

Short Communication

Detection and Identification of Tridemorph Fungicide by Gas-Liquid and Thin Layer Chromatography: A Case Study

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ABSTRACT

Tridemorph is a widely used fungicide for crops, and is fairly frequently encountered in forensic toxicology. The aim of this paper is to describe the postmortem changes in a case of death due to tridemorph poisoning, the procedure of extraction from forensic samples, and detection and identification by thin layer and gas-liquid chromatography using a new solvent system and spray reagent, on the basis of an actual case of tridemorph fungicide exposure death.

Key Words: Tridemorph, Fungicide, Chromatography

Introduction

The German multinational company BASF developed tridemorph as a fungicide in the 1960's under the trade name 'Calixin' in Europe. Tridemorph is a synthetic heterocyclic fungicide used in the cultivation of numerous crops in many countries around the world. It is used to control the fungus *Erysiphe graminia* in cereals, *Mycosphaerella* species in banana, and *Ceticum solmonicolor* in tea. Tridemorph is also used against powdery mildew on cereals and roots.

Tridemorph is a synthetic heterocyclic fungicide (2,6-Dimethyl-4-tridecyl morpholine) having the chemical formula $C_{19}H_{39}NO$.

The acute oral LD_{50} for Tridemorph is said to be 650 mg/kg for rats.¹ Various chromatographic procedures

(TLC and GLC) have been recommended for the identification of this fungicide in samples.²⁻⁵ In the present study, a routine thin layer and gas-liquid chromatographic method has been suggested for the identification of tridemorph, using a new solvent system and spray reagent.

Materials and Methods

Extraction Procedure: The stomach along with its contents, small intestine with contents, and lung pieces were ground in a mixer. The ground viscera was refluxed with n-hexane and acetone (5:5) for 6 hours at controlled temperature. The refluxed solvent was cleaned up by alumina-packed glass column, using ethyl alcohol as solvent. The ethyl alcohol portion was then dried in a water bath, and diluted with more ethyl alcohol to obtain 0.5 mL purified sample.

Thin Layer Chromatography: 0.25 mm thick plates were prepared using slurry of 20 g silica gel 'G' (BDH 13% calcium sulphate as binder) by dissolving in 40 mL de-ionised water. The plates were dried in air for ten minutes, and activated for an hour at 110°C. The solvent system and locating reagent used were respectively n-hexane: acetone: diethyl ether (70: 20: 10), and methanolic solution of 0.2% fast blue "B" salt. Tridemorph fungicide of BASF India Limited was diluted with ethyl alcohol and used as the standard. Both the standard sample and the clean-up concentrated sample from viscera in ethyl alcohol were applied on silica gel "G". The TLC

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chromatogram was developed with solvent system n-hexane: acetone: diethyl ether (7: 2: 1) at a height of 10 cm. The plate was dried at room temperature, sprayed with prepared fast blue "B" salt, and heated at 100°C for 10 minutes.

Gas-liquid Chromatography: Gas chromatograph of Perkin Elmer (model F-11) with FID detector was used for the identification of the fungicide, with the constants shown in **Table 1**. The gas-liquid chromatogram was developed for 5 minutes, for the extracted clean-up and standard sample in ethyl alcohol.

Table 1 Constants for gas-liquid chromatography

S No.	GLC Constants	Conditions
1	Flow of nitrogen	30 in/lb ²
2	Flow of hydrogen	20 in/lb ²
3	Sensitivity	50x10 ²
4	Chart speed	5 mm/min
5	Oven temperature	110°C
6	Injection temperature	80°C
7	Sample size std	1 microlitre
8	Clean up sample from viscera	1 microlitre
9	Detector	FID
10	Column	OV-17

Results and Discussion

The viscera of the deceased were found acidic in nature, and the postmortem changes were similar to those seen in insecticide poisoning. The Rf values were calculated for the standard and extracted cleanup sample. These values are listed in **Table 2**.

The solvent system used in this case was found to be quick acting, with good resolving power. The location reagent gave multicoloured spots for the differentiation. The Rf values obtained for standard sample and purified sample corresponded with each other. The gas-liquid chromatogram showed two peaks for each standard and clean up sample at retention time (RT) 3.01 min and 4.1 min. The first peak obtained was found as a shoulder peak, while the second peak at 4.1 min was a sharp peak. There is little data about tridemorph in the public domain in spite of several years of use. The solvent system developed in this case, locating reagent, and gas-liquid chromatography data can be recommended for the routine identification of tridemorph fungicide from viscera.

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Table 2 Rf values and colour of TLC and retention time for GLC

S No.	Sample	Rf Values & Colour of the Spot	Rt for GLC in min.
1	Tridemorph std.	0.06 (Y), 0.13(B), 0.16(B), 0.24(Y), 0.26(Y), 0.04(Y), 0.66(Y), 0.77(O)*	3.01, 4.1
2	Purified Sample	0.06 (Y), 0.13(B), 0.16(B), 0.24(Y), 0.26(Y), 0.04(Y), 0.66(Y), 0.77(O)*	3.01, 4.1
*Y=Yellow, B=Blue, O=Orange			