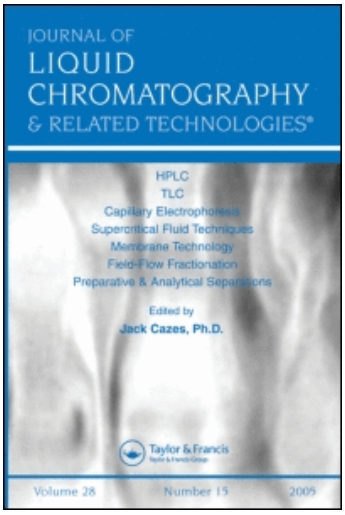

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Detection of Phomenone by High-Performance Liquid Chromatography in Tomato Plants Infected by *Phoma Destructiva* Plowr

Renato Capasso[a]; Elvira Corrado[a]; Giacomino Randazzo[a]; Antonio Bottalico[b]
[a] Istituto di Chimica Organica e Biologica dell'Università di Napoli, Napoli, Italy [b] Istituto Tossine e Micotossine da parassiti vegetali del Consiglio Nazionale delle Ricerche, Bari, Italy

To cite this Article Capasso, Renato , Corrado, Elvira , Randazzo, Giacomino and Bottalico, Antonio(1984) 'Detection of Phomenone by High-Performance Liquid Chromatography in Tomato Plants Infected by *Phoma Destructiva* Plowr', Journal of Liquid Chromatography & Related Technologies, 7: 5, 935 — 942
To link to this Article: DOI: 10.1080/01483918408074015
URL: http://dx.doi.org/10.1080/01483918408074015

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(5), 935-942 (1984)

DETECTION OF PHOMENONE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN TOMATO PLANTS INFECTED BY PHOMA DESTRUCTIVA PLOWR.[+]

Renato Capasso, Elvira Corrado and Giacomino Randazzo

Istituto di Chimica Organica e Biologica dell'Università di Napoli, Via Mezzocannone 16, 80134 Napoli, Italy.

Antonio Bottalico

Istituto Tossine e Micotossine da parassiti vegetali del Consiglio Nazionale delle Ricerche, Via G. Amendola 197/F, 70126 Bari, Italy.

ABSTRACT

Identification and quantitative determination of phomenone in ethyl acetate extracts from Tomato plants infected by Phoma destructiva Plowr., was carried out by high-performance liquid chromatography with UV detection, and ethanol-water (30/70, v/v) as mobile-phase on Perkin-Elmer RP-18/10 stainless column, at 20°C and 0.9 ml min^{-1} flow rate. Detection limit was 5.5 ng, with standard deviation of ± 3%, and retention time of 6.22 min. Analysis of extracts from spiked tomato fruits shows the same parameters. The method appears to be adequate for detection and quantitation of phomenone in contaminated tomato leaves and fruits using a pre-column packed with Lichroprep RP-18/25-40 μm.

[+]This research has been supported by the Italian National Research Council. Special "ad hoc" program "Chimica Fine e Secondaria" - Subprojiect Ca.

 0148-3919/84/0705-0935$3.50/0

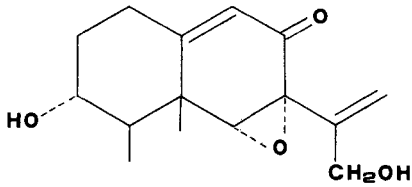

Fig. 1 - Chemical structure of phomenone.

INTRODUCTION

Phomenone (Fig. 1) is an eremophilanic type sesquiterpene produced by some phytopathogenic Phoma species (1,2,3). It was recently identified as the phytotoxin of Phoma destructiva Plowr., the causal agent of a wilt disease of Tomato, and found in wilted leaves of infected Tomato plants (4-6).

Because of the toxicity of phomenone to plants and animals (7,8) and of its potential occurrence in agricultural commodities infected by Phoma species and their fruit-rotting isolates (9-13) the development of a simple and rapid analytical method is of importance. This paper reports on the identification and quantification of phomenone by high-performance liquid chromatography (HPLC) in infected tomato leaves and fruits. A method based on three steps, i.e.: extraction of toxin by ethyl acetate; purification by a Lichroprep RP-18 column; and chromatographic separation and quantification by HPLC was developed.

EXPERIMENTAL

Reagent. All reagents were analytical-grade chemicals. HPLC grade water and ethanol for eluting solvents were purchased from Fluka

AG. A pure sample of phomenone was purified from culture filtrates of P. destructiva as previously described (5). A solution of 4.3 mg of toxin in 100 ml methanol was used as standard.

Extraction and preliminary purification of phomenone from Tomato plants.

The infected material was obtained from artificially infected Tomato plants as reported elsewhere (6). The lyophilized plant material was extracted 4 times, with ethyl acetate (32 ml/g), at room temperature, in a Warring blendor, for 5 min. The combined ethyl acetate extracts were filtered through a sintered glass funnel and then evaporated under reduced pressure to give a green powder. This residue was dissolved in a small volume of methylene chloride, and the solution mixed with Lichroprep RP-18 (Merck, particle size 25-40 µm). Then the mixture was evaporated and the dry residue was loaded on a Lichroprep RP-18 small column (cm 10 x 0,8), which was eluted under vacuum water-pump with a single volume of water-ethanol (70:30, v/v). The colourless extract was concentrated and analyzed by HPLC.

Extraction and preliminary purification of phomenone from tomato fruits.

A pure sample of phomenone (0.1 mg) and ethyl acetate (192 ml) were added to lyophilized tomato juice (6g). This mixture was treated as described above for the plant material.

Thin-layer chromatography.

Analytical TLC was performed on silica gel plates (Merck, Kieselgel 60, F_{254}, 0.25 mm, eluted with ethyl acetate-n-hexane, (90:10); or with chloroform-methanol (85:15), and on K/C_{18} F plates (Whatman, 0.25 mm, eluted with water-ethanol (70:30), or with acetonitrile-water (90:30). After elution, the chromatograms were air dried and observed under 254 nm UV light. Phomenone appeared as quenched fluorescence spot and was visualized as blu-green spot on chromatograms sprayed with 5% sulforic acid and 3% phosphomolybdic acid in methanol, and heated for 10 min at 110°C.

High-performance liquid chromatography.

A Perkin - Elmer series 3B microcomputer controlled pump module, equipped with a variable wavelenght Perkin-Elmer LC-75 Spectrophotometric Detector set at 240 nm was used in connection with a Perkin-Elmer Sigma 10B chromatografic data station. Liquid chromatografic separations were performed on a prepacked Perkin--Elmer RP-18 (particle size 10 μm) column (cm 25x0.46, stainless steel). Analyses were performed at 20°C, employng a mixture of water-ethanol (70:30, v/v) as mobile-phase at 0.9 ml min^{-1} flow rate.

RESULTS AND DISCUSSION

Before performing the analysis on the previously decolorized tomato extracts, the optimum conditions of phomenone analysis were determined.

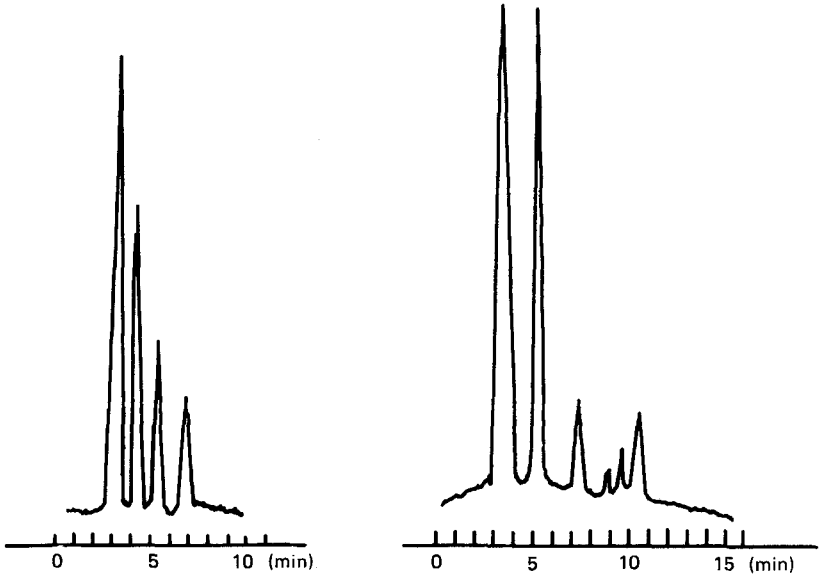

Fig. 2 - a) Chromatogram of extract from healty tomato fruits.
b) Chromatogram of extract from healty Tomato plant.
Column: Perkin-Elmer RP-18 (cm25x0.46, 10 μm) at 20°C. Mobile phase: water-ethanol (70:30, v/v) at 0.9 ml min^{-1} flow rate. Detector: UV absorption at 240 nm.

The minimum detectable amount of the phytotoxin was 5.5 ng, and the retention time was 6.22 ± 0.05 min. The standard deviation calculated from eight repeated injection with four different volumes, was in the range of ± 3%. Linear calibration curve from 0-50 ng.

The analysis performed on healthy tissue extracts no peak with retention time as phomenone (Fig. 2).

Furthermore, the analysis of healthy plant and fruit material spiked with phomenone led to a recovery of 85%. Finally, the

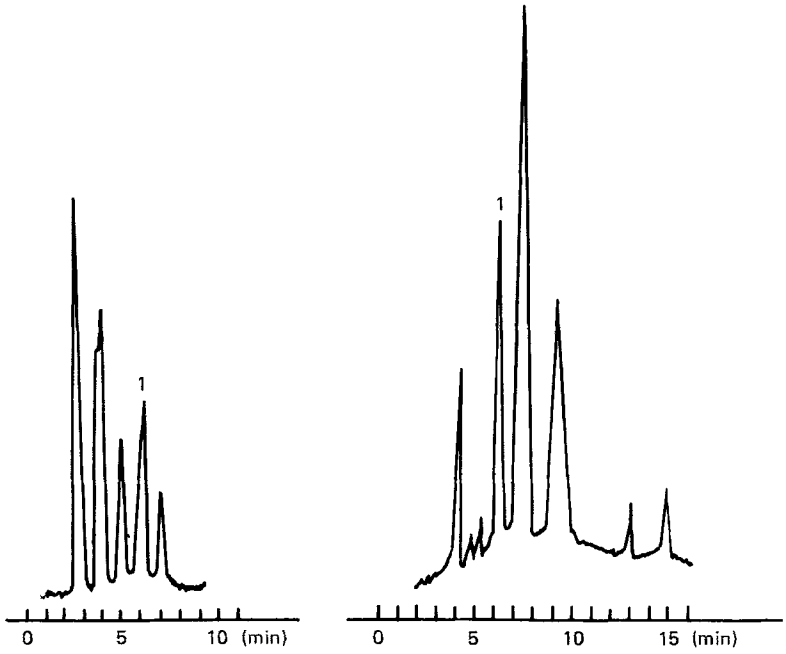

Fig. 3 - a) Chromatogram of extract from tomato fruits added with phomenone.

b) Chromatogram of extract from Tomato plant infected by Phoma destructiva.

1, phomenone (6,22 min). Column: Perkin-Elmer RP-18 (cm 25x0.46, 10 µm) at 20°C. Mobile phase: water-ethanol (70: 30, v/v), at 0.9 ml min^{-1} flow rate. Detector: UV absorption at 240 nm.

analysis performed on the extracts from Tomato plants infected by P. destructiva and on the extracts from tomato fruits spiked with phomenone led to identification of phomenone, by the appearance of an individual peak at 6.22 min (Fig. 3).

The nature of the compound responsible of this signal was confirmed by co-injection of the positive extracts with a reference sample of

phomenone. The spectroscopic, physicochemical and chromatographic properties of a purified fraction of infected plant extracts collected from the liquid chromatograph at the phomenone retention time were identical to those of a reference sample of toxin. Moreover, the contaminated extracts of the Tomato plants, treated with a mixture of acetic anhydride and pyridine, showed, by HPLC analysis, a compound with chromatographic properties identical to a pure sample of acetylphomenone.

In Tomato plants infected by P. destructiva, phomenone was found in amount up to 0.096 mg of toxin per Kg of plant, fresh weight.

Conclusions

High-performance liquid chromatography represents a feasible method for separation and determination of phomenone in infected tomato leaves and fruits.

This technique can be regarded as applicable to agricultural commodities infected by toxigenic Phoma species.

REFERENCES

(1) J.F.Bousquet, M.Barbier, Phytopath.Z. 75, 365(1972).

(2) G.F.Bousquet, Ann.Phytopathol. 5, 289(1973).

(3) C.Riche, C.Pascard-Billy, M.Devys, A.Gaudemer, M.Barbier, J.F. Bousquet, Tetrahedron Lett. 32, 2765(1974).

(4) A.Bottalico, S.Frisullo, N.S.Iacobellis, R.Capasso, A.Evidente I.Iasiello, G.Randazzo, Phytopath.medit. 22, 00(1983) (in press).

(5) A.Bottalico, S.Frisullo, P.Lerario, G.Randazzo, R.Capasso, Phytopath.medit. 21, 39(1982).

(6) A.Bottalico, S.Frisullo, N.S.Iacobellis, R.Capasso, E. Corrado G.Randazzo, Phytopath.medit. 22, 00(1983) (in press).

(7) S.Moreau, Y.Moulè, J.F.Bousquet, Ann.Nutr.Alim. 31, 881(1977).

(8) Y.Moulè, S.Moreau, J.F.Bousquet, Chem.Biol.Interactions 17, 185 (1977).

(9) G.Govi, Ann. Sperim.Agr. 8, 455(1954).

(10) D.Ceci, Ind.Ital.Cons.Alim. 30, 113(1955).

(11) G.H.Boerema, M.M.J.Dorenbosch, Stud.Mycol.Baar 3,1 (1973).

(12) E.P.Obrero, E.E.Trujillo, M.Aragaki, Pl.Dis.Reptr. 52, 946. (1968).

(13) F.A.Langton, Physiol.Pl.Pathol. 1, 477 (1977).