Journal of Chromatography, 448 (1988) 424–427
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 691

Note

Simultaneous detection of trichothecenes and rosenonolactone in grape juice and wine by capillary gas chromatography

S. SCHWENK, B. ALTMAYER* and K. W. EICHHORN
Landes-, Lehr- und Forschungsanstalt für Landwirtschaft, Weinbau und Gartenbau, Abt. Phytomedizin, Breitenweg 71, D-6730 Neustadt/W. (F.R.G.)
(First received May 19th, 1987; revised manuscript received June 3rd, 1988)

The fungus *Trichothecium roseum* Link ex Fr. produces several mycotoxins of the trichothecene group, trichothecin and trichothecolone being the major toxins[1,2]. Both are toxic against fungi, some bacteria, viruses, eucaryotic cell cultures and mammals[1,3–6]. In addition *T. roseum* produces some diterpenelactones, of which rosenonolactone is the main component[6]. The biological activity of this metabolite has been reported recently[7].

Because of the increasing occurrence of *T. roseum* on graphes during the last few years it was necessary to examine the contamination of grape juices and wines with these mycotoxins. Trichothecin inhibits the alcoholic fermentation. It is stable during the usual treatment or storage of wine[8]. A method for simultaneous detection of trichothecene and rosenonolactone contaminations in grape juice and wine was developed.

EXPERIMENTAL

Materials

Trichothecin and rosenonolactone were extracted from culture filtrates of *Trichothecium roseum* Link ex Fr. (isolate 5388) and purified by liquid chromatography on silica gel. Trichothecolone was prepared from trichothecin by alkaline hydrolysis with 1 *M* potassium hydroxide. The substances were identified by mass spectroscopy and ^{1}H NMR spectroscopy.

All chemicals (analytical grade), silica gel (40 μm), octadecylsilane bonded silica gel disposable columns (SPE, volume 6 ml) and the vacuum extraction system were obtained from J. T. Baker (Phillipsburgh, NJ, U.S.A.).

The capillary gas chromatography was carried out with a Model 8320 instrument (Perkin-Elmer, Überlingen, F.R.G.) equipped with a capillary column (CPMS, OV-225, 0.32 mm I.D.) and flame ionization detection (FID). Hydrogen served as the carrier gas (150 kPa) and the split mode of injection (20 ml/min) was used. The column temperature was increased from 140 constant (for 5 min) to 240°C constant (for 15 min) at 10°C/min, then to 280°C (constant for 1 min). The temperature of the injector and the determination zone were 230 and 300°C, respectively. For the peak integration, a Perkin-Elmer Sigma 10B data system was used.

0021-9673/88/$03.50 © 1988 Elsevier Science Publishers B.V.

Extraction

An octadecyl extraction column was conditioned with methanol and water according to the instructions of the supplier. A sample of 25 ml grape juice or wine was aspirated through the column using the vacuum extraction system. The lipophilic toxins were eluted from the dry column with 1.5 ml chloroform into a graduated vial, concentrated by evaporation of the chloroform and redissolved in methanol to a final volume of 200 μl. These extracts were used for all analyses without further clean-up. For the quantitative analysis of trichothecolone, samples of 25 ml were extracted two times each with 10 ml chloroform in a separation funnel and the methanol extract was obtained as described before. Recovery experiments were carried out by adding 113–2800 μg/l trichothecin, 106–158 μg/l trichothecolone and 100–500 μg/l rosenonolactone to untreated grape juice and wine. To investigate the stability of the toxins, methanolic extracts of different toxin concentrations were stored at 4°C for 2 weeks.

Analysis

A linear calibration graph was obtained by measuring a number of toxin standards with an increasing concentration from 10 to 300 μg/ml. The toxins in the methanolic extracts of grape juice and wine (5 μl injected) could be quantified in a range of 50–2600 μg/l for trichothecin, 50–800 μg/l for trichothecolone and 100–480 μg/l for rosenonolactone by peak area integration. The limits of the detectable amounts of toxins were found by adding 100, 50 and 20 μg/l of each toxin to grape juice and wine. To detect substances interfering with the toxins in the analysis, extracts from 20 wine varieties, *e.g.*, Riesling, Silvaner were analysed.

RESULTS AND DISCUSSION

The rapid method using reversed-phase silica gel for the extraction of the liphophilic toxins from grape juice and wine was suitable for quantitative separation of trichothecin and rosenonolactone from the matrix. To detect trichothecolone, which was recovered in a range between 30 and 40%, the method is only suitable for qualitative analysis. The quantitative extraction of trichothecolone was accomplished by means of a separation funnel (Table I).

The gas chromatographic method allowed the separation of the toxins from the multi-component matrix without any derivatization of the extracts. The reproducibility was measured by injecting ten times the same extract with the toxins at about

TABLE I

RECOVERY (%) OF THE TOXINS FROM GRAPE-JUICE AND WINE

Means and standard deviations from twelve eperiments.

	Grape juice	*Wine*
Trichothecin*	77.0 (8.9)	93.2 (5.6)
Trichothecolone**	64.7 (9.1)	75.4 (11.7)
Rosenonolactone*	82.5 (7.8)	93.7 (3.2)

* Column extraction (C_{18}).
** Separation funnel method.

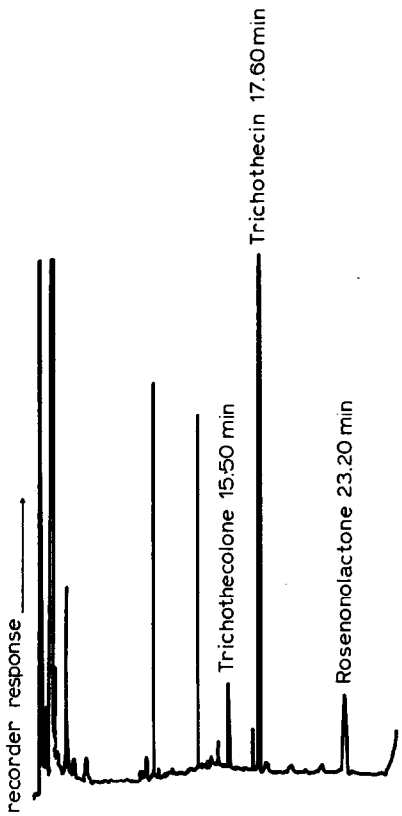

Fig. 1. Capillary gas chromatogram of wine extract (Ruländer), contaminated with mycotoxins.

200 μg/ml, which resulted in analysis differences of less than 7%. The detection limit was 50 μg/l. The analysis of every wine extract was done twice. The retention times were 15.50 min for trichothecolone, 17.60 min for trichothecin and 23.30 min for rosenonolactone (Fig. 1).

All toxins were stable in methanolic extracts for 2 weeks (differences less than 5%), which was important for storage in laboratory routine analysis.

In the extracts from the twenty wine varieties, no substances were found to interfere with the toxins.

Now we are preparing an extensive wine research programme, in which the C_{18} extraction and the gas chromatographic method will be used to search for trichothecin and rosenonolactone in wines from the German wine growing region "Rheinpfalz".

REFERENCES

1 Y. Ueno, *Trichothecenes*, Elsevier, Amsterdam, 1983.
2 J. Lacey (Editor), *Trichothecenes and Other Mycotoxins*, Wiley, Chichester, 1985.
3 A. P. Snyder, *J. Food Prot.*, 49 (1986) 544.
4 G. G. Freeman, *J. Gen. Microbiol.*, 12 (1955) 213.

5 J. R. Bamburg, *Adv. Chem. Ser.*, 149 (1975) 144.
6 B. Achilladelis and J. R. Hanson, *Phytochemistry*, 8 (1969) 765.
7 P. Flesch, G. Stockinger and I. Voigt-Scheuermann, *WeinWiss.*, 3 (1986) 182.
8 S. Schwenk and B. Altmayer, *Z. Lebensm.-Unters.-Forsch.*, 181 (1985) 304.