UNCERTAINTY EVALUATION OFPARAMETERS USED IN LC-MS/MSSYSTEM SUITABILITY FOR THE DETERMINATION OF PESTICIDE RESIDUES

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Abstract- Parameters used to assess LC-MS/MS system suitability for the determination of the concentration of pesticide residues in food are the retention time, the ion ratio, the signal to noise ratio and the slope of the calibration line.

The EU tolerance values of the first two of these parameters have changed more than one time during the last years in the SANTE Document (last edition 11945/2015), they are differentiated from the respective tolerances mentioned in the Commission Decision 657/2002, and have been subject of debate.

In the present study system suitability parameters of LC-MS/MS were evaluated under the same LC-MS/MS conditions at concentration 0.01μg/mL, over 28 months' period. Six representative compounds were used for this purpose and the respective control charts were constructed. The uncertainty values of these parameters were evaluated and compared to respective values of the literature.

1. INTRODUCTION

The purpose of this study is through long term LC-MS/MS system suitability data of 28 months, to evaluate the variability of retention time and ion ratio at 0.01mg/kg, which is the limit of detection (LOD). This was attained by the use of 6 representative compounds and the respective control charts. In Table 1, the physicochemical properties of the pesticides used for the System Suitability Test are presented, from which it is seen that the pesticides selected belong to different chemical classes, and have a great range of values of physicochemical properties.

Table 1: Physicochemical properties of pesticides used for the System Suitability Test (Pesticide Manual and Data Pool of the EURL for Residues of Pesticides).

Analyte	Chemical class	Solubility in H2O mg/l	pkow	V.p. mPa
acetamiprid	Neonicotinoid	4250	0.8	< 1x10^{-6}
diazinon	Organophosphorous	60	3.3	1.2 x10^{-2}
etoxazole	Various	0.075	5.6	7.0 x10^{-6}
metalaxyl M	Amides	26000	1.7	3.3 x10^{-3}
oxamyl	Carbamate	280000	-0.44	5.1 x10^{-5}
pirimicarb	Carbamate	3000	1.7	4.0 x10^{-4}

The accepted tolerance values for the ion ratio and the retention time, as described in Council Directive 96/23/EC and the EU Guidance Documents for pesticide residues (SANCO N°12495/2011, SANCO No 12571/2013 and SANTE 11945/2015) are presented in Table 2.

Table 2. Tolerance values (LC-MS/MS) for the ion ratio and for the retention time as described in in Council Directive 96/23/EC and in the EU Guidance Documents for pesticide residues.

Document	R.T.	Ion ratio
Directive 96/23/EC	± 2,5 % (as relative)	± 20 % (for ratio > 50 %) ± 25 % (for ratio > 20 % - 50 % ± 30 % (for ratio > 10 % - 20 %) ± 50 % (for ratio ≤ 10 %)
SANCO N°12495/2011	Same as above	Same as above
SANCO N°12571/2013	±0.2 min	± 30 %
SANTE 11945/2015	±0.1 min	± 30 %

As seen from this Table, the tolerance values have changed during the last years more than one time and scientific arguments have been expressed on whether all laboratories can reach the new strict tolerances, especially when working without sophisticated and expensive equipment.

2. EXPERIMENTAL

Pesticide reference standards (purity>98%) of analytes were purchased from Ehrenstorfer GmbH. All solvents, namely acetonitrile, methanol and water were of HPLC grade. Stock solutions of each of the 6 pesticides used for the System Suitability Test were prepared at 1000 µg/mL in acetone and were stored at -20°C up to 3 years. The different stock solutions were combined into a standard solution of intermediate concentration, containing the six analytes, each at 1 µg/mL in acetonitrile. This intermediate concentration solution was stored at -20°C up to 6 months. From this solution a working solution of the 6 pesticides was prepared every day of use, at concentration 0.01µg/mL.

An Agilent 1200 Liquid Chromatograph combined with a Waters Quattro Premier Triple Quadrupole Mass Spectrometer, operating at the ESI^+ mode, with an Eclipse XDB C-18, 2.1x150mm column were used. Gradient elution with solvent A: 0.1% HCOOH, 5mM $HCOONH_4$, 20% methanol in water and solvent B: 0.1% HCOOH, 5mM $HCOONH_4$ in methanol was used as in the Table 3 below. Column temperature was set at 40°C. Flow rate was set at 0.25mL/min and 5µL of sample were diluted in the autosampler with 20µL of water before injection, so that the injected solvent to simulate the initial composition of the mobile phase. MS/MS was operated at the MRM mode.

Table 3.

Time (min)	% Solvent A	% Solvent B
0.0	100	0
2.0	100	0
12.0	50	50
30.0	0	100
40.0	0	100
40.5	100	0

3. RESULTS AND DISCUSSION

The stability of the retention time and the ion ratio at the limit of detection over 28 months' period, as system suitability control of LC-MS/MS was studied under same conditions. LC-MS/MS system was assessed for its suitability for everyday use, by injecting the mixture of the 6 representative analytes at 0.01µg/mL, which is the limit of detection.

The data collected for the retention time (RT) and ion ratio of these analytes were monitored in control charts. Long term data collected for 28 months were then statistically treated, in order to evaluate the variability of retention time and ion ratio. Since the same column was used throughout the whole period of the 28 months, the signal to noise ratio (S/N) and the shape of the peaks were also monitored, in order to detect any column deterioration and to take the proper corrective actions, as cleaning the frit, flush back flush of the column with proper solvents, etc.

It was found that the S/N at 0.01mg/L, and the shape and the width of the peaks did not present significant differences through the 28-months period. As seen from Table 4, for the retention timewithin the 28 months period, the typical uncertainty values of the analytes were found between 0.09 and 0.15min and the expanded uncertainty between 0.18 and 0.30 min at 95% confidence level. Therelative typical uncertainty values, expressed as %RSD, were found between 0.49% and 1.06%, except a value 3.18% for the very polar and early eluting compound oxamyl, probably due to its poorchromatography. It is also seen that RSD values decreasedwith increasing retention time.

Table 4.

	oxamyl	**acetamiprid**	**pirimicarb**	**metalaxyl**	**diazinon**	**etoxazol**
RT_{aver}, min	4.03	10.8	14.5	17.9	24.1	28.9
s	0.128	0.115	0.168	0.090	0.118	0.15
%RSD	3.18	1.06	1.16	0.50	0.49	0.519

Table 5.

	oxamyl	**acetamiprid**	**pirimicarb**	**metalaxyl**	**diazinon**	**etoxazol**
IR (aver)	0.576	0.287	0.341	0.248	0.445	0.121
s	0.114	0.043	0.087	0.041	0.132	0.027
%RSD	19.8	15.1	25.6	16.5	29.7	22.3

As for the second critical confirmation criterion in LC-MS/MS, which is the ion ratio (IR), as it is seen from Table 5, the RSD values during 28 months data, were found between 19.8% and 29.7%, not showing any dependence on the relative intensity of the product ion, a criterion that was set in the Council Directive 96/23/EC and the SANCO Document 12495/2011, but not in the following SANCO12571/2013 and SANTE 11945/2015Documents.

REFERENCES

[1] SANCO N°12495/2011, Method validation and quality control procedures for pesticide residues analysis in food and feed.

[2] SANCO No 12571/2013, Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed.

[3] SANTE 11945/2015, Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed.

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