης ως 0.167 μg/mL. Από τις 32 ενώσεις που εμφάνισαν carry over, οι 28 απαιτούσαν περισσότερες από 2 εγχύσεις λευκού δείγματος για να επιτευχθεί αποδεκτή μείωση του φαινομένου. Σε σχέση με τη φύση των ουσιών που εμφάνισαν Carry over, προέκυψε ότι πρόκειται για μη πολικές ενώσεις, με τιμές του δείκτη πολικότητας $\log k^{ow}$ μεταξύ 4 και 7 και με πολύ μικρή διαλυτότητα στο νερό, στην πλειονότητα μεταξύ 0.001 και 2 mg/L. Αντίθετα, η τάση ατμών δε φαίνεται να σχετίζεται εμφανώς με το φαινόμενο, αφού οι ουσίες με carry over είχαν ποικίλες τιμές τάσης ατμών, από 10^{-12} ως 0,2 mP.

Λέξεις ευρετηρίασης: *Υπολείμματα φυτοφαρμάκων, Φαινόμενο Carry over, LC-MS/MS*

INTRODUCTION

The phenomenon of the transportation of a substance from a sample to the next one (carry over effect) is a major problem for the analysts either in Liquid or Gas Chromatography; especially when it has to be determined at a low concentration. The Guidance Document KO1-KPITE of the Hellenic Accreditation System (ESYD), explains that carry over is the "system overload" and there is demand for the Accredited Laboratories to check the chromatographic systems for carry over, among other parameters (SANTE, 2015)

Carry over is determined as a peak that should not appear, of an analyte in the chromatograph of blank injection that follows an injection of a sample where the analyte was present. This can cause false positives results; either qualitative or quantitative, especially when there is no blank sample between the standards and the samples. This means that it affects the precision and accuracy of the method and it is important to be realized and solved on time.

The laboratories deal with different matrices of unknown origin, which means that it is impossible to eliminate carry over effect during routine analysis. According to the literature, the acceptable carry over from sample to sample should be less or equal than 1%. Blank samples are injected in regular basis aiming to detect the phenomenon, but it is possible to prevent it only by optimizing the injection volume, checking and cleaning the connections, replacing the rinsing solution and its vial, improving the parameters of the elution system etc.

The effect can be result of contamination during the sample extraction, in the autosampler or the chromatography column. In order to determine the source of the contamination, the first step of sample extraction is omitted and only blank solution is injected. If the chromatograph does not show any peaks, while the one of the blank matrix does, then the problem is targeted in the extraction procedure. If this is this case, peaks of the same area usually appear in all chromatographs. The most usual contamination source is reusable glass equipment, pipettes, the rotary evaporator, etc., and the way to determine where the problem is, is to check separately each different step.

Carry over that is a result of retention in the column

is highly related to the interactions between the static phase and the analytes, e.g. basic substances are attracted from active acidic sites of the static phase. In this kind of carry over the analyte response is affected a lot, especially at low concentrations, however gradient elution is usual effective for its reduction (Dolan, 2001).

In the most cases, the injector of the autosampler is the reason for the phenomenon, and there are two mechanisms for this: dilution and combination of dilution-adsorption. In the first one, the sample is attached in some parts of the injector and eluted with the next injections. This is the reason that the consecutive injections decrease carry over, and as a result the peak can be insignificant after the third or the fourth injection. On the contrary, in the second mechanism the sample extract is connected to some parts of the injector from which it is difficult to be removed (Shimanzu, 2008). The main reasons are the interactions of the sample with some parts of the system, the absorption of ionic substances from the metals of the system and the absorption of lipophilic substances due to hydrophobic reactions with the plastic materials of the system, such as membranes, filters, tubes, vial caps etc. In this case the phenomenon is more complicated and the substances are difficult to remove (Anonymous, 2013).

The most usual source of contamination is the vial that contains the sample solution, and the elution solution of the injector, that must be replaced in regular base. Probably there is the need to increase the number of elutions or the volume of the elution solution. But the chemical properties are still the same so it is mandatory to increase the power of the elution solution by using new solution with more parts of methanol or acetonitrile. Moreover the elution solution should have even higher or lower pH value, by adding formic or acetic acid or base in a low concentration (0.1-1% v/v), in order to dissolve the sample. On the other hand, the use of buffers or salts is not proposed, as they remain in the injector parts (Dolan, 2001).

Every manufacturer designs in a different way the auto sampler, and so there are differences in the mechanism of the elution. The vial where the needle is eluted is a usual source of contamination and should be replaced regularly and the same should apply for the septum, where residues may remain. Even the elution solvent can facilitate sample adsorption in the different parts of the chromatographic system. This mainly occurs if this solvent is water or a buffer. The addition of small quantity of an organic solution e.g. 5% (v/v) methanol or acetonitrile, decreases this adsorption.

The present study is dealing with the carry over effect during pesticide residues analysis by the use of LC-MS/MS. In total 128 pesticides that belong to 13 different chemical groups were chosen, in order to investigate the mechanism of carry over and the cases that is significant.

MATERIALS AND METHODS

Reference materials from Ehrenstorfer, Sigma-Aldrich and ChemService were used and their purity was >98%, while acetonitrile, water and methanol were HPLC grade. PSA 40 μm, Bondesil was from Varian Inc., USA, and Magnesium Sulphate from Acros Organics (EN 15662:2008). Standard stock solutions at 1000 μg/mL were prepared in acetone and working solutions of the 128 pesticides in acetonitrile, at concentrations <1 mg / mL. The solutions were kept at -20°C.

The analysis was performed with an Agilent 1200 LC-MS/MS and a triple quadrupole Waters Quattro Premier, in positive ESI mode with an Eclipse XDB C-18, 2.1 x 150 mm analytical column. For the gradient elution two solvents were used: A=0.1% (v/v) HCOOH, 20% (v/v) methanol in water and B=0.1% (v/v) HCOOH and 5 mM HCOONH4 in methanol. The gradient elution program is shown in Table 1 (Anagnostopoulos and Miliadis, 2013).

Table 1. Gradient program of the mobile phase

Time (min)	% solvent A	% solvent B
0.00	100.0	0.0
2.00	100.0	0.0
12.00	50.0	50.0
30.00	0.0	100.0
40.00	0.0	100.0
40.50	100.0	0.0

The column temperature was 40°C, the flow rate 0.25 mL/min and the sample volume 5 μL, was diluted with 20 μL of water and injected into the autosampler, in order to have the same composition of the injected sample with the initial mobile phase. The triple quadrupole was operated at the multiple reaction monitoring (MRM) mode.

RESULTS AND DISCUSSION

In total 128 representative pesticides that belong to 13 different chemical groups were chosen in order to cover the whole range of physicochemical properties of each group. Table 2 describes the target analytes and their properties. Taking into consideration that all analyzed pesticides had the same concentration, it was expected that the response was much higher for some of them, and as a result the same happened to the carry over effect. 32 of the 128 analytes (25%) presented carry over effect, at concentrations between the LOD and 0.167 μg/mL.

Moreover, it was found that injections of blank matrix extract were more efficient to decrease carry over, than injections of pure solvent. Flour extract was used for this purpose and the number of injections that eliminated the phenomenon was the criterion to evaluate the extent of carry over. As critical levels for the reduction of carry over, three different levels were selected, at 10%, 1% and 0% of the area of the initial peak. Table 3 shows the 32 analytes that presented carry over and the number of blank matrix injections required to reduce carry over at the 3 selected levels. The standard solution injected prior to this testing included all 128 pesticides at 0.167 μg/mL.

For 19 of the 32 pesticides only one blank matrix

Table 2. Analyzed chemical substances and range of their physicochemical properties

Chemical group	Analyzed substances	Range		
		Solubility in H_2O, mg/L	$logk_{ow}$	Vapor Pressure, mPa
Amides	5	$0.9 – 26x10^3$	0.67 - 2.17	$2x10^{-4} – 3.3x10^{-3}$
Aryloxyalkanoic acid	2	0.05-7.9	4-4.5	$5.5x10^{-2}$
Benzoylureas	3	0.004 - 111	2.28 - 4	$6.52x10^{-12} – 1.2x10^{-4}$
Benzimidazoles	3	8 - 30	1.5 - 2.4	$8.8x10^{-6} – 1.5x10^{-4}$
Carbamates	14	$7.74 - 28x10^4$	-0.44 – 4.6	$7.7x10^{-8} – 1.3x10^{-2}$
Neonicotinoids	4	185 - 4250	-0.13 - 1.26	$4x10^{-10} – 1x10^{-6}$
Organophosphates	20	$1 – 10^6$	-0.9 - 3.85	$1.03x10^{-5} – 0.123$
Pyrethrins	6	0.35 - 1038	2.85 - 5.62	$4.6x10^{-7} – 2.02x10^{-5}$
Sulfonylureas	8	3.7 - 3293	-0.78 – 0.646	$4.2x10^{-11} – 2.8x10^{-6}$
Strobilurin	3	1.9 - 6	2.5 – 3.99	$1.1x10^{-10} – 2.3x10^{-6}$
Triazine	5	$6.2 – 13x10^3$	-0.1 – 3.21	$4.48x10^{-7} – 1.5x10^{-4}$
Triazoles	8	0.2 - 156	3.08 – 4.1	$2.2x10^{-10} – 0.056$
Phenylureas	8	0.06 - 735	1.6 – 5.76	$5x10^{-6} – 4.3x10^{-3}$
More	39	$0.075 – 2x10^5$	-0.5 – 5.6	$7.9x10^{-7} – 0.267$

Table 3. Carry over of 128 different chemical substances (at 0.167 μg/mL), and required repeated injections of blank sample in order to gradually reduce the phenomenon at 10, 1 and 0%.

Chemical Substance	Number of blank sample injections for reducing arry over (%)		
	10%	1%	0% (not detectable)
Chlorpyrifos Ethyl	2	4	4
Cinerin I	1	2	2
Coumaphos	1	3	4
Diazinon	1	2	2
Etofenprox	3	8	8
Ethion	2	4	6
Ethirimol	1	1	2
Etoxazole	2	4	6
Fenarimol	1	2	2
Fenazaquin	2	4	4
Fenoxycarb	1	2	4
Fluazifop-P-butyl	2	3	7
Flufenacet	1	2	3
Flufenoxuron	3	6	8
Fluopicolide	1	3	3
Haloxyfop Methyl	1	2	3
Metolachlor	1	1	5
Pendimethalin	2	4	5
Phosalone	1	3	7
Picoxystrobin	1	2	3
Piperonyl Butoxide	1	2	8
Pirimiphos methyl	1	2	4
Pyrethrin I	2	3	3
Pyrethrin II	1	2	3
Pyraclostrobin	1	3	8
Pyrazophos	1	2	6
Pyriproxyfen	2	4	8
Quinoxyfen	2	4	7
Spirodiclofen	2	5	7
Tebufenpyrad	2	3	6
Trifloxystrobin	1	3	8
Zoxamide	1	2	4

injection was enough to decrease the effect by 10%, while for the others the max number was 3 (Table 3). In order to reach 1% decrease of the initial peak, that is considered satisfactory, 2 or more injections were necessary for 30 out of 32 pesticides. The 32 pesticides that presented mostly carry over were very lipophilic, non-polar compounds with polarity as logkow values between 4 and 7. As a result, they were found to have low solubility in water, mainly between 0.001 mg/L and 2 mg/L. On the other hand, the vapor pressure does not seem to affect the carry over, as it was found to vary from 10^{-12} to 0.2 mP.

According to these results, the carry over effect's mechanism in this study was the dilution-adsorption, in which the sample is chemically adhered to some parts of the injector from which it is difficult to be removed. The high lipophilicity of the substances that present carry over results in hydrophobic interactions with the plastic parts of the system, membranes, filters, tubes, vial caps etc.

CONCLUSION

The carry over effect is usual in the daily routine analysis, fact which is amplified from the high number of substances that cause it. First, there is the need to realize this problem, in order to avoid the report of false positive results. Then, it is mandatory to find a way to overcome the problem, so that the routine in the lab will not be interrupted. Beyond the above mentioned measures that reduce the phenomenon, there is another practical one, the preparation of calibration solutions differing in concentration, so that analytes with significant carry over are at a lower concentration from other analytes. As a result of this study, the 32 analytes that appear more intense carry over are prepared so as to have ten times less concentration than the others 96 compounds. In this way much fewer blank sample extract injections will be required in order to reduce the carry over to acceptable levels.

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