1. SUMMARY

- The method of analysis presented here allows the quantification of residues of the fungicide ZK 100 309 in water and wine.

- Residues of ZK 100 309 were extracted from water or wine by solid phase extraction (SPE) using C18 reversed phase. After enrichment the residue was eluted with hexane/ethyl acetate (9:1). Sample clean up was done by use of florisil cartridges. Finally residues were quantified by RP/HPLC with UV-detection.

- Analysis of a batch of 6 samples takes up to 1 day from extraction of water or wine samples to preparation of the final solutions for HPLC determination.

2. INTRODUCTION

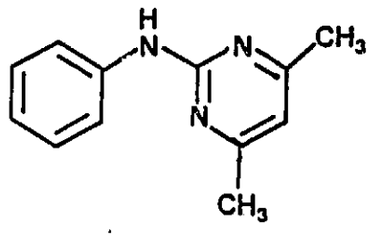
ZK 100—309 is a fungicide, which has excellent preventative activity on Botrytis cinerea in vines and strawberries and very good activity on Ventura inaequalis in apples.

According to the EEC-guideline for drinking water a maximum residue level of 0.1 µg/l drinking water per pesticide is admissible. For the determination of residues in wine also no analytical method was available. Therefore it was necessary to develop a residue method for ZK 100 309 in these matrices.

3. PHYSICAL AND CHEMICAL DATA

Active ingredient:	ZK 100 309		
Chemical name: (IUPAC)	2-anilino-4,6-dimethylpyrimidine		
(CA)	4,6-dimethyl-N-phenyl-2-pyrimidinamine		
Empirical formula:	$C_{13}H_{13}N_3$		
Molecular weight:	199.2		
Melting point:	99 -101 °C		
Solubility:	water (25 °C)	0.121	g/l
	dichloromethane	1000.2	g/l
	ethyl acetate	616.9	g/l
	toluene	412.3	g/l
	acetone	388.8	g/l
	methanol	175.9	g/l
	n-hexane	23.7	g/l

Structural formula:



4. PRINCIPLE OF METHOD

Residues of ZK 100 309 were extracted from water or wine by solid phase extraction (SPE) using C18 reversed phase. After enrichment the residue was eluted with hexane/ethyl acetate (9:1). Sample clean up was done by use of florisil cartridges. Finally residues were quantified by RP/HPLC with UV-detection.

5. SAMPLING, SAMPLE PREPARATION AND STORAGE

Sampling and storage of the samples should be done following procedures mentioned in the relevant guidelines (1,2,3).

Storage of water and wine samples should be done at + 4 °C.

6. ANALYTICAL METHOD

6.1 Standard solutions

Prepare a stock solution by dissolving about 20 mg of analytical standard of ZK 100 309 in 20 ml acetonitrile. Prepare working solutions from the stock by further dilution.

Reprepare working solutions from the stock in monthly intervals.

6.2 Apparatus

Standard laboratory glassware
Rotary vacuum evaporator with water bath
Sample concentrator SC-3 (Techne LTD, GB)
Disposable syringes
Vacuum sample preparation unit (Supelco. Inc.)
Ultrasonic bath
Chromatography column, 400 x 16 mm, glass
HPLC system with UV-detector

6.3 HPLC conditions

Pump:	Gynkotec HPLC Pump 300	
Injector:	Autosampler Kontron 460	
Detector:	ABI Spectroflow 773 Absorbance Detector	
Column:	Nucleosil 5 µm, C18; 25 cm	
Mobile phase:	acetonitrile	600 ml
	water	400 ml
Flow rate:	1.0 ml/min	
Wave length:	268 nm	
Retention time:	7.4 min	
Injection volume:	10 µl	

6.4 Reagents and materials

- Florisil: SEP-PAK cartridges (Waters, USA)
- Syringe-filter: Acrodisc LC PVDF, 0.45 µm
  ø 25 mm (Gelman, USA)
- Acetonitrile, p.a. (Baker, Netherlands)
- Methanol, p.a. (Merck, Darmstadt, FRG)
- Hexane, distilled, b.p. 69 °C
- Ethyl acetate, distilled, b.p. 77 °C
- Bondesil sorbent, C18, 40 µm (ict, FRG)
- ZK 100 309, analytical standard (Schering AG, Berlin)

6.5 Laboratory steps

6.5.1 Extraction

Pour 1000 ml water or 50 ml wine into a 1000 ml separatory funnel. Fortify at this stage for recovery experiments. Pass the sample through a C18-column (2 ml disposable syringe filled with 1.5 g Bondesil sorbent, activated with 50 ml p.a. methanol and 100 ml distilled water) by suction. In case of wine analysis rinse the separatory funnel twice with ca. 25 ml distilled water, each, and pass the water through the C18-column, too.

Dry the C18-adsorbent by passing air through the column using vacuum for about 2 hours.

6.5.2 Clean up

Connect the C18-column with a SEP-PAK florisil cartridge and a syringe-filter. Elute any residues of ZK 100 309 with 30 ml hexane/ethyl acetate (9:1) and collect the eluate.

Reduce the eluate to approximately 1 ml using a rotary evaporator (40 °C, vacuum) and transfer the solution to a 10 ml test-tube. Rinse the flask two times with ca. 2 ml hexane/ethyl acetate (9:1). Combine the solutions and reduce to dryness (SC-3 sample concentrator, N_2, 45 °C). Dissolve the residue in 1 ml or 2 ml acetonitrile depending on the sensitivity requirements (use an ultrasonic bath). This solution is ready for quantitation with HPLC (see 6.3).

7. CALCULATION

7.1 Calculation of results

For establishment of a calibration curve solutions with known amounts of ZK 100 309 in acetonitrile are injected into the HPLC-system.

Peak areas measured by electronic integration are plotted vs. the amount of ZK 100 309 yielding a straight line, following the general equation $y = a + b \cdot x$. The amount of ZK 100 309 in a sample may be taken directly from this calibration curve. Residue concentrations of ZK 100 309 are calculated as follows:

$$\text{Residue } (\mu g \text{ ZK 100 309/l}) = \frac{A \cdot V \cdot F}{W}$$

A Amount of ZK 100 309 obtained from calibration curve (ng/µl)
V Volume of the final solution (ml)
W Weight of analysis sample (l)
F Dilution factor

7.2 Determination of recovery efficiency

$$\text{Recovery } (\%) = \frac{A \cdot V \cdot F}{W_R} \cdot 100$$

W_R Amount of ZK 100 309 added (µg/ml), other abbreviations see 7.1.

8.2 Apparent residues

In water control samples so far analyzed no apparent residues of ZK 100 309 could be detected (<0.02 µg/l, limit of detection). In wine control samples apparent residues of < 0.004 mg/l (limit of detection) up to 0.03 mg/l could be detected.

8.3 Limit of determination and detection

The limit of determination was established at 0.05 µg/l for water and at 0.05 mg/l for wine. The lower limit of detection was found to be in the order of 0.2 ng per injection.

9. ARCHIVES

All raw data belonging to this method and a copy of this report are stored under study number PF-R 89 095 (ZK 100 309 in water) or PF-R 91 078 (ZK 100 309 in wine) in the archives of HA PF-UPSR at Berlin-Frohnau.

10. REFERENCES

1. Richtlinien für die Prüfung von Pflanzenschutzmitteln im Zulassungsverfahren Teil IV,
"Prüfung des Rückstandsverhaltens - Untersuchungen an Traubenmost und Wein -"
3-3.4 (Dez. 1990), Biologische Bundesanstalt für Land- und Forstwirtschaft der Bundesrepublik Deutschland.

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Mitteilung VI der Senatskommission für Pflanzenschutz-, Pflanzenbehandlungs- und Vorratsschutzmittel "Rückstandsanalytik von Pflanzenschutzmitteln" Abschnitt IX, 1979

3. IVA-Leitlinie "Rückstandsversuche", Teil IB, 1990
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