

Separating and identifying pesticides in milk through Thin Layer Chromatography and Mass Spectrometry

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Abstract

We separated several pesticides (imidacloprid, deltametrin, bifentrin, fenilbutation oxide, captan prepaquizofos, benflutamid, amitraz, hexachlor benzene, metachlor 960, triflurex 400, stamp) on chromatographic plates with diatomitic earth modified chemically C-8, using as a mobile phase a mixture of methanol and water in different ratios. Identifying pesticides was done through UV light examination at 254 nm. The pesticide amitraz was added to the milk and we extracted it 24 h later with methylene hexane-chloride (1:1, v/v) and the extract was applied on the plates together with the standards. Identification was done with the standards and through mass spectrometry.

Keywords: pesticides, milk, thin layer chromatography, mass spectrometry

1. Introduction

Pesticides are chemical substances developed to kill pests such insects, rodents, weeds, fungi, etc. They have been used since times immemorial: thus, sulphur was used 4,000 years ago, in Sumer (Ancient Mesopotamia), 3,000 years ago in China, as a fumigant to destroy certain fungi, and in the 19th century to destroy pulverulent mould on fruits, in Europe. Sulphur is still used nowadays for the same purposes in California (U.S.A.). In the 16th century, the Japanese used a mixture of whale oil and vinegar to destroy the larvae of rice crop pest insects. In the 17th century, tobacco extracts were used to kill certain insects and seeds of *Strychnos nuxvomica* (strychnine) were used to control rodents. Arsenic trioxide was used in the 19th century to destroy weeds and rat poison was used to kill the Colorado beetle.

Again in the 19th century, they used for the first time the Bordeaux mixture (copper sulphate, lime, and water) to control downy mildew produced by *Plasmopara viticola* and sulphuric acid 10% o destroy weeds. After World War II, the production and diversity of pesticides increased steadily.

Though they have used pesticides and other methods to control pests, losses caused by pests in the U.S.A. alone represent 30%, i.e. about 33,000,000,000 UD\$ per year [1]. The synthesis of organ chloride pesticides after 1950, of organophosphoric pesticides after 1960, of carbamates after 1970, of pyrethroids after 1980, and of biopesticides after 2000, resulted in the name of “pesticide era” [2].

The increase of resistance of bacteria to antibiotics and of pests to pesticides threatens to destroy some of the most remarkable progresses achieved in the fields of public health and of agriculture during the 20th century. To compensate the decrease of pesticide activity, they use larger and larger amounts. This, as well as the fact that most pesticides are also toxic for humans, is a major issue for mankind.

Thus, World Health Organisation (W.H.O.) shows that in 1990 there were 3,000,000 poisonings by pesticides resulting in 220,000 deaths. In the U.S.A. alone, there are 80,000 pesticide poisonings per year, most of the victims being agriculturists [3]. Besides these poisonings, pesticides can contaminate food produce and the environment and, thus, damage human health; this is why they have prohibited some pesticides in certain countries.

A basic requirement nowadays is to produce foods in as large amounts as possible, with diminution of pesticide concentration in foods, fodder, and environment. The level of residues in foods or fodder is a danger for the consumers and for the entire food chain [4].

Thus, such international organizations as the United Nations Organization for Food and Agriculture (F.A.O.) the World Health Organization (W.H.O.), and the Codex Alimentarius Commission have developed standards for pesticide concentration in foods and tap water. The Directive of the Council of Europe 98/83/EC decided that total concentration of pesticides in the tap water is between 0.1 µg/L and 0.5 µg/L. Present legislation as well as health issues ask for analysis methods that are specific, selective, and sensitive to monitor pesticides [5].

Traditional analysis methods of trace pesticides need extraction and purifying. These time- and solvent-consuming procedures have been simplified lately. Thus, they take into account the nature of the samples to be analysed, i.e. fat foods should be processed differently from non-fat foods or from vegetal samples with higher or lower sugar or natural colorant contents [6]. Analysing pesticides residues covers methods that allow the identification of a compound or of several pesticides in a sample. This situation is common in international trade with foods and feeds where quality control should be done in accordance with the multi-residue method.

These methods need to be once or twice more sensitive than the maximum limit for pesticide residues established by the health authorities of a country, be characterised by a high capacity of recovery, and by a good reproducibility to be acknowledged internationally [7]. Organ chloride pesticides and their metabolites create serious problems because of their toxicity and persistence [8], which lead to their prohibition some 40-50 years ago in several countries. However, some organ chloride pesticides (DDT and similar compounds) are still used to control the tsetse fly in Africa and malaria. Gas chromatography and liquid chromatography, together with mass spectrophotometry have been used to analyse pesticides in different samples [9]. Organophosphoric pesticides in leak have been analysed through gas chromatography with mass spectrophotometry and flame photometry detection [10], and over 90 pesticides have been identified in fresh fruit through mass gas chromatography-spectrophotometry [11].

Pesticides in water, soil, and food products have been analysed through multi-dimensional gas and liquid chromatography [12]. Thus, Dalluge *et al.* [13] have determined pesticides in food extracts through 2-D gas chromatography using mass spectrometry in detection. By coupling column chromatography with plane chromatography [14,15], Tuzimski separated pesticides from water. Tigae *et al.* [16] separated seven pesticides through high-productivity thin layer chromatography (HPTLC), using as a mobile phase a mixture of heptane-chloroform-ethyl-acetate (30:19:1, v/v) in the first direction and heptane-chloroform-ethyl-acetate (30:10:10, v/v) in the second direction. Liquid chromatography and mass spectrometry were used in food safety [17].

Thin-layer chromatography (TLC) has been the most used method of separating and identifying pesticides in different tap water [18,19], soil [20], milk [8], meat and meat products [21,22], fruit, grains, and vegetable samples [22,23].

In this paper, we present a new method of separating and identifying amitraz in milk through thin-layer chromatography.

2. Material and Method

Pesticide solutions 0.1% were prepared in methanol (imidacloprid, deltamethrin, bifenthrin, diquat, metachlor, trifluralin, pendimethalin) and toluene (fenbutation, captan, paraquizaop, hexachlorbenzene, amitraz) (Table 1).

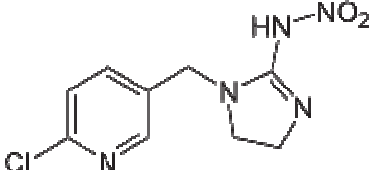

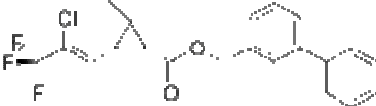
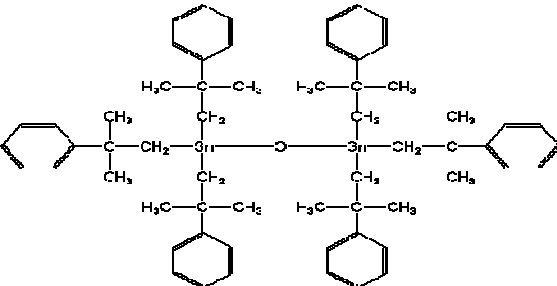
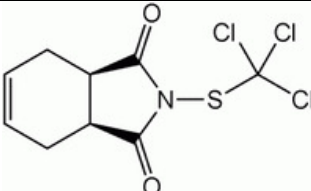
We prepared chromatographic plaques with C-8 chemically modified diatomitic earth by mixing diatomitic earth powder (100 g) with a fluorescence indicator made up of barium and magnesium aluminate activated with europium (4 g) and with yttrium aluminate activated with cerium (3 g).

The homogenised mixture was suspended in a solution of 150 ml ethylic alcohol and 5 g polyvinylpyrrolidone.

The paste thus obtained was set on previously degreased clean glass plaques 0.3 mm thick and let to dry out at room temperature [24]. The plaques have white fluorescence within the UV range. A volume of 5 μ L of the pesticide solutions were applied on the chromatographic plaques with a Brand micro-pipette. The plaques were developed in normal chromatographic rooms using as a mobile phase mixtures of methanol and water in different ratios. The pesticides were visualized by examination in UV light at 254 nm using a Camag lamp.

Table 1. Studied food colorants

Table 1. Studied pesticides

Nr.	Chemical formula	Chemical name	Trade name
1		<i>N</i> -[1-[(6-Chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl]nitramide	Imidacloprid
2		[cyan-(3-phenoxyphenyl)-methyl] 3-(2,2-dibromoethyl)-2,2-dimethyl-cyclopropane-1-carboxylate	Deltamethrin
3		2-Methyl-3-phenylphenyl)methyl (1 <i>S</i> ,3 <i>S</i>)-3-[(<i>Z</i>)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropane-1-carboxylate	Bifenthrin
4		bis[tris(2-methyl-2-phenylpropyl)tin] oxide	Fenbutation-oxide
5		(3 <i>aR</i> ,7 <i>aS</i>)-2-[(trichloromethyl)sulfanyl]-3 <i>a</i> ,4,7,7 <i>a</i> -tetrahydro-1 <i>H</i> -isoindole-1,3(2 <i>H</i>)-dione	Captan

6		2-isopropylideneamino oxyethyl(<i>R</i>)-2-[4-(6-chloroquinoxalin-2-yl)oxy]propionate	Propaquizafop
7		6,7-Dihydrodipyrido[1,2-a:2',1'-c]pyrazinediium dibromide	Diquat
8		Hexachlorbenzene	Hexachlorbenzene
9		<i>N,N'</i> -[(methylimino)dimethylidene]di-2,4-xylylidine	Amitraz
10		2-Chloro- <i>N</i> -(2-ethyl-6-methyl-phenyl)- <i>N</i> -(1-methoxypropan-2-yl)acetamide	Metolachlor
11		2,6-Dinitro- <i>N,N</i> -dipropyl-4-(trifluoromethyl)aniline	Trifluralin
12		3,4-Dimethyl-2,6-dinitro- <i>N</i> -pentan-3-yl-aniline	Pendimethalin

A volume of 5 mL standard solution of amitraz – a pesticide with insecticide and acaricide action used in the control of ectoparasites in cattle and honeybees and that releases, through hydrolysis, 2,4-dimethylamine, a very toxic product for humans and animals [25] – was added to 100 mL milk. After 24 hours, we added 2g NaCl and 4g anhydric MgSO₄ and the mixture was stirred for 10 minutes after which we extracted amitraz in two volumes of 25 mL with hexane-methylene chloride (1:1, v/v). The organic fraction was dry evaporated in a rotavapour. The pesticide residue was solved in 0.5 mL toluene.

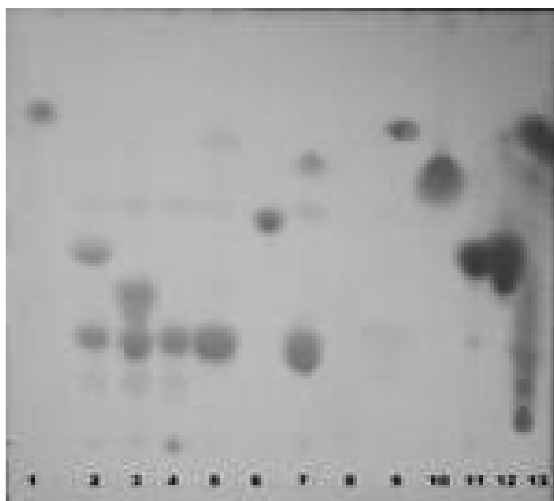


Figure 1. Chromatogramme of pesticide mixture separated on C-8 chemically-modified diatomitic earth with a mobile phase: methanol-water (95:5, v/v); 1- imidacloprid; 2-deltametrin; 3-bifentrin; 4-fenilbutation-oxide; 5-captan; 6-paraquizafof; 7-diquat; 8-hexacolbenzen; 9-amitraz; 10-metaclor; 11-trifluralin; 12-pendimentalin (stamp); 13-milk sample

A volume of 5 μ L of this residue was applied together with the standards and separated on the chromatographic plaques (Figures 1-3).

The first plaque was eluted with the mixture of methanol and water (95:5, v/v) (Figure 1), the second one with a mixture of methanol and water (90:10, v/v) (Figure 2) and the third one with a mixture of methanol and water (85:15, v/v) (Figure 3).

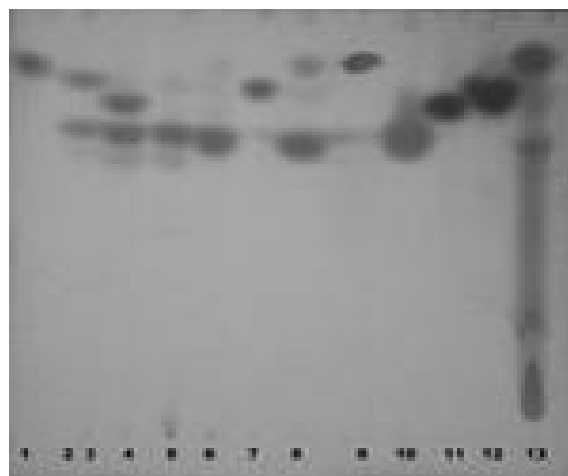


Figure 2. Chromatogramme of pesticide mixture separated on C-8 chemically-modified diatomitic earth with a mobile phase: methanol-water (95:10, v/v); 1-imidacloprid; 2-deltametrin; 3-bifentrin; 4-fenilbutation-oxide; 5-captan; 6-paraquizafof; 7-diquat; 8-hexacolbenzen; 9-amitraz; 10-metaclor; 11-trifluralin; 12-pendimentalin (stamp); 13-milk sample.

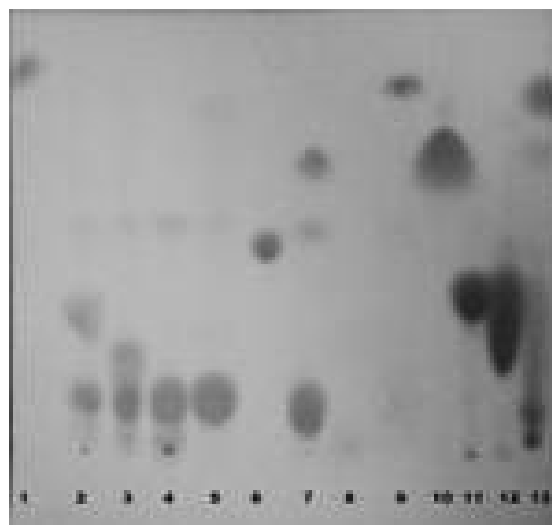


Figure 3. Chromatogramme of pesticide mixture separated on C-8 chemically-modified diatomitic earth with a mobile phase: methanol-water (95:15, v/v); 1-imidacloprid; 2-deltametrin; 3-bifentrin; 4-fenilbutation-oxide; 5-captan; 6-paraquizafof; 7-diquat; 8-hexacolbenzen; 9-amitraz; 10-metaclor; 11-trifluralin; 12-pendimentalin (stamp); 13-milk sample.

The studied pesticide (amitraz) separated through thin-layer chromatography was analysed through gas chromatography after being extracted from the spot using a Hewlett-Packard 5890 chromatograph with mass spectrophotometer (MS-5972).

The gas chromatograph with mass spectrophotometer was equipped with a capillary column (30 m, 0.25 mm ID) with an inner film of 0.25 μm 5% phenyl-polydimethylsiloxane. The carrier gas was helium with a constant flow of 1.0 mL/min, starting temperature 50°C with an increase rate of 5°C/min. Mass spectra were compared with those in the computer library (Woley library 275).

3. Results and Discussion

The best separations were done by using the mobile phase methanol-water (90:10, v/v) (Figure 2): the higher the water concentration, the closer to the front the compounds analysed are. Some pesticides have several spots denoting that the standards are not pure or that they decayed in time. Spot 13 is an amitraz extract from milk.

There are several spots in its composition that represent front amitraz while the rest are hydrolysis products: 2,4-dimethylphenylformamide (DMF), 2,4-dimethylaniline (2,4-DMA) and 3-methylcatecol (3-MC) [26]. The compound 2,4-DMA is genotoxic, hepatotoxic and carcinogenic [27].

The compound having the same value R_F as amitraz was extracted from the spot and analysed through CG-MS. This compound is amitraz, which has also been proved by the mass spectrophotometer and which is identical to that of the Woley spectra library (Figure 4).

4. Conclusions

The method developed can be used in the separation and identification of pesticides in milk. C-8 chemically modified diatomitic earth can be used as a stationary phase in thin-layer chromatography to analyse different substance classes.

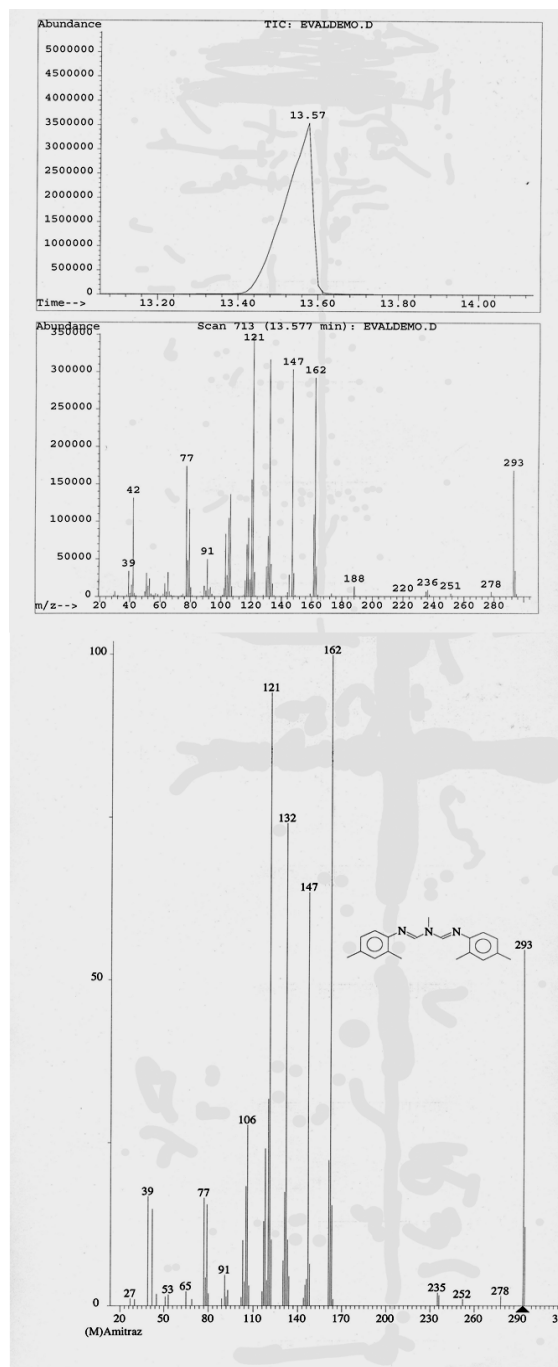


Figure 4. Chromatogramme and mass spectrum of amitraz

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