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METHOD VALIDATION FOR DETERMINATION OF ORGANOCHLORINE PESTICIDE RESIDUES IN FOOD AND FEED

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Abstract

Validation of analytical methods ensures the reliability and accuracy of analytical results. To get a reliable result we performed a validation of the method taking into account all factors that may affect of the result. In this paper we optimized QuEChERS method for food and feed sample preparation as well as instrumental method using gas chromatography with mass spectrometry (GC-MS), and we obtained a method for successful determination of pesticides with calibration through the matrix. In this way, applying calibration through matrix, we satisfied the requirements for precision and reproducibility of the method being less than 20%, the accuracy rate in the range of 70-130% and method linearity throughout the range of interest.

Keywords: validation, matrix, GC-MS, QuEChERS

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VALIDACIJA METODE ZA UTVRĐIVANJE REZIDUA ORGANOHLORNIH PESTICIDA U NAMIRNICAMA I HRANI ZA ŽIVOTINJE

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Kratak sadržaj

Validacijom analitičkih metoda se obezbeđuje pouzdanost i tačnost analitičkih rezultata. Da bismo dobili pouzdane rezultate prilikom validacije metode smo uzeli u obzir sve faktore koji mogu uticati na rezultate ispitivanja. U ovom radu pokazana je optimizacija metode za pripremu uzoraka hrane i hrane za životinje QuECHERs metodom pripreme kao i optimizacija instrumentalne metode gasne hromatografije (GCMS) za određivanje pesticida u različitim matriximа. Na ovaj način. kalibracijom kroz matrix, dobili smo rezultate preciznosti i ponovljivosti koji su manji od 20%, tačnost se kretala u opsegu od 70-130% a metoda je bila linearna u celom opsegu od interesa.

Ključne reči: validacija, matrix, GCMS, QuECHERS

INTRODUCTION

Pesticides are chemical compounds, which are used for the removal, suppression and destruction of plant and other pests. Unlike the majority of pollutants that are introduced into the environment without specific targets, pesticides are introduced with the intention to help the man, to increase nutrition and to protect the environment in the "fight" against harmful microorganisms and numerous pests (Stajkovac. 2009). Analysis of organochlorine pesticides (OCP) in food and feed samples comprises application of the number of methods in order to prepare samples for analysis and determination, depending on the type of the samples. Anastassiades et.al (2003) were the first who developed Quick, Easy, Cheap, Effective, Rugged and Safe approach (QuEChERS) method for sample preparation, which gave fast, efficient and reliable result of preparation in optimal time. To eliminate the influence of matrix, calibration through matrix that does not contain pesticide was performed as well. Identification of pesticide residues is complex procedure, and monitoring of low concentrations requires the use of highly sensitive instrumental analytical techniques,gas chromatography with mass spectrometry(GC-MS) or high performance liquid chromatography (HPLC).GC-MS predominates in the testing's due to its greater selectivity and sensitivity as compared to other analytical methods. The aim of this study was to validate the method for determination of organochlorine pesticides set down by regulations in Serbia ("Official Gazette"RS. 29/2014) in food and feed by GC-MS technique after sample preparation applying QuEChERS method.

MATERIALS AND METHOD

Solvents and chemicals that were used in the validation process were HPLC gradient grade (Merck. Germany). Calibrant solutions were prepared using the pesticides mix of 20 pesticides (organochlorine pesticides mixture, manufacturer Ultra Scientific. lot CL-1069).Spiked samples were used to the purpose of internal control of the following parameters: recovery, precision, limit of quantification (LOQ) and limit of detection (LOD). Pesticides mix that contains 19 pesticides (Chlorinated Pesticides-herbicides, AccuStandard. Inc. lot: 213091108) was used for spike preparation. In order to eliminate the influence of the matrix, calibration through matrix blank sample was performed as well (SANCO, 2014).

Matrix	Representative sample matrix	Number of samples	Sample amount (g)	Expected concentration in matrix (mg/kg)
Meat and meat products	Meat	10	3	0.005
Milk and milk products	Whey powder	10	3	0.005
Eggs and egg products	Melange	10	3	0.005
Feedstuff	Fishmeal	10	3	0.005

Table 1 The matrix and the amount of working solution for LOQ and LOD determination.

Table 2 Representative matrix and the amount of working solution for spike with the aim of determining the precision and reproducibility

Matrix	Representative sample matrix	Number of samples	Sample amount (g)	Expected concentration in matrix (mg/kg)
Meat and meat products	Meat	20	3	0.05
Milk and milk products	Whey powder	20	3	0.05
Eggs and egg products	Melange	20	3	0.05
Feedstuff	Fishmeal	20	3	0.05

This method of sample preparation is based on the extraction with acetonitrile in the presence of anhydrous magnesium sulfate (MgSO4) and anhydrous sodium acetate (CH3COONa). Sample (3 g) is measured and transferred into centrifuge tube, 3 mL of water is added and 3 mL of acetonitrile. After intensive stirring on a vortex, 3 g of anhydrous magnesium sulphate and 1 g of anhydrous sodium acetate were added. Exothermic reaction occurred within 1 min after the intense stirring on vortex. The sample was then centrifuged until 5 min at 3000 rpm. 1 mL of upper acetonitrile extract is transferred into the 5 ml tube, which contained 150 mg of anhydrous magnesium sulphate, 100 mg of Primary and Secondary Amine (PSA) and 50 mg of C18 (Anastassiades et al. 2003). The tube content was centrifuged for 5 min at 3000 rpm. After centrifuging, purified and clear extract is obtained. Then, 0.5 mL of the extract is evaporated in nitrogen and reconstituted with hexane. A sample prepared in this way is ready for the analysis on GC-MS (Agilent 7890B/5977A).

Organochlorine pesticides are separated on DB-5MS column (30 m· 0.25 µm· 0.25 mm). Sample volume of 4 µL (splitless mode) was injected at the constant pressure of 11.36 psi and and flowed through the column of the carrier gas at the flow rate 1.2 mL/min. The list of analyzed OCP as well as retention time, molecule weight, ions important for the analysis of HCH isomer are shown in Table 3. The target and qualifier abundances were determined by injecting the mixture of pesticide standards under the same chromatographic conditions using full scan with the mass/charge ratio ranging from 60 to 500 m/z. Standards were prepared in blank matrix extracts to counteract the matrix effect

(SANCO. 2014). With the aim of obtaining more reliable results, further pesticide quantification was performed in SIM mode. Pesticide quantification was performed according to mass spectra and characteristic ions defined in SIM mode (Table 4), as well as the retention time of exit components, pesticides (Selvi et al. 2012). The processing of the obtained data was performed applying Mass Hunter Software. The analysis of the method performance is performed in a calibration range from 0.005 to 0.1 mg/kg.

Table 3 Retention time (RT), molecule mass (MW), primary (target) ion (T) and secondary and tertiary ion (Qualifier Ions, Q1, Q2).

Pesticide	RT(min)	MW	T	Q1	Q2
α HCH	11.28	290.8	181	181	219
β HCH	12.47	290.8	183	181	183
γ HCH (lindane)	12.57	290.8	181	183	109
δ HCH	13.74	290.8	109	219	183
heptachlor	15.74	370	272	235	237
aldrine	17.40	362	263	220	291
heptachlorepoxide	19.55	386	353	81	355
trans-chlordane	20.88	406	373	375	-
alpha endosulfan	21.46	404	195	159	133
cis chlordane	21.71	406	373	375	-
pp'DDE	22.84	378	79	277	239
dieldrine	23.09	316	246	176	211
endrin	23.80	378	263	191	226
endosulfan	24.26	404	195	157	159
pp'DDD	24.90	318	235	165	237
endrin aldehyde	25.065	378	67	345	-
endosulfansulfate	25.97	420	272	274	387
pp'DDT	26.26	352	235	165	200
methoxychlor	26.88	344	227	165	184
endrin ketone	27.46	240	317	67	-

Table 4 SIM program was used for the analysis and confirmation (m/z. total dwell time)

Group	Time (min)	Pesticide	m/z	Total dwell time
1	10.78	α HCH, β HCH, γ HCH, δ HCH	181, 219, 109	150
2	14.98	heptachlor	100, 237, 272	150
3	16.66	aldrine	66, 263, 293	150
4	18.84	heptachlor epoxide	81, 353, 237, 263	200
5	20.37	Cis, trans-chlordane, endosulfan I	373, 237, 272, 195, 237, 170	300
6	22.38	Dieldrine. pp´DDE	79, 263, 246, 176, 318	250
7	23.45	Endrin, Endosulfan II	81, 67, 263, 245, 195, 237, 243	350
8	24.62	pp´DDD. endrin aldehyde	235, 165, 67,173, 250	250
9	25.29	pp´DDT. endosulfansulfate	165, 235, 237, 275, 387, 422	300
10	26.56	methoxychlor	227, 152	100
11	27.14	endrin ketone	67, 317, 345	150

RESULTS AND DISCUSSION

Based on tests conducted on five representative matrices and implemented to the internal controls, we obtained the results shown in Tables 5 and 6. Validation plan included determination of linearity, precision, reproducibility, accuracy and LOQ and LOD.

Linearity that was determined by setting a calibration curve was tested by regression analysis to establish the mathematical relationship between concentration and results in a set range of resultant values.

The precision of the method represents an agreement between values obtained in a series of repeated measurements of the same homogenous sample under the same determination conditions by at least 5 repeated measurements of the representative spiked matrix.

The reproducibility of the method represents matching results obtained by successive measurements of the same samples, but under many different conditions, determination is accomplished by analyzing at least 5 spiked samples (representative matrix).

The accuracy represents the mean value of the obtained results and actual or accepted value of the results. It is expressed as the yield (recovery), calculated on the spiked sample in relation to the expected theoretical value of the results.

LOQ is the lowest concentration that can be determined with reasonable accuracy. It is calculated as the sum of the mean values of 10 repetitions and 3 standard deviations.

LOD , granica detekcije , je najniza koncentracija analita koja može biti dokazana ali ne i određena. Izračunava se kao zbir srednje vrednosti od deset ponavljanja na matrix spajku , koji odgovara prvoj tački kalibracije, i 10 standardnih devijacija.

Table 5 The average values of accuracy, reproducibility, accuracy, linearity, LOQ and LOD for all matrices

Pesticides	Precision (%)	Reproducibility (%)	Accuracy (%)	Linearity (R^2)	LOQ (mg/kg)	LOD (log/kg)
α HCH	4.35	5.22	96.07	0.9990	0.0047	0.0014
β HCH	17.91	8.88	99.14	0.9918	0.0019	0.0006
γ HCH (lindane)	8.98	8.32	99.64	0.9980	0.001	0.0003
δ HCH	0.8	18.2	100.32	0.9993	0.0042	0.0003
heptachlor	3.39	14.64	88.08	0.9979	0.001	0.0003
aldrine	3.57	3.44	98.3	0.9904	0.0046	0.0014
heptachlorepoxide	3.52	3.36	94.37	0.9973	0.0016	0.0005
trans-chlordane	4.37	8.22	90.16	0.9981	0.0012	0.0004
alpha endosulfan	9.32	8.7	87.27	0.9977	0.0028	0.0009
cis chlordane	4.3	8.22	91.55	0.9993	0.0039	0.0012

Pesticides	Precision (%)	Reproducibility (%)	Accuracy (%)	Linearity (R^2)	LOQ (mg/kg)	LOD (log/kg)
pp'DDE	3.14	3.87	96.87	0.9965	0.0048	0.0014
dieldrine	3.52	3.36	94.37	0.9924	0.005	0.0015
endrin	8.51	16.21	83.41	0.9942	0.0031	0.0009
endosulfan	7.77	10.25	91.24	0.9986	0.0049	0.0015
pp'DDD	5.69	14.29	81.57	0.9991	0.0039	0.0012
endrin aldehyde	5.57	10.46	85.62	0.9979	0.0044	0.0013
endosulfansulfate	13.7	15.3	116.3	0.9990	0.0046	0.0014
pp'DDT	3.52	3.36	94.37	0.9918	0.0048	0.0014
methoxychlor	7.67	1.84	106.09	0.9980	0.0021	0.0006
min	**0.8**	**1.84**	**81.57**	**0.9904**	**0.001**	**0.0003**
max	**17.91**	**18.2**	**116.3**	**0.9993**	**0.005**	**0.0015**

Table 6 Data obtained from internal quality control for the different matrices (mean values Xsr, STD, RSD, Bias Recovery, N number of measurements for each pesticide)

Matrix	N	Xsr (mg/kg)	STD	RSD (%)	Bias (%)	Recovery (%)
Fishmeal	20	0.053	0.010	19.438	7.095	106.330
Whey powder	20	0.055	0.005	9.818	9.697	109.183
Meat	20	0.048	0.010	18.807	11.176	96.607
Melange	20	0.047	0.008	16.052	7.814	93.916
Honey	20	0.052	0.005	10.523	3.960	103.960
Xsr	**20**	**0.051**	**0.0076**	**14.927**	**7.948**	**101.999**

According to study of Maštovská et al. (2005), as compared to matrix-matched standardization, the analyte protectant approach offers a more convenient solution to the problems associated with calibration in routine GC/MS analysis of pesticide residues and possibly other susceptible analyte types in diverse samples. In a study on Alternative calibration techniques for counteracting the matrix effects in GC–MS-SPE pesticide residue analysis, Rimayi (2015) shows descriptive and inferential statistics proving that the matrix-matched internal standard calibration was the best approach for samples of varying matrix composition since it produced the most precise average mean recovery of 87% across all matrices tested. We demonstrated the same in our testing. Kartalovic et al. (2015) suggested application of gas chromatography with mass detector for the determination of pesticide traces, as it provides us with a confirmation of result reliability by comparing the obtained spectrum with that from the library. GCMS analysis offers good precision and recovery rate for determination of pesticides in hake fillets when applying matrix calibration (Kartalovic et al., 2015b).

CONCLUSION

Based on the conducted research and appropriate preparation, calibration and verification of the representative matrix we can conclude that the method for determination of pesticide residues in food and feed meets the eligibility criteria required by SANCO (2014). The method is linear in the range of 0.005 to 0.1 mg/kg. The linearity factor (R^2) is higher than 0.99. The precision and reproducibility rate for pesticide determination is a greater than 20%. The accuracy of the method is in the range 70-130%. The method can be successfully used for the determination of pesticide residues in food and feed.

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