International Workshop
Combining and reporting analytical results. The role of metrological traceability and measurement uncertainty for comparing analytical results

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Collaborative study for pesticides residues determination in water samples (Method 5060 APAT-IRSA CNR)

Project 4b L. 93/01

APAT; APPA Trento; ARPA Campania; ARPA Emilia Romagna; ARPA Lazio; ARPA Lombardia; ARPA Piemonte; ARPA Puglia; ARPA Sicilia

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Collaborative study

to verify the performance characteristics and validate a multi residue analytical method

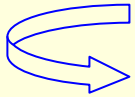
APAT – IRSA CNR

Manuals and Guidelines 29/2003: Water Analytical Methods

- ✓wastewater
- ✓freshwater
- ✓groudwater
- ✓drinking water

Selection criteria for active substances

- ✓ Priority Index (AAAF)



based on
sales data
use
degradation of active substances

- ✓ Results of previous monitoring activities
- ✓ List of dangerous substances (D.M. 367/03)
- ✓ Historical persistent substances
- ✓ Information on pesticide metabolites
- ✓ Applicability of the selected substances to chosen multi residual method (5060 – APAT- CNR IRSA).

Selected pesticides

SIMAZINA	ALACLOR	METOLACLOR
OXADIXIL	TERBUTILAZINA	METALAXIL
MOLINATE	OXADIAZON	PROPIZAMIDE
PENDIMETALIN	PROMETRINA	TERBUMETON
LINURON	CLORPIRIFOS	LINDANO
ATRAZINA	ATRAZINA DESETIL	TERBUTILAZINA DESETIL

Selected pesticides

Pesticides		M.W.	Water solubility (mg/L)	v.p. (Pa)	$logK_{ow}$	DT50 (d)
ALACHLOR	HERB	269.8	170.31	2.10E-03	3.09	30
ATRAZINE	HERB	215.7	33	3.85E-05	2.5	41
CLHORPYRIFOS	INS	350.6	1.4	2.70E-03	4.7	120
LINDANE	INS	290.8	8.52	4.40E-03	3.5	400
LINURON	HERB	249.1	63.8	5.10E-05	3	100
METALAYIL	HERB	279.3	8400	7.50E-0.4	1.75	70
METOLACHLOR	HERB	283.8	488	4.20E-03	2.9	46
MOLINATE	HERB	187.3	990	0.746	2.88	21
OXADIAZON	HERB	345.2	1.0	1.00E-04	4.91	180
OXADIXYL	FUN	278.3	3400	3.30E-06	0.65	270
PENDIMETHALIN	HERB	281.3	0.3	4.00E-03	5.18	488
PROMETRYNE	HERB	241.4	33	1.65E-04	3.1	90
PROPYZAMIDE	HERB	256.1	15	5.80E-05	3.1	60
SIMAZINE	HERB	201.7	6.2	2.94E-06	2.1	180
TERBUMETON	HERB	225.3	130	2.70E-04	3.04	300
TERBUTHYLAZINE	HERB	229.7	8.5	1.50E-04	3.21	60
	minimum	187.3	0.3	2.94E-06	0.65	21
	maximum	350.6	8400	0.746	5.18	488

Determination of pesticides residues. Method with solid phase extraction and gas-chromatographic analysis by selective detectors

CARTRIDGE ACTIVATION

Polymeric Cartridges (divinylbenzene/N-vinylpyrrolidone) (60mg/3ml) SPE1
or
C18 Cartridge (500mg/6ml) SPE2

- ✓Ethyl Acetate 5 ml
- ✓Methyl alcool 5 ml
- ✓Water 10 ml

(flux of 8 ml/min)
Leave an over layer

EXTRACTION

filtered eventually on glass fiber

process standard FENCHLORFOS

Sample (500-1000 ml) + MeOH (5ml/l)

SPE Actived column

Wash 10 ml distilled water

If in serie :

anhydrous Na_2SO_4 or diatomaceous earth 10 ml

Remotion of residual water

3-5 ml EtOAc

Eluate

Rotative evaporator and/or under N_2 (T= 40°C

0.5-1.0 ml STD INT ethion

ANALYTICAL DETERMINATION

GC:
ECD, NPD, MS

Collaborative study protocol

All laboratories had to strictly observe a set of detailed instructions to handling standards and analytical material. The instructions were agreed among laboratories before starting experimental phase. This in order to minimize all the variables and obtained data useful for validation.

Some laboratories did the analysis with both kind of cartridges

Laboratories: 10

Analytical material for study

18 selected substances

concentration around

10 mg/l in EtOAc

standard (1 mg/l)

Each laboratory

Calibration solutions (AcEt or Is):

(range 0.02 - 0.8 mg/l)

Spiking solution (Acetone):

0.1 ug/l e 0.5 ug/l

10 litres of spiked water for level 1 (0.1 µg/l) and 10 litres for level 2 (0.5 µg/l)

REC1 (0.1 ug/l) - REC2 (0.5 ug/l)

Each fortified water sample was divided in 10 different and independent samples of 500 ml

Each laboratory

Extraction had to be done

in the same way

with the same analytical apparatus

with the same lot of material (solvent, cartridges, etc..)

and all at the same time

Statistical data analysis

Statistical data analysis was done on the basis of
UNI EN ISO 5725

Statistical elaboration phases had been:

- ✓Normal data distribution tests: Shapiro –Wilk and Kolmogorov-Smirnof
- ✓Graphic elaborations (scatter plot, box plot, h and k Mandel plots)
- ✓Statistical tests for outliers: Grubbs test and Cochran test.
- ✓Evaluation of statistical parameters obtained after elimination outlier laboratories.

Data from C18 cartridges and data from polymeric cartridges were treated separately according to the described procedures. If this was true they were treated like a unique set of data.

All data belong or not to the same population

!

comparison (f-test, t-test) on variance and average

After verified their belonging to a unique population the data were treated all together

F test

$$\frac{1}{F_{0,95;n_{SPE1};n_{SPE\,2}}} \leq \frac{s^2_{SPE1}}{s^2_{SPE2}} \leq F_{0,95;n_{SPE1};n_{SPE\,2}}$$

$\nu = \text{lab}SPE_i - 1$

T test

$$\frac{\left|\overline{x}_{SPE\ 1} - \overline{x}_{SPE\ 2}\right|}{\sqrt{\frac{s^2_{SPE\ 1}}{n_{SPE\ 1}} + \frac{s^2_{SPE\ 2}}{n_{SPE\ 2}}}} \leq t_{0,95;n}$$

ν = real degree of fredom (Welch-Satterhwaite)

Here we report some results obtained. The data were relative to five substances, so selected:

- ✓A metabolite (Desethylterbutylazine)
- ✓Chemical-Physical properties (Pendimenthalin: lowest solubility; Oxadixil: highest solubility, lowest Kow).
- ✓Presence in a proficiency test (organized by APAT) and performed during same period from the same laboratories (Chlorpyrifos, Metolachlor)

All pesticides were at a concentration of 0,1 µg/l (this value is closed to the MRL for ground water). Only for oxadixil were showed the results at both concentrations (i.e. 0,1 and 0,5 µg/l).

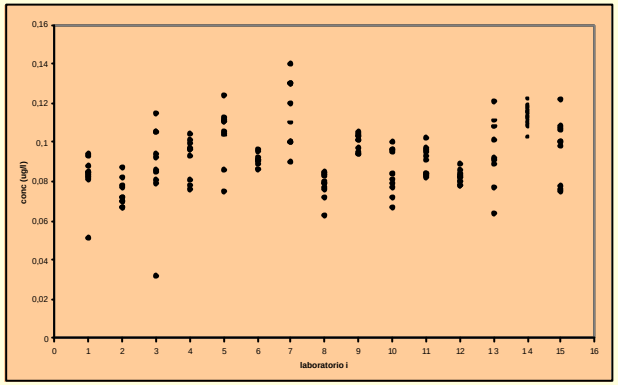
T-test and F-test (p-level < 0.05) (data SPE1 and data SPE2 separately)

Concentration 0.1 ug/l						
Residue	F-test (repeatability)		F-test (average)		t-test (average)	
	F sper	F tab	F sper	F tab	t sper	t tab
Chlorpyrifos	1,62	1,49	1.25	4.82	1.64	2.18
Metolachlor	1.42	1.52	1.67	4.82	0.61	2.17
Oxadixil	1.76	1.77	12.34	19.35	0.82	2.36
Pendimenthalin	*3.07**	1.54	1.20	4.39	*3.62**	2.26
Desethyl terbutylazine	1.01	1.54	2.70	4.88	0.94	2.20
Concentration 0.5 ug/l						
Residue	F-test (repeatability)		F-test (average)		t-test (average)	
	F sper	F tab	F sper	F tab	t sper	t tab
Oxadixil	1.51	1.52	1.46	4.82	0.31	2.20

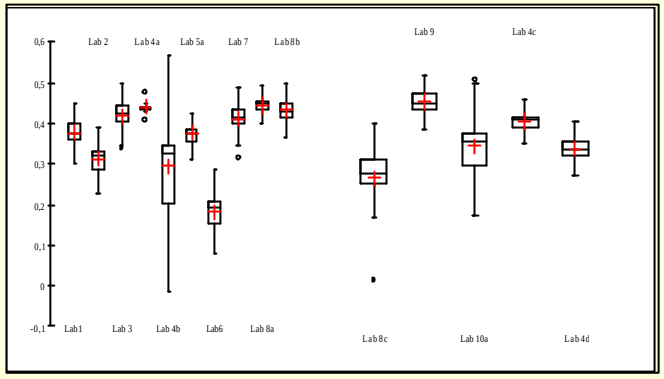
Grafical analysis:

- Before to discard outlier laboratories, we represented all results using scatter plot, box plot, h and k Mandel plots.
- Scatter plot and box plot were useful to check outlier data within the data set of each laboratory.
- Information from Mandel plots were useful to put in evidence outlier data of single residue from each laboratory. Anyway, their presence were confirmed by Cochran and Grubbs tests for elaboration according to ISO 5725.
- As example we reported graphic of Metolachlor at concentration 0.1 µg/l

Graphical representation of data

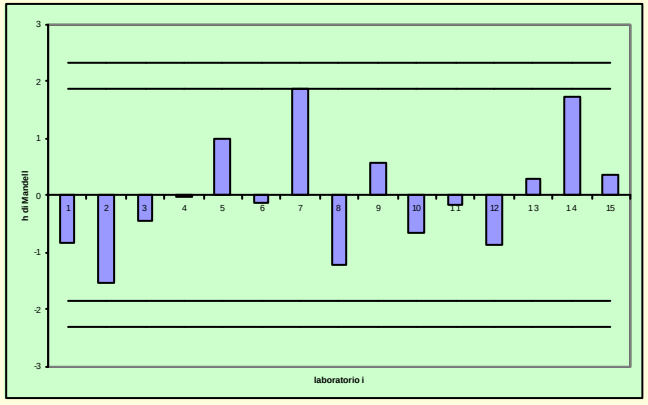


Metolachlor 0.1ug/l Scatter plot

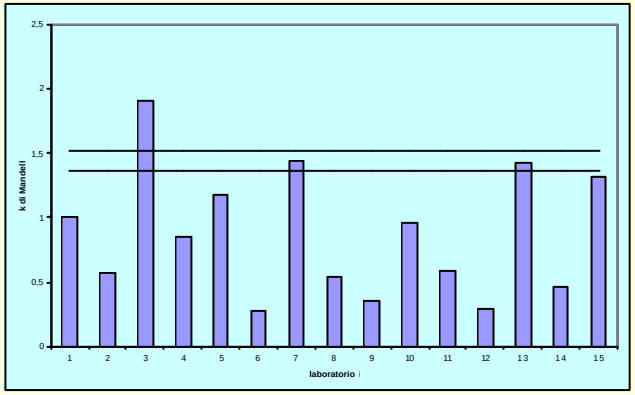


Metolachlor 0.1ug/l Box plot

Metolachlor 0.1ug/l - Mandel h plot



Metolachlor 0.1ug/l Mandel k plot



Results obtained from elaboration of the data after remotion of outliers laboratories for specific residue.

	Chlorpyriphos	Metolachlor	Oxadixil	Oxadixil	Pendimetalin	Desethyl terbutylazine
Concentration (ug/l)	0,103	0,103	0,101	0,507	0,100	0.102
Number of laboratories	15	14	11	12	14	15
Repeatability (ug/l)	0,031	0,032	0,034	0,144	0,025	0,030
Reproducibility (ug/l)	0,057	0,046	0,074	0,269	0,050	0,050
Average (ug/l)	0,075	0,093	0,101	0,462	0,076	0,091
Recovery (%)	73	90	100	91	76	90

Results from SPE1 data set and SPE2 data set

	Chlorpyriphos	Metolachlor	Oxadixil	Oxadixil	Pendimetalin	Desethylterbutyl azine
Concentration	0,103 ug/l	0,103 ug/l	0,101 ug/l	0,507 ug/l	0,100 ug/l	0.102 ug/l
SPE1						
Repeatability (ug/l)	0,027	0,038	0,033	0,186	0,015	0,027
Reproducibility (ug/l)	0,058	0,053	0,085	0,317	0,047	0,060
Average (ug/l)	0,081	0,094	0,094	0,457	0,087	0,095
Recovery(%)	78	91	93	90	87	93
SPE2						
Repeatability (ug/l)	0,035	0,032	0,025	0,228	0,026	0,026
Reproducibility (ug/l)	0,058	0,043	0,033	0,335	0,054	0,041
Average (ug/l)	0,067	0,090	0,086	0,441	0,065	0,088
Recovery(%)	65	88	85	87	65	86

Conclusion

- Evaluation of outlier data. There were full correspondence among results obtained by statistical elaboration of Grubbs and Cochran tests and the estimation by graphical technique on coherence (h and k Mandel test).
- Comparison between SPE used for extraction. Results, obtained from F-test and t-test, confirmed the hypothesis of equivalence between phases (polymeric and C18). The hypothesis was based on experience of analyst participating to the study and on method indications. However, results suggest better performances in recovery percentages of the polymeric phase, at least for studied residues.
- Analytical method. First results here showed confirmed how method fits for residues with very different chemical-physical properties. Repeatability values were in agreement with some specific rules (i.e. D.Lgs. 31/2001 or Directive 98/83/CE for quality of drinkable water)

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Network:

APAT (Damiano Centioli); APPA TRENTO (Michele Lorenzin); ARPA CAMPANIA (Maria Cristina Manca); ARPA EMILIA ROMAGNA (Marco Morelli); ARPA LAZIO (Bruno Bencivenga); ARPA LOMBARDIA (Marco Volante); ARPA PIEMONTE (Elio Sesia); ARPA PUGLIA (Francesco Fiume); ARPA SICILIA (Maria Antoci, Vittoria Giudice, Sabrina Finocchiaro)

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