Linee guida per un approccio semplificato alla validazione del metodo multiresiduo

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Raccomandazione della Commissione delle Comunità Europee del 3 marzo 1999 (G.U. L 128 21/5/99) : "Linee direttrici per i procedimenti di controllo della qualità per l'analisi di residui di antiparassitari"

…….I dati sui residui di antiparassitari possono essere impiegati per verificare la conformità ai limiti massimi di residui (LMR) stabiliti per i residui stessi, quale base di azioni coercitive o per valutare l'esposizione del consumatore agli antiparassitari.

L'analisi dei residui è difficoltosa, ed è essenziale disporre di appropriati procedimenti di controllo della qualità che permettano di dimostrare la validità del risultato e servano al tempo stesso ad evitare spese superflue…..

PERCHE' VALIDARE UN METODO ANALITICO?

- Esigenza sentita dalle principali organizzazioni internazionali
- Richiesto dalla nuova norma EN 17025
- Richiesto da alcune norme nazionali

VALIDAZIONE DI UN METODO ANALITICO……?

- "Linee guida per l'applicazione delle BPL e l'assicurazione e il controllo della qualità nell'analisi di residui di prodotti fitosanitari", Rapporto Istisan 97/24
- "Quality control procedures for pesticides residues analisys - Guidelines for residues monitoring in the European Union", Second edition, 1999-2000, Document No.SANCO/3103/2000
- " The fitness for purpose of analytical methods - A laboratory guide to method validation and related topics " , EURACHEM Guide 1998
- " Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC technical report), Pure Appl. Chem., vol.74, n.5, pp.835-855, 2002

PARAMETRI CARATTERISTICI DI UN METODO ANALITICO

- Specificità e selettività
- Limite di rilevabilità (LOD)
- Limite di quantificazione (LOQ)
- Intervallo di lavoro ed intervallo di linearità
- Accuratezza
- Precisione (ripetibilità e riproducibilità)
- Sensibilità
- Robustezza
- Incertezza
- Esattezza-Recupero

“Guidelines for in -house validation of analytical methods for pesticide residues in food and animal feeds”

A. Hill, S. Reynolds, Analyst 1999 124 (953-958)

…….It is usually impossible to validate all combinations of analyte, analyte concentration and sample matrix to which the method may be applied…….

……..proposal of minimum requirements for method validation in terms of the parameters studied and criteria for acceptability with the aim that the requirements should be simple, rational and affordable……...

International Workshop on Principles and Practices of Method Validation

4-6 novembre 1999, Budapest

Organizzato da AOAC, FAO, IAEA, IUPAC

“Guidelines for single laboratory validation of analytical methods for trace-level concentration og organic chemicals”

(www.iaea.org/trc/ sito FAO/IAEA Training & Reference Centre for Food and Pesticide Control, Vienna)

GUIDELINES FOR SINGLE-LABORATORY VALIDATION OF ANALYTICAL METHODS FOR TRACE-LEVEL CONCENTRATIONS OF ORGANIC CHEMICALS

The Guidelines consists of an overview and 6 Appendices which can be accessed independently.

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Overview of method validation

Guidelines for Single-laboratory Validation of Analytical Methods for Trace-level Concentrations of Organic Chemicals

Table 2. Within Laboratory Method Validation Criteria for Analysis of pesticide residues and veterinary drugs[1]

Concentration	Repeatability		Reproducibility		Trueness[2]
	CV_A%[3]	CV_L%[4]	CV_A%[3]	CV_L%[4]	Range of mean % recovery
≤1 µg/kg	35	36	53	54	50–120
> 1 µg/kg ≤ 0.01 mg/kg	30	32	45	46	60–120
> 0.01 mg/kg ≤ 0.1 mg/kg	20	22	32	34	70–120
> 0.1 mg/kg ≤ 1 mg/kg	15	18	23	25	70–110
> 1 mg/kg	10	14	16	19	70–110

1. With multi-residue methods, there may be certain analytes where these quantitative performance criteria cannot be strictly met. The acceptability of data produced under these conditions will depend on the purpose of the analyses e.g. when checking for MRL compliance the indicated criteria should be fulfilled as far as technically possible, while any data well below the MRL may be acceptable with the higher uncertainty.
2. These recovery ranges are appropriate for multi-residue methods. Stricter criteria may be necessary for some purposes e.g. methods for single analytes or veterinary drug residues (see Codex V3, 1996).
3. CV_A: Coefficient of variation for analysis excluding sample processing. The parameter can be estimated from tests performed with reference materials or analytical portions spiked before extraction. A reference material prepared in the laboratory may be used in the absence of a certified reference material.
4. CV_L: Overall coefficient of variation of a laboratory result, allowing up to 10% variability of sample processing.

Annex 1

Table 4. Representative commodities/samples for validation of analytical procedures for pesticide residues

Group	Common properties	Commodity group	Representative species
Plant products			
I.	High water and chlorophyll content	Leafy vegetables Brassica leafy vegetables Legume vegetables	spinach or lettuce broccoli, cabbage, kale green beans
II.	High water and low or no chlorophyll content	Pome fruits Stone fruits Berries Small fruits Fruiting vegetables Root vegetables	apple, pear peach, cherry strawberry grape, tomato, bell pepper, melon mushroom potato, carrot, parsley
III.	High acid content	Citrus fruits	orange, lemon
IV.	High sugar content		raisins, dates
V.	High oil or fat	Oil seeds Nuts	avocado, sunflower seed, walnut, pecan nut, pistachios
VI.	Dry materials	Cereals Cereal products	wheat, rice or maize grains wheat bran, wheat floor
	Commodities requiring individual test		e.g. garlic, hops, tea, spices, cranberry
Products of animal origin			
		Meats	Cattle meat, chicken meat
		Edible offals	Liver, kidney
		Fat	Fat of meat
		Milk	Cow milk
		Eggs	Chicken egg

Note: The method should be validated with representative analytes for each commodity, which is difficult to analyse and requires individual tests (e.g. garlic)

Annex 3 : Worked example for validation of a MRM for Pesticide residue analysis

Table 1. Summary of physico-chemical properties[8,9] of selected representative compounds

Active ingredient	Water solubility		$LogP_{ow}$		Vapour pressure		Hydrolysis	
	mg/l	pH; °C		°C	mPa	°C	DT50 [day]	pH' °C
DDE-p,p	0.065	24						
Permethrin	0.2	30	6.1	20	0.045	25	>720	4, 50
Endosulfan a	0.32	22	4.74	PH 5	0.83	25[a]		
Chlorothalonil	0.81		2.89		0.076	25		
Chlorpyrifos	1.4	20	4.7		2.7	25	water, 1.5	8, 25
Lindane	7.3	25			0.051	25	191	7, 22
Iprodione	13	20	3	PH 3, 5	0.0005	25	1 to7	7
Dimethoate	23.3	5, 20	0.704		1.1	25	12	9, -
Azinphos-Methyl	28	20	2.96		0.18	20	87	4, 22
Diazinon	60	20	3.3		12	25	0.49 185	3.1, 20 7.4, 20
Progargite	632	25	3.73		0.006	25	800	7, -
Methamidophos	200,000	20	-0.8	20	2.3	20	657	4, 22

Notes: (a) 2:1 mixture of a and b isomers

Annex 3

Table 5. Summary of recovery (Q%) data in orange

	Fortification level [mg/kg]								
	0.02		0.3		1		2		
	Q	CV_A	Q	CV_A	Q	CV_A	Q	CV_A	Qa
Methamidophos	72.5	14.5	75	11	77.5	10.5	85.2	13.2	77.55
Dimethoate	78.5	14	79	12	83	10	84.8	12.7	81.33
Propargite	80	13	85	8	84	9.5	83.5	12	83.12
Chlorpyrifos	83	12	88	9	82	10	86.8	11.7	84.96
Azinphos-methyl	85	10	92	12	90.5	11	82.6	11.2	87.52
Ethion	83	10.5	93	13	91.5	13.5	84.9	12.7	88.11
Diazinon	83.5	11	95	13	93.5	12	82.3	12.2	88.57
Iprodione	88.5	13	96	14	91.5	9.5	82.2	12	89.54
Lindane	82.5	10	102	8	89	11.5	85.9	11.5	89.84
Endosulfan I	90	10	98	10	93.5	10	89.6	10.7	92.78
p,p' DDE	88	11.5	103	7	93	11.5	89.6	12.2	93.4
Permethrin	95	11	99	9	101	11	88.5	11.7	95.76
Average	84.13		92.08		86.08		83.91		86.5
CV_{typ}		12.4		11.1		11.3		12.6	
Lsd	13.3		13.02		12.8		13.7		

Notes: see Table 3

Q = media 5 recuperi replicati a un livello

Qa = media recuperi tutti i livelli

Annex 3

Table 8. Summary of recoveries and their variation in samples of high water content at 0.3 mg/kg fortification level

	Cabbage				Apple				Orange			
	Q	CV_A	S_A	V_A	Q	CV_A	S_A	V_A	Q	CV_A	S_A	V_A
Azinphos-methyl	85	10	8.5	72	95	8	7.6	58	92	12	11.04	122
Chlorpyrifos	87	14	10.4	109	78	6	4.68	22	88	9	7.92	63
p,p' DDE	95	6	5.7	32	100	12	12	144	103	7	7.21	52
Diazinon	90	14	12.6	159	97	9	8.73	76	95	13	12.35	153
Dimethoate	75	10	7.5	56	81	13	10.5	111	79	12	9.48	90
Endosulfan I	85	8	6.8	46	91	6	5.46	30	98	10	9.8	96
Chlorothalonil	90	13	11.7	137	93	10	9.3	86	93	13	12.09	146
Lindane	80	6	4.8	23	95	8	7.6	58	102	8	8.16	67
Iprodione	95	16	15.2	231	110	11	12.1	146	96	14	13.44	181
Methamidophos	72	14	10.1	102	74	12	8.88	79	75	11	8.25	68
Permethrin	105	7	7.35	54	103	10	10.3	106	99	9	8.91	79
Propargite	88	11	9.68	94	91	11	10	100	85	8	6.8	46
Sum				1115				1016				1162
Cochran 5/12=0.34				0.21				0.14				0.16
CV ave(arithm.)		10.6				9.67				10.5		
CV_{typ}		11				10				11		

Annex 3

Table 10 Typical recovery values estimated for commodity groups

Representative analyte	Groups I, II & III.		Cereals	Oil seeds
Azinphos-methyl	87.7		94.4	77.9
Chlorpyrifos	83.5		90.7	75.6
p,p' DDE	92.7		99.5	82.5
Diazinon	89.7		100.5	85.6
Dimethoate	80.4		85.7	83.3
Endosulfan I	90.9		94.6	83.7
Ethion	88.5		90.7	79.2
Lindane	86.7		93.6	82.0
Iprodione	82.7		91.6	88.9
Permethrin	97.3		102.5	86.8
Propargite	85.3		88.7	83.2
Dimethoate		80.4	85.7	83.3
Methamidophos		76.1	88.7	80.3
Typical for the group	**89.6**		**93.4**	**82.4**

4 CHARACTERISATION OF THE METHOD

In conclusion, the method may be characterised as:

The method is applicable within the analytical range of 0.02 and 2 mg/kg for a wide range of GC amenable pesticides, the physico-chemical properties of which are within the ranges of representative compounds given in Table 1. The typical recoveries from plant commodities of high water content are about 90 % (except the highly water soluble compounds such as methamidophos), cereals 93% and oil seeds 82% with a typical CV of ≤12%.

The typical or average residue values and typical CV-s characterise the trueness and precision of the method in general. The particular values characterise the method for specific pesticide/commodity combinations. For the represented, but not tested, pesticide/ commodity combinations, the typical values for the group should be used as an initial guidance. Their performance characteristics should be checked during the extension of the method.

The daily performance of the method must be checked with appropriate internal quality control procedures during the routine use of the method. The performance characteristics established during method validation should be adjusted, if necessary, based on the results of internal quality control/performance verification tests.

Principi attivi rappresentativi Metodo M/P/AL/001/FE (multiresiduo ortofrutta)

Composto	Solubilità in acqua (mg/l)	LogPOW	Tensione di vapore (mPa)	Idrolisi - DT50 (giorni)
Lambda cialotrina	0.005	7	0.0002 (20°C)	da 1 a 15
DDE – p,p'	0.065			
Endosulfan alfa	0.32	0,22	0.045(25°C)	
Clorotalonil	0,05625	0,15	0.83(25°C)	
Diclofluanide	1.03	3.07	0.014(20°C)	10min(pH9) - 15
Clorpirifos etile	1.04	4.07	2.7(25°C)	1,5
Procimidone	4.05	3.14	18(25°C)	
Lindano	7.03		0.051(25°C)	191
Iprodione	13	3	0.0005(25°C)	da 1 a 7
Dimetoato	23.03	0,49	1.1(25°C)	12
Azinfos metile	28	0,15	12(25°C)	87
Diazinone	60	3.03	12(25°C)	0,5(pH3) ,185 (pH7)
Penconanazolo	73	0,175	0.37(25°C)	
Metolaclor (ortaggi)	400	2.09	4.2(25°C)	>200
Pirimicarb	3000	1.07	0.4(20°C)	<1
Metamidofos	200000	-0.8	2.3(20°C)	657

Principi attivi rappresentativi Metodo M/P/AL/008/FE (multiresiduo cereali e derivati)

Composto	Solubilità in acqua (mg/l)	LogPOW	Tensione di vapore (mPa)	Idrolisi - DT50 (giorni)
Lambda cialotrina	0.005	7	0.0002 (20°C)	
DDE – p,p'	0.065			
Pendimetalin	0.30	0,21	4.0 (25°C)	< 21
Clorpirifos etile	1.04	4.07	2.7(25°C)	1.05
Lindano	7.03		0.051(25°C)	191
Pirimifos metile	9.00	4,2	2.0 (20°C)	da 1 a 35
Dimetoato	23.03	0,49	1.1(25°C)	12
Diazinone	60	3.03	12(25°C)	0.5 – 185
Metolaclor	400	2.09	4.2(25°C)	>200
Metamidofos	200000	-0.8	2.3(20°C)	657