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Journal of Chromatography A, 1103 (2006) 362–367
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

A comparison of a gas chromatographic with electron-capture detection and a gas chromatographic with mass spectrometric detection screening methods for the analysis of famoxadone in grapes and wines

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Received 9 May 2005; received in revised form 3 December 2005; accepted 6 December 2005

Abstract

Famoxadone is a recent oxazolidinedione fungicide widely used in viticulture and in Integrated Pest Management strategies. In this work, after a simple and fast liquid–liquid extraction (LLE), two new gas chromatographic methods were developed to analyze famoxadone residues in grapes and wines, one with electron-capture detection (GC-ECD) and the other with mass spectrometry (GC–MS). Global uncertainties for validation parameters of both methods were compared. Limits of detection (LODs) were 0.06 and 0.02 mg/L, precision was not above 11.7 and 6.8% and recoveries were, on average, 103% ± 12 and 96% ± 12, respectively, for the GC-ECD and GC–MS methods. Similar expanded uncertainties in the range from 0.25 to 1.00 mg/L were below 35%, with increasing values for lower levels of famoxadone. GC–MS method had a lower LOD and a lower uncertainty if compared with the GC-ECD method.

Keywords: Famoxadone residues; Grapes; Wine; Method validation; Uncertainty; GC-ECD; GC–MS

1. Introduction

Famoxadone (3-anilino-5-methyl-5-(4-phenoxyphenyl)-1,3-oxazolidine-2,4-dione) is a new oxazolidinedione fungicide announced in 1996 and first sold in 1997, arriving later to different countries [1]. The chemical structure, the octanol–water partition coefficient (log *P*) and the solubility in water of famoxadone are presented in Fig. 1 [2].

This active ingredient is effective against a broad spectrum of plant pathogenic fungi. Famoxadone is used in grape, cucurbit, potato, tomato, to control downy mildew, late and early blights, wheat leaf, glume blotch and barley net blotch. The fungicidal activity of famoxadone consists in the inhibition of the mitochondrial respiration by binding at the so-called Q_0 site of cytochrome *b*, located in the inner mitochondrial membrane of the fungi [3]. This fungicide has similarities in terms of resistance, chemistry and biochemical mode of action with an important fungicide class widely used in Europe named strobilurins [4].

The maximum residue limit (MRL) set for famoxadone in grapes is 2 mg/kg [5].

The strobilurin fungicides have been analyzed by different analytical methodologies in different matrices, such as grapes and wines, mainly comprising LLE [6,7], solid-phase extraction (SPE) [8,9] and solid-phase microextraction (SPME) [9,10], prior to chromatographic techniques. Residues analysis of this class of fungicides has been carried out by GC-ECD [11,12], GC-NPD [6,12], GC–MS or liquid chromatography with mass spectrometry (LC-MS) techniques [7,8,10].

To our knowledge, only one analytical method is reported in the literature to determine famoxadone residues in grapes and wines. This method is characterized by a laborious and time-consuming LLE sample preparation, centrifugation, evaporation, gel permeation chromatography (GPC) steps followed

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doi:10.1016/j.chroma.2005.12.022

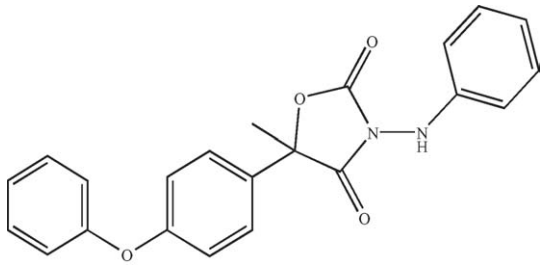

Fig. 1. Chemical structure, molecular weight, log *P* and solubility in water of famoxadone MW = 374.4; log *P* at 20 °C = 4.6; solubility in water at 20 °C = 52 μg/L.

by the GC-ECD analysis and, requiring the use of non-environmental friendly solvents [13].

Presently, simpler methods, more easily implemented by quality control laboratories, are advised, aiming the reduction of the use of organic solvents and tedious steps and, consequently, the improvement of the uncertainty associated with the final results.

Therefore, in this work, a simple extraction methodology using a single LLE step without further cleanup was developed. Sample extracts were analyzed using the two chromatographic methods and a comparative study of THE uncertainties associated to these new analytical methods was performed. The methods developed are simpler, faster and cheaper if compared with the previous described methodology. Being aware that simpler methods may have disadvantages in terms of, for example, selectivity and sensitivity, conducting to the presence of interfering compounds and giving poorer chromatographic resolution, special care was focused on that issue.

2. Experimental

2.1. Chemicals and materials

The analytical standard of famoxadone (99.4% purity) was obtained from the manufacturer DuPont Crop Protection (Newark, DE, USA). Acetonitrile LiChrosolv isocratic grade for GC (≥99.8% purity) was purchased from Merck (Darmstadt, Germany). Hexane (≥95% purity) and ethyl acetate (≥99.8% purity) were Pestanal Grade for residue analysis from Riedel-de Haën (Seelze, Germany).

2.2. Standards preparation

A fungicide stock solution (1 g/L) and a working solution (100 mg/L) were prepared in acetonitrile and stored in dark glass vials, at −20 and 4 °C, respectively. Calibration standard solutions (0.05, 0.1, 0.25, 0.50, 0.75 and 1.00 mg/L) for the GC-ECD and GC–MS analysis were prepared daily for calibration purposes, evaporating to dryness portions of the working solution under gentle nitrogen stream and taking up the remained residue with the extract obtained from the matrix (untreated grapes and wine without famoxadone). A calibration standard of 0.50 mg/L was prepared daily, in the same way, for control quality assessment.

2.3. Samples extraction

Fresh grapes were stemmed manually and crushed using a blender. For extraction procedure, grape and wine samples were measured (5 g and 5 mL, respectively) in 40 mL screw-capped tubes and 10 mL of ethyl acetate/hexane (50:50, v/v) was added. Tubes containing the mixture were agitated for 15 min in a rotary shaker (FALC Instruments, Bergamo, Italy) at 9 rpm and at room temperature. The phases were allowed to separate, and the organic phase was removed and injected for the chromatographic analysis.

For recovery evaluation purposes, grape and wine samples were spiked with 0.10, 0.50 and 1.00 mg/L of famoxadone and analyzed in triplicate.

2.4. Apparatus and chromatography

For the GC-ECD analysis of famoxadone, a ThermoQuest CE Instruments (Milan, Italy) HRGC 8560 Mega 2 Series gas chromatograph, consisting of an AS 800 autosampler, a SSL71 capillary split-splitless injector and an ECD 850 detector, was used. This system was coupled with a Hewlett-Packard (Avondale, PA, USA) HP 3396 Series II integrator. The analytical column used was a J&W Scientific DB-17MS fused silica capillary column (Folsom, USA) with 15 m of length, 0.25 mm of internal diameter and 0.15 μm of film thickness. The temperatures of the injector and of the detector were set at 200 and 300 °C, respectively. The oven temperature programme was as follows: initial temperature was kept at 150 °C for 1 min, increased to 280 °C at 10 °C/min and held at this temperature for 7 min. Total run time was 21.0 min. The volume of sample was 2 μL, injected in split mode (1:10). Helium (99.998% purity) was used as carrier gas and nitrogen (99.999% purity) as makeup gas, at constant head pressure of 100 and 150 kPa, respectively.

The GC–MS analysis of famoxadone was carried out on a Hewlett-Packard (Palo Alto, CA, USA) HP 5890 Series II gas chromatograph system, equipped with a HP 7673 GC/SFC autosampler, a 19251A capillary split-splitless injector and a GC–MS HP 5971 Series detector. The separations were performed using the same column used in the GC-ECD determinations. The injector temperature was set at 300 °C. The oven temperature program was as follows: initial temperature was kept at 200 °C for 1 min, increased to 300 °C at 10 °C/min and held at this temperature for 2 min. Total run time was 13.0 min. The volume of sample was 2 μL, injected in splitless mode with splitless time of 1 min. Helium (99.9999% purity) was used as carrier gas at constant flow rate of 0.8 mL/min. The mass spectrometer was operated in electron impact (65 eV of ion energy), with 10 min solvent delay, the interface temperature was kept at 300 °C, and the ion source temperature was kept at 180 °C. The dwell time for the ion monitoring was 100 ms per ion. Selected monitoring ion (SIM) mode was used. The ions utilized for quantification of famoxadone were *m/z* 196, 224, 330 and 374 amu.