HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY FOR CONVENIENT ANALYSES OF POLAR PESTICIDES

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Pesticides are used in plant protection products. Once applied appear the question of their dosing both in plants intended for consumption and the environment. Liquid chromatography may be successfully used for the analysis of nonvolatile and thermolabile pesticides. The most common type used is reversed phase (RP-HPLC). However, very polar and ionic pesticides shall not be retained in RP-HPLC. Therefore, a convenient solution is HILIC. Lipophilic Hydrophilic Chromatography is a type of liquid chromatography that used mobile phases for RP-HPLC on normal-phase stationary phases. This paper aims to highlight applications of HILIC in the analysis of a polar pesticide using liquid chromatography coupled with mass spectrometry.

Keywords: HILIC, liquid chromatography-mass spectrometry, pesticides residues.

INTRODUCTION

Modern analytical chemistry is dominated by separation techniques. For pesticides' analysis of have been posted two techniques for separating: gas chromatography and liquid chromatography. Gas chromatography, as the first separation technique with a long tradition, may be successfully applied to the analysis of volatile and thermostable pesticides (Niessen, 2001). It is worth noting that the first generation pesticides consist with those possibilities analysis by gas chromatography. Besides the main organochlorine pesticides (DDT, lindane) are persistent organic pollutants. A measure of the polarity of organic molecules is the octanol-water partition coefficient (K_{ow}) expressed as logaritm (Leo *et al.*, 1971). This parameter has been defined in particular for toxicological reasons to measure the extent of penetration of substances that pass the brain barrier. Then this parameter was correlated with year retention on reversed-phase liquid chromatography. Fosetyl is a very polar molecule, characterized by the log P = -2.7, that means that is highly soluble in water and practically insoluble in any organic solvent. It is used extensively as a fungicide for fruit and vegetables. The main advantage of fosetyl toxicity is low toxicity (Tomlin, 2003). As a general principle of retention in reversed phase liquid chromatography on C18 column, the retention time increases with increasing of log K_{ow} (or logP). This is true in general for logP > 1 But there are many highly polar organic substances (sugars, water soluble vitamins) or ionic (amino acids, nucleotides) that have logP <1 or negative. There is no retention on reverse-phase liquid chromatography for these substances. For this reason for the ionic substances it is preferable ion exchange chromatography. Fosetyl was successfully analysed by this method (Metrohm). However, ion-exchange chromatography is especially suitable when we use conductivity detection and most times it is necessary to suppress the mobile phase noise. To obtain retention of very polar substances and ionic in liquid chromatography we can use a very elegant mode with stationary phases for normal phase liquid chromatography with mobile phases for reversed phase. Such conventional silica column with mobile phase gradient from 97% acetonitrile, 3% water (100 mmol of ammonium formate pH 3 with formic acid) was successfully used to analyze free amino acids in aqueous solutions (Majors, 2014). The most often used for formulated plant protection products analysis is high performance liquid chromatography with ultraviolet-visible detection. Unfortunately, fosetyl has no UV

chromophore and is therefore difficult to analyze from formulated products due to interference of coformulants and other active substances that are present in products mix. As an alternative to UV-VIS detection mass spectrometry is used more extensively for polar and ionic analytes due to suitable interfaces such as electrospray (ESI). In this paper we aimed to develop a method for the analysis of fosetyl-based products by high performance liquid cromatografy coupled with mass spectrometry, and a method applicable to scale traces.

EXPERIMENTAL, RESULTS AND DISCUSSION

High performance liquid chromatography was carried out with a liquid chromatograph consisting of a ternary pump ProStar 240 SDM, an automatic injector Prostar 430 and a mass spectrometer 1200 L / MS / MS with electrospray interface, all from Varian. Ultrapure water was obtained from a DirectQ purifier (Millipore), acetonitrile and formic acid from Sigma, ammonium formate from Roth. Chromatography column was Polaris Amino (Varian) 150x4,6 mm (Lxi.d.) and 3 µm particles. The mobile phase consisted of water (100 mM ammonium formate pH = 3.7 with formic acid) 75% and 25% acetonitrile at a flow rate of 2 ml/min. Before entering the ESI interface was made a division of the flow of mobile phase 1/10, so to the reach only 0.2 ml / min of mobile phase. Drying gas was air at 21 psi and 400^0C, nebulizer gas was nitrogen at 42 psi, ESI needle was subjected to a voltage of -4500 V. The mass spectrometer was programmed in MRM (Multiple Reactions Monitoring) using Ar as collision gas. Fosetyl reference and sample Profiler 71.1 WG were from Bayer. Calibration of the mass spectrometer was performed with solutions between 5.3-28.5 µg/ml for macro analysis and 113-576 ng / ml for traces. Before all of that was performed parameters optimization by infusion of a solution containing ~ 1 µg/ml fosetyl. Breakdown curves from fig. 1 were used as basis of MRM merged program for chromatographic aquisition using three MRM (109 to 81 12 CE, 109 to 79 20 CE, 109 to 63 22CE).

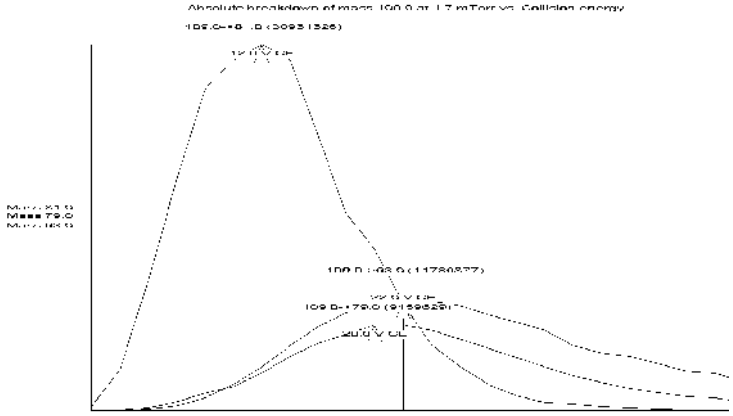

Figure 1. Breakdown curves for molecular ion [M-H]- of fosetyl m/z=109 to give main fragments m/z=81 (ethene loss), m/z=63 (ethylene oxide loss)

By replicated injections of calibrations solutions for *macro* analysis was obtained a linear calibration curve with a good parameters, comparable by one obtained in HPLC-UV detection presented in fig. 2.

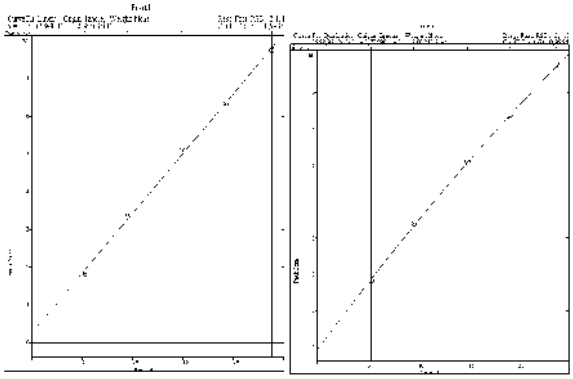

Figure. 2 Calibration curves with linear and quadratic fitting model for macro analysis

Relative standard deviation of response factors is good for a LC-MS external standardization method with a good correlation coefficient. Product *Profiler 71.1 WG* is a granular material consisting in a mixture of 66.67 % fosetyl, 4.44 % fluopicolid and some detergents for dispersing. By analyzing a *Profiler* sample, using instead MRM a full-scan MS program we can see that the potential interferences were removed by filtering information. Reconstructed ion chromatogram (RIC) for ion *m/z* = 109, corresponding to fosetyl, and for ions *m/z* = 381, 383, 385 corresponding to fluopicolid are presented in figure 3. Sample analysis is biased with 5 % because results were between 70-74%. A possible cause could be that calibration was *external.*

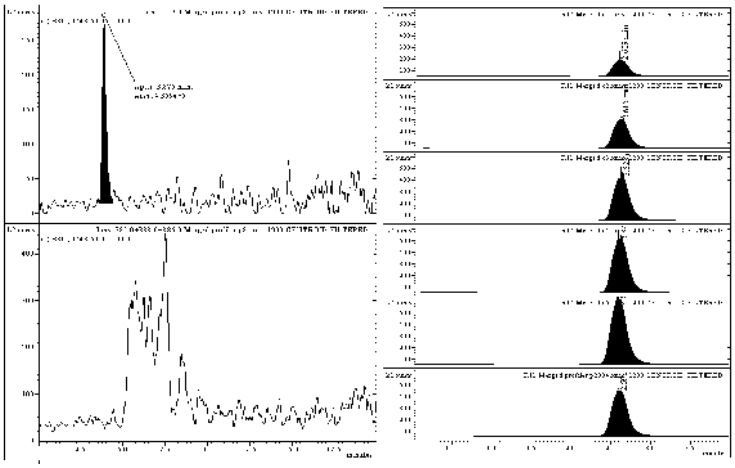

Figure 3. Extracted ion chromatogram for m/z=109 (fosetyl) and m/z=381,383,385 (fluopicolid) and stacked peaks from filtered MRM analyses

For traces calibration curve was not so easy to obtain because of highly noise baseline. However, the most diluted solution still gave a chromatographic peak with a signal/noise ratio S/N=52. Despite that detection limit was defined as the concentration that will generate S/N=3, it was very difficult to obtain such as peak. This time was obtained as most fitted model a quadratic curve, demonstrating the range of analyses was near the start of dynamic range. So we consider that as quantification limit 100 ng/ml because the chromatographic peak still remain with a good shape and exceed the noise (figure 4). Fragmentations pattern is almost the same for the first and for the last calibration level.

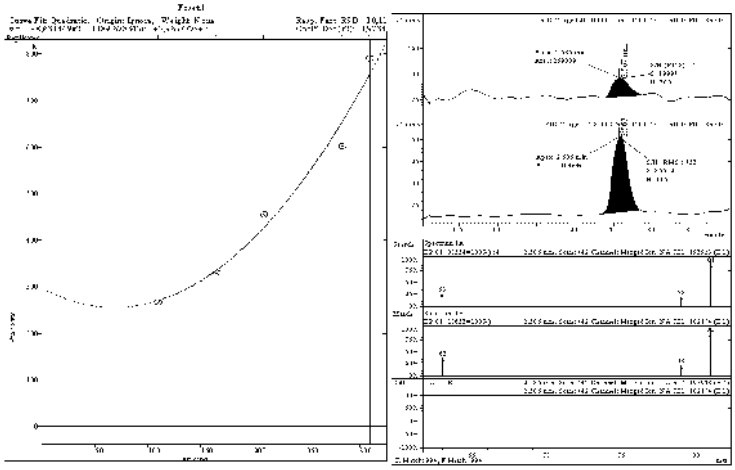

Figure 4. Trace analysis quadratic curve and fosetyl first and last calibration chromatographic peaks. Fragmentations patterns was maintained for all calibration levels

CONCLUSIONS

Using HILIC MS/MS for fosetyl anlysis from formulated product, were obtained comparable parameters with that from an HPLC-UV analysis. Main advantage was selectivity and the main disdvantage was the biased results. Probably using an appropriate internal standard and checking bias with another reference material the method will gain trueness. For residues from water or extracts from soils and plants was developed a trace analysis. Possible improvements could be using a narrow-bore column and concentration step before determination.

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