95-0696

Identification of Pesticides Separated by HPLC with on-line UV PDA and ThermaBeam MS Detectors.

Screening of Pesticides in Water

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Summary: the newly available Integrity system, combining a low dispersion HPLC unit with a high performance diode array UV detector and a ThermaBeam mass detector was tested for the detection and identification of polar and non polar pesticides in water samples.

Using HPLC conditions compatible with MS detection, the photodiode array detector provides information on peak purity and allows detection, identification (UV library) and quantitation in the ppt range after extraction.

The ThermaBeam detector combines in a small instrument an enhanced particle beam interface with an electon impact ionization and a quadrupole mass filter. The interface is compatible with 100 % aqueous buffers and thus can be used even for very polar pesticides.

When used in scan mode, it allows the positive identification of most of the pesticides at 50 ng on column, corresponding to sub ppb levels before extraction.

When used in SIM mode, the ThermaBeam detector allows peak identity confirmation for most of the pesticides at 5 ng on column

Objectives

The objectives of our work is

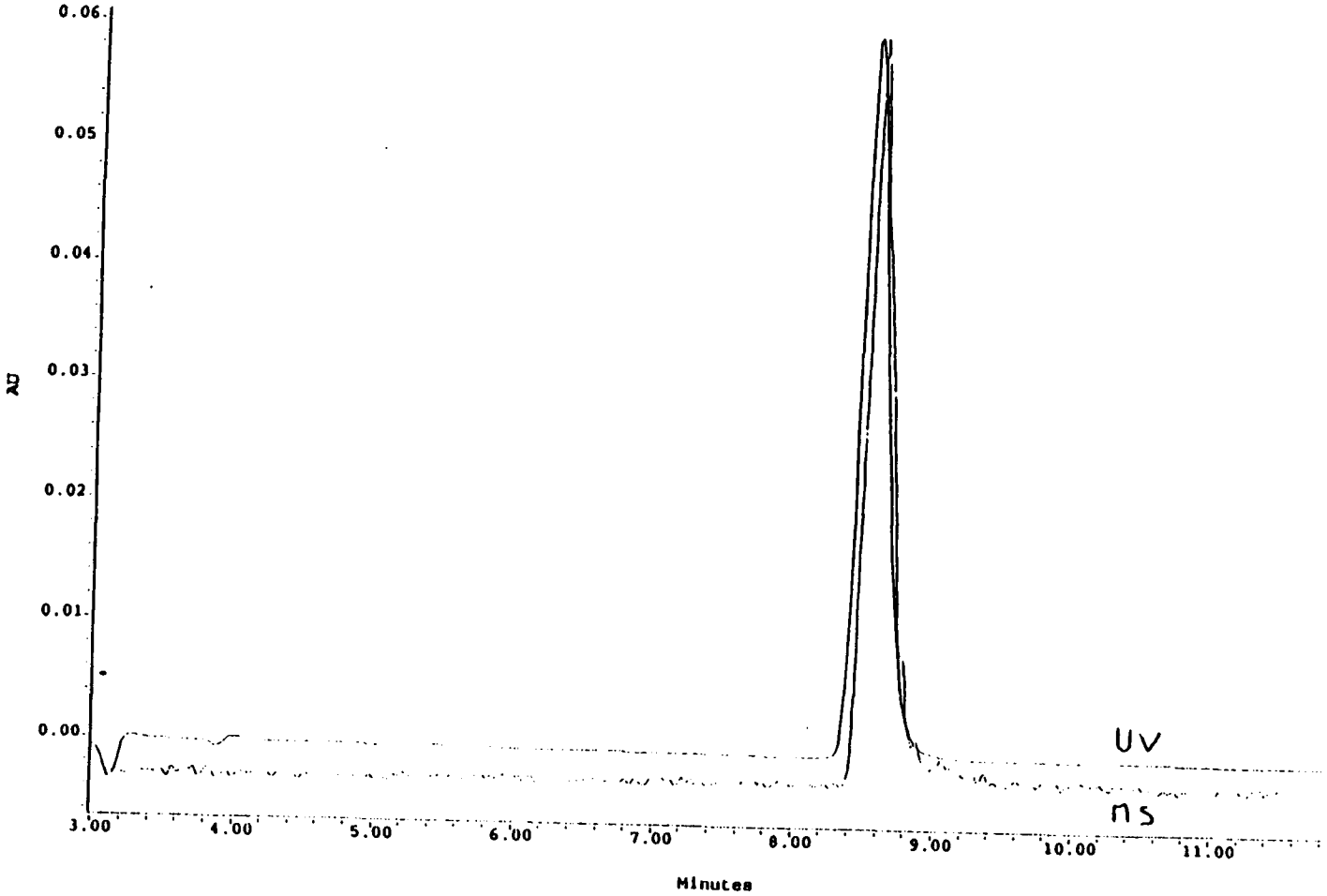
-to develop an HPLC separation method for the screening of pesticides in water
- to use both photodiode UV detection and mass detection in line to get the following information:

- peak purity verification (UV)
- peak identification with UV library
- quantitation with UV
- positive peak identification with electon impact mass spectra
- peak confirmation using Sim mode

- to apply solid phase extraction to real samples and to verify the performances of the system

- to verify the absence of interference from humic acids for the mass detection

Integrity system efficiency: dispersion test



The overlay of the UV chromatogram at 230 nm (Simazine peak, 100 ng on column), and MS chromatogram (mass extracted at 201 amu, smoothed by 31 point Savitsky-Golay) shows a very small delay volume and a minimal dispersion between the two detectors.

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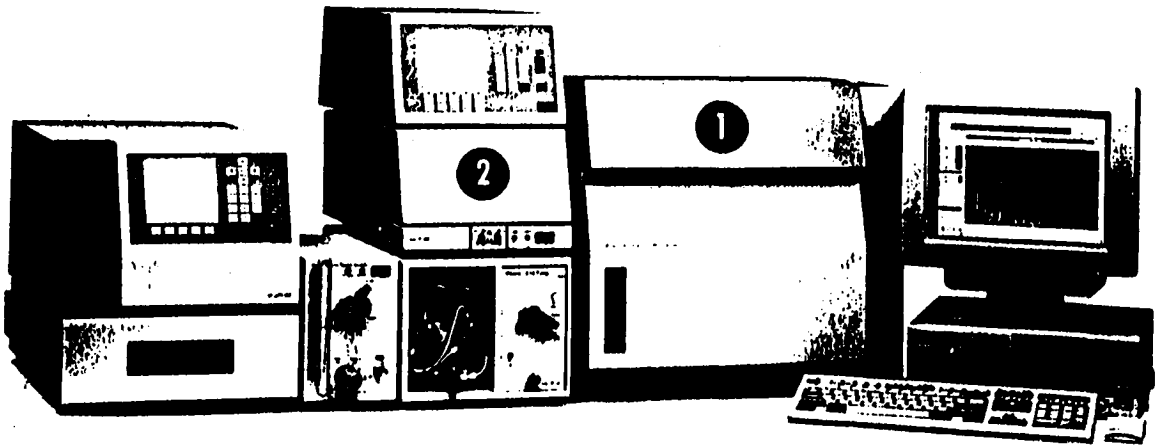
Integrity system photograph



(1) ThermaBeam mass detector

(2) 996 photodiode array UV detector

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HPLC conditions

Integrity system, consisting in:

- M 616 quaternary gradient pump
- M717 automatic injector
- column bypass module
- M996 diode array detector
- ThermaBeam Mass detector

All modules and plumbing connections are optimized for minimum dead volumes and dispersion

- Millennium 2010 acquisition and control software

Column: Novapak C18 2 mm x 300 mm

Solvents: flow rate: 0.3 ml/mn
solvent A: 0.1 M ammonium acetate (Aldrich)
solvent B: 100 % acetonitrile (Baker)
See chromatograms for gradient profiles

Detectors conditions

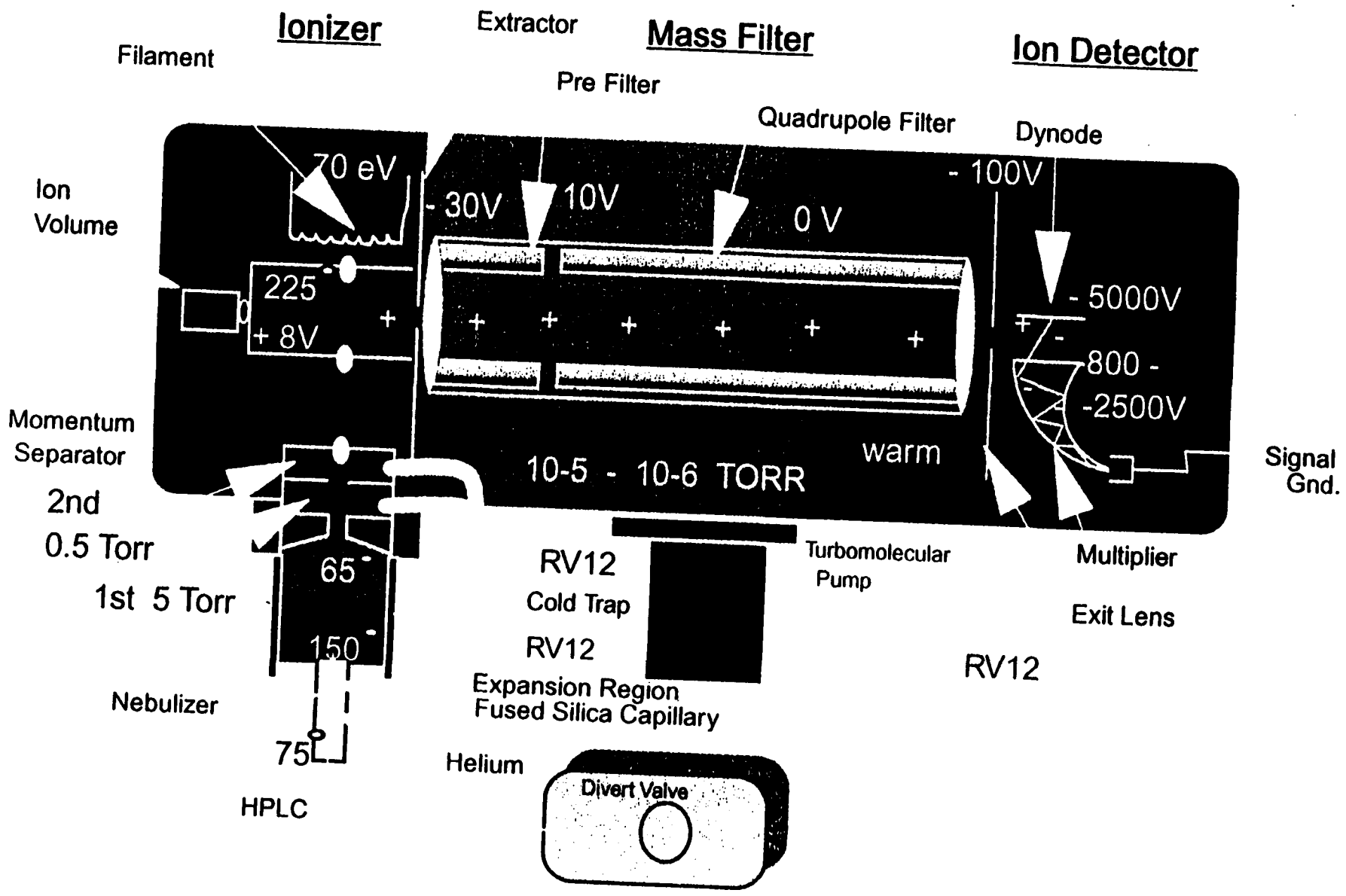
UV detection:

- Acquisition range: 200 to 350 nm
- Resolution: 2 nm
- Acquisition rate: 2 spectra per second

Mass detection:

- Interface: nebulizer from 58 to 70 °C, depending on eluent composition
- Expansion region: 65 °C
- MS: vacuum level: 1.2 x 10-4 torr, HPLC eluent and helium on
- ion volume: 230 °C
- Scan range: 65 to 350 AMU, 1 spectrum per second, or SIM mode

ThermaBeam Mass Detector



Solvent compatibility with MS and UV detection

Eluent volatility:

Pesticide screening is usually made in gradient HPLC using a phosphate type buffer as solvent A, and acetonitrile as solvent B.

The phosphate being non compatible with MS, it was replaced by a 0.1 M ammonium acetate buffer.

As a consequence, we have observed a negative drift on the UV signal during the gradient. However, detection and identification limits were still excellent, even for compounds such as triazines, which present a maximum absorbance at 221 nm.

Compatibility of the ThermaBeam interface with highly aqueous buffer: by optimizing the interface parameters (nebulizer temperature), it was possible to run fully aqueous buffers. During gradient separation, we have noticed a decrease of sensitivity for high concentration in organic solvent. For that reason, we prefer actually to use two different sets of conditions, depending if the main focus is on very polar pesticides or not.

Solid phase extraction for batch screening of pesticides

- SepPak Vac (syringe) 3 ml, containing 0.5 g of tC18 material

- Cartridge conditioning
  - 4 ml MeOH-CH3CN (70-30)
  - 6 ml of 2% acetic acid in water

- Sample extraction
  - -sample volume: 400 ml
  - -flow rate: 9 ml/mn

- Elution: 1.5 ml MeOH-CH3CN (70-30)

- 2 times dilution for injection on column,
  or evaporation to dryness for additional preconcentration.
  The new SepPak dry cartridge (PN WATO54265) is useful for the elimination of the residual water before drying

Results of batch extraction of pesticides: Recovery study on 8 selected pesticides

Sample	2,4-D	Warfarin	Dinoseb	Atrazine	Carbaryl	Diuron	Propazine	Linuron
MilliQ water spiked at 10 ppb	79	93	86	95	90	95	94	94
MilliQ water spiked at 0.4 ppb	90	105	112	113	117	120	115	113
Tap Water spiked at 0.4 ppb	44	20	107	123	109	114	107	110

Calculations are made without considering the internal standard

Results of batch extraction of pesticides: Recovery study on 8 selected pesticides, corrected according to propazine recovery

Sample	2,4-D	Warfarin	Dinoseb	Atrazine	Carbaryl	Diuron	Propazine	Linuro
MilliQ water spiked at 10 ppb	84	99	91	101	96	102	100	100
MilliQ water spiked at 0.4 ppb	79	92	98	98	102	104	100	98
Tap Water spiked at 0.4 ppb	41 *	19 *	100	114 **	102	106	100	103

* Abnormally low values, due to the presence of humic acids in the sample (see page 289 of J. of Chrom., 665 (1994) 283-293

** abnormally high value, due to the presence of atrazine at about 0.1 ppb in the tap water

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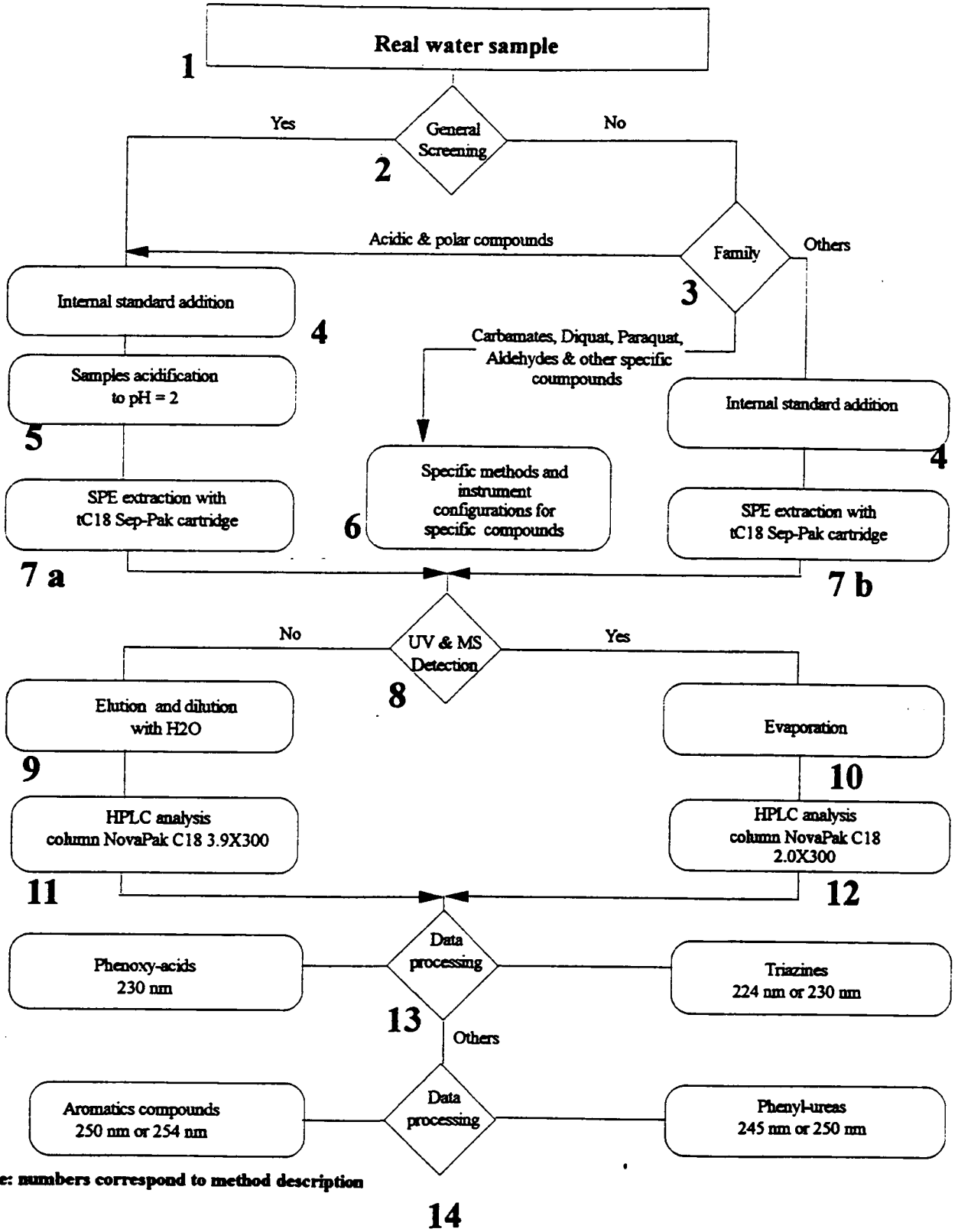
October 1994

Pathway for pesticides determination in water samples
with Waters HPLC



Note: numbers correspond to method description

Detection and identification limits (ng on column) for PDA and MS detectors

	Benta-zone	Meta-mitron	Chlori-dazone	2,4-D	MCPA	Meco-prop	Dichlor prop	Sima-zine	Atra-zine	Diuron	Propa-zine	Linu-ron
PDA det. limit	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.09	0.09	0.09	0.09	0.09
PDA id.limit	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.09	0.09	0.09	0.09	0.09
TMD det. limit, scan	20	10	10	20	20	20	20	*10	*30	*10	*10	*30
TMD id. limit, scan	60	30	30	60	60	60	60	*30	*60	*30	*90	*90
TMD det. limit, sim	2	2	2	2	2	2	2	*1	*3	*1	*3	*3

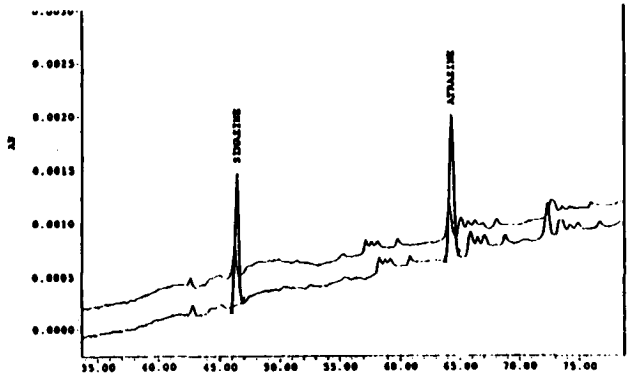
Remarks: detection limits are expressed as « amounts on column »., and given for synthetic samples obtained by spiking Milli Q water. After extraction (i.e. 100 ml sample with solid phase on line extraction) , limits are in the 0.003 ppb range for UV detection and in the 0.03 ppb range for TMD (sim mode) in the synthetic sample.
In real samples, interfering compounds (humic acids) will impact the UV limits. Depending on the sample, UV limits might be multiplied by a factor of 10 or even 20. MS limits in sim mode are normally not affected, but MS positive identification limits in scan mode might be impacted by interfering compounds

Conditions: ammonium acetate-acetonitrile gradient, excepted *: water -acetonitrile gradient

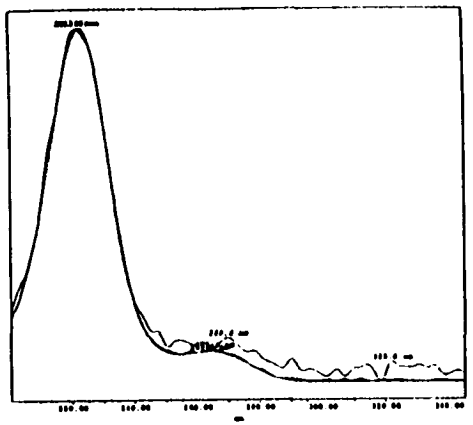
PDA detection limit: limit of detection (3 S/N) for the 996 UV detector, scan from 200 to 350 nm , resolution 2 nm, chromatogram extracted at full resolution
PDA identification limit: limit of identification for the 996 detector. The detector allows peak purity determination and peak identification in the users built library at that concentration.
TMD detection limit, scan: limit of detection in scan mode. The extracted chromatogram will show the peak with a S/N ratio =3.
TMD identification limit, scan: limit of identification in scan mode. At this limit, the TMD provides on a synthetic sample a library searchable electron impact spectra.
TMD detection limit, sim: limit of detection in selected ion monitoring mode. S/N =10. At this limit, it is possible to confirm the UV identification.

Triazine identification limits with the M996 photodiode array detector

Non UV absorbing eluent: 1 and 5 ppb atrazine and simazine, 150 µl direct injection on column **(no preconcentration)**

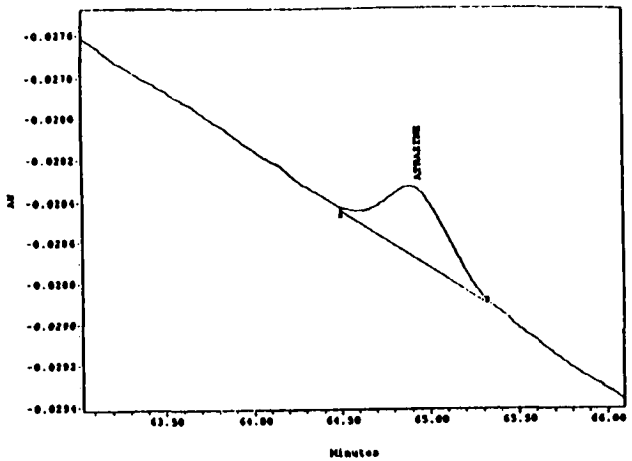


Overlay of chromatograms extracted at 230 nm

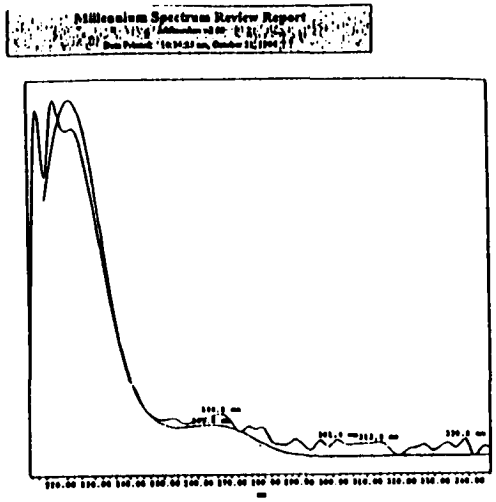


Overlay of simazines UV spectra at 500, 5 and 1 ppb

Ammonium acetate eluent: 1 ppb atrazine, 150 µl direct injection on column **(no preconcentration)**

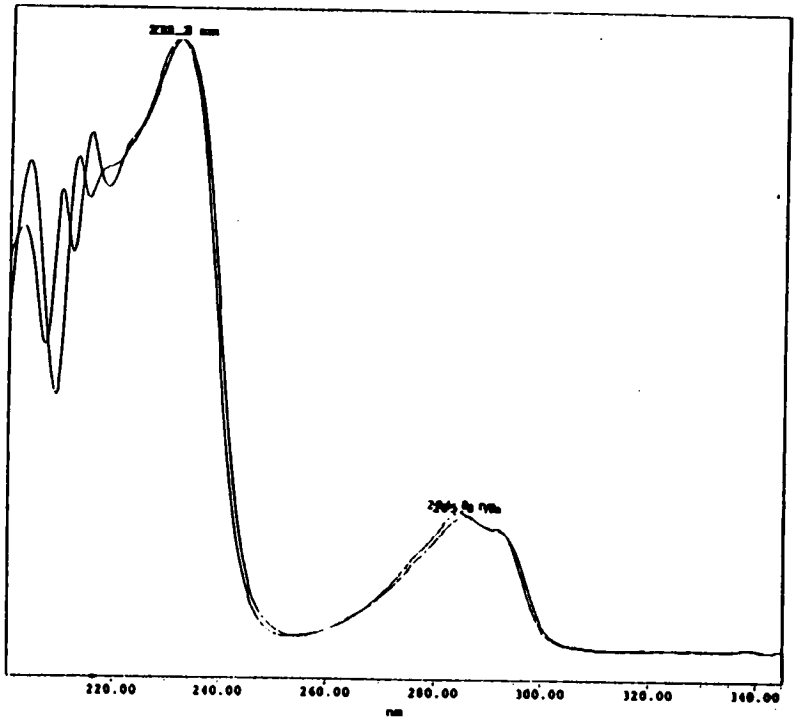


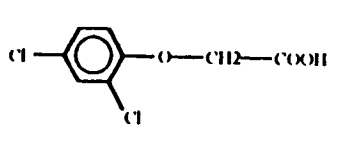
Chromatogram at 230 nm: peak close up view



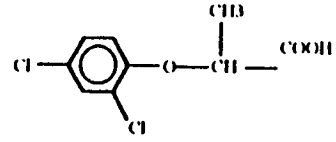
Overlay of atrazine UV spectra at 40 and 1 ppb

Exemple of PDA identification: 2,4-D and Dichlorprop 6 ng injected on column

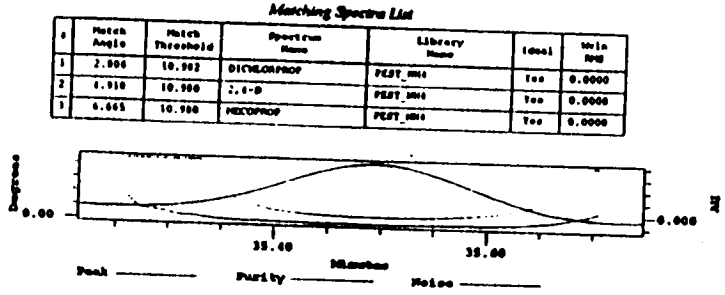




2,4-D



Dichlorprop



2,4-D and dichlorprop: 40 ppb, 150 µl injected, Novapak C18 2mm x 300mm

Ammonium acetate gradient

Detector parameters: filter 2, resolution 2.4nm

Library parameters: search threshold 20

The good spectral resolution and spectral sensitivity allows peak identification using UV library only, even at very low concentration and in presence of ammonium acetate

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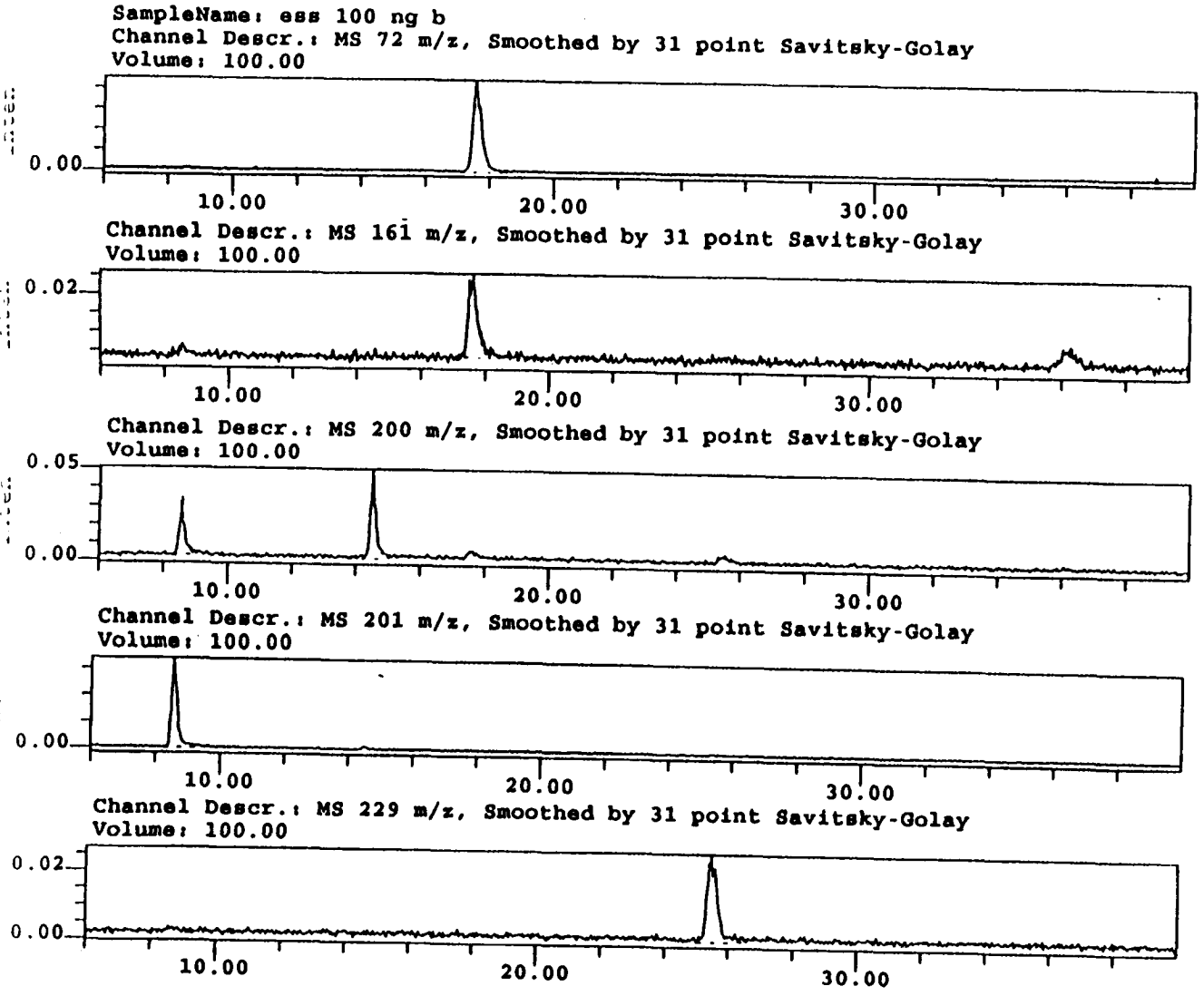
Mass chromatograms extacted for polar pesticides, Scan mode, each pesticide at 100 ng on column



Conditions: see UV chromatogram

Scan mode from 65 to 350 amu

Chromatograms extracted at:

Mass 72: diuron at 17.57 mn

Mass 161: linuron at 36.14 mn

Mass 200: atrazine at 14. 52mn

Mass 201: simazine at 8.59 mn

Mass 229: propazine at 25.47 mn

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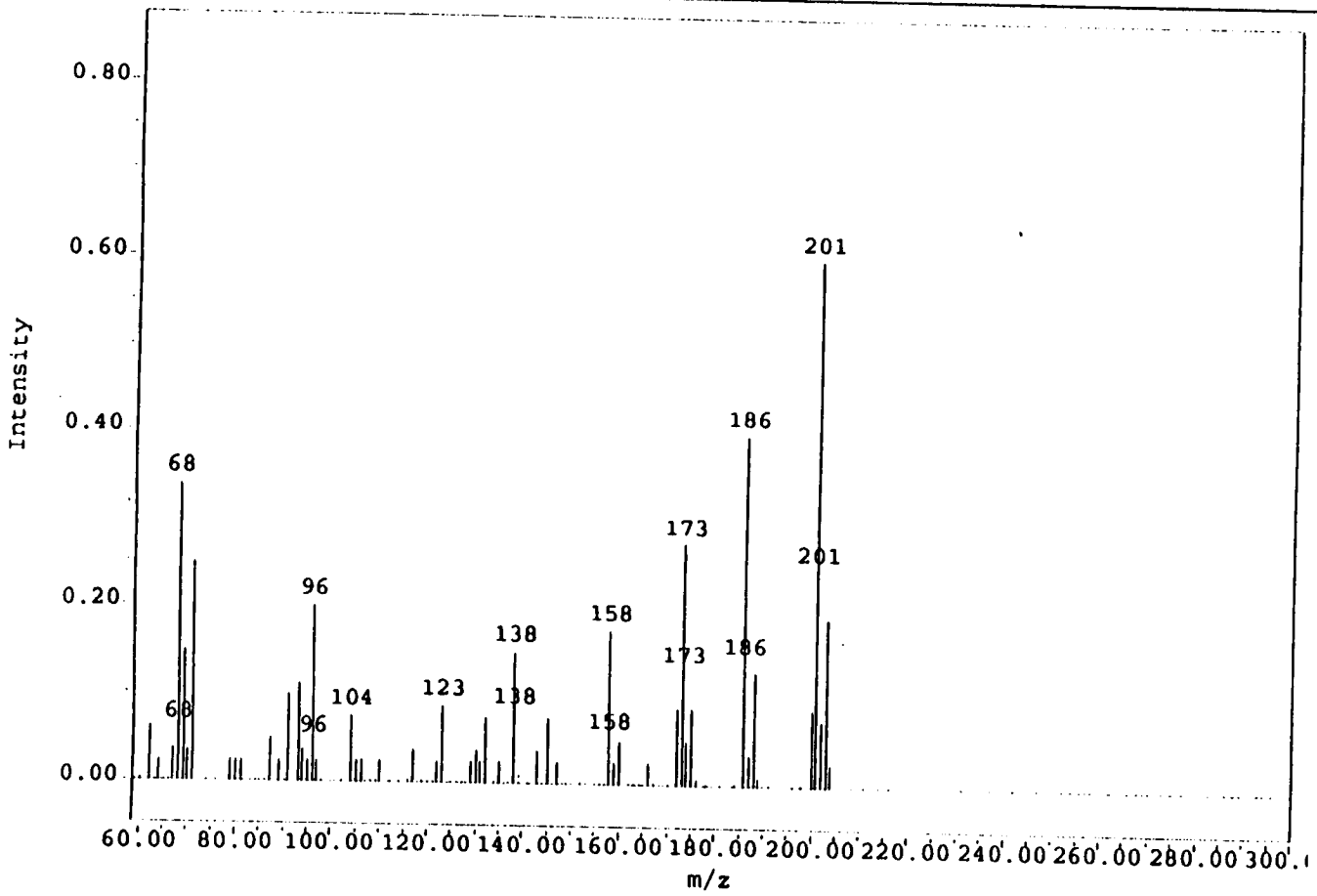
Millennium Spectrum Review Report

Millennium v2.20HotTempers

Date Printed: 11:55:30 AM, May 14, 1995



8.579 minutes, 50 - 300 m/z, from ess 100 ng b
1,3,5-Triazine-2,4-diamine, 6 0.000 minutes, 40 - 205 m/z, from WILEY®

Positive identification of simazine at 100 ng on column with the Wiley library:

Spectral Table

#	Retention Time	Source	Spectrum Name	Baseline Correct	Searchable
1	8.579	ess 100 ng b		On	Yes
2	0.000	WILEY®	1,3,5-Triazine-2,4-diamine, 6	Off	Yes

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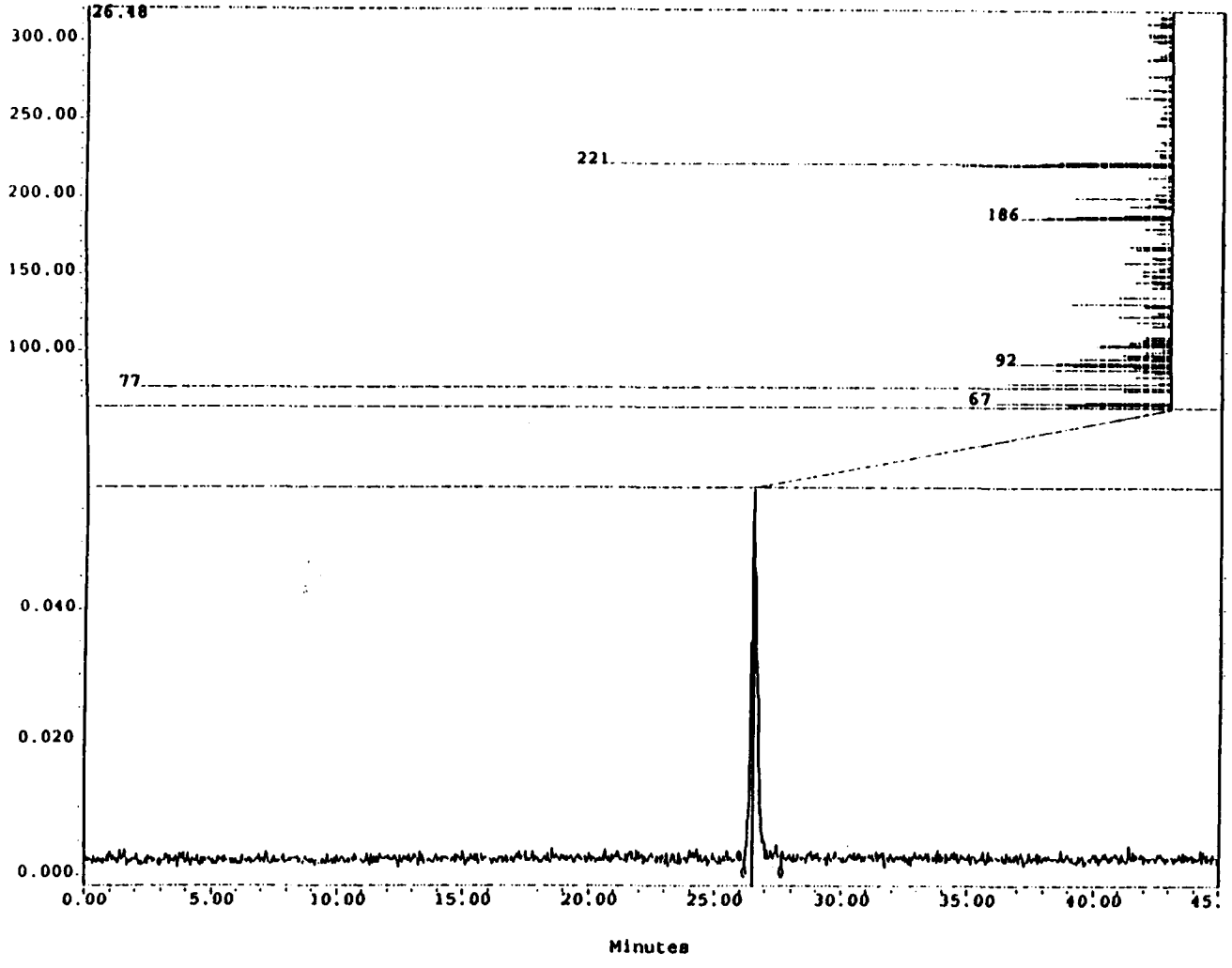
Mass chromatogram for polar pesticides, Scan mode for chloridazone



Millennium PDA Spectrum Index Plot - SampleName 40 ng, 221 - MS 221 m/z, Smoothed by 31 point Savitsky-Golay

Conditions:gradient ammonium acetate -acetonitrile (95-5 to 85-15)

Chromatogram extracted at m/z 221

40 ng on column

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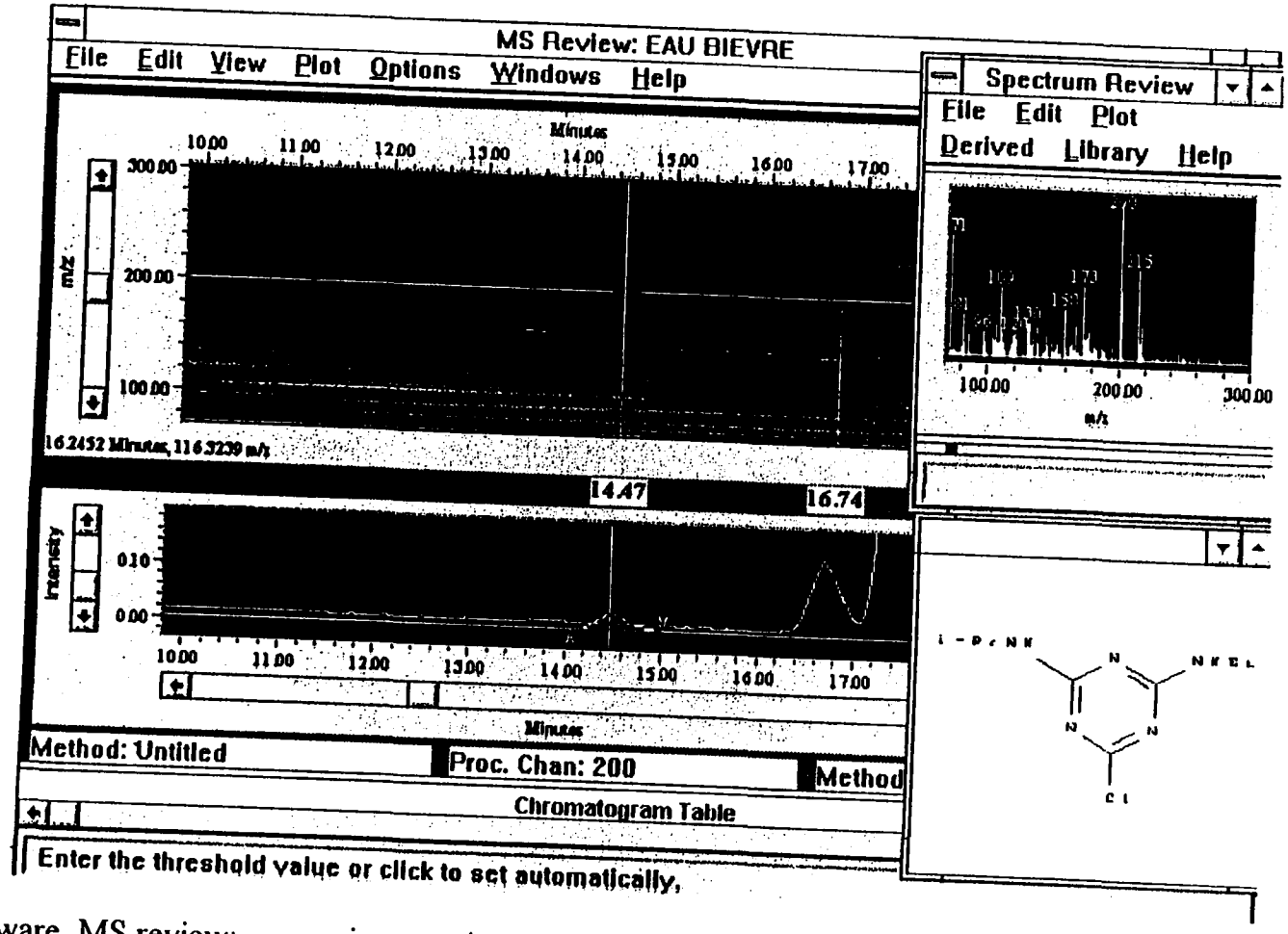
River water sample: atrazine positive identification in MS scan mode at 0.6 ppb



Millennium software, MS review: x axis: retention times Y axis: m/z colors: intensity
Chromatograms extracted at m/z 72 (yellow) and m/z 200 (white). Atrazine peak at 14.47 mn
The atrazine peak is first identified using the UV photodiode array detector (retention time and UV spectrum).
The identification is confirmed by the ThermaBeam mass detector used in Scan mode.
Conditions: preconcentration factor (solid phase extraction): 2500

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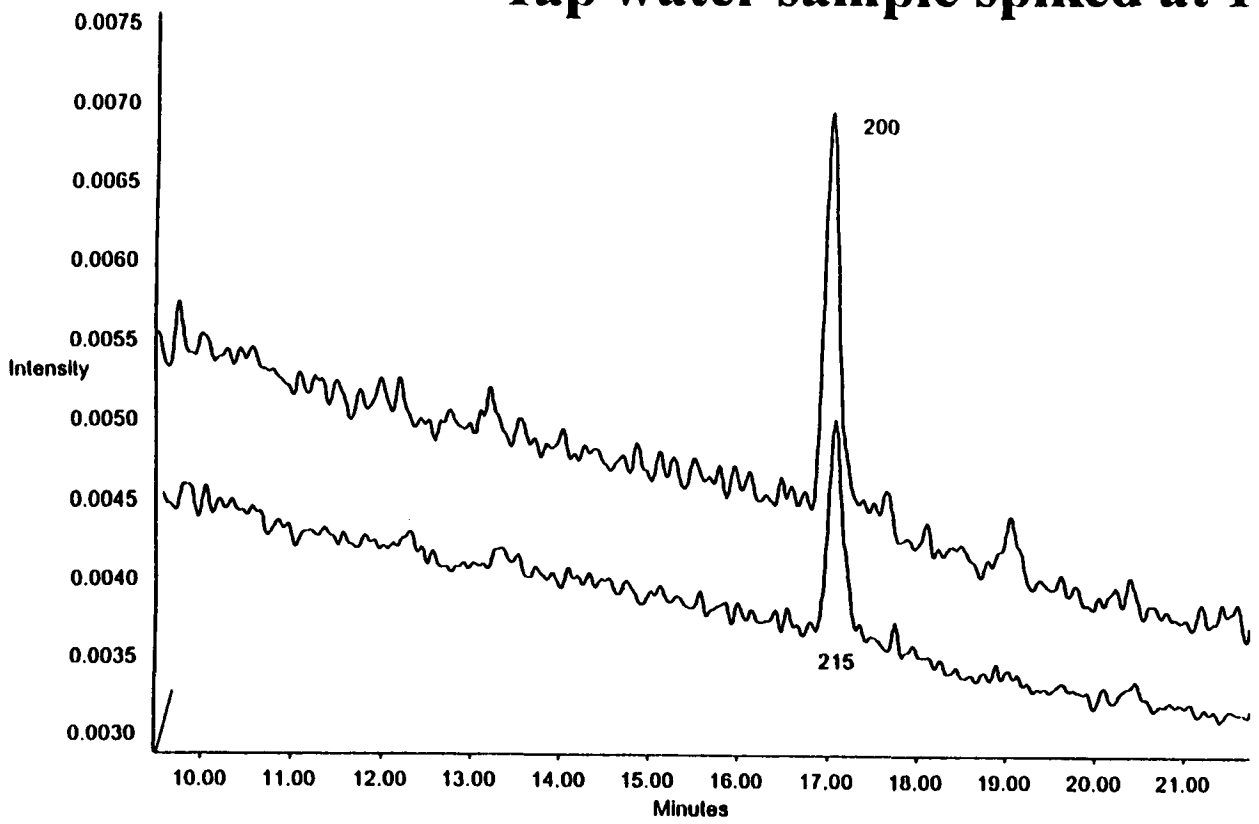
Identity confirmation using Sim mode: Tap water sample spiked at 1 ppb



The atrazine peak is first identified using the UV photodiode array detector (retention time and UV spectrum). The identification is confirmed by the ThermaBeam mass detector used in SIM mode.

Conditions: preconcentration factor (solid phase extraction): 500
injected volume: 10 µl (5ng on column)

Sim channels: m/z: 200 and 215

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Conclusion (1)

The combination of UV diode array detection and MS-PB-EI is a powerful tool for the HPLC of pesticides.

The 996 UV detector provides information on peak purity and peak identification, even in presence of ammonium acetate.

The ThermaBeam mass detector provides either positive peak identification (scan mode), or peak confirmation (Sim mode), even with highly aqueous eluents (95% aqueous).

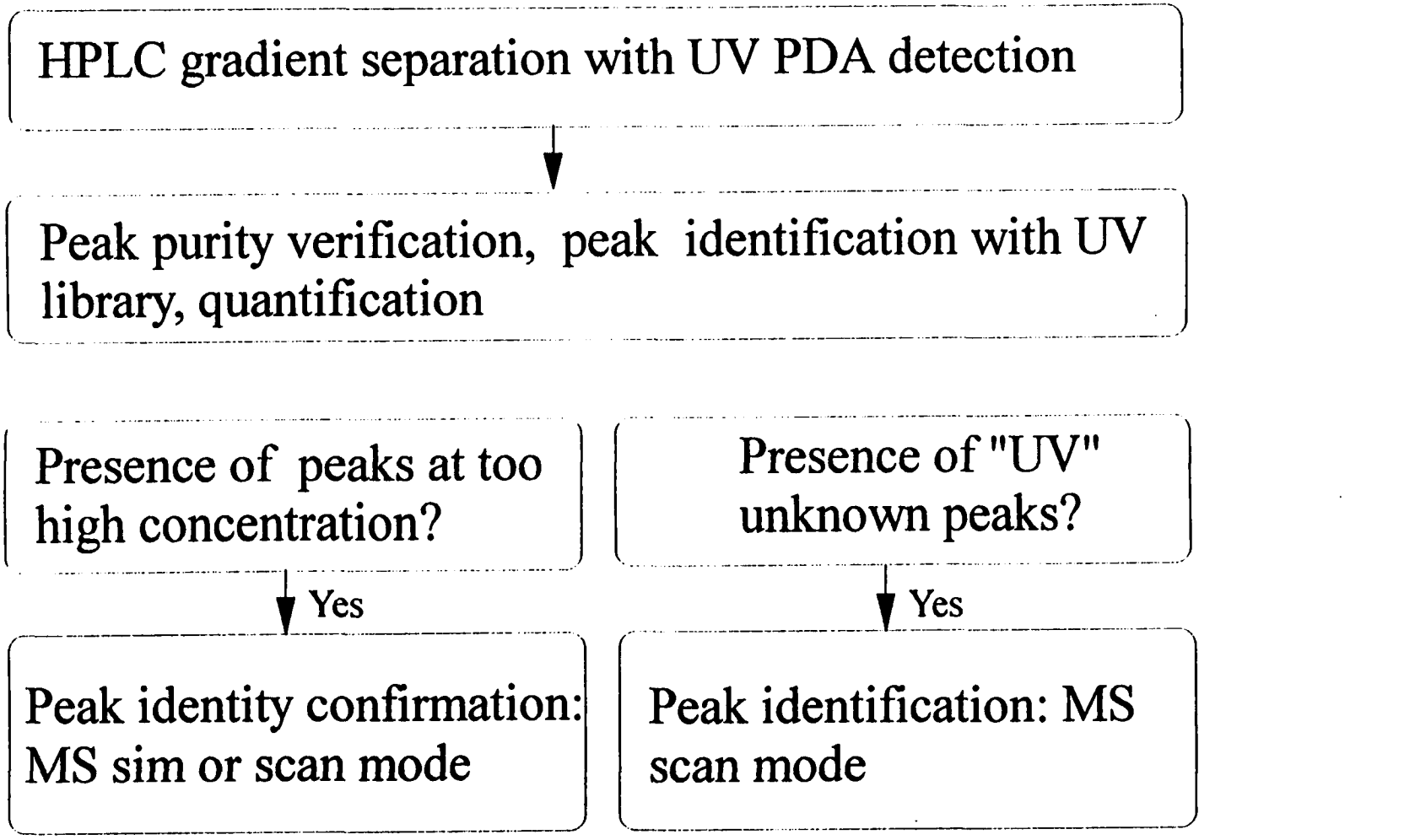
Detection and identification limits are typically better than 0.5 ng on column in UV, 50 ng on column in scan mode, and 5 ng on column in sim mode.

After pesticide extraction, both detectors will give identification (and quantification) of pesticides at the maximum concentration given by the EEC directive for drinking water.

Regarding interferences (humic acids), the selectivity of MS is obviously an advantage.

Conclusion (2)

Our HPLC analytical approach for pesticides monitoring is as follows:



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