

DEFINING SEVERAL KEY PERFORMANCE CHARACTERISTICS OF THE GC-MS METHOD FOR DETERMINATION OF ORGANO-CHLORINATED PESTICIDES IN WATER

MIHAELA PANĂ¹, ALEXANDRU PANĂ², GABRIELA RĂU³,
GEORGE DAN MOGOȘANU^{4*}

¹*Quality of Water Laboratory, National Agency of Romanian Waters,
6 Remus Bellu Street, 240204 Râmnicu Vâlcea, Romania*

²*Quality Control Laboratory, S.C. Oltchim S.A., 1 Uzinei Street, 240050
Râmnicu Vâlcea, Romania*

³*Department of Organic Chemistry & Medicinal Chemistry*

⁴*Department of Pharmacognosy & Phytotherapy*

*University of Medicine and Pharmacy of Craiova, Faculty of Pharmacy,
2-4 Petru Rareș Street, 200349 Craiova, Romania*

**corresponding author: mogosanu@umfcv.ro*

Abstract

The paper presents the results of investigations carried on the determination of organo-chlorinated pesticides as aldrin (C₁₂H₈Cl₆), endrin (C₁₂H₈Cl₆O), *p,p'*-DDT (C₁₄H₉Cl₅ - dichlorodiphenyl trichloroethane) and γ -HCH (C₆H₆Cl₆ - hexachlorocyclohexane) in water using the GC-MS technique. The aim of this work was to prove that the new analytical procedure GC-MS fits for the purpose (establishing the optimal separation parameters and the validation parameters). The most important validation parameters were established in this method: specificity and selectivity, repeatability, linearity, precision, recovery, limits of detection and quantification.

Rezumat

Lucrarea prezintă rezultatele cercetărilor efectuate în scopul determinării pesticidelor organoclorurate ca: aldrin (C₁₂H₈Cl₆), endrin (C₁₂H₈Cl₆O), *p,p'*-DDT (C₁₄H₉Cl₅ - diclorodifenil tricloroetane) și γ -HCH (C₆H₆Cl₆ - hexaclorociclohexan), din ape, utilizând tehnica GC-MS. Obiectivul lucrării îl constituie demonstrarea faptului că noua metodă analitică GC-MS este adecvată scopului (stabilind parametrii optimi de separare și parametrii de validare). Cei mai importanți parametri de validare stabiliți prin această metodă sunt: specificitatea și selectivitatea, repetabilitatea, linearitatea, precizia, regăsirea, limita de detecție și de cuantificare.

Keywords: gas chromatography – mass spectrometry (GC-MS); organo-chlorinated pesticides; aldrin; endrin; *p,p'*-DDT; γ -HCH.

Introduction

By organo-chlorinated pesticides (OCP) we understand lipophilic and persistent compounds, such as aldrin, endrin, *p,p'*-DDT (dichlorodiphenyl trichloroethane), and γ -HCH (hexachlorocyclohexane). Due to their lipophilic properties and high persistence, these pesticides are

bio-accumulated in the food chains and therefore they have been banned in most countries throughout the world. The residues and metabolites of these compounds may still be determined in different environmental or food samples.

Usually, analyses of residual organo-chlorinated pesticides are performed using the gas chromatography technique with two capillary columns with different polarities and an Electron Capture Detector (ECD). The majority of standardized methods approach this opportunity. The GC–MS (gas chromatography mass spectrometry) method was selected because it has become a recommended technique for residual analysis for many years. The technique supports the analyst not only in collecting important and complete information on mass spectra, thus contributing to the elucidation of the structure of unknown compounds, but also in the analysis of traces due to the high sensitivity and selectivity of the SIM (Selected Ion Monitoring) operational mode [9].

It is often noticed that, irrespective of the residual analysis method applied, when using multi-residual procedures with ECD or NPD (Nitrogen and Phosphorous Detector) type detectors, are detected signals whose retention time does not correspond with those of reference compounds. In this case, the GC–MS technique may well serve to confirm the results, when the peak we are looking for is an active substance or, when interferences of the matrix, which due to the high concentration may also send a signal to detectors mentioned above, occur.

By means of the SIM technique, fragments with relatively intense abundance and ions with high signal/noise ratio are investigated, situation in which, due to the considerably longer period for scanning each fragment, clearer chromatograms are obtained for the compounds to be determined and which contain at least a specific fragment selected for analysis.

The aim of the paper is to evaluate the validation parameters (specificity, linearity, accuracy, precision, detection and quantification limit) in order to validate the method, and to provide proof that once the validation protocol for the analytical method is applied, the types of analytes and a certain range of concentrations are defined, the protocol is adequate for the proposed aim.

Materials and methods

The method consists in extracting pesticides from water by means of petroleum ether, concentrating the extract in a rotary evaporator and injecting it on a GC–MS 6890N/5973MSD Agilent Technologies System. Water samples of 500 mL were subjected to successive extractions in three

phases, with volumes of 25 mL / 25 mL / 15 mL of petroleum ether, under agitation for 10 minutes. Extracts were filtered and dried on successive layers of glass wool and anhydrous sodium sulphite, then all together were concentrated in a Laborota 4010 digital Heidolph rotary evaporator at 500-mbar vacuum and a temperature of 60°C of the water bath. After concentration, the extract was washed with 2 mL of petroleum ether and transferred in a porcelain capsule. After evaporation at room temperature, the concentrate was transferred with 1 mL of petroleum ether in a vial of 2mL and injected in the auto-injector [13].

Instrument control parameters

Injector working conditions

- Splitless injection.

Oven working conditions

- HP-5MS capillary column of 25 m × 3 mm × 0.25 µm;
- Initial temperature: 50°C; time: 1 min.; rate: 25°C/min.; intermediary temperature: 100°C; time: 0 min.; rate: 5°C/min.; final temperature: 300°C; time: 0 min., analysis period: 43 min.;
- Carrier gas: helium; pressure at column end: 9.38 psi; column flow rate: 1.2 mL/min.; purging capacity: 30 mL/min.

Mass spectrometer detector working conditions

- Interface temperature: 310°C; temperature at MS quadrupole: 150°C; temperature at MS source: 230°C; electron multiplying voltage: 952.9 V; solvent delay: 3 min.;
- Target ions for SIM mode: mass ions 182.9 for γ -HCH from 20.43 to 21.43 min.; 262.9 for aldrin, from 24.86 to 25.86 min.; 316.9 for endrin, from 29.82 to 30.82 min.; 235 for *p,p'*-DDT, from 32.01 to 33.01 min.

Auto injector working parameters

- Injection volume: 1 µL; syringe volume: 10 µL; washing with samples outside the vial: five times;
- Washing with samples in the vial (pumping): three times.

Supelco certified the reference materials used for the calibration of the instrument. The purities certified for each component were: endrin 99.1%, aldrin 96.8%, γ -HCH 99.7%, and *p,p'*-DDT 98.2%.

The following solutions were prepared:

M stock solution: 0.05 g γ -HCH, 0.0506 g aldrin, 0.05045g endrin and 0.0516 g *p,p'*-DDT were weighed and brought to the mark with acetone

in a 100 mL flask; concentrations of 500 ng/ μ L γ -HCH, 489.8 ng/ μ L aldrin, 504.4 ng/ μ L endrin, and 506.7 ng/ μ L *p,p'*-DDT were obtained.

Intermediate solution I: From the stock solution, 2 mL were drop pipetted into a 100 mL calibrated flask and brought to the mark with isooctane. The pesticide concentrations obtained after preparation were: 10 ng/ μ L γ -HCH, 9.796 ng/ μ L aldrin, 10.088 ng/ μ L endrin, and 10.134 ng/ μ L *p,p'*-DDT.

Reference solutions A, B, C, and D for multipoint calibration, with concentrations of 0.2 ng/ μ L, 0.4 ng/ μ L, 0.8 ng/ μ L, 1 ng/ μ L and 2 ng/ μ L, were prepared in petroleum ether at 50 mL flasks of the intermediate solution.

The content of organo-chlorinated pesticides in water was calculated from the relation:

$$\text{Organo-chlorinated pesticides (mg/L)} = C \frac{V \times 100}{V_a \times R \times 100}$$

where:

C – result given by the instrument (ng/ μ L);

V – volume of petroleum ether used for redissolving the sample concentrate (mL);

V_a – volume of water sample analyzed (L);

R – pesticides' recovery coefficient (%).

Results and discussion

Selectivity and specificity of the method

While in the case of other gas chromatography techniques method selectivity mainly based on the retention time attributed to each analyte can be quoted, in the case of GC–MS technique the identity of the analyte can be demonstrated by comparing the mass spectrum obtained with the standard spectrum to be found in the spectra library.

In order to demonstrate the selectivity and specificity, first, a standard mixture of organo-chlorinated pesticides at a concentration of 2 ng/ μ L was injected and the chromatogram was acquired in SCAN Mode.

The retention times for compounds of interest were: γ -HCH 20.92 min., aldrin 25.36 min., eldrin 30.32 min., and *p,p'*-DDT 32.51 min. Subsequently, the chromatogram of the mixture of compounds was plotted in SCAN Mode (Figure 1) and thus, by comparison to spectra in NIST spectra library, organo-chlorinated pesticides were found with probabilities of 98% for γ -HCH, 99% for aldrin, 95% for endrin, and 99% for *p,p'*-DDT.

The high probability of identifying the monitored pesticides demons-

trates, on the one hand, the validity of working conditions chosen for the determination method and, on the other hand, the lack of interferences due to the matrix, or the extraction solvent, respectively.

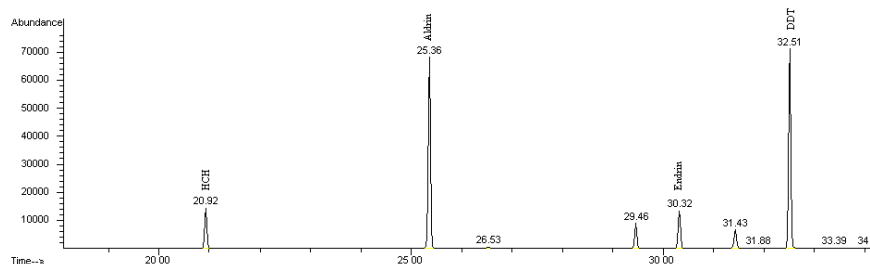


Figure 1

Chromatogram of the most concentrated standard mixture in SCAN mode.

The spectra obtained from the chromatogram acquired in SCAN mode gave us the possibility to select the target ions for subsequent quantifications in SIM mode: 182.9 mass ion for γ -HCH, 262.9 mass ion for aldrin, 316.9 mass ion for endrin, and 235 mass ion for p,p' -DDT.

In order to demonstrate the method selectivity, it was necessary to check the resolution between the compounds, which should have values greater than 1–1.5 [2]. Chromatographic resolution globally characterizes the quality of separation, which can be measured by the parameters defining the chromatographic peak [5].

The values of the calculated resolutions were very high, pointing to an appropriate chromatographic separation of the compounds (Table I).

Table I
Chromatographic resolutions (R_s) calculated for each compound

No.	Compound	Retention time (min.)	Base line width (min.)	R_s
1.	γ -HCH	20.92	0.049	92.55
2.	Aldrin	25.36	0.048	97.45
3.	Endrin	30.32	0.054	20.74
4.	p,p' -DDT	32.51	0.047	21.40

Moreover, six replicated injections were performed, in order to demonstrate that the relative standard deviation of the retention time for each peak should be less than 1%. Thus, the following values were obtained for the relative standard deviations (RSD): γ -HCH 0.00023%, aldrin 0.0002657%, p,p' -DDT 0.0003045%, and endrin 0.000208%.

Linearity of MS detector response

In order to demonstrate the good correlation between the analyte concentration and the detector response, the application of the following criteria

was taken into consideration [1, 3, 6]:

- determining the correlation coefficient of the calibration curve (R);
- plotting a curve, which represents the ratio between the detector signal and the analyte concentration function of the analyte concentration logarithm;
- carrying out the statistical test of linearity (Fisher–Snedecor).

In order to demonstrate the linearity of detector signal, five synthetic mixtures with concentrations of 0.2, 0.4, 0.8, 1 and 2 ng/μL in petroleum ether were prepared and from each one three replicating injections were made. The calibration curve for each component was computed assuming first a linear regression and then a quadratic regression and the correlation coefficients of the curves (R) and the residual standard deviations S_y were calculated (Table II).

Table II
Data used for calibration curves drawing

No.	Standard solution (ng/μL)	Response			
		Aldrin	Endrin	<i>p,p'</i> -DDT	<i>γ</i> -HCH
1.	0.2	19289	3692	7942	7313
2.	0.4	36627	7915	13882	19669
3.	0.8	75407	15268	29486	33758
4.	1	91405	18965	35365	41804
5.	2	188084	42326	66245	95356
R linear		1.0	0.997	0.997	0.997
Sy_1^2 linear		1923769	1020100	715716	8797156
R quadratic		0.9997	0.999	0.997	0.997
Sy_2^2 quadratic		1752976	150544	1430416	4468996

It may be noticed that values obtained for the correlation coefficients are close to 1, which points to a linear regression in the predicted range of work. In the case of endrin, whose correlation coefficient is 0.997 in the linear representation and 0.999 in the quadratic representation, in order to determine which of the two types of graphical representation is more appropriate, a statistical test of linearity was carried out [11]. Thus, the difference of DS^2 dispersions was calculated for the two representations:

$$DS^2 = (N-2) Sy_1^2 \text{ linear} - (N-3) Sy_2^2 \text{ quadratic}$$

where:

N – the number of concentration levels;

Sy_1^2 linear – the residual standard deviation of linear representation;

Sy_2^2 quadratic – the residual standard deviation of quadratic representation.

The PG-value necessary for the F-test was calculated from the relation:

$$PG = \frac{DS^2}{Sy_2^2}$$

The number of degrees of freedom in the case of a linear model is $f_{\text{linear}} = n-2$, and $f_{\text{quadratic}} = n-3$ respectively, for a non-linear model. From the comparison between the test value $PG = 18.32$ and the value $F_{(3,2,99)} = 30.81$, results that $PG < F(f_{\text{linear}}, f_{\text{quadratic}}, \alpha)$, consequently the quadratic representation is not more appropriate than the linear one, the latter providing a better data correlation. When plotting a curve in which the ratio between the detector signal and the analyte concentration function of the analyte concentration logarithm, horizontal lines parallel to the concentration axis throughout the range of concentrations are obtained. The points of concentration are randomly distributed as compared to the detector signal/concentration ratio average values (Figure 2) [7].

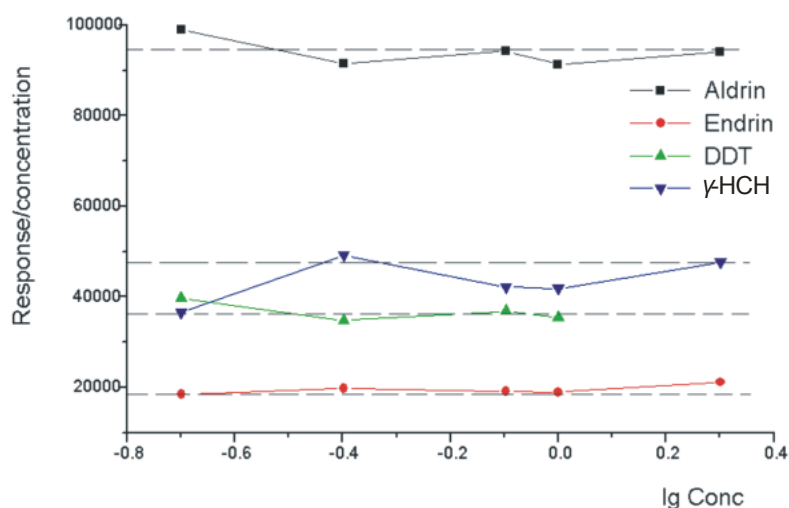


Figure 2

Graphical representation of the ratio between the detector signal and the analyte concentration, function of the analyte concentration logarithm.

Precision

The precision of a method represents the degree of correspondence between the results of a series of measurements; therefore, it can reflect the type of variability to be obtained, when a sample is analyzed in duplicate [10].

In this study, only two out of the three existing types of accuracy were

analyzed, i.e. repeatability precision (intra-run precision) and intermediate precision (inter-run precision), as it was impossible to make an inter-comparison study to determine reproducibility precision (inter-laboratory precision) as well.

Repeatability was expressed as relative standard deviation (RSD - %), and its maximum admissible level was determined based on the modified Horwitz relation [8]:

$$\text{RSD}_r < 0.6 \times 2^{(1-0.5 \log C)}$$

RSD_r – repeatability relative standard deviations (%).

In order to determine repeatability, 10 injections of a standard reference (external standard – 1 ng/ μL), were made successively by one analyst, on the same instrument, in a short period of time (one day). In order to determine intermediate precision, 10 injections of a standard reference (external standard), prepared daily at the same concentration (ng/ μL) were made in ten different days. The analyses were made by two different analysts, on the same instrument [12].

The values obtained for repeatability and intermediate precision are shown in Table III.

Table III

Repeatability and intermediate precision

Precision	γ -HCH	Aldrin	Endrin	<i>p,p'</i> -DDT
	RSD (%)			
Repeatability	3.36	0.86	2.06	2.23
Intermediate precision	4.60	2.05	4.14	3.35

RSD – relative standard deviation.

The values obtained under conditions of repeatability for the relative standard deviations were in the range of 0.86–3.36%, below the maximum admissible level of 9.6%, which demonstrates a good repeatability of measurements. In this case, values obtained for RSD were in the range of 2.05–4.6%, below the maximum admissible level of 9.6%, which demonstrates a good intermediate precision of measurements.

In order to prove the fact that during determination of intermediate precision, except for random errors, there was no occurrence of systematic errors, which could impair the results, for each of the analyte of interest diagrams of X_{medium} -type were plotted. As attention limit, the maximum value of relative standard deviation (9.6%) was taken in consideration, calculated according with Horwitz equation. Measured values and this maximum value of relative standard deviation taken into account for drawing and interpretation of these diagrams, make sure that there were no systematic errors to affect the results of the analytical measurements.

Accuracy

The accuracy of a method may vary throughout the range of measurement, and therefore it must be determined at different fortification levels. The criterion, on which the accuracy of a method is accepted, is represented by the recovery obtained, which in its turn depends on the sample matrix, on the sample processing procedure and on the analyte concentration. In the present paper, in order to determine the accuracy, three pesticide standards in water were prepared, at 500 mL flask of the intermediate solution I (10 ng/μL); after all the filtration, extraction, concentration and transfer in 1 mL of petroleum ether operations, theoretical concentrations of 0.2, 1 and 2 ng/μL in petroleum ether were obtained. This alternative of enriching a blind sample of distilled water instead of enriching real water samples with pesticide content was selected, for the following reasons:

- in this manner, it is certain that the matrix is absolutely homogenous;
- the concentration resulted from the enrichment shall certainly be in the linearity range of the method;
- the concentration of the matrix in organo-chlorinated pesticides is below the detection limit.

In order to calculate the recovery, the following relation was used:

$$\text{Recovery \%} = \frac{C_{bimb} - C_b}{C_{bimb}} \times 100$$

where:

C_b – concentration of the blind sample (ng/μL);

C_{bimb} – concentration found after enriching the blind sample (ng/μL).

In view of an accurate method, the average value of recovery depending on the concentration should be in the ranges presented in table IV [10].

Table IV

Accepted values for the average recovery depending on the concentrations

Analyte concentration (%)	Analyte proportion	Unit	Average recovery (%)
0.001	1.00E-05	10 ppm	80–110
0.0001	1.00E-06	1 ppm	80–110
0.00001	1.00E-07	100 ppb	80–110
0.000001	1.00E-08	10 ppb	60–115
0.0000001	1.00E-09	1 ppb	40–120

Practical concentrations obtained for the blind samples from distilled water were: 0.01 ng/μL γ-HCH, 0.01 ng/μL aldrin, 0.01 ng/μL endrin, and 0.03 ng/μL *p,p'*-DDT.

Table V presents the values for the average recovery. The average values for the degree of recovery were in the range of 80–108%, observing the requirement imposed for the concentration levels studied, stating that the degree of recovery must be in the range of 80 – 110%. This fact demonstrates that the method is accurate.

Table V

The results obtained in the accuracy (recovery) study

	<i>γ</i> -HCH	Aldrin	Endrin	<i>p,p'</i> -DDT
$C_{bimb}-C_b$ (ng/μL)	0.16	0.18	0.15	0.17
C_{bimb} (ng/μL)	0.2	0.2	0.17	0.2
Recovery (%)	80	90	88.2	85
$C_{bimb}-C_b$ (ng/μL)	0.94	0.94	0.8	0.8
C_{bimb} (ng/μL)	1.01	1.01	1	0.99
Recovery (%)	93	93	80	99
$C_{bimb}-C_b$ (ng/μL)	1.69	1.9	1.86	2.18
C_{bimb} (ng/μL)	2.01	2.05	2.27	2.08
Recovery (%)	84	92.5	82	108

C_b – concentration of the blind sample; C_{bimb} – concentration found after enriching the blind sample.

Detection and quantification limits

Two methods may be applied in order to calculate the detection limit (LoD - ng/μL) [8]:

(a) minimum six independent blind samples, each of them measured only once:

$$\text{LoD} = x_m(\text{blank}) + 3S$$

where:

$x_m(\text{blank})$ – mean value of blank (ng/μL);

S – standard deviation of repeatability (ng/μL).

(b) minimum six independent blind samples, fortified at the lowest concentration accepted, each of them measured only once:

$$\text{LoD} = 0 + 3S$$

In order to calculate the detection limit, the first method was used. For each component, an injection from six independent blind samples, previously subjected to all the steps of the method (filtration, extraction, concentration, and transfer in 1 mL of petroleum ether operations), was made. Blind samples were chosen since they yielded measurable values of the pesticides content.

In order to calculate the quantification limit (LoQ - ng/μL), an injection from six independent blind samples, previously subjected to all the steps of the method, was made.

The following relation was applied:

$$\text{LoQ} = x_m(\text{blank}) + 10S$$

Values obtained for detection and quantification limits of γ -HCH, aldrin, endrin, and *p,p'*-DDT in water are presented in Table VI.

Table VI
Values of detection and quantification limits in water

Compound	LoD (ng/ μ L)	LoD _{water} (ng/ μ L)	LoQ (ng/ μ L)	LoQ _{water} (ng/ μ L)
γ -HCH	0.037	0.000074	0.123	0.000246
Aldrin	0.019	0.000037	0.063	0.000127
Endrin	0.022	0.000044	0.073	0.000147
<i>p,p'</i> -DDT	0.0574	0.000115	0.191	0.000382

LoD – detection limit; LoD_{water} – detection limit in water; LoQ – quantification limit; LoQ_{water} – quantification limit in water.

The values of detection limits for organo-chlorinated pesticides using gas chromatography with electron capture detector GC–ECD, according to standard SR EN ISO 6468/1996, are in the range of 0.001–0.01 μ g/L, which means a sensibility of ECD-detector and implicitly, of the method, 10 times greater than that of the MS-detector [4].

Conclusions

All the performance characteristics of the GC–MS technique, i.e. selectivity, specificity, linearity, repeatability, detection limit and quantification limit and accuracy followed the conditions required for the validation of the method. Although superior conditions regarding the method selectivity are obtained, some of the performance characteristics, such as the detection and quantification limits are much higher than in the case of the GC–ECD technique, even if mass spectrometry with SIM-mode was used.

References

1. Doerffel K., Statistik in der analytischen Chemie, 5 Auflage, Deutscher Verlag für die Grundstoffindustrie, Leipzig, 1990.
2. Majors R.E., Carr P.W., Glossary of liquid-phase separation terms, *LC GC North America*, 2001, 19(2):124–162.
3. Mandel J., The statistical analysis of experimental data, Dover Publications, New York, 1984.
4. Pană M., Analytical control of pesticides, PhD Thesis, University of Bucharest, 2006 (in Romanian).
5. Rebscher H., Pyell U., In-column versus on-column fluorescence detection in capillary electrochromatography, *Journal of Chromatography A*, 1996, 737(2):171–180.
6. Sachs L., Statistische Auswertungsmethoden, 3 Auflage, Springer-Verlag, Berlin–Heidelberg–New York, 1972.
7. Thompson M., Ellison S.L.R., Wood R., Harmonized guidelines for single-laboratory

- validation of methods of analysis (IUPAC Technical Report), *Pure and Applied Chemistry*, 2002, 74(5):835–855.
8. Verheij E.R., Tjaden U.R., Niessen W.M.A., Van der Geef J., Pseudo-electrochromatography–mass spectrometry: a new alternative, *Journal of Chromatography A*, 1991, 554(1–2):339–349.
 9. Wittkowski R., Matissek R., Capillary gas chromatography in food control and research, Technomic Publishing Company, Pennsylvania, 1993.
 10. ***, *ICH-Q2A*. Guideline for Industry: text on validation of analytical procedures, 1995 (<http://www.fda.gov/cder/guidance/index.htm>).
 11. ***, Calitatea apei. Etalonarea și evaluarea metodelor de analiză și estimarea caracteristicilor de performanță. Partea I: Evaluarea statistică a funcției de etalonare, SR ISO 8466–1:1999.
 12. ***, The fitness for purpose of analytical methods. A laboratory guide to method validation and related topics, 1st edition, EURACHEM Guide, 1998.
 13. Gheorghe M., Bălălu D., Ilie M., Baconi D.L., Ciobanu A.M., Component analysis of illicit heroin samples by GC-MS method, *Farmacia*, 2008, LVI(5), 577–582.

Manuscript received: February 25th 2010